UNIVERSITÀ DELLA CALABRIA

UNIVERSITA' DELLA CALABRIA

Dipartimento di Farmacia e Scienze della Salute e della Nutrizione

Dipartimento di Chimica e Tecnologie Chimiche - CTC

Dottorato di Ricerca in

Medicina Traslazionale

CICLO

XXX

TITOLO TESI

Design, Synthesis and Characterization of Biologically Active Heterocyclic Organic Compounds

Settore Scientifico Disciplinare CHIMICA ORGANICA (CHIM/06)

Coordinatore: Ch.mo Prof. Sebastiano Andò

Supervisore/Tutor: Ch.mo Prof. Antonio De Nino

Firma

Dottorando: Dott. Vincenzo Algieri

Firma

...ai miei Genitori,

a Cristina...

CONTENTS

SUPERVISOR COMMENTS

UNIVERSITÀ DELLA CALABRIA
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Dottorato di Ricerca in Medicina Traslazionale, indirizzo Progettazione Molecolare, (XXX CICLO)
Giudizio del Supervisore sull'attività del dottorando **Vincenzo Algieri**

Il dott. Vincenzo Algieri ha iniziato il corso di dottorato in Medicina Traslazionale (Indirizzo Progettazione molecolare) il 04/11/2014 con un progetto di ricerca avente come oggetto la sintesi e la caratterizzazione di composti eterociclici attivi biologicamente. Durante i tre anni l'attività ha riguardato lo studio di:

- Nuovi agenti farmacologici mediante reazioni di cicloaddizione 1,3-dipolare.
  - I. Nuclei indolil o ossindolil spiroisossazolidine *N,O*-Nucleosidiche.
  - II. Acidi isossazolidin-*gem*-bisfosfonici.
  - III. 1,2,3-Triazoli.
- Reazioni Organocatalizzate
- Reazioni Solvent-Free e coadiuvate da microonde

Il dott. Vincenzo Algieri ha dimostrato di possedere un'eccellente preparazione di base nelle scienze chimiche e durante il triennio del suo dottorato ha acquisito una profonda conoscenza delle strategie sintetiche nel campo della chimica organica e delle metodologie di purificazione e caratterizzazione di prodotti organici complessi. La sua formazione è stata completata attraverso la frequentazione di tutti i corsi ed i seminari organizzati nell'ambito della Scuola di Dottorato in Medicina Traslazionale.
Il dott. Vincenzo Algieri ha svolto un anno di ricerca (Gennaio- Dicembre 2016) presso il "Laboratorio de Sintesis Asimetrica" del Prof. Pedro Merino, presso la Universidad de Zaragoza, Departamento de Quimica Organica & Departamento de Sintesis y Estructura de Biomoleculas, Instituto de Sintesis Quimica y Catalisis Homogenea (ISQCH), Facultad de Ciencias, Zaragoza (Spagna).
L'attività di ricerca ha consentito la pubblicazione dei seguenti lavori su riviste internazionali.

1. Loredana Maiuolo, Giordana Feriotto, Vincenzo Algieri, Monica Nardi, Beatrice Russo, Marialuisa Di Gioia, Emilia Furia, Matteo Antonio Tallarida, Carlo Mischiati, Antonio De Nino. Antiproliferative Activity of Novel Isatinyl/Indanyl Nitrones (INs) as Potential Spin Trapping of Free Radical Intermediates. *MedChemComm*, **2018**, 000, Impact Factor 2.608. DOI: 10.1039/C7MD00537G.
2. Loredana Maiuolo, Pedro Merino, Vincenzo Algieri, Monica Nardi, Marialuisa Di

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Gioia, Beatrice Russo, Ignacio Delso, Matteo Antonio Tallarida, Antonio De Nino. Nitrones and Nucleobase-Containing Spiro-Isoxazolidines Derived from Isatin and indanone: Solvent-Free Microwave-Assisted Stereoselective Synthesis and Theoretical Calculations. *RSC Advances*, **2017**, 7, 48980-48988, Impact Factor 3.108, DOI: 10.1039/c7ra09995a.

3. Loredana Maiuolo, Antonio De Nino, Vincenzo Algieri, Monica Nardi. Microwave-Assisted 1,3-Dipolar Cyclo-addition: Recent Advances In Synthesis of Isoxazolidines. *Mini-Reviews in Organic Chemistry*, **2017**, 14 (2), 136-142, Impact Factor 1.095 DOI: 10.2174/1570193X14666170206123513.
4. Pedro Merino, Loredana Maiuolo, Ignacio Delso, Vincenzo Algieri, Antonio De Nino, Tomas Tejero. Chemical approaches to inhibitors of isoprenoid biosynthesis: targeting farnesyl and geranylgeranyl phyrophosphate synthases. *RSC Advances*, **2017**, 7, 10947-10967, Impact Factor 3.108, DOI: 10.1039/c6ra28316k.
5. Antonio De Nino, Loredana Maiuolo, Pedro Merino, Monica Nardi, Antonio Procopio, David Roca-López, Beatrice Russo, Vincenzo Algieri. Efficient Organocatalyst Supported on a Simple Ionic Liquid as a Recoverable System for the Asymmetric Diels-Alder Reaction in the Presence of Water. *ChemCatChem*, **2015**, 7, 830-835, Impact Factor 4.803, DOI: 10.1002/cctc.201402973.

L'attività svolta dal dott. Vincenzo Algieri è stata esposta, tramite sette (7) comunicazioni poster e cinque (5) comunicazioni orali, in congressi scientifici nazionali ed internazionali.
Il dottorando Vincenzo Algieri ha dimostrato di aver raggiunto la completa autonomia scientifica sia nel proporre soluzioni ai problemi incontrati che nella razionalizzazione dei risultati ottenuti. Inoltre ha dimostrato di saper lavorare in gruppo in completa armonia con i colleghi di laboratorio.
Il giudizio complessivo sulla formazione raggiunta, sull'attività di ricerca svolta nel corso del dottorato, sulla personalità scientifica e l'attitudine alla ricerca del Dott. Vincenzo Algieri è, pertanto, ampiamente positivo.
Il Supervisore giudica quindi Il dott. Vincenzo Algieri meritevole del titolo di Dottore di Ricerca in Medicina Traslazionale, indirizzo Progettazione molecolare.

Rende 02/02/2018

Il Supervisore
Prof. Antonio De Nino

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REFEREE COMMENTS

Doctoral School in Translational Medicine
University of Calabria
Italy

PhD THESIS ASSESSMENT REPORT

CANDIDATE: *VINCENZO ALGIERI*

TITLE OF THE THESIS: *Design, Synthesis and Characterization of Biologically Active Heterocyclic Organic Compounds*

Criterion 1: Scientific, technical, humanistic, legal or artistic quality (as applicable).

The thesis aims to the synthesis of a series of pharmacologically interesting heterocyclic compounds, such as indolyl or indanyl spiroisoxazolidine N,O-Nucleosides, isoxazolidin-gem-bisphosphonic acids and 1,2,3-Triazoles. Modern, creative solvent free and green methods are implemented for the various syntheses, which is of key potential for the improvement of such procedures and contributes to the advancement of knowledge.

Criterion 2: Novelty, originality and translational outcomes of the thesis.

The thesis in the various subchapters reviews the state-of-the-art in the field and goes beyond contributing in the fields of asymmetric synthesis, catalysis and green methods. Regarding the novelty and originality of this thesis is given and apart my personal opinion is confirmed by the original publications of the results in respectable peer-reviewed journals.

Criterion 3. Results obtained.

The thesis is well organized in separate subchapters focusing on the state-of-the-art in each sub-field followed by the experimental and discussion of the results. The nature of the obtained results is absolutely coherent with the hypothesis of the thesis and contribute to the advancement of knowledge in the field. I would say that the experimental work done and the findings are more than the expected for the period of thesis and it reflects to the quality of supervision of both Prof. De Nino and Prof. Merino.

Criterion 4. Editing and use of language.

The language and spelling of the thesis are of high quality. The list of contents is very detailed and in coherence with the text. Technical and scientific terms are correctly used and the references are adequate and up to date. The quality of the figures, tables and legends is also very good and in coherence with the text referred to them.

FINAL RECOMMENDATIONS CONCERNING THE DEFENCE OF THIS THESIS

I recommend that the thesis should be defended as it is (with no changes)

Reviewer's name and surname(s): Prof. Constantinos Athanassopoulos/ Associate Professor

Date: **Signature**

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ UNIVERSITY OF PATRAS
Digitally signed by Konstantinos Athanasopoulos
Date: 2018.01.28 17:09:10 +02'00'

REFEREE COMMENTS

DEPARTMENT OF ORGANIC CHEMISTRY
ISQCH
CSIC-UNIVERSITY OF ZARAGOZA

The PhD Thesis presented by Vincenzo Algieri to achieve the Doctorate Degree by the Calabria University is an excellent piece of work which comprises several parts in which have been involved various contributions carried out in laboratories of several countries. I would like to emphasize this aspect of the Thesis because it shows the capability and determination of Vincenzo to learn new research methodologies and at the same time adapt to new languages, customs……... I am completely sure that the experience Vincenzo has made during his Doctorate will have a very positive impact in his personal and professional future, especially now that students hardly want to assume the risk of leaving the security of their parents or friends.

The diversity of topics comprised in this work has, in my opinion, additional merit and importance in the academic development made by Vincenzo. All topics are collected in several manuscripts some of them revised by Merino from Zaragoza University where Vincenzo has made a doctoral stay. This very general title includes a quite remarkable contribution in the chemistry of some heterocycles compounds. Most of the one part of the work was developed in the University of Zaragoza (Spain) under the supervision of Prof. Merino, Prof. Tejero and Dr. Delso and it was oriented to cover an important gap in the chemistry of isoxazolidines obtained from dipolar 1,3-cycloaddition. These compounds were prepared for first time a few years ago and since then, they have been synthesised and used in almost any imaginable way. They are particularly important when considering the opening reaction of the heterocyclic ring which allows the synthesis of many interesting compounds, such as amino acids or amino alcohols and products of biological interest. Additionally, the reactivity of the double or triple bond has been exploited for the synthesis of pharmacological interesting compounds. It is important to say that most pharmaceuticals are based on heterocycles. An inspection of the structures of the top-selling brand-name drugs in 2007 reveals that 8 on the top 10 and 71 on the top 100 drugs contain heterocycles. However, although many reactions which involve the use of some intermediates have been studied, only some investigations have dealt with catalysts, including asymmetric organocatalysts and theoretical calculations. The present work pretends to explore this gap which could open enormous possibilities to be further explored. I find particularly attractive the results achieved Lewis catalysts which improve the rate and stereochemistry of the reaction, although first it is important to do a separation of enantiomers. The metal is able to activate a certain position allowing, in that manner, the major rate of the cycloaddition and Diels Alder reaction. The original compounds obtained, the novel reactivity studied and the new amino acids that could be diverted and explored opens many perspectives for future research. It is worth mentioning the introduction where some interesting perspectives of the Diels Alder reaction and the dipolar 1,3-cycloaddition is made. It is important to note that the combination of reactive is so plain than the number of possibilities is enormous. It is worth mentioning, also, the good level in the bibliography used, the detailed experimental provided and the correct treatment of spectroscopical data of compounds.

Vincenzo, although I have not been able to attend the presentation and defence of the Thesis due to a car accident, I am completely convinced of the success of the presentation. I would like to thank your effort and enthusiasm to learn new reactions, products and biological applications and to open new possibilities in organic chemistry. At the same time I would like to wish you a very good luck in your personal and professional future.

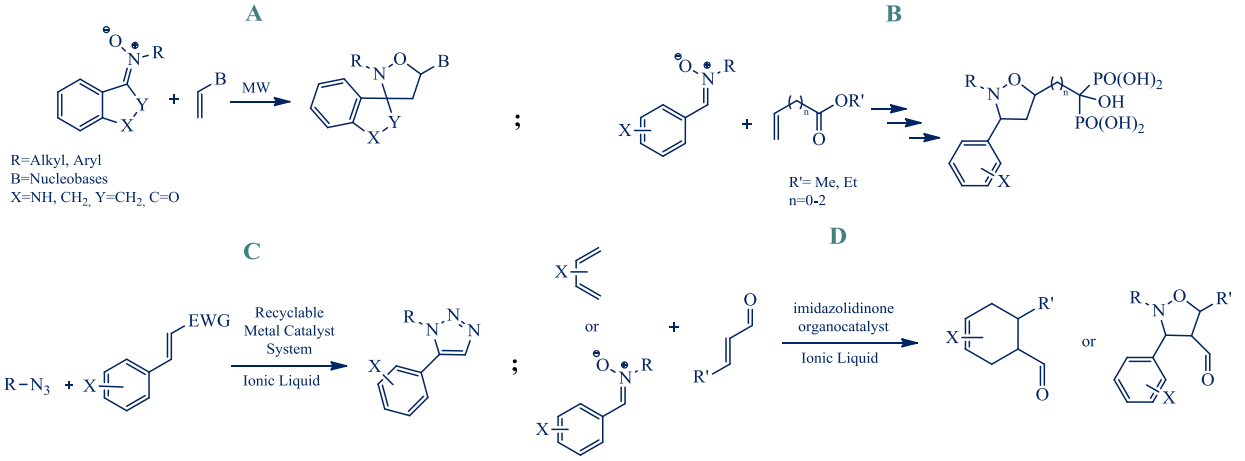
Professor Carlos Cativiela
Zaragoza, February 20th 2009

Doctoral Thesis Abstract: Design, Synthesis and Characterization of Biologically Active Heterocyclic Organic Compounds.

Candidate: Vincenzo Algieri,[a] Supervisor: Prof. Antonio De Nino,[a] Doctoral Coordinator: Prof. Sebastiano Andò.[b]

[a] *Dipartimento di Chimica e Tecnologie Chimiche – CTC, Università della Calabria, Ponte P. Bucci, Cubo 12C, 6° Piano, 87036, Arcavacata di Rende, Italy.* [b] *Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Università della Calabria, Edificio Polifunzionale, 87036, Arcavacata di Rende, Italy.*

The discovery of new pharmacological agents is one of the biggest challenges for the current research. In recent years, the synthesis of heterocycles has been developed by pericyclic reactions[1] in which the product cycloadduct is an interesting pharmacophore that could be the future in biology and pharmacology fields. These pharmacologically active heterocycles can be indolyl or indanyl spiroisoxazolidine *N*,*O*-Nucleosides, isoxazolidin-*gem*-bisphosphonic acids and 1,2,3-Triazoles. Isoxazolidines are five-membered cyclic molecules that mimic natural nucleosides exerting antiviral and antitumoral activity. The addition of a *gem*-bisphosphonate group on the isoxazolidine ring increases the cytotoxicity of the obtained substrates that can be applied in clinical treatment of bone metastases and osteoporosis. The 1,2,3-triazole nucleus represents a significant class of biologically active nitrogen compounds that exhibit the important biological properties, such as antibacterial, anticancer, antivirus, and antituberculosis. In addition, organocatalysis exploits the small organic molecules to increase the speed of the reactions; expecially the covalent catalysis mediated by iminium ion has been used in various applications.



In this doctoral thesis numerous synthesis of all classes of molecules descrived above are reported. In particular, the Solvent-Free Microwave assisted 1,3-dipolar cycloaddition reaction for the synthesis of indolyl or indanyl spiroisoxazolidine *N*,*O*-Nucleosides (**A)** is described.[2] The reactions are conducted with short time (order of minutes), high diastereoselectivity and excellent yields. Moreover, the ketonitrone precursors are synthesized with same methodology.[3,4] In addition, the synthesis of the isoxazolidin-*gem*-bisphosphonic acids is carried out by a multistep reactions in which the isoxazolidine ring is obtained by solvent-free microwave assisted 1,3-dipolar cycloaddition (**B**).[5] The 1,2,3-triazoles are obtained by using of a eco-friendly recyclable system ($Er(OTf)_3$/Ionic Liquid/H_2O) in high yields and excellent regioselectivity, furnishing only 1,5 regioisomers (**C**). Moreover, the system can be reused for several cycles without loss of catalytic activity. Finally, the synthesis of a new imidazolidinone organocatalyst namely (5*S*)-2,2,3-trimethyl-5-thiobenzyl-4-imidazolidinone is described. The eco-friendly catalytic system is represented by organocatalyst/ionic liquid/H_2O that was tested on Diels-Alder and 1,3-dipolar cycloaddition betweed an α,β-unsaturated aldehyde and various dienes or 1,3-dipoles, isolating target products in high yields and excellent diastereo- and enantioselectivity (**D**).[6] Some of this compounds are been biologically tested and have demonstrated good results while others are currently being tested.

1. Loredana Maiuolo, Antonio De Nino, Vincenzo Algieri, Monica Nardi, *Mini-Reviews in Organic Chemistry*, **2017**, 14 (2), 136-142.
2. Loredana Maiuolo, Pedro Merino, Vincenzo Algieri, Monica Nardi, Beatrice Russo, Maria Luisa Di Gioia, Ignacio Delso, Antonio De Nino, *Eur. J. Med. Chem.*, **2018**, submitted.
3. Loredana Maiuolo, Pedro Merino, Vincenzo Algieri, Monica Nardi, Marialuisa Di Gioia, Beatrice Russo, Ignacio Delso, Matteo Antonio Tallarida, Antonio De Nino, *RSC Advances*, **2017**, 7, 48980-48988.
4. Loredana Maiuolo, Giordana Feriotto, Vincenzo Algieri, Monica Nardi, Beatrice Russo, Marialuisa Di Gioia, Emilia Furia, Matteo Antonio Tallarida, Carlo Mischiati, Antonio De Nino, *MedChemComm*, **2018**, 9, 299-304.
5. Pedro Merino, Loredana Maiuolo, Ignacio Delso, Vincenzo Algieri, Antonio De Nino, Tomas Tejero, *RSC Advances*, **2017**, 7, 10947-10967.
6. Antonio De Nino, Loredana Maiuolo, Pedro Merino, Monica Nardi, Antonio Procopio, David Roca-López, Beatrice Russo, Vincenzo Algieri, *ChemCatChem*, **2015**, 7, 830-835.

DOCTORAL RESEARCH ACTIVITY OF THE CANDIDATE

Vincenzo Algieri (born in Acri (CS), Italy) was graduated in Chemistry (2014) at the "Università della Calabria". 04/11/2014 started the XXX Cycle of the Research Doctorate in Translational Medicine belonging to the "Dipartimento di Farmacia e Scienze della Salute e della Nutrizione" situated in the "Università della Calabria (Arcavacata di Rende (CS), Italy)" and Coordinate to the Professor Sebastiano Andò.
His main research interests are: Synthesis and Characterization of Heterocyclic Compounds at Biological Activity, Enantioselective Organocatalysis in non-conventional solvent, Metal-Catalysis in a recyclable eco-frienfly systems and Solvent-Free Microwave-Assisted 1,3-Dipolar Cycloadditions.
Your Doctoral Research Activity belongs to the disciplinary Organic Chemistry scientific sector (SSD CHIM/06) and is supervised by Professor Antonio De Nino (Dipartimento di Chimica e Tecnologie Chimiche – CTC, Università della Calabria, Cubo 12C, 6° Piano, 87036 Arcavacata di Rende (CS), Italy).
Your triennial Doctoral reseach activity is took place:

- in the first year (04/11/2014-31/12/2015) in LaborSy Laboratory direct by Professor Antonio De Nino and situated in the Dipartimento di Chimica e Tecnologie Chimiche – CTC, Università della Calabria, Cubo 12C, 2° Piano, 87036, Arcavacata di Rende (CS), Italy;
- in the second year (01/01/2016-31/12/2016) in the Laboratory of "Sintesis Asimetrica" direct by Professor Pedro Merino and situated in the "Departamento de Quimica Organica & Departamento de Sintesis y Estructura de Biomoleculas, Instituto de Sintesis Quimica y Catalisis Homogenea (ISQCH), Facultad de Ciencias, Universidad de Zaragoza", Calle de Pedro Cerbuna, 12, 50009, Zaragoza, Spain;
- in the final third year (01/01/2017-30/10/2017) in LaborSy Laboratory direct by Professor Antonio De Nino and situated in the Dipartimento di Chimica e Tecnologie Chimiche – CTC, Università della Calabria, Cubo 12C, 2° Piano, 87036, Arcavacata di Rende (CS), Italy.

Currently, his research activity during PhD studies has led to the publication of 4 articles an 2 submission (in under revision) on international journals with Impact Factor. This articles are follow listed:

1. Loredana Maiuolo, Pedro Merino, Vincenzo Algieri, Monica Nardi, Beatrice Russo, Maria Luisa Di Gioia, Ignacio Delso, Antonio De Nino. 1,3-Dipolar Cycloaddition of Indanyl Nitrones to Vinylnucleobases: Synthesis, *in vitro* Anticancer Evaluation and Docking Study of Diastereomerically Pure Nucleobase-Containing Spiro-Isoxazolidines. *Eur. J. Med. Chem.*, **2018**, submitted, Impact Factor 4.519.
2. Loredana Maiuolo, Giordana Feriotto, Vincenzo Algieri, Monica Nardi, Beatrice Russo, Marialuisa Di Gioia, Emilia Furia, Matteo Antonio Tallarida, Carlo Mischiati, Antonio De Nino. Antiproliferative Activity of Novel Isatinyl/Indanyl Nitrones (INs) as Potential Spin Trapping of Free Radical Intermediates. *MedChemComm*, **2018**, 9, 299-304, Impact Factor 2.608, DOI: 10.1039/C7MD00537G.
3. Loredana Maiuolo, Pedro Merino, Vincenzo Algieri, Monica Nardi, Marialuisa Di Gioia, Beatrice Russo, Ignacio Delso, Matteo Antonio Tallarida, Antonio De Nino. Nitrones and Nucleobase-Containing Spiro-Isoxazolidines Derived from Isatin and indanone: Solvent-Free Microwave-Assisted Stereoselective Synthesis and Theoretical Calculations. *RSC*

Advances, **2017**, 7, 48980-48988, Impact Factor 3.108, DOI: 10.1039/c7ra09995a.

4. Loredana Maiuolo, Antonio De Nino, Vincenzo Algieri, Monica Nardi. Microwave-Assisted 1,3-Dipolar Cyclo-addition: Recent Advances In Synthesis of Isoxazolidines. *Mini-Reviews in Organic Chemistry*, **2017**, 14 (2), 136-142, Impact Factor 1.095 DOI: 10.2174/1570193X14666170206123513.
5. Pedro Merino, Loredana Maiuolo, Ignacio Delso, Vincenzo Algieri, Antonio De Nino, Tomas Tejero. Chemical approaches to inhibitors of isoprenoid biosynthesis: targeting farnesyl and geranylgeranyl phyrophosphate synthases. *RSC Advances*, **2017**, 7, 10947-10967, Impact Factor 3.108, DOI: 10.1039/c6ra28316k.
6. Antonio De Nino, Loredana Maiuolo, Pedro Merino, Monica Nardi, Antonio Procopio, David Roca-López, Beatrice Russo, Vincenzo Algieri. Efficient Organocatalyst Supported on a Simple Ionic Liquid as a Recoverable System for the Asymmetric Diels-Alder Reaction in the Presence of Water. *ChemCatChem*, **2015**, 7, 830-835, Impact Factor 4.803, DOI: 10.1002/cctc.201402973.

In addition, all the works were presented at 13 interregional, national and international congress as follow listed:

1. Loredana Maiuolo, Antonio De Nino, Pedro Merino, Vincenzo Algieri, Beatrice Russo, Ignacio Delso, Matteo A. Tallarida, Emilia Furia, Giordana Feriotto. "*Isatinyl/Indanyl nitrones (INs)as Important Precursors of Heterocycles: Synthesis and Antioxidant/Biological Activity".* Giornate Scientifiche dei Borsisti Del Consorzio Interuniversitario Nazionale Metodologie e Processi Innovativi di Sintesi - CINMPIS (17th EDITION) (15-16/12/2017), Cagliari, ORAL COMUNICATION, Atti OC-09.

2. Vincenzo Algieri, Antonio De Nino, Loredana Maiuolo, Beatrice Russo, Pedro Merino. "*Asymmetric 1,3-dipolar cycloadditions catalyzed by a new imidazolidinone organocatalyst".* XXVI National Congress of the Società Chimica Italiana - SCI 2017 (10-14/09/2017), Paestum (SA), ORAL COMUNICATION, Atti ORG-OR62.

3. Antonio De Nino, Loredana Maiuolo, Vincenzo Algieri, Monica Nardi, Maria Luisa Di Gioia, Matteo Antonio Tallarida. *"Er(Otf)$_3$ in Ionic Liquid catalyzed [3+2] cycloaddition of azides with electron-deficient dipolarophile: regioselective synthesis of substituted 1,2,3-triazoles".* XXVI National Congress of the Società Chimica Italiana - SCI 2017 (10-14/09/2017), Paestum (SA), POSTER COMUNICATION, Atti ORG-PO03.

4. Loredana Maiuolo, Antonio De Nino, Vincenzo Algieri, Beatrice Russo, Monica Nardi, Matteo Antonio Tallarida, Ignacio Delso, Pedro Merino. "*Synthesis of isoxazolidinyl gem-bisphosphonic acids and study of protein-ligands interactions".* XXVI National Congress of the Società Chimica Italiana - SCI 2017 (10-14/09/2017), Paestum (SA), POSTER COMUNICATION, Atti ORG-PO57.

5. Vincenzo Algieri. "*Sintesi di molecole farmacologicamente attive e preparazione di composti organici ad alto valore aggiunto".* La Chimica e le Tecnologie Chimiche: formazione_ricerca_lavoro, 2nd edition (event ID CS01-2017), Dipartimento di Chimica e Tecnologie Chimiche-CTC, Università della Calabria, Sala Stampa "Centro Congressi" (15/06/2017), ORAL COMUNICATION, LabOrSy Laboratory research activity presentation.

6. Vincenzo Algieri, Antonio De Nino, Loredana Maiuolo, Beatrice Russo, Monica Nardi, Pedro Merino, Ignacio Delso. "*Innovative Synthetic Approaches to Indanoyl, Indoxyl and Isatin Spiroisoxazolidines: New N,O-Nucleosides Used as MDM2 Inhibitors".* Workshop delle Sezioni Sicilia e Calabria 2016-17 of the Società Chimica Italiana (09-10/02/17), Messina, ORAL COMUNICATION, Atti CO-26.

7. Antonio De Nino, Vincenzo Algieri, Beatrice Russo, Monica Nardi, Pedro Merino, Ignacio Delso, Loredana Maiuolo. "*Spiroisoxazolidines as Promising Anticancer Agents through Protein/non-peptide Small-Molecule Interactions".* XXXVII Nazional Conress of the Divisione di Chimica Organica of the Società Chimica Italiana – CDCO 2016 (18-22/09/16), Venezia, ORAL COMUNICATION, Atti OC19.

8. Antonio De Nino, Vincenzo Algieri, Beatrice Russo, Monica Nardi, Pedro Merino, Ignacio Delso, Loredana Maiuolo. "*Spiroisoxazolidines: Promising Scaffolds for Anticancer Agents through Protein/non-peptide Small-Molecule Interactions".* 11th Spanish-Italian Symposium on Organic Chemistry – SISOC XI (13-15/07/16), Donostia-San Sebastián, POSTER COMUNICATION, Atti POSTER-28.

9. Vincenzo Algieri, Antonio De Nino, Loredana Maiuolo, Beatrice Russo, Ignacio Delso, Pedro Merino. "*Synhesis of isoxazolidinyl-gem-bisphosphonic acid as FPPS ligands".* 11th Spanish-Italian Symposium on Organic Chemistry – SISOC XI (13-15/07/16), Donostia-San Sebastián, POSTER COMUNICATION, Atti POSTER-1.

10. Ignacio Delso, Mattia Ghirardello, Tomás Tejero, Pedro Merino, Vincenzo Algieri, Loredana Maiuolo, Antonio De Nino. *"Synthesis of new FFPS Ligands. Characterization of Protein-Ligand interactions".* III BIENNAL MEETING OF THE CHEMICAL BYOLOGY GROUP, XII CARBOHYDRATE SYMPOSIUM. (14-16/03/16) Madrid, POSTER COMUNICATION, Atti PC24. I. Delso.

11. Vincenzo Algieri, Antonio De Nino, Loredana Maiuolo, Monica Nardi, Beatrice Russo. "*Sintesi di Acidi Aril-Isossazolidin-gem-bifosfonati a potenziale attività farmacologica".* Conjunct Congress of the sezioni Calabria e Sicilia of the Società Chimica Italiana - SCI 2015 (03-04/12/15), Catanzaro, ORAL COMUNICATION, Atti Relazione orale 3.

12. Vincenzo Algieri, Antonio De Nino, Loredana Maiuolo, Monica Nardi, Beatrice Russo. "*Solvent-Free Synthesis and Characterization of Spiro-isoxazolidines at Potential Biological Activity".* XXXVI Nazional Congress of the Divisione di Chimica Organica of the Società Chimica Italiana – CDCO 2015 (13-17/09/15), Bologna, POSTER COMUNICATION, Atti PC47.

13. Vincenzo Algieri, Antonio De Nino, Loredana Maiuolo, Monica Nardi, Antonio Procopio, Beatrice Russo. "*Organocatalizzatore Imidazolidinonico in Liquido Ionico ed Acqua come Sistema Riciclabile per reazioni di Diels-Alder Asimmetriche".* Conjunct Congress of the sezioni Sicilia e Calabria of the Società Chimica Italiana - SCI 2014 (01-02/12/14), Palermo, ORAL COMUNICATION, Atti O12.

The PhD Candidate has followed 8 specialization courses:

1. *Elucidation of Organic Reaction Mechanisms through Topological Approaches.* Advanced couse of 24h, held by Prof. Pedro Merino (Universidad de Zaragoza), followed by Universidad de Zaragoza. The main subject of the course is the develope of the new

theoric-experimentals methodologies that permit to discover the reaction mechanisms.

2. *Chemical Challenges for the Design of Enzyme Inhibitors: Computational and Spectroscopic Approaches.* Advanced couse of 24h, held by Dott. Ignacio Delso (Universidad de Zaragoza), followed by Universidad de Zaragoza. The main subject of the course is the design and the develop of new enzymatic inhibitors through Computational study of Docking and Molecular dinamic followed to experimental confirms by STD-NMR study carried out on real samples.
3. *La Spettroscopia NMR applicata alla Metabonomica.* Advanced couse of 6h, held by Dott. Amerigo Beneduci (Università della Calabria), on definition of the Metabonomic Science and the application of the NMR Spectroscopy in the identification of the pathology metabolites marckers in the biological fluids.
4. *Capacità sequestrante di leganti naturali nei confronti di metalli biodisponibili.* Advanced couse of 6h, held by Dott.ssa Emilia Furia (Università della Calabria), on the particular characteristics of the heterocyclic organic compounds used as chelating ligands of bioavailable metals.
5. *Antioxidant Reaction Mechanisms and Oxidative Stress.* Advanced couse of 6h, held by Dott.ssa Gloria Mazzone (Università della Calabria), on the definition of antioxidants and theoretical-computational rating of some mechanisms in which are involved molecular oxidative process associated to the stress.
6. *Farmaci Liquido-Cristallini.* Advanced couse of 24h, held by Prof. Fiore Pasquale Nicoletta (Università della Calabria), on the definition, synthesis, characterization and application of thermotrophic and liotropic liquid crystals in pharmacological field.
7. *NMR for organic and biological chemistry: Old experiments for new applications*. *Theoretical and practical overview.* Advanced couse of 16h, held by Dott. Ignacio Delso Hernàndez (Universidad de Zaragoza), on the use of bidimensional NMR tecniques used to discovery of the molecular organic configuration.
8. Diploma de *Curso Práctico de manejo de espectrómetros de RMN, nivel básico, organizado por el Istituto de Sintesis Quimica y Catálisis Homogénea*. Course Director Dr. Esteban P. Urrolabeitia, Madrid, 04/03/2016.

The PhD student has partecipated to the following seminars and workshop as provided in the study plan:

"CONDITIONAL TARGETED SOMATIC MUTAGENESIS IN THE MOUSE".

Seminar held in seminars room of the Sanitary Centre of the Università della Calabria by Prof. Daniel Metzger Research Director of the Istituto di Genetica e Biologia Molecolare e Cellulare (IGBMC9) from Strasburgo (F) , 12/06/2017.

"*RECENT ADVANCES TOWARDS PERSONALIZED CHEMOTERAPHY* ".

Seminar held in Dipartimento di Chimica e Tecnologie Chimiche of the Università della Calabria by Prof. Tamer Shoeib of the Department of Chemistry of the The American University in Cairo, New Cairo 11835, 04/05/2017.

"*FAST 2D-NMR EXPERIMENTS AND CONFIGURATIONAL ANALYSIS: NOVEL DEVELOPMENTS IN NMR SPECTROSCOPY*".

Seminar held in Dipartimento di Chimica e Tecnologie Chimiche of the Università della Calabria by

Prof. Burkhard Luy of the Institute of Organic Chemistry and Institute for Biological Interfaces 4 – Magnetic Resonance, 27/02/2017.

"*THE MANY FACES OF BRAIN AROMATASE*".

Seminar held in Dipartimento di Farmacia e Scienze della Salute e della Nutrizione of the Università della Calabria by Prof. Chuck Roselli of the Department of Physiology & Pharmacology of Oregon Health & Science University, 21/02/2017.

"*SALUTE DELL'AMBIENTE E SALUTE UMANA: IL CASO DEL DESTINO AMBIENTALE DELLE DIOSSINE IN CAMPANIA*".

Seminar held in Dipartimento di Farmacia e Scienze della Salute e della Nutrizione of the Università della Calabria by Dott. Nic Pacini, confirmed researcher of the Dipartimento di Ingegneria per l'Ambiente ed il Territorio ed Ingegneria Chimica of the Università della Calabria, 08/10/2015.

"QUANTUM CHEMISTRY APPLIED TO ASYMMETRIC HOMOGENEOUS AND ENZIMATIC CATALYSIS".

Seminar held in Dipartimento di Chimica e Tecnologie Chimiche-CTC of the Università della Calabria by Prof. Fahmi Himo of the Department of Organic Chemistry Arrhenius Laboratory, Stockholm University, 11/09/2015.

"LA CHIMICA SCIENZA DELLA SICUREZZA E DELLO SVILUPPO SOSTENIBILE".

Congress organized by Dipartimento di Chimica e Tecnologie Chimiche-CTC of the Università della Calabria in collaboration with the ordine dei Chimici, Società Chimica Italiana, ARPACAL and Regione Calabria, carried out in the Aula Magna "Beniamino Andreatta" of the Università della Calabria, Rende (CS), 22-23/06/2015.

"ACCREDITAMENTO DEI LABORATORI E SICUREZZA DEGLI ALIMENTI-SICUREZZA DEGLI ALIMENTI: IL RUOLO DI ACCREDIA".

Seminar held in Dipartimento di Farmacia e Scienze della Salute e della Nutrizione of the Università della Calabria by Dott. Federico Pecoraro and by Dott.ssa Silvia Tramontin of the Dipartimento dei Laboratori di Prova ACCREDIA, 17/06/2015.

"INTERACTION OF GRAPHENE WITH BIOLOGICAL AND BIOMIMETIC MEMBRANES".

Seminar held in Dipartimento di Farmacia e Scienze della Salute e della Nutrizione of the Università della Calabria by Dott. Aravind Vijayaraghavan which has developed hers research activity in the Università di Manchester, lecturer in Graphene Science and Nanotechnology School of Materials, 15/06/2015.

"EPIGENETIC ALTERATION AND MICRORNA DYSREGULATION IN HUMAN CANCERS".

Seminar held in Dipartimento di Farmacia e Scienze della Salute e della Nutrizione of the Università della Calabria by Dott. Domenico Zito which has developed hers research activity in the Dipartimento di Virologia Molecolare, Immunologia e Genetica Medica of the The Ohio State University, Columbus OH, USA, 17/04/2015.

"ORGANIC REACTIONS THAT ARE NOT WHAT THEY SEEM".

Seminar held in Dipartimento di Chimica e Tecnologie Chimiche of the Università della Calabria by

Prof. Pedro Merino of the Dipartimento di Chimica Organica of the Università di Saragozza, Spagna, 04/02/2015.

"*RATIONAL DESIGN OF GLYCOMIMETIC COMPOUNDS TARGETING FUNGAL TRANSGLYCOSYLASES"*.

Seminar held in Dipartimento di Chimica e Tecnologie Chimiche of the Università della Calabria by Prof. Pedro Merino of the Dipartimento di Chimica Organica of the Università di Saragozza, Spagna, 03/02/2015.

"*UPDATING ENGLISH TESTING FRAMES FOR SPECIALISED LANGUAGE SKILLS*". Workshop held in Dipartimento di Farmacia e Scienze della Salute e della Nutrizione of the Università della Calabria by Professors: Sebastiano Andò, Anna Franca Plastina, Domenico Sturino and Fabrizia del vecchi, 27/01/2015.

"*RECENT ADVANCES IN COMPUTATIONAL PROTEOMICS*".

Seminar held in Dipartimento di Chimica e Tecnologie Chimiche of the Università della Calabria by Prof. Pedro A. Fernandes of the Università di Porto, 11/12/2014.

Various attestations of partecipations in activities has been obtained in the course of the last three years:

1. ATTESTATION OF PARTECIPATION TO UPDATE CONFERENCE "*LA MEDICINA DI LABORATORIO DELL'ETA' PEDIATRICA"* organized by Società Italiana di Patologia Clinica e Medicina di Laboratorio at the cittadella Regionale Germaneto (CZ), 06/04/2017.

2. ATTESTATION OF PARTECIPATION TO CONFERENCE "*LA SICUREZZA AMBIENTALE IN CALABRIA: CHIMICI E UNIVERSITA' IN SUPPORTO ALLA MAGISTRATURA"* organized by Ordine dei Chimici della Calabria in collaboration with the Università della Calabria and the Università Magna Graecia di Catanzaro at the Università Magna Graecia di Catanzaro, 06/04/2017.

3. ATTESTATION OF PARTECIPATION TO "*FAO WORLD FOOD DAY – L'ALIMENTAZIONE SCOLASTICA TRA PROTEZIONE SOCIALE ED EDUCAZIONE ALIMENTARE"* organized by Dipartimento di Chimica e Tecnologie Chimiche-CTC of the Università della Calabria in collaboration with the Consiglio per la ricerca in Agricoltura e l'analisi dell'economia Agraria-CREA, Agenzia Nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile-ENEA, Camera di Commercio di Cosenza, at the aula Magna of the Università della Calabria, Arcavacata di Rende, 23/10/2015.

4. ATTESTATION OF PARTECIPATION TO UPDATE COURSE "*LA GESTIONE DELL'IGIENE NELL'INDUSTRIA ALIMENTARE"* organized by centro di informazione e formazione, tecnica e scientifica (apotema forum, divisione Calsystem) at the Saporito di Rende (CS), 09/07/2015.

5. ATTESTATION OF PARTECIPATION TO FORMATION COURSE "*NORMATIVA SULL'ATTIVITÁ DEL CHIMICO E DEONTOLOGIA PROFESSIONALE"* organized by Ordine dei Chimici della Calabria, at the Dipartimento di Chimica e Tecnologie Chimiche

dell'Università della Calabria, 31/01/2015.

He is participant of the research project "Design of glycomimetics as enzyme inhibitors" afferent to "Ministerio de Economia, Industria y Competitividad – MINECO", scientific site from Spain. The project is developed at the "Departamento de Quimica Organica & Departamento de Sintesis y Estructura de Biomoleculas, Instituto de Sintesis Quimica y Catalisis Homogenea (ISQCH), Facultad de Ciencias de la Universidad de Zaragoza". Project code: Spain: 2016_CTQ2016-76155R

During the last three years the candidate has carried out various international mobility activities as follow reported:

- Visiting PhD Student in the" Laboratorio de Sintesis Asimetrica" of the Prof. Pedro Merino, situated in the "Universidad de Zaragoza, Departamento de Quimica Organica & Departamento de Sintesis y Estructura de Biomoleculas, Instituto de Sintesis Quimica y Catalisis Homogenea (ISQCH), Facultad de Ciencias", Zaragoza (Spain), (6/10/2017-15/10/2017).
- Visiting PhD Student in the "Laboratorio de Sintesis Asimetrica" of the Prof. Pedro Merino, situated in the "Universidad de Zaragoza, Departamento de Quimica Organica & Departamento de Sintesis y Estructura de Biomoleculas, Instituto de Sintesis Quimica y Catalisis Homogenea (ISQCH), Facultad de Ciencias", Zaragoza (Spain), (01/01/2016-31/12/2016).

In addition, various disciplinary tutoring activities were carried out as follow reported:

- Disciplinary Tutoring Activity for the course of the "Sintesi organiche avanzate e laboratorio" (SSD CHIM/06) of the course of "Laurea Magistrale in Chimica". Teacher of the course: Prof. Antonio De Nino, site: Università della Calabria, Dipartimento di Chimica e Tecnologie Chimiche (CTC), Arcavacata di Rende (CS). From 02/11/2017 to actually in course.

- Disciplinary Tutoring Activity for the organic chemistry laboratory (SSD CHIM/06) for the "Progetto Piano Lauree Scientifiche – PLS". Teacher of the course: Prof. Antonio De Nino, site: Università della Calabria, Dipartimento di Chimica e Tecnologie Chimiche, Arcavacata di Rende (CS). Academic years: 2015/2016, 2016/2017, 2017/2018.

- Disciplinary Tutoring Activity for the organic chemistry laboratory (SSD CHIM/06) for the "Progetto Piano Lauree Scientifiche – PLS". Teacher of the course: Dott.ssa Loredana Maiuolo, site: Università della Calabria, Dipartimento di Chimica e Tecnologie Chimiche, Arcavacata di Rende (CS). Academic years: 2015/2016, 2016/2017, 2017/2018.

- Disciplinary Tutoring Activity for the course of the "Chimica Generale ed Inorganica" (SSD CHIM/03) of the course of "Laurea Magistrale a ciclo unico in Chimica e Tecnologie Farmaceutiche (CTF)". Teacher of the course: Prof. Giovanni De Munno, site: Università della Calabria, Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Arcavacata di Rende (CS). From 01/12/2015 to 30/06/2016.

- Disciplinary Tutoring Activity for the course of the "Chimica Generale ed Inorganica" (SSD CHIM/03) of the courses of "Laurea: Triennale in Biologia e Triennale in Scienze e

Tecnologie Biologiche". Teacher of the course: Prof. Giovanni De Munno, site: Università della Calabria, Dipartimento di Biologia, Ecologia e Scienze della Terra (DIBEST), Arcavacata di Rende (CS). From 12/12/2014 to 30/09/2015.

- Disciplinary Tutoring Activity for the course of the "Chimica Organica Superiore e Laboratorio" (SSD CHIM/06) of the course of "Laurea Magistrale in Chimica". Teacher of the course: Dott.ssa Loredana Maiuolo, site: Università della Calabria, Dipartimento di Chimica e Tecnologie Chimiche (CTC), Arcavacata di Rende (CS). From 01/12/2014 to 30/09/2015.

INTRODUCTION

INTRODUCTION

The discovery of new pharmacological agents is one of the biggest challenges for the current scientific community. The synthesis of new pharmacological active heterocyclic organic molecules is one of the goals most wanted by research group directed by Prof. Antonio De Nino.
In recent years, the various functionalized heterocyclic compounds are subject of important research in the field of pharmaceutical chemistry, since over the last decade, because they have demonstrated their pharmacological activity on animal biological systems, including humans.
Heterocycles are compounds having a cyclic structure in which there are at least two different types of atoms. Generally, the cycle is composed by carbon atoms and one or more heteroatoms of which the most common are: nitrogen, oxygen and sulfur. There are two different types of heterocyclic compounds:

- Aliphatic Heterocycles: Saturated and Unsaturated rings;
- Aromatic Heterocycles.

The applications of these compounds in the clinical field are constantly increasing due to their high pharmacological activity due to the great ability to respond to the biochemical system requirements by the formation of covalent bonds and / or the formation of weak interactions with the biomolecules.[1] This excellent and versatile reactivity is due to the electronic distribution within the cycle, in fact, the introduction of heteroatoms to the corresponding carbocycle leads to a remarkable increase in the physical-chemical properties of the molecule. For example, depending on the pH of the medium, the heterocycles can act as acids or bases because they form cations and anions. Some can easily interact with electrophiles, others with nucleophiles, others with both. Some are easily oxidable but resistant to reduction, others can be easily reduced, for example subjected to hydrogenation but stable to oxidation. There are also amphoteric heterocycles that simultaneously exhibit all of these properties and, in addition, the presence of heteroatoms in the cycles act so that there is tautomerism.[2]
In the world, many heterocyclic compounds of various nature are synthesized daily, among which a promising class of biologically active molecules are isoxazolidines.
Due to the chemical-physical properties of heterocyclic compounds, isoxazolidines perfectly imitate natural nucleosides, exercising antiviral and antitumor activity according to the following characteristics:

- Competition in reverse transcriptase;
- Termination of the viral DNA chain;
- Termination of the damaged DNA chain;
- Anti-metabolitic action;
- Competition with physiological nucleosides;
- Competition with a large number of intracellular targets;
- Induction of cytotoxicity;
- Enzymatic inhibitions;
- Enzymatic regulation;
- Damaged DNA reparations.

This is apparent in their different applications, for example, furanopyrimidines *N*,*O*-nucleosides

inhibit DNA and RNA replication of Polyovirus 1, Human Ecovirus 9, Coxsackie Virus B4, Type 2 Adenovirus, Types 1 and 2 of the Herpes Simplex and Cytomegalovirus.[3]

The acquired knowledge in recent years on viral and cellular reproduction has made it possible to identify some molecular portions that, when bound to the isoxazolidine nucleus, are able to selectively act on viral functions. This guarantees to the isoxazolidines a unique pharmacological versatility because, depending on the group that is inserted into the cycle, the biological activity of the latter changes. For example, the addition of a nucleobase to the isoxazolidine ring leads to the formation of highly modified *N*,*O*-nucleosides[4] with high cytotoxicity and variable functionality. Indeed, the latter, besides comprehending a large class of antiviral agents, are excellent candidates for anticancer drugs. Indeed, was synthesized of the isoxazolidine *N*,*O*-nucleosides which have showed an enhanced *in vitro* antiproliferative activity on cell lines such as SKOV3 and SW480.[5]

If instead of the nucleobase is added a geminal bisphosphonic group linked on the ring by a variable carbon chain, an important isoxazolidine class is obtained and is used to treat:

- Osteoporosis;
- Imperfect osteogenesis;[6]
- Fibrous dysplasia;
- Prostate Cancer.[7]

This biological activity is due to the inhibition effect that these molecules exert on Farnesyl Pyrophosphate Syntase (FPPS),[8] Geranylgeranyl Pyrophosphate Syntase (GGPPS), and the chelating activity that diphosphate groups exert on calcium ions.

In general, in most cases, the biological activities of these molecules are associated with enzymatic inhibitions so that the asymmetric character of heterocycles possessed, for example by spiro compounds, is one of the most important pharmacological factors. Based on these latter considerations, the spiroisoxazolidinyl oxindole derivatives play a crucial role in the clinical field, in fact they are used as:

- Antimicrobials;
- Anticancer;
- Antibiotics;
- Antivirals.

Spiro-compounds are molecules characterized by the presence of one or more hybridized sp^3 chiral carbon atoms in common between two different cycles. This latter is called spiro-atom or also linked-atom. The tetrahedral nature of spiro carbon causes the two planes of the connected rings to be closely perpendicular to each other.

These molecules are an important part of many alkaloids present in nature such as: alstonisine, coerulescine, formosanine, spirotriprostatin A and B, with proven pharmacological activity.[9]

The indolic compounds are of further interest because their derivatives are good anti-tumor agents, antibiotics, NK-1 receptor inhibitors, p53 protein interaction inhibitors involved in DNA damage repair processes, and the MDM2 enzyme which regulates this protein.[10]

These nucleuses are a base of 1-indanone and isatine. 1-Indanone and its derivatives are drug reaction intermediates. For example, 5,6-dimethoxy-1-indanone is a portion of the active molecular principle of Donepezil®, an anti-demential. In addition, some 1-indanone derivatives are potent antiviral agents used against virus hepatitis C (HCV) as they act on the replication system of the virus by means of a mechanism that involves non-structural proteins.[11]

An *in vivo* study, however, has allowed to observe the potent anticancer activity that 1-indanone exerts against cancer of Ehrlich, a cancer that attacks the connective tissue. Isatin is a basic constituent of many alkaloids and drugs. Several derivatives of isatin have various activities such as antibacterial, antimycotic, antiviral, anti-HIV, anti-tuberculosis, anticancer, anti-inflammatory and anticonvulsant.
Based on the above, some of the works done in this thesis have the objective to the synthesis of a new series of variously functionalized isoxazolidines called:

- INDANYL AND ISATINYL SPIROISOXAZOLIDINE *N,O*-NUCLEOSIDES (**Figure 1a**)
- ARYL-ISOXAZOLIDIN-*Gem*-BISPHOSPHONIC ACIDS (**Figure 1b**)

compounds capable of encompassing all pharmacological properties of the individual classes of molecules described above.

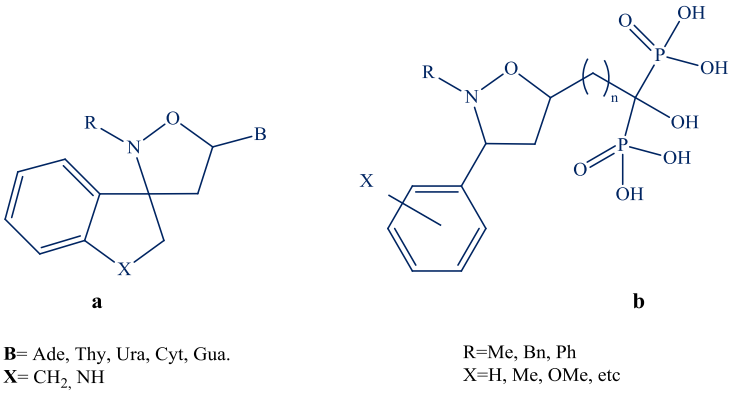
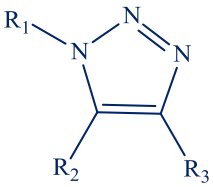


Figure 1 *a) Structure of a spiroisoxazolidine N,O-Nucleoside. b) Structure of an Aryl-isoxazolidin-gem-bisphosphonic acid.*

The synthesis of these compounds is carried out with simple and solvent-free methodologies. The reaction is a Microwave-assisted 1,3-dipolar cycloaddition which takes place between a dipolarophile and a 1,3-dipole and leads to five-membered heterocycles formation in a highly regioselective- and diastereoselective way. For the synthesis of the spiroisoxazolidine *N,O*-nucleosides are used stable *N*-Me, *N*-Bn or *N*-Ph ketonitrones derived from 1-indanone and isatin that react with vinyl-nucleobases. For the synthesis of the aryl isoxazolidin-*gem*-bisphosphonic acids are used the stable aldonitrones derived from various aryl-substituted benzaldehydes that react with dipolarophiles consisting of a vinyl group bonded to an ester group through an alkyl chain having a number of variable carbon atoms, to give the esther isoxazolidine in which multistep reactions are carried out, introducing bisphosphonic function on the heterocycle.
Another class of the heterocyclic organic compounds very important from a clinical point of view are the 1,2,3-triazoles. Triazoles are heterocyclic organic compounds containing five-membered ring with three nitrogen and two carbon atoms in according to the structure reported in **Figure 2**.



R_1=Bn, Octyl, Ph
R_2=Me, Aryl, R_3=H, COMe

Figure 2 *Structure of a 1,2,3-triazole.*

Triazoles can also be used as a linker and show bioisosteric effects on peptide linkage, aromatic ring, double bonds and an imidazole ring. Some unique features like hydrogen bond formation, dipole-dipole and π stacking interactions of triazole compounds have increased their importance in the field of medicinal chemistry as they bind with the biological target with high affinity due to their improved solubility.

The 1,2,3-triazole based heterocycles have been well exploited for the generation of many medicinal scaffolds exhibiting anti-HIV, anticancer, antibacterial activities, etc.

The synthesis of this compounds is carry out by an eco-friendly methodology in which an alkyl or aryl azide react with a deactivated dipolarophile, consisting in an enaminone or ω-nitrostirene, in a recovery catalytic system composed by $Er(OTf)_3$/ionic liquid/H_2O. This latter is a homogeneous system that can have an industrial application because when the reaction is stopped the product is isolated by extraction with dichloromethane and the catalytic system can be reused for more times. More important is the fact that the reaction is very regioselective; in fact only 1,5-products are obtained in good yield.

The last part of this thesis is focused on the synthesis and application of a new imidazolidinone organocatalyst applied in Diels-Alder and 1,3-Dipolar Cycloadditions to obtain, in eco-friendly manner, six-membered carbocycles and five-membered heterocycles enantiomerically enriched.

The enantiomeric pure products are compounds that have excellent biological properties.

Paradoxically it is known that under certain biological conditions an enantiomerically pure species can be more active, not active or even detrimental to the other molecule, its specular image.

The organocatalyst used in this cycloaddition reactions exploits its capacity to form the iminium ion with a carbonylic group to accelerate the reaction. In addition, the enantioselection should be facilitated through the formation of a reaction intermediate having a configuration such as to prevent access on an enantioface of the dienophile (or dipolarophile) to the diene (or 1,3-dipole).

The organocatalyst synthesized are reported in **Figure 3**.

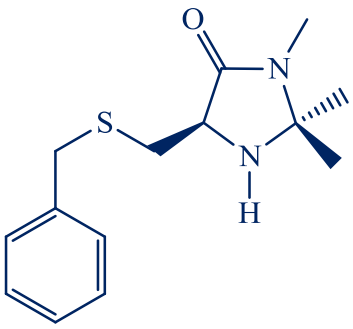


Figure 3. *Imidazolidinone organocatalyst applied in Diels-Alder and 1,3-Dipolar Cycloaddition Reactions.*

Considering that the purpose of this dissertation concerns the synthesis of new heterocyclic compounds with pharmacological action, all the synthesized products have been tested, or are being tested, with both enzymatic and *in vitro* tests to evaluate their pharmacological activity.

CHAPTER 1

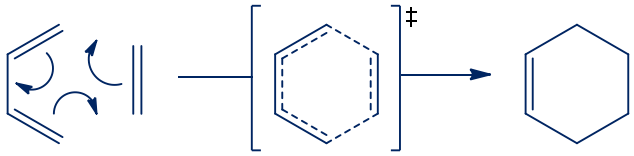
CHAPTER 1 – DIELS-ALDER REACTION

1.1 INTRODUCTION

The formation of new chemical bonds between molecules is one of the key challenges in the development of drug delivery systems and biomaterials.

The Diels–Alder reaction (DA), discovered by Otto Diels and Kurt Alder in 1928, is a key reaction in organic synthesis. Its popularity can be attributed to the reaction's simplicity, fast speed, extensive applicability, and mild reaction conditions. Moreover, these transformations as key steps have been frequently used for the construction of complex biologically active molecules and natural product synthesis.[12]

The Diels-Alder reaction is one of the most important reactions for the synthesis of cyclic compounds in organic chemistry because of its high regio- and stereo-selectivity. In this reaction, a diene reacts with a dienophile to form a cyclic product. Two σ bonds and one π bond are formed from three π bonds as depicted in **Scheme 1.1**.[13]



Scheme 1.1 *Classic Diels-Alder Reaction*

The cycloaddition of alkenes and dienes is a very useful method for forming substituted cyclohexenes. A concerted mechanism requires that a single transition state, and therefore no intermediate, lie on the reaction path between reactants and adduct. The transition state has six delocalized π electrons and it's aromatic in character, having some of the special stabilization of benzene. You could look at it as a benzene ring having all its π bonds but missing two σ bonds. The simple TS reported in **Scheme 1.1** is fine as far as it goes, but it is incomplete. Below we will perform a more detailed orbital analysis.

In the terminology of orbital symmetry classification, the Diels-Alder reaction is a $[4\pi_s+2\pi_s]$ cycloaddition, an allowed process. There have been a large number of computational studies of the D-A reaction, and as it is a fundamental example of a concerted reaction, it has frequently been the subject of advanced calculations.[14] These studies support a concerted mechanism, which is also supported by good agreement between experimental and calculated (B3LYP/6-31G*) kinetic isotope effects.[15] The TS for a concerted reaction requires that the diene adopt the *s-cis* conformation. The diene and the dienophile (substituted alkene) approach each other in approximately parallel planes. The symmetry properties of the π orbitals permit stabilizing interactions between C(1) and C(4) of the diene and the dienophile. Usually, the strongest bonding interaction is between the HOMO of the diene and the LUMO of the dienophile. The interaction between the frontier orbitals is depicted in **Figure 1.1**.

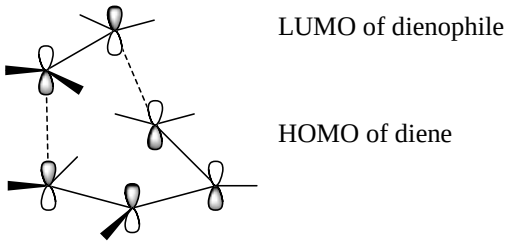


Figure 1.1 *Interaction between* LUMO *of dienophile and* HOMO *of diene in the DA Reaction.*

We will now discuss of concerted cycloaddition reactions by exploring how the orbital symmetry requirements distinguish between reactions that are favorable and those that are unfavorable.

1.2 THE WOODWARD-HOFFMANN RULES

Woodward and Hoffmann formulated the orbital symmetry principles for cycloaddition reactions in terms of the frontier orbitals. An energetically accessible TS requires overlap of the frontier orbitals to permit smooth formation of the new σ bonds. If it is assumed that the reactants approach one another face-to-face, as would be expected for reactions involving π orbitals, the requirement for bonding interactions between the HOMO and LUMO are met for [2+4] but not for [2+2] or [4+4] cycloadditions. More generally, systems involving [4n+2] π electrons are favorable (allowed), whereas systems with 4n π electrons are not (**Figure 1.2**).

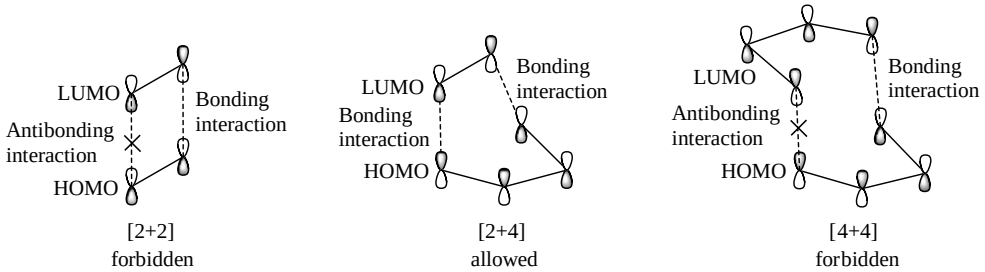


Figure 1.2 *Allowed and forbidden interactions in cycloaddition reactions.*

The generalized Woodward-Hoffmann rules for cycloaddition are summarized in **Table 1.1**.

m+n	supra/supra	supra/antara	antara/antara
4n	Forbidden	Allowed	Forbidden
4n+2	Allowed	Forbidden	Allowed

Table 1.1 *Orbital Symmetry Rules for m+n Cycloaddition Reaction.*

In general, for pericyclic reactions Woodward-Hoffmann rule states that a ground-state pericyclic change is symmetry allowed when the total number of $(4n+2)_s$ and $(4n)_a$ components is odd.

The orbital symmetry principles can also be applied by constructing an orbital correlation diagram.[16] Let us construct a correlation diagram for the addition of butadiene and ethene to give

cyclohexene. For concerted addition to occur, the diene must adopt the *s-cis* conformation. Because the electrons that are involved are the π electrons in both the diene and dienophile, the reaction occurs via a face-to-face rather than an edge-to-edge orientation. When this orientation of the reacting complex and TS is adopted, it can be seen that a plane of symmetry perpendicular to the planes of the reacting molecules is maintained during the course of the cycloaddition.

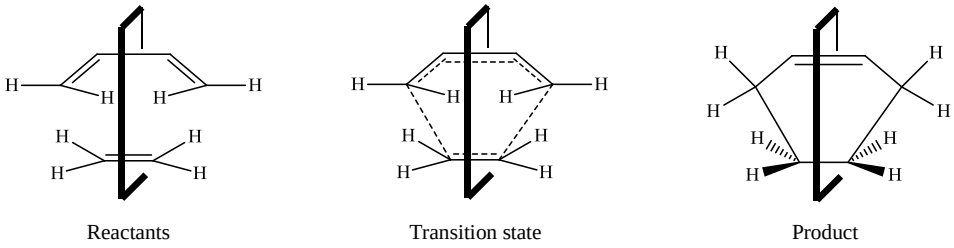


Figure 1.3 *Planes of the reacting molecules.*

An orbital correlation diagram can be constructed by examining the symmetry of the reactant and product orbitals with respect to this plane, as shown in **Figure 1.4**. An additional feature must be taken into account in the case of cyclohexene. The cyclohexene orbitals σ_1, σ_2, σ_1^*, and σ_2^* are called symmetry-adapted orbitals. We might be inclined to think of the σ and σ* orbitals as being localized between specific pairs of carbon atoms, but this is not the case for the MO treatment because localized orbitals would fail the test of being either symmetric or antisymmetric with respect to the plane of symmetry.

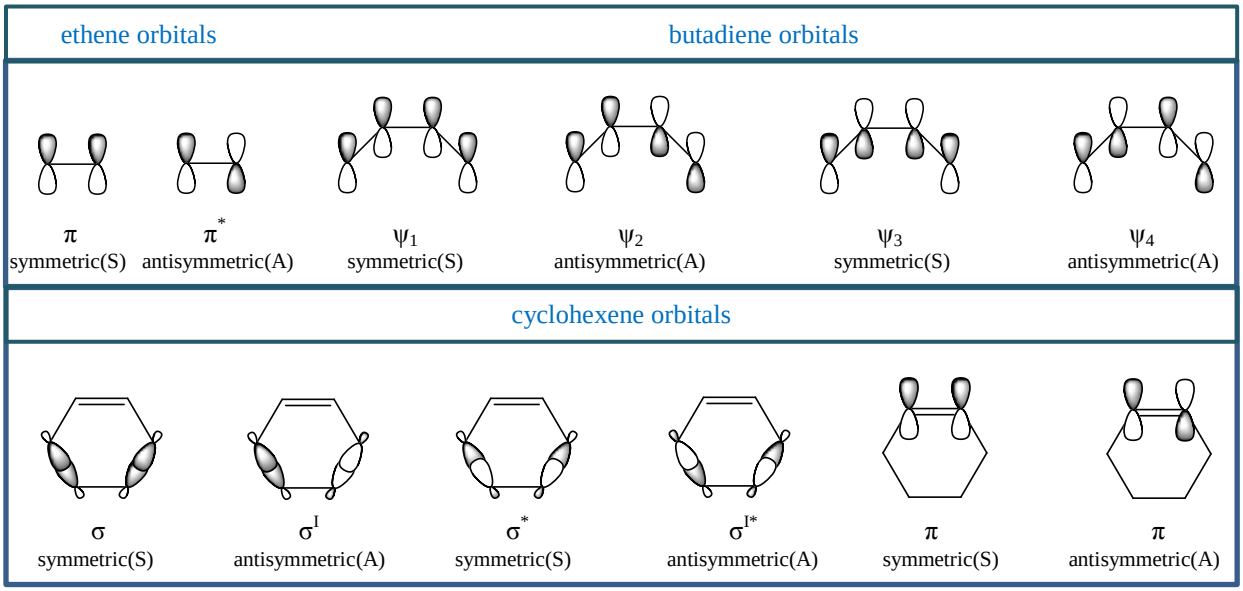


Figure 1.4 *Symmetry properties of ethane, butadiene and cyclohexene orbitals with respect to a plane bisecting the reacting system.*

In the construction of orbital correlation diagrams, all of the orbitals involved must be either symmetric or antisymmetric with respect to the element of symmetry being considered.
When the orbitals have been classified with respect to symmetry, they are arranged according to

energy and the correlation lines are drawn as in **Figure 1.5**. From the orbital correlation diagram, it can be concluded that the thermal concerted cycloaddition reaction between butadiene and ethylene is allowed. All bonding levels of the reactants correlate with product ground state orbitals. Extension of orbital correlation analysis to cycloaddition reactions with other numbers of π electrons leads to the conclusion that suprafacial-suprafacial addition is allowed for systems with [4n+2] π electrons but forbidden for systems with 4n π electrons.

The frontier orbital analysis, basis set orbital aromaticity, and orbital correlation diagrams can be applied to a particular TS geometry to determine if the reaction is symmetry allowed. The orbital symmetry rules can be generalized from conjugated polyenes to any type of conjugated π system. Conjugated anions and cations such as allylic and pentadienyl systems fall within the scope of the rules. The orbital symmetry considerations can also be extended to isoelectronic systems containing heteroatoms. Thus the C=C double bonds can be replaced by C=N, C=O, C=S, N=O, N=N, and other related multiple bonds.

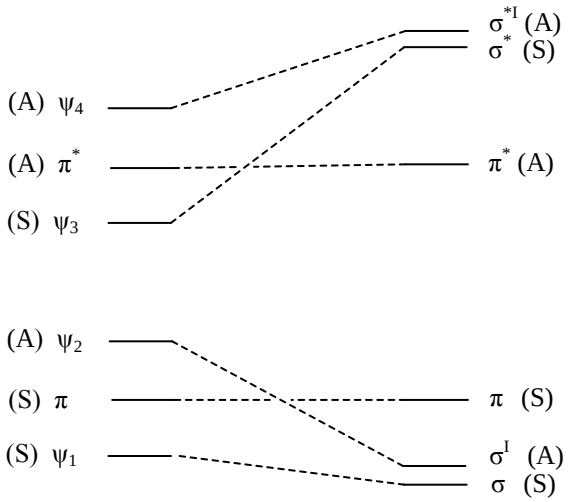


Figure 1.5 *Orbital symmetry correlation diagram for [π2$_s$ + π4$_s$]cycloaddition of ethene and 1,3-butadiene.*

1.3 REACTIVITY AND REGIOSELECTIVITY

There is a strong electronic substituent effect on the D-A cycloaddition. It has long been known that the reaction is particularly efficient and rapid when the dienophile contains one or more EWG and is favored still more if the diene also contains an ERG. Thus, among the most reactive dienophiles are quinones, maleic anhydride, and nitroalkenes. α,β-Unsaturated esters, ketones, and nitriles are also effective dienophiles. The D-A reaction between unfunctionalized alkenes and dienes is quite slow. For example, the reaction of cyclopentadiene and ethene occurs at around 200°C.[17] These substituent effects are illustrated by the data in **Table 1.2**. In the case of the diene, reactivity is increased by ERG substituents. Data for some dienes are given in **Table 1.3**. Note that ERG substituents at C(1) have a larger effect than those at C(2). **Scheme 1.2** gives some representative examples of dienophiles activated by EWG substitution.[18]

It is significant that if an electron-poor diene is utilized, the preference is reversed and electron-rich alkenes, such as vinyl ethers and enamines, are the best dienophiles.

Dienophile	Relative rate[a]
Tetracyanoethene	43.000.000
1,1-Dicyanoethene	450.000
Maleic anhydride	56.000
p-Benzoquinone	9.000
Z-1,2-Dicyanoethene	91
E-1,2-Dicyanoethene	81
Dimethyl fumarate	74
Dimethyl maleate	0,6
Methyl acrylate	1,2
Cyanoethene	1,0

a. From second order rate constants in dioxane at 20°C.[19]

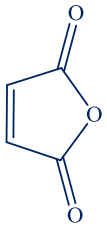
Table 1.2 *Relative Reactivity towards Cyclopentadiene in the Diels-Alder Reactions.*

Such reactions are called inverse electron demand Diels-Alder reactions, and the reactivity relationships are readily understood in terms of frontier orbital theory. Electron-rich dienes have high-energy HOMOs that interact strongly with the LUMOs of electron-poor dienophiles. When the substituent pattern is reversed and the diene is electron poor, the strongest interaction is between the dienophile HOMO and the diene LUMO. The FMO approach correctly predicts both the relative reactivity and regioselectivity of the D-A reaction for a wide range of diene-dienophile combinations.

Diene Substituents	Dienophile Tetracyanoethene	Dienophile Maleic anhydride
None	1	1
1-Methyl	103	3,3
2-Methyl	45	2,3
1,4-Dimethyl	1.660	-
1-Phenyl	385	1,65
2-Phenyl	191	8,8
1-Methoxy	50.900	12,4
2-Methoxy	1.750	-
1,4-Dimethoxy	49.800	-
Cyclopentadiene	2.100.000	1.350

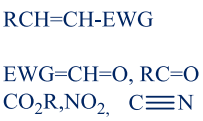
Table 1.3 *Relative Reactivity of Some Substituted Butadienes in the Diels-Alder Reaction.*[20]

a) substituted alkenes

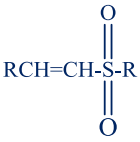


Maleic anhydride

Benzoquinone

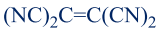


α,β-unsaturated aldehydes, ketones, esters, nitriles and nitro compounds



α,β-unsaturated sulfones

α,β-unsaturated phosphonates



tetracyanoethene

b) substituted alkines

Esters of acetylene-dicarboxylic acid

Dibenzoyl-acetylene

Dicyanoethyne

c) Heteroatomic dienophiles

Esters of azodicarboxylic acids

N-substituted 1,2,4-triazoline-3,5-diones

iminocarbonates

Scheme 1.2 *Representative Electrophilic Dienophiles.*

The question of regioselectivity arises when both the diene and alkene are unsymmetrically substituted. Generally, there is a preference for the 1,2 (named “Ortho”) and 1,4 (named “Para”) orientations, respectively, as in the examples shown in **Figure 1.6**.[21]

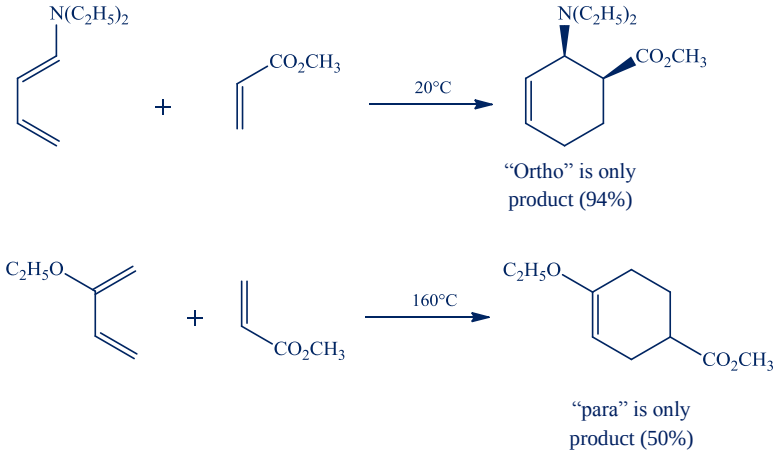


Figure 1.6 *Regioselectivity in some Diels-Alder Reactions.*

The regioselectivity of the D-A reaction is determined by the nature of the substituents on the diene and dienophile. FMO theory has been applied by calculating the energy and orbital coefficients of the frontier orbitals.[22] When the dienophile bears an EWG and the diene an ERG, the strongest interaction is between the HOMO of the diene and the LUMO of the dienophile, as indicated in **Figure 1.7**. The reactants are preferentially oriented with the carbons having the highest coefficients of the two frontier orbitals aligned for bonding. **Scheme 1.3** shows the preferred regiochemistry for various substitution patterns. The combination of an electron donor in the diene and an electron acceptor in the dienophile gives rise to cases **A** and **B**. Inverse electron demand D-A reactions give rise to combinations **C** and **D**. In reactions of types **A** and **B**, the frontier orbitals will be the diene HOMO and the dienophile LUMO.

The strongest interaction is between ψ_2 and π^* because the donor substituent on the diene raises the diene orbitals in energy, whereas the acceptor substituent lowers the dienophile orbitals. In reaction types **C** and **D**, the pairing of the diene LUMO and dienophile HOMO is the strongest interaction.

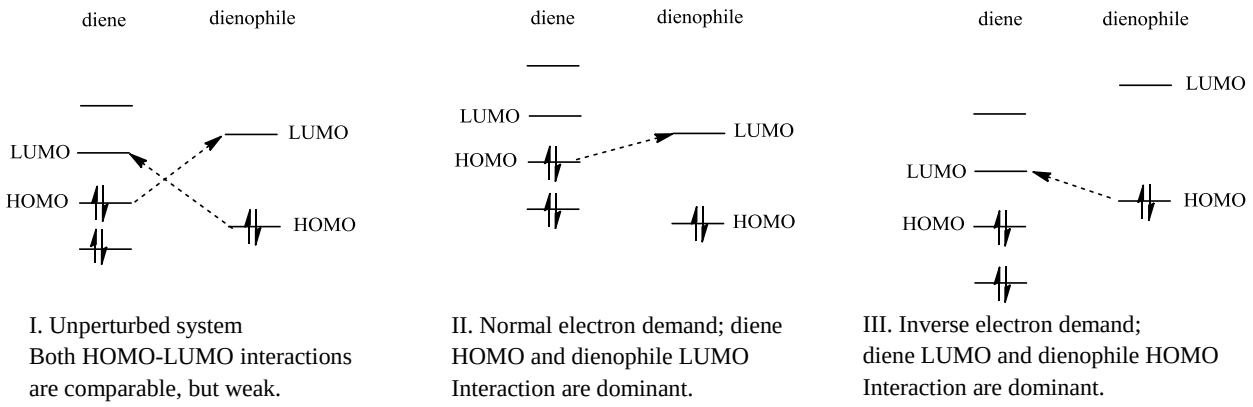
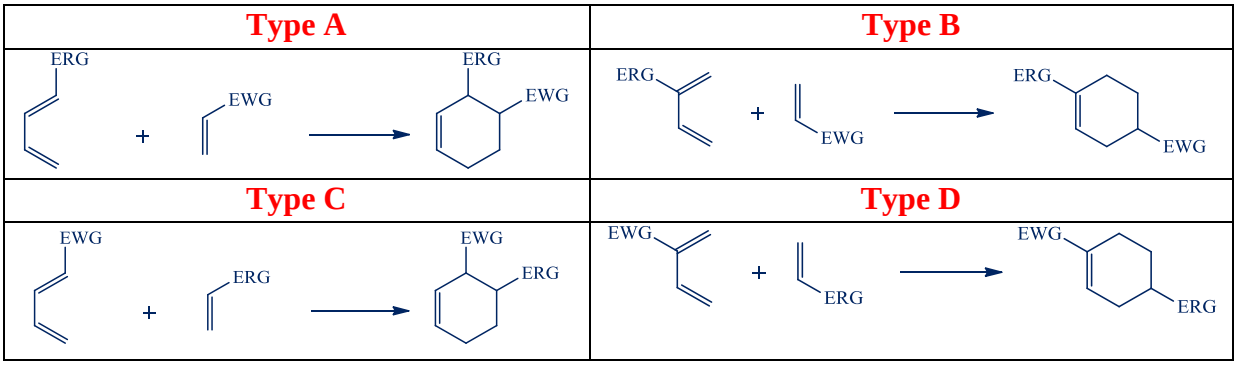


Figure 1.7 *Frontier Orbital interactions in Diels-Alder reactions.*

The regiochemical relationships summarized in **Scheme 1.3** can be understood by considering the atomic coefficients of the frontier orbitals. **Figure 1.8** gives the approximate energies and

orbital coefficients for the various classes of dienes and dienophiles.[23] 1-ERG substituents (X:) raise the HOMO level and increase the coefficient on C(4) of the diene. 2-ERG substituents raise the HOMO and result in the largest HOMO coefficient at C(1). For EWG substituents, the HOMO and LUMO are lowered in energy. For dienophiles, the largest LUMO coefficient is at C(2).



Scheme 1.3 *Regioselectivity of the Diels-Alder Reaction*

The regiochemistry can be predicted by the generalization that the strongest interaction is between the centers on the frontier orbitals having the largest orbital coefficients. For dienophiles with EWG substituents, π^* has its largest coefficient on the β-carbon atom. For dienes with ERG substituents at C(1) of the diene, the HOMO has its largest coefficient at C(4). This is the case designated Type **A** in **Scheme 1.3**, and is the observed regiochemistry for the type **A** Diels-Alder addition. A similar analysis of each of the other combinations in **Scheme 1.3** using the orbitals in **Figure 1.8** leads to the prediction of the favored regiochemistry. Note that in the type **A** and **C** reactions this leads to preferential formation of the more sterically congested 1,2-disubstituted cyclohexene. The predictive capacity of these frontier orbital relationships for D-A reactions is excellent.[24]

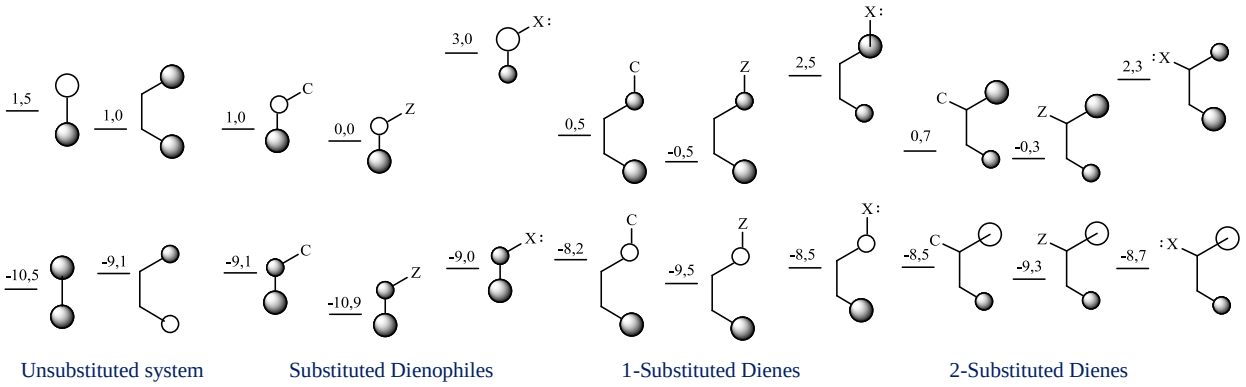


Figure 1.8 *Coefficients and relative energies of dienophiles and diene frontier MOs.*[a]

[a] Orbital energies are given in eV. The sizes of the circles give a relative indication of the orbital coefficient. Z stands for a conjugated EWG, e.g., CO, NO_2, CN; C is a conjugated substituent without strong electronic effect, e.g., phenyl, vinyl; X in a conjugated ERG, e.g., OCH_3, NH_2.

From these ideas, we see that for substituted dienes and dienophiles there is charge transfer in the process of formation of the TS. The more electron-rich reactant acts as an electron donor (nucleophilic) and the more electron-poor reactant accepts electron density (electrophilic). It also seems from the data in **Table 1.2** and **Table 1.3** that reactions are faster, the greater the extent of charge transfer. The reactivity of cyclopentadiene increases with the electron-acceptor capacity of the dienophile. Note also that the very strongly electrophilic dienophile, tetracyanoethene, is more sensitive to substituent effects in the diene than the more moderately electrophilic dienophile, maleic anhydride. These relationships can be understood in terms of FMO theory by noting that the electrophile LUMO and nucleophile HOMO are closer in energy the stronger the substituent effect, as illustrated schematically in **Figure 1.9**.

The FMO considerations are most reliable when one component is clearly more electrophilic and the other more nucleophilic. When a diene with a 2-EWG substituent reacts with an electrophilic dienophile, the major product is the 1,2 product ("para"), even though simple resonance consideration would suggest that the 1,3 product ("meta") might be preferred. Two examples are reported in **Figure 1.10**.

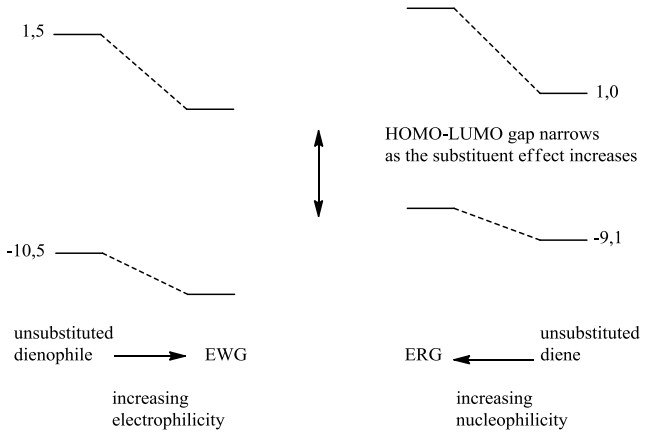


Figure 1.9 *Schematic diagram illustrating substituent effect on reactivity in terms of FMO theory.*[b]

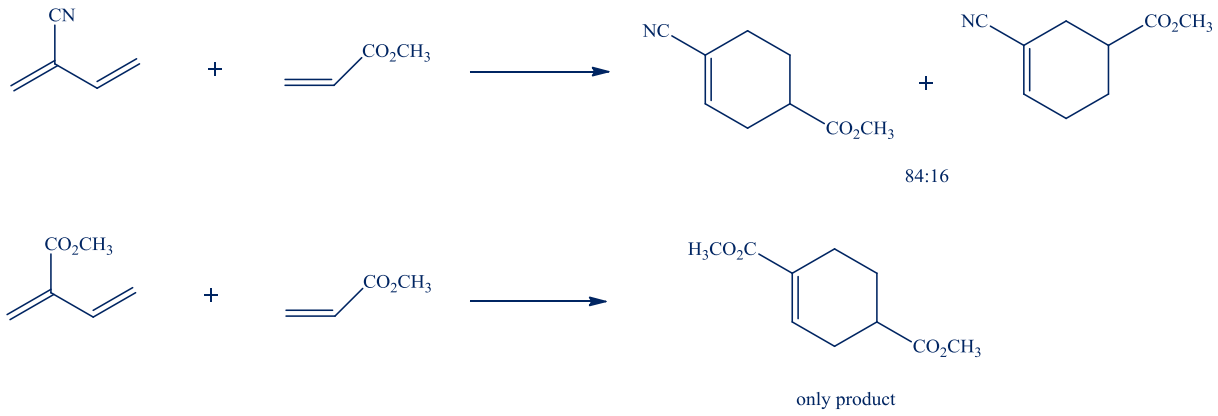


Figure 1.10 *Diels-Alder Reactions with diene 2-EWG substituted.*

[b] HOMO-LUMO gap narrows, transition state is stabilized and reactivity is increased in normal electron-demand Diels-Alder reaction as the nucleophilicity of diene and the electrophilicity of dienophile increase.

1.4 STEREOCHEMISTRY

Diels-Alder reaction has been the object of extensive mechanistic and computational study, as well as synthetic application. For most systems, the reactivity pattern, regioselectivity, and stereoselectivity are consistent with a concerted process. In particular, the reaction is a stereospecific *syn* (suprafacial) addition with respect to both the dienophile and the diene. This stereospecificity has been demonstrated with many substituted dienes and alkenes and also holds for the simplest possible example of the reaction, ethene with butadiene, as demonstrated by isotopic labeling (**Figure 1.11**).[25]

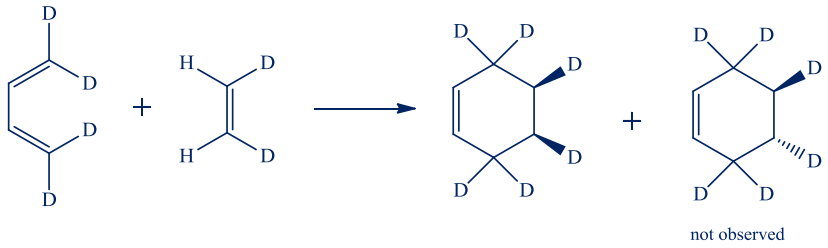


Figure 1.11 *Diels-Alder Reaction with isotopically-labeled diene and dienophile.*

The issue of the concertedness of the D-A reaction has been studied and debated extensively. It has been argued that there might be an intermediate that is diradical in character.[26] D-A reactions are almost always stereospecific, which implies that if an intermediate exists, it cannot have a lifetime sufficient to permit rotation or inversion. The prevailing opinion is that the majority of D-A reactions are concerted reactions and most theoretical analyses agree with this view.[27] It is recognized that in reactions between unsymmetrical alkenes and dienes, bond formation might be more advanced at one pair of termini than at the other. This is described as being an asynchronous process. Loss of stereospecificity is expected only if there is an intermediate in which one bond is formed and the other is not, permitting rotation or inversion at the unbound termini as shown in **Figure 1.12**.

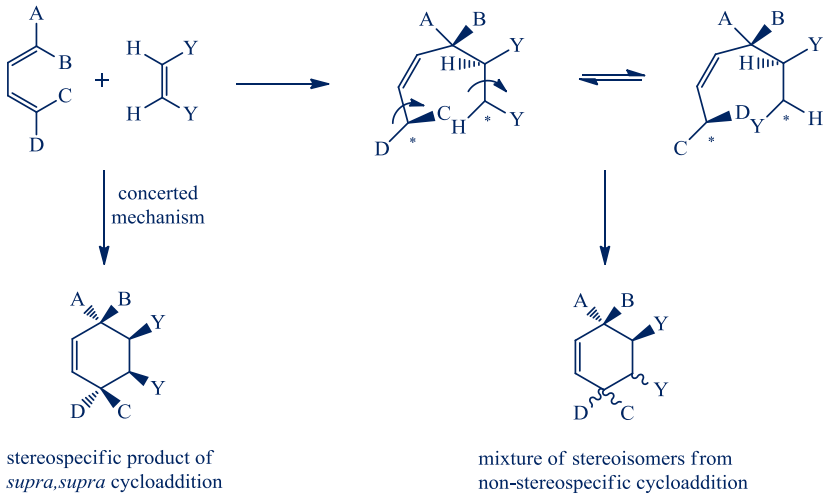
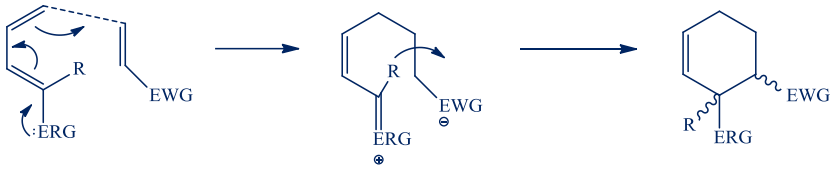


Figure 1.12 *Different Reaction Mechanisms in Diels-Alder Reaction.*

Loss of stereospecificity is observed when ionic intermediates are involved. This occurs when the reactants are of very different electronic character, with one being strongly electrophilic and the other strongly nucleophilic. Usually more than one substituent of each type is required for the ionic mechanism, as shown in **Scheme 1.4**, to occur.



Scheme 1.4 *Hypothetical Ionic Mechanism for Diels-Alder Reaction.*

For a substituted dienophile, there are two possible stereochemical orientations with respect to the diene. In the *endo* TS the reference substituent on the dienophile is oriented toward the π orbitals of the diene. In the *exo* TS the substituent is oriented away from the π system. The two possible orientations are called *endo* and *exo*, as illustrated in **Figure 1.13(a)**. For many substituted butadiene derivatives, the two TSs lead to two different stereoisomeric products. The *endo* mode of addition is usually preferred when an EWG substituent such as a carbonyl group is present on the dienophile. This preference is called the Alder rule. Frequently a mixture of both stereoisomers is formed and sometimes the *exo* product predominates, but the Alder rule is a useful initial guide to prediction of the stereochemistry of a D-A reaction. The *endo* product is often the more sterically congested. For example, the addition of dienophiles to cyclopentadiene usually favors the *endo*-stereoisomer, even though this is the sterically more congested product as shown in **Figure 1.13(b)**.

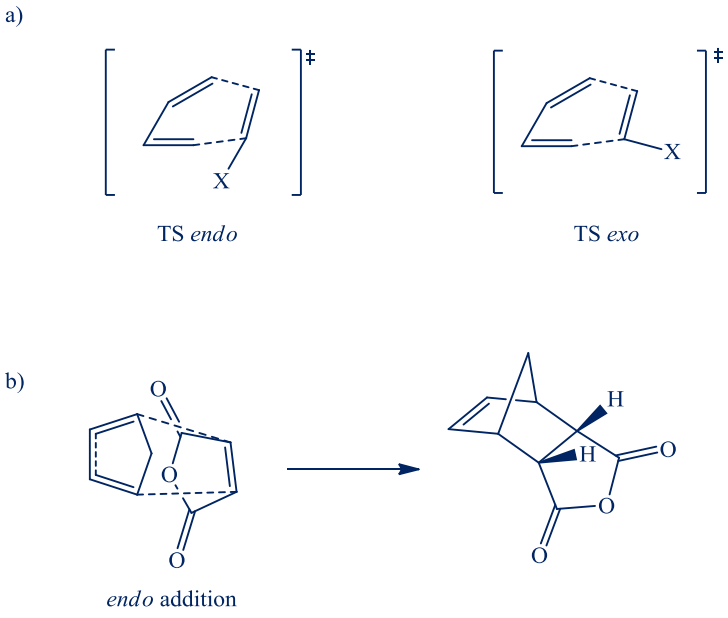


Figure 1.13 *a) Exo and endo transition structures for the Diels-Alder reaction; b) addition of maleic anhydride to cyclopentadiene.*

The preference for the *endo* mode of addition is not restricted to cyclic dienes such as cyclopentadiene. By using deuterium labels it has been shown that in the addition of 1,3-butadiene and maleic anhydride, 85% of the product arises from the *endo* TS as shown in **Figure 1.14**.[28]

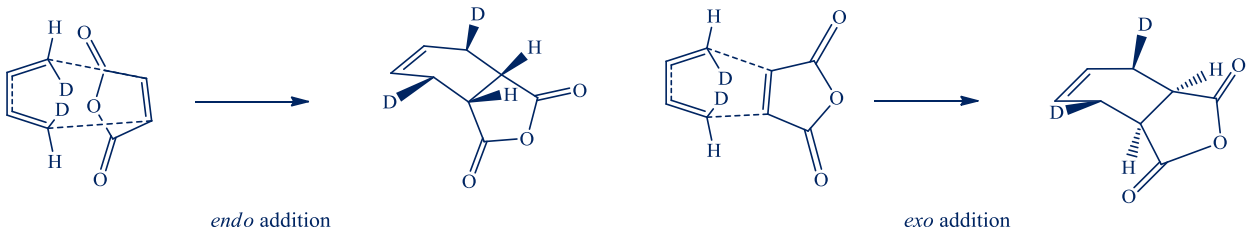
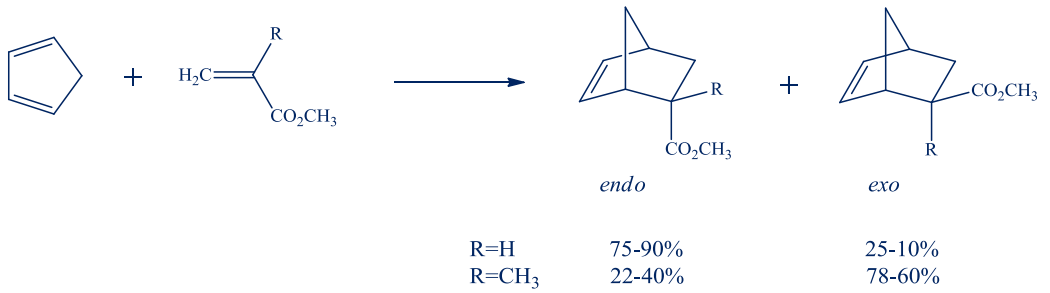


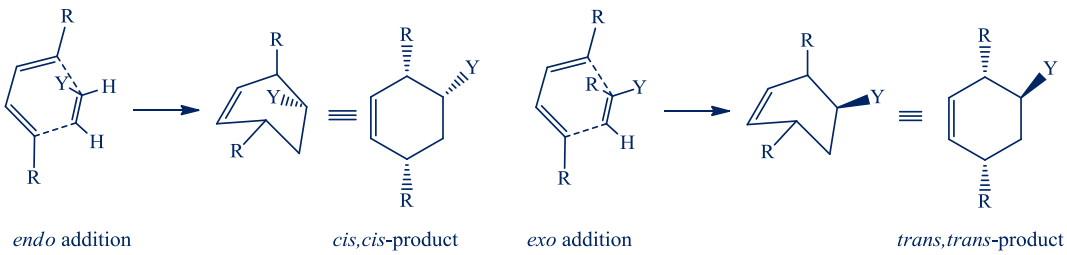
Figure 1.14 *Cycloaddition between deuterium labeled 1,3-butadiene and maleic anhydride.*

The stereoselectivity predicted by the Alder rule is independent of the requirement for suprafacial-suprafacial cycloaddition because both the *endo* and *exo* TSs meet this requirement. There are many exceptions to the Alder rule and in most cases the preference for the *endo* isomer is relatively modest. For example, although cyclopentadiene reacts with methyl acrylate in decalin solution to give mainly the *endo* adduct (75%), the ratio is solvent sensitive and ranges up to 90% *endo* in methanol. When a methyl substituent is added to the dienophile (methyl methacrylate) the *exo* product predominates (**Scheme 1.5**).[29]



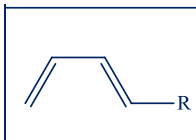
Scheme 1.5 *Diels-Alder reaction between cyclopentadiene and methyl acrylate or methyl methacrylate.*

Stereochemical predictions (**Scheme 1.6)** based on the Alder rule are made by aligning the diene and dienophile in such a way that the unsaturated substituent on the dienophile overlaps the diene π system.



Scheme 1.6 *Stereochemical product predictions of the generic Diels-Alder reaction.*

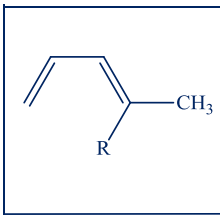
There are probably several factors that contribute to determining the *endo*:*exo* ratio in any specific case, including steric effects, electrostatic interactions, and London dispersion forces.[30] Molecular orbital interpretations emphasize secondary orbital interactions between the π orbitals on the dienophile substituent(s) and the developing π bond between C(2) and C(3) of the diene.
D-A cycloadditions are sensitive to steric effects. Bulky substituents on the dienophile or on the termini of the diene can hinder the approach of the two components to each other and decrease the rate of reaction. This effect can be seen in the relative reactivity of 1-substituted butadienes toward maleic anhydride as observed in **Table 1.4**.[31]



R	K_{rel} (25°C)
H	1
CH_3	4,2
$C(CH_3)_3$	<0,05

Table 1.4 *Kinetic constants of the substituted 1,3-butadiene in reaction with maleic anhydride.*

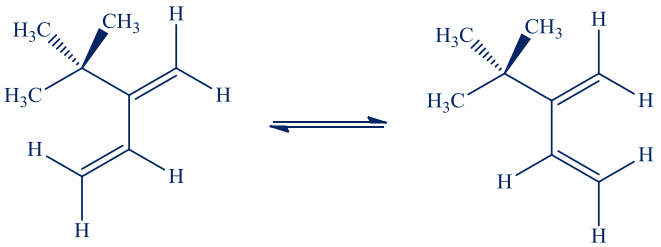
Substitution of hydrogen by methyl results in a slight rate increase as a result of the electron-releasing effect of the methyl group. A t-butyl substituent produces a large rate decrease because the steric effect is dominant.
Another type of steric effect has to do with interactions between diene substituents. Adoption of the *s-cis* conformation of the diene in the TS brings the *cis*-oriented 1- and 4-substituents on diene close together. *trans*-1,3-Pentadiene is 103 times more reactive than 4-methyl-1,3-pentadiene toward the very reactive dienophile tetracyanoethene, owing to the unfavorable steric interaction between the additional methyl substituent and the C(1) hydrogen in the *s-cis* conformation (**Table 1.5**).[32]



R	K_{rel}
H	1
CH_3	10^{-3}

Table 1.5 *Kinetic constants of the monosubstituted 1,3-pentadiene in reaction with tetracyanoethene.*

Relatively small substituents at C(2) and C(3) of the diene exert little steric influence on the rate of D-A cycloaddition. 2,3-Dimethylbutadiene reacts with maleic anhydride about ten times faster than butadiene because of the electron-releasing effect of the methyl groups. 2-t-Butyl-1,3-butadiene is 27 times more reactive than butadiene. The t-butyl substituent favors the *s-cis* conformation because of the steric repulsions in the *s-trans* conformation (**Scheme 1.7**). The presence of a t-butyl substituent on *both* C(2) and C(3), however, prevents attainment of the *s-cis* conformation, and D-A reactions of 2,3-di-(t-butyl)-1,3-butadiene have not been observed.[33]



Scheme 1.7 *s-trans and s-cis equilibrium of the 2-t-butyl-1,3-butadiene.*

Steric effects play a dominant role with more highly substituted dienes. Hexachlorocyclopentadiene, for example, shows a higher *endo* preference than cyclopentadiene because the 5-chlorine causes steric interference with *exo* substituents (**Figure 1.15**).[34]

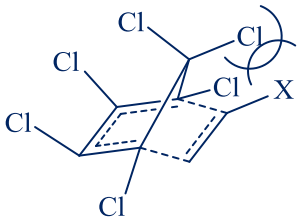
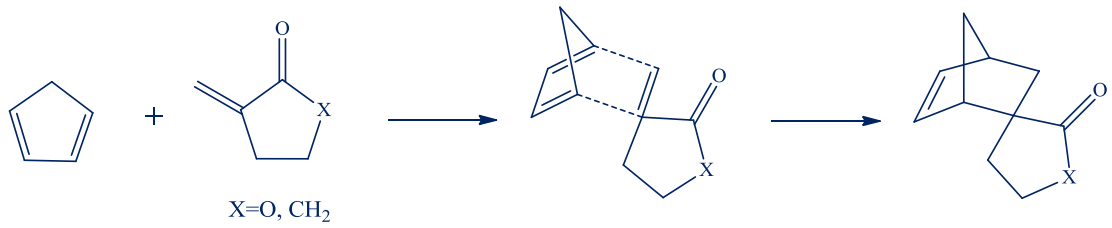


Figure 1.15 *Steric interaction with 5-chlorine of the cyclopentadiene and the exo substituents.*

Cyclic α-methylene ketones and lactones, in which the *syn* conformation is enforced, give predominantly *exo* adducts (**Scheme 1.8)**.[35]



Scheme 1.8 *Diels-Alder between cyclopentadiene and generic α-methylene ketone or lactone.*

It has been suggested that this is due to a more favorable alignment of dipoles in the *exo* TS.[36]

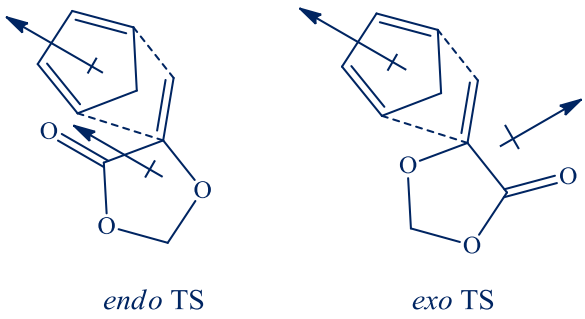


Figure 1.16 *Dipole Moment in Transition State.*

1.5 CATALYSIS AND ENANTIOSELECTION

Diels-Alder reactions are catalyzed by many Lewis acids, including $SnCl_4$, $ZnCl_2$, $AlCl_3$, and derivatives of $AlCl_3$ such as $(CH_3)_2AlCl$ and $(C_2H_5)_2AlCl$.[37] A variety of other Lewis acids are effective catalysts. The types of dienophiles that are subject to catalysis are typically those with carbonyl substituents. Lewis acids form complexes at the carbonyl oxygen (**Figure 1.17**) and this increases the electron-withdrawing capacity of the carbonyl group. The basic features are well modeled by HF/3-21G level computations on the TS structures.[38]

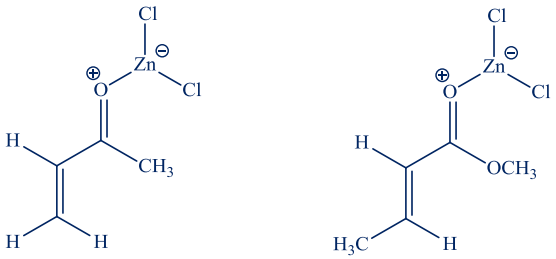
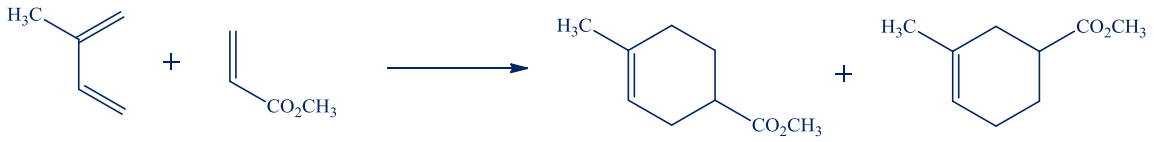


Figure 1.17 *TS structures modeled by HF/3-21G level computations of the dienophile Zinc dichloride coordinated.*

This complexation accentuates both the energy and orbital distortion effects of the substituent and enhances both the reactivity and selectivity of the dienophile relative to the uncomplexed compound (**Scheme 1.9**).[39] Usually, both regioselectivity and *exo,endo* stereoselectivity increase. Part of this may be due to the lower reaction temperature. However, the catalysts also shift the reaction toward a higher degree of charge transfer by making the EWG substituent more electrophilic.



	1,4-product ("para")	1,3-product ("meta")
uncatalyzed reaction, 120°C, 6h	70%	30%
$AlCl_3$ -catalyzed, 20°C, 3h	95%	5%

Scheme 1.9 *D-A Reaction uncatalyzed and catalized with $AlCl_3$.*

The stereoselectivity of any particular D-A reaction depends on the details of the TS structure. The structures of several enone–Lewis acid complexes have been determined by X-ray crystallography.[40] The site of complexation is the carbonyl oxygen, which maintains a trigonal geometry, but with somewhat expanded angles (130°–140°). The Lewis acid is normally *anti* to the larger carbonyl substituent. Boron trifluoride complexes are tetrahedral, but Sn(IV) and Ti(IV) complexes can be trigonal bipyramidal or octahedral. The structure of the 2-methylpropenal-BF_3 complex is illustrative in **Figure 1.18**.[41]

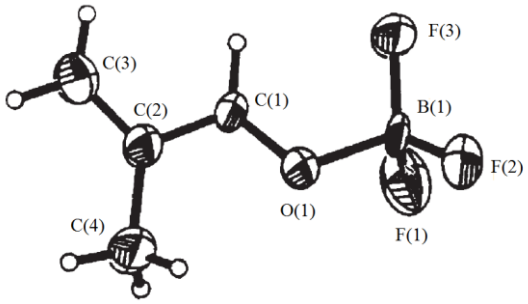


Figure 1.18 *Structure of the 2-methylpropenal-BF_3 complex.*

Chelation can favor a particular structure. For example, O-acryloyl lactates adopt a chelated structure with $TiCl_4$ as shown in **Figure 1.19**.[42]

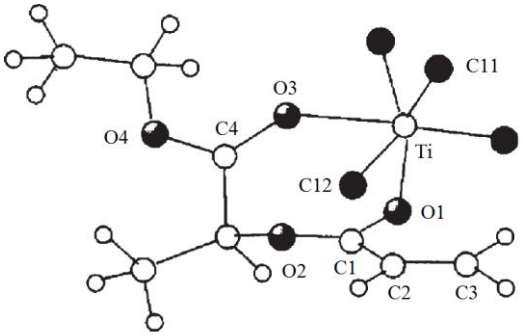
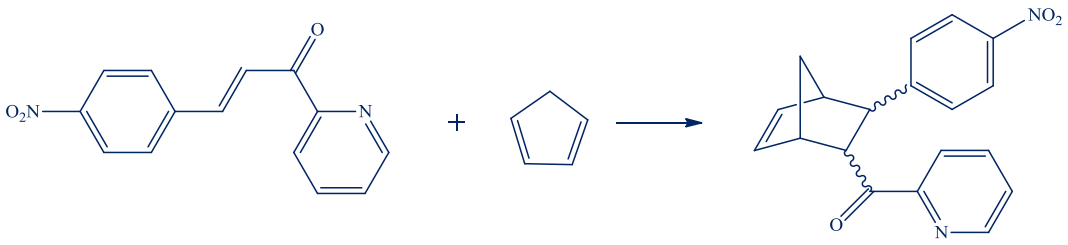


Figure 1.19 *O-acryloyl lactate chelated with $TiCl_4$.*

Lewis acid catalysis can also be applied to inverse electron demand D-A reactions, but with the proviso that the strongest interaction must be with the diene in this case (**Scheme 1.10**).[43]

Scheme 1.10 *Lewis acid catalysis applied to inverse electron demand D-A reactions.*

Metal cations can catalyze reactions of certain dienophiles. For example, Cu^{2+} strongly catalyzes addition reactions of 2-pyridyl styryl ketones as depicted in **Figure 1.20**, presumably through a chelate.[44] DFT (B3LYP/6-31G*) computations indicate that this reaction shifts to a stepwise ionic mechanism in the presence of the Lewis acid.[45]



Solvent	Rate ($M^{-1}s^{-1}$)	Relative Rate
acetonitrile	$1,3x10^{-5}$	1
ethanol	$3,8x10^{-5}$	2,9
water	$4,0x10^{-3}$	310
Water + 0.01M $Cu(NO_3)_2$	3,25	250.000

Figure 1.20 *D-A reaction between cyclopentadiene and 2-pyridyl-styryl ketones catalyzed by* Cu^{2+}.

The solvent also has an important effect on the rate of D-A reactions. The traditional solvents were nonpolar organic solvents such as aromatic hydrocarbons. However, water and other highly polar solvents, such as ethylene glycol and formamide, accelerate a number of D-A reactions.[46] The accelerating effect of water is attributed to "enforced hydrophobic interactions."[47] That is, the strong hydrogenbonding network in water tends to exclude nonpolar solutes and forces them together, resulting in higher effective concentrations. There may also be specific stabilization of the developing TS.[48] For example, hydrogen bonding with the TS can contribute to the rate acceleration.[49]

Hydrogen-bonding interactions can be designed into reaction systems. For example, the reactants **1** and **2** reported in **Figure 1.21** were found to react much more rapidly than the corresponding ester and to give exclusively the *exo* product.[50] Molecular mechanics and spectroscopic studies indicate that the hydrogen-bonding pattern is responsible.

To summarize the key points, D-A reactions are usually concerted processes. The regio- and stereoselectivity can be predicted by applying FMO analysis. The reaction between electron donor dienes and electron acceptor dienophiles is facilitated by Lewis acids, polar solvents, and favorable hydrogen-bonding interactions. The D-A reaction is quite sensitive to steric factors, which can retard the reaction and also influence the stereoselectivity with respect to *exo* or *endo* approach.

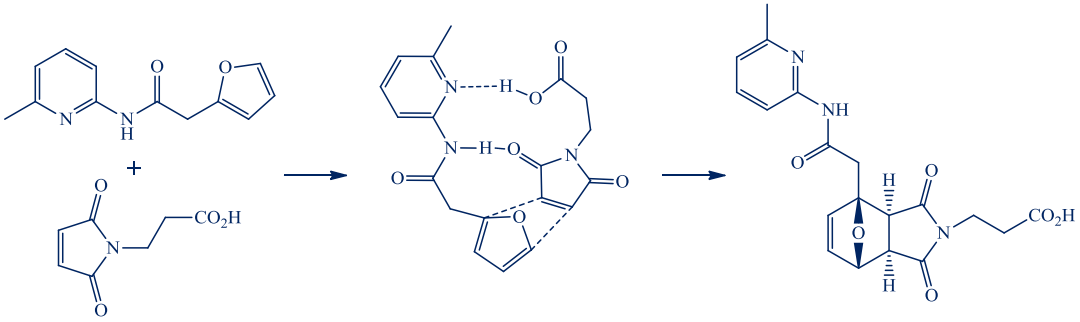
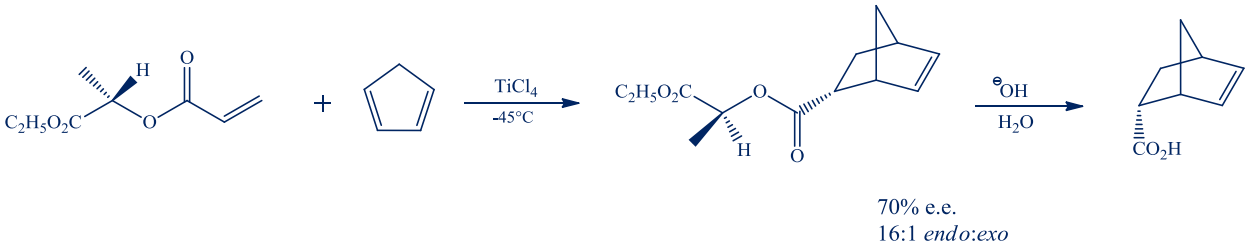


Figure 1.21 *Hydrogen-bonding interaction in Diels-Alder reaction.*

The highly ordered cyclic TS of the D-A reaction permits design of diastereo or enantioselective reactions. One way to achieve this is to install a chiral auxiliary.[51] The cycloaddition proceeds to give two diastereomeric products that can be separated and purified. Because of the lower temperature required and the greater stereoselectivity observed in Lewis acid–catalyzed reactions, the best diastereoselectivity is observed in catalyzed reactions. Several chiral auxiliaries that are capable of high levels of diastereoselectivity have been developed. Chiral esters and amides of acrylic acid are particularly useful because the auxiliary can be recovered by hydrolysis of the purified adduct to give the enantiomerically pure carboxylic acid (**Scheme 1.11**).[52] Early examples involved acryloyl esters of chiral alcohols, including lactates and mandelates. Esters of the lactone of 2,4-dihydroxy-3,3-dimethylbutanoic acid (pantolactone) have also proven useful.



Scheme 1.11 *Chiral ester hydrolysis to give enantiomerically pure carboxylic acid.*

Prediction and analysis of diastereoselectivity are based on steric, stereoelectronic, and complexing interactions in the TS.[53] In the case of the lactic acid auxiliary, a chelated structure promotes facial selectivity. In the $TiCl_4$ complex of O-acryloyl ethyl lactate, one of the chlorines attached to titanium shields one face of the double bond as shown in **Figure 1.22** (see also **Figure 1.19**).

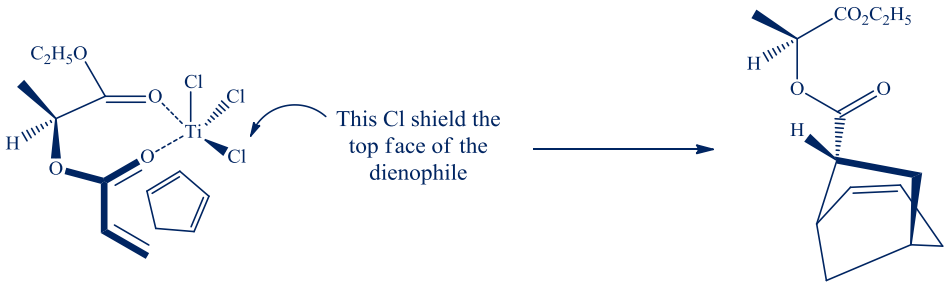


Figure 1.22 *Selectivity of O-acryloyl ethyl lactate-$TiCl_4$ complex to Cyclopentadiene.*

Table 1.6 gives some other examples of use of chiral auxiliaries in D-A reactions.[54] Entries 1 and 2 show two chiral auxiliaries developed from terpene precursors. The acrylate shown in Entry 1 gave excellent enantioselectivity with cyclopentadiene and 1,3-butadiene, but introduction of a methyl substituent on the dienophile (crotonyl derivative) resulted in a very slow reaction owing to steric problems. Entry 2 involves another sultam auxiliary. The chirality of the product is consistent with approach of the diene from the *re* face of a conformation in which the carbonyl oxygen is *syn* to the sulfonyl group. Entry 3 shows a carbohydrate-derived

auxiliary with SnCl4 as the Lewis acid. This dienophile also gives good enantioselectivity using TiCl4 as the Lewis acid. Entry 4 is a proline-derived oxazolidinone auxiliary used in conjunction with ZrCl4. The observed diastereoselectivity is consistent with a chelated TS having an *s-cis* conformation at the carbonyl group.

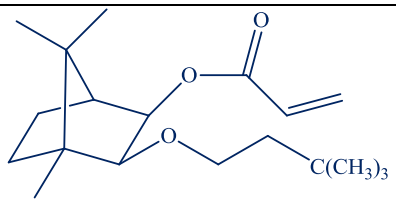
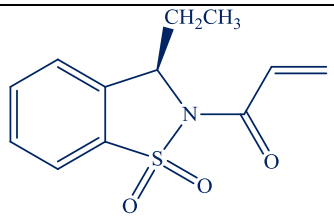
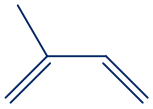
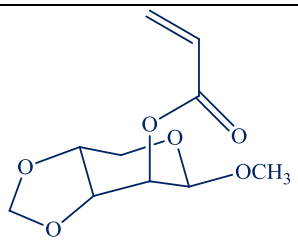
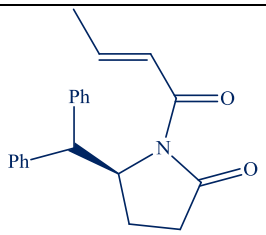
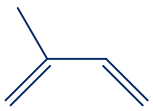
Entry	Dienophile	Diene	Catalyst, Temperature	Yield (%)	*dr*
1			$TiCl_2(i\text{-}OPr)_2$, -20°C 1,5 equiv	90	>99:1
2			$(C_2H_5)_2AlCl$, -40°C	94	98:2
3			$SnCl_4$, -78°C 2,0 equiv	93	96:4
4			$ZrCl_4$, -78°C	86	>99:1

Table 1.6 *Diels-Alder Reactions with Chiral Auxiliaries.*[55]

Enantioselectivity can also be achieved with chiral catalysts. Several examples of catalytic enantioselective D-A reactions are given in **Table 1.7**. Entries 1 to 6 involve *N*-acyloxazolidinones and *N*-acylthiazolidinones as dienophiles. Note that there are no stereogenic centers in the reactants, so racemic mixtures would result from reaction in the absence of a chiral catalyst. The metal ions used in these reactions can accommodate two additional ligands in addition to those present in the catalyst. The reactions are believed to involve a chelated TS. The phenyl substituents and the tetrahedral coordination geometry at magnesium give rise to a well-defined geometry. Note that the catalyst has c_2 symmetry. In entry 1 the phenyl substituents cause differential facial shielding. The enantioselectivity of this catalyst, which is prepared as the iodide salt, is somewhat dependent on the anion that is present. If $AgSbF_6$ is used as a cocatalyst, the iodide is removed by precipitation and the e.e. increases from 81 to 91%. These results indicate that the absence of a coordinating anion improved enantioselectivity. In Entry 2 Cu^{2+} as the metal, the coordination geometry is square planar. The complex exposes the *Re* face of the

dienophile. Entry 3 involves a catalyst derived from (R,R)-*trans*-cyclohexane-1,2-diamine. The square planar Cu^{2+} complex exposes the *Re* face of the dienophile. As with the BOX catalysts, this catalyst has c_2 symmetry. In Entry 6, the catalyst was prepared using Ti(O-*i*-Pr)$_4$ and $SiCl_4$. In this catalyst, the aryl groups are carry 3,5-dimethyl groups. The 3,5-di-CF_3 and 3,5-di-Cl derivatives, which were also studied, gave high *exo*:*endo* ratios, but much reduced enantioselectivity. This is thought to be due to the reduced π donor character of the rings with EWG substituents. The presence of chlorides at the Ti center is also probably an important factor in the reactivity of the catalyst.
Entry 9 uses the oxaborazolidine catalysts with 2-bromopropenal as the dienophile. The aldehyde adopts the *exo* position in each case.

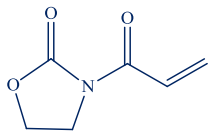
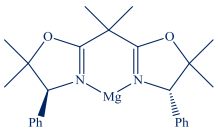
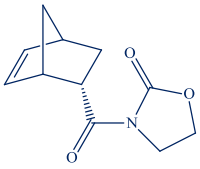
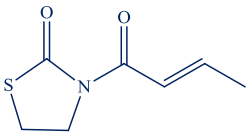
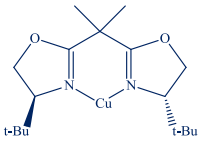
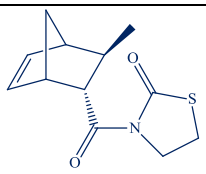
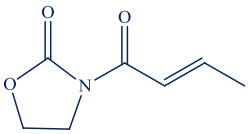
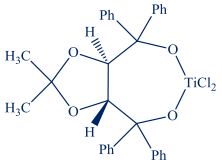
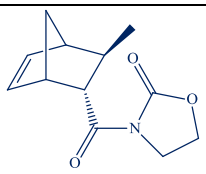
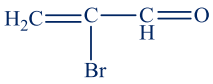
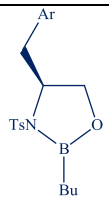
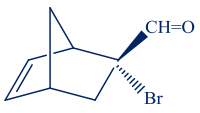
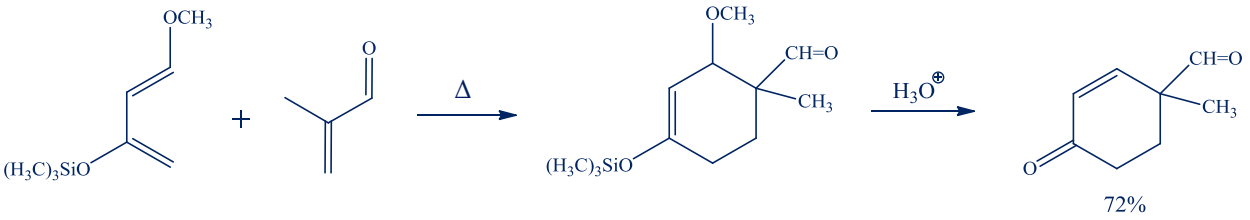
Entry	Dienophile	Diene	Catalyst	Amount	Product	Yield (%)	*e.e.*
1				10%		82	95
2				10%		79	94
6				20%		92	93
9			 Ar= 3-indolyl	5%		>99,5	>99,5

Table 1.7 *Catalytic Enantioselective Diels-Alder Reactions.*[56]

1.6 SYNTHETIC APPLICATIONS

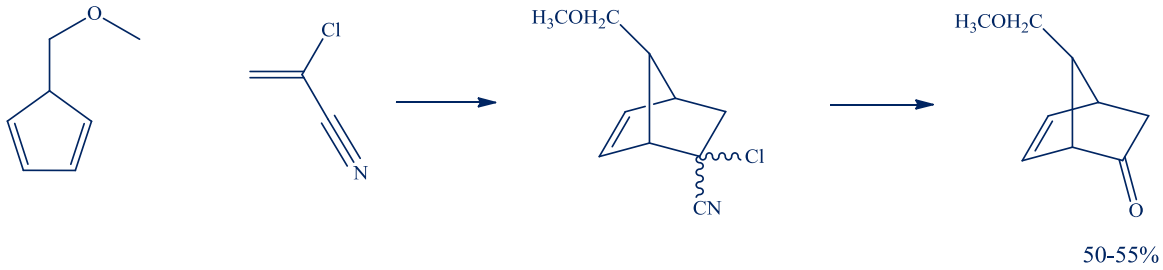
The D-A reaction is frequently used in synthesis and can either be utilized early in a process to construct basic ring structures or to bring together two subunits in a convergent synthesis. The intramolecular version, for example, can be used to construct two new rings. The virtues of the D-A reaction include its ability to create a cyclohexene ring by formation of two new bonds with predictable regiochemistry. The reaction can also create as many as four contiguous stereogenic

centers. The stereoselectivity is also often predictable on the basis of the *supra-supra* stereospecificity and considerations of the preference for the *endo* or *exo* TS. The synthetic value of D-A reactions can be enhanced in various ways. In addition to hydrocarbon dienes, substituted dienes can be used to introduce functional groups into the products. One example is illustrated in **Scheme 1.12**.



Scheme 1.12 *Functionalized product of the Diels-Alder Reaction.*

The two donor substituents provide strong regiochemical control. The D-A adducts are trimethylsilyl enol ethers that can be readily hydrolyzed to ketones. The β-methoxy group is often eliminated during hydrolysis, resulting in formation of cyclohexenones.[57]
The synthetic utility of the D-A reaction can be expanded by the use of dienophiles that contain masked functionality and are the synthetic equivalents of unreactive or inaccessible compounds. For example, α-chloroacrylonitrile shows, in **Scheme 1.13**, satisfactory reactivity as a dienophile. The α-chloronitrile functionality in the adduct can be hydrolyzed to a carbonyl group. Thus, α-chloroacrylonitrile can function as the equivalent of ketene, $CH_2=C=O$,[58] which is not a suitable dienophile because it has a tendency to react with dienes by [2+2] cycloaddition, rather than the desired [4+2] fashion.



Scheme 1.13 *Diels-Alder reaction between cyclopentadiene derivative and ketene equivalent.*[59]

The discovery of new pharmacological agents is one of the biggest challenges for current research. In this context the Diels-Alder reaction play an excellent and important role.
In **Table 1.8** are reported some products at biological activity obtained for kay-step Diels-Alder reaction.

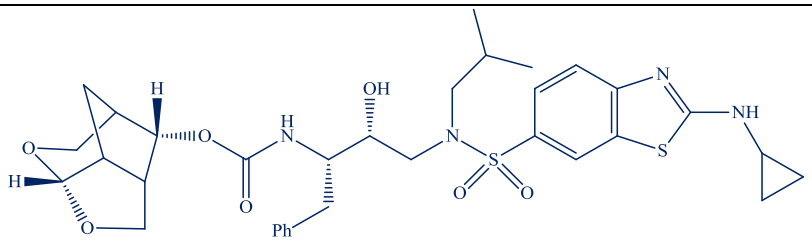
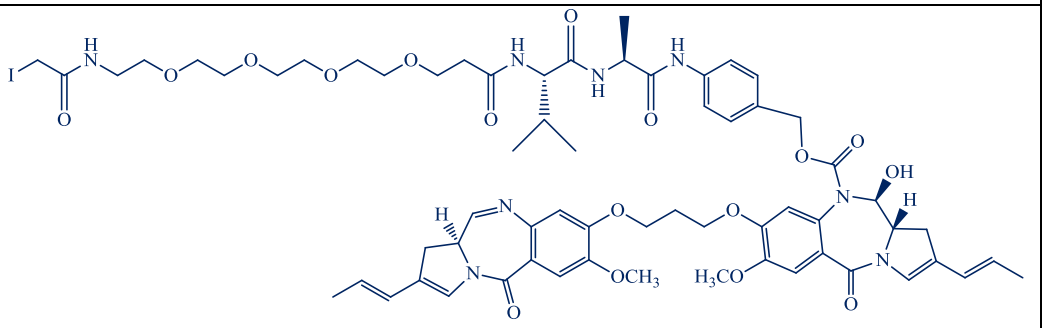
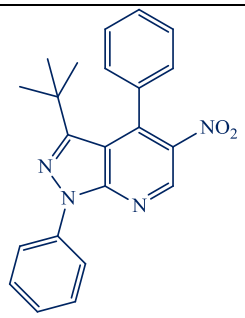
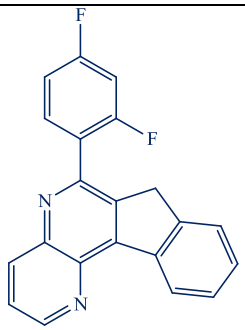
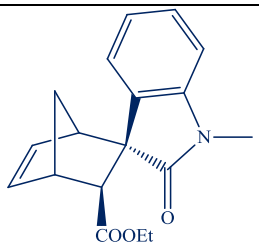
Entry	Product
1	
2	
3	
4	
5	

Table 1.8 *Some product at biological activity obtained with Diels-Alder reactions.*

In Entry 1 an exceptionally potent HIV-1 protease inhibitor is reported. Generally, the introduction of protease inhibitors (PIs) in combination with antiretroviral therapies (ART) improve the treatment of patients with HIV-1 infection and AIDS. This inhibitor have a inhibition constant and a inhibition concentration very low (K_i=0,04 nM (HIVP), IC_{50}=0,26 nM as antiviral). A portion of this molecule has been synthesized efficiently in an optically active

form using a enantioselective Diels−Alder cycloaddition providing a key intermediate in high enantiomeric purity.[60]

In Entry 2 a compound named SG3227 is reported, this was rationally designed based on the naturally occurring antitumour compound sibiromycin[c]. SG3227 was synthesized from a dimeric core in an efficient fashion. An unexpected room temperature Diels-Alder reaction occurred during the final step of the synthesis and was circumvented by use of an iodoacetamide conjugation moiety in place of a maleimide. It has exhibited potent activity against a HER2 human cancer cell line in vitro.[61]

A pyrazolo[3,4-*b*]pyridines (entry 3) were prepared by a microwave-assisted aza-Diels–Alder reaction between pyrazolylformimidamides derivative and β-nitrostyrenes. This procedure provides a simple one-step and environmentally friendly methodology with good yields for the synthesis. This compounds was tested for antifungal activity against two clinically important fungi *Candida albicans* and *Cryptococcus neoformans*, the most common causes of opportunistic fungal infections.[62]

In Entry 4 a compound that inhibit Topoisomerase I activity is reported. The product is obtained in two steps in which the first consist in Diels-Alder cycloaddition. In vitro test has demonstrated that this product exhibited a cytotoxic effect on cell lines derived from human breast cancer (BT 20), human lung adenocarcinoma (A 549) or human ovarian carcinoma (SKOV3).[63]

In Entry 5 a spiro-oxindole obtained with high yield and good diastereosiomeric ratio is showed. It was tested *in vitro* on Cytocrome P450 enzyme from *Mycobacterium tuberculosis* and was established that the Diffusion Constant is very low (K_D=357μM).

[c] Sibiromycin is the most potent naturally occurring pyrrolobenzodiazepine (IC_{50} L1210, leukaemia = 2.9 nM). It contains an amino sugar derivative at the C7 position and a *trans*-propenyl group at the C2 position that interacts favourably with the minor groove of DNA.

CHAPTER 2

CHAPTER 2 – 1,3-DIPOLAR CYCLOADDITION

2.1 INTRODUCTION

Dipolar cycloaddition reactions (1,3-DPCA) are useful for synthesis of heterocyclic compounds and for carbon-carbon bond formation. There is a large class of reactions known as 1,3-dipolar cycloaddition reactions that are analogous to the Diels-Alder reaction in that they are concerted [$\pi 4_s + \pi 2_s$] cycloadditions.[64] 1,3-DPCA reactions can be represented as shown in the following **Figure 2.1**. The entity a-b-c is called the 1,3-dipole and d-e is the dipolarophile.

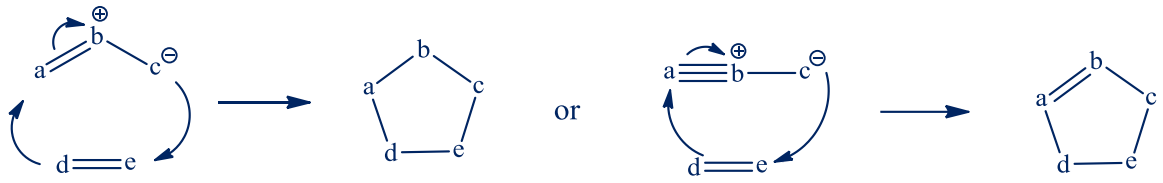


Figure 2.1 *General representation of the 1,3-dipolar cycloaddition.*

The 1,3-dipoles have a π-electron system consisting of two filled and one empty orbital and are analogous with the allyl or propargyl anion. Each 1,3-dipole has at least one charge-separated resonance structure with opposite charges in a 1,3-relationship. It is this structural feature that leads to the name 1,3-dipole for this class of reactants. The dipolarophiles are typically substituted alkenes or alkynes but all that is essential is a π bond, and other multiply bonded functional groups such as carbonyl, imine, azo, and nitroso can also act as dipolarophiles. The reactivity of dipolarophiles depends both on the substituents present on the π bond and on the nature of the 1,3-dipole involved in the reaction. Owing to the wide range of structures that can serve either as a 1,3-dipole or as a dipolarophile, the 1,3-DPCA is a very useful reaction for the construction of five-membered heterocyclic rings. **Table 2.1** gives some examples using both ethenyl and ethynyl dipolarophiles.

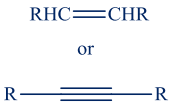
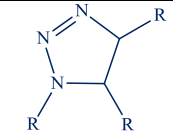
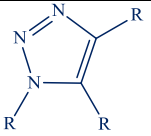
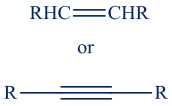
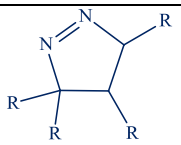
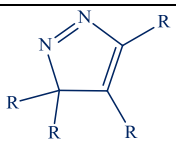
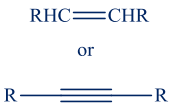
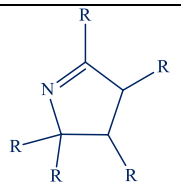
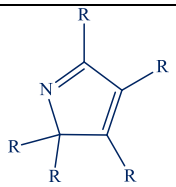
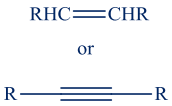
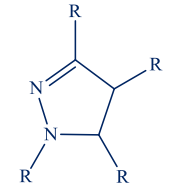
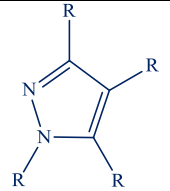
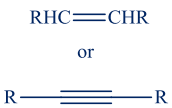
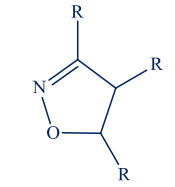
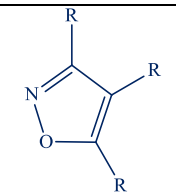
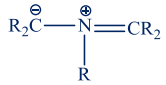
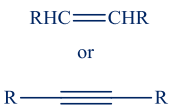
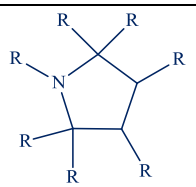
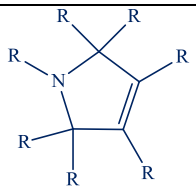
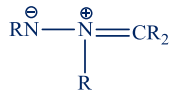
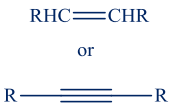
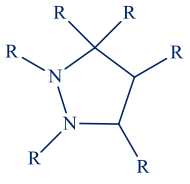
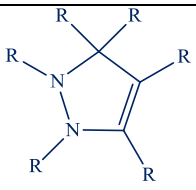
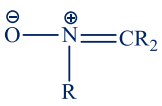
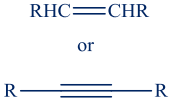
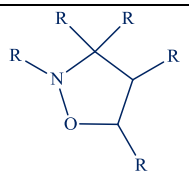
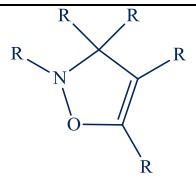
1,3-Dipole	Dipolarophile	Alkenyl derived	Alkinyl derived
 AZIDE		 1,2,3-TRIAZOLINE	 1,2,3-TRIAZOLE
 DIAZOALKANE		 1-PYRAZOLINE	 3H-PYRAZOLE
 NITRILE YLIDE		 1-PYRROLINE	 2H-PYRROLE
 NITRILE IMINE		 2-PYRAZOLINE	 PYRAZOLE
 NITRILE OXIDE		 2-ISOXAZOLINE	 ISOXAZOLE
 AZOMETHINE YLIDE		 PYRROLIDINE	 3-PYRROLINE
 AZOMETHINE IMINE		 PYRAZOLIDINE	 3-PYRAZOLINE
 NITRONE		 ISOXAZOLIDINE	 4-ISOXAZOLINE

Table 2.1 *Class of heterocyclic compounds obtained for reaction of different 1,3-dipoles with dipolarophile.*

The bonding changes for 1,3-DPCA reactions involve four π electrons from the 1,3-dipole and two from the dipolarophile. In most cases, the reaction is a concerted [$\pi 2_s + \pi 4_s$] cycloaddition.[65] As in the D-A reaction, the reactants approach one another in parallel planes. There is interaction between the complementary HOMO-LUMO combinations (**Figure 2.2**), and depending on the combination, either reactant can be the electrophilic or the nucleophilic component. Generally speaking, the reactant 1,3-dipoles are more polar than the TS or the reaction product. The rate of reaction is not strongly sensitive to solvent polarity.

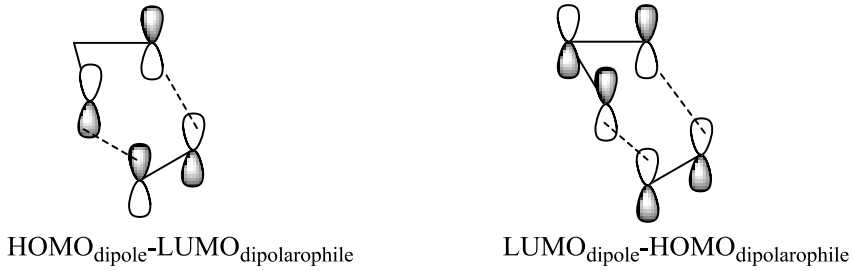


Figure 2.2 *FMO interaction between 1,3-dipole and dipolarophile.*

There have been many computational analyses of 1,3-DPCA TSs, and they are generally regarded to be aromatic in character. Typical TSs are characterized by aromatic NICS values.[66] The ring current associated with this aromaticity is primarily due to the six electrons undergoing bonding changes.[67] The orbital interactions in the cyclic TS serve as the focal point for discussion of relative reactivity, regioselectivity, and stereoselectivity of 1,3-DPCA reactions.

The most widely applied interpretation of substituent effects on relative reactivity is based on FMO theory. According to FMO theory, interacting orbitals are most stabilized when they are closest in energy. Substituent effects on dipolar cycloadditions can be interpreted in terms of matching of HOMO and LUMO orbitals of the two reactants.[68] This is the same concept used in applying FMO theory to D-A reactions. In the D-A reaction, it is fairly clear which reactant is electrophilic and which is nucleophilic, and the interpretation of substituent effects follows directly. This choice is not always so obvious for 1,3-DPCA reactions. In fact, for several of the 1,3-dipoles both EWGs and ERGs in the dipolarophile enhance reactivity. These 1,3-dipoles are called ambiphilic. Let us look carefully to see why they have this property.

Much of the relative reactivity data on 1,3-DPCA reactions has been tabulated and discussed in reviews by R. Huisgen, a pioneer researcher in the field.[53] Some representative data are presented in **Table 2.2**. The dipolarophiles are shown in decreasing order of electrophilicity. The data from these monosubstituted dipolarophiles should be relatively free of steric influences on reactivity. Note that for phenyl azide and benzonitrile oxide, reactivity is at a minimum for unfunctionalized alkenes and is increased by both donor and acceptor substituents.

CH_2=CHX	Ph_2CN_2	PhN_3	PhC≡NO	$PhCH{=}NCH_3$	PhC≡NNPh	CH_2N_2
Dimethyl fumarate	996	31	94	18.3	283	
Dimethyl maleate	27.8	1.25	1.61	6.25	7.94	
Ethyl acrylate	288	36.5	66	11.1	48.2	175
Ethyl crotonate	1.0	1.0	1.0	1.0	1.0	1.0
Norbornene	1.15	700	97	0.13	3.12	3.3×10^{-2}
1-Alkene		0.8	2.6	0.072	0.146	6.9×10^{-4}
Styrene	0.57	1.5	9.3	0.32	1.60	6.9×10^{-2}
Cyclopentene		6.9	1.04	0.022	0.128	4.2×10^{-4}
Cyclohexene			0.055		0.011	1.6×10^{-5}
Vinyl ether		1.5	15			8.5×10^{-6}
Vinyl amine		$\sim 1 \times 10^5$				

Table 2.2 *Representative Relative Rate Data for 1,3-Dipolar Cycloadditions.*[69]

In addition to the electronic effects of substituents, several other structural features affect the reactivity of dipolarophiles. Strain increases reactivity. Norbornene, for example, is consistently more reactive than cyclopentene in 1,3-dipolar cycloadditions. Cyclopentene is also more reactive than cyclohexene. Conjugating substituents, such as the phenyl group in styrene, usually increase reactivity of dipolarophiles.

Sustmann and Trill118 summarized these and related reactivity relationships in terms of FMO theory and pointed out that 1,3-DPCA reactions could be of three types, depending on relative placement of the frontier orbitals: (**A**) $HOMO_{dipole}$-$LUMO_{dipolarophile}$ dominant; (**B**) $LUMO_{dipole}$-$HOMO_{dipolarophile}$ dominant; (**C**) both HOMO-LUMO interactions are significant. The first type should be accelerated by ERG in the dipole and EWG in the dipolarophile. The second type should be facilitated by an EWG in the dipole and an ERG in the dipolarophile. These relationships suggest a parabolic substituent effect as the Type **C** reactions shift from $LUMO_{dipolarophile}$ to mixed to $HOMO_{dipolarophile}$ controlled (**Figure 2.3**).

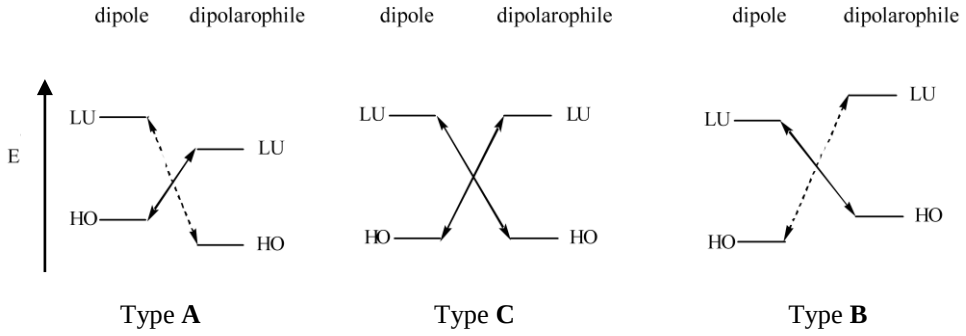
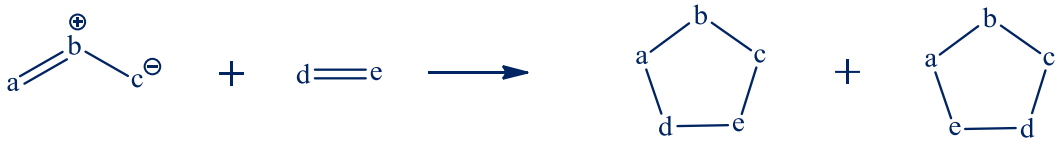


Figure 2.3 *The classification of 1,3-DC reactions on the basis of the FMOs.*

2.2 REGIOSELECTIVITY

1,3-Dipolar cycloaddition can be regioselective as shown in **Scheme 2.1**.



Scheme 2.1 *Generic regioisomer products in 1,3-dipolar cycloaddition.*

The observed regioselectivity is controlled by steric as well as electronic factors. Sometimes pure cycloadducts are isolated and occasionally a mixture of isomers is obtained.

Addition of terminal alkenes to the sterically crowded 1,3-dipoles generally leads to the formation of 5-substituted isomers. Electronic effects sometimes preponderate over steric effects. For example, the cycloaddition reaction between nitrone and terminal alkenes, with EDG in the dipolarophiles, leads to the formation of 5-substituted regio-isomer. On the other hand, terminal alkene with EWG leads to the formation of 4-substituted isomer.[70] The former reaction is mostly controlled by $LUMO_{dipole}$-$HOMO_{dipolarophile}$ interaction. The $LUMO_{dipole}$ has largest coefficient at the carbon atom an the $HOMO_{dipolarophile}$ has largest coefficient at the terminal carbon atom. Thus, the nitrone and alkene combine in a regioselective manner to give the 5-isoxazolidine.

When the reaction is mainly controlled by the $HOMO_{dipole}$-$LUMO_{dipolarophile}$ interaction, the $HOMO_{dipole}$ has largest coefficient at the oxygen atom whereas $LUMO_{dipolarophile}$ has largest coefficient at the terminal carbon atom. This favours formation of the 4-isomer, but when steric effects preponderate over electronic effects a mixture of regioisomers is often obtained.[71] In the case of the reaction between nitrone and 1,2-disubstituted alkene with EWG, steric factor is eliminated, leading to the formation of 4-*EWG*-substituted isomer as the sole product.

In General, the analysis of the regioselectivity of a 1,3-dipolar cycloaddition by FMO theory requires information about the energy and atomic coefficients of the frontier orbitals of the 1,3-dipole and the dipolarophile.

Figure 2.4 gives estimates of the energies of the HOMO and LUMO orbitals of some representative 1,3-dipoles. By using these orbital coefficients and calculating or estimating the relative energies of the interacting orbitals, it is possible to make predictions of the regiochemistry of 1,3-DPCA reactions.

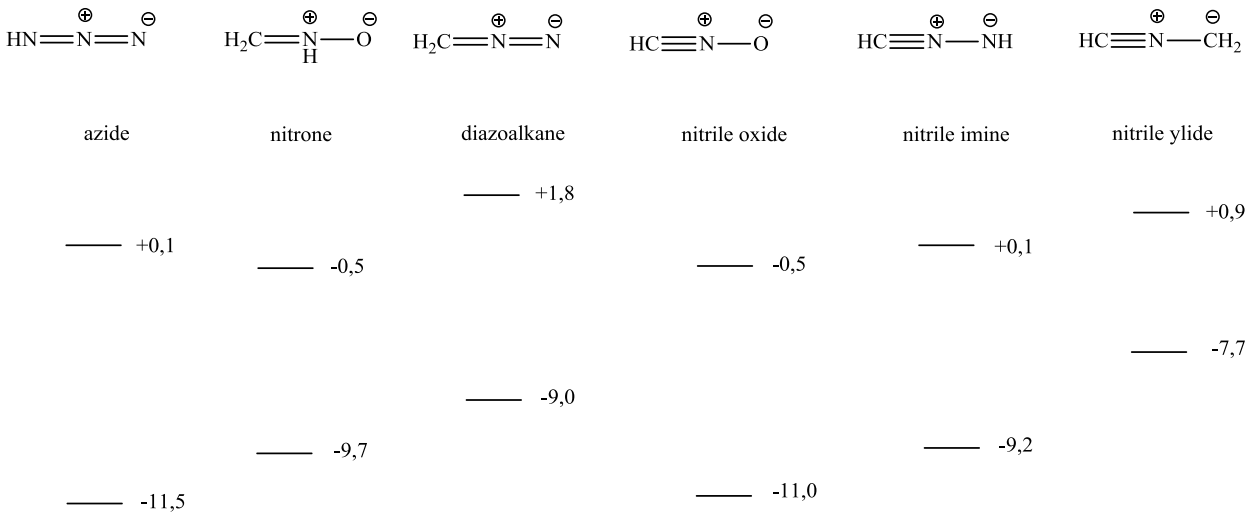


Figure 2.4 *Estimated energies (eV) of π FMOs for some 1,3-dipoles.*

The most important dipolarophiles are the same types of compounds that are dienophiles in the D-A reaction. The orbital coefficients can be used in analyses of 1,3-DPCA reactions to establish if the HOMO-LUMO combination will interact most strongly for a given pair of reactants.

This procedure is illustrated for two specific cases in **Figure 2.5**. The reaction of a nitrile oxide with an alkene is considered on the left. The smallest energy gap is for the alkene HOMO and the 1,3-dipole LUMO. This is qualitatively reasonable in that the atoms in the 1,3-dipole are more electronegative than those in the dipolarophile. The LUMO coefficient is largest at carbon for the nitrile oxide group. The largest coefficient for a terminal alkene HOMO is at C(1). The matching of the largest coefficients of the 1,3-dipole LUMO and the dipolarophile HOMO leads to the predicted (and observed) product. The same procedure can be applied to the case shown at the right of **Figure 2.5**. In this case, the 1,3-dipole is the nucleophile and the dipolarophile is the electrophile. The largest coefficient of the nitrone HOMO is at oxygen and the largest coefficient for the acrylate ester LUMO is at the β-carbon.

Although the FMO approach provides a good foundation for understanding the regioselectivity of 1,3-cycloadditions, there are many specific cases in which it fails to provide a complete understanding. Steric factors are not considered by the FMO analysis and in many instances steric factors control regiochemistry. 1,3-DPCA can be broadly classified as sterically controlled or electronically controlled. There may also be specific interactions in the TSs that are not considered by the FMO analysis and that they have to be evaluated case by case.

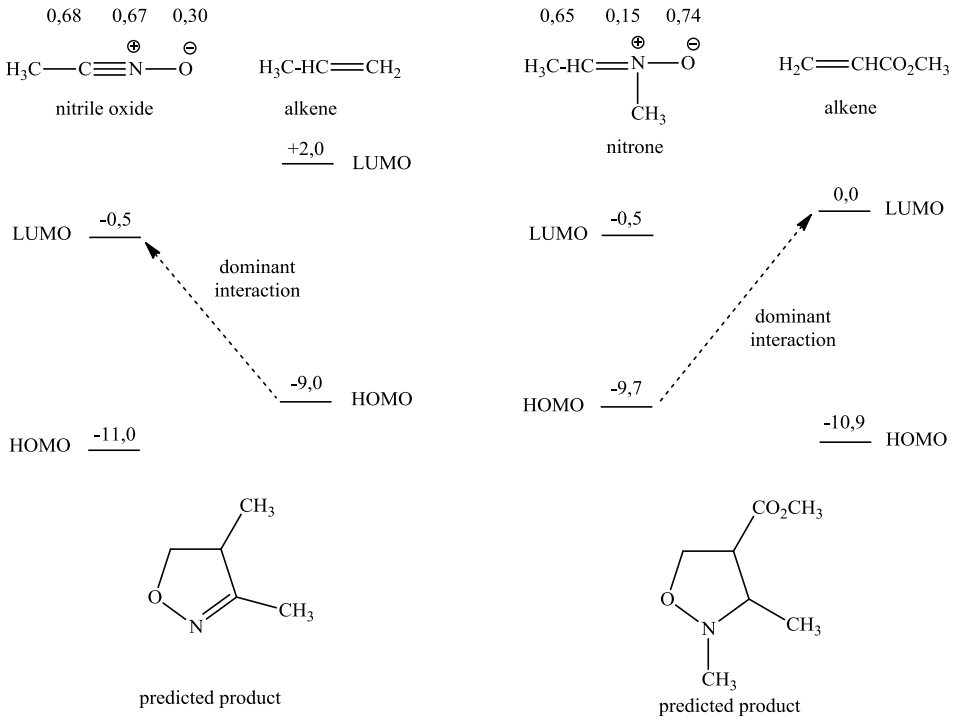


Figure 2.5 *Prediction of the regioselectivity of 1,3-dipolar cycloaddition reactions on the basis of FMO interactions.*[d]

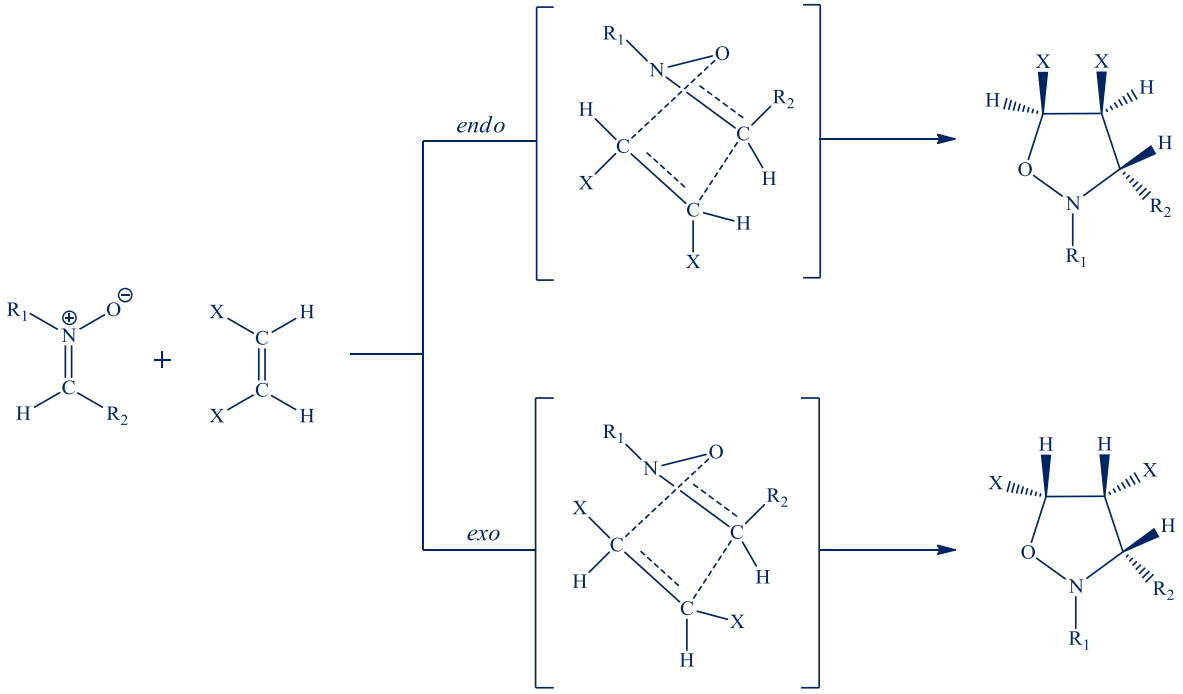
In broad terms, there is similarity in the reactivity and regiochemistry relationships for 1,3-DPCA and those of the D-A reaction. The most favorable reactions are those with the most complementary electronic character, that is, high nucleophilicity in one reactant with high electrophilicity in the other. Such reactions have high charge transfer character, early TS, and lower TS energy. The best match between HOMO and LUMO predicts the preferred regiochemistry. Relative reactivity trends should also be governed by these criteria.

2.3 STEREOCHEMISTRY

In general, the preferential formation of one stereoisomer over another in a chemical reaction is known as stereoselectivity. When the stereoisomers are enantiomers, the phenomenon is called enantioselectivity and is quantitatively expressed by the enantiomer excess (*ee*); when they are diastereoisomers, it is called diastereoselectivity and is quantitatively expressed by the diastereoisomer excess (*de*). Stereospecificity is an important criterion for the concertedness of cycloaddition. As long as the 1,3-dipole and dipolarophile are configurationally stable compounds, no rotation about the crucial bonds is conceivable during the concerted formation of new σ-bonds. That is, stereospecificity of the cycloaddition has been cited as evidence supporting the concerted reaction: *cis*-1,2-disubstituted dipolarophiles give *cis*-substituted pentacycles, and *trans*-1,2-disubstituted dipolarophiles give *trans*-substituted pentacycles. This stereospecific nature of 1,3-dipolar cycloaddition rules out the diradical mechanism proposed by Firestone.[72]

[d] The orbital energies of the reactants are indicated in eV.

When 1,2-disubstituted alkenes are involved in 1,3-dipolar cycloaddition reaction with 1,3-dipoles, two new chiral centers can be formed in a stereospecific manner due to the *syn* attack of the dipole on the double bond. If the alkene and 1,3-dipole, containing a chiral center approach is an *endo* or *exo* fashion, they give rise to a pair of diastereoisomers; each of them exist as a mixture of two enantiomers. The *endo* isomer arises from the reaction in which nitrogen atom of the dipole points in the same direction as the substituent of alkene, whereas, the *exo* isomer arises from the reaction in which the nitrogen atom of the dipole points in opposite direction as the substituent of alkene (**Scheme 2.2**).



Scheme 2.2 *Diastereoisomers of the two different possible transition states endo and exo.*

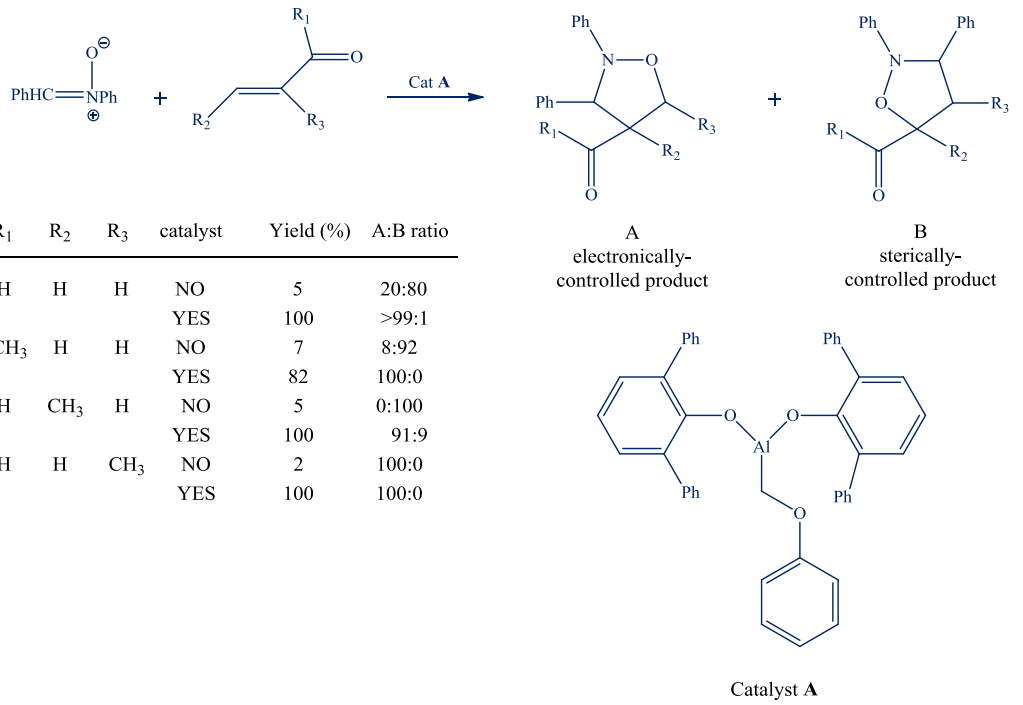
Since the secondary orbital interactions are very weak the *endo*/*exo* selectivity (or occasionally *cis*/*trans* selectivity) in 1,3-dipolar cycloaddition reaction is mainly controlled by the structure of substrates and the presence of catalysts.

It is know that nitrones having an electron-withdrawing group at the α position are configurationally unstable and they can be found as a mixture of *E*/*Z* isomers. The equilibrium between these isomers has been studies in solution and a dependence on the polarity of the solvent has been found. As a consequence of the interconversion between *E*/*Z* isomers, parallel models are always proposed for cycloaddition reactions of nitrone. In all cases it is possible to explain the obtention of the *trans* isomer by invoking either an *endo* approach to the *Z*-isomer an *exo* approach to the *E*-isomer. Similarly, the obtention of *cis* isomers can be explained through an *exo* approach to the *Z*-isomer or an *endo* approach to the *E*-isomer. The reaction of nitrones having an electron-withdrawing group in the α position with electron-deficient alkenes usually give *trans* adduct preferentially. It has been invoked that these reactions take place through an *E*-*exo* approach due to the higher stability of the *E*-isomer.[73] However, it is also possible to propose that the reaction undergoes through an *endo* approach (preferred in all cycloaddition reactions

with electron-deficient alkenes) to the more reactive *Z*-isomer.[74] The corresponding parallel can be done with electron-rich alkenes, too. The stereochemistry of cycloaddition reactions is in function of different factors, such as: steric effects, secondary orbital interactions, formation of hydrogen bonds and electrostatic interactions.

2.4 CATALYSIS AND ENANTIOSELECTION

The role of catalysts in 1,3-DPCA reactions is similar to that in D-A reactions. Most catalysts are Lewis acids. Effective catalysts include $Yb(O_3SCF_3)_3$ with BINOL,[75] Mg^{2+}-*bis*-oxazolines,[76] and oxazaborolidines.[77] The catalysts function by enhancing the reactivity of the more electrophilic component of the reaction. Although the diene is often nonpolar and inert to Lewis acids in D-A reactions, that is not the case for 1,3-DPCA. Consideration of catalysts must include the potential interaction with both the dipole and dipolarophile. Catalyst interaction with the 1,3-dipole is likely to be detrimental if the dipole is the more nucleophilic component of the reaction. For example, with nitrones and enones, formation of a Lewis acid adduct with the nitrone in competition with the enone is detrimental. One approach to this problem is to use highly substituted catalysts that are selective for the less substituted reactant.
Bulky aryloxyaluminum compounds are excellent catalysts for nitrone cycloaddition and also enhance regioselectivity.[78] The reaction of diphenylnitrone with enones is usually subject to steric regiochemical control. With the catalyst **A** high electronic regiochemical control is achieved and reactivity is greatly enhanced, but the catalyst does not strongly influence the *exo*:*endo* selectivity, which is 23:77 for propenal. In **Figure 2.6** is reported the reaction scheme and the related data for this reaction.



R_1	R_2	R_3	catalyst	Yield (%)	A:B ratio
H	H	H	NO	5	20:80
			YES	100	>99:1
CH_3	H	H	NO	7	8:92
			YES	82	100:0
H	CH_3	H	NO	5	0:100
			YES	100	91:9
H	H	CH_3	NO	2	100:0
			YES	100	100:0

Figure 2.6 *Reaction of diphenylnitrone with enones catalyzed by aryloxyaluminium catalyst* ***A****.*

Domingo examined computationally the effect of Lewis acid catalysis in the reaction of a nitrone with a vinyl ether, a combination in which the nitrone is the electrophilic reagent.[79] The results are summarized in **Figure 2.7**.

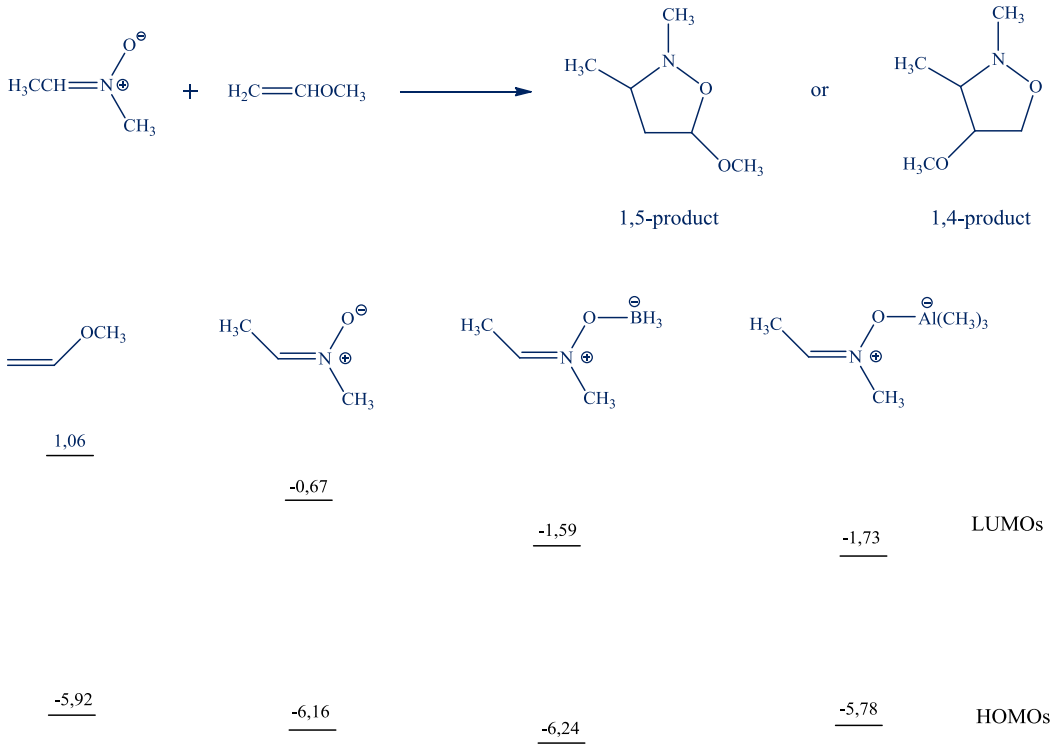
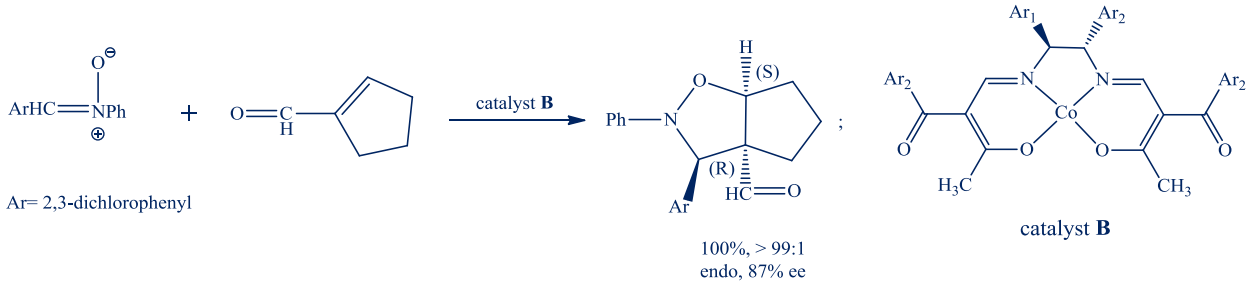


Figure 2.7 *Shift in FMO energy levels (in eV)(B3LYP/6-31G*) on complexation with* BH_3 *or* $(CH_3)_3Al$ *with nitrone reactant.*

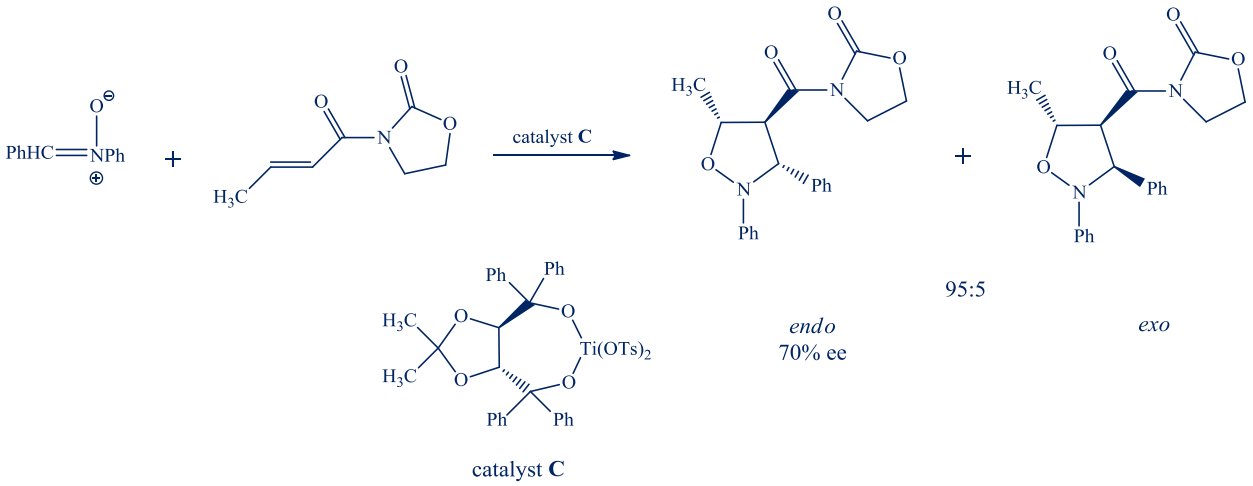
The Lewis acid, modeled by BH_3 or $Al(CH_3)_3$, is attached at the nitrone oxygen. The Lewis acid decreases the E_a for 1,5-addition, while increasing it for 1,4- addition. The catalyst also increases the selectivity for the *exo* TS. These results are consistent with the energy changes of the FMO of the reactants. The catalyst lowers the energy of the nitrone LUMO, enhancing its interaction with the vinyl ether HOMO. The reaction takes on enhanced charge transfer character in the presence of the catalyst. These overall effects are similar to those found in D-A reactions catalyzed by Lewis acids.

As with D-A reactions, it is possible to achieve enantioselective cycloaddition in the presence of chiral catalysts.[80] Many of the catalysts are similar to those used in enantioselective D-A reactions. The catalysis usually results from a lowering of the LUMO energy of the dipolarophile, which is analogous to the Lewis acid catalysis of D-A reactions. The more organized TS, incorporating a metal ion and associated ligands, then enforces a preferred orientation of the reagents. For example, the bulky aryl groups in the catalyst **B** (**Scheme 2.3**) favor one direction of approach of the nitrone reactant.[81]



Scheme 2.3 *Enantioselective 1,3-DPCA reaction catalyzed by catalyst **B**.*

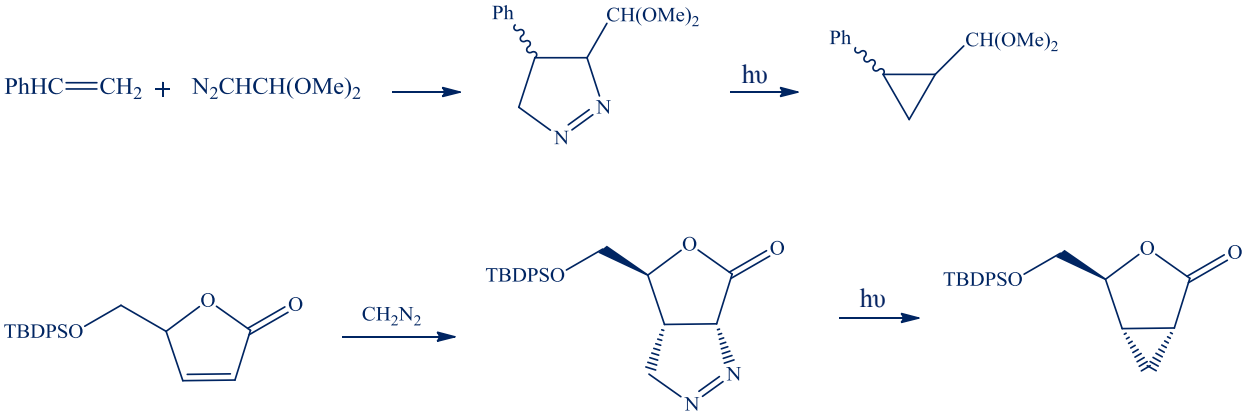
The Ti(IV) TADDOL catalyst **C** (**Scheme 2.4**) leads to moderate to high enantioselectivity in nitrone cycloaddition with *N*-acyloxazolidinones.[82]



Scheme 2.4 *Enantioselective 1,3-DPCA reaction catalyzed by catalyst **C**.*

2.5 SYNTHETIC APPLICATIONS

1,3-DPCA reactions are an important means of synthesis of a wide variety of heterocyclic molecules, some of which are useful intermediates in multistage syntheses.
Pyrazolines, which are formed from alkenes and diazo compounds, for example, can be pyrolyzed or photolyzed to give cyclopropanes as shown in **Scheme 2.5**.[83]



Scheme 2.5 *Synthesis of cyclopropanes derivatives mediated by 1,3-dipolar cycloadducts.*

Table 2.3 gives some examples of 1,3-DPCA reactions.

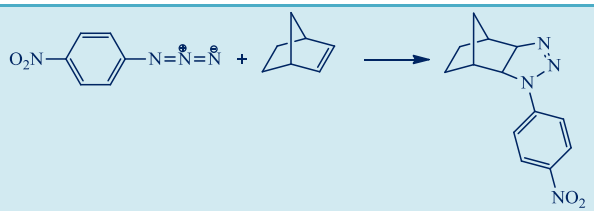
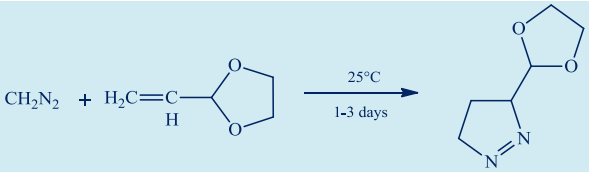
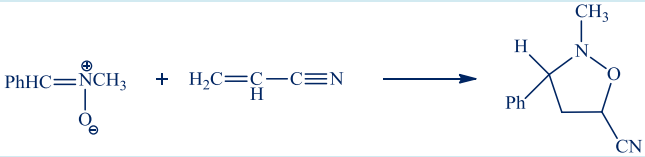
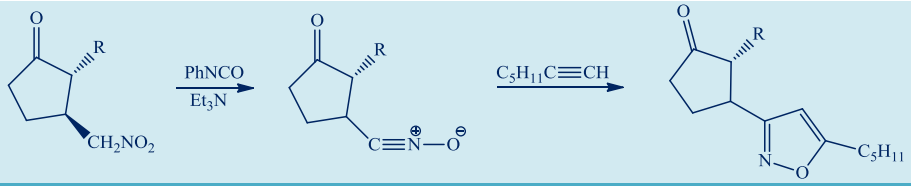
Entry	Reaction
1	
2	
3	
4	
5	

Table 2.3 *Typical 1,3-Dipolar Cycloaddition Reactions*

Entry 1 is an addition of an aryl azide to norbornene. The EWG nitro group is rate enhancing and the reaction occurs with a rate constant of $6{,}3x10^{-3}$ M^{-1} s^{-1} at 25°C. Owing to steric approach control, the product is the *exo* stereoisomer. Entry 2 involves an acetylenic dipolarophile and gives an aromatic triazole as the product. Entry 3 is an addition of diazomethane to the dioxolane derivative of acrolein. The reaction is carried out in a closed vessel at room temperature. Entry 4 involves a nitrone as the 1,3-dipole. Nitrone cycloadditions are particularly useful in synthesis because a new carbon-carbon bond is formed and the adducts can be reduced to β-amino alcohols. Nitrile oxides, which are formed by dehydration of nitroalkanes or by oxidation of oximes with hypochlorite,[84] are also useful 1,3-dipoles. They are highly reactive, must be generated in situ,[85] and react with both alkenes and alkynes. The product in Entry 5 is an example in an isoxazole that was eventually converted to a prostaglandin derivative.

2.6 SYNTHETIC APPROACHES USED TO 1,3-DPCA

1,3-dipolar cycloadditions can be performed with different methods. The methods presents in the literature are as follows:

- Classic or Conventional method (with solvent at reflux);
- Solvent-free method assisted by microwave irradiation;
- Solvent-free method assisted by ultrasounds;
- Use of non-conventional solvent method (Ionic Liquids).

In the following subparagraphs are illustrate the principles on which the various methods mentioned above are based.

2.6.1 Classic or Conventional method (with solvent at reflux)

Classic or Conventional method involves the use of solvents such as toluene, ethanol, methanol, chloroform or acetonitrile to reflux. This method has several disadvantages such as:

- Non eco-friendly solvent are used;
- Reaction times very long with the possibility of the product degradation;
- Low regioselectivity reactions.
- High probability of the product racemization.

All of these factors have made this technique obsolete.

2.6.2 Solvent-free method assisted by microwave irradiation

Over the last few decades, microwave heating has become one of the most widely used activation methods and, between the various technologies applied to synthesis methodologies, has been one that has undergone strong innovation.

While the use of microwave in the inorganic chemistry field dates back to the end of the 1970s, organic microwave utilization has only increased in the mid-1980s. The reason for this slow development lies in the poor controllability and reproducibility that the syntheses present, but above all in the lack of knowledge of theories based on so-called "dielectric heating" and in the existence of various risks such as the inflammability of organic solvents and possibility of

explosions. Most studies on the use of microwaves showed a significant increase in reaction rate and yields, sometimes also selectivity, compared to synthesis reactions conducted by classical or conventional heating. A relevant reason why an incredible spread of microwave technology has been witnessed is the ability to conduct a large number of reactions in the absence of solvent.[86]
There are several and obvious advantages that result from the possibility to conduct the reaction synthesis of organic compounds in the absence of solvent and through microwave heating, for example:

- In the absence of solvent the radiation is directly absorbed by the reagents, consequently the effect of the microwaves is greater;
- efficiently solid supports can be used, including mineral oxides, which, while not being good heat conductors, effectively absorb microwaves;
- This methodology can be used in combination with other synthesis methodologies.[87]

Furthermore, the use of "solvent-free" conditions is very advantageous from an environmental point of view, in favor of the notorious "Green Chemistry", thanks to a significant reduction of pollution resulting from the suppression and disposal of chemicals. This is possible in function of factors such as:

- A drastic decrease in solvent volumes for the various synthesis procedures;
- Work-up procedures considerably simplified;
- Use of recyclable solid supports.

Solvent-free reactions can be carried out using both acidic and basic solid supports, such as silica, alumina or zeolites, or, alternatively, directly on the solid state reagent mixture; in this case the radiation is directly absorbed by the reagents and this causes a considerable increase in the rate of reaction, yield and purity of the obtained products. [88]
Generally, all organic synthesis reactions that need heat may be effected by dielectric heating, i.e. with the aid of microwaves. There are, however, substantial differences between traditional and microwave heating. While in the first case the heat source acts on the external surface of the system and diffuses into the interior of the body by convection, in the latter case the heat is generated at the center of the product by dielectric loss of polar molecules and propagates outwards.
The main advantage of this heating lies in the fact that it is possible to reach higher temperatures than conventional ones, but above all that it is possible to reach the same temperatures at extremely rapid times; this results in a drastic reduction in reaction times, a decrease in collateral products, and an increase in yields. Many of the reactions that, with conventional heating, yield nothing or almost nothing, by heating with the microwaves have yielded good and sometimes even high yields. However, dielectric heating requires greater control respect to over the traditional one because a sharp thermal increase jumble can occur, coincident with the so-called "heat break point", specific for each substance: at this temperature value, the thermal increase can reaching values between 200 and 300 °C in just one minute. It is also necessary to consider the possible formation of so-called "hot and cold spots" within the system that is to be heated: sometimes the heating is not uniform and the system may exhibit hottest or colder points that generate disomogeneity from one point of thermal view. This is one of the reasons why sometimes the results obtained by this technology have less reproducibility.
It has just been said that the benefits of microwave heating, such as increased reaction rate, yields and sometimes selectivity, are due to the rapid and intense increase in temperature that

occurs within the reaction mixture. However, some authors attribute these advantages not to the mere thermal effect but to specific effects induced by the microwaves, such as the variation of thermodynamic parameters, in particular the free activation energy (ΔG) as a result of a modification of system entropy . However, anyway, the existence of specific microwave effects has not been demonstrated and in most publications they are considered irrelevant.[75]

2.6.3 Solvent-free method assisted by ultrasounds

Recently, they advance the new methodologies that replace old systems, one of these is the use of ultrasound.

Sonication is a term used to describe the use of acoustic waves, particularly ultrasonic, for various purposes. Ultrasounds are mechanical waves that cause a pressure variation with sine-wave andament in the propagation medium. The peculiarity of ultrasounds is that when they propagate in a liquid they cause not only the pressure variation; ultrasonic frequency waves cause in a liquid the acoustic cavitation phenomenon. The acoustic waves provide the medium, in the case of a liquid, mechanical energy. This energy is transmitted and causes vibrational excitation of the molecules: the vibrational energy surplus causes heating with the formation of microscopic gas bubbles that initially grow in size but then, as a result of increased pressure of the medium, are compressed violently with resulting implosion. There are three physical theories concerning the sonochemistry cavitation:

- Hot-point theory (**Figure 2.8**);
- Electric theory;
- Plasma theory.

The most widely used is the hot point theory that affirm that during bubble collapse the temperature rise is adiabatic.[89] When a solution is crossed by sufficiently high intensity ultrasounds, these can alter and destroy the structure of the solvent because, under such conditions, intramolecular forces cannot maintain cohesive molecules. This results in the formation of gas bubbles in the solution, called cavitation bubbles. These bubbles are generated by gases dissolved in the exame liquid and are formed in a point of the space called cavitation threshold. Cavitation bubbles have two destinies: they swing around a balance dimension, or grow rapidly and subsequently collapse. The bubbles collapse creates drastic pressure and temperature conditions: locally it comes at pressures of 1800 atmospheres and temperatures in the range 2000-5000 K.[90]

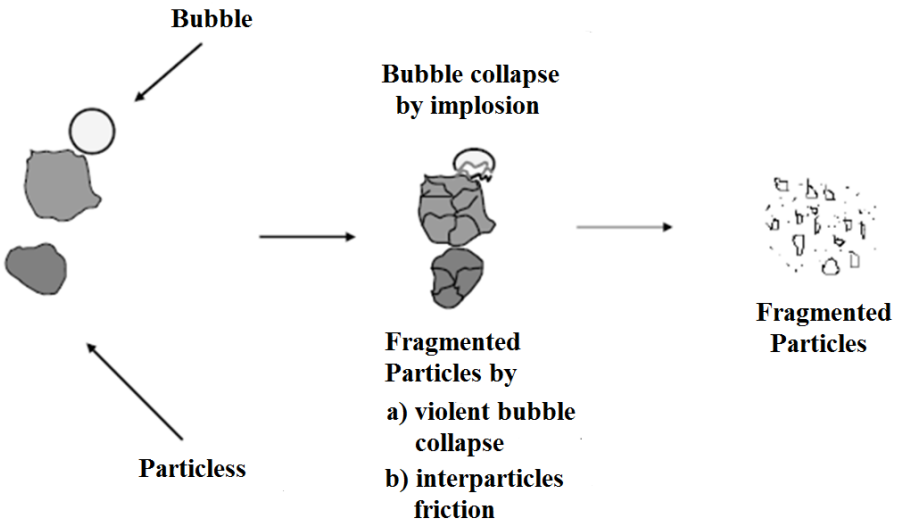


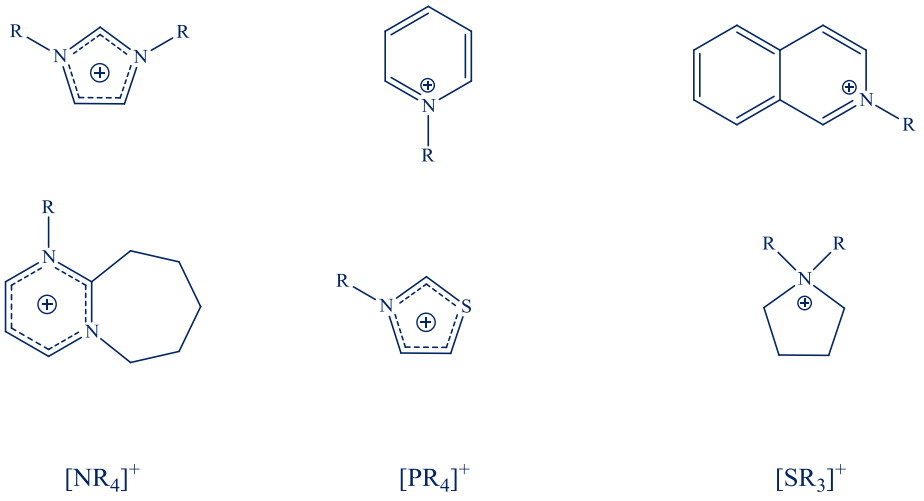
Figure 2.8 *Description of the sonication effect according to Hot-point theory.*

The sonication allows to speed the dissolution of the solutes in certain solvents and, in the case of cycloadditions, dissolves and disperses the dipole and the dipolarophile in the solvent employed by favoring the interaction between them.

2.6.4 Use of the Ionic Liquids as non-conventional solvent method

Ionic liquids can be considered, depending on the point of view, both as a new and promising class of solvents, as well as a type of substance that has a long and interesting history. The increased interest in these compounds is clearly due to the progressive increase in the production of these substances, previously used for specialized electrochemical applications, in order to improve their utility and effectiveness just as reaction solvents.[91]
However, very simply, ionic liquids can be defined as liquids exclusively composed of ions. Furthermore, this term contains a further special definition, so that it can be distinguished from the classic definition of "molten salt".[92] While the latter are generally regarded as high melting salts, extremely viscous and corrosive, ionic liquids are low temperature liquids (<100 ° C) and have a relatively low viscosity.[93] Most ionic liquids are made up of organic cations and an inorganic polyatomic anion. The most used cations are: tetraalkylammonium ions, tetraalkylphosphonium, *N*-alkyl-pyridinium, 1,3-dialkyl-imidazolium, trialkylsolphonium as shown in **Figure 2.9**.[94]

Cations



$[NR_4]^+$ $[PR_4]^+$ $[SR_3]^+$

Anions

BF_4-; $(CF_3SO_2)_2N^-$; PF_6^-; $CF_3CO_2^-$; SbF_6^-; $HexBEt_3^-$; $CH_3CO_2^-$; TsO^-; HSO_4^-; $AuCl_4^-$; NO_3^-; $AlCl_4^-$; NO_2^-; Carborane anions; $CF_3SO_3^-$.

Figure 2.9 *Common cations and anions used as Ionic Liquids.*

They are also commonly expressed in the form of abbreviations, for example the ionic liquid 1-alkyl-3-methylimidazole triflate, reported in **Figure 2.10**, is indicated by the abbreviation $[C_n\text{mim}]^+[CF_3SO_3]^-$, where *n* denotes the number of carbon atoms in the alkyl linear chain in position 1; the linear chain, as well as with the term C_n, can also be indicated by normal alphabet letters, preferably in lowercase: "e" if the alkyl chain is an ethyl, "b" is a butyl and so on. Obviously, "m" refers to the methyl located on the ring in position 3 and "im" to the imidazole.

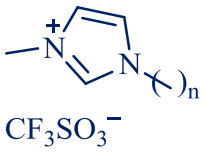


Figure 2.10 *$[C_nmim]^+[CF_3SO_3]^-$ Ionic Liquid.*

The most commonly used alkyl chains are methyl, ethyl, butyl, hexyl, octyl and decyl. The list of ionic liquids is enriched almost daily, so that in recent years the number of potential ionic liquids has become enormous, but the determination of their utility as solvents requires a more substantial investment, especially in the determination of physical and chemical properties. During the '70s and '80s ionic liquids were mainly studied for electrochemical applications; from the mid-1980s, those with low melting point were instead proposed as organic synthesis solvents by Fry and Pienta[95] and Boon and other colleagues.[96] One of the main characteristics of ionic liquids is to solubilize a wide variety of organic, inorganic and organometallic compounds, being the ideal solvent for reactions that occur under catalyzed conditions. It is interesting to note that, depending on the coordinating properties of the anion, the ionic liquid can be used as a normal

inert solvent or as a co-catalyst.
In the specific case of reactions catalyzed by organometallic derivatives or transition metals ionic liquid with tetrafluoroborate or hexafluorophosphate ions are considered as inert solvents: in such cases the role of the ionic liquid is only to provide a weak polar coordination medium for the catalyst and also to provide excellent solubilization of reagents and products. The use of ionic liquids often makes it possible to appropriately combine the properties of the solvent itself, which cannot be achieved by using water or any other common organic solvent.
The ionic liquid formed by the reaction of a halide with a Lewis acid (for example chloraluminated or chlorinated salts) generally act as co-catalysts. The reason lies in the acidity or the basicity of Lewis, which results in strong interactions with the complex catalyst. In many cases, the Lewis acidity of an ionic liquid is used to convert the precursor of the neutral catalyst into the corresponding cationic active form. Some examples relate to the activation of $[Cp_2TiCl_2]$[97] in the salts (melts) of the chloroaluminate acids and the activation of $[(PR_3)_2PtCl_2]$ in the chlorostannates salts:

$$[Cp_2TiCl_2] + [catione]^+Al_2Cl_7^- \rightleftharpoons [Cp_2TiCl]^+AlCl_4^- + [catione]^+AlCl_4^- \quad \textbf{Eq. 1.1}$$

$$[(PR_3)_2PtCl_2] + [catione]^+Sn_2Cl_5^- \rightleftharpoons [(PR_3)_2PtCl]^+SnCl_3^- + [catione]^+SnCl_3^- \quad \textbf{Eq. 1.2}$$

There are several good reasons to consider ionic liquids alternative solvents in well-known reactions catalyzed by transition metals. In addition to the advantage of their non-volatile nature, particular interest is the search for new biphasic reactions with a phase consisting of an ionic catalyst. The possibility of improving the solubilization properties, through the different cation / anion combination, allows a systematic optimization of the biphasic reaction itself, for example regarding the selectivity of the product. An important alternative to increasing selectivity in multiphase reactions is the preferential solubility of a single reagent in the solvent catalyst or the in situ extraction of the reaction intermediates outside the catalyst layer.[98]
Ionic liquids may be superior to water or common organic solvents in transition metal catalysis, particularly when ionic complexes are used as catalysts. In these cases, it is possible to achieve a significant increase in the stability and activity of the catalyst.
In the case of 1,3-dipolar cycloadditions it is obvious that the use of ionic liquids as co-catalysts increases the rate of reaction and selectivity.

CHAPTER 3

CHAPTER 3 – BIOLOGICAL ACTIVITY OF THE SPIROOXINDOLE COMPOUNDS

3.1 INTRODUCTION

Carcinogenesis is a highly complex multi-step process induced by a number of carcinogens which leads to the development of cancer.[99] In cancer, cells divide and grow uncontrollably, forming malignant tumors, which may invade nearby normal cells, even spread to more distant parts of the body through the lymphatic system or bloodstream. Many natural and also synthetic anticancer agents such as paclitaxel and cisplatin are available in the market and some are in clinical trials. However, these drugs are always associated with serious side effects such as bone marrow depression, alopecia and nephrotoxicity owing to their non-selective action. Although some antiproliferative drugs like tamoxifen are highly selective to cancer cells. These agents are not very effective to kill the existing tumor cells. More importantly, their prolong use may develop uterine and endometrial cancers.[100] Hence, developing the new therapeutic drugs for cancer treatment is much more important to improve the efficiency and efficacy of the drugs on the cancer cells.

Spiro compounds have always been prevalent in organic synthesis due to the pronounced biological activities.[101] In particular, the spirocyclic oxindoles have emerged as attractive synthetic targets because of their prevalence in numerous natural products and biologically active molecules.[102] The key structural characteristic of these compounds is the spiro ring fused at the C3 position of the oxindole core with varied heterocyclic motifs as shown in **Figure 3.1**. These spirooxindoles seem to be promising candidates for drug discovery, since they incorporate both oxindoles and other heterocyclic moieties simultaneously.

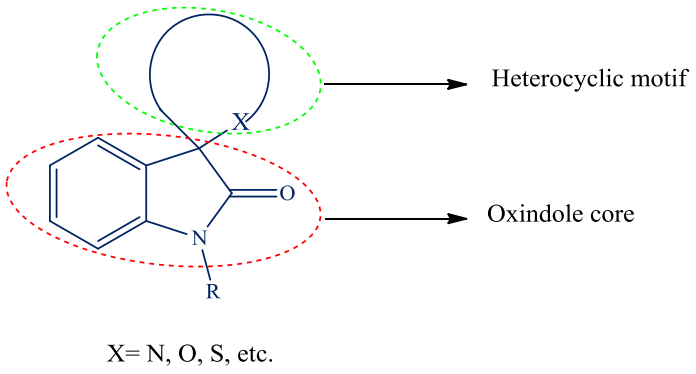


Figure 3.1 *Scaffold of Spyrociclic Oxindoles.*

Two representative examples are NITD609 (**1**) and MI-888 (**2**) (**Figure 3.2**), which are currently in preclinical evaluation for the treatment of malaria and human cancer, respectively.

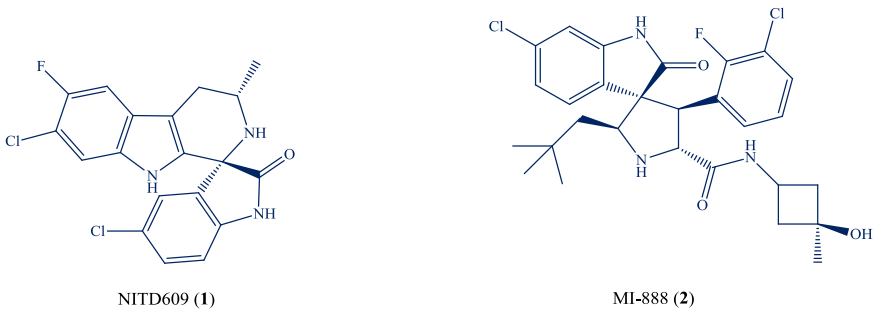


Figure 3.2 *Two representative examples of Spirooxindoles, NITD609 and MI-888.*

The synthesis, especially asymmetric synthesis of spirooxindoles has always been a research field of great interest due to their biological activity and applications for pharmaceutical lead discovery. The general strategies for the synthesis of such compounds are summarized in **Figure 3.3**.

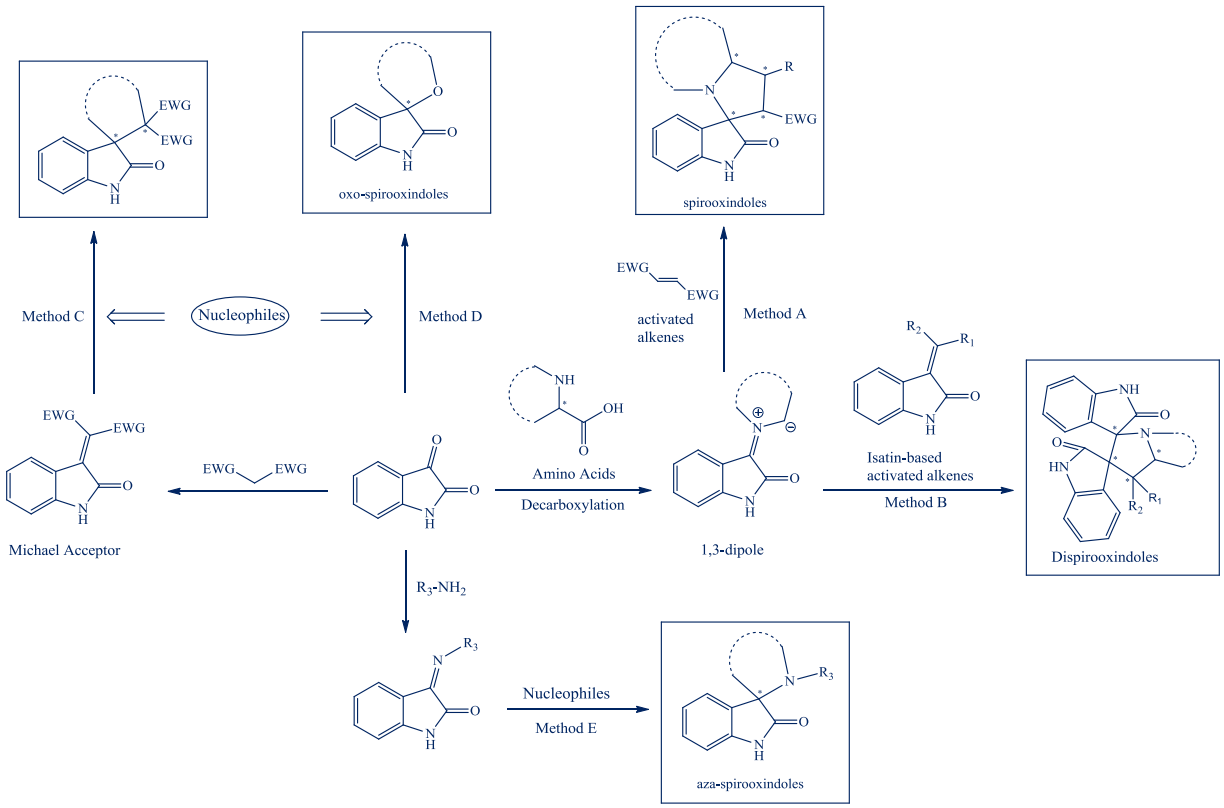


Figure 3.3 *The general synthetic strategies of spirooxindoles*

In **Figure 3.3** is possible to observe four general synthetic strategies for spirooxindoles as follow described.

- Reactions based on 1,3-dipolar cycloaddition: the azomethine ylides (1,3-dipoles), which are always generated in situ from isatin derived compounds and α-amino acids through the thermal decarboxylation, react with activated alkenes (dipolarophiles), giving pyrrolidine-containing spirooxindoles with high regioselectivity and stereoselectivity (Method A). Alternatively, cycloaddition can also be fulfilled between azomethine ylides

and electron-withdrawing Knoevenagel alkenes originated from isatin (Method B).

- Reactions based on domino Knoevenagel-Michael-cyclization: due to the high reactivity of the C3 carbonyl group in isatin, the Knoevenagel condensations always occur in the presence of nucleophilic methylene substrates, forming corresponding Knoevenagel alkenes. These electron-deficient alkenes (Michael acceptors) are capable of initiating further domino Michael-cyclization (Method C).
- Reactions based on Michael-cyclization of isatins: due to the high reactivity of the C3 carbonyl group, nucleophilic attack to the C3 carbonyl group of isatin, followed by further cyclization gives the oxo-spirooxindoles (Method D).
- Reactions based on C3 aminalization: aminalization of isatins at C3 position leads to corresponding aminals, which are subjected to further transformation to give nitrogen-containing spirooxindoles (Method E).

The type of reactions above described give to the spirooxindoles with different degrees of anticancer activities mainly based on the spiro rings fused at the C3 position of oxindole scaffold and substituents on the oxindole nucleus. Below are reported the synthetic methods and their related biological activities of the more common classes of spirooxindoles.

3.2 SPIRO-PYRROLIDINYL OXINDOLES

Synthesized spiro-pyrrolidinyl oxindoles have drawn wide attention as non-peptide small molecule inhibitors of p53-MDM2 interaction for cancer therapy since the structural basis of the p53-MDM2 interaction has been established by X-ray crystallography.[103] X-ray crystal structure analysis showed that the interaction between p53 and MDM2 was primarily mediated by three hydrophobic residues (Phe19, Trp23 and Leu26) of p53 peptide and a small but deep hydrophobic cleft in MDM2. Since the indole ring of the Trp23 residue of p53 is buried deeply inside the hydrophobic cavity in MDM2 and its NH group forms a hydrogen bond with the backbone carbonyl in MDM2, Trp23 appears to be the most critical for binding of p53 to MDM2.[104] Based on this fact, a library of spirooxindoles as p53-MDM2 inhibitors were initially discovered using a *de novo* structure based design strategy, two alkaloids (Spirotryprostatin A and Alstonisine) contained the spiro-pyrrolidinyl oxindole core that was used as a scaffold to design possible inhibitors by docking into the MDM2 structure using the GOLD program (**Figure 3.4**).[105] These spirooxindoles can closely mimic the Trp23 of p53 in both hydrogen-bonding formation and hydrophobic interactions with MDM2, and the spiro-pyrrolidinyl oxindole ring provides a rigid scaffold from which two hydrophobic groups can be projected to mimic the side chain of Phe19 and Leu26.

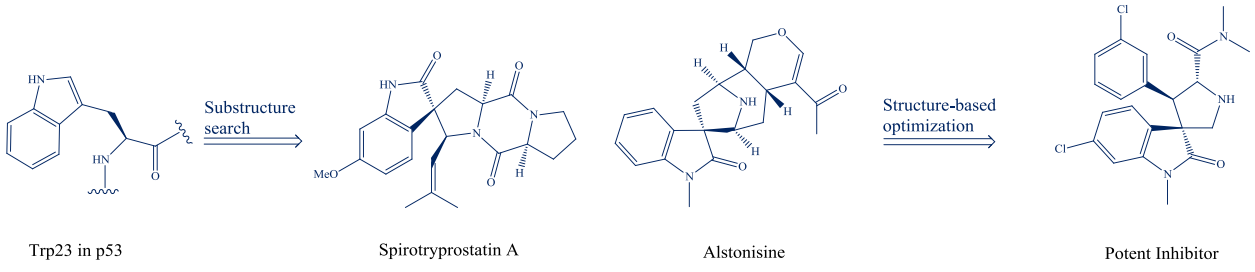
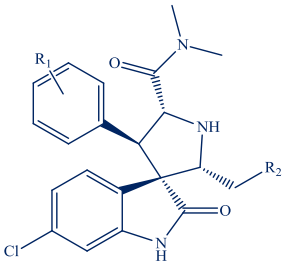


Figure 3.4 *Spirotryprostatin and alstonisine-based design of potent inhibitors of p53-MDM2 interaction.*



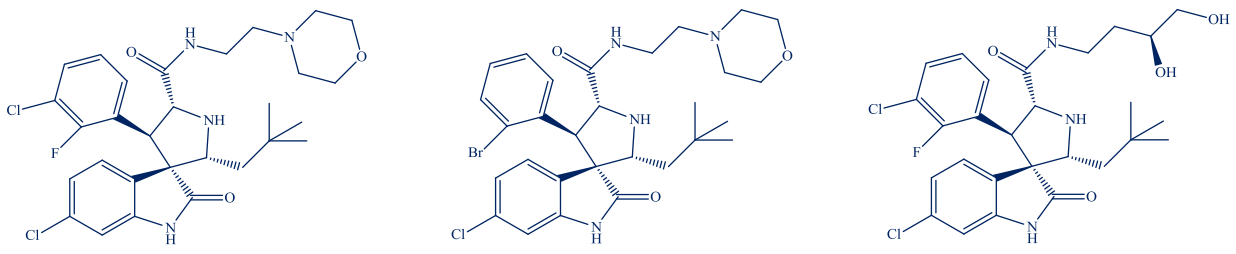
3	R_1=H, R_2=*i*-Pr	K_1=8,46 μM
4	R_1=Cl (*m*), R_2=*i*-Pr	K_1=0,30 μM
5	R_1=Cl (*p*), R_2=*i*-Pr	K_1=7,68 μM
6	R_1=Cl (*m*), R_2=*t*-Bu	K_1=0,086 μM
7	R_1=Cl (*m*), R_2=Et	K_1=0.65 μM
8	R_1=Cl (*m*), R_2=*i*-Bu	K_1=0.39 μM
9	R_1=Br (*m*), R_2=*t*-Bu	K_1>10 μM

Figure 3.5 *Several spirooxindoles that inhibit p53-MDM2 interaction.*

Spirooxindoles **3-9**, indicated in **Figure 3.5**, displayed different levels of activity in an FT-based assay with K_i values ranging from 0,086 μM to 8,46 μM (1,52 μM for natural p53 peptide). Compound **3** bond to MDM2 with a K_i value of 8,46 μM due to the fact that it fitted poorly into the binding pocket occupied by the side chain of Phe19 and Leu26, respectively. However, compound **3** provided a template for further optimization. Further modifications focusing on the variations of R_2 group and the position of the chlorine atom at the phenyl ring yielded compounds **4-9**. Among them, compound **6** showed the best affinity to MDM2 (K_i=0,086 μM). Docking studies suggested that the chlorooxindole ring in compound **6** occupied the Trp23 pocket, the 3-chlorophenyl group projected into the Phe19 pocket, and the t-butyl group filled the Leu26 pocket. Besides, compound **6** represented excellent inhibition against LNCaP cells with wild-type p53 (IC_{50}=0,086 μM) but relatively weak inhibition against human prostate cancer PC-3 cells with a deleted p53 (IC_{50}=22,5 μM) and had good selectivity between cancer and normal cells with wild-type p53. Compound **6** had an IC_{50} value of 10,5 μM in inhibition of normal human prostate epithelial cell growth, 13 times less toxic than to LNCaP cancer cells.

Compared to compound **6**, compound **9** (known as MI-61, **Figure 3.5**) with a bromo atom at the meta-position of the phenyl ring showed a significantly decreased activity (K_i >10 mM), indicating that substituent on the oxindole core played an important role in its binding to MDM2.[106]

Although these spirooxindoles achieved high binding affinities to MDM2, they were still significantly less potent than the most potent peptide-based inhibitors probably due to the fact that the additional interaction between MDM2 and peptide-based inhibitors was still not captured by these spirooxindoles. Analysis of the X-ray structure of the MDM2-p53 complex showed that Leu22 was also crucial in the overall interaction between MDM2 and p53.[91] Structure-based optimization of this class of compounds as inhibitors of MDM2-p53 interaction was further carried out, generating new effective inhibitors (**Figure 3.6**), which captured the additional interaction between Leu22 in p53 and MDM2.[107]



MI-63 MI-43 MI-219

Figure 3.6 *New inhibitors that capture the interaction between Leu22 in p53 and MDM2.*

MI-63 bond to MDM2 with high affinity[95] and even effectively induced apoptosis ex vivo in chronic lymphocyte leukemia patient samples with functional p53,[108] MI-63 had a poor PK profile and was not suitable for in vivo evaluation, extensive modifications of MI-63 yielded MI-219 with a desirable PK profile.[109]

It is well known that the stereochemistry play an important role in the drug synthesis. Drug chirality is now a major theme in the design, discovery, development, launching and marketing of new drugs.[110]

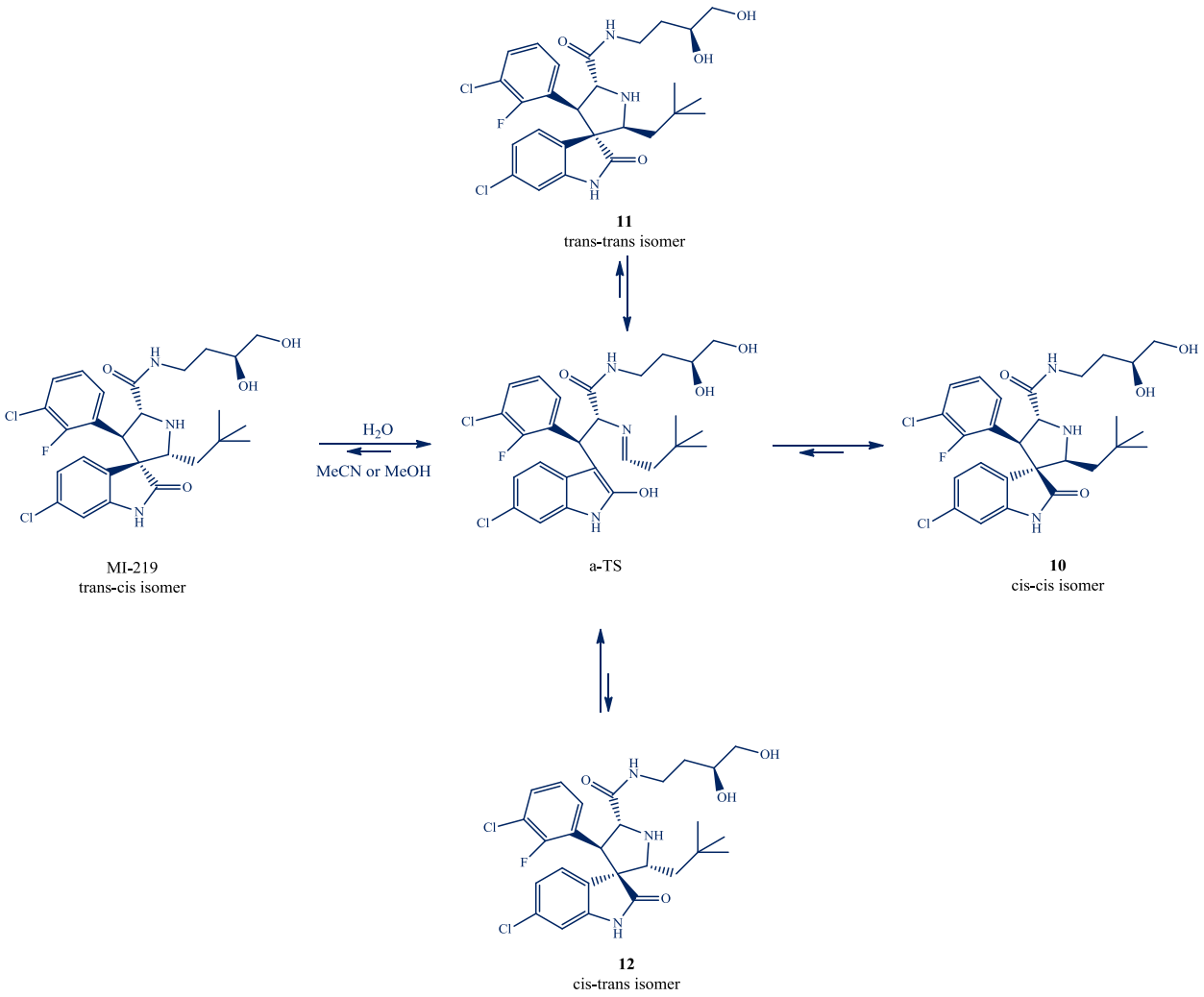


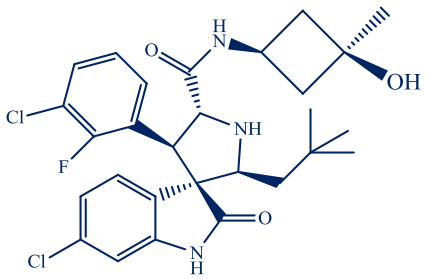
Figure 3.7 *Isomerization of MI-219 in polar solvent.*

For example, an excellent work regarding the relationship between the stereochemistry and binding affinities to MDM2 of spirooxindoles was carried out by Wang and co-authors.[111] Three isomers (**10-12**) were obtained when MI-219 was treated with MeOH, MeCN or H_2O (**Figure 3.7**). In aqueous solution, MI-219 underwent a reversible retro-Mannich reaction affording the respective ring-opened transition state (a-TS) in which the pyrrolidine ring was opened. Recyclization of a-TS afforded four stereoisomers. Binding kinetics of MI-219, **10-12** to MDM2 were investigated using the fluorescence-polarization assay, showing that isomer 10 had the highest binding affinity, followed by isomer **11**, MI-219 and **12**.

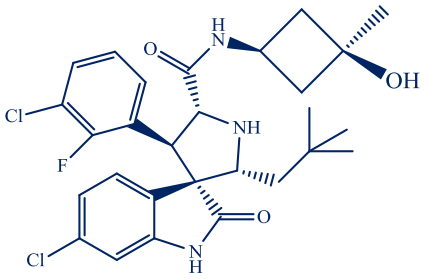
Isomer 10 was >300-times more potent than isomer **12** and was 18-times more potent than

isomer MI-219. Interestingly, there was no significant difference in the activity against cellular growth of the SJSA-1 cell line due to the rapid isomerization of both isomer MI-219 and **10** in the cell culture medium. Isomer **10** was more potent than isomer MI-219 in vivo in activation of p53 and induction of apoptosis and had much better in vivo anticancer activity in the SJSA-1 xenograft model than isomer **10**. Besides, no significant weight loss or other signs of toxicity for either isomer was observed.

Although isomer **10** had a strong antitumor activity and shrank tumors by 91% in the SJSA-1 xenograft model, it failed to achieve complete tumor regression probably due to its less ideal pharmacokinetic properties. Extensive modifications of such compounds ultimately led to the identification of MI-888 (**Figure 3.8**), which was capable of achieving rapid and complete tumor regression in animal models of human cancer upon oral administration and bond to MDM2 with a K_i value of 0,44 nM, being 10-times more potent that its cis-trans isomer **13**.



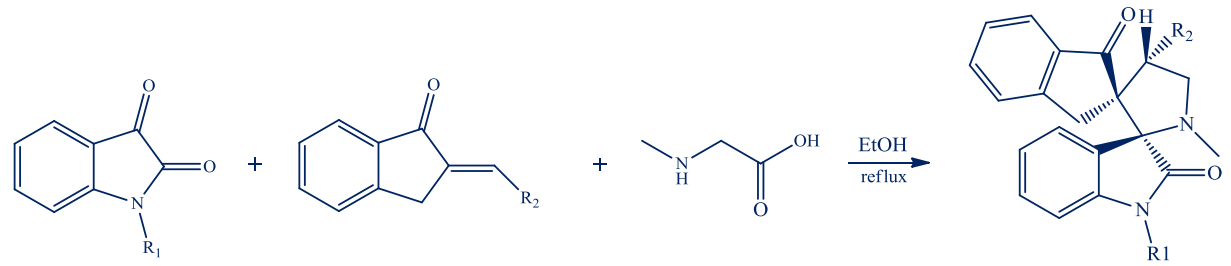
MI-888



13

Figure 3.8 *MI-888 and its cis-trans isomer.*

When the number of the spiroatom in the heterocyclic ring increase the biological activity of the compound further increase. Dispirooxindoles **14-19** were efficiently synthesized in a highly regioselective manner via a 1,3-dipolar cycloaddition reaction between 2-(arylmethylene)-2,3-dihydro-1H-inden-1-ones and azomethine ylides, which were generated in situ via decarboxylative condensation of sarcosine and isatins (**Table 3.1**).[112] These compounds were further evaluated for their anticancer activity against 56 human cancer cell lines (leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate and kidney). The tested compounds showed moderate or no activity at all against most of the tested cancer cell lines. Interestingly, these compounds exhibited selective inhibition against SK-MEL-2 cell lines. SARs studies showed that the methyl group attached to the nitrogen of the amide was essential to the observed anticancer activity. The 4-chlorophenyl group attached to the pyrrolidine ring was much more beneficial to the anticancer activity than the 4-methoxyphenyl and thienyl groups.



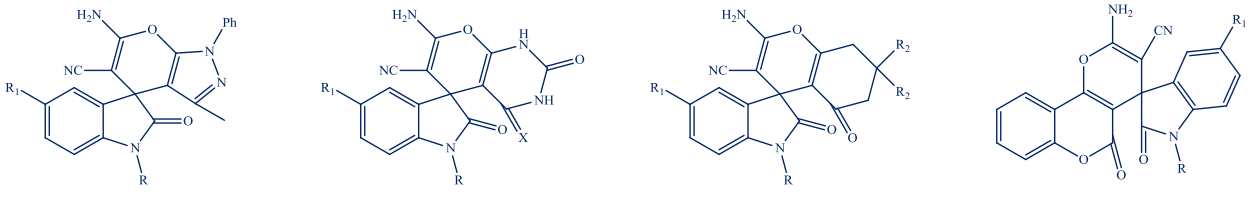
14-19

Compound	R_1	R_2	Percentage grown of SK-MEL-2
14	H	4-ClC_6H_4	-6,14
15	CH_3	4-ClC_6H_4	-11,05
16	H	4-$MeOC_6H_4$	8,70
17	CH_3	4-$MeOC_6H_4$	-6,92
18	H	2-thienyl	6,58
19	CH_3	2-thienyl	-5,11

Table 3.1 *Antitumor property of the tested compounds against selected melanoma cancer cell line (SK-MEL-2) at a dose of 10 µM.*

3.3 SPIRO-PYRAN OXINDOLES

Pyrans and oxindoles have been extensively studied before because of their biological potentials and synthetic utilities. However, the reports about the pharmaceutical evaluation of spiropyran oxindoles are really rare. The first report that described the I_2-catalyzed three-component one-pot synthesis of spiro-pyran oxindoles (**Figure 3.9**) and their in vitro cytotoxic activities against U87 human glioma cells was published in 2012.[113] Compound **22a** (R_1=H, R_2=Me, R=$CH_2(CH_2)_9Br$) had comparable cytotoxic activity (IC_{50} = 2.5 µg/mL) with the control drug Carmustine (IC_{50} = 3.9 µg/mL), about 40 times more potent than compound **22b** (R_1 = H, R_2 = Me, R = H) (IC_{50} = 40 µg/mL). This finding indicated that the lipophilic moiety attached to the nitrogen atom of oxindole nucleus was beneficial to the anticancer property probably due to the increased lipophilicity of molecules.



20 21 22 23

Figure 3.9 *Four types of spiro-pyran oxindoles.*

5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one (also known as kojic acid) has been reported to possess diverse pharmaceutical activities. Considering its biological potentials, Perumal et al.

reported the $Cu(OTf)_2$ catalyzed one-pot synthesis of kojic acid tethered spirooxindoles (**Figure 3.10**) and evaluated their anticancer potency towards A549 human lung cancer cell lines using MTT assay.[114] All these compounds showed moderate cytotoxicity against A549 cells (IC_{50} > 50 µM).

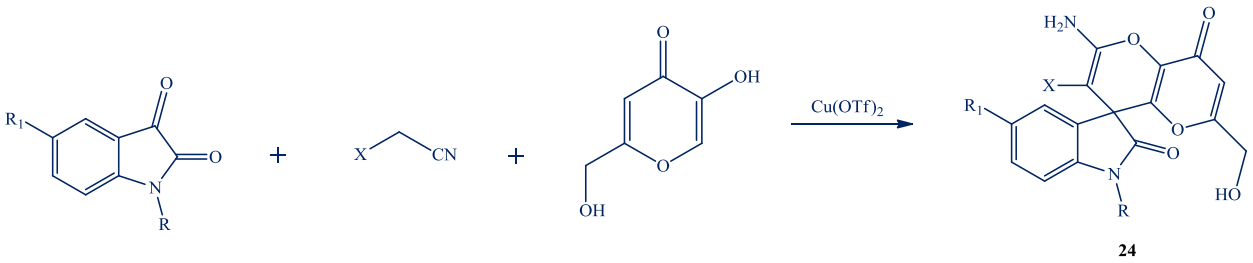


Figure 3.10 *Cu-catalyzed synthesis of Kojic acid rethered spirooxindoles.*

Tao et al. reported catalyst-free one-pot synthesis of novel 4H-pyran-fused pyrrolidinones and evaluated their cytotoxicity against Raji cells with Cisplatin as a positive control (**Figure 3.11**) [64]. Most of these compounds (**25-28**) showed remarkable cytotoxicity against Raji cells with the IC_{50} values ranging from 17 to 75 µM.

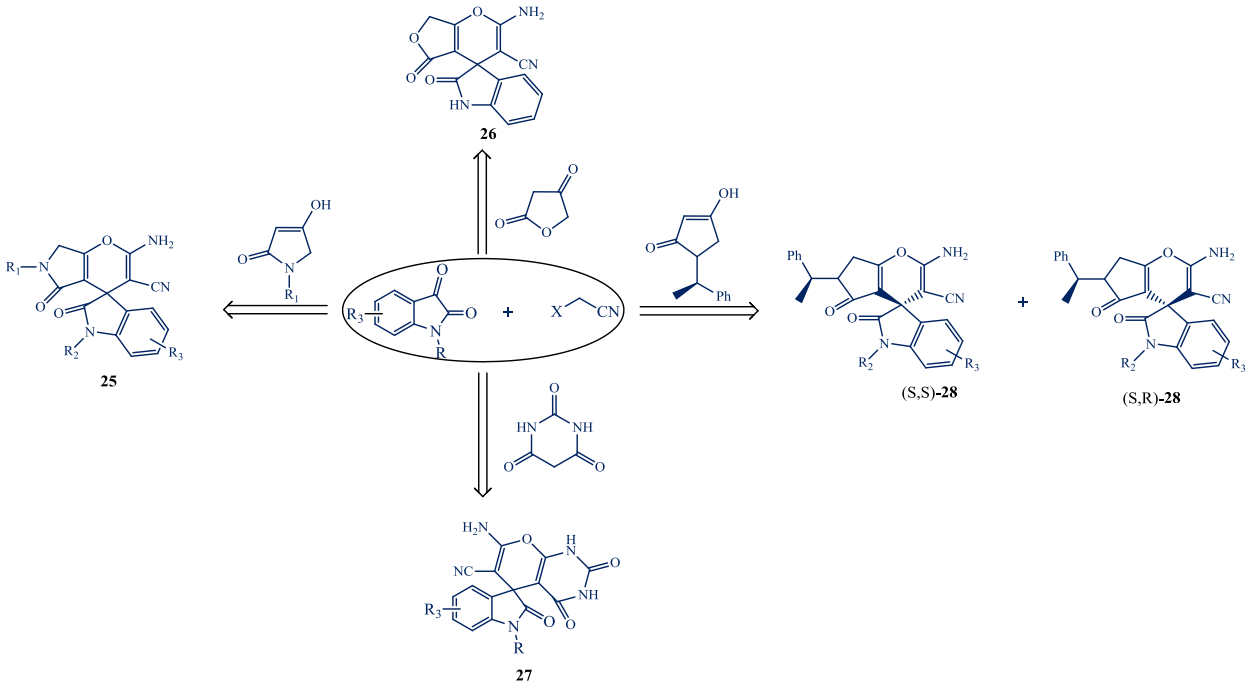


Figure 3.11 *Four types of 4h-pyran-fused pyrrolidinones.*

When R_1 was changed from benzyl group to the phenylethyl group, the cytotoxicity decreased significantly. The electronic characteristicsof substituents and their position on the benzene ring had remarkable effect on their bioactivity. Except for trifluoromethyl substituted derivatives, compounds with an electron-withdrawing group exhibited decreased cytotoxicity against Raji cells. For halogenated derivatives, the bioactivity depended on the positionof halo atom on the

benzene ring and brominated derivatives had better cytotoxicity than chlorinated ones. Generally, the trends in cytotoxicity followed this sequence (6-X > 5-X > 4-X; X = Br or Cl).
The synthesis of chiral molecules with structural complexity and diversity has played a critical role in the discovery of drug candidates. Inspired by the diverse and excellent bioactivities of pyranopyrimidines, Wang and co-workers developed an intramolecular asymmetric Michael/cyclization reaction of coumarins with isatylidene-malononitriles in the presence of the rosin-derived thiourea catalyst, leading to the generation of pyran-fused spirooxindoles. These intermediates were subjected to further transformation, giving chiral spirooxindole-pyranopyrimidines **29a-b**, **30a-g** and **31a-b** (**Figure 3.12**).[115] Anticancer evaluation of these compounds against MDA, U937, Jurkat, Hela and EJwas carried out using MTT assay. Compounds **29a-b** and **31a-b** showed weak or no cytotoxicity. In contrast, most of compounds **30a-g** showed good cytotoxicity against the tested cancer cells (only the IC_{50} values for **30a** and 108g are listed in **Table 3.2**). Compound **30a** (R_1 = H and R_3 = H) was first screened out with good cytotoxicity against five different cancer cell lines. In particular, compound **30a** was about twice more potent than Camptothecin (IC_{50}: 11,004 µM vs 23,401 µM). However, the other enantiomer *(S)*-**30a** had slightly decreased cytotoxicity.

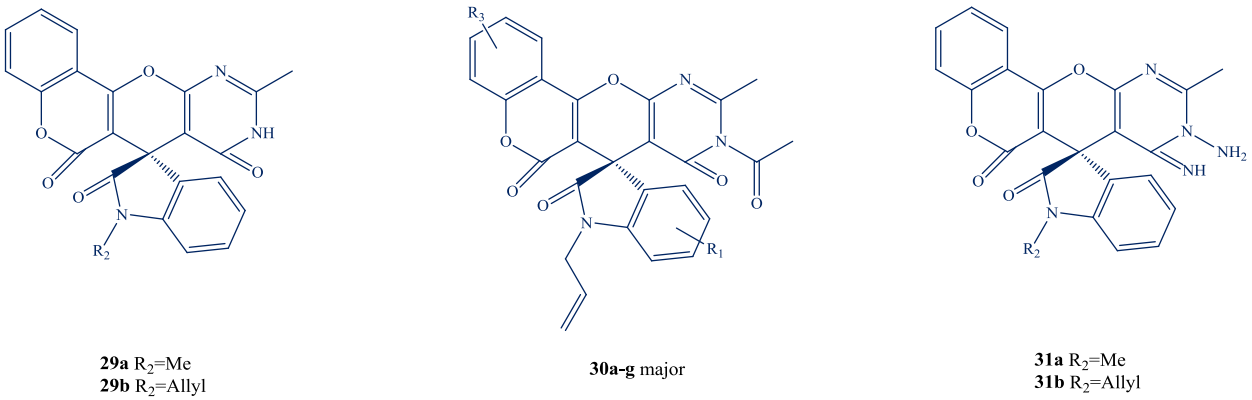


Figure 3.12 *Pyran-fused spirooxindoles and pyranopyrimidines synthetized by Wang and co-workers.*

Compounds	MDA (IC_{50})	U937 (IC_{50})	Jurkat (IC_{50})	Hela (IC_{50})	Ej (IC_{50})
30a	11,004± 2,677µM	7,711± 3,444µM	8,225± 2,854µM	20,394± 7,143µM	11,844± 0,987µM
30g	6,952± 2,654µM	6,296± 0,460µM	6,548± 1,228µM	6,373± 0,652µM	10,381± 3,008µM
Camptothecin	23,401± 3,210µM	0,015± 0,004µM	0,021± 0,002µM	25,226± 3,987µM	0,026± 0,013µM

Table 3.2 *In Vitro anticancer activities of compounds **30a** and **30g**.*

3.4 SPIROOXINDOLE-BASED 2,5-DIHYDROPYRROLES

Considering the versatile bioactivities of spirooxindole and 2,5-dihydropyrrole, Tan et al. designed and synthesized a series of novel spirooxindole-based 2,5-dihydropyrroles with structural diversity via an efficient three-component reaction of isatin, amino ester and alkyne (**Figure 3.13**).[116] In vitro cytotoxicity of these spirodihydropyrroles against MCF-7 cells was carried out at three different concentrations (1, 10, 100 µg/mL) using doxorubicin hydrochloride

as the control. Six compounds showed slightly inhibition against MCF-7 cells at 1 μg/mL, while doxorubicin hydrochloride had no effect towards MCF-7 cells at this concentration. At 100 μg/mL, all 16 compounds showed remarkable cytotoxicity against MCF-7 cells. Among them, compound **32a** was the most potent to inhibit the growth of MCF-7 cells to 28.0%, which was comparable to the positive control. Further mechanism study showed that compound **32a** induced the apoptosis of MCF-7 cells by the MAPK pathway as indicated by the increase of phosphorylated ERK1/2, p38 and JNK levels.

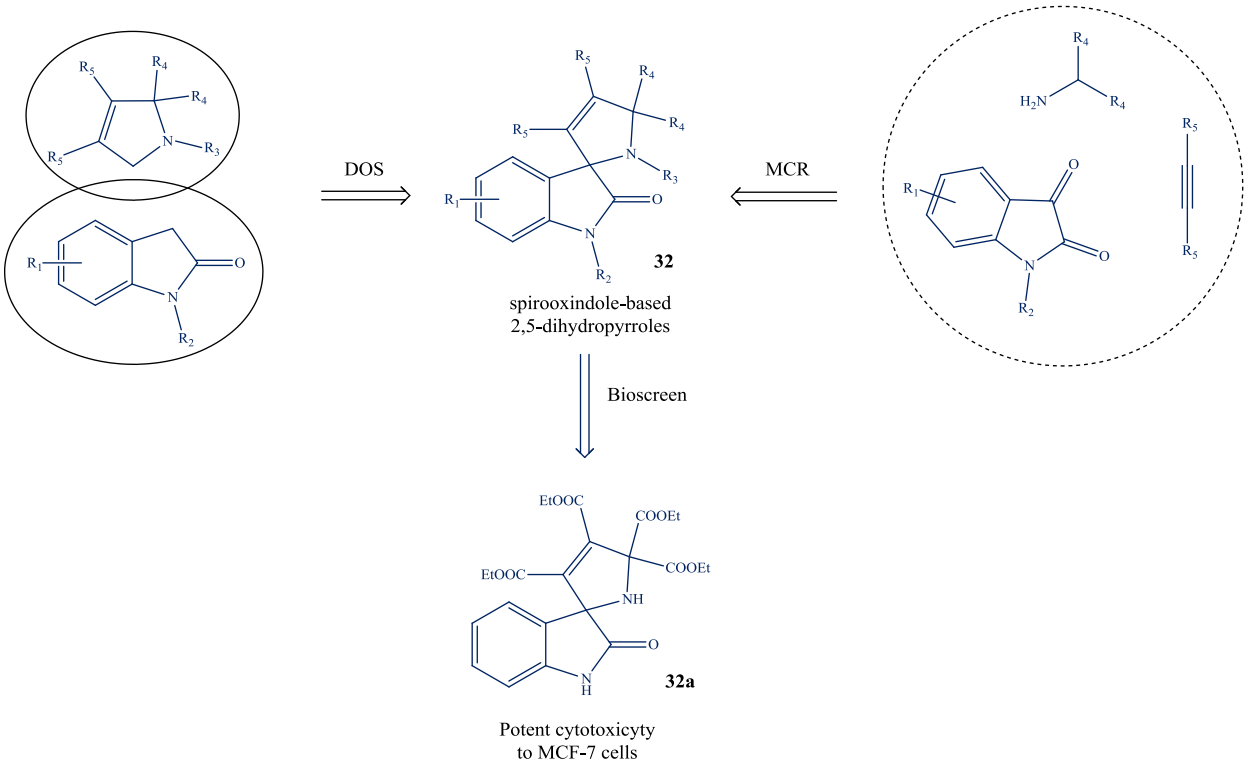


Figure 3.13 *Novel spirooxindole-based 2,5-dihydropyrroles with structural diversity.*

3.5 SPIROISOXAZOLINE OXINDOLES

As shown in the known inhibitors of p53-MDM2 interaction (Nutlin, MI-888 and the recently discovered AM-8553), all these molecules detain a rigid heterocyclic scaffold, from which three lipophilic groups are projected into p53 pocket in MDM2 mimicking the three pivotal residues (Phe19, Trp23 and Leu26).[117] With this in mind, Santos et al. designed and synthesized a library of novel spiroisoxazoline oxindoles (**33**, **Figure 3.14**) as inhibitors of p53-MDM2 interaction and evaluated their antiproliferative effect towards human hepatocellular carcinoma cell line (HepG2) with wild type p53.[118] The SARs study showed the substituents on the three phenyl rings played an important role in the activity, compounds with no substituents on the phenyl rings were inactive. Any substituents introduced at position R_2 and R_4 yielded more active compounds. Introducing a methoxyl group at R_3 abrogated the cytotoxicity, regardless of the pattern of substituents in other positions. Different substituents at R_4 in para position (methoxy,

nitro and methyl) and ortho-methoxy were well tolerated, not affecting substantially potency. A halogen atom (Br or Cl) at position 6 of the oxindole moiety was beneficial to the activity, as exemplified in compound **33d**, which had the best cytotoxicity against HepG2 cells (GI_{50} = 29,11 µM). Compounds **33a**, **33b** and **33d** can inhibit p53-MDM2 interaction in a cell-based bimolecular fluorescence complementation assay. Compounds **33a** and **33d** induced a significant dose-dependent increase of cleaved PARP and active caspase-3, which are reliable markers of the apoptotic process. Moreover, compounds **33b** and **33g** showed good stability in 7,4 phosphate buffer and plasma, and had moderate susceptibility towards NADPH-dependent rat microsomal enzymes. Similarly, Ung and co-workers reported that isoxazolidine **33h** had appreciable cytotoxicity against H460, MCF-7 and SF-268 cell lines (GI_{50} = 2,6 µM against MCF-7 cells).[119]

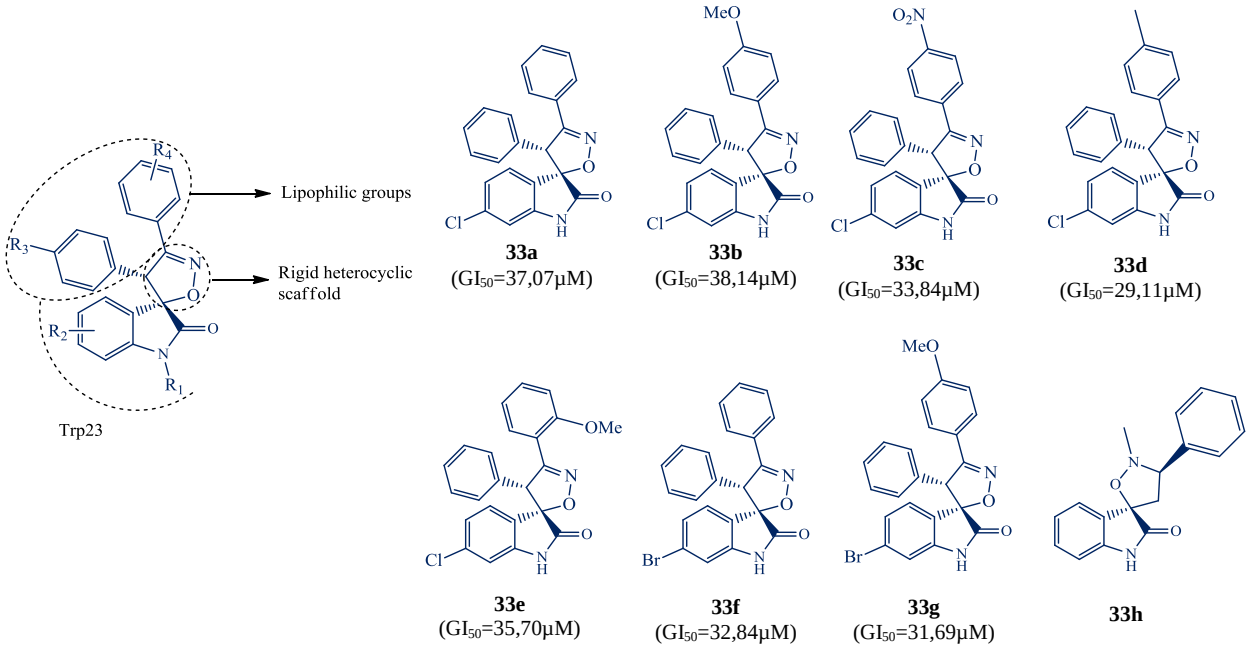


Figure 3.14 *Spiroisoxazoline oxindoles from recent synthesis.*

3.6 SPIROPYRAZOLINE OXINDOLES

Santos et al.[120] designed a new series of spiropyrazoline oxindoles (34) containing a pyrazoline ring with one more aromatic substituent (R_4), in which the oxygen atom in isoxazoline ring was replaced by a *N*-Ar group (**Figure 3.15**). In vitro cytotoxicity against MCF-7 cells was evaluated using MTT assay. The general SARs are listed as below. (a) No activity against MCF-7 cells was observed when R_4=H (GI_{50}>100 µM); (b) Compared to the spiroisoxazoline oxindoles (**33d**: GI_{50} =29,11 µM, **Figure 3.14**), six compounds from the library of spiropyrazoline oxindoles had GI_{50} values of less than 12 µM, indicating that the substitution of an isoxazoline ring by a pyrazoline ring led to an increased cytotoxicity; (c) The inhibitory effect towards MCF-7 cells significantly depended on the nature of R_2 group. When R_2 = Ph, the activity against MCF-7 cells depended on the nature of R_1 and varied in the following order (Br > Cl > H); (d) For 6-Br spirooxindoles

containing phenyl groups at R_2 and R_4, the activity against MCF-7 cells was almost the same regardless of the R_3 group. However, for 7-Cl spirooxindoles containing phenyl groups at R_2 and R_4, the activity varied with the R_3 group. Compounds with the GI_{50} values of less than 30 µM against MCF-7 cells were further tested on human MDA-MB-231 cell line and normal human HEK293T cell line. Interestingly, all these compounds showed low or no cytotoxicity against HEK293T cells at concentration up to 100 µM. Among them, compound **33a** with the most potent activity against MCF-7 cells (GI_{50} = 7,0 µM) also had good cytotoxicity against MDA-MB-231 cells (GI_{50} = 28,6 µM) and no cytotoxicity against nHEK293T cells (GI_{50} > 100 µM), respectively.

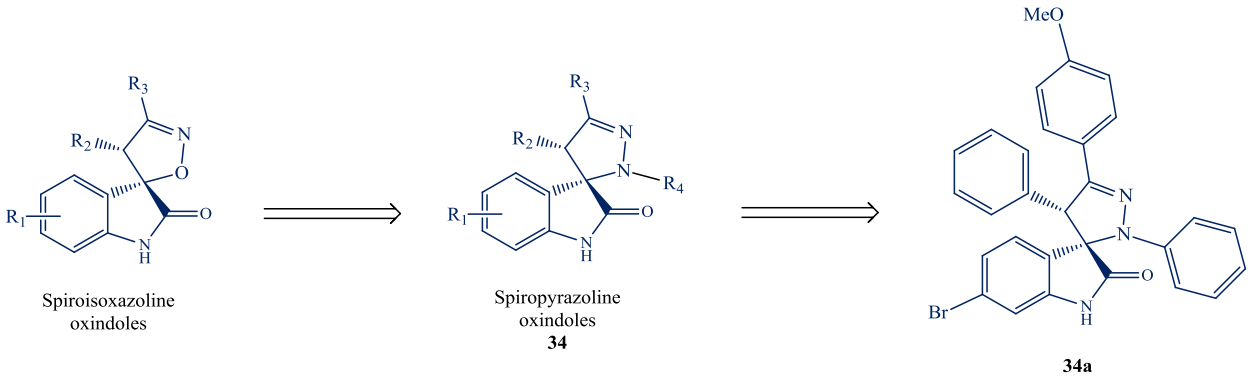


Figure 3.15 *Spiroisoxazoline oxindoles based design of spiropyrazoline oxindoles.*

Due to the drawbacks of current anticancer drugs, the research for more effective and selective anticancer agents has always been the hot topic in medicinal chemistry. Spirooxindoles have emerged as privileged scaffolds because of their prevalence in numerous natural products and biologically active molecules. Structure (spirotryprostatin A and alstonisine) based drug discovery has been proved as an efficient strategy to find new anticancer agents (e.g. the discovery of MI-888). For spirooxindoles, their anticancer potency mainly depends on the type of cyclic ring fused on the C3 position of oxindole core and the stereochemistry. Among them, spiro-pyrrolidinyl oxindoles seem to be the most promising ones that act as potent inhibitors of p53-MDM2 interaction. Of course, substituents attached to the oxindole core should never be escaped from our attention. However, numerous spirooxindoles with structural novelty reported have not been subjected to anticancer evaluation, which restricts the discovery of new anticancer agents. In spite of the interesting work on spirooxindoles as anticancer drugs that was described in this chapter, there is still a great need and opportunity for medicinal chemists to further explore the biological activity of such scaffolds through extensive SAR efforts.

CHAPTER 4

CHAPTER 4 – CHEMICAL APPROACHES TO INHIBITORS OF ISOPRENOID BIOSYNTHESIS: TARGETING FARNESYL AND GERANYLGERANYL PYRO-PHOSPHATE SYNTHASES

4.1 INTRODUCTION

Isoprenoids (also known as terpenoids) are considered the most ancient and diverse class of natural products. They have been found in sediments from 2,5 billion years ago and more than 40.000 representatives have been found in all kingdoms of life. They participate in a great variety of basic biological functions in plants (e.g. growth regulation, pigments) and mammals (e.g. steroids metabolism, cellular signaling, antioxidants), and have been used in the food, pharmaceutical, chemical and biofuel industries.

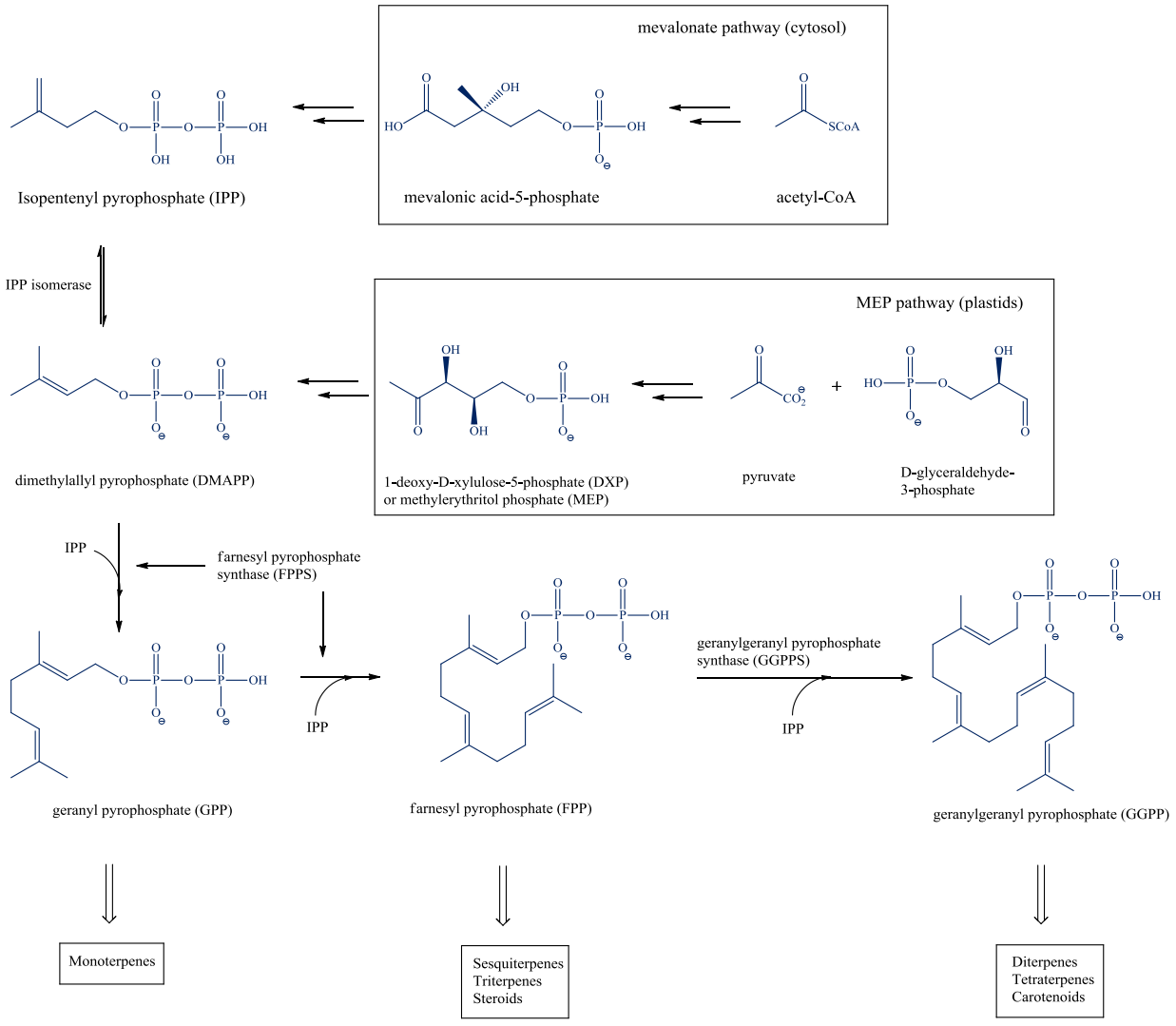


Figure 4.1 *Biosynthesis of isopreoids by FPPS and GGPPS.*

Isoprenoids are biosynthesized ubiquitously in eubacteria, archaebacteria and eukaryotes by the consecutive condensation of the five-carbon monomer isopentenyl diphosphate (IPP) to its isomer dimethylallyl pyrophosphate (DMAPP) (**Figure 4.1**). Whereas in mammals and yeast IPP is synthesized in the cytosol and endoplasmic reticulum from acetyl-CoA through mevalonic acid (mevalonate pathway), in higher plants and other microorganisms IPP is synthesized in the plastids by the condensation of pyruvate with glyceraldehyde-3-phosphate through 1-deoxyxylulose-5-phosphate (DXP) – also called methylerythritol (MEP pathway).

Two consecutive condensations of IPP and DMAPP catalyzed by farnesyl pyrophosphate synthase (FPPS) provide geranyl diphosphate (GPP) and farnesyl diphosphate (FPP). The former is the precursor of monoterpenes and the latter of sesquiterpenes, triterpenes and sterols (via squalene biosynthesis) as well as other important secondary metabolites like ubiquinones and dolichols. An additional condensation of FPP with IPP, catalyzed by the enzyme geranylgeranyl pyrophosphate synthase (GGPPS), furnishes geranylgeranyl pyrophosphate (GGPP) precursor of di- and tetraterpenes and carotenoids **(Figure 4.1**).

Given the importance of the metabolites accessible from isoprenoid biosynthesis, the enzymes involved in the process are excellent drug targets. The non-mevalonate pathway (MEP pathway) is not present in mammalian systems; consequently the enzymes involved in MEP pathway are attractive drug targets for the development of herbicides, antimicrobial drugs and fighting against pathogenic microorganisms like P. falciparum (malaria), T. cruzi (Chagas disease) and M. tuberculosis. Enzymes in the MEP pathway, IspG and IspH, are anti-infective drug targets and HMG-CoA reductase involved in the synthesis of IPP is the primary target of hypocholesterolemic drug therapy.

Protein prenylation, in particular farnesylation and geranylgeranylation, is one of the essential post-translational protein modification in the eukaryote. Therefore inhibition and/or modulation of the enzymes FPPS and GGPPS will affect not only to essential secondary metabolites derived from isoprenoid biosynthesis but also to the functionality of prenylated proteins. FPPS has been identified as a target for a series of drugs acting as anticancer, antimicrobial and antiparasitic agents. In particular, human FPPS is a drug target for cancer, osteoporosis and related diseases, Paget's disease, metastatic diseases, and cardiovascular disorders. Inhibition of human GGPPS has been reported as a new route to bone antiresorption and the same enzyme from Plasmodium has been identified as a target against malaria.

The multiple sequence alignment of FPPS and GGPPS found in different organisms makes that the same potential inhibitor should be tested against both enzymes from diverse biological sources. The main group of inhibitors is constituted by bisphosphonates (BPs), structural analogues of DMAPP considered chemically stable analogues of inorganic pyrophosphate. Bisphosphonates, known from more than 40 years are being used clinically in the treatment of osteoporosis and malignant bone diseases (**Figure 4.2**).[121]

Simple bisphosphonates correspond to analogues of pyrophosphate **1** with therapeutic properties in which the bridging oxygen atom has been replaced by a methylene group that can incorporate non-nitrogenated substituents. Typical examples are etidronate **2** and cloronate **3** and their mechanism of action consists of being incorporated to non-hydrolyzable analogues of ATP, inducing osteoclast apoptosis.[122]

On the other hand, nitrogen-containing bisphosphonates (e.g. alendronate **4**, pamidronate **5**, ibandronate **6**, risedronate **7**, zoledronate **8**) showed to be more than 10000 times more active

than non-nitrogenated derivatives. These analogues have a diverse mechanism of action causing (i) disruption of normal function of essential signaling proteins and (ii) accumulation of IPP which is incorporated into a toxic nucleotide metabolite. More recently, a variety of non-nitrogenated bisphosphonates like tiludronate **9** and others bearing arylsulfonium and phosphonium groups showed cytotoxicity against cancer, inhibition of TpFPPS and stimulation of T-cells in the human immune system revealing that the presence of a nitrogen atom is not strictly necessary. In addition to bisphosphonates, other inhibitors including quinoline and salicylic acid derivatives, have been reported. These non-bisphosphonate inhibitors bind to an allosteric site on FPPS identified by X-ray crystallography.[123]

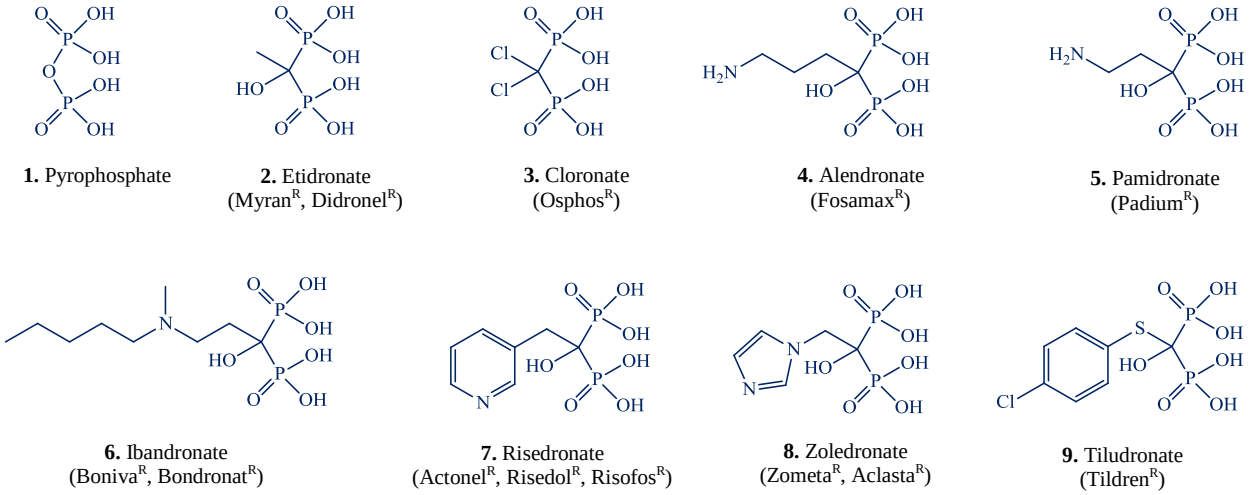


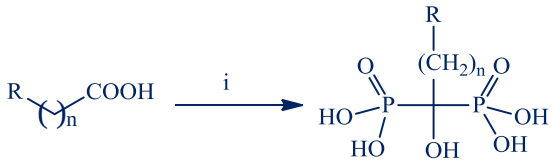
Figure 4.2 *FDA-approved protonated-bisphosphonates clinically used for the treatment of bone disorders.*

In this chapter is reported the highlight synthetic methodologies directed to the preparation of FPPS and GPPS inhibitors and their therapeutic effects.

4.2 BISPHOSPHONATES COMPOUNDS

4.2.1 Reaction of carboxylic acids with phosphorous reagents

The most general and attractive approach for preparing 1,1-bisphosphonates is the reaction of a carboxylic acid, easily accessible by conventional methods, with an inexpensive phosphorous reagent like phosphorous trichloride. Due to high therapeutic interest of 1,1-bisphosphonates, the first reports regarding their synthesis were published as patents and no clear experimental details were given, the reaction being difficult to scale up and lacking of reproducibility. Indeed, no mechanism of the reaction had been determined and a great variability was observed in reactants ratio, temperature, reaction time and work-up. In 1995, Kieczykowski and Jobson, from Merck and Co., Inc., reported a general procedure for reacting a carboxylic acid with phosphorous acid and phosphorous trichloride in the presence of methanesulfonic acid (**Scheme 4.1**).[124]

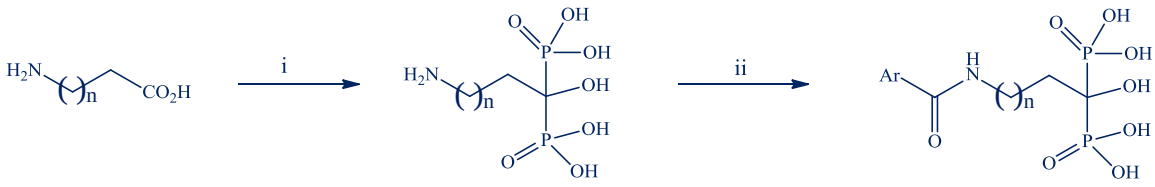


10 **11**

R	n	Yield (%)
NH_2	2	57
NH_2	3	89
NH_2	4	78
NH_2	5	89
Me	10	95
Cl	1	31
3-imidazoyl	1	38
3-pyridyl	1	26
4-$H_2NC_6H_4$	3	30

Scheme 4.1 *Reagents and conditions: (i) H_3PO_3 (1 equiv.), $MeSO_3H$ (1 equiv.), PCl_3 (2 equiv.), 65°C, 16-20h; then H_2O, reflux; then pH=4, 50% NaOH.*

Since then, this method has been the most widely used for the synthesis of 1-hydroxy-1,1-bisphosphonates although in some particular case it has been reported contamination of the product with the methanesulfonate salt, that can be avoided by performing the reaction without solvent.[125] The procedure is amenable of being used with several substrates including those bearing an amino functionality. For instance, alendronate **4** and pamidronate **5**, have been prepared by this route and served as precursors of substituted analogues like compounds **13** (**Scheme 4.2**).[126]

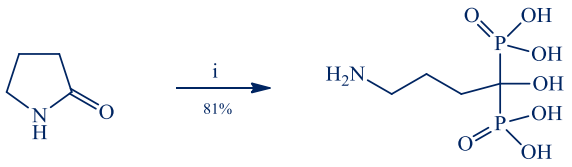


12 **4** n=2 **5** n=1 **13**

Ar= Ph, 2,3-diOMeC$_6$H$_3$, 4-MeC$_6$H$_4$, 3-MeOC$_6$H$_4$, 2-MeC$_6$H$_4$, 4-MeOC$_6$H$_4$

Scheme 4.2 *Reagents and conditions: (i) H_3PO_3, PCl_3. (ii) ArCOCl, NaOH.*

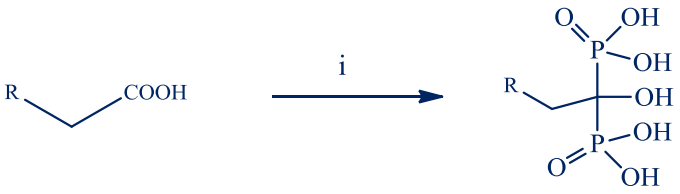
Alendronate **4** can also be obtained in a straightforward manner from pyrrolidone (**Scheme 4.3**).[127] Hydrolysis of **14** in aqueous methanesulfonic acid followed by reaction with phosphorous trichloride and hydrolysis with water furnished compound **4**.



14 **4**

Scheme 4.3 *Reagents and conditions: (i) H_3PO_3, PCl_3. (ii) ArCOCl, NaOH.*

Good yields in the synthesis of heterocyclic (risedronate **7**, zoledronate **8**) and aminoalkyl bisphosphonates were obtained in just 20 min using microwaves and sulpholene as a solvent in the first stage of the reaction (**Scheme 4.4**).[128]



15 **4, 5, 7, 8, 16**

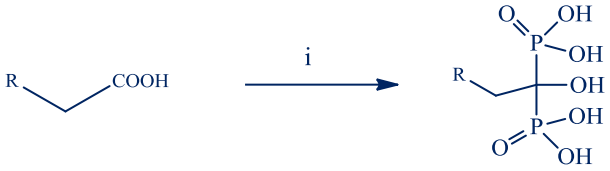
Compound	R	Yield (%)
8	3-imidazoyl	74
7	3-pyridyl	70
5	aminomethyl	64
4	aminoethyl	41
16	aminobutyl	53

Scheme 4.4 *Reagents and conditions: (i) H_3PO_3 (3 equiv.), PCl_3 (4 equiv.), sulpholene, 65°C, 3-7 min; MWI 200-400 W max, then H_2O, MWI, 450-500 W.*

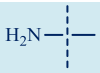
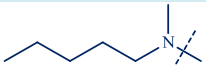
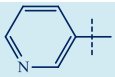
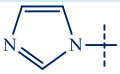
Pyridinium fluorescently-labeled conjugates of risedronate **7** were synthesized using an epoxide linker which was bonded to the pyridyl nitrogen. The conjugates were used for fluorescence imaging of Bacillus subtilis.[129]

Despite this synthetic activity that demonstrate the utility of the carboxylic acid-approach, various inconsistent procedures appeared in patents concerning the synthesis of the most clinically used zoledronic and risedronic acids which has been the matter of some controversy regarding the reagents and the ratio between them to be used. In 2011, Keglevich and co-workers finally established that the best conditions correspond to the use phosphorous trichloride in 1: 3,2 molar ratio in methanesulfonic acid.[130] When the reaction is carried out in the presence of paraformaldehyde, bisphosphonic acids can be obtained without the addition of water, the use of microwaves enhancing the yield of the reaction. By using their conditions, Keglevich and co-workers reported the synthesis of etidronate **2**, alendronate **4**, pamidronate **5**, ibandronate **6**,

risedronate and **7** zoledronate acid **8** (**Scheme 4.5**).[131]

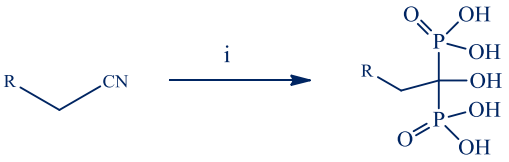


15 **2, 4-8**

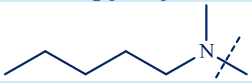
Compound	R	t, T	Yield (%)
2	Me	24h, 75°C	36
4		24h, 75°C	57
5		12h, 75°C	57
6		12h, 75°C	46
7		3h, 80°C	74
8		3h, 80°C	53

Scheme 4.5 *Reagents and conditions: (i) PCl_3 (3.2 equiv.), $MeSO_3H$; then H_2O, 4-5h, 105°C; then 50% aq NaOH to pH=1,8-2,0.*

Nitriles **17** can also be used as an alternative to carboxylic acids and the reaction can be performed under similar conditions but without the presence of phosphorous acid (**Scheme 4.6**).[132] Risedronate **7** prepared by this route was further labeled with ^{99}Tc by forming the corresponding complex.[133]



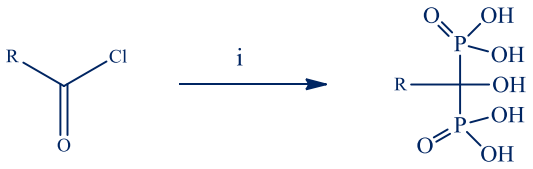
17 **18-20**

Compound	R	Yield (50%)
7	3-pyridyl	85
18	2-pyridyl	71
19	4-pyridyl	75
6		65
20		64

Scheme 4.6 *Reagents and conditions: (i) CH_3SO_3H, 8h, 100°C; then PCl_3, 70°C, 5h; then H_2O, 15h, 98°C.*

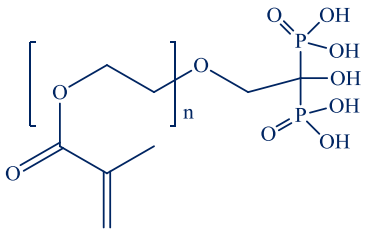
4.2.2 Reaction of acylphosphonates with dialkyl phosphites

In some instances, the direct reaction between a carboxylic acid and phosphorus trichloride takes place with low yields or fails completely as in the case of sterically hindered α-aminoacids. An alternative is the use of the acyl chloride as starting material. The Arbuzov-type reaction[e] with a di-, trialkyl phosphite provides an α-ketophosphonate (acylphosphonates) capable of reacting with another molecule of di-, trialkyl phosphite to give the corresponding 1-hydroxy-1,1-bisphosphonate. The first report on this approach described the use of tris(trimethylsilyl)phosphite as the only phosphorylating reagent (**Scheme 4.7**).[134] The reaction proceeds under mild conditions rendering the process compatible with labile substrates including bile acids.[135] Acrylic ester bisphosphonates with numerous potential applications in biomedicine are also accessible through this approach.[136]



21 **22**

R	Solvent	T (°C)	Yield (%)
Me	None	25	98
C_5H_{11}	None	25	97
$C_{11}H_{23}$	None	25	97
$C_6H_5CH_2$	None	25	90
C_6H_5	None	25	91
4-$MeOC_6H_4$	THF	25	90
4-$NO_2C_6H_4$	THF	-70	85
3-pyridyl	THF	25	61



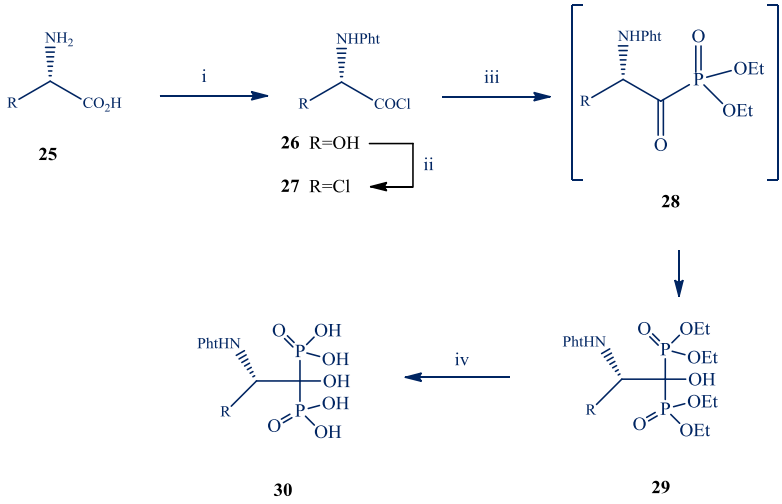
23 n=5
24 n=8

Scheme 4.7 *Reagents and conditions: (i)P(OSiMe$_3$)$_3$, 0°C then rt; then MeOH, 1 h, 25°C.*

The direct use of trialkyl phosphites is also possible. A series of α-amino acid derived bisphosphonates **42** has been prepared in good yield by using as starting materials N-

[e] Actually, the Arbuzov reaction (or Michaelis–Arbuzov reaction) is the reaction of a trialkyl phosphite with an alkyl halide to form a phosphonate. In this case, the reaction takes place with an acyl chloride to give a ketophosphonate.

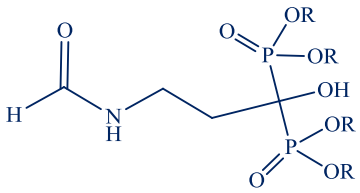
phthalimido-protected amino acids and using sequentially tri- and diethyl phosphite as P-reagents (**Scheme 4.8**).[137] Although the addition of phosphites is consecutive, the reaction is carried out in a one-pot procedure without isolating the intermediate ketophosphonate **40**. Final deprotection of the amino group was made with 6N HCl. The use of hydrazine is also possible and better yields are obtained. By using this variation, alendronate **4**, pamidronate **5**, and neridronate have been prepared as mono- and diesters, which were soluble in water at physiological pH.[138] The use of tri- and dimethylphosphite led to shorter reaction times.



Amino acid 25	26 (Yield %)	27 (Yield %)	29 (Yield %)	30 (Yield %)
Phenylalanina	88	88	30	50
Leucine	81	90	25	90
Tryptophane	95	93	50	88
methionine	92	85	44	95
Aspartic	55	88	18	40
Glutamic	83	89	48	99
Lysine	80	80	18	90
Ornithine	26	92	15	82
Valine	81	86	18	90

Scheme 4.8 *Reagents and conditions: (i) N-(ethoxycarbonyl)phtalimide, $NaHCO_3$, 0°C, 5 min; (ii) $SOCl_2$, CH_2Cl_2, 5h, reflux; (iii) triethyl phosphite, toluene, 1h, 0°C, then diethylphosphite, Et_3N, 1-3h, 0-5°C; (iv) 6N HCl, reflux overnight, then 5N NaOH pH=4,4.*

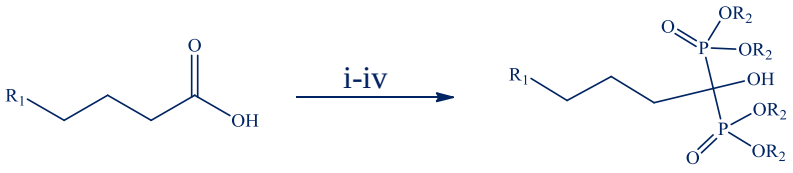
In **Figure 4.3** is reported the Compound **31** was used as a contrast agent in image-guided surgery of large animals.[139]



31

Figure 4.3 *Bisphosphonate used in image-guided surgery of animals clinic treatments.*

The use of dimethylphosphite also afforded better results in the synthesis of alendronate **4** reported by Seki in which both P- reagents were compared. Differences were found in the final hydrolysis of the phosphate esters. Ethyl esters showed a lower reactivity towards hydrolysis (**Scheme 4.9**).[140]



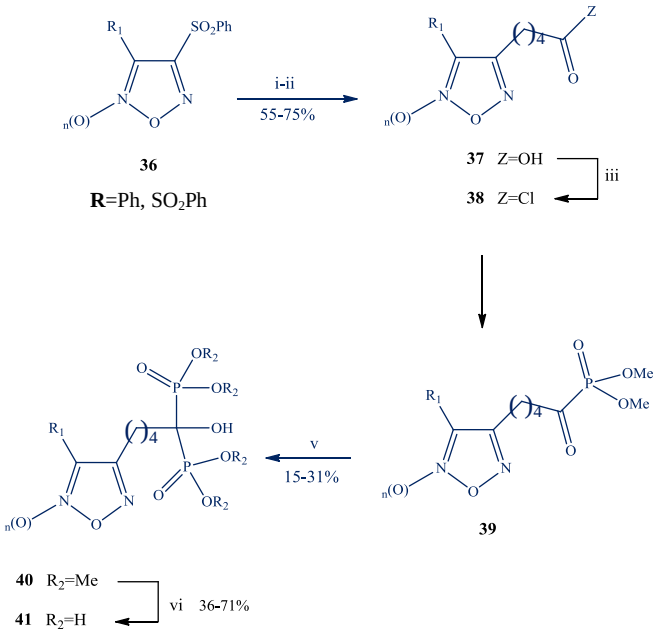
32 R_1=AcNH
33 R_1=Me
34 R_1=PhtN

35

Compound	R_1	R_2	Method	Yield (%)
35a	AcNH	Et	A	0
35b	AcNH	Et	B	0
35a	AcNH	Et	C	22
35b	Me	Me	A	Quant.
35a	AcNH	Me	A	70
35b	PhtN	Me	A	12

Scheme 4.9 *Reagents and conditions: (i) CH_2Cl_2, DMF (cat.), 3h, rt.; (ii) $(R_2O)_3P$, 1h, rt; (iii) $(R_2O)_3P$, TMSBr, 1h, rt; (iv) method A: aq 5M HCl, 17h, reflux; method B: $MeSO_3H$, 17h, reflux; method C: 48% HBr, 17h, reflux.*

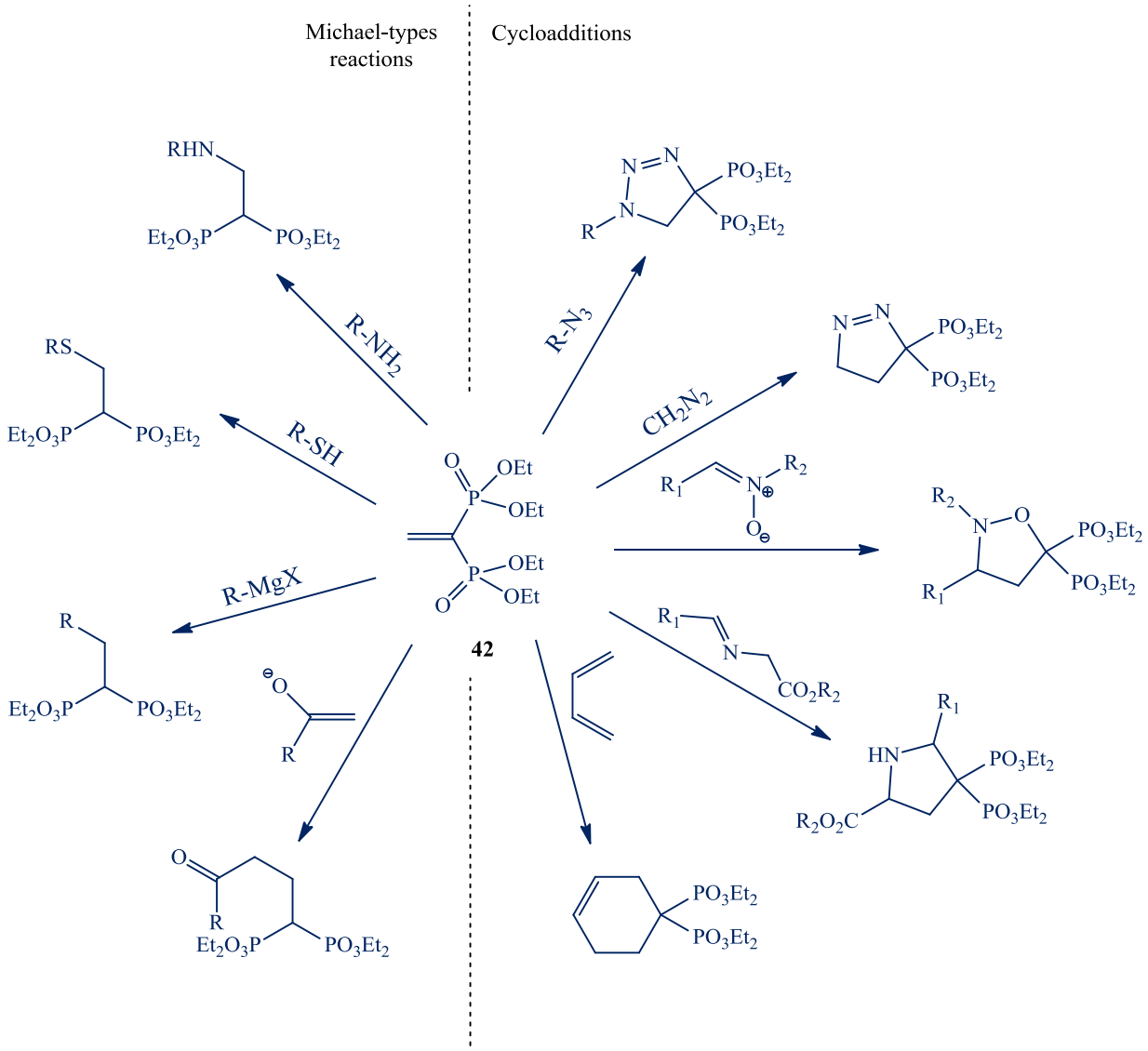
A series of bisphosphonates bearing either the nitrogen-containing *N,O*-donor furoxan (1,2,5-oxadiazole 2-oxide) or the related furazan (1,2,5-oxadiazole) systems in the lateral chain has been prepared by using trimethylphosphite as P-reagent (**Scheme 4.10**).[141]



Scheme 4.10 *Reagents and conditions: (i) 1,5-pentanediol, NaOH 50% w/w, THF; (ii) Jones reagent, acetone, 0°C to rt; (iii) $SOCl_2$; (iv) $P(OMe)_3$, dry THF, 0°C to rt; (v) $HPO(OMe)_2$, Et_2NH, dry THF, 0°C to rt; (vi) TMSBr, CH_2Cl_2, then MeOH, 0°C to rt.*

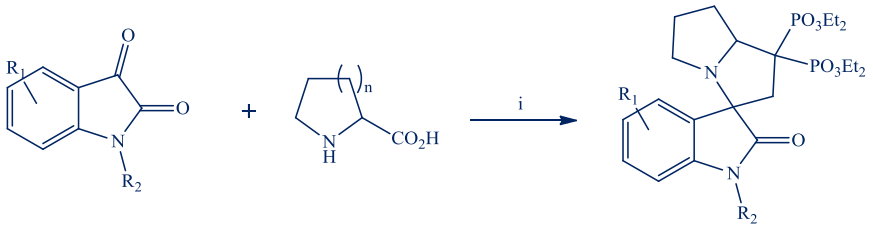
4.2.3 Michael addition to teraethylvinylidenebisphosphonate

Tetraethyl vinylidenebisphosphonate **42** is an easily available Michael acceptor and electron-poor dipolarophile/dienophile that results an excellent synthetic intermediate for the synthesis of 1,1-bisphosphonates (**Scheme 4.11**).



Scheme 4.11 *Synthetic utility of tetraethyl vinylidenebisphosphonate* ***42****.*[142]

A series of spiro[indole-pyrrolizine], spiro[indole-indolizine],and spiro[indole-pyrrolidine] *gem*-bisphosphonates were synthesized through multicomponent reactions between isatins **43**, amino acids **44** and **42** in the presence of montmorillonite (**Scheme 4.12**).[143] Acyclic aminoacids can also be used.

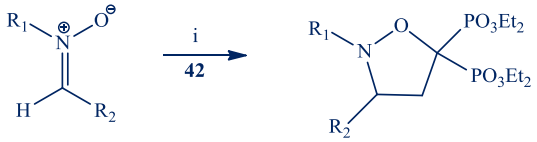


43 44 45

R_1	R_2	n	t (h)	dr	Yield (%)
H	H	1	0,5	100:14	80
4-F	H	1	0,5	100:6	92
4-Cl	H	1	0,5	100:22	95
4-Br	H	1	0,5	100:20	93
3-Cl	H	1	2	100:0	57
4-Me	H	1	1	100:24	45
4-Me	Bn	1	2	100:17	47
H	Bn	1	2	100:18	44
H	H	2	2	100:46	65
4-F	H	2	2	100:8	45
4-Cl	H	2	2	100:0	40
4-Br	H	2	2	100:4	45
4-Me	Bn	2	2	100:5	40
H	Bn	2	2	100:8	35

Scheme 4.12 *Reagents and conditions: (i) montmorillonite, MeCN, 0,5-2h, 80°C; (ii) TMSBr, 18h, rt, then water, 4h, rt.*

The cycloaddition of aromatic nitrones **46** with **42** furnished spiro(isoxazolino)bisphosphonates **47** (**Scheme 4.13**).



46 47

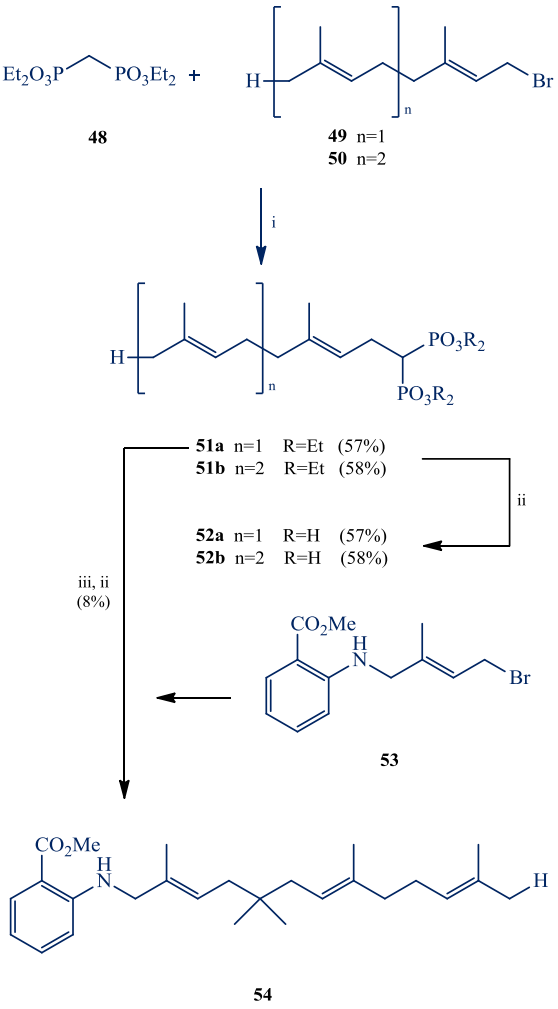
R_1	R_2	t (min)	Yield (%)
Me	Ph	10	75
Me	2-ClC_6H_4	12	77
Me	3-pyridyl	13	3
Me	2-furyl	15	68
Bn	4-OHC_6H_4	14	74
Bn	Ph	18	83
Bn	2-ClC_6H_4	18	85
Bn	2-FC_6H_4	20	86

Scheme 4.13 *Reagents and conditions: (i) neat, microwave irradiation 200W.*

The reaction, carried out in the absence of solvent and under activation with microwaves takes place in several minutes with good yields.[144] In all these cases the bisphosphonates prepared by this route lack the 1-hydroxyl group but consist of interesting structurally constrained analogues.

4.2.4 Alkylation of tetralkylmethyl bisphosphonate

Alkylation of tetraethyl bisphosphonate **48** is an expeditious way of preparing bisphosphonates lacking the 1-hydroxy groups. However, precise reaction conditions must be used in order to avoid elimination reactions.[145] Alkylation of **48** with farnesyl and geranyl bromides **49** and **50** using sodium hydride as a base provided bisphosphonates 51a,b. Further hydrolysis furnished free bisphosphonates **52a,b** (**Scheme 4.14**).[146]



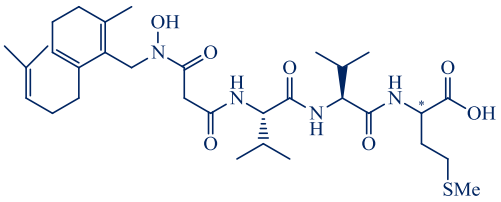
Scheme 4.14 *Reagents and conditions: (i) NaH, THF, 0°C, 1h, then 1 day, rt; (ii) TMSBr, collidine, 1 day, rt, then 0.5M NaOH 16 days, rt; (iii)* ***53****, NaH, THF, 15-crown-5, overnight, rt.*

On the other hand, using potassium hydride an undesired elimination reaction was observed.[133] By oxidizing the allylic position of the terminal tri-substituted double bond in **49**, it was possible

to introduce additional substituents at the end of the isoprenoid unit.[147] Further alkylation of compound **49** furnished, after hydrolysis, fluorescent anthranilate analogue **54** that showed some inhibition in geranylgeranylation.[148]

4.3 NON-BISPHOSPHONATE DERIVATIVES

Non-bisphosphonate antiresorptive agents are represented by molecules with variable dimensions and functional groups.
The presence of a hydroxamate moiety induces a major attitude in terms of metal chelation.[149] Thus, the hydroxamic group is expected to improve the ionic and/or metal chelation interactions with the active site of FPT (Farnesyl Protein Transferase). In **Figure 4.4** is reported a hydroxamic derivative **55** with improved inhibition activity against FPT.



55

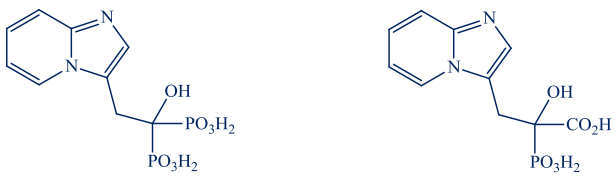
Figure 4.4 *Hydroxamic acid derivative to improved inhibition FTP activity.*

In 1993, the chaetomellic acids A and B **56** and **57**, classified as alkyl cis-dicarboxylates, were discovered to be potent inhibitors of FPTase because of analogy with the active site of FPP (**Figure 4.5**).[150]

56 **57**

Figure 4.5 *Chaetomellic acids.*

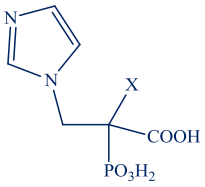
Minodronic acid **58** (**Figure 4.6**) was the first bisphosphonate developed and approved for osteoporosis treatment in Japan and today is available in a number of countries worldwide.



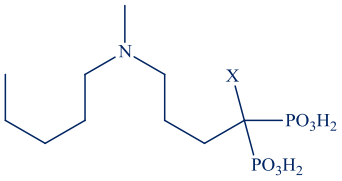
58 **59**

Figure 4.6 *Minodronic acid and analog.*

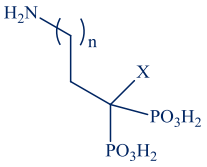
McKenna and co-workers reported, in 2010, the synthesis of the analogue **59** (**Figure 4.6**) starting from imidazo[1,2-a]pyridine.[151] Resolution of **59** enantiomers by chiral HPLC furnished the (+)-**59** isomer, which revealed a potent inhibitory activity of RGGT.
Recently, Coxon and colleagues used various approaches among those described to synthesize phosphonocarboxylates **60–63** showing some structural diversity (**Figure 4.7**).[152]



60a X=H
60b X=F
60c X=OH



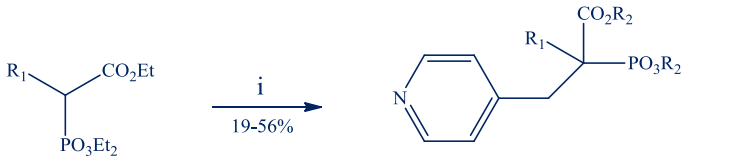
61a X=H
61b X=F
61c X=OH



62a n=1 X=H
62b n=1 X=F
62c n=1 X=OH
63a n=2 X=H
63b n=2 X=F
63c n=2 X=OH

Figure 4.7 *Phosphonocarboxylates.*

Interestingly, the exchange of hydroxyl group with an alkyl chain of different length increased in the hydrophobicity enhancing the activity against GGPPS and FPPS. The synthesis of derivatives **66** was carried starting from α-alkyl phosphonoacetate **64** through an Arbuzov reaction and subsequent alkylation with picolyl chloride (**Scheme 4.15**). The corresponding free acids **66** were obtained upon hydrolysis with a 12 M solution of HCl at reflux.



64

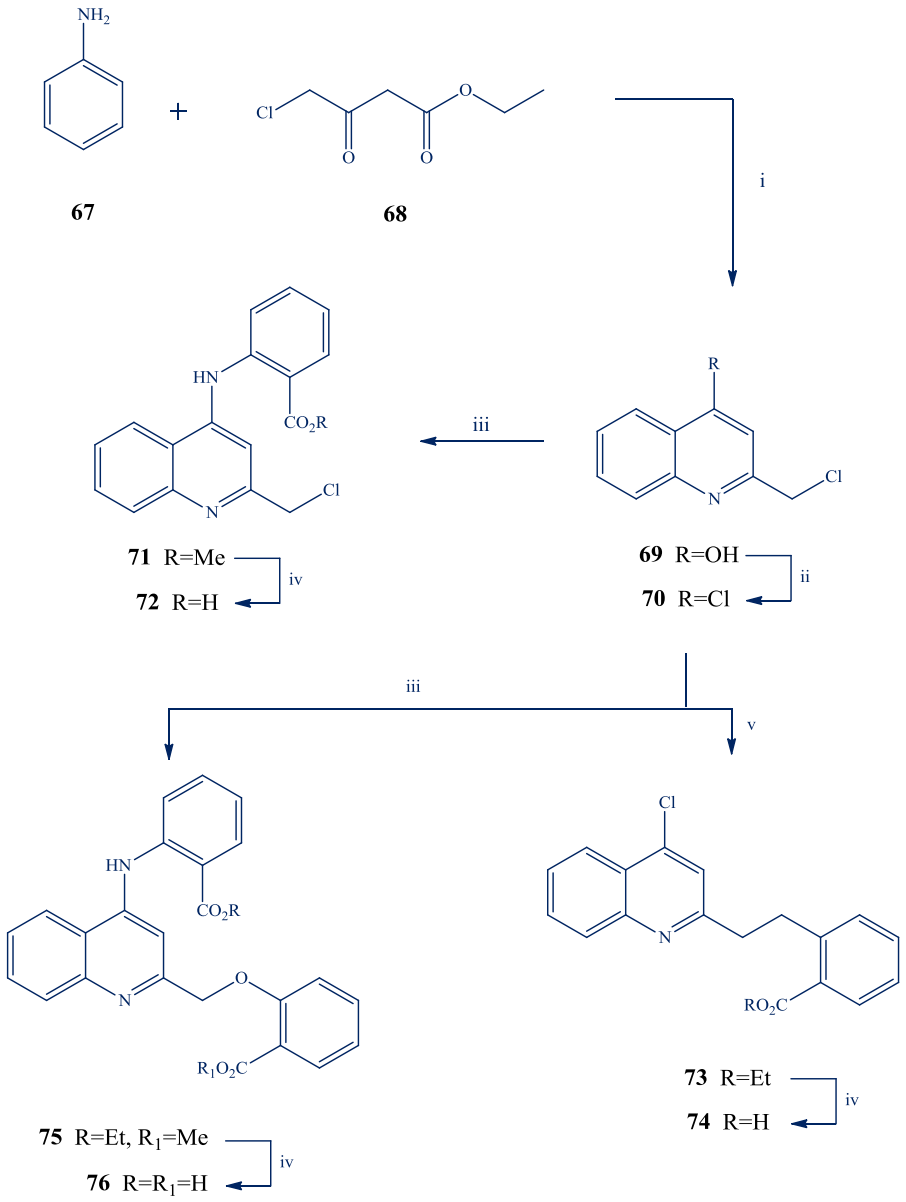
65 R_2=Et
66 R_2=H
ii (33-72%)

R_1= Me, n-Pr, n-pentyl, n-hexyl, n-octyl

Scheme 4.15 *Reagents and conditions: (i) picolyl chloride, NaH, DMF/THF; (ii) 12M HCl, reflux.*

Quinolines and salicylic derivatives have also been shown to be inhibitors of FFPS. In particular, the combination of quinolines with zoledronate seems to amplify the inhibition effect respect to the individual inhibitor. Therefore, a series of quinolone derivatives was synthesized starting from aniline **67** and ethyl 4-chloroacetoacetate **68** in PPA at 130°C (**Scheme 4.16**).[153]
The crude mixture was directly chlorinated by POCl3 at 100°C. The resulting intermediate **70**

was used without further purification in the following reaction with methyl 2-aminobenzoate **71** or ethyl 2-hydroxybenzoate **73**. Different reaction routes allowed obtaining three different quinoline analogues **72**, **74** and **76**.



Scheme 4.16 *Reagents and conditions: (i) PPA, 130°C, 1h; (ii) $POCl_3$, 100°C, 3h; (iii) methyl 2-aminobenzoate, C_2H_5OH, conc. HCl, 5h; (iv) THF/MeOH, LiOH, 3h; (v) ethyl 2-hydroxybenzoate, K_2CO_3, CH_3CN, 90°C, 5h.*

4.4 CONCLUDING REMARKS

Historically, bisphosphonates are benchmark drugs for the treatment of a variety of bone disorders including osteoporosis and bone metastasis. Their inhibitory activity of the isoprenoid biosynthesis resulted in other important applications as modulators of the metabolism of several

protozoa parasites thus also being potential therapeutic agents for the treatment of trypanosomiasis (Chagas disease), leishmaniasis, toxoplasmosis and malaria. Bisphosphonates could also be useful in the treatment of other diseases such as breast cancer, myeloma multiple and progeria. Both clinical success in bone disorders and expectative for other diseases prompted the enormous synthetic activity directed to the preparation of bisphosphonates and more recently, nitrogen-containing bisphosphonates which are demonstrated better biological properties. In general, the reaction of carboxylic acids with phosphorous reagents like $POCl_3$ is the preferred approach to bisphophonates. The reaction, however, is very sensitive to steric hindrance. More recently, the use of tetraethylvinylidenebisphosphonate as the source of phosphorylated part of the molecule has facilitated enormously the access to a variety of bisphophonates. Moreover, the use of such a reagent presented a high tolerance to a variety of functional groups. For the particular case of bisphosphonates lacking the hydroxyl group the alkylation of tetralkylmethyl bisphosphonate is preferred; however, elimination reactions are common undesired lateral processes that, on the other hand, can be eliminated by using precise reaction conditions.

The presence of two phosphate units, in addition to difficult manipulation and purification, limits the oral bioavailability and contribute to undesired side-effects. In this respect, novel bisphosphonate analogues that selectively target FPPS and GGPPS enzymes might provide notable advantages over the currently used drugs and this is now the subject of intense investigation in medicinal chemistry and chemical biology.

Achieving this target implies to have at disposition a series of synthetic strategies that allow not only the preparation of the target compound but also structural variations of the parent compound that provide lead compounds for further studies for treatment of the several diseases related to isoprenoid biosynthesis.

CHAPTER 5

CHAPTER 5 – MEDICINAL ATTRIBUTES OF 1,2,3-TRIAZOLES

5.1 INTRODUCTION

There are many heterocyclic ring structures, which have been designed in such a way that their binding efficiency with the receptor increases after structural modifications. This medicinal chemistry is a boon to the researchers and provides long-term advancement in the medical field. One of the motif is triazoles, which have been explored widely and still its scope is inevitable.[154] Triazoles are heterocyclic organic compounds containing five-membered ring with three nitrogen and two carbon atoms. Generally, 1,2,3-triazoles are further subdivided into three main class, namely, monocyclic 1,2,3-triazoles, benzotriazoles and 1,2,3-triazolium salts as depicted in **Figure 5.1**. Monocyclic 1,2,3-triazoles and benzotriazoles are remarkably stable towards hydrolysis, oxidative/reductive conditions, and enzymatic degradation but reductive cleavage occurs under forcing conditions leading to the formation of triazolium salts.[155]

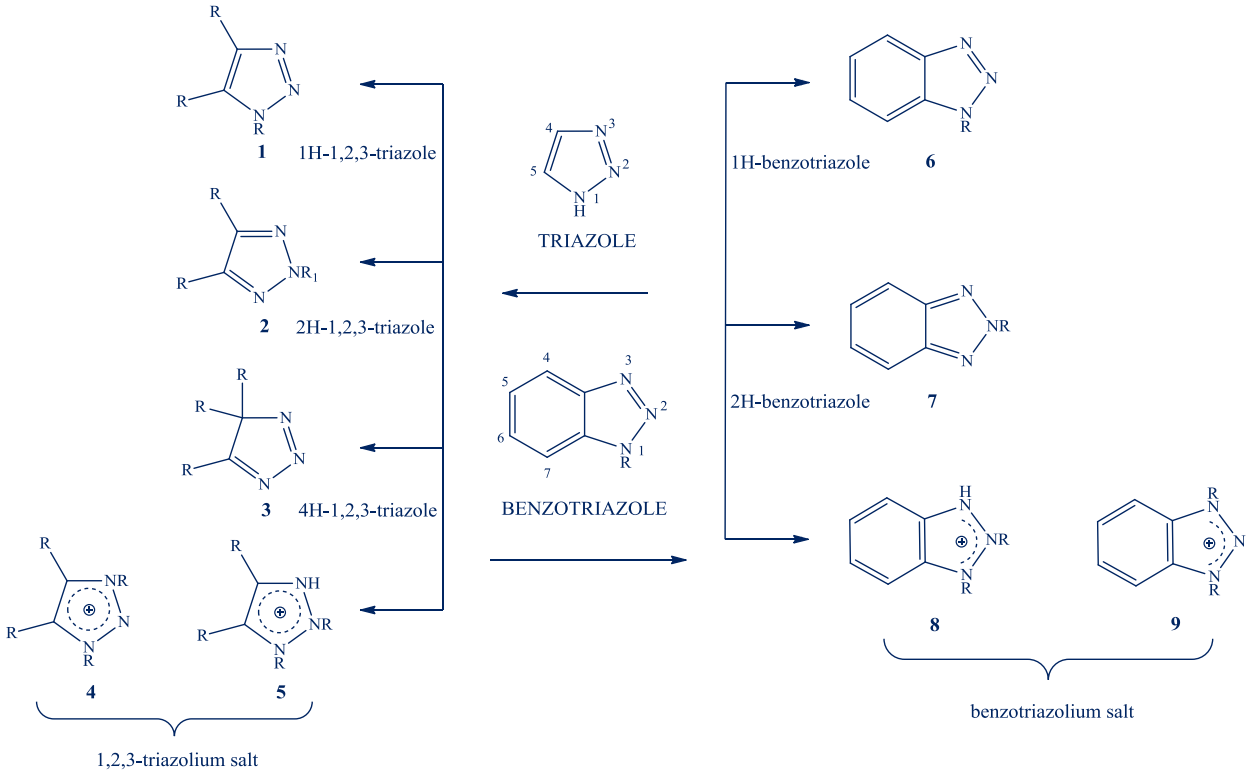


Figure 5.1 *Classification of 1,2,3-triazoles.*

Triazoles can also be used as a linker and show bioisosteric effects on peptide linkage, aromatic ring, double bonds and an imidazole ring. Some unique features like hydrogen bond formation, dipole-dipole and π stacking interactions of triazole compounds have increased their importance in the field of medicinal chemistry as they bind with the biological target with high affinity due to their improved solubility.

The 1,2,3-triazole based heterocycles have been well exploited for the generation of many medicinal scaffolds exhibiting anti-HIV, anticancer, antibacterial activities, etc.[156] Numerous compounds bearing this moiety are also well acknowledged for therapeutic effects elucidated in **Figure 5.2**.

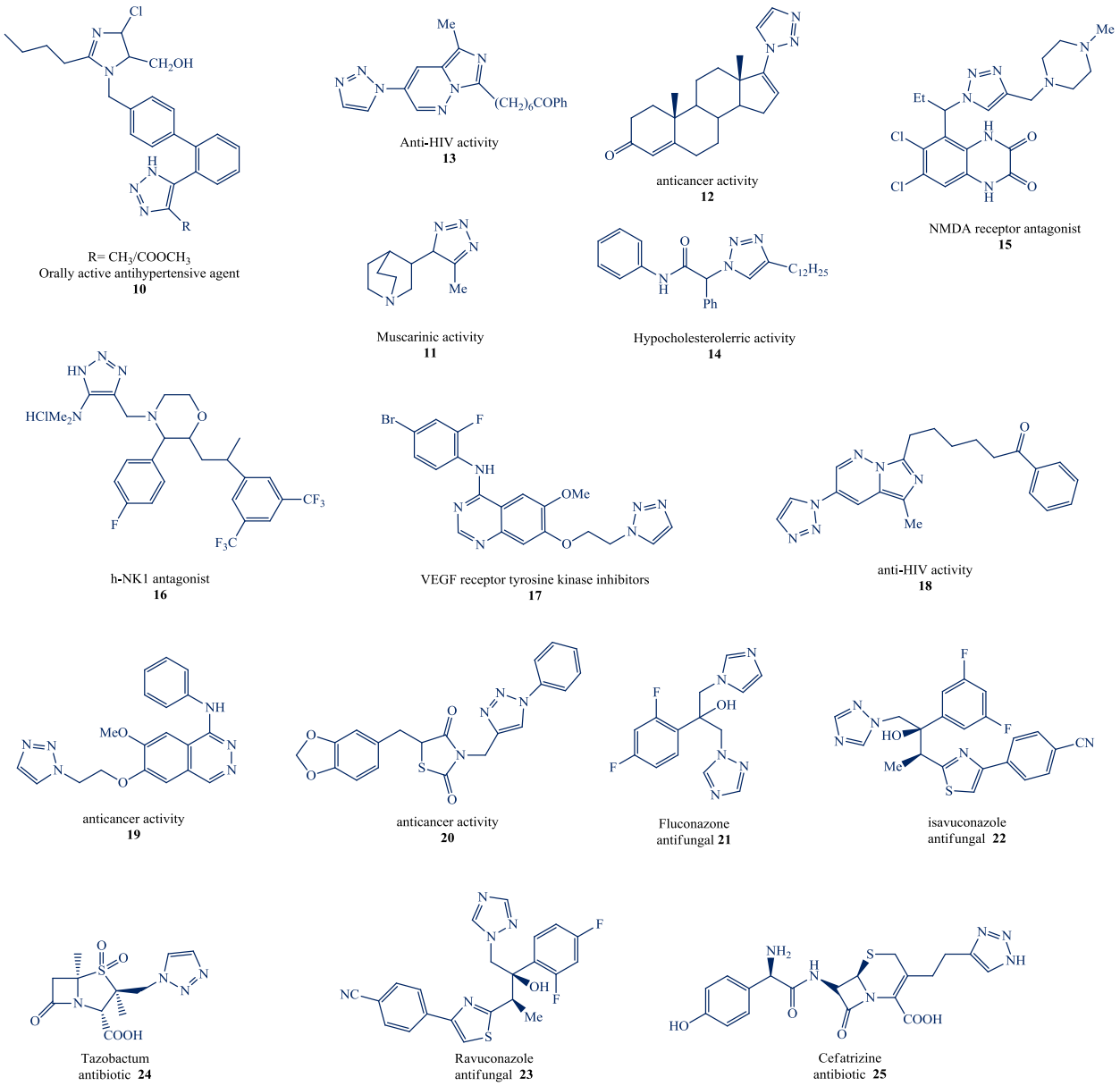


Figure 5.2 *Some examples of triazole containing commercial drugs and bioactive molecules.*

Divergent synthetic methodologies for 1,2,3-triazoles have been carried out rationally leading to the construction of novel molecules promoted in the field of organic synthesis, drug discovery efforts, polymer chemistry in addition to several other disciplines of material science.

Nevertheless, a well-directed database describing the azide-dipolarophile cycloaddition methodologies developed over the recent years has been reported a short while back. Consequently, these central structural motifs of biological interest when coupled to another heterocyclic ring have manifested therapeutic potential in different domains: antitumor, anti-

inflammatory, analgesic, anti-HIV, anticandidal (antifungal), antimicrobial, anticonvulsant, antioxidant, antitubercular, antiparasitic, antithrombotic, antidepressant and many other antiviral activities. 1,2,3-Triazole based derivatives have also been used as various enzyme inhibitors such as histone deacetylase, PDE4, alkaline phosphatase, cysteine protease and acetylcholinesterase and have witnessed tremendous progress in recent years. Apart from above applications, these motifs have also been applied in the preparation of glycoconjugates, active glycosidase inhibitors, and even protein and DNA modifications such as deoxynojirimycin based imino sugars and nucleoprotein inhibitors.

There are numerous amount of biologically active molecules containing 1,2,3-triazole motif and were synthesized well before the 'click chemistry' approach became popular. A few examples are shown in **Figure 5.2**. The 1,2,3-triazole moiety also serves as key synthetic intermediates in many industrial applications such as agrochemicals,[157] corrosion inhibitors,[158] additives,[159] supramolecular chemistry,[160] dendrimers, polymers,[161] liquid crystals,[162] photostabilizers,[163] pigments[164] and metal chelators.[165] Therefore, it is envisaged pertinent in the current scenario to update the medicinal chemistry of this privileged framework.

5.2 SYNTHETIC STRATEGIES

1,2,3-Triazole ring system has been a subject of intense research due to its versatile potential to interact with diverse biological systems. In recent years, many synthetic methodologies have been developed for the synthesis of this ring system. The most popular reaction to produce the 1,2,3-triazole moiety is the 1,3-Dipolar cycloaddition between an azide and a terminal alkyne, under thermal conditions. A copper (I)-catalyzed cycloaddition between azide and alkyne (CuAAC) is a click chemistry approach, invented by Sharpless, and consists in the production of a large number of 1,4-disubstituted 1,2,3-triazoles in very high yields.[166] Over the past few decades, the various synthetic methodologies has received a lot of attention and offered new opportunities for medicinal chemists. The various metal free and metal catalyzed approaches explored for the preparation of triazole framework are illustrated in **Figure 5.3 and Figure 5.4**.

5.2.1 Metal free synthesis of 1,2,3-triazoles

Metals are known to be associated with cellular toxicity, metabolic disruption and oxidative damage in biological systems.[167] A number of metal-free [3+2] cycloaddition approaches have been developed up to date with the aim to synthesize diversely functionalized 1,2,3-triazoles. As a consequence, significant research efforts have been directed towards the development of metal-free procedures to generate triazoles under mild conditions.[168] In the field of metal free approaches for the synthesis of 1,2,3-triazole, primary work has been carried out by Bertozzi's lab, where they showed strain-promoted azide-alkyne cycloaddition (SPAAC).[169] Besides the frequently used cyclo-octyne, various other dipolarophiles, such as enamines, enolates, and activated alkenes were demonstrated as a useful precursor for triazole formation. Amino acids such as proline have been utilized to catalyze the synthesis of 1,2,3-triazoles from activated carbonyl compound.[170] A similar protocol was adopted for the formation of benzo-annulated

triazole products.[171]

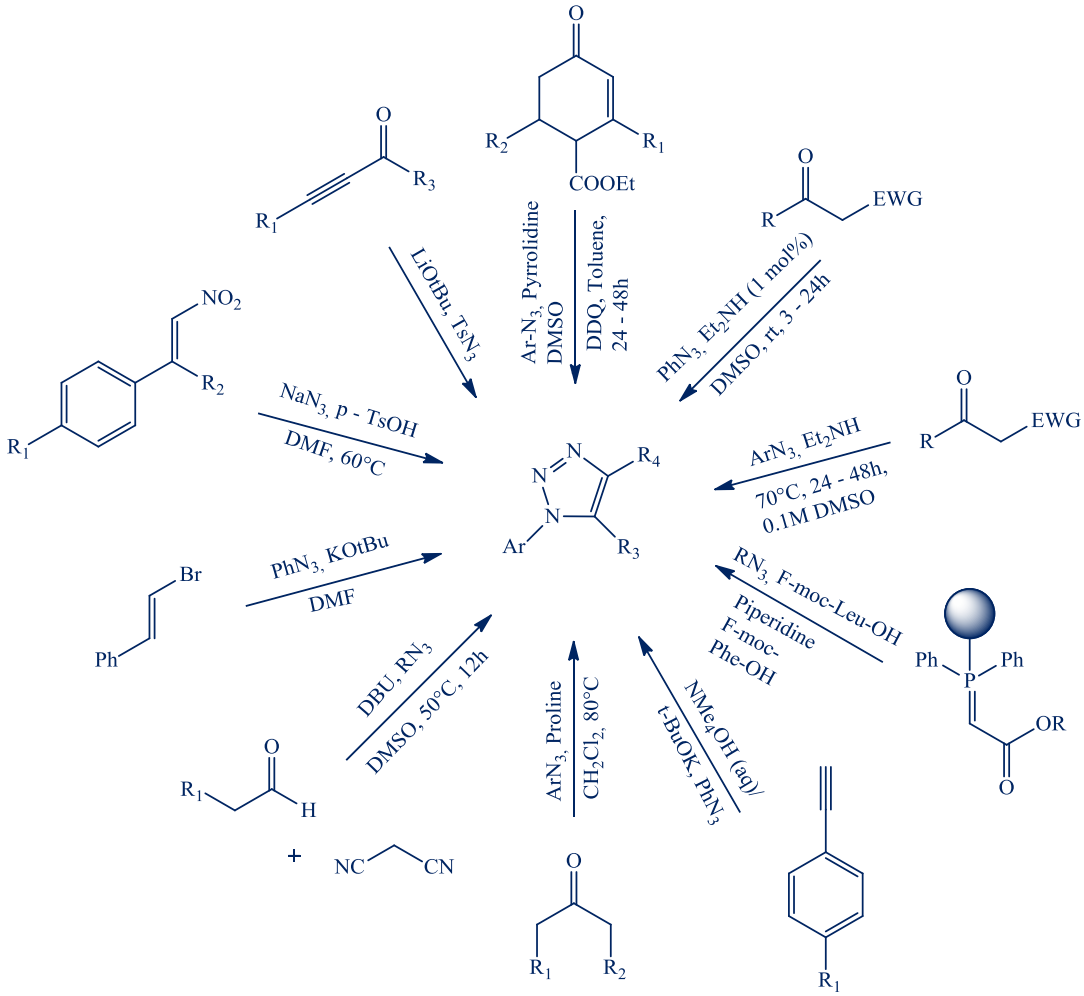


Figure 5.3 *A summary of various metal free approaches toward synthesis of 1,2,3-triazoles.*

In another synthetic methodology, alkynes were utilized to form in-situ reactive acetylide intermediates resulting in 1,5-disubstituted triazoles.[172] Interestingly, triazoles has been explored widely by Paixao group and reported an effective one-pot protocol for the highly regioselective metal-free synthesis of 1,4-disubstituted-1,2,3-triazoles. This method was found applicable to various alkylidene malononitriles and aromatic azides. It was further discovered that azides with α-chiral center could also be used.[173] Later an appealing method was discovered for obtaining selective triazole substituent patterns with the help of an appropriate azide. This organo-catalytic method was found to be highly regioselective due to the presence of substituents on the (*E*)-vinyl sulfone moiety that further initiated the results of the reaction.[174] In another intriguing methodology, a single step multicomponent type reaction was designed including sequential nitroalkene formation and the addition of organic azides to generate 1,4,5-trisubstituted triazoles.[175] Exploring the triazole concept, a highly regioselective library of 1,4-disubstituted triazoles were synthesized exclusively in 2012. Surprisingly, this methodology was found to be highly chemoselective and requiring no protection for carboxy-, hydroxyl- and other functional groups.

5.2.2 Metal-catalyzed synthesis of 1,2,3-triazoles

Since, Huigsen and co-workers developed method of azides to alkynes cycloaddition, it has gained much attention to yield a wide variety of triazoles with structural diversity[176] but still there exist some limitations such as requirement of high temperature for this transformation and formation of a mixture of the 1,4 and 1,5 regioisomers caused a problematic scenario. Therefore, it was always demanding to develop regiospecific approaches with mild conditions for triazole formation. Fortunately, Sharpless and Meldal in 2002 discovered Cu(I) catalyzed reaction (CuAAC) for regioselective synthesis of 1,4-isomer.[177] In this work, the Huisgen reaction worked well under mild conditions, giving the desired triazoles with high yield and good regioselectivity. Since then, the so-called field of ‘‘click chemistry” has been used extensively. Over the years different catalysts were discovered mainly ruthenium catalyzed 1,4-disubstituted 1,2,3-triazoles and ruthenium catalyzed a reaction for 1,5-regioisomer (RuAAC).[178] The unique features of CuAAC reactions display high regioselectivity, vast substrate scope, easy handling, excellent yield, insensitivity towards oxygen and water. A similar type of other representations in this field has been depicted in **Figure 5.4**. Various stabilizing ligands or additives have also been investigated in order to enhance the performance of the reaction and regioselectivity. In fact, cuprous oxide on charcoal was described firstly in 2013, which catalyzes the formation of 1,4-disubstituted 1,2,3-triazoles in excellent yields.[179]

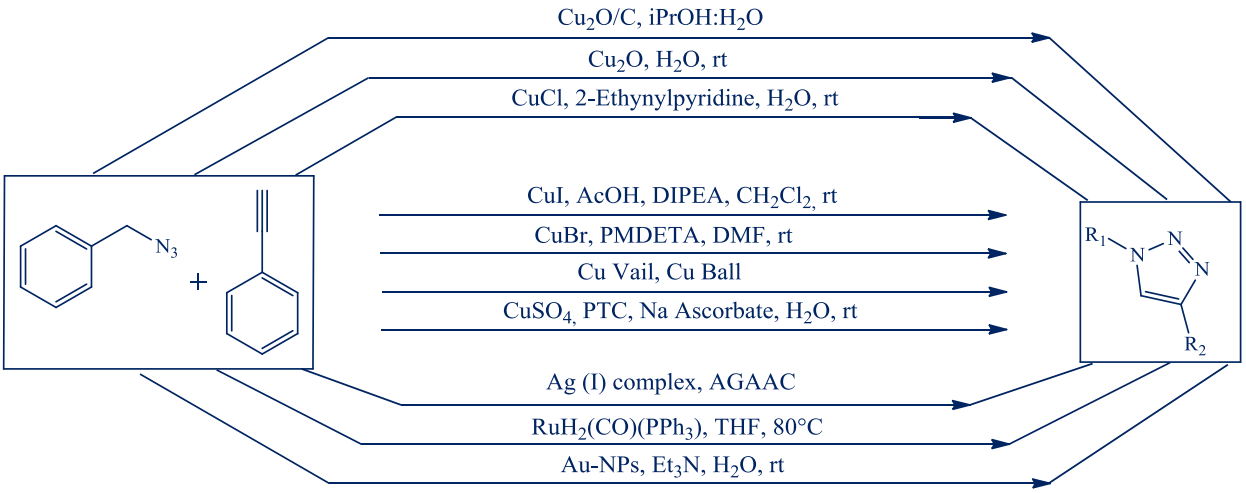


Figure 5.4 *A summary of transition metal catalyzed synthesis of 1,2,3-triazoles.*

On the same note, Wang et al. pioneered the CuAAC reaction in water.[180] Various stabilizing ligands or additives have also been used in CuAAC in order to increase the efficacy of reaction. Using 2-ethynylpyridine as an additive with copper chloride, Hiroki et al. synthesized the diverse triazole moieties.[181] Copper iodide was also explored in presence of N,N’-diisopropylethylamine and acetic acid in dichloromethane at room temperature.[182] Similarly, another metal combination of copper salt, copper bromide was employed in combination with PMDETA in dimethylformamide for cycloaddition reaction.[183] Cu(0) and Cu(II) precatalysts have been employed individually with an oxidizing agent while the latter with a reducing agent in the azide reactions.[184] Copper ball has also been utilized in Cu(0) form along with an oxidizing agent

which catalyzes the CuAAC reactions magnificently.[185] On the other hand, one-pot protocol by using copper sulfate and sodium ascorbate has been used by many groups in processing metalcatalyzed triazole formation.[186] Extending their scope, a combination of Cu(0), Cu(I), and Cu(II) has also been examined by various researchers in a catalytic form. Regarding this, Appukkuttan et al. encompass Cu(0) and CuSO4 in a mixture of water, trimethylamine and tert-butanol at 100°C and the protocol resulted into good yielded adduct.[187] Some of the ligands used in combination with copper salts are tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA), tris[(1-hydroxypropyl-1H-1,2,3-triazol-4-yl)methyl]amine (THPTA) and many more.[188] Other metal catalysts such as Ag, Ru and Au have also been analyzed for azide-alkyne cycloaddition reaction (Ag-AAC),[189] (Ru-AAC),[190] and (Au-AAC).[191]

5.3 BIOLOGICAL ACTIVITY

Tremendous demands for new chemical materials and biologically active molecules are constantly growing in the market. In the present scenario, better drugs in shorter times is always challenging for medicinal chemists. However, synthesis of new patentable molecules, combining high activity and selectivity, drug-likeness and good pharmacokinetic properties is equally fascinating.[192] 1,2,3-Triazole moieties are attractive prototypes, as they offer high stability even under strong oxidative and reductive environment and their propensity to form hydrogen bonding increases their solubility which favor binding towards biomolecular targets.[193] It has been revealed that triazole derivatives exhibit wide range of biological properties through different mechanism of action like enzymatic action or receptor-mediated mechanism.

5.3.1 Anti-cancer activity

1,2,3-Triazoles have been derivatized since long and have been explored mainly for their potential in anti-tumor chemotherapy. In **Figure 5.5** are reported a series of 1,2,3-triazoles at anti-cancer activity. Many groups have carried out focused research in this particular area. Odlo et al. discovered a series of cis-restricted 1,5-disubstitued 1,2,3-triazole analogs of combretastatin A-4 (CA-4). The triazole, 2-methoxy-5-(1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-5-yl)aniline **26**, inhibited tubulin polymerization with IC_{50} = 4,8 µM.
Recently, 3-(4-(4-phenoxyphenyl)-1H-1,2,3-triazol-1-yl)benzo[d]isoxazole (PTB) **27** was found to be one of the most potent antiproliferative agent with an IC_{50} of 2 µM against MV4-11 cells using MTT assay. Notably, PTB-induced cytotoxicity in MOLM13, MOLM14 and MV4-11 cells with selectivity over normal bone marrow cells (C57BL/6).
Similarly, 4-[phenyl-1-(1-phenyl-ethyl)]-1H-1,2,3-triazole motif **28** displayed good cytotoxic activity against HL 60 cells with IC_{50} values of 1,15 µM.[194] In another study, Philip et al. designed and synthesized 3-(4,5-substituted)-4H-1,2,4-triazol-3-ylthio)-Nisopropylpropan-1-amine derivatives for their *in vitro* cytotoxicity which was further analyzed by Trypan blue exclusion assay against EAC cells with IC_{50} values ranging from 0,55 to 0,59 µM. Carrying forward, DNA fragmentation study was enrolled to study the mechanism of cell death which revealed that triazole compounds **29** and **30** having lowest IC_{50} values induced DNA damage

through apoptosis which was probably the biochemical basis for excellent cytotoxicity of these compounds.[195] Two compounds namely, 3-[1N-(2-cyanophenyl)-1H-1,2,3-triazol-4yl]-methyloxy betulinic acid **31** and 3[1N-(5-hydroxy-naphth-1yl)-1H-1,2,3-triazol-4yl]-methyloxy betulinic acid **32** displayed impressive IC_{50} = 2,5 and 3,5 µM respectively against leukemia cell line HL-60, in short, showing 5–7-fold higher potency than betulinic acid.[196]

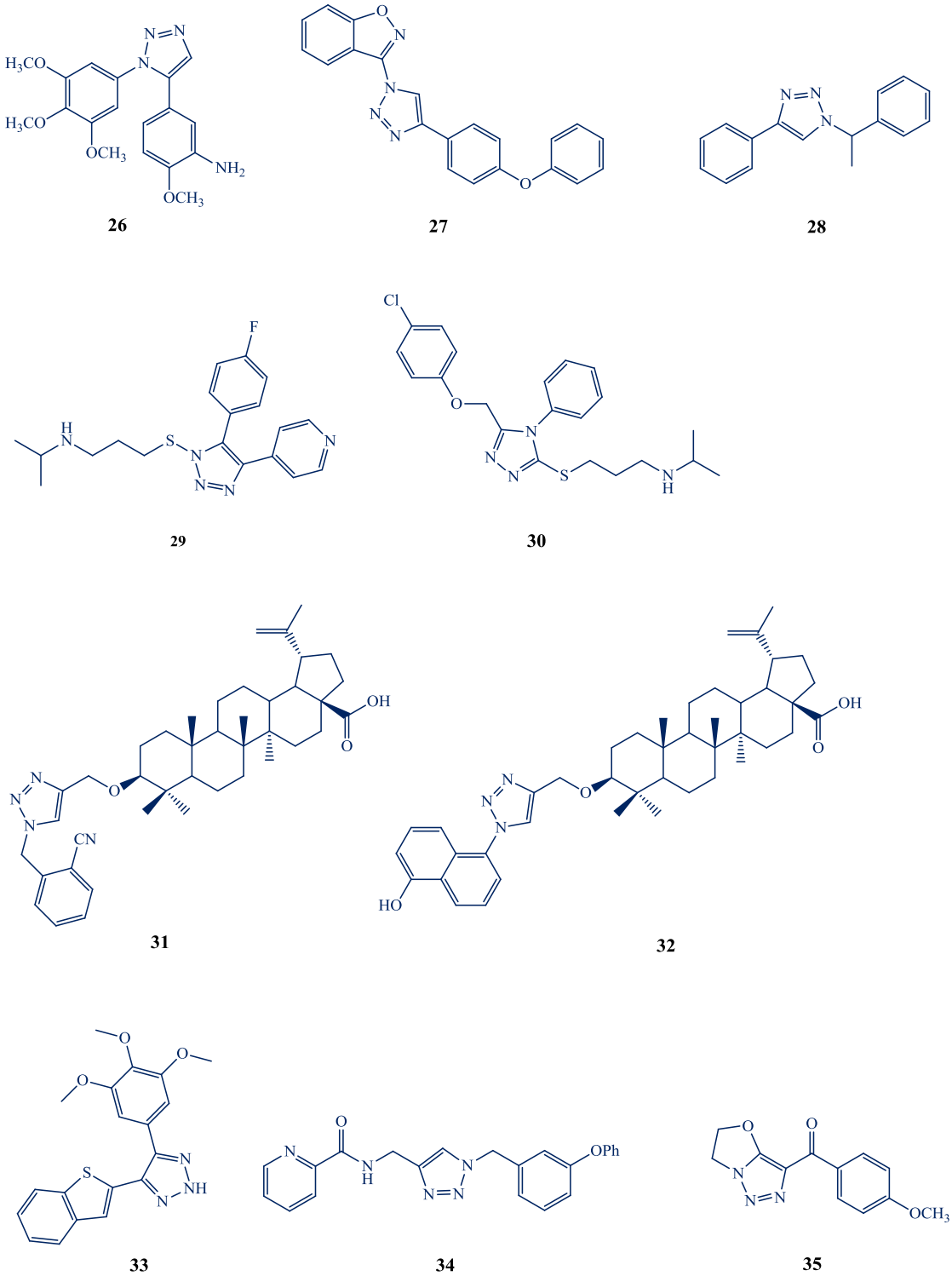


Figure 5.5 *Representative 1,2,3-triazoles as anti-cancer agets.*

All the compounds were tested in a panel of 60 human cancer cell lines and compound **33** was found to be the most potent against triple negative Hs578T breast cancer cell lines and was also the most effective inhibitor of tubulin polymerization.[197] In a similar but complementary approach, a series of N-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)acrylamide were synthesized with CuAAC, and **34** was the foundational compound for further study which induced M-phase arrest in HeLa cells at 5 μM concentration.[198] The compound, **35** was found to be the most potent derivative with IC_{50} values lower than 1,9 μg/ml against A431 and K562 human tumor cell lines.[199]

5.3.2 Anti-inflammatory activity

Inflammation is an extremely complex biological process, which includes arachidonic acid (AA) release from membrane-bound phospholipids, undergoing further biotransformation processes via cycloxygenase (COX) and 5-lipoxygenase (5-LOX) pathways. Many non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, indomethacin, and naproxen blocks AA metabolism by inhibiting COX.[200] However, the prolonged use of traditional NSAIDs cause serious side effects such as kidney failure, ulcer and extended bleeding time after injury. Therefore, exigency for new inflammatory drugs is in demand in the current scenario.[201] In **Figure 5.6** are reported a series of recent synthetized 1,2,3-triazoles at anti-inflammatory activity.

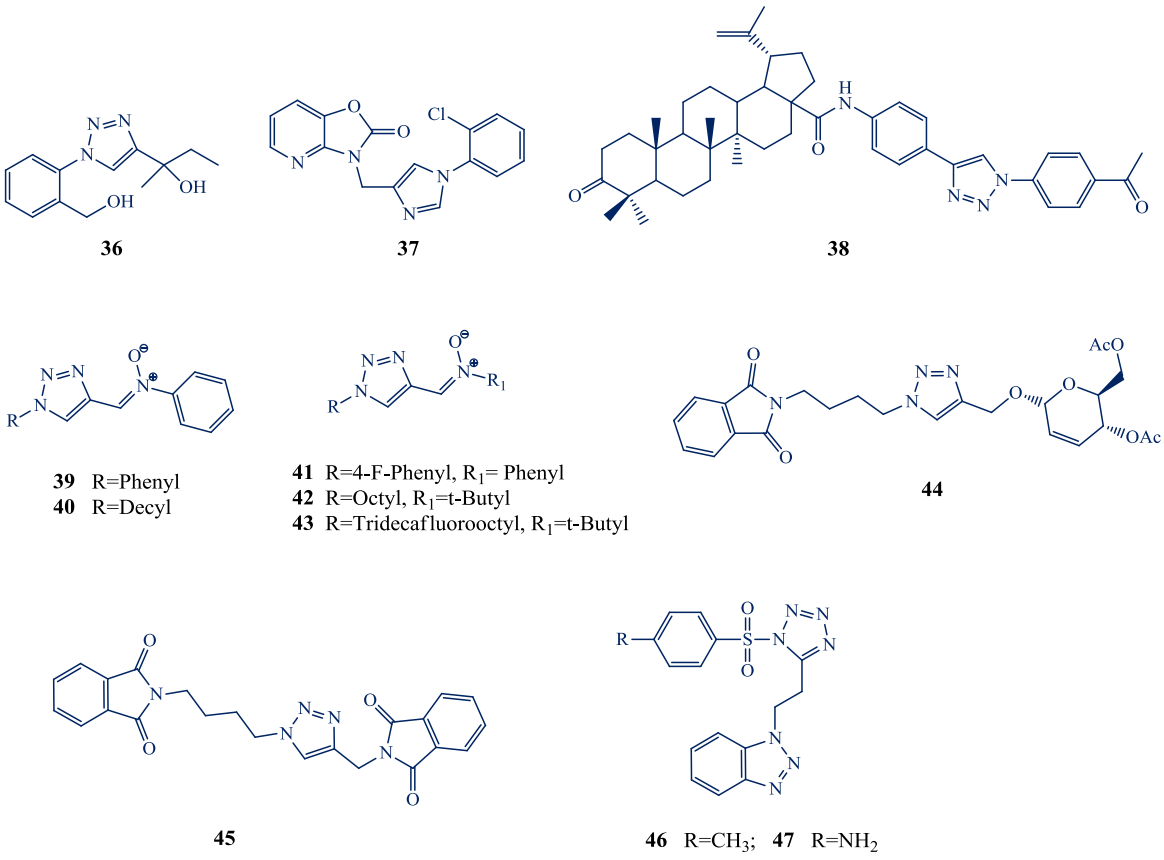


Figure 5.6 *Representative examples of 1,2,3-triazoles with anti-inflammatory activity.*

Kim et al. efficiently synthesized phosphonate and carboxylic acid analogs of phenyl-1H-1,2,3-triazole for anti-inflammatory activities and assessed their potential by using the xylene-induced ear edema model in mice. The studies revealed that addition of analog **36** enhanced TNFa-induced COX-2 expression (Western blotting) and proved to be the best on the molecular level, compared to the reference drug, diclofenac.[202]

Interestingly, analog **37** showed maximum inhibition with IC_{50} value of 0,19 µM. Most of the compounds exhibiting good GSK-3b inhibition were experimented for *in vivo* anti-inflammatory activity in rat paw edema model. **37** showed pronounced activity (76,36% inhibition) and was found to inhibit the inflammatory cytokines TNF-a, IL-1b, and IL-6 substantially in comparison to indomethacin, the standard anti-inflammatory drug and also a GSK-3b inhibitor, SB216763.[203]

It was observed that betulonic conjugate **38** caused a significant anti-inflammatory effect when introduced at the highest dose compared to indomethacin. According to SAR predictions, the probability of the anti-inflammatory effect in triazoles increased upon changing of hexyl substituent at the triazole moiety into a benzyl and to a larger extent, acetyl phenyl and methoxyphenyl substituent.[204] Similarly, new 1,2,3-triazole substituted *N*-phenyl nitrone derivatives and 1,2,3-triazole substituted *N*-alkyl nitrone derivatives were prepared rationally and screened for anti-inflammatory and anticancer activity against various cancer cell lines. Molecules **39–43** revealed significant inhibition of IL-1b secretion, a measure of anti-inflammatory activity.[205] New 1,2,3-triazole phthalimide derivatives were synthesized by 1,3-dipolar cycloaddition reaction from N-(azido-alkyl) phthalimides and terminal alkynes and in continuation, the anti-inflammatory activity was determined by injecting carrageenan through the plantar tissue of the right hind paw of Swiss mice to produce inflammation. The best activity was found for the compounds **44** and **45** which decreased 69% and 56,2% carrageenan-induced edema in mice.[206]

Widening the scope of triazole moieties, eight new leads comprising of 1-[2-(1H-tetrazol-5-yl)ethyl]-1H-benzo[d][1,2,3]triazoles were proposed for potent anti-nociceptive and anti-inflammatory activities. The title compounds were evaluated for anti-inflammatory activity using carrageenan induced paw edema method in which **46** and **47** elicited superior bioactivity compared to reference drug.[207] Results from carrageenan-induced hind paw edema showed that compounds possessed significant anti-inflammatory activity as compared to the standard drug ibuprofen.[208]

5.3.3 Anti-tubercular activity

There has been a recent substantial increase in incidences of tuberculosis especially, through drug-resistant Mycobacterium tuberculosis. The anti-tubercular drugs namely, isoniazid, pyrazinamide, ethambutol, rifampicin, and streptomycin are very often insufficient.[209] In fact, tuberculosis in HIV-infected patients has been a great matter of concern which initiates the urgent need for new active agents.[210] In search of such new active molecules against M. tuberculosis (MTB) H37Ra and M. bovis BCG, a small library of benzothiazinone based 1,2,3-triazoles were efficiently developed via click chemistry method by Shaikh et al. and his coworkers. In **Figure 5.7** are reported a series of recent synthetized 1,2,3-triazoles at anti-inflammatory activity.

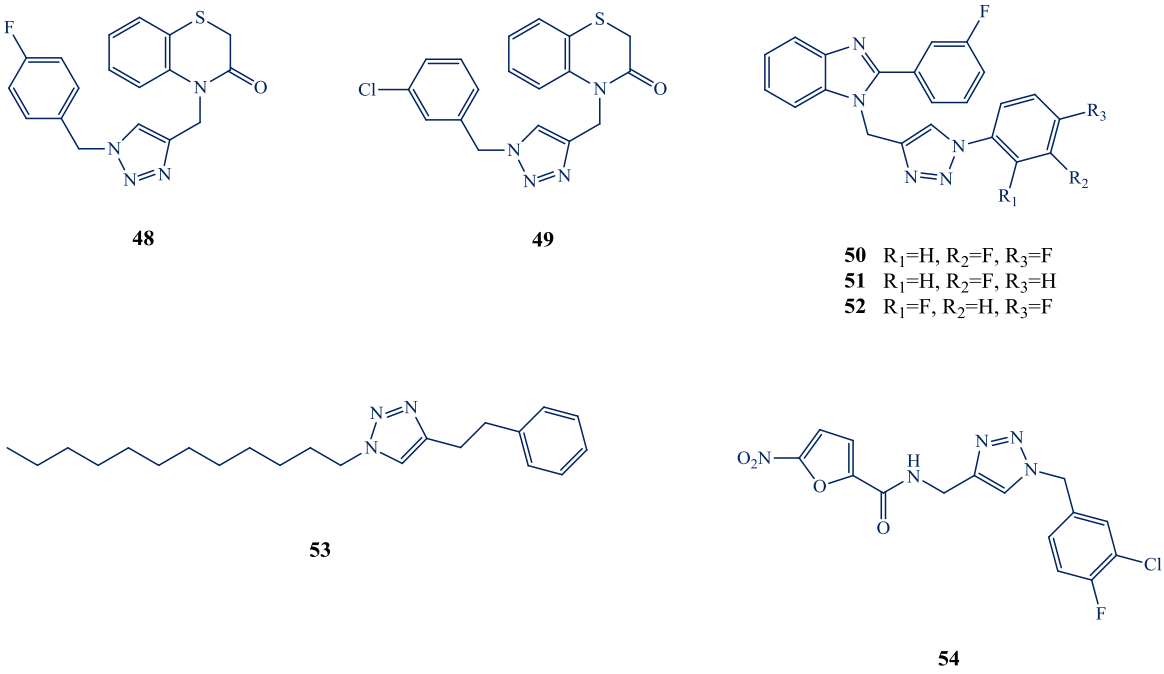


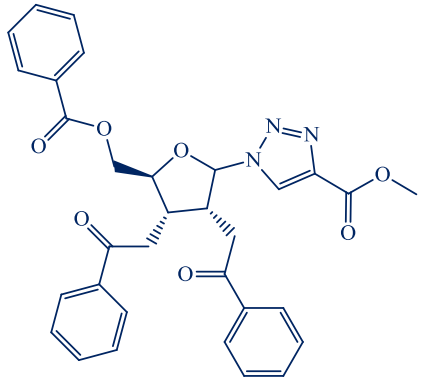
Figure 5.7 *Representative examples of 1,2,3-triazole derivatives with anti-tubercular activity.*

Compounds **48** and **49** were found to be most active against MTB and M. bovis BCG characterized by lower MIC values (27, 34–29,37 µg/ml). Lately, a novel approach was clubbed using 1,2,3-triazoles with fluorine benzimidazole series of H37Rv strain inhibitors. These derivatives were potentially useful for the treatment of tuberculosis and mainly **50–52** showed better activity compared to standard rifampicin. Compound **53**, namely 1-dodecyl-4-phenethyl-1H-1,2,3-triazole displayed a minimum inhibitory concentration inferior to 2 µg/ml against M. tuberculosis H37Rv.[211] In a similar study, different 20 quinoline derivatives were synthesized possessing triazolo, ureido and thioureido substituents for antimycobacterial activity. Among all the screened derivatives, **54** showed promising bioactivity against tuberculosis with MIC value of 0.25 µg/mL.[212]

5.3.4 Antiviral activity

Viral diseases are capable of infecting practically all organs and systems of a host organism, causing latent, acute, and chronic forms of infection.[213] Vaccination is a reliable tool to fight viruses, but it is only available against few viruses. The exigency of antiviral agents is still inevitable.[214] In fact, more than half of the clinical drugs for treating viral diseases are nucleoside analogs.[215] Among triazole based antiviral agents, ribavirin is a broad class of compound with reported activities against herpes simplex virus (HSV), human immunodeficiency virus (HIV-1), influenza virus, respiratory syncytial virus, and hepatitis C virus, among other viruses. But most tragically, routine clinical use of ribavirin is limited because this compound is considerably cytotoxic.

Compound **55** (**Figure 5.8**) was found to be superior to ribavirin in terms of antiviral activity against influenza A replication and HIV-1 RT (reverse transcriptase) activity.



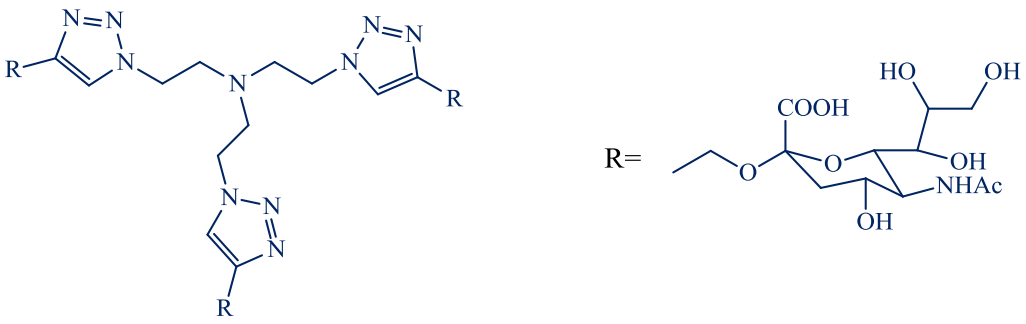
55

Figure 5.8 *An example of 1,2,3-triazole based nucleoside analog as anti-viral agent.*

Compound **55** (R = CO_2CH_3) was the most effective analog, with IC_{50} values 14 and 3,8 µM for influenza A and HIV-1 RT, respectively.[203]

Bioactivity against cytomegalovirus (CMV) and varicella-zoster virus (VZV) in human embryonic lung cell cultures were evaluated, however, all compounds showed low antiviral activity.[216] In fact, the investigation of drug-DNA interaction is important for understanding the molecular mechanism of drug action and especially for the design of specific DNA-targeted drugs and anti-viral agents are one of them. Therefore, sugar-[1,2,3-triazole] based uracil appended sugar-imine derivatives were designed and synthesized and their application in DNA binding studies were highlighted.

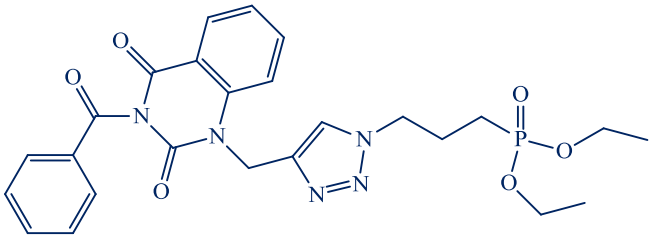
Adenovirus type 37 (Ad37) is one of the principal agents responsible for epidemic kerato-conjuctivitis (EKC), a severe ocular infection that persists without any available treatment. In an extraordinary achievement, new trivalent sialic acid derivatives were designed, synthesized and their inhibitory properties against Ad37 infection of the human corneal epitheal cells were investigated. The best compound **56** (**Figure 5.9**) was found to be over three orders of magnitude more potent in a cell-attachment assay with IC_{50} = 1,4 nM and about 140 times more potent in a cell-infection assay with IC_{50} = 2,9 nM.[217] An efficient synthesis of acyclonucleotide analogs was reported with a 1,2,3-triazole linker by Glowacka et al. Several O,O-diethylphosphonate acyclonucleotides were transformed into their respective phosphonic acids.



56

Figure 5.9 *Representative 1,2,3-triazole with antiviral activity.*

Acyclonucleotide **57** (**Figure 5.10**) containing a three carbon linker between the phosphorous atom and the 1,2,3-triazole ring substituted at the C-4 position with 3-*N*-benzoyl-1-*N*-methylquinazoline-2,4-dione exhibited a moderate activity against both herpes simplex viruses (HSV-1, HSV-2) in HEL cell cultures with EC_{50} = 17 µM and feline herpes virus with EC_{50} = 24 µM in CRFK cell cultures.[218]



57

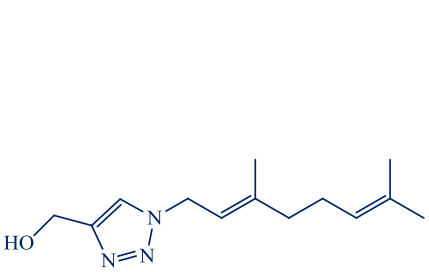
Figure 5.10 *Another representative 1,2,3-triazole with antiviral activity.*

5.3.5 Antibacterial activity

1,2,3-triazole framework has already been reported for antibacterial properties. Bacterial β–lactamases **21** and **22** are the examples of 1,2,3-triazole based drugs shown in **Figure 5.2**.

Derivatives of 6-bromobenzo[d]thiazol-2(3H)-one containing 1,2,3-triazole evaluated for antibacterial activity, results showed that majority of compounds have good to moderate activity when compared with positive control drug streptomycin. Also in vitro, cytotoxic studies were carried out on various cell lines and results were remarkable when compared with standard drug cisplatin.[219]

The antibacterial activity of the 1,2,3-triazole compounds using geraniol as the precursor was assessed by the micro-broth dilution technique against a panel of Gram-positive Staphylococcus aureus, Bacillus cereus and Gram-negative E. coli and Pseudomonas aeruginosa. Compound **58** (**Figure 5.11**) was tested on E. Coli FabH and showed an mayor antibacterial activity respect to standard ciprofloxacin.[220]



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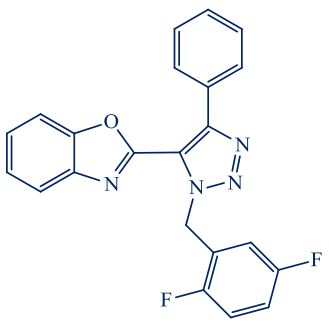

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Figure 5.11 *Representative 1,2,3-triazoles with antibacterial activity.*

Regioselective multicomponent approach for the construction of benzoxazole-linked triazoles was initiated by Srivastava et al. for further synthesizing benzoxazole-triazole scaffolds subjected to in vitro antibacterial and anticancer evaluation. While most of the compounds showed good Gram-negative activity, compound **59** (**Figure 5.11**) has been identified to have potent cytotoxic effects and was comparable to control daunomycin.[221]

CHAPTER 6

CHAPTER 6 – ASYMMETRIC SYNTHESIS: THERMAL CYCLOADDITION MEDIATED BY IMINIUM ION CATALYST

6.1 GREEN CHEMISTRY

Chemistry, and all its fields of application, has become an integral part of everyday life and has brought enormous benefits to modern society. Chemicals are indispensable for most of the anthropological activities, as they are used daily in many activities such as house cleaning and even in foods such as preservatives and additives. Some of the chemicals that are used are not biodegradable and tend to accumulate in the environment; others, being very soluble in lipophilic media, such as the adipose tissue of animals, tend to accumulate in the human body; others may be responsible for various pathologies affecting the population, such as, for example, allergies, asthma, reproductive system dysfunction and, in the most serious cases, cancer or death.
In recent decades, we are trying to minimize or, even better, eliminate the danger associated with polluting organic substances in the environment. In this context, "Green Chemistry" (also known as green chemistry or eco-compatible chemical) plays a key role. Green Chemistry uses "clean chemical processes" that are based on the reduction or elimination of hazardous substances, including the consumption of solvents, thus reducing the negative impact on human health and the environment. Green Chemistry therefore forces the replacement of old technologies with new, cleaner, and eco-compatible processes.
The basic principles on which Green Chemistry is based are:

- Preventing pollution more than reducing it after its production;
- The synthesis methods should be designed to maximize the incorporation of all the atoms, used in the process, within the final product;
- The synthesis of chemicals should be designed, where possible, to use and produce substances that have a low or no toxicity to human health or the environment;
- Chemicals should be synthesized so as to maintain their desired function and effectiveness by reducing toxicity;
- The use of solvents and auxiliary products must be minimized;
- The energy consumption of chemical processes must be reduced for economic and environmental reasons. If possible, the synthesis should be carried out at room temperature and pressure;
- Whenever technically and economically feasible, raw materials and natural resources must come from renewable sources;
- Unnecessary derivations need to be eliminated or minimized because these steps require additional reagents and generate by-products and residues;
- Catalytic reagents are preferable to stoichiometric reagents;
- Chemicals must be designed so that at the end of their useful life they are not persistent and their decomposition products are harmless;

- Analytical methods must be developed to allow real-time monitoring and monitoring of processes before the formation of unwanted substances;
- The substances and the form in which they are used in chemical processes must be chosen in such a way as to minimize the risk of chemical accidents.

Green Chemistry provides the perfect synergy between the need to adopt eco-friendly technologies and market needs. For this reason, some activities such as Sustainable Chemistry (Paris, February 1998) promote the development of eco-compatible processes and products, identifying the areas of Green Chemistry[222] development at the following points:

- Harmless reagents use;
- Alternative solvents use;
- Natural processes use;
- Alternative storage materials use;
- Chemical Safety Programming;
- Development of alternative reaction conditions;
- Minimizing energy uses.

However, for have a lesser waste of chemical reagents, one of the best solutions is the replacement of inorganic reagents, used in stoichiometric quantities in the classical organic synthesis, with reagents in catalytic quantities. In addition, chemical reactions become more and more efficient if they exhibit greater selectivity of the reaction product and provide excellent yields. There is a high number of chemical reactions that proceed at extremely low rate. It is therefore necessary to find solutions to increase such speeds so that the greatest benefit from these reactions can be gained, especially at the industrial level. From this point of view, the concept of catalysis plays a very important role. A catalyst is a chemical species or any substance that increases the rate of a chemical reaction without undergoing transformation and without being present in stoichiometric quantities. The catalysts allow for new pathways and therefore offer reagents and products the ability to interconnect according to a new path, characterized by a lower Energy Efficacy (E_{cat}) than the original (**Figure 6.1**).

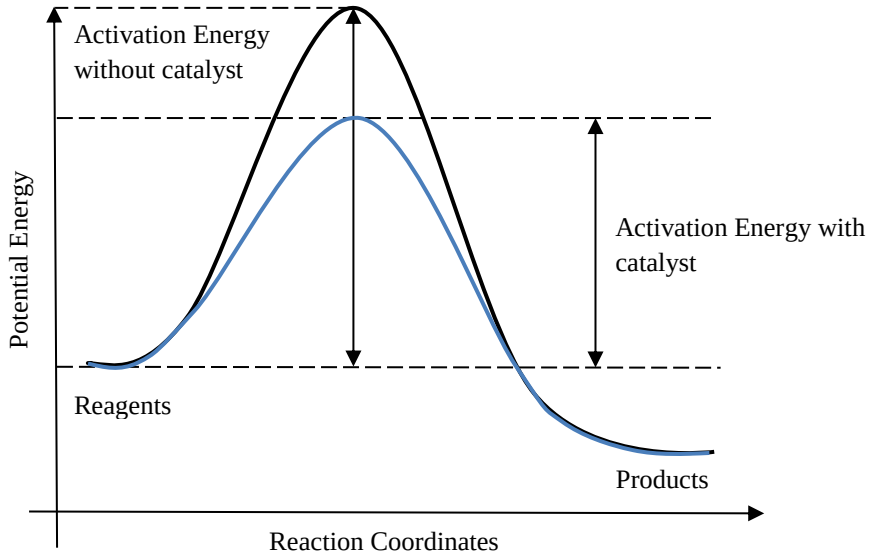


Figure 6.1 *Chemical reaction andament without catalyst use(black curve) and with catalyst use (blue curve).*

From **Figure 6.1** it is noted that the non-catalyzed and catalyzed processes occur in a single stadium. In reality, the catalyzed reactions proceed through the succession of multiple stages, all of which have lower activation energy than the stage determining the reaction rate of the uncatalyzed process. Another caracteristic of the catalyzed processes is the increased reaction selectivity. It should also be remembered that the use of catalysts allows the development of many reactions at moderate temperatures and in stiffer conditions. Thanks to all these features, catalysis has become one of the foundamental bases for the use and development of Green Chemistry. Recently, in the organic synthesis new catalytic methods have been introduced such as Lewis' s acid catalysis, which has low environmental impact, and, as regards asymmetric synthesis, the use of organic catalysts is identified with organocatalysis that use organic molecules as catalysts.

6.2 ORGANOCATALYSIS: GENERAL CONCEPTS

Asymmetric catalysis, for some decades, has played a key role both in academic ambit and industry, so as to become an indispensable tool for modern organic chemistry. The extensive use of catalysis is justified by some factors such as eco-sustainability, economicity and efficiency of the process. In addition, the growing demand in the world market for enantiomerically pure pharmaceuticals, agriculture and natural products makes enantioselective catalytic processes very interesting. Before the diffusion of asymmetric catalysts, two general categories of catalysts were used to make the stereoselective synthesis of organic molecules: coordination compounds of transition metals and enzymes. Recently, however, as has already been mentioned, the use of organocatalysts has been enormously developed. Organocatalysis is a type of catalysis that uses small organic molecules to increase the speed of some chemical processes. Before 1998, asymmetric catalysis was based on the almost exclusive use of some transition metal chiral complexes, allowing for a large number of reductions, oxidations, insertions on sigma bonds, activation of π bonds, and Lewis acid-catalyzed reactions. However, their use had drawbacks regarding the toxicity of the metals used, their high cost and their sensitivity to air and humidity.
Organocatalysis is another innovative way to achieve asymmetric synthesis. The main advantages of organocatalysts derive from three important factors:

- Organic molecules are generally inert to the air and humidity of the atmosphere, so it is not necessary to use dry solvents and anhydrous reagents;
- Many organic reagents, such as amino acids, carbohydrates and hydroxylyc acids, are available from natural sources as individual enantiomers;
- In according to some researchers in the industrial field, the use of organocatalysts can be limited due to the low number of "turnover".[f]

It should be remembered, however, that in any catalytic process the main considerations concern costs and safety. Being the cheaper organocatalysts of metal catalysts, at the same price, they can be used in greater quantities than metal-containing catalysts. Moreover, it is well known that in industrial production processes the disposal of toxic catalysts, present as impurities in discharges, has a greater economic impact respect to the number of catalyst turnover. [223]

[f] "Turnover" is defined as the number of times a particular chemical substances is used.

Organocatalysis is subdivided into "covalent catalysis" and "non-covalent catalysis". Covalent catalysis is involved in those processes leading to the formation of covalent adducts between the catalyst and the substrates during the catalytic cycle, while non-covalent catalytic processes are a hydrogen bonds or ionic pairing . The formation of the substrate-catalyst adducts can be via Lewis acid and Lewis bases interactions in a single step, or, through multi-step reactions, such as the formation of enamines from the reaction between aldehydes and secondary amines (**Figure 6.2**). [224]

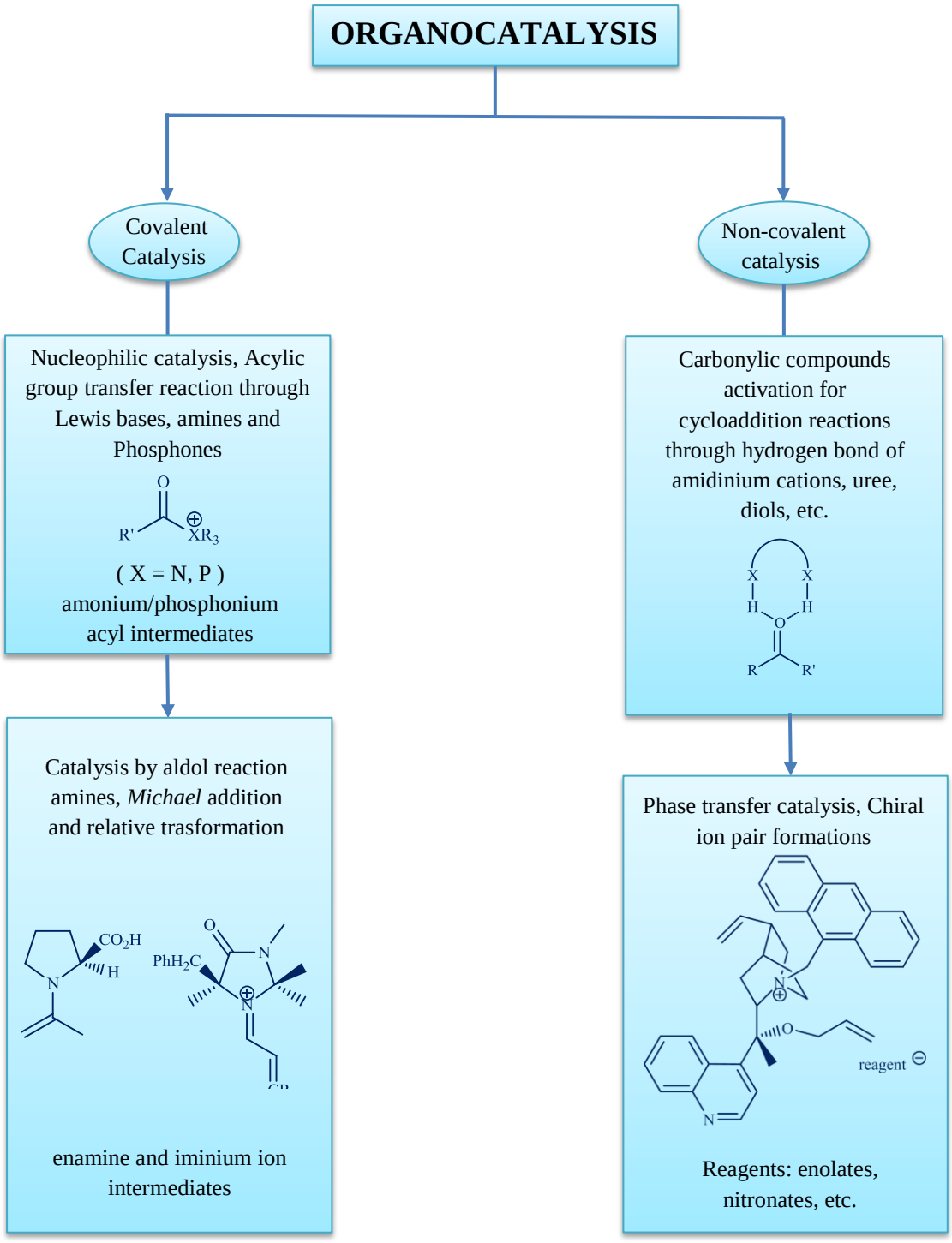


Figure 6.2 *Summary scheme, exemplary, of organocatalysis suddivision.*

There are four major classes of organic catalysts:

- Lewis Bases;
- Lewis Acids;
- Brønsted Bases;
- Brønsted Acids.

These catalysts perform their catalytic function by providing or removing electrons or protons from a substrate or transition state. In organocatalysis the catalysts most commonly used are Lewis bases, such as, for example, amines and carbenes, while Lewis acids, such as carbonyl compounds, are rarely used.[225]

A particular type of organocatalysis is the iminium cationic ion catalysis, which is used as Lewis base catalysts.

6.3 IMINIUM ION CATALYSIS

The condensation of aldehydes or ketones with primary amines is a reversible reaction that leads to the formation of immines (**Figure 6.3**).

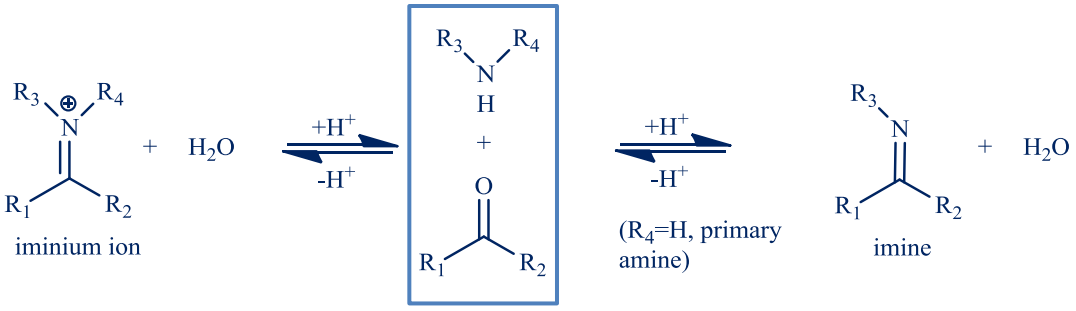


Figure 6.3 *Iminium ion and enamine formation.*

As can be seen from the scheme in **Figure 6.3**, the synthesis of the immines, performed for the first time by Schiff in 1984, is carried out using as primary amine reagents and aldehydes or ketones. Imine are basic substances (pKa ≈ 7) and, for this reason, are defined Schiff bases. These imines, in acidic environment, exist in the iminium ions form. It is also noted that secondary amines can condense with aldehydes and ketones directly forming iminium ions. In this case, deprotonation cannot occur and, consequently, iminium cation can only be isolated as salt of a strong acid. In the catalysis mediated by the iminium ion, both primary amines and secondary amines can be used, although these are the most widely used ones. Iminium ion salts are much more electrophilic than the corresponding aldehydes or ketones, so the reversible formation of this ion activates the carbonyl component toward the nucleophilic attack. This type of activation is similar to activation induced by Lewis or Brønsted acids but, it is much more general and may be different depending on the nucleophilic-electrophilic interaction type. Structural studies on iminium systems have shown a greater reactivity of these compounds than the corresponding non-activated systems.[226] Examples of possible imminent activation modes are given in **Figure 6.4**.

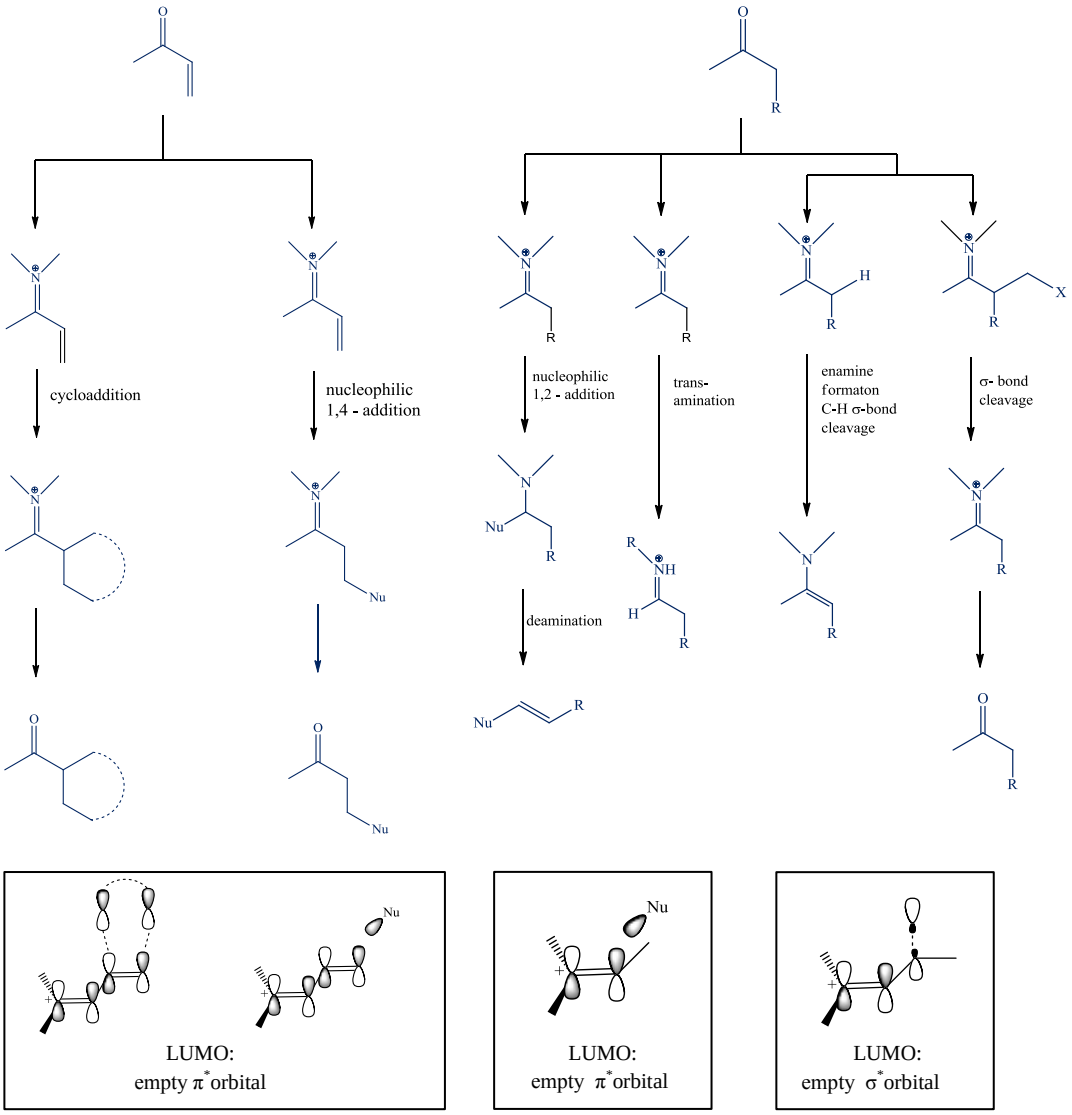


Figure 6.4 *Possible ways of activating iminium ion.*

It must be remembered, however, that the reactions activated by the imine ion are only catalytic if the amine (catalyst) is released in the final hydrolysis or in the elimination step. For example, the nucleophilic hydride addition of a C=N double bond ion involves the formation of an iminium intermediate and, therefore, is called the iminium-activated reaction but it cannot be defined as imminio-catalyzed reaction since the amine is not eliminated.

6.4 DEVELOPMENT OF IMINIUM ION CATALYSIS

It is impossible to attribute a precise date to the introduction of catalysis by iminium ion formation.

The first example of iminium ions catalyzed reaction is the Knoevenagel's condensation mediated by primary or secondary amines. Knoevenagel's reactions can also be catalyzed by

tertiary amines, so iminium ion formation is only one of the possible mechanisms.[227]
The first reaction that has postulated the iminium formation as intermediate is the decarboxylation reaction of β-chetocarboxylic acids described by Pollak in 1907 (**Figure 6.5**).[228]

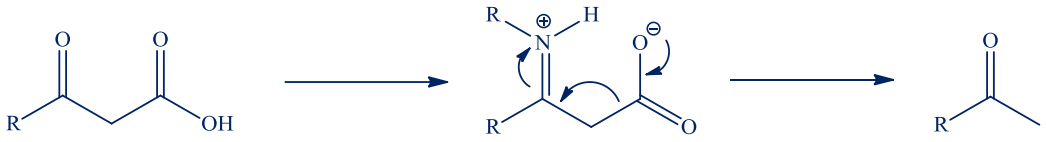


Figure 6.5 *Generic decarboxylation reaction of the β-chetocarboxylic acids.*

In 1934, Pedersen demonstrated that the β-chetoacid decarboxylation, catalyzed by amines, occurs through a mechanism involving iminium ion.[229]
The first conjugated addition catalyzed by iminium ion was carried out by Langenbeck and other collaborators in 1937 (**Figure 6.6**).[230]

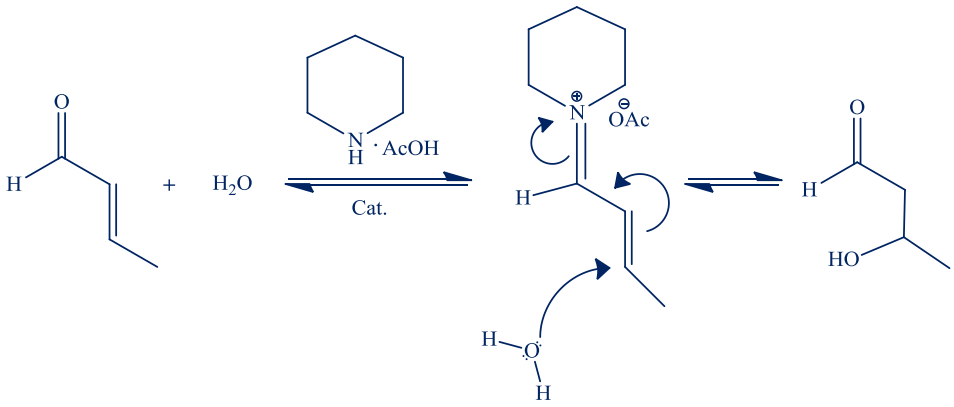


Figure 6.6 *Conjugated addition catalyzed by iminium ion.*

In 1976 Baum and Viehe have demonstrated that iminium ion salts accelerate the Diels-Alder reactions (**Figure 6.7**).[231]

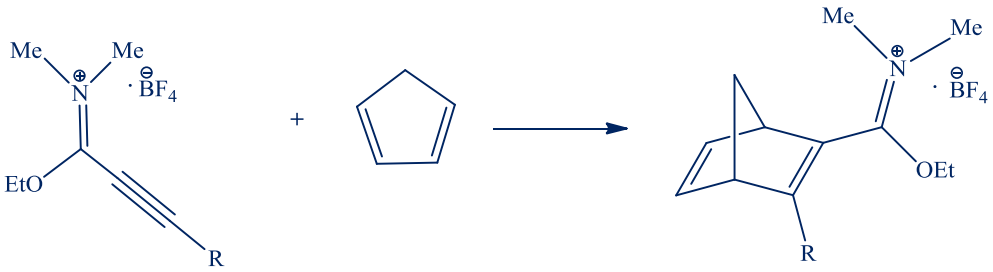


Figure 6.7 *Iminium ion catalized Diels-Alder Reaction.*

Woodward et al., in 1981, used Proline aminoacid as an asymmetric catalyst in erythromycin synthesis (**Figure 6.8**).

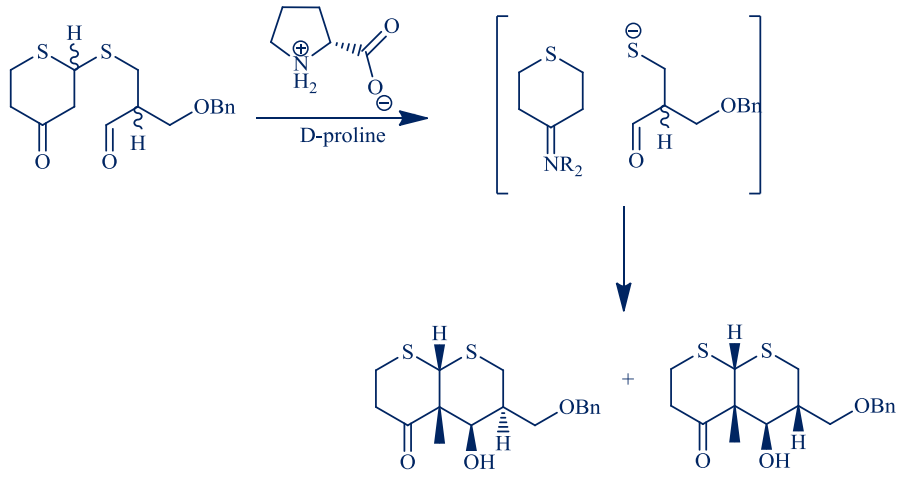


Figure 6.8 *Stadium where (D)-Proline aminoacid is used in Erythromycin synthesis*

The first asymmetric catalysis activated by iminium ion in the conjugated addition reactions was discovered by Yamaguchi and collaborators (**Figure 6.9**).[232]

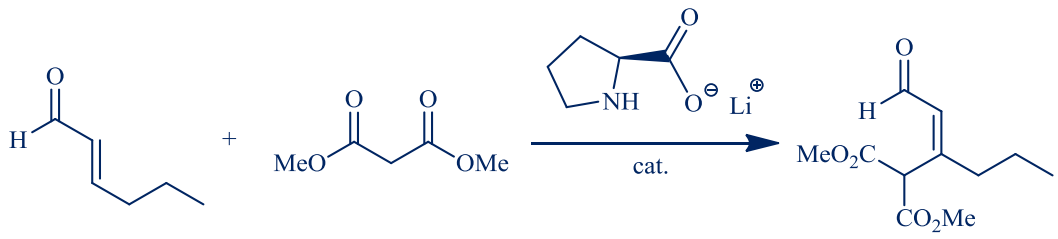


Figure 6.9 *The first asymmetric catalysis activated by iminium ion in the conjugated addition reactions.*

In 2000, MacMillan and collaborators have made the first enantioselective synthesis by iminium ion formation in Diels-Alder reactions (**Figure 6.10**).[233]

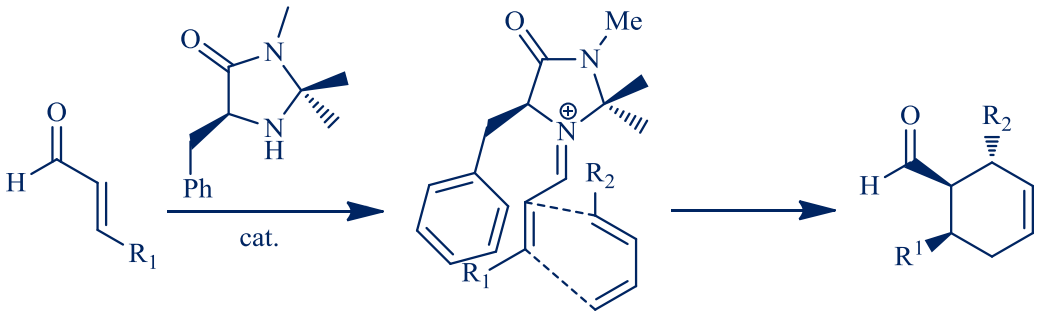


Figure 6.10 *Enantioselective Diels-Alder Reaction catalyzed by iminium ion.*

MacMillan was the first to write iminium ion catalysis using the term "LUMO-lowering catalysis" for describe the common catalytic strategy of Lewis acids and amines as catalysts.

6.5 CATALYSTS

Many secondary amines (**Figure 6.11**) are used as catalysts, as they lead to imminent ion formation. Between all the reactions, these that most commonly use these type of catalysis are the cycloaddition reactions and the condensation reactions.
Secondary amines most commonly used are:

- Proline and its derivatives;
- Imidazolidinones;
- Substituted Pyrrolidine;
- Diarylpropynol silyl ether.

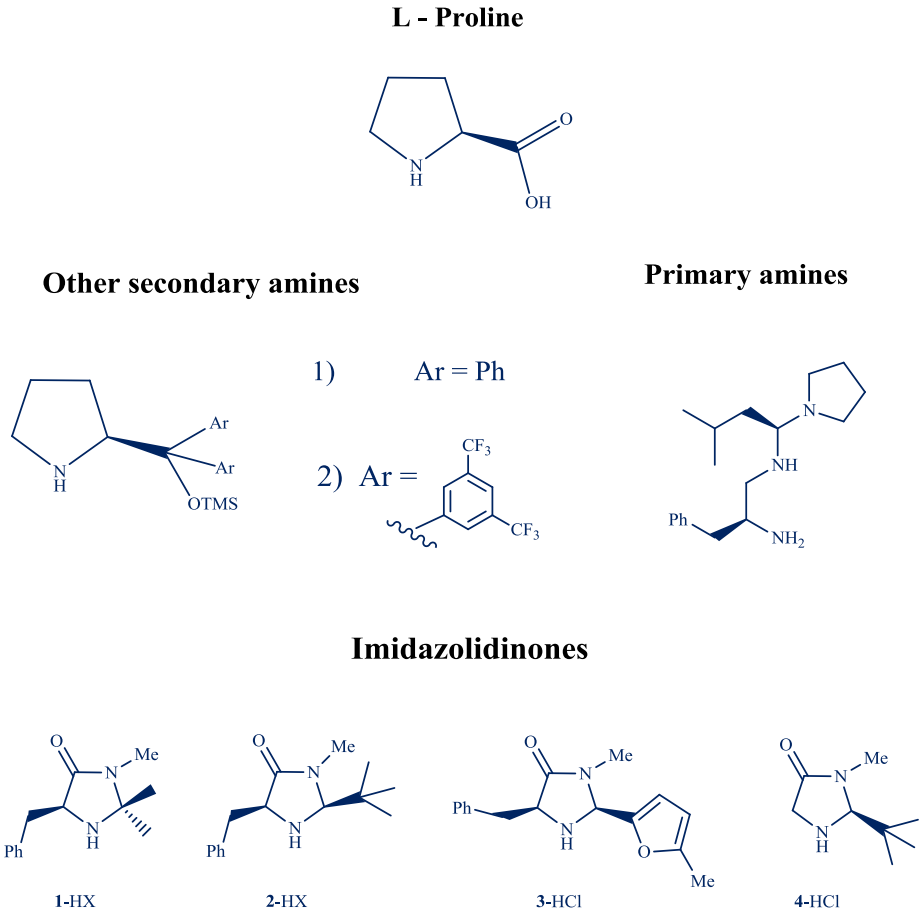


Figure 6.11 *Molecules used as catalysts.*

Primary amines, used at lesser respect to secondary amines, are used, above all, in the reactions of alpha-substituted enals, because they are too bulky to provide satisfactory speed and selectivity with secondary amines.
In some cases chiral triamines as catalysts have also been used.
Between the iminium cation and a generic anion there is a close interaction, therefore, the use of a chiral anion can affect the enantioselectivity of the iminium-catalyzed process.[234]

6.6 IMINIUM ION ACTIVATION: DEVELOPMENT AND CATALYSIS

For many years, Lewis acids have been used in condensation reactions to activate π systems toward nucleophilic attack, or to increase the electrophile power of such systems. The activation mechanism of such systems, via Lewis acid, occurs through the reversible binding of the same acid with the electrophilic substrate. During this interaction, the LUMO (Lowest Unoccupied Molecular Orbital) of the π system is lowered, this resulting in an increase of the electrophilic power of the substrate, i.e. there is a decrease of the potential energy of the LUMO. Consequently, if the LUMO's potential energy of the substrate is lower, greater is the predisposition of that substrate to the nucleophilic attack. Secondary amines carry the same activation type of the Lewis acids (**Figure 6.12**).

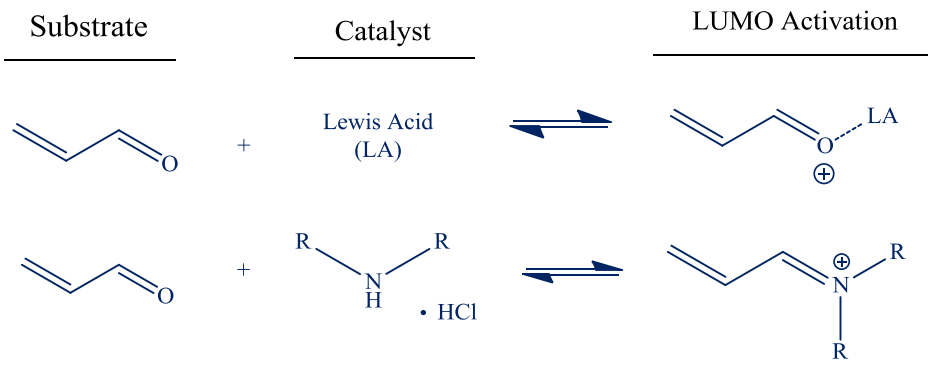


Figure 6.12 *Comparison between the interaction of Lewis acid and secondary amine with the electrophile.*

In the Diels-Alder reactions the formation of imminic ion leads to the activation of the LUMO of the substrate-catalyst complex (alkene) by lowering the potential energy. This electronic redistribution results in a decrease of the difference of the energy between the LUMO of the electrophile (alkene) and the HOMO (Highest Occupied Molecular Orbital) of the nucleophile (conjugated diene), so the reaction is favored. In summary, if the potential energy of the substrate LUMO is lower, greater is the predisposition of this substrate to the nucleophilic attack. In **Figure 6.13** is reported a reaction in which the (L)-Proline catalyst lower the LUMO of the electrophile.

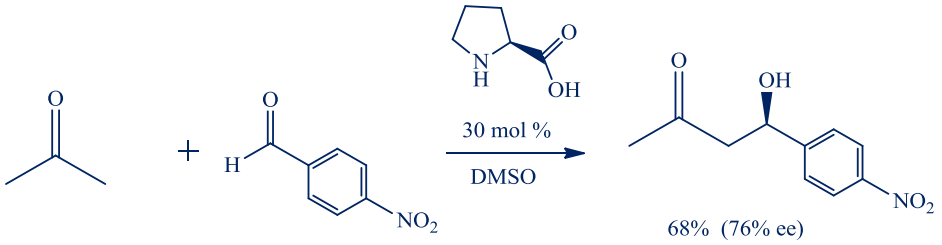


Figure 6.14 *Aldol condensation reaction catalyzed by (L)-Proline.*

In order to obtain a enantioselective nucleophilic attack on an activated iminium system, certain conditions must be met. The catalyst must be able to control the iminium ion geometry. In **Figure 6.15** is reported the geometry of an iminium ion.

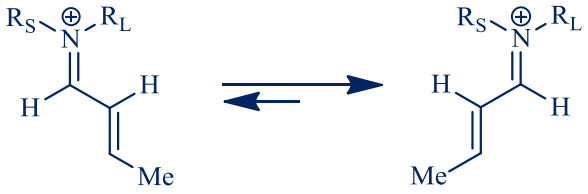


Figure 6.15 *Iminium ion geometry.*

The catalyst must be capable of differentiate an enantiface, *Re* or *Si*, of the π system, and to protect that enantioface from the nucleophilic attack by steric obstruction and by π–π interaction. Many examples in the literature show that good results are obtained when Proline is used as a catalyst.[235]

6.7 IMIDAZOLIDINONES

Prior to MacMillan's studies, the iminium-catalysis strategy had never been documented. However, several established methodological investigations provided the experimental basis for this organocatalytic approach.

First, one of the most utilized transformations in organic synthesis is represented by reductive amination.[236] In this process, an aldehyde and an amine reversibly combine to generate an iminium ion in equilibrium quantities that rapidly undergoes hydride reduction to provide the corresponding alkyl amine. Second, the studies of Jung and co-workers[237] in their Diels–Alder investigations during the late 1980s revealed that α,β-unsaturated iminium ions are significantly more reactive as dienophiles than α,β-unsaturated aldehydes, acid chlorides, ketones, nitriles, or esters. This is an essential criterion for the amine-catalyzed strategy in that a significant rate acceleration of the enantioselective bond-forming step must accompany iminium ion formation.

In order to test the iminium-activation strategy, MacMillan first examined the capacity of various amines to enantioselectively catalyze the Diels–Alder reaction between dienes and α,β-unsaturated aldehyde dienophiles.[238] Preliminary experimental findings and computational studies proved the importance of four objectives in the design of a broadly useful iminium-

activation catalyst: (1) the chiral amine should undergo efficient and reversible iminium ion formation; (2) high levels of iminium geometry control and (3) selective π-facial discrimination of the iminium ion should be achieved in order to control the enantioselectivity of the reaction, and (4) in addition, the ease of catalyst preparation and implementation would be essential for the widespread adoption of this catalytic technology.

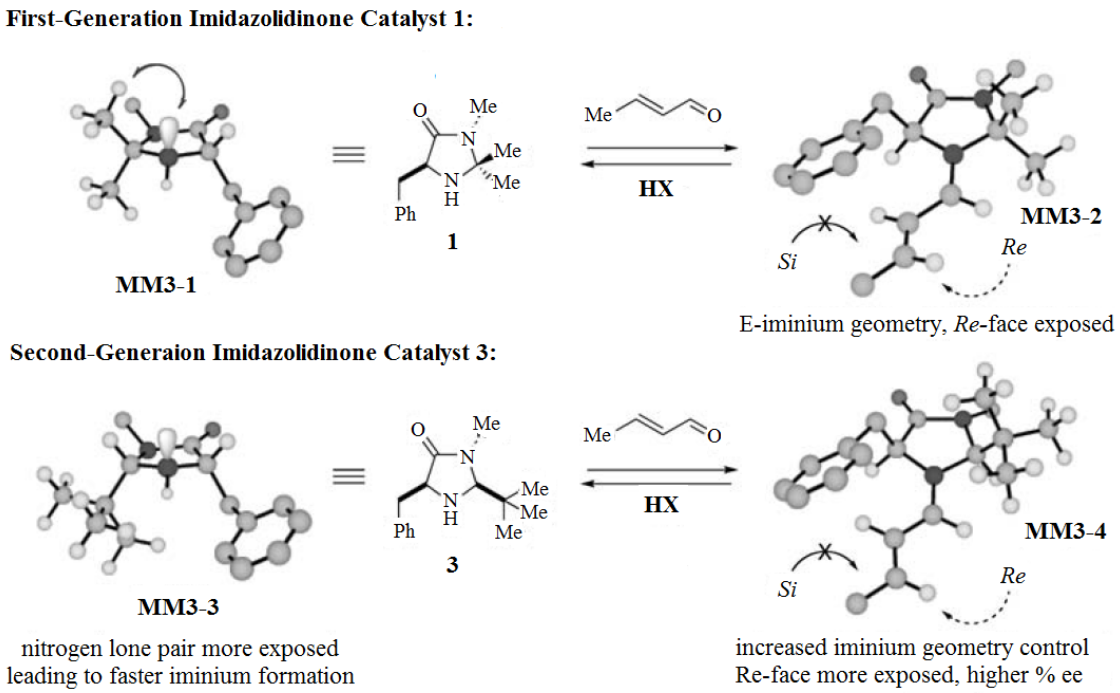


Figure 6.16 *Computational studies of the first- and second-generation imidazolidinone catalyst (**1** and **3**) and of the corresponding iminium ions.*

The first catalyst to fulfill these requirements was imidazolidione **1** (**Figure 6.16**, top). As indicated from computational studies, the catalyst-activated iminium ion MM3-2 was expected to form with only the (E)-conformation to avoid nonbonding interactions between the substrate double bond and the *gem*-dimethyl substituents on the catalyst framework. In addition, the benzyl group of the imidazolidinone moiety should effectively shield the iminium-ion *Si*-face, leaving the *Re*-face exposed for enantioselective bond formation. The efficiency of chiral amine **1** in iminium catalysis was demonstrated by its successful application in several transformations such as enantioselective Diels–Alder reactions,[227] nitrone additions,[239] and Friedel–Crafts alkylations of pyrrole nucleophiles.[240]

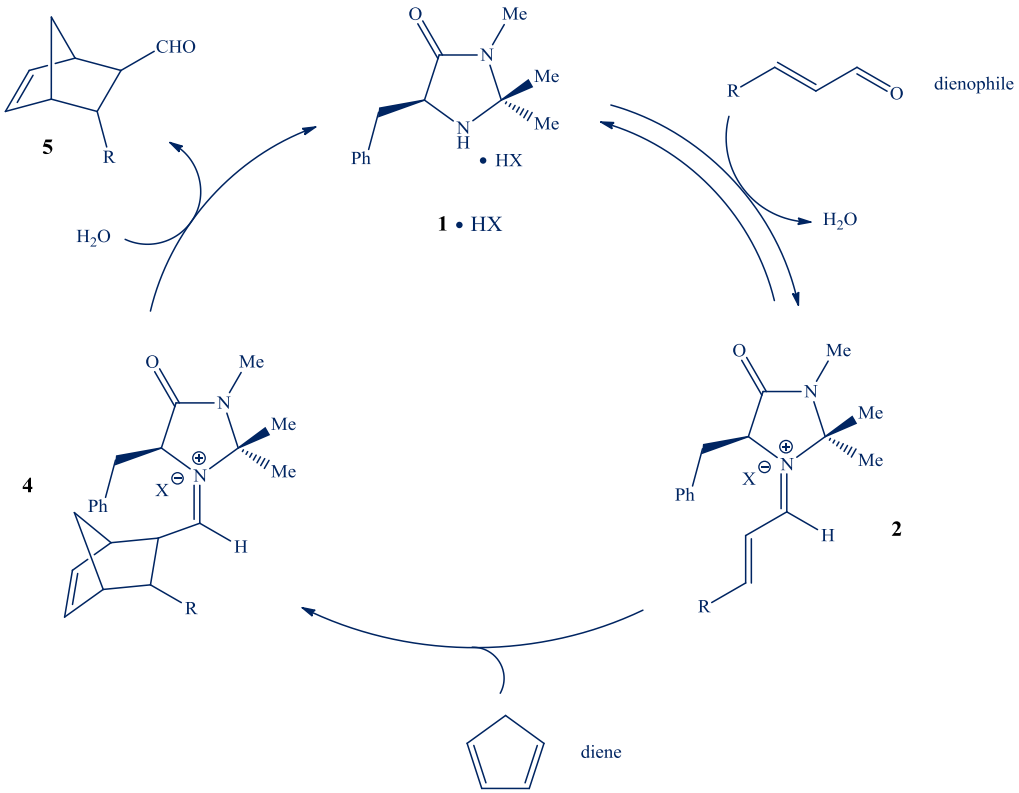
This led ultimately to the discovery of the ‘‘second-generation’’ imidazolidinone catalyst **3** (**Figure 6.16**, bottom).[241]

Preliminary kinetic studies with imidazolidinone catalyst 1 suggested that the overall rates of iminium-catalyzed processes were influenced by the efficiency of both the initial iminium ion and the C-C bond-forming steps. With the help of computational modeling, it was hypothesized that reduced reactivity of amine **1** in several iminium-catalyzed reactions was due to its diminished nucleophilicity towards carbonyl addition as the participating nitrogen lone pair is positioned adjacent to an eclipsing methyl group (**Figure 6.16**, top). In order to overcome this unfavorable interaction, MacMillan postulated that the replacement of the methyl group with a

hydrogen substituent would enable catalysts such as **3** to rapidly engage in iminium formation. At the same time, replacement of the cis-methyl group of 1 with a tert-butyl functionality as in **3** was proposed to increase iminium geometry control, providing better coverage of the blocked *Si*-enantioface (**Figure 6.16**, bottom). Since its introduction in 2001, imidazolidinones of type 3 have been successfully applied to a broad range of transformations, which include cycloadditions,[242] conjugate additions,[243] hydrogenations,[244] epoxidations, and cascade reactions.[245]

6.8 DIELS-ALDER REACTIONS CATALYZED BY IMINIUM ION

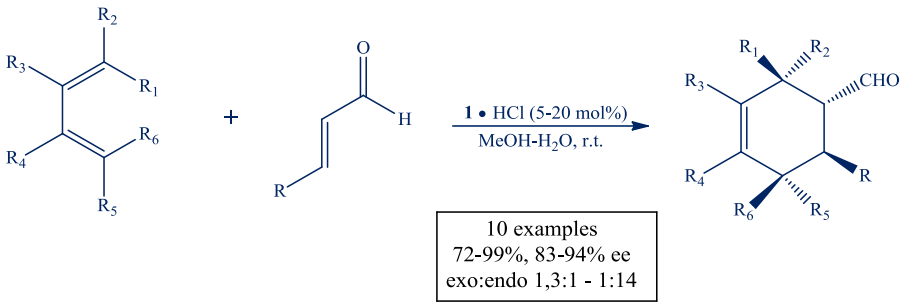
In line with the mechanistic rationale of LUMO-lowering iminium activation, MacMillan hypothesized that intermediate **2**, generated from the secondary amine **1** and an α,β-unsaturated aldehyde, could be activated towards cycloaddition with an appropriate diene (**Scheme 6.1**). The Diels–Alder reaction would form iminium ion cycloadduct **5** that, in the presence of water, would hydrolyze to yield the enantioenriched product **6** and regenerate the chiral imidazolidinone catalyst **1**.



Scheme 6.1 *Catalytic cycle of the MacMillan catalyst.*

In 2000, the first highly enantioselective amine-catalyzed Diels–Alder reaction was disclosed,[227] in which the addition of a range of α,β-unsaturated aldehydes (dienophiles) to a variety of dienes (symmetrical and unsymmetrical) in the presence of catalytic amounts of imidazolidinone **1** (5–20 mol%) afforded the corresponding cycloadducts in good yields (72–99%), and high regio-

and enantioselectivities (**Scheme 6.2**).



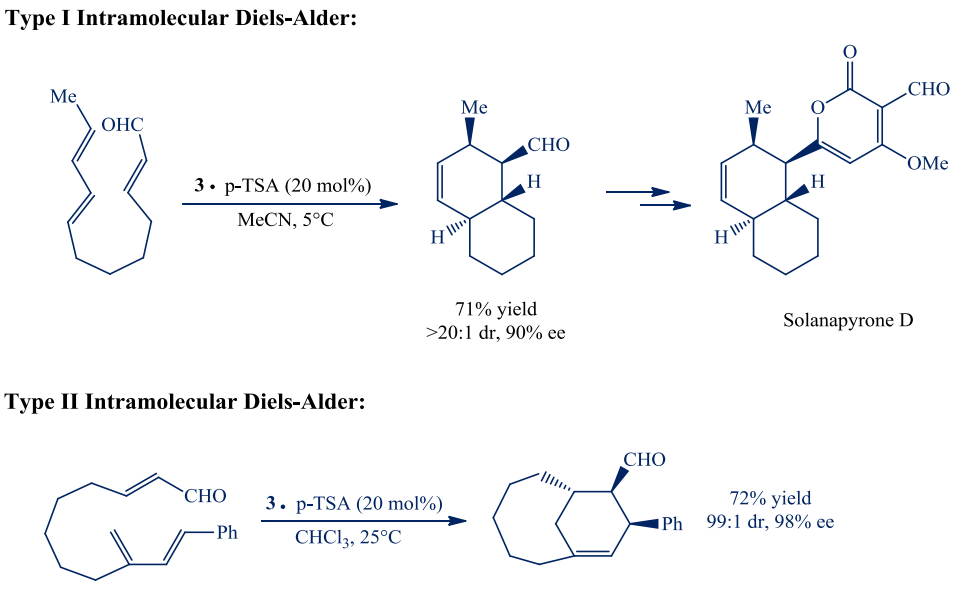
Scheme 6.2 *Examples of Diels-Alder reactions catalyzed by MacMillan catalyst.*

Remarkably, the presence of water showed beneficial effects on both reaction rates and selectivities, while facilitating the iminium ion hydrolysis step in the catalytic cycle. Computational studies suggest an asynchronous mechanism for the reaction,[246] where the attack of the diene to the β-carbon atom of the iminium ion is the rate-limiting step,[235] and the π,π-interaction between the phenyl ring of the catalyst's benzyl group and the olefinic π-system of the iminium ion accounts for the selectivity of the reaction.[227,247]

A limitation of MacMillan's approach towards iminium-activated Diels–Alder reactions has been the use of α-substituted α,β-unsaturated aldehydes as dienophiles.

Another important application of the iminium catalysis concept has been the development of enantioselective Type I and Type II intramolecular Diels–Alder reactions (IMDA).[248]

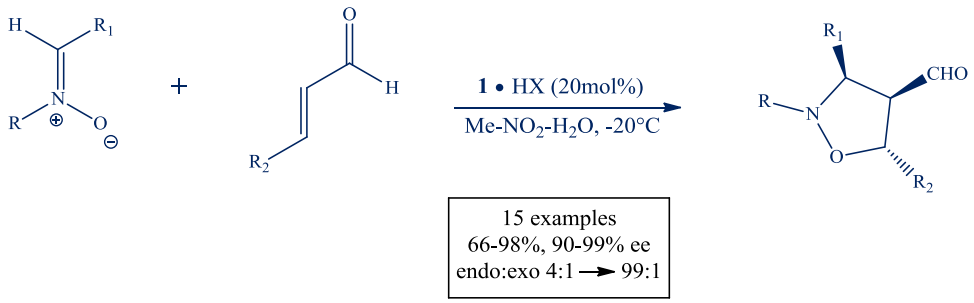
For these transformations, both catalysts **1** and **3** proved to be highly efficient, as demonstrated by both the short and effective preparation of the marine methabolite solanapyrone D via Type I IMDA (**Scheme 6.3**, top) and the development of an early example of an enantioselective, catalytic Type II IMDA reaction (**Scheme 6.3**, bottom).[249] Importantly, cycloadducts incorporating ether and quaternary carbon functionalities could be efficiently produced.



Scheme 6.3 *Two Type of Diels-Alder Reactions catalyzed by MacMillan catalyst **3**.*

6.9 1,3-DIPOLAR CYCLOADDITIONS CATALYZED BY IMINIUM

MacMillan and co-workers applied the LUMO-lowering activation strategy to the first organocatalytic [3+2]-cycloaddition (**Scheme 6.4**).[228] Transformation of several α,β-unsaturated aldehydes with a variety of *N*-alkylated nitrones in the presence of catalytic amounts of imidazolidinone **1** afforded the corresponding isoxazolidines in high yields and good diastereo- and enantioselectivities. The reaction appears quite general with respect to the nitrone structure (>66% yield, >92:8 endo:exo, >91% ee). Variation inthe N-alkyl group (R = Me, Bn, Allyl) is possible without loss in enantioselectivity.



Scheme 6.4 *1,3-Dipolar Cycloaddition reactions catalyzed by MacMillan catalyst.*

The reaction is also tolerant to a range of aromatic substituents on the dipole (R_1=Ph, p-Cl-Ph, p-OMe-Ph, p-Me-Ph, 2-naph, c-hex). Moreover, excellent levels of diastereo- and enantioselectivities can be achieved with an alkyl-substituted nitrone (R_1=*c*-hex, 99:1 endo:exo, 99% ee). In contrast, only limited variation of the dipolarophile can be achieved, crotonaldehyde (R_2=Me) and acrolein (R_2=H) generate cycloadducts in good yields and selectivities, but other β-substituted enals are largely unsuccessful due to the sluggish nature of these reactions.

CHAPTER 7

CHAPTER 7 – SYNTHESIS OF INDANYL AND ISATINYL SPIROISOXAZOLIDINES N,O-NUCLEOSIDES AT BIOLOGICAL ACTIVITY: RESULTS AND DISCUSSION

7.1 INTRODUCTION

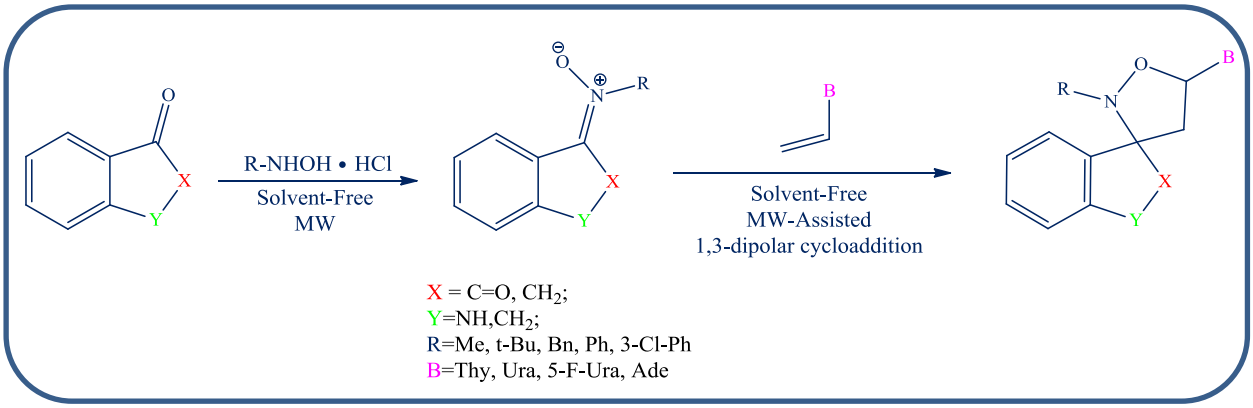
The p53 tumor suppressor protein is a transcriptional factor that plays a key role in the regulation of several cellular processes, including apoptosis, DNA repair, and angiogenesis.[250] The murine double minute 2 (MDM2) protein is the primary cellular inhibitor of p53, functioning through direct interaction with p53,[251] in fact tumoral cells show an overexpression of MDM2 which suppresses the functions of the p53 protein.[252]

The design of non-peptide, small-molecule inhibitors that block the MDM2-p53 interaction has been sought as an attractive strategy to activate p53 for the treatment of cancer and other human diseases.[253] Major advances have been made in the design of lipophilic small-molecule inhibitors of the MDM2-p53 interaction in recent years, and several compounds have moved into advanced preclinical development or clinical trials.[254] Potent MDM2-p53 inhibitors such as Nutlin-3 [255] and the spiro-oxindoles, for example MI-63 and MI-219,[256] as reported in Chapter 3 of this thesis (**Figure 3.6**), have demonstrated cellular activity consistent with inhibition of MDM2-p53 binding and have shown in vivo antitumor activity.[257] However, the spiro-oxindole scaffold is the characteristic structural core of numerous alkaloids and unnatural biologically active compounds.[258] These latter have demonstrate the versatility of the spiro-isoxazolidinic scaffold as MDM2-p53 inhibitor and show that significant improvements in potency may be gained by modest structural modifications.[259]

The introduction of the structural diversity into the oxindole scaffold can represent an important approach towards the design of new chemotherapeutics, antivirals or antibiotic drugs.

Inspired by these important factors, a number of methods to synthesize isatin and oxindole derivatives were realized during the last few years. In fact, the biological importance of compounds containing spiro-carbon at the C-3 position of the indoline or oxindole skeleton has recently been emphasized in the literature.[260] In particular, spiro-oxindoles and spiro-isoxazolidines possess antiviral activity for various human diseases, inhibiting poxvirus,[261] ectromelia,[262] rhinovirus,[263] HIV-1[264] and as very strong inhibitors of MDM2-p53 interaction.[265]

On the basis of these considerations, it has designed a route towards a new class of potential MDM2-p53 inhibitors, through the construction of the spiro-isoxazolidine *N,O*-Nucleoside system. The synthetic scheme (**Scheme 7.1**) exploits the strategy of the 1,3-dipolar cycloaddition of nitrones on the vinylnucleobases, obtained by a recent methodology that use the Solvent-Free Microwave-Assisted synthesis. The 1,3-dipolar cycloaddition between a ketonitrone and a vinylic substrate may be considered a common procedure for the preparation of these compounds.[266]



Scheme 7.1 *General multi-step reaction for the Solvent-Free Microwave-assisted synthesis of the spiro-isoxazolidine N,O-Nucleosides.*

In present work, firstly a facile synthesis of isatinyl and indanyl nitrones is described via microwave-assisted reaction between ketones and hydroxylamines under solvent-free conditions. It possible to observe very short reaction times and a reduced formation of by-products and, in addition, the expected products are isolated in high yields and excellent stereoselectivity. Successively, for the first time a regio- and diastereomeric synthesis of nucleobase-containing spiro-isoxazolidines with an indoline or an indane ring is reported, obtained in excellent yields via solvent-free MW-assisted 1,3-dipolar cycloaddition. All products are tested *in vitro* to evaluate the excellent biological activity.
Moreover, all reactions and experimental data were supported by theoretical-computational calculations that confirmed the excellent reactivity of the different substrates, the diverse involved reaction mechanisms and the various enzyme-substrate interactions.

7.2 SYNTHESIS OF KETONITRONES USED AS 1,3-DIPOLES FOR SPIRO-ISOXAZOLIDINES PREPARATIONS

A broad range of methodologies to the synthesis of aldonitrones is available today,[267] in contrast, the preparation of ketonitrones is not always accomplished by simple condensation reaction.[268] A thorough search of the relevant literature yielded only few articles related to synthetic procedures of isatin and oxindole nitrone.[269] In particular, a methodology regards the preparation of *N*-substituted isatinyl nitrones via a multi-step reaction sequence.[270]
Recently, the synthesis of (*Z*)-*N*-aryl oxindole nitrones was performed through a *N*-arylation of 3-(hydroxyimino)indolin-2-ones with diaryliodonium salts, demonstrating the current interest in having efficient routes towards those sort of nitrones.[271] On the contrary, methodologies of indanoyl nitrones are not present in literature.
As result, the development of a simple and convenient method for the synthesis of isatinyl and indanyl nitrones realized from ketones and hydroxylamines through mild conditions could be highly desirable.
In recent years, the use of microwave technology in organic chemistry, allows to prepare organic compounds very fastly, with high purity and better yield respect to other conventional methods.[272]

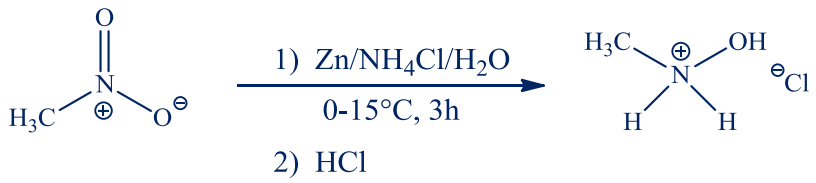
Recently, it is described a green approach to synthesize aldo- and ketonitrones by solvent-free condensation of alkyl- or aryl-hydroxylamines hydrochlorides with aromatic aldehydes and ketones under microwave irradiation, bypassing the critical results obtained with ketonitrones.[273] Then, in present paragraf, a facile synthesis of isatinyl and indanyl nitrones via microwave-assisted reaction between ketones and hydroxylamines under solvent-free conditions is described. In this approach, it is observed very short reaction times and a reduced formation of by-products, isolating the expected products in high yields and excellent stereoselectivity.[274]

7.2.1 Synthesis of the hydroxylamines used as nitrones precursors

Hydroxylamines are the most common precursors for the synthesis of the nitrones. In general, these compounds react with an aldehyde or ketone to give the nitrones by direct condensation.
In this work the hydroxylamines used as nitrones precursors are:

- *N*-methylhydroxylamine (**1a**)
- *N*-tert-butylhydroxylamine (**1b**)
- *N*-benzylhydroxylamine (**1c**)
- *N*-phenylhydroxylamine (**1d**)
- *N*-(3-chloro)-phenylhydroxylamine (**1e**)

All this compounds are commercially available but their instability doesn't allow a long storage, for example, the *N*-methylhydroxylamine is very hygroscopic, the *N*-phenylhydroxylamine is very unstable at room temperature, etc. Moreover, their purchase cost is very high and, consequently, it is more convenient to synthesize them in the laboratory before they are used, given the low cost of precursors.
N-methylhydroxylamine hydrochloride is the most simple and commonly used alkyl-hydroxylamine and has a current high purchase cost. It is synthetized in according to **Scheme 7.2**.

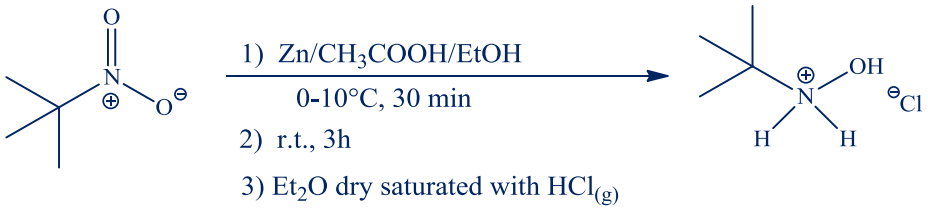


Scheme 7.2 *Synthesis of the N-methylhydroxylamine hydrochloride.*

In the first step of the reaction, when nitromethane reacts with a zinc dust in aqueous weak acid ambient it is reduced. If the reaction is conduct at room temperature the final product is the volatile methylamine because the amine group is the maximum reduced-state of the nitro group obtainable with this metodology. However, *N*-methylhydroxylamine is an intermedium isolable product, in fact, the synthesis of this compound is carry out in a temperature range of 0-15°C for a maximum time of 3 hours. It is necessary to monitor the reaction in continuous way by TLC to avoid the formation of the undesirable methylamine. The second step of the reaction consists in a hydrochloride salt formation of the hydroxylamine group with hydrochloride acid by lowering

the pH solution.

N-tert-butylhydroxylamine hydrochloride is an alkyl-hydroxylamine characterized to a large size of t-butyl group. It is commercially available at the current high purchase cost and is synthetized in according to **Scheme 7.3**.



Scheme 7.3 *Synthesis of the N-tert-butylhydroxylamine hydrochloride.*

If the reaction is initially conducted at room temperature the final product is the *t*-butylamine. However, *N*-*t*-butylhydroxylamine is an intermedium isolable product, in fact, the synthesis of this compound is carry out in a temperature range of 0-10°C for 30 minutes with consequently increase at room temperature for a maximum of 3 hours. It is necessary to monitor the reaction by TLC to avoid the formation of the undesirable *t*-butylamine. The second step of the reaction consist in a hydrochloride salt formation of the hydroxylamine group with diethyl ether dry $HCl_{(g)}$ saturated. This methodology consist in a dissolution of the *N*-*t*-butylhydroxylamine reaction crude in diethyl ether dry $HCl_{(g)}$ saturated because the instantaneous salified product is separable as precipitate by filtration and the undesirable by-product are dissolved in ether phase.

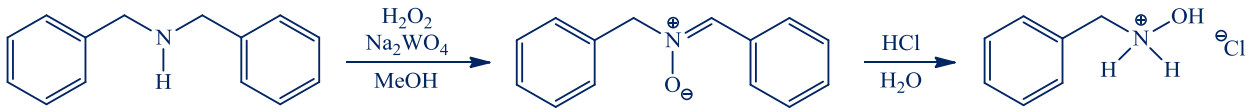


Figure 7.4 *Synthesis of the N-benzylhydroxylamine hydrochloride.*

N-Benzylhydroxylamine has become one of the most popular reagents in nitrone chemistry. Its condensation with an aldehyde or a ketone and its addition to an activated triple bond are among the most common methods to *N*-benzylnitrones.[275]

1,3-Dipolar cycloaddition of these nitrones with alkenes provides *N*-benzylisoxazolidines in which the benzyl group can be easily removed from nitrogen in order to perform further manipulations at this atom.

The retail price of *N*-benzylhydroxylamine hydrochloride is notably high for such a simple molecule. During the course of our research on new methodological developments concerning the 1,3-dipolar cycloaddition of nitrones, a large quantity of *N*-benzylhydroxylamine was used as the starting material.

In the literature, some methods for the preparation of this compound have been reported. Direct benzylation of the hydroxylamine results in a complex mixture of polybenzylated products, in which *N*,*N*-dibenzylhydroxylamine is the major product.[276] Reduction of benzaldoxime by $NaBH_3CN$ in buffered diethyl ether solution affords the desired *N*-benzylhydroxylamine in moderate and hardly reproducible yields possibly due to a pH change during the course of the

process.[277] Moreover, use of $NaBH_3CN$ is highly toxic and can generate deleterious side products after workup.

The preparation of *N*-benzylhydroxylamine by acid hydrolysis of *N*-benzyl-*C*-phenyl-nitrone and subsequent neutralization attracted the attention. Based on this result, we thought that nitrone abovementioned could be an interesting precursor for *N*-benzylhydroxylamine, provided that the nitrone could be obtained in a high-yielding and simple way. Our attention was directed to the preparation of nitrone by oxidation of dibenzylamine, as it can be easily made by the reaction between NH_3 and benzyl chloride in industrial scale.

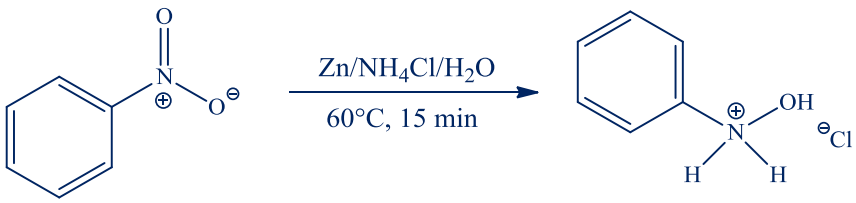
This transformation was previously carried out by using H_2O_2 in the presence of a catalyst such as Na_2WO_4,[278] SeO_2,[279] or $MeReO_3$,[280] or by using dimethyldioxirane,[281] and *N*-phenylsulfonyl-*C*-phenyloxaziridine.[282] Among the methods reported on a large-scale, Murahashi's procedure using Na_2WO_4 (2 mol%) seems to be the most economic and practical as low-cost reagents (H_2O_2, Na_2WO_4) and solvent (MeOH) was used, and the desired nitrone could be obtained in excellent yield (85–96% after recrystallization).

However, in the original Murahashi's procedure,[267] the crude reaction mixture was subjected to methanol evaporation without prior removal or treatment of the residual oxidative agent H_2O_2. Despite the high yield, this method seemed to be unsafe, especially for a larger scale synthesis.

Following a modified procedure, simple addition of ice was found to precipitate the nitrone from the crude reaction mixture. By simple filtration, we could recover the nitrone in high yield (~80%) and avoid hazardous manipulations. Moreover, this modification of the procedure prevents the use of a large amount of CH_2Cl_2, previously used for extraction of nitrone. Since water is not detrimental for the next reaction, we used the wet nitrone directly in the subsequent acidic hydrolysis step. Treatment of this crude mixture with an aqueous hydrochloric acid solution (20%) followed by steam distillation under reduced pressure in order to eliminate PhCHO afforded pure BnNHOH·HCl as colorless crystals after only one recrystallization from hot methanol with good overall yield (72%) (**Scheme 7.4**).

In conclusion, here a simple, economic, and safe procedure for the preparation of *N*-benzylhydroxylamine hydrochloride is described. This two-step method requires only low-cost starting materials (Bn_2NH, H_2O_2, Na_2WO_4, HCl) and solvents (H_2O, MeOH) with highly simple purification (recrystallization, evaporation, and steam distillation) in good overall yield.

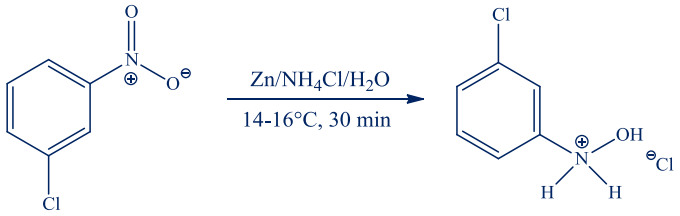
N-phenylhydroxylamine is the most simple and commonly used aryl-hydroxylamine and has a current high purchase cost. It is synthetized in according to **Scheme 7.5**.



Scheme 7.5 *Synthesis of the N-phenylhydroxylamine.*

The zinc-reduction reaction is conduct at 60°C for a short time period in contraposition to the most literature procedures that report the use of the low temperature.[283] It is verified that if the reaction is conduct at 0°C, the obtained product is the nitrosobenzene, a very degradable by-

product that makes difficult the hydroxylamine purification.
N-(3-chloro)-phenylhydroxylamine isn't commercially available so must be prepared in laboratory before its utilize. It is synthetized in according to **Scheme 7.6**.

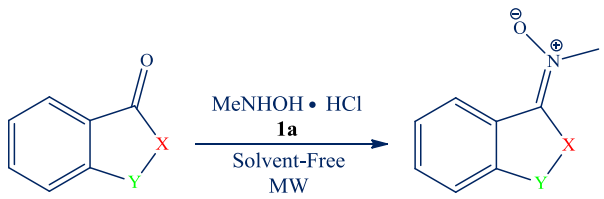


Scheme 7.6 *Synthesis of the N-(3-chloro)-phenylhydroxylamine.*

The zinc-reduction reaction is conduct at 14-16°C for a short time period and must be monitored in continuous by TLC stopping the reaction before by-products formation.

7.2.2. Microwave-Assisted Solvent-Free Synthesis of the Nitrones

To find the optimal conditions for the solvent-free microwave-assisted synthesis of nitrones, we chose *N*-methylhydroxylamine hydrochloride **1a** and 1-isatin **2a** and indanone **3a** as ketones. The methodology consists of the co-grinding of the ketone and hydroxylamine in a mortar, followed by transfer of the mixture in a sealed vessel and further mixing by a vortex; finally the mixture is placed in a microwave oven. The optimization study is collected in **Table 7.1**.



2a X = C=O, Y=NH
3a X = Y = CH_2

4a X = C=O, Y=NH
5a X = Y = CH_2

Entry	Ketone[b]	MW (W)	T (°C)	Time (min)	Product	Yield (%)
1[c]	**2a**	200	180	10	**4a**	33
2[c]	**2a**	400	180	10	**4a**	42
3	**2a**	400	180	10	**4a**	53
4	**2a**	400	180	20	**4a**	61
5	**2a**	600	180	10	**4a**	97
6[d]	**2a**	600	180	10	**4a**	80
7[e]	**2a**	-	rt	1440	**4a**	70
8	**3a**	600	180	10	**5a**	37
9	**3a**	600	180	15	**5a**	51
10	**3a**	400	180	10	**5a**	25
11	**3a**	400	180	30	**5a**	82
12[d]	**3a**	-	rt	1440	**5a**	78

[a] Reaction conditions: 2,0 eq. of **1a** were used unless otherwise indicated. [b] 1,0 eq. was used. [c] 1,0 eq. of **1a** was used. [d] 1,0 eq. of NaOAc was added. [e] 1:1 EtOH/water was used as a solvent and 2,0 eq. of NaOAc were added.

Table 7.1 *Optimization of isatinyl and indanyl nitrones synthesis.*[a]

The best conditions for both ketones (entries 5 and 11) were those corresponding to an irradiation of 600W (T=180 °C) for 10 min for ketone **2a** and an irradiation of 400W (T=180°C) for 30 min for ketone **3a**. The corresponding products **4a** and **5a** were obtained in 97% and 82% chemical yield, respectively. In both cases, nitrones were obtained as single *E* isomers as confirmed by NOESY experiments reported in **Figure 7.1**.

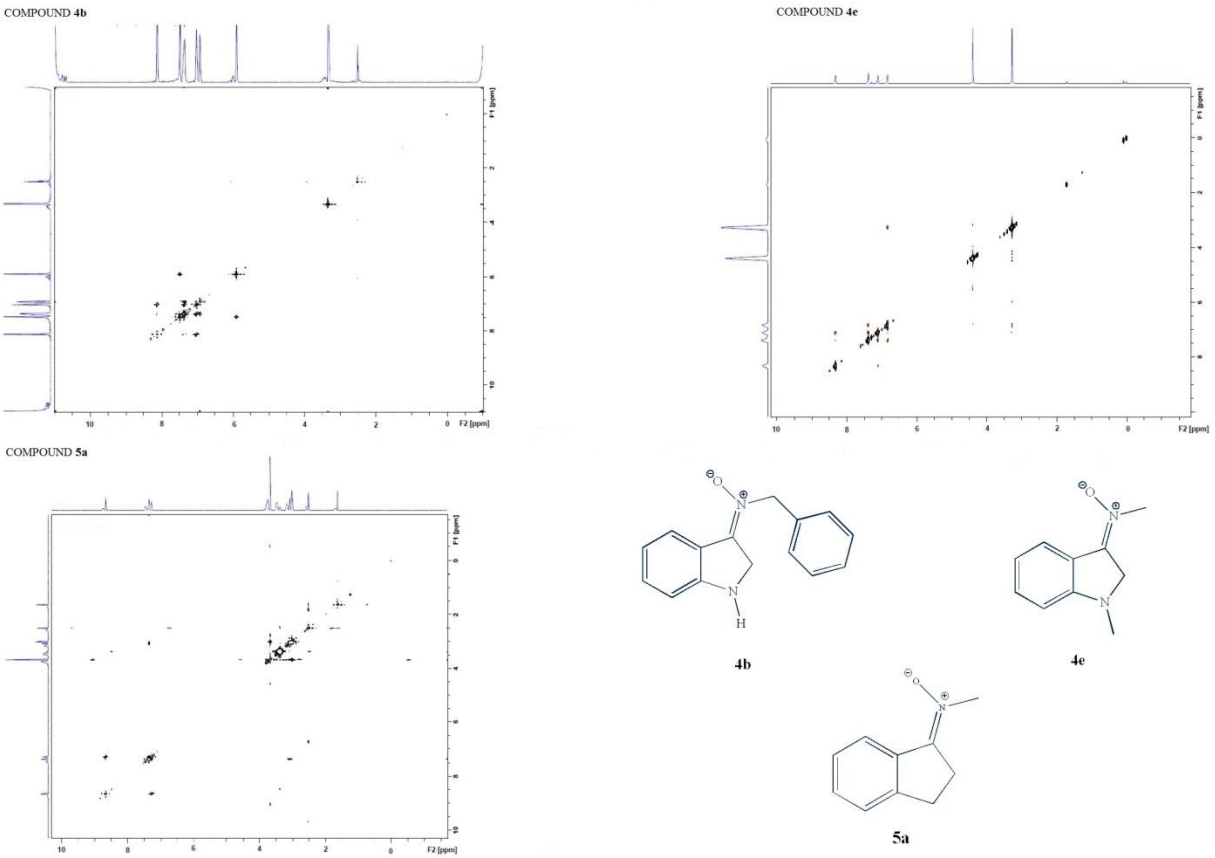
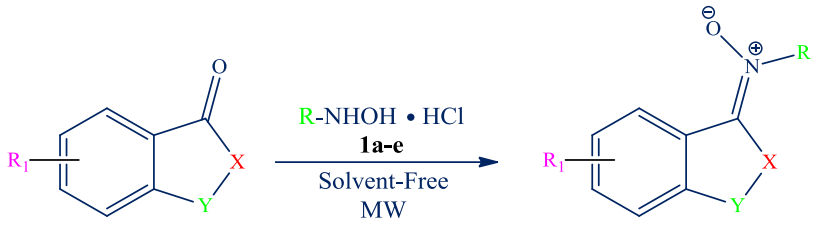


Figure 7.1 *NOESY experiments of the **4b**, **4e**, **5a** compounds.*

In all three cases reported above isn't impossible to observe the NOESY interaction between alkyl group bonded to nitrone-nitrogen atom and aromatic proton, interaction presumable present in nitrones with *Z* configuration.

When the reaction was carried out in EtOH/H_2O as a solvent and in the presence of 2 equivalent of sodium acetate as reported in literature on similar substrates,[284] compounds **4a** and **5a** were obtained in 70% and 78% chemical yield, respectively, in 24 hours (entries 7 and 12). Moreover, the presence of sodium acetate in reaction mixture does not significantly change the trend of the reaction (entry 6) and therefore we chose not to use it.



2a-e; 3a-j **4b-p; 5b-n**

Entry	Hydroxylamine	R	Ketone	X	Y	R_1	Time (min)	Product	Yield (%)
1	**1b**	Bn	**2a**	C=O	NH	H	12	**4b**	95
2	**1c**	Ph	**2a**	C=O	NH	H	10	**4c**	92
3	**1d**	3-Cl-Ph	**2a**	C=O	NH	H	10	**4d**	87
4	**1a**	Me	**2b**	C=O	NMe	H	10	**4e**	90
5	**1b**	Bn	**2b**	C=O	NMe	H	12	**4f**	92
6	**1c**	Ph	**2b**	C=O	NMe	H	10	**4g**	90
7	**1d**	3-Cl-Ph	**2b**	C=O	NMe	H	10	**4h**	88
8	**1a**	Me	**2c**	C=O	NH	5-Br	10	**4i**	95
9	**1b**	Bn	**2c**	C=O	NH	5-Br	10	**4j**	93
10	**1c**	Ph	**2c**	C=O	NH	5-Br	10	**4k**	92
11	**1a**	Me	**2d**	C=O	NH	5-NO_2	10	**4l**	95
12	**1b**	Bn	**2d**	C=O	NH	5-NO_2	13	**4m**	92
13	**1c**	Ph	**2d**	C=O	NH	5-NO_2	10	**4n**	92
14	**1a**	Me	**2e**	C=O	NH	5,7-Cl	25	**4o**	80
15	**1e**	t-Bu	**2a**	C=O	NH	H	60	**4p**	-
16	**1b**	Bn	**3a**	CH_2	CH_2	H	30	**5b**	85
17	**1a**	Me	**3b**	CH_2CH_3	CH_2	H	30	**5c**	61
18	**1a**	Me	**3c**	CH_2	CHMe	H	35	**5d**	78
19	**1a**	Me	**3d**	CH^nPr	CH_2	H	32	**5e**	20
20	**1a**	Me	**3e**	CH_2	CH_2	5-Br	30	**5f**	88
21	**1a**	Me	**3f**	CH_2	CH_2	5-F	28	**5g**	83
22	**1a**	Me	**3g**	CH_2	CH_2	4-Br-7-OH	60	**5h**	67
23	**1b**	Bn	**3g**	CH_2	CH_2	4-Br-7-OH	48	**5i**	78
24	**1e**	t-Bu	**3a**	CH_2	CH_2	H	60	**5j**	-
25	**1a**	Me	**3h**	CH_2	CH_2CH_2	H	50	**5k**	traces
26	**1b**	Bn	**3h**	CH_2	CH_2CH_2	H	50	**5l**	traces
27	**1a**	Me	**3j**	CH_2	CH_2CH_2	7-F	45	**5m**	traces
28	**1a**	Me	**3j**	CH_2	CH_2CH_2	6-OMe	55	**5n**	-

[a] Ketone/phenylhydroxylamine ratio 1:3

Table 7.2 *Synthesis of nitrones* ***4*** *and* ***5****.*

In an effort to expand the scope of the reaction, coupling of substituted ketones with *N*-methylhydroxylamine hydrochloride (**1a**) *N*-benzylhydroxylamine hydrochloride (**1b**),[285] *N*-phenylhydroxylamine (**1c**),[286] *N*-(3-chloro)-phenyl-hydroxylamine (**1d**) and *N*-t-butyl-hydroxylamine hydrochloride (**1e**) is explored (**Table 7.2**).

The reaction works well with various isatin ketones **2** and we noticed that the presence of an electron-withdrawing group (Br or NO_2) on C-5 of phenyl ring of isatin does not significantly alters their performance (entries 8–13). Methylation of nitrogen does not reduce the reactivity (entries 4–7). Phenylhydroxylamine **1c** was used in further excess (ratio ketone/phenyl-

hydroxylamine 1:3) because of its known degradability at temperature higher than 50 °C.[287] Attempts to reduce byproducts by lowering power of MW (200 or 400 W) or by reducing the maxim temperature were unnecessary. In all cases the *E*-isomer was the only obtained with the exceptions of compounds **4d** and **4h** for which small quantities of *Z* isomers (ratio *E*/*Z*:78:22 and 75:25 for **4d** and **4h**, respectively) were obtained. In general, ketones **3** present lower reactivity with respect to isatin ketones **2**. In all cases, the *Z*-isomer was the only obtained. When an alkyl group is present on the 2-C or 3-C of the carbon backbone of indanone ring (entries 17 and 18), the yield drops presumably due to a minor electrophilicity of carbonyl group. The propyl group introduces additional steric hindrance, and the yield drops to 20% (entry 19). The presence of Br or F in C-5 of indanone ring (entries 20 and 21) does not produce significant differences of reactivity respect to unsubstituted indanone. On the other hand, the reaction is very sensitive to the size of the cycloalkyl ring (tetralone versus indanone). Tetralone derivatives showed very little reactivity and longer reaction times than other substrates (entries 25–27) are required for obtaining just traces of products. Moreover, resonance effect in C-4 or in C-7 of isatine and indanone ring (entries 14, 22 and 23) causes major reaction times and/or minor yields because the electrophilicity of the acceptor carbonyl group is reduced. Unfortunately, the large size of t-butyl group of **1e** dramatically influences both reactions of isatin **2a** and indanone **3a**, not leading to the formation of any product (entries 15 and 24). Attempts to carry out the reactions using the conditions previously applied in entries 7 and 12 of **Table 7.1** confirmed the reaction trend, revealing that these reactions are sensitive to steric effects.

7.2.3 Theoretical calculations of the electrophilicity of the ketone reagents.

In order to correlate the reactivity of ketones with hydroxylamines we evaluated the electrophilicity of both reagents by calculating ω reactivity index. The ω index establishes an absolute scale of electrophilicity in the sense that the hierarchy of electrophilicity is built up from the electronic structure of molecules. The electrophilicity hierarchy may be systematically rationalized on the basis of substituent effects. As a result, electron-withdrawing groups lead to electrophilic activation, and electron-releasing groups lead to electrophilic deactivation.

Accordingly, by establishing a scale in which electrophiles and nucleophiles are present, couples of reagents (ketones and hydroxylamines in our case) presenting large differences of electrophilicity (i.e. $\Delta\omega > 2{,}0$) will be the more reactive.

Calculations were carried out at b3lyp-d3bj/def2svp level of theory according to the procedure described elsewhere. We considered the experimentally studied ketones **2a–e** and **3a–j** but also ketones **2f–k**, **3k–3p** were calculated in order to predict an extended reactivity. The results are illustrated in **Figure 7.2**.

Among the ketones the most electrophilic one is predicted to be **2d**, which has been studied experimentally. Among the hydroxylamines the most nucleophilic one resulted **1a**, also experimentally studied. Consequently, the reaction between **2d** and **1a** (Table 2, entry 11) is the most favoured as, indeed, is experimentally demonstrated (high yield and only 10 min of reaction). In general, isatin derivatives are the most reactive with values of $\Delta\omega > 2$ with some exception. The lower reactivity predicted ($\Delta\omega < 2$) for indanyl and tetralonyl derivatives is experimentally confirmed by the longer reaction times required and lower yields obtained. In

fact, the absence of product obtained for the reaction between ketone **3j** and hydroxylamine **1a** correlates with a low difference in electrophilicity (Δω=1,11).

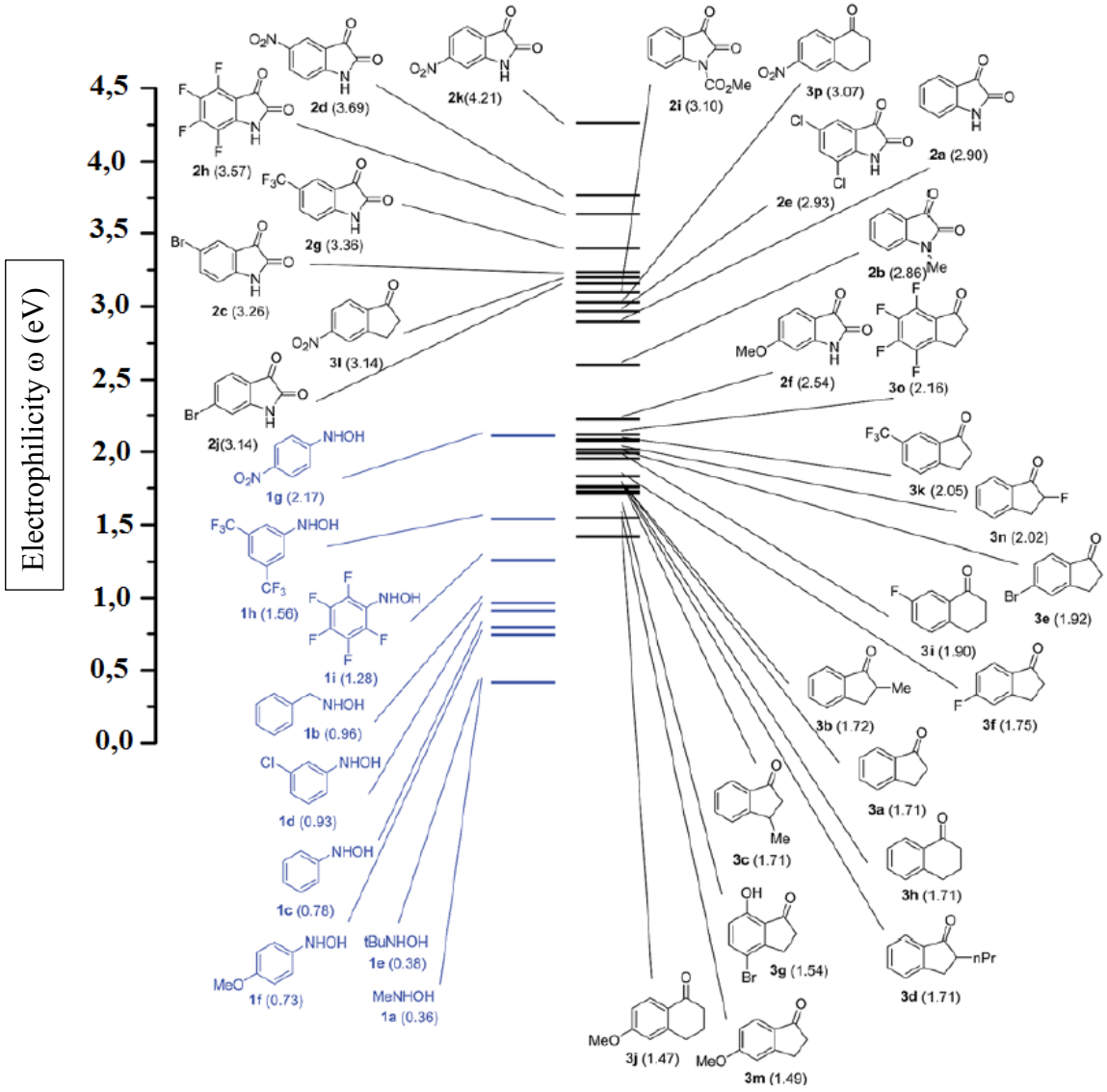


Figure 7.2 *Electrophilicity (ω in eV)scale for ketones and hydroxylamines.*

7.2.4 Antiproliferative and antioxidant activities of ketonitrones

Small organic molecules have proven to be invaluable tools for investigating biological systems, but there is still much to learn from their use.[288]

Nitrones are small molecules that have general chemical formula XCH=NO-Y, as shown in previous paragraph. Their structural nature confers them their "*spin-trap*" ability for trapping free radical intermediates (R•), forming stable radical adducts (X-CHR=NO•-Y).[289] In fact, studies of spin trapping methods involved the reaction of nitrones with reactive free radicals such as hydroxyl (•OH), lipid alkoxy (•OL) or lipid hydroxyperoxyl (•OOL) radicals, observing the formation of more stable radicals that can be classified and quantified by Electron Spin Reso-

nance (ESR) or Electron Paramagnetic Resonance (EPR) spectroscopy.[290]

Considering a) that the biological systems may actively produce reactive oxigen species (ROS) and reactive nitric oxide species (RNS), b) that specific oxidation products are produced from reaction between biological molecules and ROS and RNS, c) that ROS and RNS play an important role in many pathologic diseases,[291] nitrones and, in particular, PBN-nitrones where X is a phenyl group and Y is a *tert*-butyl group were recently noted as therapeutics for their wide spread anti-cancer activity.[292] The mechanistic basis of their anti-cancer action is not known. It is probable that the ability to scavenge radical intermediates that are produced during disease processes, including cancer, is the basis for their anti-cancer activity. Moreover, their action on important membrane enzymes and as anti-inflammatory agents seem to contribute to the magnification of their antioxidant activity.

Nitrones prepared according to the procedure reported in **Paragraph 7.2.2** (Isatinyl/Indanyl Nitrones INs) were evaluated for their antiproliferative activity against human osteosarcoma (MG63 and TE85)[293] and chronic myeloid leukemia (K562)[294] cell lines. Cells were cultured for three days in the absence or in the presence of increasing concentrations of nitrones and then their vitality was evaluated by dosing the activity of the oxidative metabolism by the 3-(4,5-dimethylthiazolyil-2)-2,5-diphenyltetrazolium bromide (MTT) assay.[295] In **Figure 7.3** the proliferation of the treated cells was expressed as percentage compared to un-treated control cells.

The data indicated that not all the nitrones had antiproliferative activity, since the **5a**, **5b**, **5g** and **4c** compounds were completely inactive.

Compounds **4b**, **4f**, **4m** inhibited the proliferation of osteosarcoma MG63 cells compared to untreated cells (**Figure 7.3 A**, IC_{50} about 77 μM).

In **Figure 7.3 B** are shown the antiproliferative activities of **4l** and **4b**, **4m** nitrones on TE85 cells (IC_{50} about 32 μM). The active compounds are less effective in inhibiting the proliferation of K562 cells, with the exception of **4f** nitrone with an IC_{50} of about 78 μM (**Figure 7.3 C**).

These results together have shown that the indanyl derivatives do not possess antiproliferative activity on the tumour cells analyzed, as well as the derivative **4c** with the phenyl substituent. By contrast, isatinyl nitrones containing methyl or benzyl groups possess antiproliferative activity.

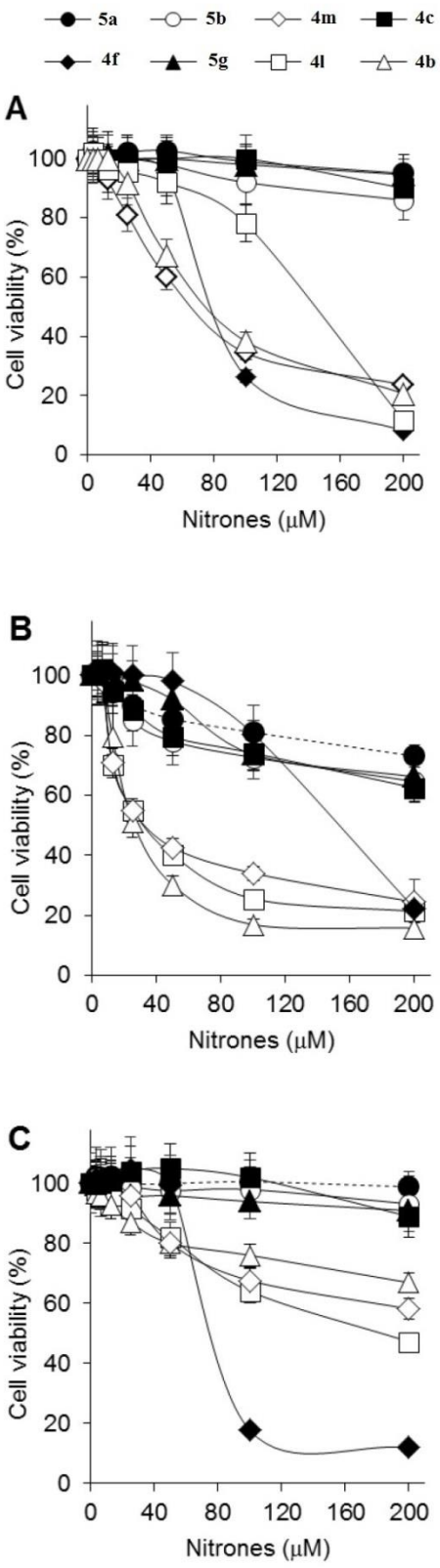


Figure 7.3 *Effect of nitrones* ***4(b, c, f, l, m)*** *and* ***5(a, b, g)*** *on cell proliferation. MG63 (A), TE85 (B) and K562 (C) cells were incubated for three days in the presence of the indicated compounds. Viable cells were measured by MTT test and re-ported as % of untreated control. The arithmetic mean value ± standard deviation of three experiments performed in triplicates is shown.*

The *in vitro* antioxidant activity of these ketooxindole nitrones **4(b, c, f, l, m)** and **5(a, b, g)** was evaluated by using the stable organic free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity.[296]

When DPPH reacts with a radical scavenger its maximum absorbance decreases. A freshly prepared DPPH solution displayed a deep purple color with an absorption maximum at 517 nm. The ethanolic solution of DPPH was added to the solution of the synthesized compounds in ethanol.

After 10-min incubation in dark, the absorbance was measured by using absolute ethanol as a blank. BHT (butylated hydroxytoluene) was used as reference. In the presence of antioxidant the DPPH absorbance decreases. The antioxidant activity was calculated as radical scavenging activity (RSA%) as ex-pressed in the equation 1:

$$RSA\ \% = [(A_0-A_i)/A_0] \times 100 \qquad \text{(eq. 1)}$$

where A_0 and A_i are the DPPH absorbance at 517 nm in the absence and presence of the synthesized compounds, respectively.

The RSA% for ketonitrones **4(b, c, f, l, m)** and **5(a, b, g)** at five different concentrations (i.e. 1,15, 2,45, 4,00, 6,25 and 9,10, 10^{-6} mol/L) of the tested compounds with DPPH at 517 nm was reported in **Table 7.3**.

	CONCENTRATIONS (10^{-6} mol/L)					
Compound	**1,15**	**2,45**	**4,00**	**6,25**	**9,10**	**EC_{50}**
4b	1,1±0,5	1,1±0,5	1,2±0,5	1,2±0,5	78±2	1,8±0,1
4c	1,1±0,5	1,2±0,5	1,2±0,5	69±2	80±2	1,3±0,2
4f	1,2±0,5	60±1	68±2	70±2	79±2	0,6±0,3
4l	-	-	1,1±0,5	72±2	79±2	1,2±0,2
4m	-	-	60±1	67±2	80±2	1,0±0,2
5a	-	-	1,1±0,5	60±1	78±2	1,3±0,2
5b	1,1±0,5	1,2±0,5	1,3±0,5	70±2	79±2	1,2±0,2
5g	-	-	1,1±0,5	1,2±0,5	79±2	1,8±0,1
BHT	-	4,6±0,5	11,7±0,5	23,0±0,5	27,0±0,5	3,8±0,5

Table 7.3 *Percentage of in Vitro radical scavenging activity of INs.*

As can be seen in the **Table 7.3**, all the compounds exhibited antioxidant activity. The substances can be divided in three main groups. The first group is composed by two substances (**4f** and **4m**) with higher values of antioxidant activity than others. In particular, the greatest values were observed for **4f**, for which the RSA% is equal to 60% at a concentration of 2,45 x 10^{-6} mol/L.

Four substances compose the second group with intermediary antioxidant activity (**4c**, **4l**, **5a** and **5b**). For all of these RSA% was major than 60% at a concentration equal to 6,25 x 10^{-6} mol/L. In the third group, only two compounds with lower less antioxidant activity were included: **4b** and **5g**. For these last two compounds RSA% was 80% at a concentration equal to 9,10 x 10^{-6} mol/L.

As can be seen in the **Table 7.3** and in **Figure 7.4**, all nitrones **4(b, c, f, l, m)** and **5(a, b, g)**

exhibited a radical scavenging activity significantly major than those of the reference (BHT). It isn't investigated concentrations higher than 9,10 x 10^{-6} mol/L as the RSA% trend reaches a plateau.

The RSA% of nitrones **4(b, c, f, l, m)** and **5(a, b, g)** may be compared with the value of archetypal (*Z*) α-phenyl-*N*-tert-butylnitrone (PBN) reported in literature.[297] Its RSA% is 1,4 ±0,9 at concentration of 0,5 mM calculated through DPPH test, demonstrating at same concentration an antioxidant property comparable or highly minor (i.e**. 4f**) respect to our nitrones.

For all tested compounds the EC_{50} values were also calculated by using GraphPad Prism 5.01 program, as reported in literature (**Table 7.3**).[298] The EC_{50} is the antioxidant concentration required to obtain a 50% radical inhibition. As can be seen in **Table 7.3** the value of EC_{50} of all eight synthesized nitrones are higher than reference substance (BHT).

The antioxidant properties of INs seems to converge quite well with the biological results, confirming the essentiality of isatinyl ring. In particular, the presence both of aromatic systems and electron-donating groups (e.g. methyl group) improve the performance, as can particularly be seen for **4f** substrate.

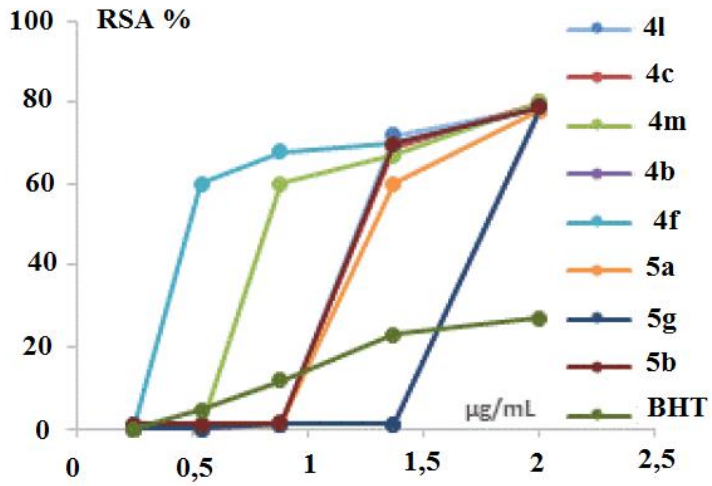


Figure 7.4 *Graphical presentation of in vitro DPPH radical scavenging activity of compounds relative to the standard antioxidant BHT.*

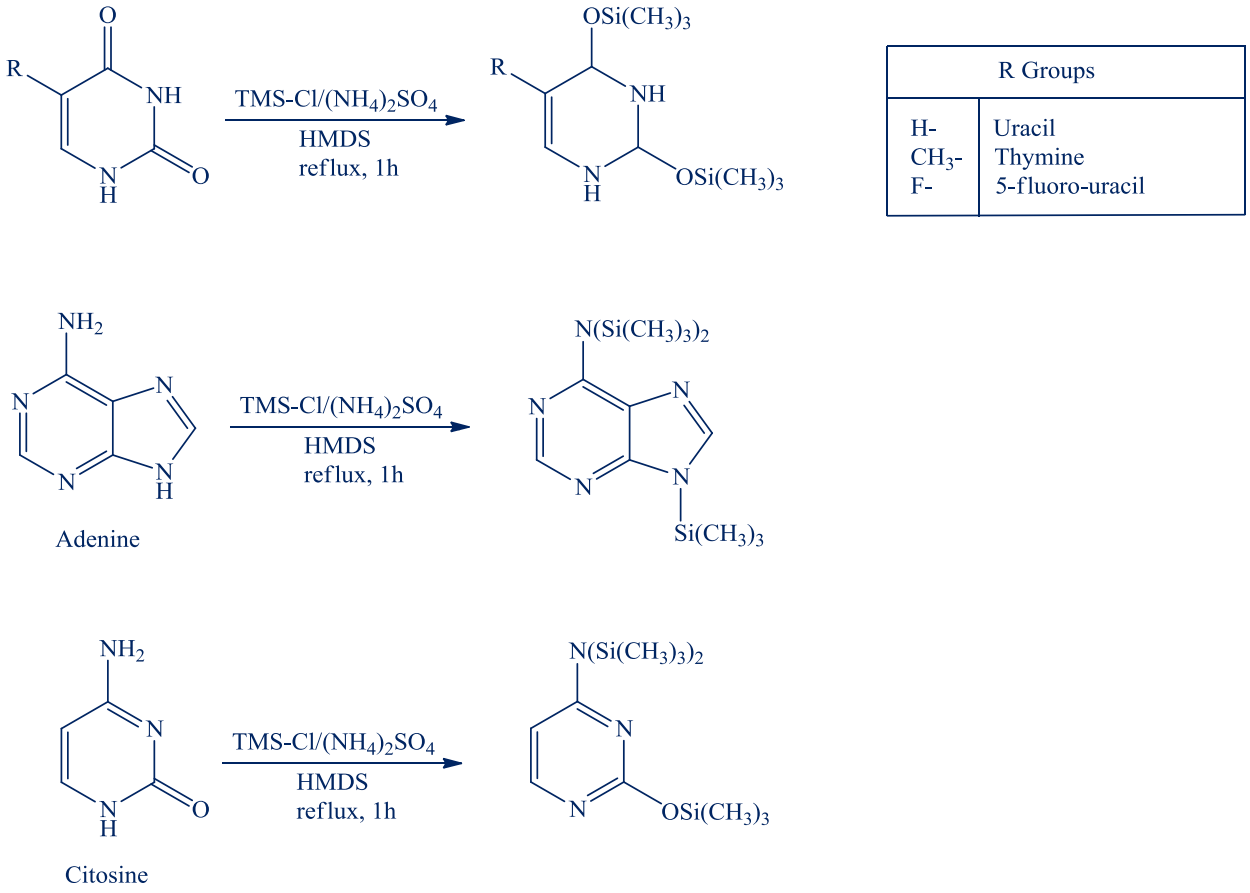
7.3 SYNTHESIS OF VINYL-NUCLEOBASES USED AS DIPOLAROPHILE FOR SPIROISOXAZOLIDINE PREPARATIONS

N-Vinyl derivatives of nucleobases are important starting materials for the synthesis of polymeric analogues of nucleic acids, useful tools in biomimetic studies on the interaction between purine and pyrimidine nucleobases,[299] and for the synthesis of nucleoside analogues recently proposed as potential antiviral drugs.[300]

Several methods have been developed for the preparation of vinyl derivatives of nucleobases, some consisting of relatively low yielding multistep procedures,[301] and some others involving the direct exchange of the acetyl group of vinyl acetate[302] with pyrimidine and purine bases or their

trimethylsilyl derivatives. Nevertheless, the latter method is faster and simpler than the former one, but it still suffers from low yields and, moreover, it is unable to produce *N*-vinyl derivatives of all nucleic acid bases.

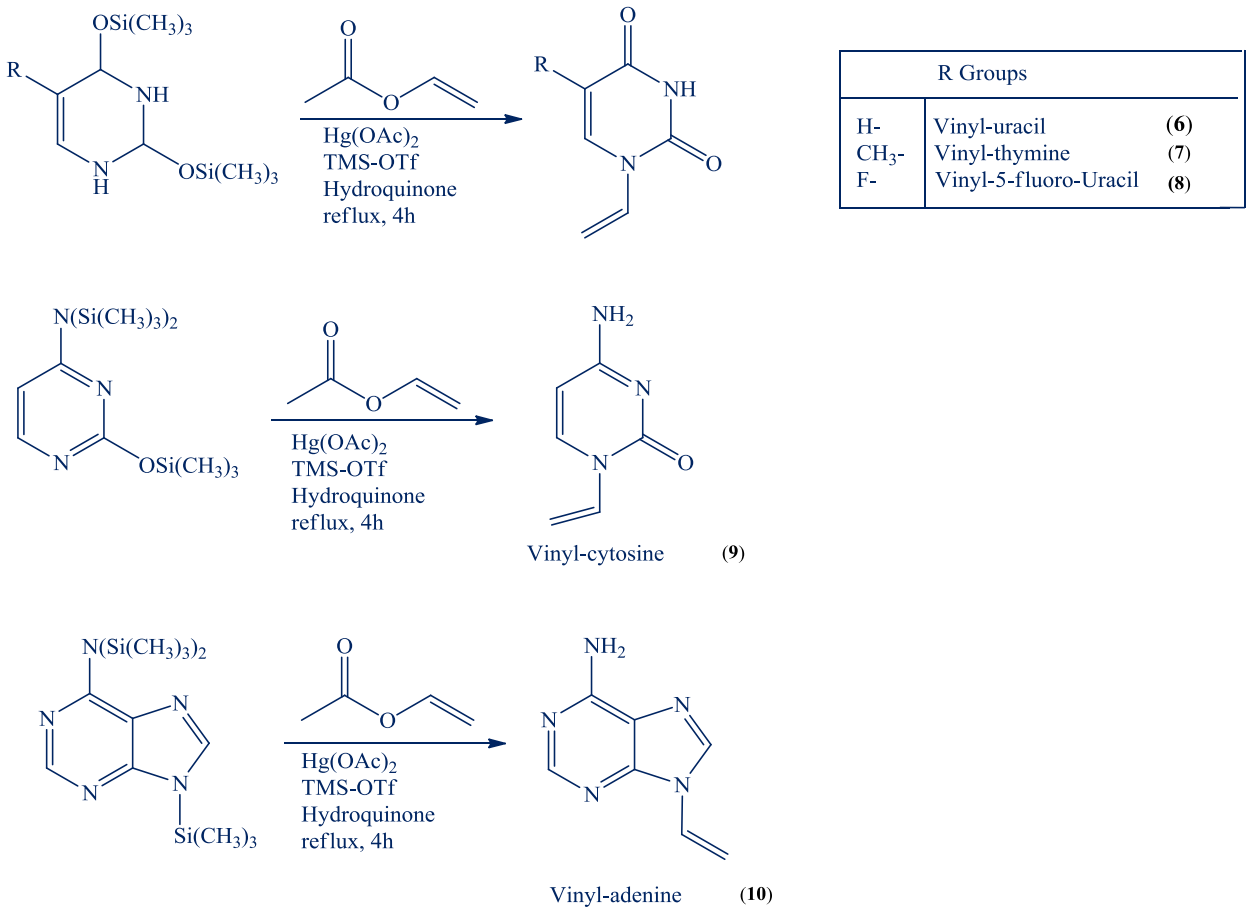
Now it is reported a simple one-pot procedure to prepare 1-vinyluracil (**6**), 1-vinylthymine (**7**), 1-vinyl-5-fluoro-uracil (**8**), 1-vinylcytosine (**9**), 9-vinyladenine (**10**), and 9-vinylguanine (**11**) using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst in direct exchange of the acetate group of vinyl acetate with pyrimidine and purine bases. It is adopted two different experimental procedures, one for the pyrimidine nucleobases (**1–3**) and vinyladenine (**4**) and the other one for the guanine nucleobase (**5**). A transient *in situ* trimethylsilyl protection of the pyrimidine and adenine nucleobases, before the vinyl exchange reaction, was sufficient to ensure good results. In **Scheme 7.7** is reported the first step of the pyrimidine and adenine nucleobases synthesis. The reaction in conducted to reflux for 1 hour in hexamethyldisylazane using trimethylsilyl chloride and traces of $(NH_4)_2SO_4$.



Scheme 7.7 *Silylation step of the synthesis of the pyrimidine nucleobases.*

The first step of reaction is the most important since the silylate formation must be carried out in an inert nitrogen atmosphere because these compounds are subject to nucleophilic attack of water present in the air which tend to hydrolyze the product.

Subsequently, the solvent is removed with rotavapor and the crude is subjected to second reaction step, under nitrogen inert atmosphere, as reported in **Scheme 7.8**.



Scheme 7.8 *Vinylation step of the synthesis of the pyrimidine nucleobases.*

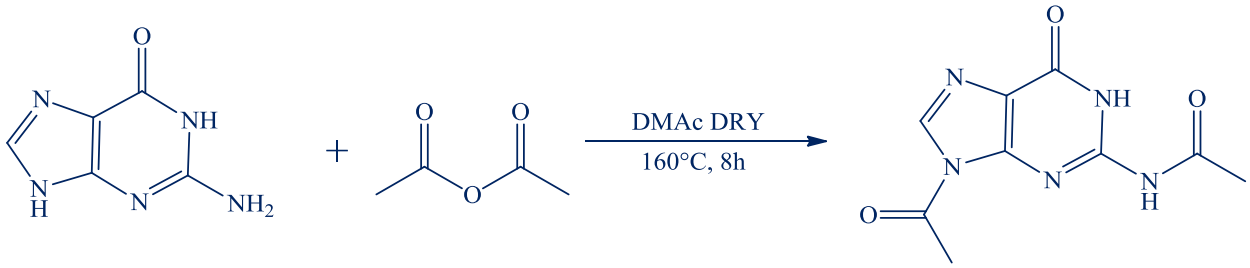
The second step of reaction leads to the formation of the five dipolarophiles with a reaction yield indicated in **Table 7.4**.

Vinyl-nucleobase	Yield (%)
1-vinyluracil (**6**)	93
1-vinylthymine (**7**)	95
1-vinyl-5-fluorouracil (**8**)	89
1-vinylcytosine (**9**)	85
9-vinyladenine (**10**)	92

Table 7.4 *Reaction yields for the synthesis of the vinyl-nucleobases.*

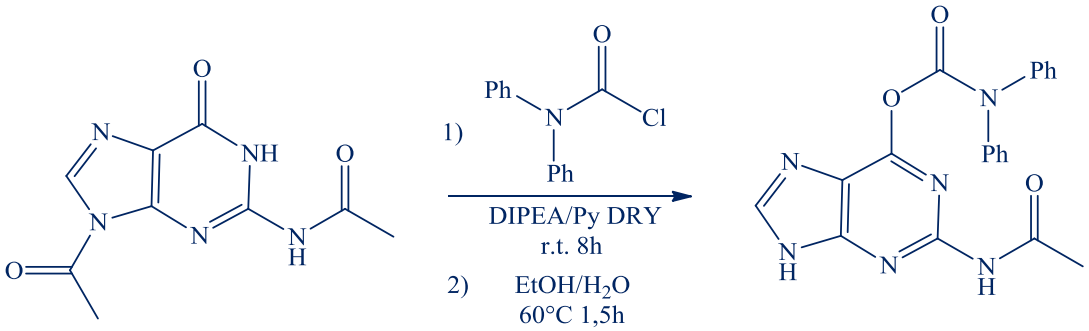
Trimethylsilyl triflate was already used as highly selective and efficient catalyst for nucleoside formation from silylated heterocycles and peracetylated sugars.[303] The use of TMSOTf as acid catalyst has permitted to obtain excellent yields for any nucleobase, especially if compared to the previously methods (2–65%) reported in literature.[304] Finally only a purification step is required with evident economic advantages. The low acidity of trimethylsilyl triflate, instead of the sulfuric acid used in the previous methods,[305] increases the selectivity of vinylation toward the

more basic nitrogen and performs generally cleaner reactions. In fact, it is obtained only low percentages of secondary reaction products such as, 1,3-divinyluracil (13%), *N*-acetyl-1-vinylcytosine (2,4%) and 7-vinyladenine (13%). The yields of byproducts drastically increase (10–60%) by the addition of small amounts of sulfuric acid.
TMSOTf is a Lewis acid able enough to promote the collapse of the intermediate formed in the equilibrium proposed as sequence of mechanism for the exchange vinyl reaction by Kaye et al.[306] Moreover, no protonated nucleobase is formed and therefore much more free nucleobase is present and more vinyl derivative is obtained. Finally, mercuric acetate is necessary to vinyl activation, in fact no reaction was obtained in the attempts to perform the vinyl exchange without it. The synthesis of the vinylguanine (**11**) is conduct by four reaction steps. The first reaction step is a protection of the amine groups by acetic anhydride in dimethylacetamide dry to 160°C for 8 hours (**Scheme 7.9**).



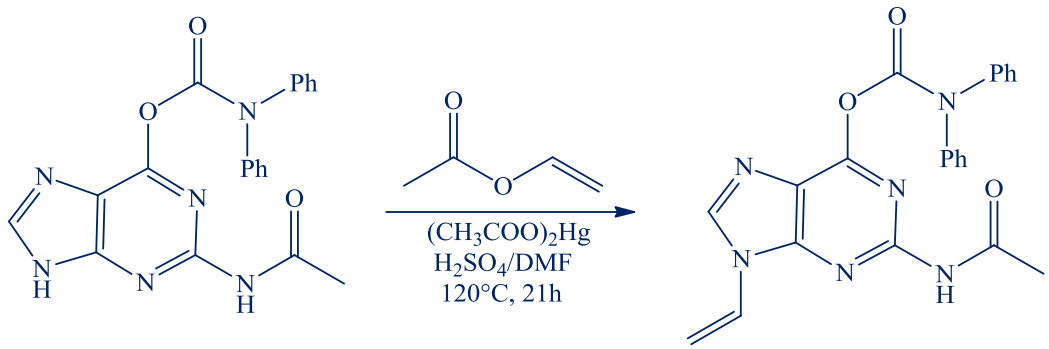
Scheme 7.9 *Synthesis of the 2,9-diacetyl-guanine.*

The second reaction step is an oxygen protection of the carbonyl group by *N*,*N*-diphenyl-carbamoyl chloride. The reaction is conduct with DIPEA in pyridine dry at room temperature for 8 hours, following to a deacetylation of the amide group in position 9 of the ring as shown in **Scheme 7.10**.



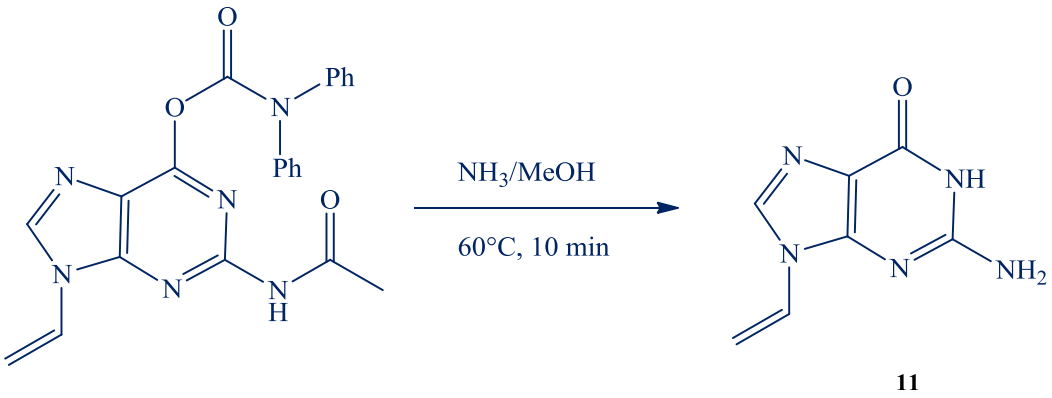
Scheme 7.10 *Synthesis of the 2-N-acetyl-6O-(N',N'-diphenylcarbamoyl)-guanine.*

The third step (**Scheme 7.11**) is the vinylation of the amine group in position 9 of the ring. This reaction in carry out using vinyl acetate as vinyl agent and mercury acetate with catalyst, the solvent is DMF dry and the acidic ambient is guaranteed by the presence of H_2SO_4.



Scheme 7.11 *Synthesis of the 2N-acetyl-9N-vinyl-6O-(N',N'-diphenylcarbamoyl)-guanine.*

The fourth step of the reaction is a deprotection of the amide group carry out by a solution of NH_3 dissolved in MeOH for 10 minutes at 60°C as shown in **Scheme 7.12**.



11

Scheme 7.12 *Synthesis of the 9N-vinyl-guanine.*

In conclusion, direct vinyl exchange of the acetate group of vinyl acetate with pyrimidine and purine bases eventually becomes a simple, fast and highly efficient procedure by the use of TMSOTf as acid catalyst. The chance to employ a nearly unique experimental method for the whole DNA and RNA base set makes, for the really first time possible, the preparation of these products from readily available starting materials.

7.4 SOLVENT-FREE AND MICROWAVE ASSISTED SYNTHESIS OF INDANYL AND ISATINYL SPIRO-ISOXAZOLIDINES *N,O*-NUCLEOSIDES

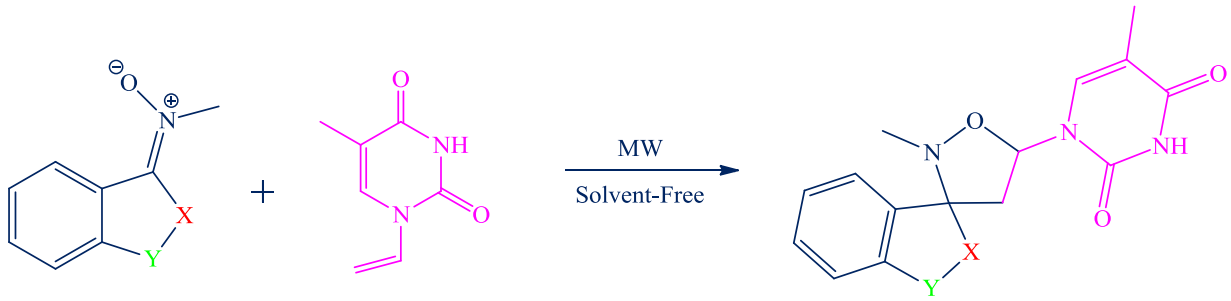
In this paragraph the MW-assisted solvent-free 1,3-dipolar cycloaddition synthesis of some nucleobase-containing spiroisoxazolidines with an indoline or an indane ring is illustred. The synthesis is obtained by reaction between nitrones as dipole and vinylnucleobases as dipolarophile. It is generally been recognized that the incorporating of different bioactive scaffolds into one molecule is the powerful strategy to construct substrates with structural

novelty and biological potential.

Considering that, some spiroindoline–isoxazolidines have shown different degrees of anticancer activity mainly based on the isoxazolidine ring fused at C-3 position of oxindole backbone,[307] it is possible to speculate that the simultaneous presence of an spiro carbon, an oxindole-like ring, an isoxazolidine portion and a nucleobase might generate novel molecular entities with amplified anticancer activity as, for example, inhibitors of MDM2-p53 interaction. Moreover, to our knowledge, in literature spiro-isoxazolidines with nucleobases and indoline or indane ring on their backbone have been never synthesized.

As is well known, the typical insertion of a nucleobase on a sugar or isoxazolidine scaffold consists of the method of Vorbrüggen,[308] but for a long time, now, in this thesis, it has developed a simple method by solvent-free MW-assisted 1,3-dipolar cycloaddition between nitrones as dipole and vinylnucleobases[309] as dipolarophile.[310]

The formation reactions between N-1-vinylthymine **7** and nitrones **4e** and **5a**, respectively, were taken as examples for optimization of reaction conditions. The target conjugates were prepared following the synthetic procedure similar to ketonitrones previously prepared, consisting of the co-grinding of the ketonitrone and vinylnucleobase in a mortar, followed by transfer of the mixture in an appropriate vessel and further mixing of the solids in a vortex without use of solvents; finally the solid mixture was placed in a microwave oven. The results are summarized in **Table 7.5**.



4e X=C=O, Y=NMe
5a X=Y=CH_2

7

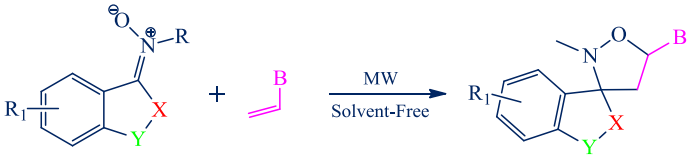
18 X=C=O, Y=NMe
12 X=Y=CH_2

Entry	Nitrone	MW (W)	T (°C)	Solvent	Time (min)	Product	Yield (%)
1	**4e**	650	180	-	30	**18**	59
2	**4e**	750	180	-	20	**18**	62
3	**4e**	850	180	-	10	**18**	85
4	**4e**	-	110	Toluene	4320	**18**	25
5	**5a**	650	180	-	10	**12**	22
6	**5a**	650	80	-	30	**12**	34
7	**5a**	650	125	-	30	**12**	38
8	**5a**	750	80	-	10	**12**	41
9	**5a**	750	100	-	30	**12**	61
10	**5a**	750	125	-	30	**12**	77
11	**5a**	-	110	Toluene	4320	**12**	20

Table 7.5 *Optimization of synthesis of nucleobase-containing spiro-isoxazolidines 17 and 12.*

It was prevalently changed the MW power that, based upon years of experience in microwave-assisted reaction, turns out to be the key parameter in MW-assisted cycloadditions, by keeping the nitrone/vinylnucleobase ratio to 2:1, respectively. The optimized conditions for the formation of **17** are listed in entry 3 (**Table 7.5**). On the contrary, the indanone nucleus obliged us to reduce maintained temperature (T=125°C) to avoid the formation of a lot quantity of byproducts (entries 5–10, **Table 7.5**). Despite everything, reaction times were major respect to isatin derivatives and yields were good (entry 10, **Table 7.5**). Finally, it was compared this results with those obtained from classical conditions of 1,3-dipolar cycloaddition, using toluene and reflux (entries 4 and 11, **Table 7.5**). It is possible to highlight that reaction times had to be extended to three days, obtaining very poor yields and many by-products.

Then, the reaction conditions of **18** and **12** were expanded to the formation of some substituted nucleobase-containing spiroisoxazolidines, using various nitrones and some vinyl-nucleobases (*N*-1-vinyluracil **6**, *N*-1-vinylthymine **7**, *N*-1-vinyl-5-F-Uracil **8**, *N*-1-vinylcytosine **9**, *N*-9-vinyladenine **10** or *N*-9-vinylguanine **11**). The results are collected in **Table 7.6**.



4 a-c, 4e, 4 i-j, 4m, 5a **6-11** **12-25**

B= ; ; ; ; ;

6 **7** **8** **9** **10** **11**

Entry	Nitrone	R	R_1	B	Product[a]	Time (Min)	Yield (%)	*exo/endo*
1	**5a**	Me	H	7	**12**	30	77	80:20
2	**5a**	Me	H	6	**13**	30	77	78:22
3	**5a**	Me	H	8	**14**	35	74	81:19
4	**5a**	Me	H	9	**15**	30	75	79:21
5	**5a**	Me	H	10	**16**	35	76	77:23
6	**5a**	Me	H	11	**17**	60	<10	-
7	**4e**	Bn	H	7	**18**	10	85	81:19
8	**4b**	Bn	H	6	**19**	12	84	83:17
9	**4c**	Ph	H	6	**20**	8	80	73:27
10	**4c**	Ph	H	7	**21**	8	80	72:28
11	**4a**	Me	H	6	**22**	10	85	82:18
12	**4i**	Me	5-Br	7	**23**	10	84	86:14
13	**4j**	Bn	5-Br	7	**24**	12	83	88:12
14	**4m**	Bn	5-NO_2	7	**25**	12	81	75:25

[a] 1.0 eq. of vinylnucleobases were used.

Table 7.6 *MW-assisted synthesis of nucleobase-containing spiro-isoxazolidines.*

All cycloadducts were isolated in good yields and short reaction times (8–30 minutes). The reaction crudes were subjected to Flash Chromatography for separate the mixture of the diasteroisomers by by-products and after, this diastereoisomers mixture was subjected to preparative direct-phase HPLC separation for obtain the isolated single diastereoisomer product. Only cycloadduct **17** (entry 6, **Table 7.6**) aren't obtained due to poor reactivity of the non-protected *N*-9-vinylguanine with the nitrones. The effect of substituents on indoline ring was also investigated, observing slightly yields in presence of bromo or nitro group as substituents because of formation of greater amount of by-products (entries 12–14, **Table 7.6**). All cycloaddition reactions were highly regioselective and goodly diasteroselective, furnishing only one regioisomer (5-substituted) and prevalent *exo*-adduct (**Table 7.6**), as confirmed through NOESY experiments depicted in **Figure 7.5**.

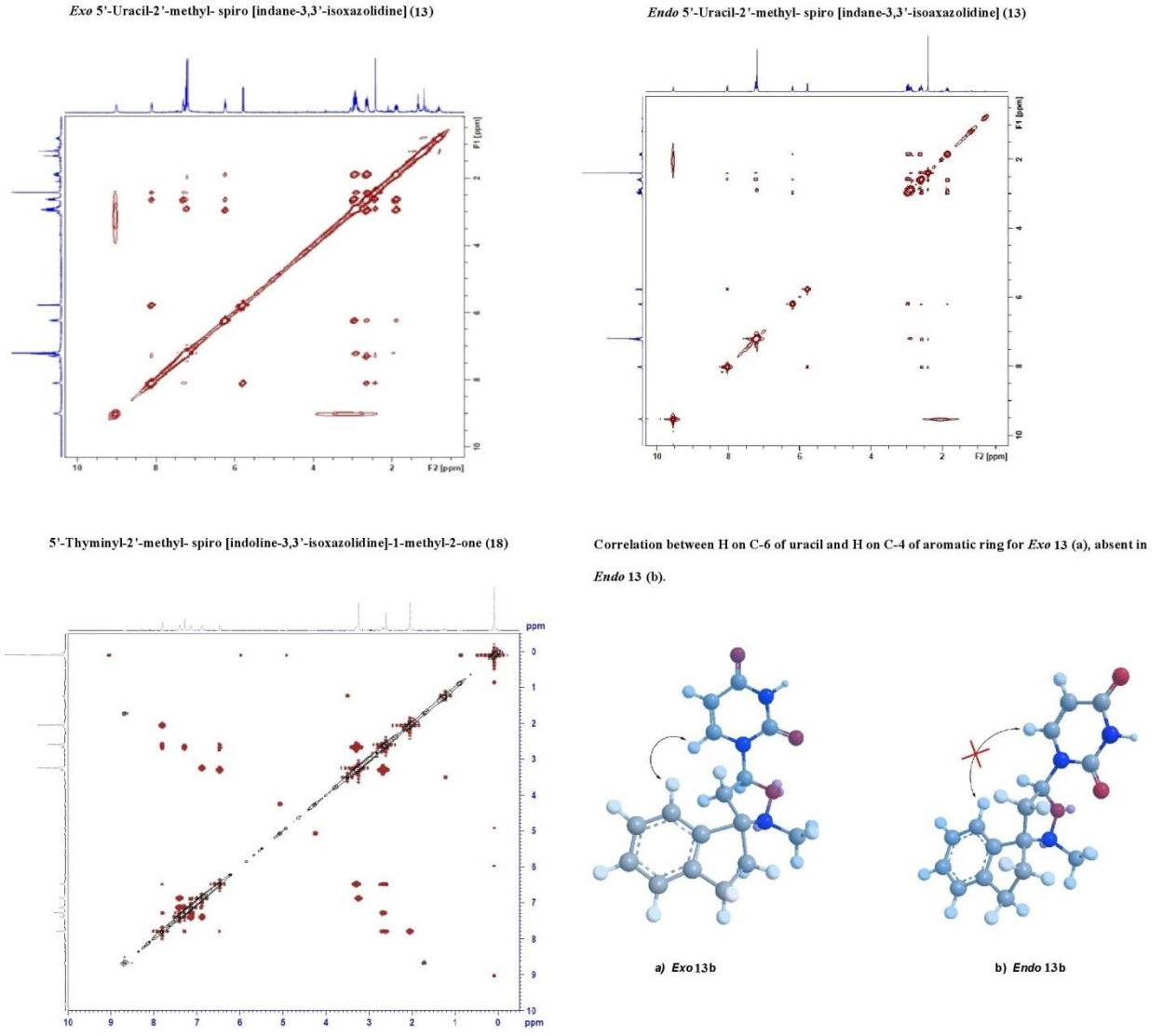


Figure 7.5 *NOESY experiments of the 13 and 18 compounds.*

In particular, we observed a specific correlation between the proton on C-6 of thymine or uracil and the proton on C-4 of aromatic ring, only possible in the *exo* approach of cycloaddition. In

fact, this approach is absent in the *Endo* product.

7.5 BIOLOGICAL ACTIVITY OF INDANOYL AND ISATINYL SPIRO-ISOXAZOLIDINES

Compounds **12**, **13**, **14**, **16 (Table 7.7**) were assayed for *in vitro* cytotoxicity against a panel of two human cancer cell lines: adenocarcinomic human alveolar basal epithelial cell line (A549) and human neuroblastoma cell line (SH-SY5Y).

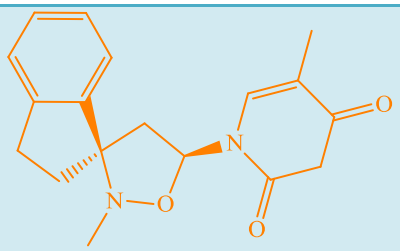
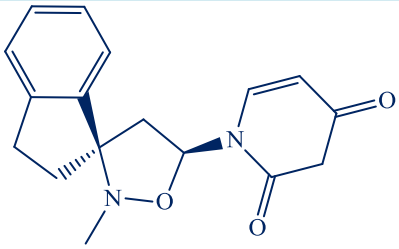
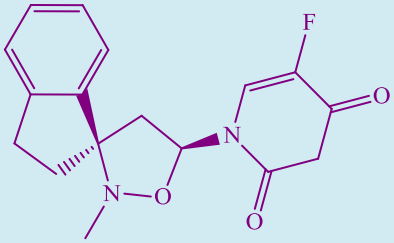
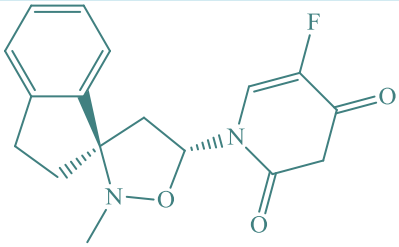
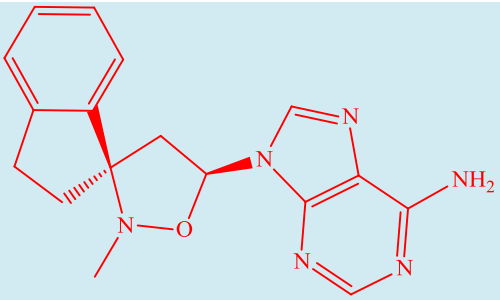
Product	Formule
12	
13	
14	
14'	
16	

Table 7.7 *Structural formules of heterocycles biologically tested.*

Initially all products *in vitro* were tested on adenocarcinomic human alveolar basal epithelial cell line (A549) to evaluate the percentage of cell viability by MTT assay.[311] The cells were cultured in assay plates and supplemented with 10% foetal bovine serum (FBS)/1% antibiotic/antimycotic solution for 24 h in a humidified incubator at 37 °C. This was followed by the addition of freshly prepared samples in medium at various concentrations (25 µM, 50 µM 100 µM 200 µM and 250 µM) with 10% dimethyl sulfoxide (DMSO) as a positive control. Following 24 h incubation, MTT solution (5 mg/mL in PBS, pH 7.4) was added to each well and incubated for 2 h at 37° C. The UV absorbance (A) was measured at 570 nm. The relative cell viability (%) was then calculated using Eq. (1):

$$\text{Cell viability (\%)} = (\text{A of treated cells}/\text{A of untreated cells}) \times 100 \quad \text{(eq. 1)}$$

Results are graphicated in **Figure 7.6**.

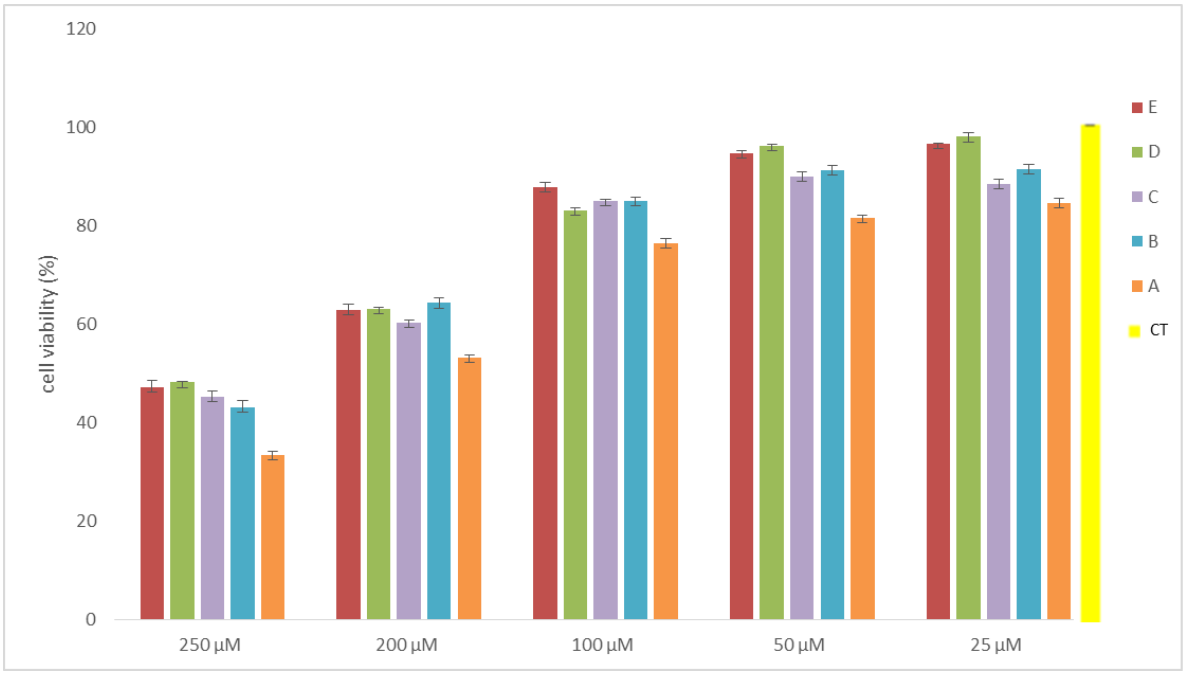


Figure 7.6 *A549 cell viability measured by MTT assay after 24 h exposure of the samples.*

When the concentration of the inhibitors is reduced the cell viability increasing. The best A549 cell inhibitors is product **12**. This latter was also tested *in vitro* on human SH-SY5Y neuroblastoma cell line by SRB assay[312] and the results are graphicated in **Figure 7.7**.
In the present screening experiment, the cells were inoculated in microtiter plates at 2 × 104 cells per well and allowed to attach overnight. Meantime, a stock solution of the drugs in dimethylsulfoxide 0,1 M was prepared. An aliquot of this stock solution was then diluted to 25 µM, 50 µM 100 µM 200 µM and 250 µM with the colture medium. After the addition of the different drug concentrations, the plates were incubated under standard conditions for 24 h; the assay was terminated by the addition of cold TCA and incubated for 60 min at 4 °C. Next, the plates were washed and then air-dried. After adding sulforhodamine B (SRB) solution at 0.4% (w/v) in 1% acetic acid to each well, the plates were incubated at room temperature for 30 min.

The bound stain was subsequently eluted with 10 mM unbuffered Tris-base solution (pH 10,5) and the plate was shaken for 15 min. The UV absorbance (A) was measured at 492 nm. The plate-by-plate examination of the test wells relative to control wells was employed to determine percent growth, and the ratio of the average absorbance in the test well to the average absorbance in the control wells × 100 was determined.

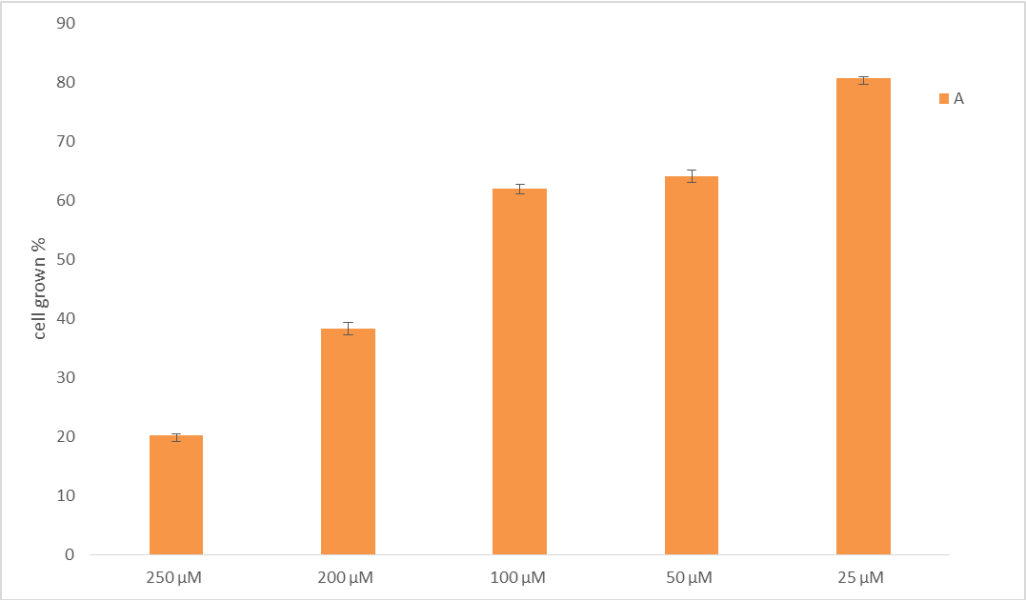


Figure 7.7 *A SH-SY5Y cell grown measured by SRB assay.*

In this case when the concentration of the inhibitors is reduced the cell viability increasing.
In conclusion, the two *in vitro* test have confirmed the good antiproliferative activity of our spiroisoxazolidines against cancer cells.

CHAPTER 8

CHAPTER 8 – SYNTHESIS OF ISOXAZOLIDIN-Gem-BISPHOSPHONIC ACIDS AT BIOLOGICAL ACTIVITY: RESULTS AND DISCUSSION

8.1 INTRODUCTION

Bisphosphonates (BPs) are a class of drugs characterized by both a structure and a stability analogue of naturally occurring pyrophosphates; they are tipified by two groups phosphonates attached to a single carbon atom, forming a P-C-P bond. They are be able to inhibit osteoclast-mediated bone resorption; therefore the BPs have been developed through many clinical trials in osteoclast-activating diseases, such as Paget's disease, hypercalcemia of malignancy and bone metastases.[313] In addition, the BPs may be used as successful antiresorptive agents in the prevention and treatment of osteoporosis, the most common senile bone disorder that is characterized by a decrease in bone mass with subsequent deterioration of in the bone skeleton.
Structurally they are also constituted of two side chains (usually defined as R^1 and R^2) covalently linked to a geminal carbon (**Figure 8.1**).[314] Generally, the strong affinity of the P-C-P moiety of bisphosphonates for bone mineral is enhanced depending of the nature of R^1 and R^2 groups.
When the bisphosphonates contain a nitrogen in side-chain R^2 (N-BPs), such in alendronate, pamindronate, risedronate and zoledronate (**Figure 8.1**),[315] BPs inhibit the mevalonate pathway and prevent the post-translational prenylation of GTP-binding proteins.[316]
Of particular importance is recent correlation between the activity of bisphonates N-BPs, especially those containing an amino heterocyclic ring,[317] and their inhibition to the farnesyl pyrophosphate (FPP) synthase enzyme.[318]

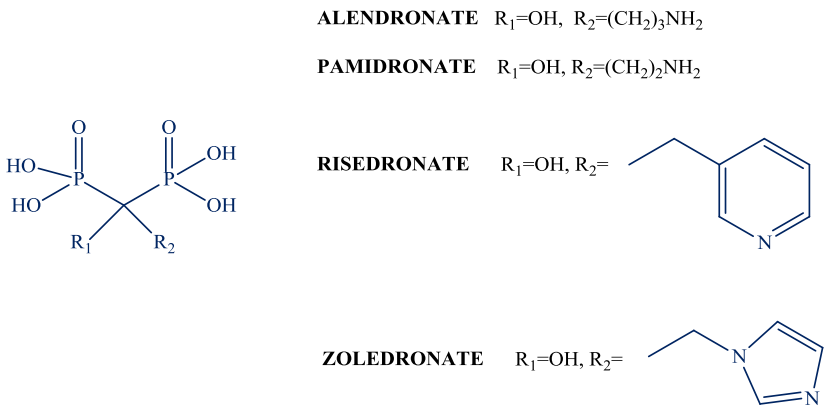


Figure 8.1 *Examples of active N-Bisphosphonates.*

Their ability to bind to bone mineral, avoiding both crystal growth and dissolution, is enhanced when a hydroxyl group is attached to the geminal carbon of the P-C-P group. In fact the presence of a *gem*-hydroxyl group in R_1 chain enhances the ability of these compounds to chelate calcium ions by tridentate rather than bidentate binding.[319] Therefore, the phosphonate groups, together a

hydroxyl group as R_1, give a high affinity for bone mineral, whereas an amine group as R_2 chain determine the biological activity of the drugs, influencing their capacity to interact with specific molecular targets (**Figure 8.2**).

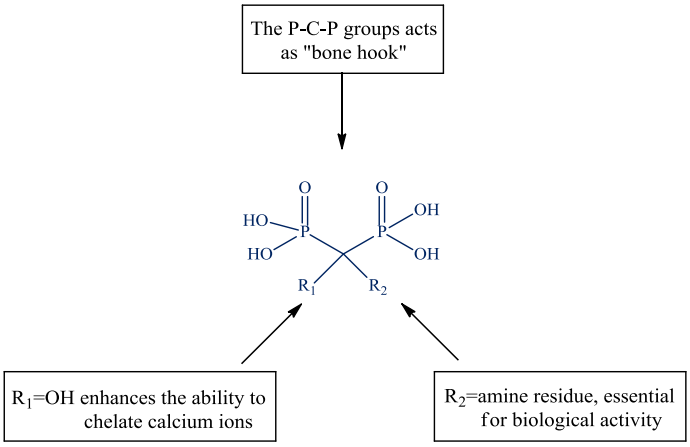
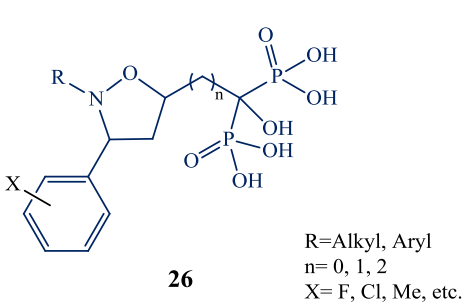


Figure 8.2 *Principal pharmacophore groups of the bisphosphonates.*

In addition, the activity seems to increase as a function of the structural relationships (distance from the P-C-P group and particular spatial configuration).[304] Moreover, the presence of an aromatic or a heterocyclic group on the backbone of BPs is also important for the biological activity, as reported in literature.[6]

All these considerations may be used to design and synthesize new and pharmacologically more active *N*-bisphosphonates based on the presence of a geminal hydroxyl group in R_1 and a variable length chain in R_2.

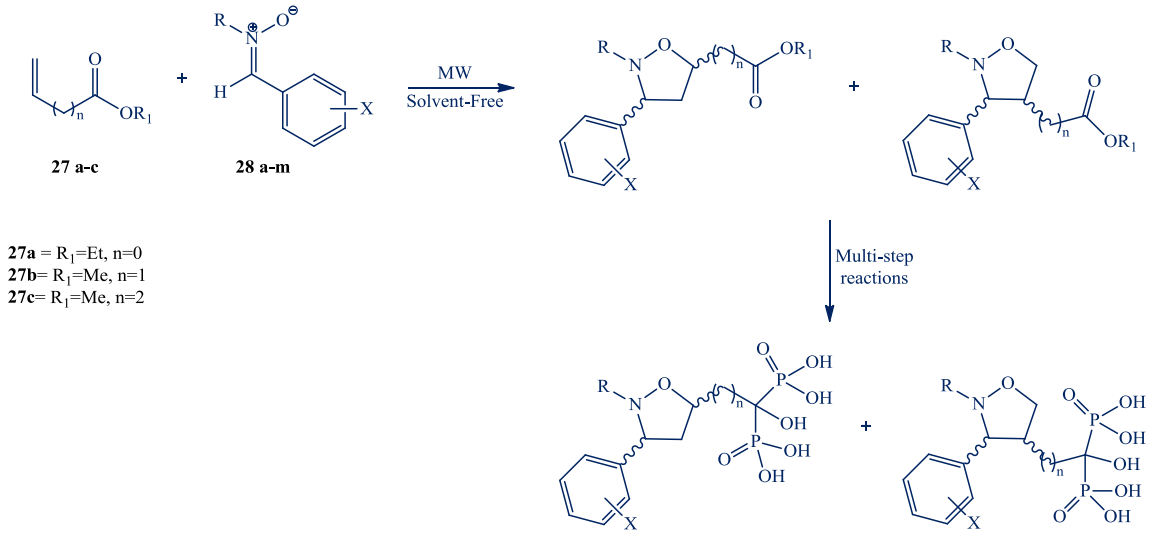
In our laboratory, leveraging from the experience and expertise in the field of thermal or microwave 1,3-dipolar cycloaddition in solvent free or green solvent condition,[320] previously a new class of isoxazolidine-substituted bisphosphonates having a cyclic nitrogen in *geminal* position was synthesized as potential biologically active molecules.[321] Consequently, with these results in hands it possible to design and realize a new type of nitrogen-bisphosphonates **26** containing both an isoxazolidine ring linked through a variable side chain to P-C-P bond and a geminal hydroxyl group to increase the chelation activity of bisphosphonates group (**Figure 8.3**). Furthermore, the presence of growing length chains and lipophilic aromatic groups on the isoxazolidine ring may also enhance the oral bioavailability that represents the major problem of the clinical utilization of bisphosphonates.[322]



26

Figure 8.3 *General structure of the isoxazolidin-gem-bisphosphonic acids.*

The synthetic route to isoxazolidine-bisphosphonates **26** involved the initial microwave-assisted 1,3 dipolar cycloaddition between vinyl esters **27a-c**, commercially availables, and appropriate nitrones **28 a-m**,[323] synthetized in according to reaction reported in paragraph 8.2, in solvent-free reaction conditions followed to multi-step reaction on the ester group to introduce the bisphosphonates function (**Scheme 8.1**).



Scheme 8.1 *Multi-step reaction synthesis of the Isoxazolidin-gem-bisphosphonic acids.*

8.2 SOLVENT-FREE SYNTHESIS OF THE ALDONITRONES USED AS 1,3-DIPOLES FOR ISOXAZOLIDINE-gem-BISPHOSPHONIC ACID PREPARATIONS

Here it wish to present a greener approach consisting in the direct synthesis of various nitrones by solvent-free condensation of primary aryl- or alkylhydroxylamine hydrochlorides with aromatic aldehydes under microwave irradiation. The advantages are short reaction times, high yields, clean reactions without formation of by-products and related procedures of purification, absence of metal catalysis, typical conveniences of microwave application.

Initially, we selected the condensation between benzaldehyde and *N*-methylhydroxylamine hydrochloride **1a** as a model reaction by using an laboratory microwave oven, in absence of solvent and base (**Scheme 8.2**).

Scheme 8.2 *Model reaction to synthesize nitrones.*

Preliminary results, collected in **Table 8.1**, were obtained by varying the main reaction factors i.e. MW power, reactants molar ratio, and reaction time.

Entry	Base[b]	Molar Ratio	MW (W)	T (°C)	Time (min)	Product	Yield (%)[a]
1	-	1:1	450	-	15	**28a**	52
2	-	1:1	600	-	5	**28a**	92
3	-	1,5:1	600	-	5	**28a**	79
4	NaOAc	1,5:1:1,3	600	-	5	**28a**	90
5[c]	-	1:1	600	-	5	**28a**	53
6[d]	-	1:1	-	160	1440	**28a**	12

[a] Isolated Yield. [b] *N*-Methylhydroxylamine hydrochloride is used. [c] *N*-Methylhydroxylamine is used. [d] Classical condition are used.

Table 8.1 *Formation conditions of N-Methyl-C-Phenyl-nitrone* ***28a****.*

The procedure is simple and consists of mixing the two components in a vortex, followed by transfer of the mixture in an apposite vessel, which is placed within a microwave oven, at 450-600 W irradiation power, for times ranging from 5 to 15 min. After the appropriate time, monitored by TLC, hot EtOAc was added to the reaction mixture, that appeared as a syrup, producing the product as a solid precipitate.

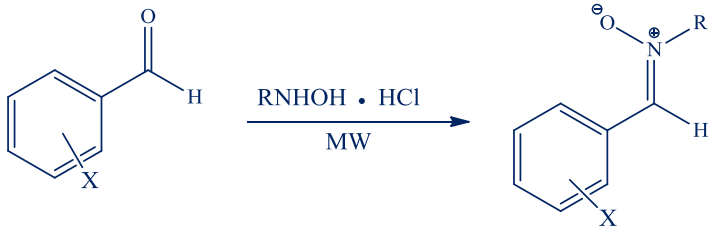
The isolated product was subjected to ^{1}H, ^{13}C-NMR spectroscopy and HRMS analysis, to confirm the formation of **28a**.

As shown in **Table 8.1**, the use of MW power at 450W (entry 1) does not lead to the complete formation of compound **28a** for which it is necessary to apply an irradiation at 600W (entries 2-5). Moreover, choosing the 1.5:1 ratio of the benzaldehyde/**1a** couple (entry 3), lower yields and formation of by-products is observed. The stoichiometric ratio of aldehyde and hydroxylamine hydrochloride actually shows three advantages: (1) quantitative yields, (2) no formation of by-products and (3) the absence of work-up and lengthy purification steps. In agreement with literature reports, the stereochemical outcome of the reaction shows the highly selective formation of the more stable Z form (Hassan et al., 1998), that we confirmed by ^{1}H-NMR spectra and by comparison with literature data.

The model reaction was confronted with the results obtained when the compound 1a was allowed to react with a slight excess of benzaldehyde (1.2 eq) and a base (1.3 eq of NaOAc) (entry 4). Although the yield was high (90%), it is possible to conclude that the use of a base is not necessary for the progress of the reaction. Moreover, when the nitrone **28a** was formed by reaction between the aromatic aldehyde and neutral *N*-methylhydroxylamine, a yield of 53% was observed, supporting the thesis that the presence of hydroxylamine hydrochloride is necessary for the complete performance of the reaction (entry 5). This result is in agreement with the well-known requirement of acidity in several nucleophilic additions to carbonyl compounds, such as the formation of an enamine. Whereas either using directly the hydroxylamine hydrochloride or in the presence of sodium acetate (the in situ formed acetic acid is present in the medium), the reaction performs well (90-92%), a lower yield (53%) is observed. In addition a further experiment was carried out by heating the reaction between benzaldehyde and *N*-

methylhydroxylamine·HCl **1a** (entry 6) without adding of solvents; in this case a very low formation of nitrone **28a** it observed, thus confirming for this method the importance of microwave irradiation.

With these results in hands, next both the effect of different substituted hydroxylamine hydrochlorides and different substituted benzaldehydes are explored by using various aromatic aldehydes and *N*-methyl (**1a**), *N*-benzyl (**1c**) and *N*-phenyl hydroxylamine hydrochloride (**1d**). The results obtained are shown in **Table 8.2**.



Entry	X	R	Product	Time (min)	Yield (%)
1	H	Me	**28a**	5	92
2	2-Cl	Me	**28b**	12	92
3	3-Cl	Me	**28c**	13	91
4	4-CN	Me	**28d**	25	80
5	2-F	Me	**28e**	10	91
6	4-F	Me	**28f**	12	92
7	4-Me	Me	**28g**	4	91
8	4-OMe	Me	**28h**	3	91
9	4-N(Me)$_2$	Me	**28i**	4	92
10	H	Bn	**28j**	4	94
11	2-Cl	Bn	**28k**	15	95
12	4-CN	Bn	**28l**	25	83
13	2-F	Bn	**28m**	15	93
14	4-Me	Bn	**28n**	5	90
15	4-OMe	Bn	**28o**	4	93
16	4-N(Me)$_2$	Bn	**28p**	4	94
17	H	Ph	**28q**	5	93
18	2-Cl	Ph	**28r**	8	97
19	2-F	Ph	**28s**	8	95
20	4-Me	Ph	**28t**	3	93
21	4-OMe	Ph	**28u**	5	92
22	4-CN	Ph	**28v**	25	82
23	4-N(Me)$_2$	Ph	**28z**	3	96

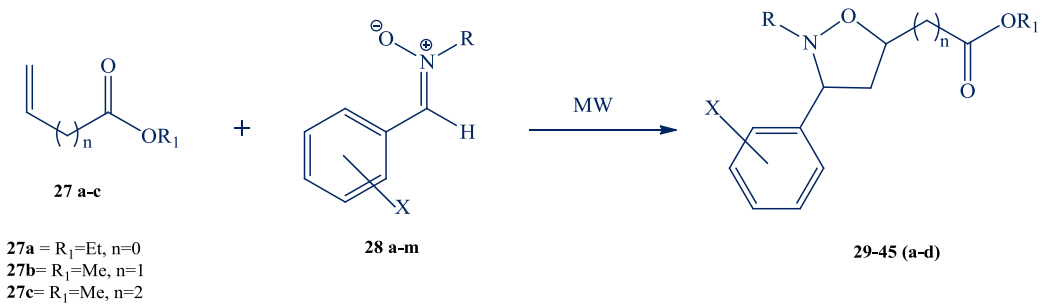
Table 8.2 *Synthesis of nitrones from various aldehydes and N-substituted hydroxylamines hydrochlorides.*

All reactions afforded the desired product in yields varying from very good to excellent and high

purity. In almost all cases the procedure does not require particular work-up and the products can be separated simply by adding hot EtOAc and a small amount of hexane if necessary. Slightly lower yields were observed only in one case (entry 22), associated with the incomplete consumption of the reagents and with the formation of uncharacterized by-products. Consequently, flash-chromatography purification are required.

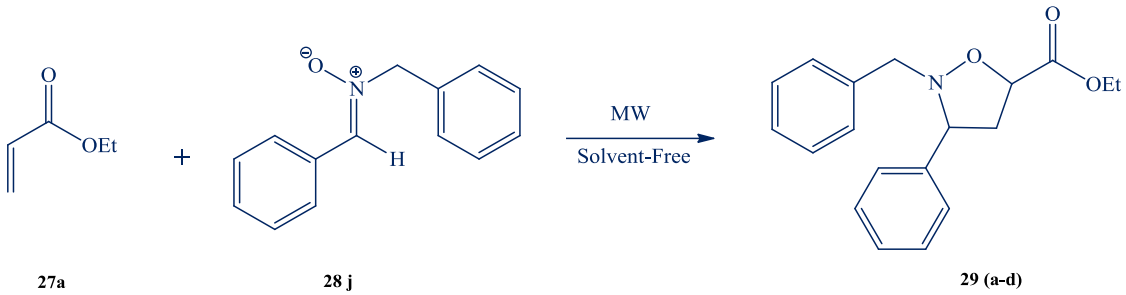
8.3 SOLVENT-FREE SYNTHESIS OF ISOXAZOLIDINE ESTERS USED AS INTERMEDIATES FOR BISPHOSPHONIC ACIDS.

With the nitrones in hand, the solvent-free microwave-assisted synthesis of aryl isoxazolidine esters was realized. The synthetic route to isoxazolidine involved the 1,3 dipolar cycloaddition between vinyl esters **27a-c** and appropriate nitrones[324] **28a-m** in solvent-free reaction conditions as shown in **Scheme 8.3**.



Scheme 8.3 *Synthesis of the Aryl-isoxazolidine esters.*

The procedure is simple and straightforward consisting of the co-mixing of the two components without use of solvents in a vessel that is placed within a microwave oven for an appropriate times, MW-power and molar ratio. Initially the reaction conditions are optimized for the target cycloaddition reported in **Table 8.3**.



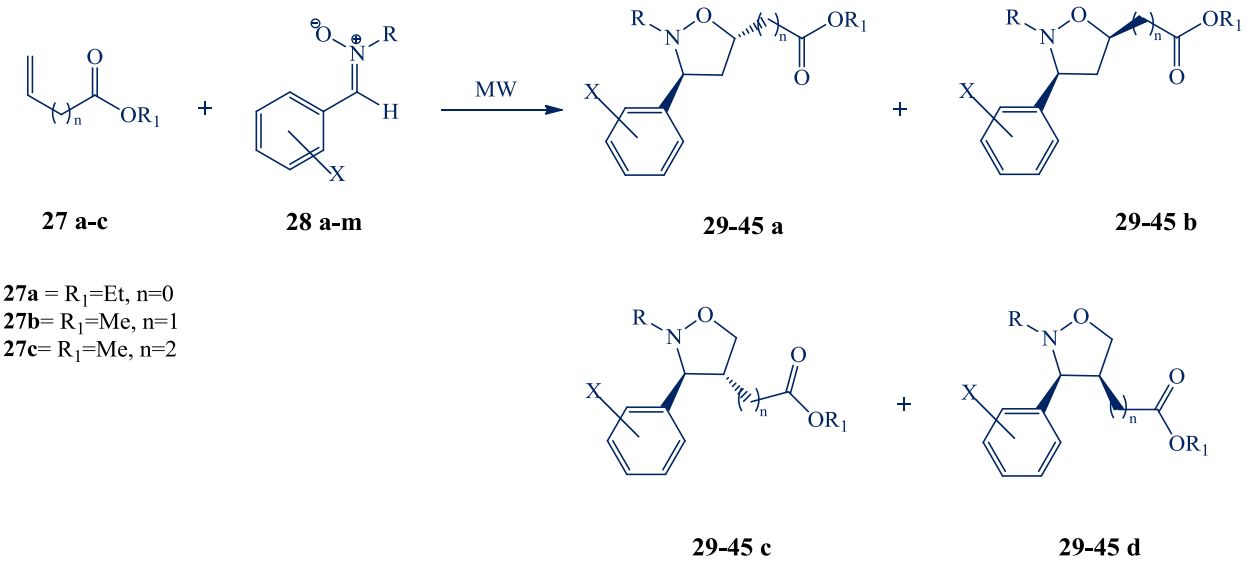
27a 28 j 29 (a-d)

Entry	P (W)	T (°C)	Time (min.)	Molar ratio	Yield (%)
1	750W	88°C	3	1:2	60
2	750W	114°C	4	1:2	72
3	750W	124°C	5	1:2	81
4	750W	138°C	6	1:2	95
5	750W	149°C	7	1:2	90
6	650W	118°C	6	1:2	73
7	600W	123°C	6	1:2	68
8	850W	150°C	6	1:2	62
9	750W	140°C	6	1:1.5	89
10	750W	138°C	6	1:3	95

Table 8.3 *Optimized condition for the target reaction.*

The experience in microwave-assisted solvent-free synthesis of the isoxazolidines dictated the use of 750W, a dipole/dipolarophile molar ratio of 1:2 for 3 minutes as initial conditions (entry 1, **Table 8.3**). The reaction yield improves when the time is increased up to a maximum of 6 min (entries 2-4), while the high temperature favors the by-product formation that cause a decrease in yields (entry 5). A mono-variate screening of the power and molar ratio is carry out and its possible to observe that the best conditions corresponding to entry 4 of the **Table 8.3**.

On the basis of these conditions different 1,3-dipolar cycloadditions have been conducted using various nitrones (**28 a-k**) as 1,3-dipoles and several vinyl esters (**27 a-c**) as dipolarophiles. The results are reported in **Table 8.4**.



27a = R_1=Et, n=0
27b= R_1=Me, n=1
27c= R_1=Me, n=2

Entry	Vinyl ester	Nitrone	Cycloadducts	Time (min)	Total Yield (%)	Ratio of Adducts a:b:c:d
1	**2a**	**28j**	**29 (a-d)**	8	98	51 : 24 : 20 : 5
2	**2a**	**28k**	**30 (a-d)**	6	98	52 : 23 : 21 : 4
3	**2a**	**28m**	**31 (a-d)**	8	97	50 : 25 : 20 : 5
4	**2b**	**28j**	**32 (a-d)**	23	95	46 : 15 : 26 : 13
5	**2b**	**28k**	**33 (a-d)**	22	96	45 : 15 : 30 : 10
6	**2b**	**28m**	**34 (a-d)**	21	97	47 : 17 : 28 : 8
7	**2c**	**28j**	**35 (a-d)**	16	97	45 : 15 : 33 : 7
8	**2c**	**28k**	**36 (a-d)**	15	96	49 : 14 : 20 : 7
9	**2c**	**28m**	**37 (a-d)**	16	95	48 : 19 : 27 : 6
10	**2a**	**28a**	**38 (a-d)**	6	97	57 : 18 : 17 : 8
11	**2a**	**28g**	**39 (a-d)**	6	94	45 : 17 : 24 : 14
12	**2a**	**28e**	**40 (a-d)**	6	98	58 : 12 : 22: 8
13	**2a**	**28f**	**41 (a-d)**	8	97	43 : 18 : 27 :12
14	**2a**	**28b**	**42 (a-d)**	6	97	60 : 15 : 17 : 8
15	**2a**	**28c**	**43 (a-d)**	6	98	59 : 17 : 16 : 8
16	**2b**	**28b**	**44 (a-d)**	21	98	48 : 12 : 28 : 12
17	**2c**	**28b**	**45 (a-d)**	15	97	46 : 14 : 28 : 12

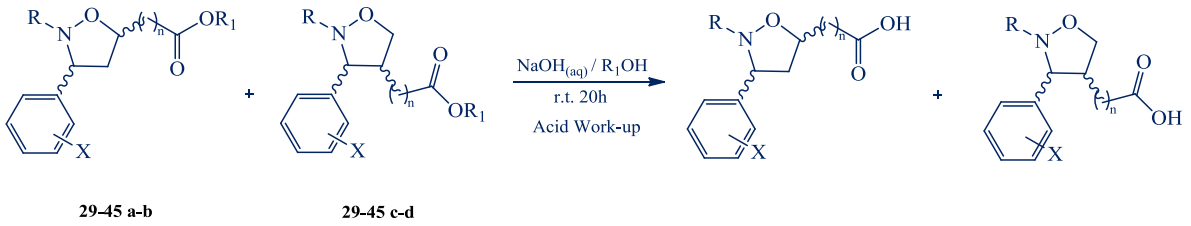
Table 8.4 *1,3-dipolar cycloadditions for several nitrones and vinyl esters.*

As shown in **Table 8.4**, the cycloadducts are formed for $(CH_2)_n$=0 as a mixture of four distereoisomers (**29-45 a,b,c and d**) of two regioisomers (5 and 4-substituted isomers respectively) with a high *trans–cis* stereoselectivity of the most abundant 5-substituted isomer, in according to literature data.[325] Alternatively, for $(CH_2)_n$ = 1, 2 *trans* and *cis* isomers of 5-substituted isoxazolidines were only observed with a remarkable abundance of *trans* isomer.[326] We chose the vinyl esters (**2a-c**) with $(CH_2)_n$ side chains until n=2, considering that the nitrogen atom in R must be a critical distance away from bisphosphonates group and that longer chains

may inactivate the substrates.
Moreover, the presence of a substituted aromatic ring deriving from nitrones **28 a-k** may ensure additional secondary interactions of BPs 1 with enzymatic target and induce possible biological activity to substrates, as demonstrated for isoxazolidines with similar substituents.[327] As it is possible to observe reaction times, yields and stereoselectivity do not seem to depend on all these kinds of variations.

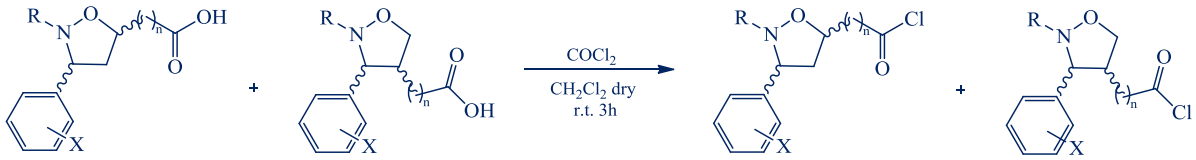
8.4 SYNTHESIS OF THE ISOXAZOLIDIN-Gem-BISPHOSPHONIC ACIDS

The no-separated regioisomeric *trans-cis* mixture of cycloadducts **29-45 a-d** were transformed in desired compounds **46-62 a-d** by conversion of the ester group in bisphosphonic acids in four synthetic steps.
First step consists in a transformation of the ester group in a carboxylic group by hydrolysis reaction as shown in **Scheme 8.4**.



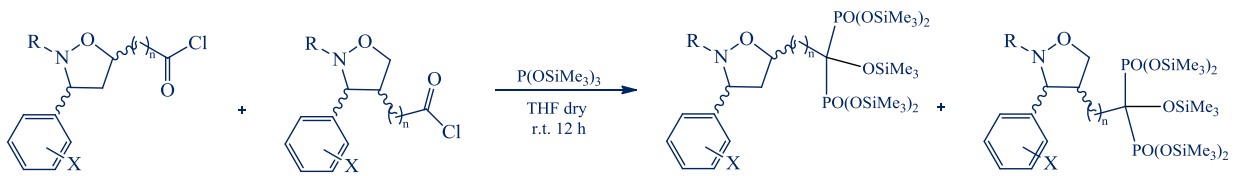
Scheme 8.4 *Hydrolysis of the ester group to carboxylic acid.*

The carboxylic acid is used without isolation to prepare the corresponding acyl chloride derivatives as shown in **Scheme 8.5**.



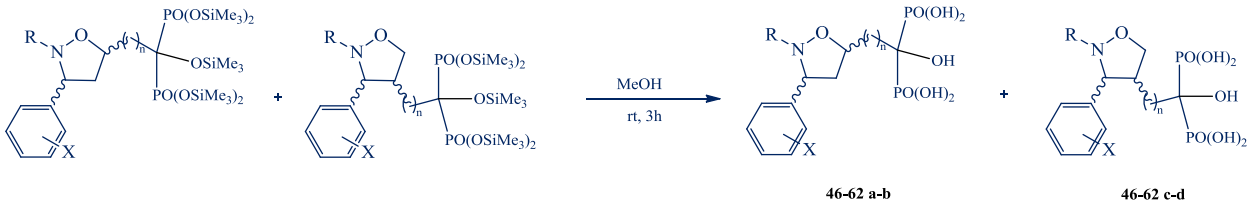
Scheme 8.5 *Formation of the acyl chloride by use of oxalyl chloride.*

The directly treatment of chloride substrates with two equivalents of tris(trimethylsilyl)posphite at room temperature give *gem*-bisphosphonic group on isoxazolidine ring as reported in **Scheme 8.6**.



Scheme 8.6 *Introduction of the bisphosphonic function on the carboxylic function.*

Fourth step of the reaction is the hydrolysis of the tris(trimethylsylil) groups with MeOH to give the isoxazolidin-*gem*-bisphosphonic acids **29-45 a-d** as shown in **Scheme 8.7**.



Scheme 8.7 *Hydrolysis of the tris(trimethylsylil) groups with MeOH.*

In the **Table 8.5** it is possible to observe the reaction yield for all product synthetized.

Entry	Ester	Compounds	Yield (%)
1	**29**	**46**	87
2	**30**	**47**	86
3	**31**	**48**	88
4	**32**	**49**	89
5	**33**	**50**	87
6	**34**	**51**	85
7	**35**	**52**	88
8	**36**	**53**	89
9	**37**	**54**	85
10	**38**	**55**	84
11	**39**	**56**	86
12	**40**	**57**	83
13	**41**	**58**	84
14	**42**	**59**	86
15	**43**	**60**	87
16	**44**	**61**	85
17	**45**	**62**	86

Table 8.5 *Reaction yield for all synthetized product.*

Purification of this products are carry out by dissolution in MeOH followed by coprecipitation with diethyl ether. For as reported in Table 8.5 all products are obtained as an unseparable mixture of regio- and diatereoisomers with good reaction yields.
At this moment the preparative HPLC is effecting to separate the isomers. Furthermore these isolated isomers will be subjected to biological *in vitro* test and enzymatic test to evaluate their pharmacological activity.

CHAPTER 9

CHAPTER 9 - SYNTHESIS OF POTENTIAL BIOLOGICAL ACTIVE 1,2,3-TRIAZOLES IN HOMOGENEOUS $Er(OTf)_3$/[mPy][OTf]/H_2O AS CATALYTIC RECICLABLE SYSTEM: RESULTS AND DISCUSSION

9.1 INTRODUCTION

1,2,3-Triazoles are five member *N*-heterocyclic compounds and are a class of compounds stable to metabolic degradation (**Figure 9.1**).

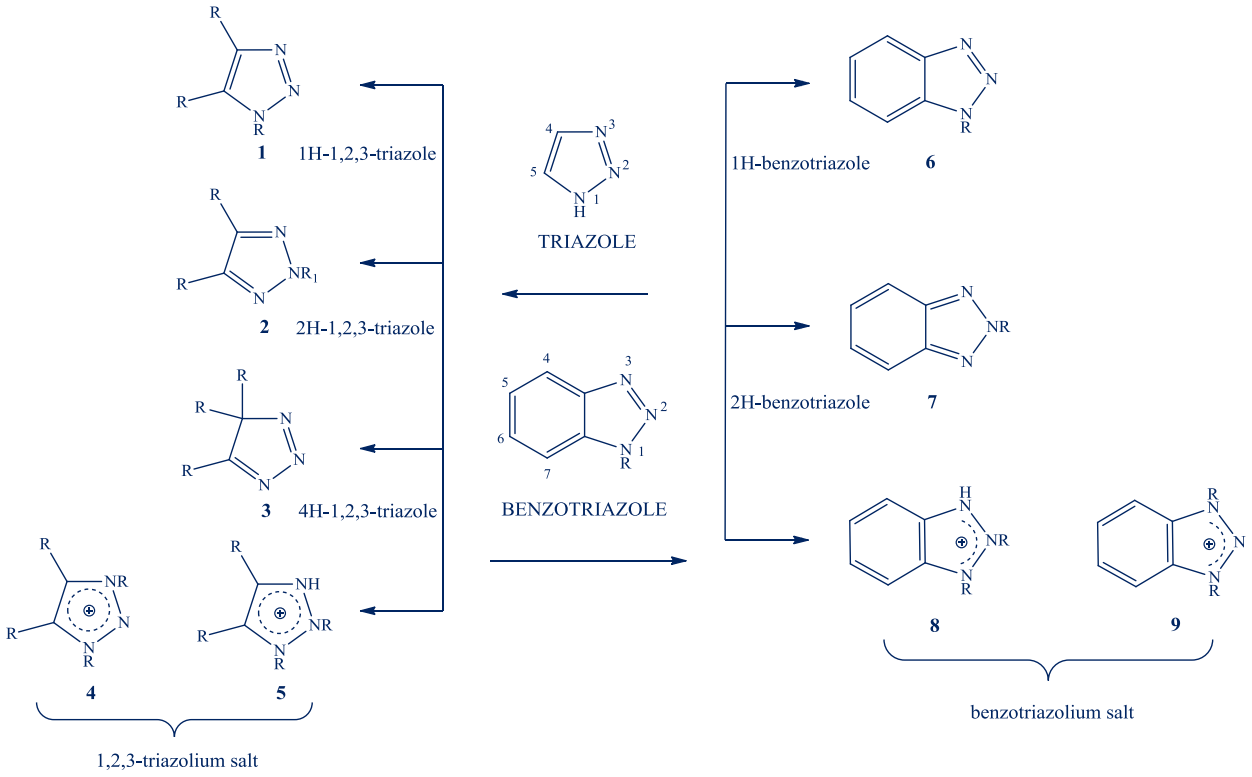
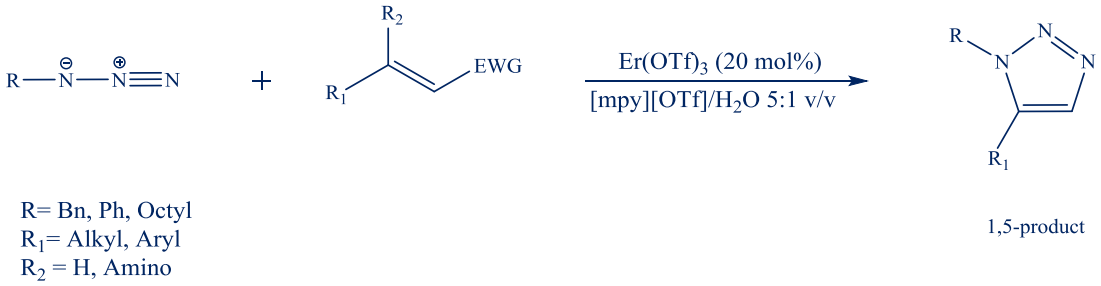


Figure 9.1 *Classification of 1,2,3-Triazoles.*

They are capable of hydrogen bonding, which can be favorable in the binding of biomolecular targets and can improve the solubility. Although absent in nature, the 1,2,3-triazoles have found a broad spectrum of biological applications such as anti-tubercular,[328] antibacterial,[329] anti-allergic,[330] anti-HIV,[331] anti-fungal,[332] anti-inflammatory,[333] anticancer,[334] and a-glycosidase inhibitor activities.[335] β-Lactum antibiotic Tazobactum, anticancer compound carboxyamidotriazole (CAI), cefatrizine are some drugs available in the market that possess 1,2,3-triazole moiety.[336] Many approaches for the synthesis of 1,2,3-triazoles have been developed so far, in which a typical click reaction, copper(I)-catalyzed azide-alkyne

cycloaddition (CuAAC) is definitely the most efficient strategies.[337]
Rare earth metal triflates $M(OTf)_3$ (M = Sc, La, Ce, Y; OTf= CF_3SO_3) have been efficiently used also to promote cycloaddition reactions with a significant increase of product yield and *endo:exo* stereoselectivity.[338] In particular some of these studies have been performed in ionic liquids,[339] with all the distinct advantages pertaining to these solvents as no measurable vapor pressure, easy catalyst and solvent recover/recycle and high solubility of the Lewis acid in these media. Ionic liquids (ILs) have received in last years a good deal of attention since classical organic reactions, including cycloadditions reactions, can be performed in these media with great advantages (yield and selectivity) as compared to conventional conditions.
A simple, catalytic, eco-friendly and efficient method for the synthesis of 1,2,3-triazoles potentially active was realized in high stereoselective way. The reaction is conducted between an aryl- or alkyl azide used as 1,3-dipole and a deactivated dipolarophile as various ω-nitrostyrenes or enaminones in a homogeneous catalytic system composed of $Er(OTf)_3$/[mPy][OTf]/H_2O (20 mol % / 5:1 v:v) (**Scheme 9.1**).



Scheme 9.1 *Generic catalyzed regioselective synthesis of 1,2,3-Triazoles.*

The synthetic strategy starts with the preparation of azides, one of the reaction partners of Erbium-catalyzed cycloaddition reaction, followed by preparation of deactivate dipolarophiles. Finally, with the starting materials in hand the triazoles are synthetized.

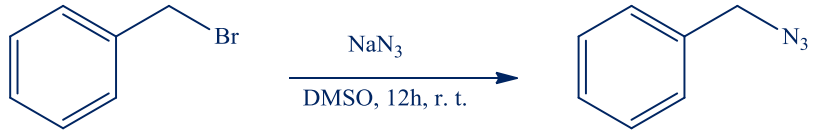
9.2 SYNTHESIS OF ALKYL- AND ARYLAZIDES USED AS 1,3-DIPOLE FOR THE 1,2,3-TRIAZOLES PREPARATION.

The azide functionality is a common progenitor for the synthesis of *N*-containing compounds and not only reacts with nucleophiles and electrophiles but also serves as a nitrene precursor upon thermolysis or photolysis.[340] Over the past decade, new perspectives have been developed for the use of azides in peptide chemistry,[341] combinatorial chemistry[342] and heterocyclic synthesis.[343] Current leading anti-HIV 1 drugs of purine and pyrimidine nucleotides contain an azide group.[344] In addition, azides can serve as precursors to biologically active compounds.[345] Recently, a set of synthetic protocols termed “Click Chemistry” has attracted enormous attention in medicinal chemistry and related areas of research, in which azides undergo 1,3-dipolar cycloaddition with terminal alkynes in the presence of a copper catalyst.[346]
Alkyl azides are generally obtained by heating an alkyl halide with sodium azide in DMSO or DMF. The work-up for this procedure involves extraction into an organic solvent (*e.g.*

DCM/$CHCl_3$/EtOAc/Et_2O) followed by evaporation to yield the azide.[347]
In this work the synthetized azides are:

- Benzylazide **46**,
- Octylazide **47**,
- Phenylazide **48**.

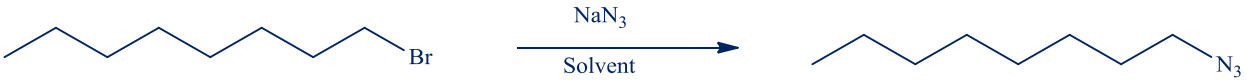
The synthesis of the benzylazide is conducted by nucleophile substitution of the benzyl bromide with sodium azide in according to **Scheme 9.2**.



46

Scheme 9.2 *Synthesis of the Benzylazide.*

The reaction is conducted to room temperature for 12 hours and give a product with a 73% yield.
The synthesis of the octylazide is conducted in according to **Table 9.1.**

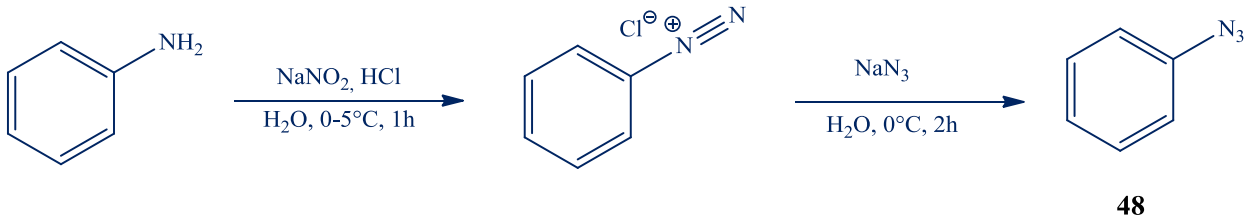


47

Entry	Solvent	Catalist (mol %)	T (°C)	t (h)	Yield (%)
1	DMSO	-	r.t.	24h	<10
2	DMSO	-	100°C	24h	35
3	H_2O/THF 8:2 v:v	-	r.t.	24h	<10
4	H_2O/THF 8:2 v:v	-	reflux	24h	25
5	DMSO	$FeCl_3$ (20)	r.t.	12h	95

Table 9.1 *Optimized reaction conditions for the synthesis of the octylazide 47.*

Initially the reaction was carried out using the same conditions tested for the benzylazide synthesis but the reaction yield was unsatisfactory (entry 1, **Table 9.1**). Therefore the reaction was conducted in the DMSO at reflux obtaining a 35% of yield (entry 2). A third experiment was attempted with different solvent. In entries 3 and 4 a mixture of H_2O/THF 8:2 v:v was used at room temperature and reflux. Unfortunatly the reaction yield was further unsatisfactory. Finally the reaction was conducted with addition of a catalyst $FeCl_3$ to improve the octyl bromide electrophilicity. The synthesis was performed in DMSO at room temperature to give the expected product with a 95% of yield.
The synthesis of the phenylazide starts with the formation of the diazonium salt intermediate from aniline. This latter reacts with sodium azide in aqueous inert ambient to give the desired phenylazide in according to the **Scheme 9.3**.



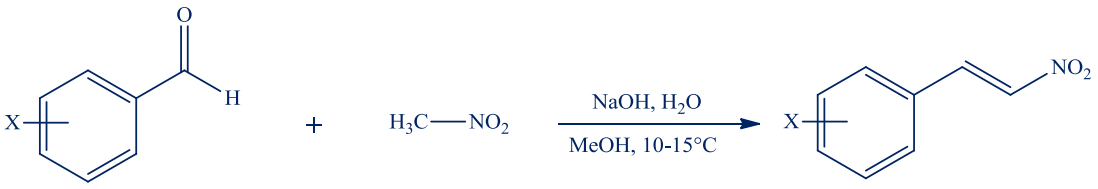
48

Scheme 9.3 *Synthesis of the phenylazide 48.*

In the first step the aniline is salified in aqueous ambient with chloride acid and next this latter reacts with sodium nitrite to give the aryl diazonium salt. This reaction must be carried out in acid ambient to prevent the undesired by-product of coupling with diazonium salt and unreacted aniline. Subsequently the aryl diazonum salt reacts with sodium azide to give the desired product with a 91% of yield.

9.3 SYNTHESIS OF THE ω-NITROSTYRENES USED AS DEACTIVATED DIPOLAROPHILES FOR THE 1,2,3-TRIAZOLES PREPARATION.

ω-Nitrostyrenes are strong electron-deficient alkenes very used in cycloaddition field. They give access to different class of heterocyclic organic compounds when are used as dipolarophile in cycloaddition reactions. Their cycloaddition products are principally applied in clinical treatments (see **Chapter 5**) and in photovoltaic field. In this thesis work they are used as deactivate dipolarophile in eco-friendly synthesis of the 1,2,3-Triazoles with potential biological activity.
The synthesis of ω-nitrostirenes variously substituted on the aromatic ring is carried out in according to the **Scheme 9.4**.



49-56

X=H, Cl, Me, OMe, NO_2

Scheme 9.4 *Generic synthesis of the ω-nitrostyrenes 49-56.*

These compounds are synthesized by direct condensation of substituted benzaldehydes with nitromethane in basic ambient. This ambient favors the formation of the anion nitromethanide which acts as nucleophile attacking the electrophilic aldehyde.
All synthesized products are reported in **Table 9.2**.

Entry	X	Reaction Time (h)	Product	Yield (%)
1	H	1	**49**	85
2	2-Cl	1	**50**	87
3	3-Cl	1	**51**	87
4	4-Cl	1	**52**	88
5	4-Me	1	**53**	86
6	4-OMe	1	**54**	89
7	2-NO_2	2	**55**	80
8	4-NO_2	2	**56**	81

Table 9.2 *Reaction conditions for the synthesis of the aryl-substituted ω-nitrostirenes.*

In **Table 9.2** is possible to observe the reaction influence of the various groups on the reactivity. When on the ring electron-donating groups are present the reaction is faster respect to the presence of the electron-withdrawing groups. Furthermore in the first case the reaction yields are mayor respect to the second. Aniway, it is possible to highlight that all products has been obtained with good reaction yields.

9.4 SYNTHESIS OF THE ENAMINONES USED AS DEACTIVATED DIPOLAROPHILES FOR THE 1,2,3-TRIAZOLES PREPARATION.

Enaminones are a class of strong electron-deficient alkenes very used in cycloaddition field. They give access to different class of heterocyclic organic compounds when are used as dipolarophile in cycloaddition reactions. In combination with azides through 1,3-dipolar cycloaddition give a series of 1,2,3-Triazoles biologically actives (see **Chapter 5**) or used as pesticides. In this thesis work they are been used as deactivate dipolarophiles in eco-friendly synthesis of the 1,2,3-Triazoles with potential biological activity.
The synthesis of the enaminones is carried out by usi of the $Er(OTf)_3$ as catalyst in according to the **Scheme 9.5**.[348]

57-65

R_1=Me, Ph, OEt
R_2=Me, Ph, Allyl, i-Pr

Scheme 9.5 *Generic synthesis of the enaminones 57-65.*

All synthesized products are reported in **Table 9.3**.

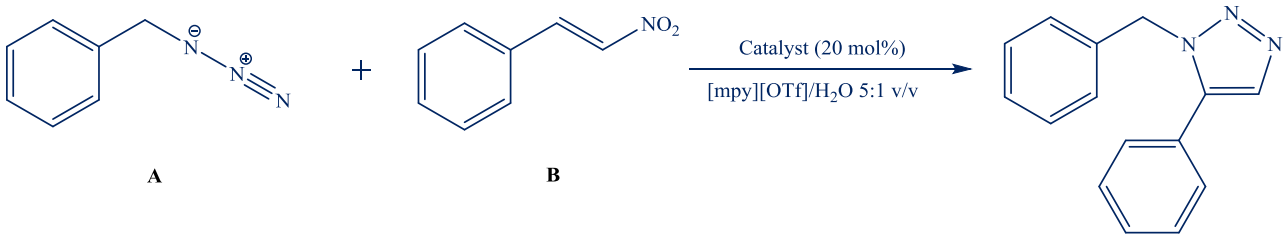
Entry	R_1	R_2	Product	Time (h)	Yield (%) in CH_2Cl_2	Yield (%) in THF
1	Me	C_6H_{13}	**57**	3	97	95
2	Me	Ph	**58**	2,5	95	93
3	Me	$PhCH_2$	**59**	23	98	98
4	Me	i-Pr	**60**	30	87	78
5	Me	Vinyl	**61**	26	96	100
6	Ph	Ph	**62**	2,5	93	92
7	Ph	$PhCH_2$	**63**	2	97	96
8	OEt	C_6H_{13}	**64**	2	100	100
9	OEt	Ph	**65**	6	71	70

Table 9.3 *Reaction data for the synthesis of the enaminones 57-65.*

The reaction is conducted in two different solvents, dichloromethane and tetrahydrofurane, and only 1 mol % of the $Er(OTf)_3$ is used. In all cases the reaction yield is good.

9.5 SYNTHESIS OF 1,2,3-TRIAZOLES IN $Er(OTf)_3$/[mpy][OTf]/H_2O AS CATALYTIC RECICLABLE SYSTEM.

After the preparation of all precursors it was possible to execute the 1,3-dipolar cycloaddition reactions in eco-friendly way. The eco-friendly approach it's guaranteed by use of a recyclable system composed by $Er(OTf)_3$/Ionic Liquid/H_2O.
In recent years metal triflates were introduced as mild and efficient Lewis acid catalysts.[349] Their Lewis acidity was related to their hydrolysis constant (K_h) and water exchange rate constants (WERC). The metals, which exhibited good catalytic activity in screening experiments, had pK_h values in the range 4-10 and WERC values grater than $3,2 \times 10^6$ $M^{-1}s^{-1}$.[335] More recently, the relative Lewis acidity of rare earth metal triflates was evaluated based on their competitive ligand dissociation from complexes using Tandem Mass Spectrometry.[350] However, metal triflates have only been applied to three-component reactions, involving carbonyl compounds and amines, where imines were not the final product.[351]
Initially the cycloaddition reaction conditions are performed in terms of catalyst, temperature, reagent molar ratio and time using the benzylazide and ω-nitrostyrene respectively in [mPy][OTf] as ionic liquid in according to the **Table 9.4.**



A B 66

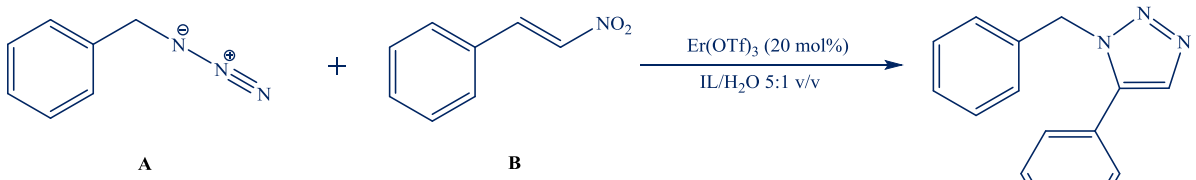
1,5-product

Entry	Catalyst	Acid Cocatalyst (%)	Molar Ratio A:B	Time (h)	T (°C)	Yield (%)	Regioisomeric Ratio 1,5 : 1,4 Products
1	$Er(OTf)_3$	-	2,5:1	48	25	<20	-
2	$Er(OTf)_3$	-	2,5:1	48	60	40	-
3	$Er(OTf)_3$	-	2,5:1	2	100	85	100:0
4	$Er(OTf)_3$	HCl (20)	2,5:1	2	100	85	100:0
5	$Er(OTf)_3$	HCl (1eq)	2,5:1	2	100	85	100:0
6	$FeCl_3$	-	2,5:1	5	100	80	100:0
7	$CeCl_3$	-	2,5:1	5	100	82	95:5
8	-	-	2,5:1	48	130	35	Mix of the elimination-non elimination product in ratio 1:1

Table 9.4 *Optimization of the cycloaddition reaction condition between benzylazide and ω-nitrostyrene.*

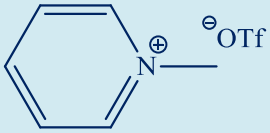
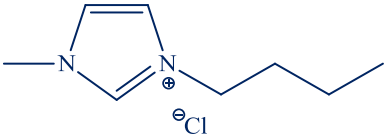
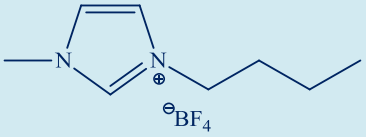
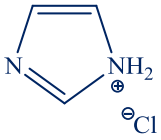
The reaction is optimized in a catalytic system composed by methyl pyridinium salt as ionic liquid in combination with water in ratio 5:1 v:v. When the reaction is conducted at room temperature for 48 hours any product is obtained (entry 1, **Table 9.4**). If the temperature increases the reaction yield is subjected to growth (entries 2 and 3). The use of the chloride acid as co-catalyst does not favor the increase of the yield (entries 4 and 5), so it can be avoided. In all cases the $Er(OTf)_3$ has been used, favoring the formation of only 1,5 regioisomer. The use of the $FeCl_3$ catalyst induces the formation of this product with a lower yield than the erbium triflate (entry 6). The situation worsens when $CeCl_3$ catalyst is use because the yield is only 82 % and the product is a mixture of the regioisomers (entry 7). This reaction is conducted with an excess of the benzylazide because it is subjected to degradation when the system is warmed. If the reaction is executed in absence of the catalyst the crude is composed by a mixture of the eliminated and non-eliminated product, rapidly decomposed by atmospheric condition. On basis of these experiments it is possible to observe that the best reaction conditions corresponding whit the entry 3.

Whereas this reaction is conducted in a recyclable system prevalently composed by ionic liquid, a screening of the reaction was carried out in several ionic liquids leaving all the other variables of the reaction unchanged in according to the **Scheme 9.5**.



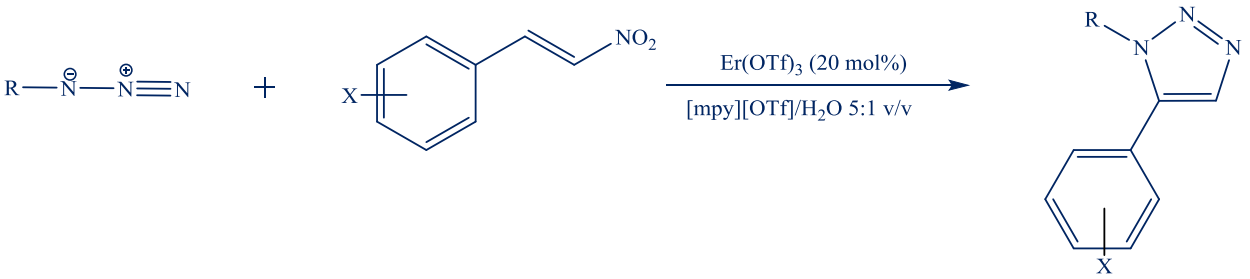
A B 66

1,5-product

Entry	Ionic Liquid	Catalyst	Molar Ratio A:B	Time (h)	T (°C)	Yield (%)
1		$Er(OTf)_3$	2,5:1	2	100	85
2		$Er(OTf)_3$	2,5:1	2	100	60
3		$Er(OTf)_3$	2,5:1	2	100	75
4		$Er(OTf)_3$	2,5:1	2	100	75

Scheme 9.5 *1,3-Dipolar Cycloaddition reaction between benzylazide and ω-nitrostirene in various Ionic Liquids.*

Four ionic liquid have been tested to evaluate the cycloaddition reactivity (**Scheme 9.5**). The ionic liquids are two different natures: methylpyridinium and imidazolium. In the first case (entry 1), when the reaction is conducted by using [mPy][OTf] the yield is very high. When the ionic liquid is changed with [bmim] the yield decreases. The counter-ion is very important for the reactivity. In fact the Cl^- ion (entry 2) disadvantages the reaction more than BF_4^- (entry 3). Finally, the imidazolium chloride salt (entry 4) does not significantly improve the yield.
Whit these results in hand the reaction is extended to various azides and ω-nitrostyrenes in according to the **Table 9.5**.



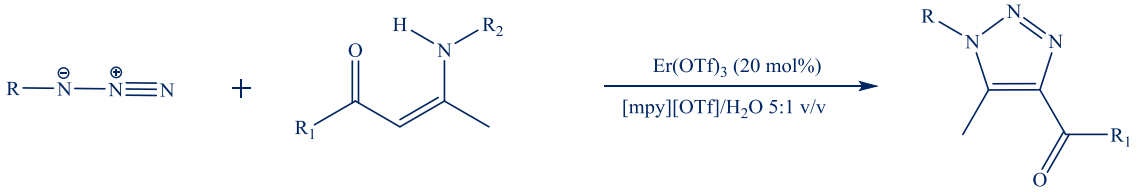
66-89

Entry	R	X	Compound	Yield (%)	Regioisomeric Ratio
1	Bn	H	**66**	85	100:0
2	Bn	2-Cl	**67**	85	100:0
3	Bn	3-Cl	**68**	86	100:0
4	Bn	4-Cl	**69**	85	100:0
5	Bn	4-Me	**70**	87	100:0
6	Bn	4-OMe	**71**	88	100:0
7	Bn	2-NO_2	**72**	81	100:0
8	Bn	4-NO_2	**73**	82	100:0
9	Octyl	H	**74**	83	100:0
10	Octyl	2-Cl	**75**	84	100:0
11	Octyl	3-Cl	**76**	83	100:0
12	Octyl	4-Cl	**77**	81	100:0
13	Octyl	4-Me	**78**	80	100:0
14	Octyl	4-OMe	**79**	82	100:0
15	Octyl	2-NO_2	**80**	79	100:0
16	Octyl	4-NO_2	**81**	78	100:0
17	Ph	H	**82**	84	100:0
18	Ph	2-Cl	**83**	86	100:0
19	Ph	3-Cl	**84**	85	100:0
20	Ph	4-Cl	**85**	86	100:0
21	Ph	4-Me	**86**	86	100:0
22	Ph	4-OMe	**87**	87	100:0
23	Ph	2-NO_2	**88**	78	100:0
24	Ph	4-NO_2	**89**	76	100:0

Table 9.5 *1,3-Dipolar Cycloaddition Reaction between alkyl and aryl azides and substituted ω-nitrostyrenes.*

All products are obtained as only one regioisomer and in good reaction yield. It is possible to observe that when the ω-nitrostyrenes containing an electron-donating group on the aryl ring the yield are mayor compared to those that have an electron-withdrawing group.

The same reaction is carried out with enaminones as deactivated dipolarophile in according to the **Table 9.6**.

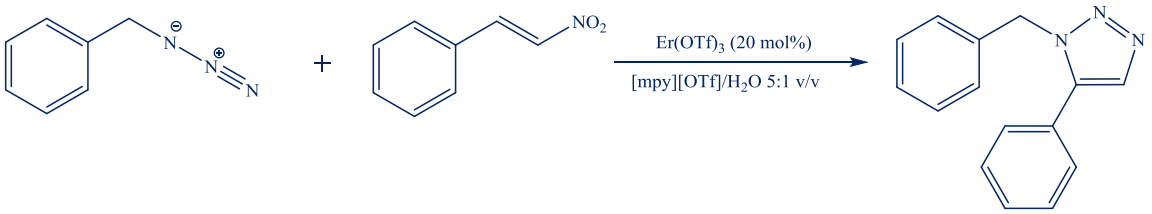


90-95

Entry	R	R_1	R_2	Product	Yield (%)	Regioisomeric Ratio
1	Bn	Me	Ph	**90**	87	100:0
2	Bn	Ph	Ph	**91**	82	100:0
3	Octyl	Me	Ph	**92**	84	100:0
4	Octyl	Ph	Ph	**93**	80	100:0
5	Ph	Me	Ph	**94**	85	100:0
6	Ph	Ph	Ph	**95**	83	100:0

Table 9.6 *1,3-Dipolar Cycloaddition Reaction between alkyl and aryl azides and enaminones.*

All products are obtained as only one regioisomer and in good reaction yield.
Finally, to enphasise the eco-friendly application of this reaction system, a recovery of the system has been conducted. At the end of the reaction the product is directly extracted from the catalytic recyclable system by CH_2Cl_2. The organic phase is evaporate in vacuo. While the catalytic system is washed with Et_2O and subjected to reuse without any treatments.
In the **Table 9.7** the recovery activity of the cycloaddition reaction between benzylazide and ω-nitrostyrene is reported.



66

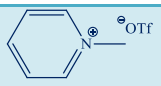
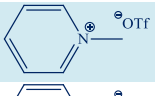
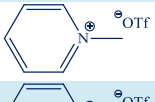
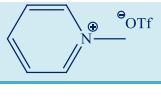
	ENTRY	IONIC LIQUID	TIME (h)	T (°C)	Recovery (%)	Yield (%)
R1			2	100	99	85
R2			2	100	98	85
R3			2	100	97	85
R4			2	100	95,5	85
R5			2	100	94	84

Table 9.7 *Recovery evaluation of the catalytic system.*

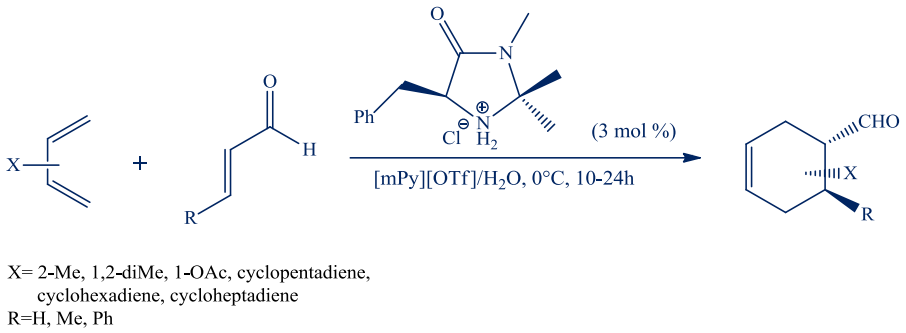
In **Table 9.7** it is possible to observe that the reaction yields are almost inhalterateduntil to five recycles. In conclusion, the system is easily recyclable considering that the lost mass is very small for every reuse.

CHAPTER 10

CHAPTER 10 - EFFICIENT ORGANOCATALYST SUPPORTED ON A SIMPLE IONIC LIQUID/WATER SYSTEM FOR ASYMMETRIC DIELS-ALDER AND 1,3-DIPOLAR CYCLOADDITIONS: RESULTS AND DISCUSSION

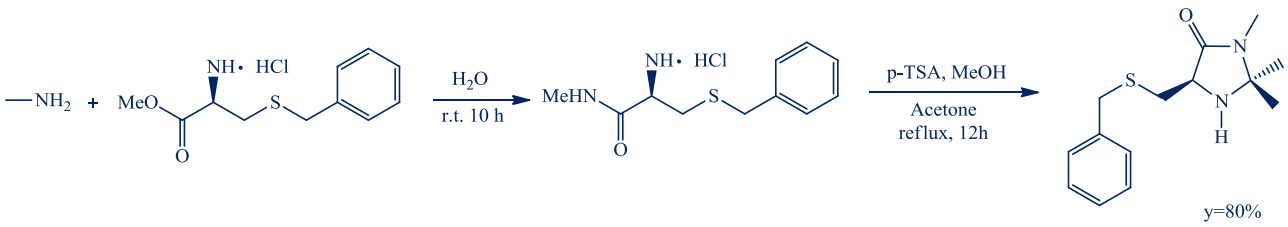
10.1 INTRODUCTION

Asymmetric organocatalysis is an attractive branch of chemistry and, in particular, the development of new chiral catalysts integrated with non-traditional solvents to form highly recyclable systems is one of the most innovative fields of chemistry to access a wide variety of chiral compounds under eco-friendly conditions.[352] Asymmetric Diels-Alder and 1,3-dipolar cycloaddition reactions provide a simple and direct access to a variety of important chiral cyclic compounds, as widely described in Chapter 1 and Chapter 2 of this thesis, and the elaboration of several efficient and reliable enantioselective catalytic methods is realized using metal-free organocatalysts.[353] In this regard, it has developed a new chiral organocatalyst and an efficient, recyclable system that promotes Diels-Alder and 1,3-dipolar cycloadditions reactions without the use of metal complexes, in accordance with the rules dictated by green chemistry. The immobilization of the organocatalyst in the polar ionic liquid (IL)/H_2O system facilitates the separation and reuse of the catalytic mixture, which promotes economic and environmental benefits. Initially, MacMillan[354] studied iminium ion organocatalysis in Diels-Alder reactions through a typical mechanism based on LUMO-lowering activation and subsequently applied it also to the 1,3-dipolar cycloaddition reactions. MacMillan's imidazolidinone catalyst is highly efficient in terms of yield and selectivity; however, it still has some drawbacks such as high catalyst loading and no possibility of recycling of the system. In this context, several attempts have been made to recycle the catalyst in recent years[355] because with this methodology, at industrial level, it is possible to have enormous economic savings. In a previous study[353a] conducted by De Nino and co-workers (**Scheme 10.1**) it was demonstrated that the use of methylpyridinium ILs, such as methylpyridinium triflate ([mPy][OTf]), as a solvent in the Diels-Alder reactions of α,β-unsaturated aldehydes with both cyclic and acyclic dienes lead to the corresponding *endo*-cycloadducts with excellent diastereoselectivities and enantioselectivities. Higher selectivity, better yields (90-95%), and shorter reaction times (10-24h) were observed in comparison with reactions performed in classical organic solvents.



Scheme 10.1 *Diels-Alder reaction in Ionic Liquid catalyzed by MacMillan catalyst.*

The synthesis of a new highly efficient organocatalyst (5S)-2,2,3-trimethyl-5-thiobenzylmethyl-4-imidazolidinone **96** was realized to use in combination with ILs. The new synthesized organocatalyst **96** bears an Sulphur atom that increases the solubility and enhances the interactions with the IL; in addition, intramolecular interactions caused by the S atom ensure the rigidity of the intermediate iminium ion and provide a high enantiofacial selectivity. Additional advantages are the facility of the synthetic procedure, good recovery of products, and recyclability of the whole IL/H_2O/organocatalyst system.
Compound **96** was prepared in excellent chemical yield from benzylcysteine methyl ester hydrochloride by adding methylamine in aqueous solution followed by treatment with acetone in methanol and p-toluenesulfonic acid (p-TSA) as shown in **Scheme 10.2**.



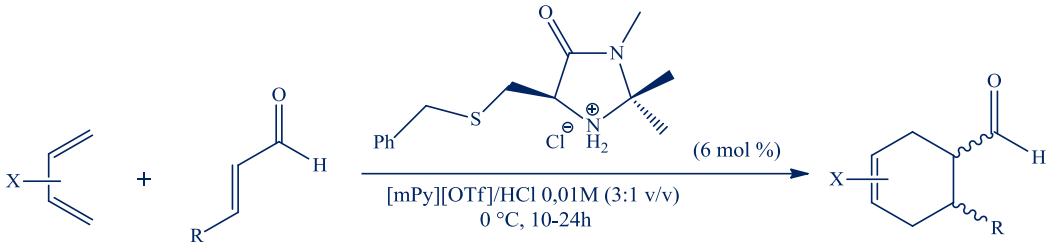
Scheme 10.2 *Synthesis of (5S)-2,2,3-trimethyl-5-thiobenzylmethyl-4-imidazolidinone.*

The reaction is carried out in two steps. In the first the S-benzyl-cysteine methyl ester hydrochloride is dissolved in water and reacts with methylamine (commercially available in aqueous 2M solution) for 10 hours at room temperature. After a basic work-up of the crude residue, consisting of *S*-benzyl-cysteine methylamide, methanol, acetone and *p*-toluenesulfonic acid are added. The reaction is conduct at reflux for 12 hours to give the desired product in high yield. The product keeps unaltered the stereocenter configuration because the reactions do not involve chiral carbon.

10.2 ASYMMETRIC DIELS-ALDER CYCLOADDITIONS

With this product in hand and with the experience of previous work, a highly efficient IL/HCl 0,01M/catalyst system is developed to promote Diels-Alder reactions of a broad range of dienes and dienophiles under mild and recyclable conditions, which afforded the corresponding cycle in

a high yield with good to high enantioselectivity (**Scheme 10.3**).



X= 2-Me, 1,2-diMe, 1-OAc, cyclopentadiene, cyclohexadiene, cycloheptadiene
R=H, Me, Ph

Scheme 10.3 *General procedure for Diels-Alder reaction catalyzed by new imidazolidinone catalyst.*

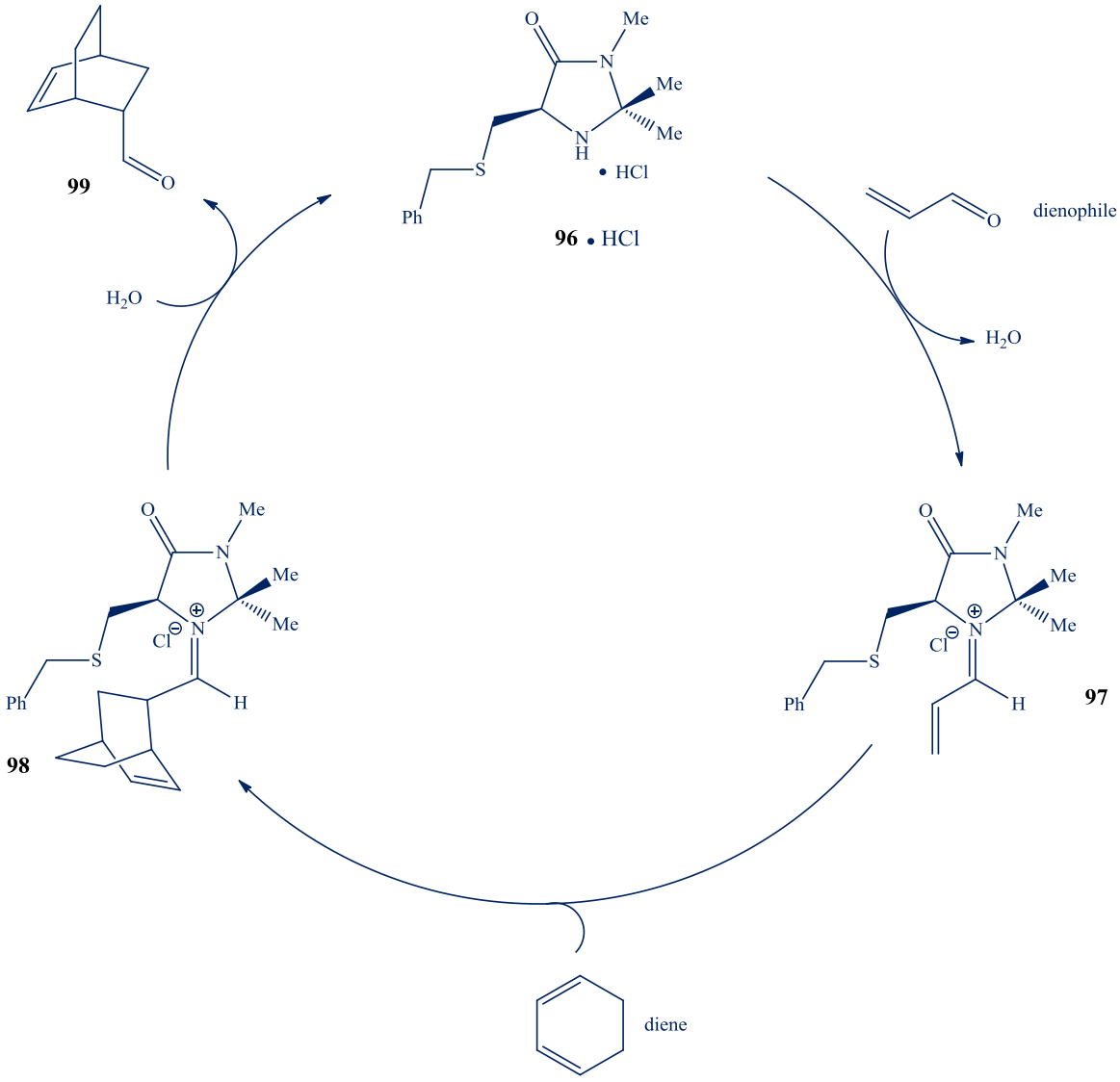
Initially, the catalytic ability of **96** in pyridinium-derived ILs and HCl 0,01M was examined in a model Diels-Alder reaction between cyclohexadiene and acrolein as shown in **Table 10.1**.

Entry	t (h)	Catalyst (mol%)	Yield (%) [a]	*endo/exo* [b]	*endo* ee [b]
1	10	3	70	98:2	92:8
2	10	6	75	98:2	95:5
3	10	10	80	98:2	95:5
4	16	6	98	98:2	95:5

[a] Isolated Yield. [b] Determined by GC analysis.

Table 10.1 *Screening of reaction time and catalyst loading.*

The cycloaddition is conveniently performed at 0 °C using 6 mol % catalyst and is completed after 16 h with the formation of cycloadduct with an excellent *endo/exo* ratio of 98:2% and an enantiomeric ratio (er) of 95:5%. The catalytic cycle is illustrated in **Scheme 10.4**.



Scheme 10.4 *Catalytic cycle for the Diels-Alder Process.*

In the catalytic cycle it is possible to observe that the catalyst imidazolidinone hydrochloride **96** reacts with acrolein to form the iminium ion with consequently water molecule loss. This system, much more reactive than free-acroleine, reacts with 1,3-cyclohexadiene to form the cycloadduct **98**. The final step restores the catalyst to its initial form releasing the product in the form of aldehyde.

In agreement with MacMillan's model, it is expected that the *E* isomer will be preferentially formed, which prevents interactions between the olefin and the methyl substituent in the geminal position. Under these circumstances, the benzyl group on the catalyst framework acts as a shield on the *Re* face of the dienophile and promotes the approach of the diene by the less hindered *Si* face.

To explore the scope of the reaction, we applied the optimized reaction conditions to various substrates. The Diels-Alder reaction that involves α,β-unsaturated aldehydes and various dienes proceeded efficiently in the system [mPy][OTf]/HCl 0,01M/catalyst to lead to the cycloadduct in good yields with good to excellent enantioselectivities (**Table 10.2**).

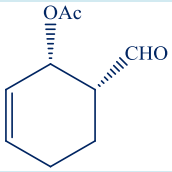
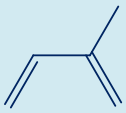
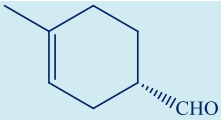
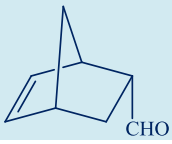
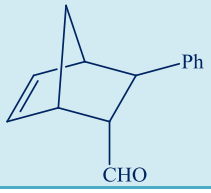
Entry	Diene	R	Product	Yield (%)	*endo/exo*	*endo* ee
1		H		98	98:2	95:5
2		H		93	94:6	97:3
3		H		97	-	96:4
4		H		90	95:5	99:1
5		H		95	86:14	94:6
6		Me		85	75:25	95:5
7		Ph		90	82:18	83:17

Table 10.2 *[mPy][OTf]-mediated Diels-Alder reaction of various dienes and dienophiles catalyzed by new imidazolidinone organocatalyst.*

In **Table 10.2** is possible to observe that the reaction yields are very high, the diastereoisomeric ratio and the enantiomeric excess are very excellent.

With the success of the reactions described above, it is studied the recyclability of the IL/HCl 0,01M/catalyst polar phase used in the Diels-Alder reaction. The recovery and recycling of the entire catalytic system can be exemplified as shown below.

After the reaction, the polar phase was extracted three times by adding Et_2O (5 mL per time) directly into the separatory funnel; the extracted crude was purified by routine silica gel column chromatography, and catalyst **93** remained dissolved in the IL/water mixture.

Therefore, IL-supported **96** can be directly reused in further reactions. As shown in the histogram, only a slight decrease in catalytic activity was observed if IL-supported **96** is recovered and reused in further reactions as shown in **Figure 10.1**.

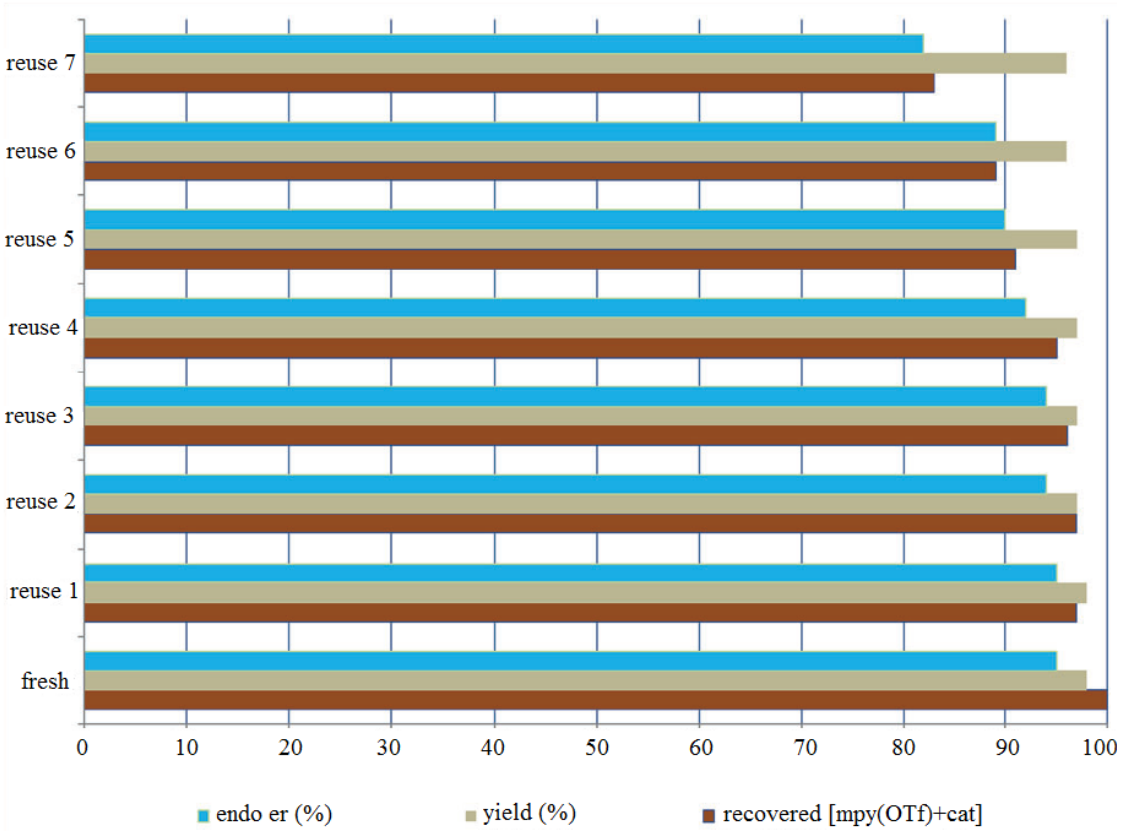
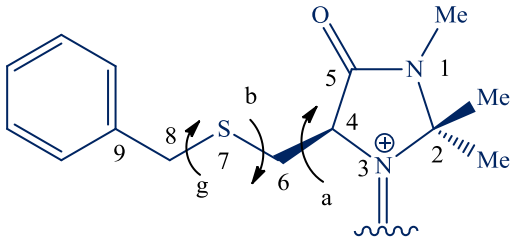


Figure 10.1 *Histogram of recovery and reuse of the IL/HCl 0.01M/catalyst system.*

The histogram shows the high percentage of the recovery system, high percentage of the reaction yields and high enantiomeric-*endo* ratio for the five reuses. Only in the six and seven reusesit is possible to observe a slight decline in performance. Based on these considerations it follows that this catalytic system can be a valid candidate for any industrial applications.

10.3 THEORETICAL CALCULATION OF THE CATALYST CONFIGURATION

The asymmetric induction observed in all cases that involves catalyst **96** is consistent with the conformation of the iminium ion **97**. The conformational preferences of the iminium intermediate were studied in detail. We considered the three dihedral angles a, b, and g illustrated in **Figure 10.2**.[356] For each dihedral angle, three staggered conformations were studied, and both the *E* and *Z* configurations of the iminium moiety were involved.



a= C5-C4-S7
b=C4-C6-C7-C8
c=C6-S7-C8-C9

Figure 10.2 *Diedral angles of compound **97**.*

Thus, a total of 54 conformers were considered of which 10 were eliminated because they led to a collision of atoms. The resulting 44 conformers were optimized fully at the CPCM=H_2O/M06-2X/6-31G(d) level.[357] A total of 42 energy minima that have different dihedral angles were obtained. In respect to the global minimum that accounts for 91,6% of all the conformations are listed in **Table 10.3**.[358] As a result of the large number of conformations studied, calculations that involve higher levels of theory were not performed for all of them. Full optimizations at the CPCM=H_2O/M06-2X/6-311+G (d,p) level of theory were performed for the above-mentioned seven energy minima. The relative energy values are also listed in **Table 10.3**.

	ΔG (2ξ level)[a]				ΔG (3ξ level)[b]			
Conformer	**α**	**β**	**γ**	**ΔΔG**	**α**	**β**	**γ**	**ΔΔG**
E-c06	-55,8	-97,1	-53,5	1,6	-54,7	-97,6	-55,4	0,0
E-c06	-66,8	79,0	164,0	1,3	-67,0	78,0	163,9	0,2
E-c21	71,5	119,5	-56,3	0,4	71,6	115,9	-56,6	0,5
E-c02	-58,3	93,8	60,7	0,0	-67,3	89,4	63,1	0,6
E-c24	64,4	-84,2	-32,9	1,5	65,5	-85,0	-33,4	1,4
E-c26	64,8	49,5	47,3	1,6	65,8	50,1	47,7	1,7
E-c04	-59,5	-93,8	-169,6	1,7	-59,5	-93,6	-168,9	1,6

[a] CPCM=H_2O/M06-2X/6-31G(d). [b] CPCM=H_2O/M06-2X/6-311+G(d,p).

Table 10.3 *Dihedral angles [°] and relative energies [kcal mol^{-1}] of the more stable conformers of iminium intermediate **97**.*

No substantial changes were observed between calculations at the 2ξ and 3ξ levels, and the observed dihedral angles are almost identical in all cases. Calculations at the highest level provided four conformations within a difference of 1,0 kcal mol¯1 (**Figure 10.3**). Of these conformations, three ((E)-c06, (E)-c07, and (E)-c02), which include the global minimum (E)-c06, present a stabilizing C_H···S interaction between one of the methyl groups at position C2 of the imidazolidinone ring and the methylthio group at position C5. The S-H distance was in the range of 2,91‑2,95 Å. The differences between these conformers correspond to the rotation of dihedral angles b and g, which corresponds to different orientations of the S-benzyl group. C-H ··· S interactions have been reported experimentally[359] and studied theoretically.[360] In

particular, theoretical calculations at the MP2/6-311+G(d,p) level[361] found an optimum S-HC(sp^3) distance of 3,095 Å. Notably, the global minimum of iminium ion **97** ((E)-c06) places the phenyl ring directly above the double bond to shield the *Re* face of the dienophile and promote approach of the diene by the less hindered *Si* face.

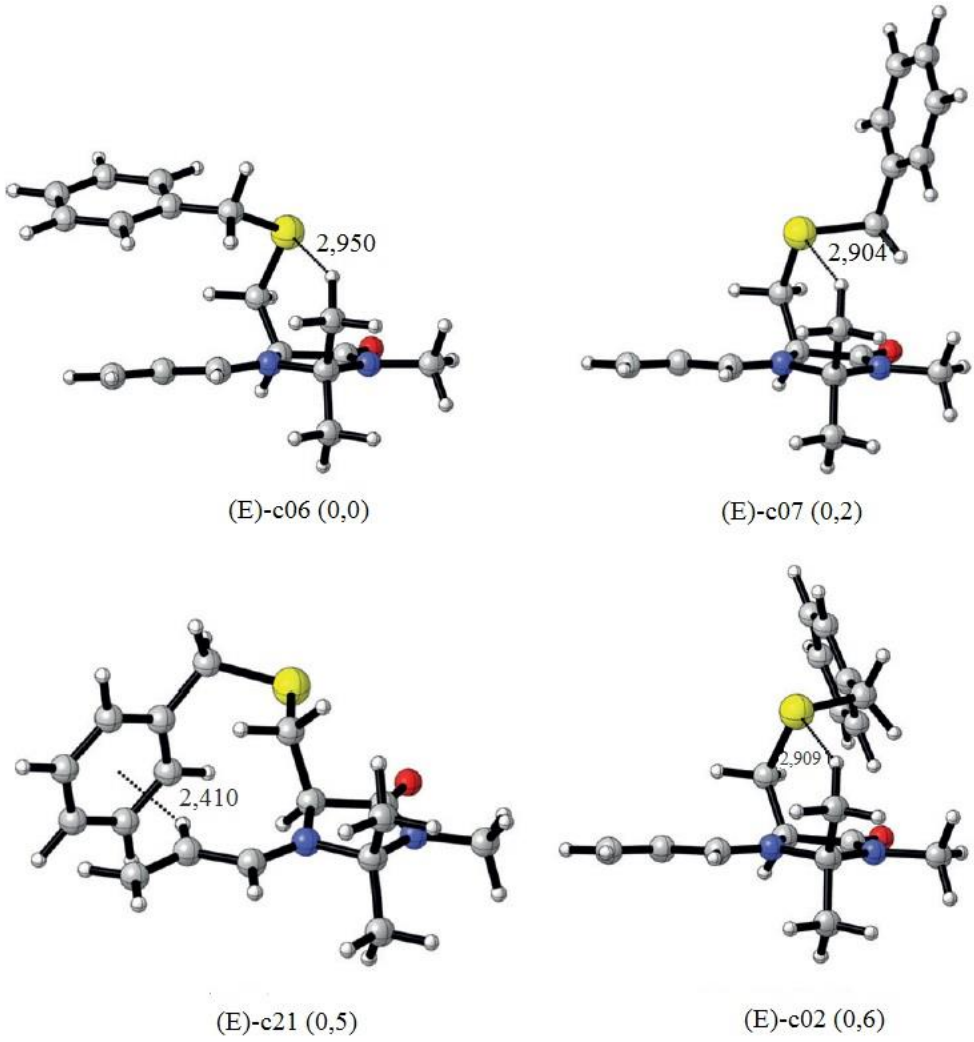


Figure 10.3 *Most stable conformers for* ***97****. (Structures optimized at the CPCM=H_2O/M06-2X/6-311+G(d,p) level; relative energies given in kcal mol^{-1}).*

Therefore, the S atom plays a major role in the high enantioselectivity observed experimentally by fixing a partial conformation (through the above-mentioned C-H···S interaction) general, *Z* isomers are less stable than the corresponding *E* conformers in agreement with previous results reported by Houk et al.[355] that demonstrated a high steric hindrance between the two methyl groups at C2 of the imidazolidinone ring and the substituent attached to *N*1. The seven energy minima below a difference of 2,0 kcal mol-1 with respect that facilitates the orientation of the phenyl ring to hinder one of the diasterofaces of the iminium intermediate **97** completely.
Conformer (E)-c21 presents a C-H···π interaction between the phenyl ring and a vinylic proton (**Figure 10.3**). This sort of stabilizing interaction has been reported in the case of MacMillan's

catalyst, for which the global minimum exhibits a stabilizing C-H···π interaction between the methyl group at C2 and the phenyl ring of the benzyl group at C5.[350,355] The same interaction has also been found for one of the more stable conformers of iminium ion **97** ((E)-c24). These interactions are illustrated in **Figure 10.4** for both the iminium ion of MacMillan's catalyst (2,42Å) and conformer (E)-c24 of the iminium ion of **96** (2,84 Å). The NCI (non-covalent interaction) analyses performed for conformers (E)-c06, (E)-c07, (E)-c21, and (E)-c24, which show three different types of interactions, are illustrated in **Figure 10.5**.

To evidence the stabilizing interactions, the M06-2X/6-311+G(d,p) wave functions were used for further NCI analysis, a semiquantitative visualization index based on the electron density and its derivatives that enables the identification of noncovalent interactions.[362] Visualization of favorable and unfavorable interactions was performed with NCI Plot Program[363] and VMD software.[364]

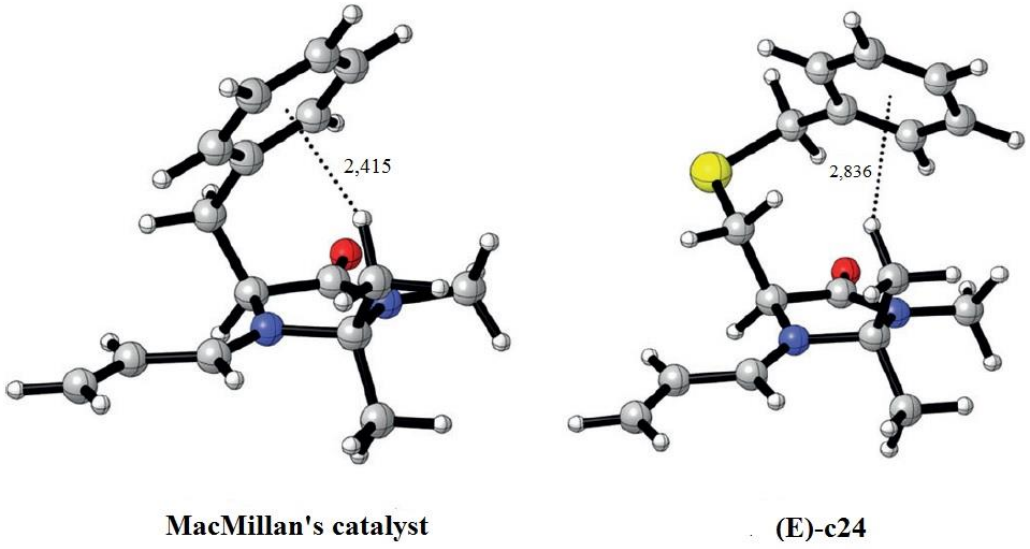


Figure 10.4 *C-H···π interactions for optimized structures (CPCM=H_2O/M06-2X/6-311+G(d,p)) of the most stable conformer of the iminium ion of MacMillan's catalyst and conformer **(E)-c24** of the iminium ion of catalyst **96**.*

Both (E)-c06 and (E)-c07 conformers show a reduced density gradient (RDG) green surface that confirms the C-H···S noncovalent stabilizing interactions. In addition, the global minimum (E)-c06 shows a green surface that corresponds to a stabilizing interaction between the phenyl ring and the double bond.

This interaction, difficult to identify by a mere visual inspection of the model, can be identified as a π-π interaction and it stabilizes the (E)-c06 conformer.

Conformers (E)-c21 and (E)-c24 show a typical RDG surface that corresponds to a C-H···π interaction in which a green conical form with a vertex that points to the center of the aromatic ring can be appreciated. As stated above, the C-H···π interaction takes place between the aromatic ring and the vinylic hydrogen atom for conformer (E)-c21 and the methyl group at C2 for conformer (E)-c24.

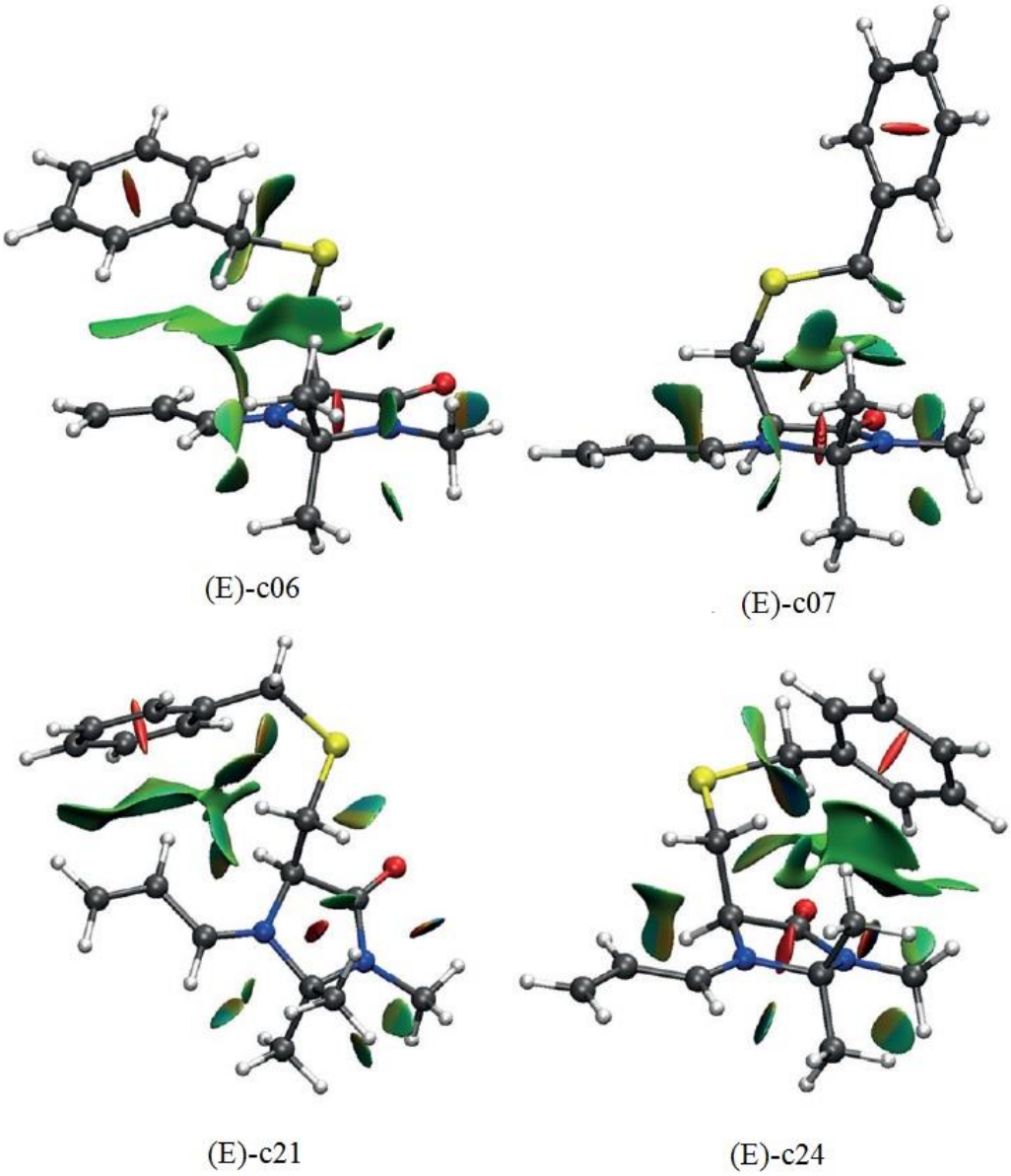


Figure 10.5 *NCI analysis showing NCI isosurfaces for conformers (E)-c06, (E)-c07, (E)-c21, and (E)-c24. Color-coded according to (λ_2)ρ, the eigenvalue of the density Hessian, to indicate attraction (green–blue) or repulsion (red). The cutoff (ρ=0,2 a.u.) was chosen to isolate purely noncovalent interactions.*

We used the most stable conformation of the catalyst (E)-c06 to locate the preferred transition structures for *endo* and *exo* approaches of cyclopentadiene by both *Re* and *Si* faces.
The geometrical features and relative energies [kcal mol^{-1}] of the four transition structures are illustrated in **Figure 10.6**.

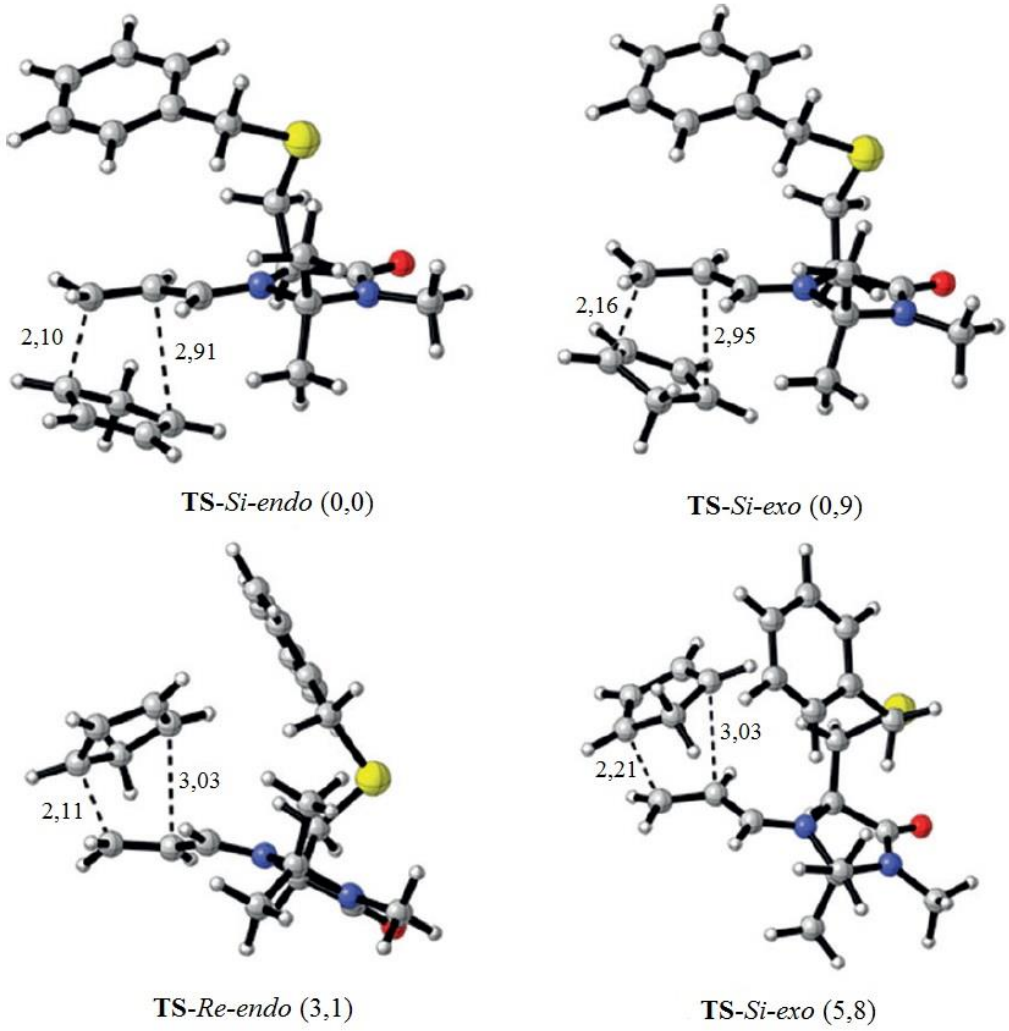
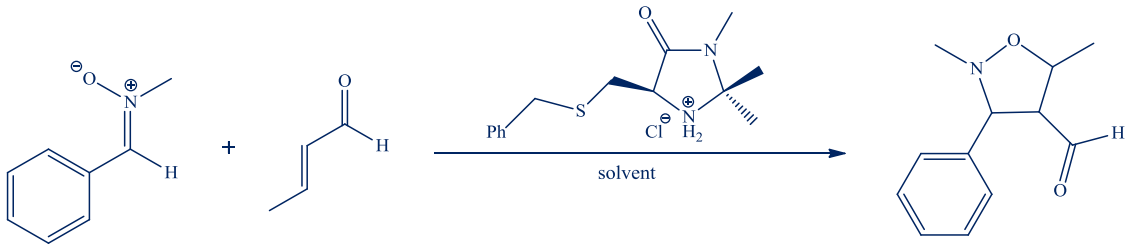


Figure 10.6 *Transition structures for the reaction between (E)-c06 and cyclopentadiene. (Structures optimized at the CPCM=H2O/M06-2X/6-311+G(d,p) level; relative energies given in kcal mol^{-1}).*

The energy difference between TS-Si-endo and TS-Si-exo is 0,84 kcal mol^{-1}, which justifies the formation of *endo/exo* mixtures in the case of the reaction with cyclopentadiene. A difference of more than 3 kcal mol^{-1} between the *Re* and *Si* approaches in the case of endo adducts (more than 5,0 kcal mol^{-1} for exo adducts) justifies the excellent enantioselectivity observed.
The bond-formation lengths are in agreement with a highly asynchronous reaction as expected for a reaction performed in a highly polar medium.

10.4 ASIMMETRIC 1,3-DIPOLAR CYCLOADDITIONS

Considering the good results obtained in Diels-Alder reaction, this catalytic approaches was extended to 1,3-dipolar cycloadditions with the scope to obtain the enantiomerically enriched five-member heterocyclic compounds.
Initially the reaction conditions were investigated on a target 1,3-dipolar cycloaddition between *N*-Methyl-*C*-Phenyl-nitrone and crotonaldehyde as reported in **Table 10.4**.

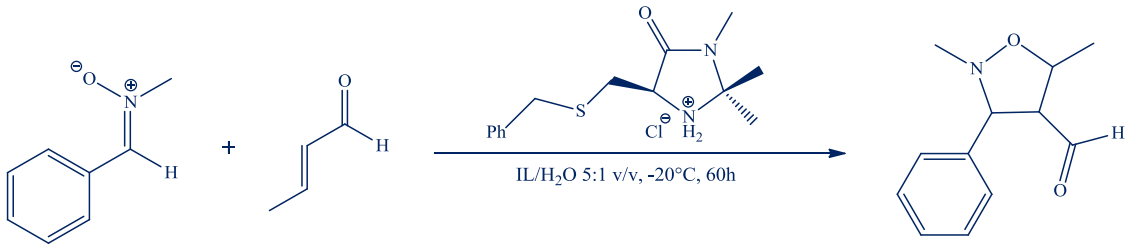


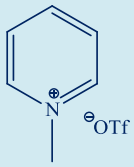
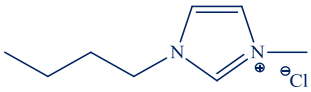
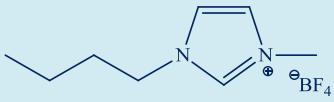
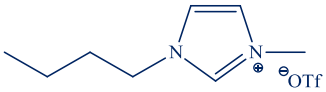
Entry	Catalyst (%)	Solvent	T (°C)	t (h)	Yield (%)	*Endo/exo*	*Endo er*
1	3 (20)	[mpy][OTf]/H_2O 3:1 v/v	25	24	72[a]	80:20[b]	70:30[c]
2	3 (20)	[mpy][OTf]/H_2O 3:1 v/v	0	48	72[a]	85:15[b]	88:12[c]
3	3 (20)	[mpy][OTf]/H_2O 5:1 v/v	0	48	85[a]	86:14[b]	89:11[c]
4	3 (20)	[mpy][OTf]/H_2O 5:1 v/v	-10	50	84[a]	90:10[b]	95:5[c]
5	3 (20)	[mpy][OTf]/H_2O 5:1 v/v	-20	60	85[a]	95:5[b]	99:1[c]
6	3 (6)	[mpy][OTf]/H_2O 5:1 v/v	-20	60	60[a]	90:10[b]	91:9[c]
7	MacMillan (20)	CH_3NO_2	-20	132	66[a]	95:5	99,5:0,5

[a] Isolated yield. [b] Determined by GC/MS analysis. [c] Determined by Chiral GC analysis.

Table 10.4 *Initial reaction conditions evaluated for the target 1,3-dipolar cycloaddition catalyzed in a recyclable system.*

In Entry 1 the reaction is conducted with 20 mol% of the catalyst at 25°C employing [mpy][OTf] as ionic liquid. The reaction time and the yield are acceptable but the diastereo- and enantiomeric ratio are low. When the temperature is lowered to 0°C (Entry 2) it is possible to obtain the same reaction yield but only a small increasing of the diastereo- and enantiomeric ratio. When the quantity of the IL/H_2O is changed the reaction yield increases further (Entry 3). If the temperature is lowered until to -20°C the diastereo- and enantiomeric ratio considerably increases (Entry 4 and Entry 5). The percentage reduction of the catalyst is reported in entry 6 and it is possible to note a reduction of the reaction yield. Finally, the comparison of best reaction conditions (Entry 5) with the results obtained to MacMillan's work (entry 7) confirms that catalyst **96** has a better catalytic activity than the MacMillan catalyst.
With the best reaction conditions in hand it is possible carried out a screening of different ionic liquid to evaluate the effect which have on the target reaction.



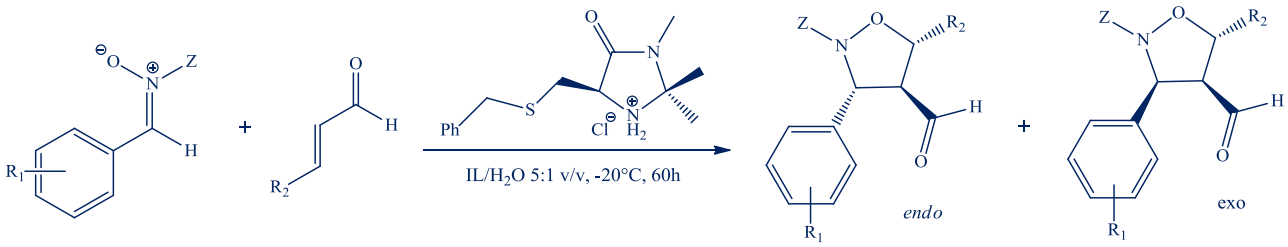
Entry	Ionic Liquid	Yield (%)[a]	*endo/exo*[b]	*endo er*[c]
1		85	95:5	99:1
2		65	60:40	70:30
3		70	70:30	80:20
4		72	90:10	95:5

[a] Isolated yield. [b] Determined by GC/MS analysis. [c] Determined by Chiral GC analysis.

Table 10.5 *1,3-Dipolar Cycloaddition catalyzed in a different recyclable system.*

In **Table 10.5** the results for the target reaction are reported in different ionic liquids. It is possible to observe that the reaction conducted in methylpyridinium triflate salt gives the best results in terms of reaction yields and diastereo- and enantiomeric ratio (Entry 1). When [mPy][OTf] is substituted with [bmim] the results are worse. In this case the anion specie have a different influence on the reaction. For example, the worst results are obtained with the [bmim][Cl] (Entry 2), followed by [bmim][BF_4] (Entry 3). The best results obtained with [bmim] are performed when the anion is triflate as shown in entry 4.

To explore the scope of the reaction, the optimized reaction conditions to various substrates are applied. The 1,3-dipolar cycloaddition reaction that involves α,β-unsaturated aldehydes and various nitrones as 1,3-dipoles proceeded efficiently in the system [mPy][OTf]/H_2O/catalyst to lead to the products in good yields with good to excellent enantioselectivities (**Table 10.6**).



Entry	Z	R_1	R_2	Yield (%)[a]	*endo*/*exo*[b]	*endo* ee (%)[c]
1	Me	2-Cl	Me	86	94:6	99:1
2	Me	3-Cl	Me	84	93:7	98:2
3	Me	4-Cl	Me	86	95:5	99:1
4	Me	2-F	Me	82	94:6	98:2
5	Me	4-F	Me	84	96:4	99:1
6	Me	4-Me	Me	87	95:5	99:1
7	Allyl	H	Me	80	93:7	97:3
8	Bn	H	Me	82	95:5	98:2
9	Bn	4-Cl	Me	84	94:6	99:1
10	Bn	4-OMe	Me	85	96:4	99:1
11	Me	4-Cl	H	83	95:5	98:2
12	Bn	H	H	80	96:4	98:2
13	Bn	4-Me	H	82	94:6	99:1
14	Bn	4-OMe	H	84	95:5	99:1

[a] Isolated yield. [b] Determined by GC/MS analysis. [c] Determined by Chiral GC analysis.

Table 10.6 *[mPy][OTf]-mediated 1,3-dipolar cycloaddition of various nitrones and dipolarophiles catalyzed by new imidazolidinone organocatalyst.*

In **Table 10.6** it is possible to observe that the reaction yields are very high, the diastereoisomeric ratio and the enantiomeric excess are very excellent.

With the success of the reactions described above, the recyclability of the IL/H_2O/catalyst polar phase is. The recovery and recycling of the entire catalytic system can be exemplified as shown below.

After the reaction, the polar phase was extracted three times by adding CH_2Cl_2 (5 mL per time) directly into the separatory funnel; the extracted crude was purified by routine silica gel column chromatography, and catalyst **96** remained dissolved in the IL/water mixture.

Therefore, IL-supported **96** can be reused directly in further reactions. As shown in the histogram, only a slight decrease in catalytic activity was observed if IL-supported **96** was recovered and reused in further reactions as shown in **Figure 10.2**.

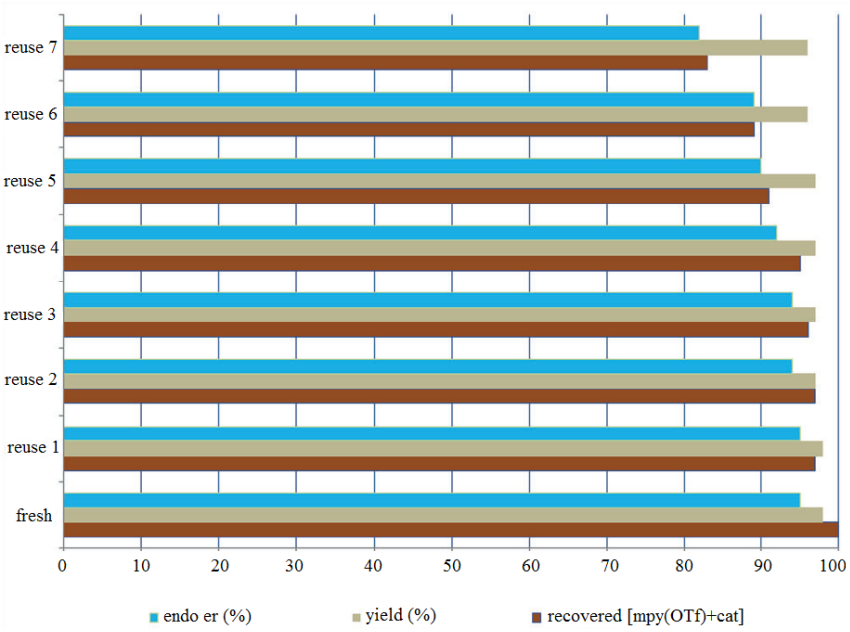


Figure 10.2 *Histogram of recovery and reuse of the IL/H_2O/catalyst system.*

The histogram shows the high percentage of the recovery system, high percentage of the reaction yields and high enantiomeric-endo ratio for five reuses. Only in the six and seven reuses it is possible to observe a slight decline in performance. Based on these considerations it follows that this catalytic system can be a valid candidate for any industrial applications.

In conclusion, a new chiral organocatalyst is described and characterized as an efficient, economical, and practical method for enantioselective Diels-Alder reactions and 1,3-dipolar cycloadditions. The reaction medium is composed of an ionic liquid (IL), water, and organocatalyst, which leads to the desired products in good yields with good to excellent enantioselectivities. Theoretical calculations confirmed the presence of C-H···π interactions similar to those found for MacMillan's catalyst. However, important C-H···S stabilizing interactions are also evidenced by both DFT calculations and NCI analysis, which justifies the high stability of conformer **(E)-c06**. In this conformer the phenyl ring is placed over the unsaturated system to render the *Si* face as the less hindered one for the attack of the dienophile. The calculated relative energies between transition structures are in good agreement with both the formation of mixtures of *endo/exo* adducts in some cases and the excellent enantioselectivity observed experimentally.

CONCLUSIONS

CONCLUSIONS

In conclusion this present doctoral thesis work has permitted to develop the synthesis of:

- Indanoyl and Isatinyl Spiro-isoxazolidine *N,O*-Nucleosides with potential biological activity derived from the reaction of *N*-Me, *N*-Bn and *N*-Ph nitrones of 1-indanone or isatin with vinyl-nucleobases. To demonstrate the presence of the pharmacopore portion on the spiroisoxazolidine nucleus the biological activity of the nitrone precursors is been tested also as antiproliferative and antioxidant agents. In addition, the interesting *in vitro* biological antiproliferative activity of spiro compounds on cellular lung cancer cells has been shown that these molecules are good candidates for future testing.
- Aryl isoxazolidin-*gem*-bisphosphonic acids derived from cycloaddition reaction between arylaldonitrones and vinyl esthers followed by a multistep reaction sequence that permit to incroduce the bisphosphonic function on the isoxazolidine ring. These compounds are in phase of testing to evaluate the potential biological activity on Farnesyl Pyrophosphate Synthase and Geranylgeranyl Pyrophosphate synthase.
- Variously substituted 1,2,3-Triazoles by homogeneous catalytic recyclable system composed by $Er(OTf)_3$/Ionic Liquid/H_2O starting from alkyl or aryl azides and deactivated dipolarophiles in regioselective way. These compounds are also undergoing biological *in vitro* tests to evaluate their biological activity.
- An enantioselective Imidazolidinone organocatalyst applied on Diels-Alder and 1,3-Dipolar Cycloaddition reactions for the synthesis of six-membered carbocyclic organic compounds and five-membered heterocyclic organic compounds. Furthermore, computational calculations demonstrated that the particular configuration of the intermedium iminium ion have a lowered LUMO that favorites the pericyclic reaction; moreover, it was possible to demonstate that the particular enantioface of this latter permit to the diene (or 1,3-dipole) to attack only from one enantioface of the system.

The synthetic cycloaddition methodology applied for all reaction in very green and eco-friendly because are used microwave-assisted and solvent-free methods or, in alternative, catalytic systems that can be reused for many cycles.

This new synthesis approach has led to several changes in the experimental conditions of the reactions (which are generally carried out under normal reflux conditions for a few days, so in the presence of organic solvents such as toluene or even benzene) not only from the point of view of reaction yields and work-up procedures, but also for a lower environmental impact. In fact, the use of microwaves allows:

- Increased reaction speed;
- Increased reaction yields;
- Higher reproducibility;
- Higher selectivity.

The use of solvent-free conditions is advantageous in favor of the so-called Green Chemistry, thanks to a significant reduction of pollution from emissions and disposal of chemicals.

Ultimately, various synthesis strategies have been proposed leading to the formation of heterocyclic compounds with increased biological activity and lower toxicity. These compounds,

in fact, consist of portions of biologically active molecules. By combining these properties, it was possible to create a new class of multifunctional molecules that, from a pharmacological point of view, may be used as antiviral and antitumor agents in clinical field. For this motif all the product that are not again tested will be subjected to biological assay to evaluate the pharmacological activity.

EXPERIMENTAL SECTION

EXPERIMENTAL SECTION

Commercial starting solid materials were used without further purification. Solvents and liquid reagents were distilled under nitrogen-inert atmosphere before use. Reactions were monitored by TLC using silica plates 60-F264, commercially available from Merk. For Flash chromatography was used the Merck Kiesgel 60 H (particle size <45µm) without additive agents as stationary phase and for mobile phase was used the same TLC elution mixture with the polar component ¼ reduced. ^{1}H and ^{13}C NMR spectra were recorded at 300, 400 and 500 MHz and 75, 100 and 125 MHz, respectively, in $CDCl_3$ and DMSO-d_6 using tetramethylsilane (TMS) as internal standard (Bruker ACP 300 MHz, 400 MHz and 500 MHz). Chemical shifts are given in parts per million and coupling constants in Hertz. The stereochemistry were established by NOESY experiments. Purity was verified by NMR and HPLC spectra.
LC-MS analysis were carried using an Agilent 6540 UHD Accurate-Mass Q-TOF LC-MS (Agilent, Santa Clara, CA) fitted with a electrospray ionisation source (Dual AJS ESI) operating in positive ion mode. Chromatographic separation was achieved using a C_{18} RP analytical column (Poroshell 120, SB-C18, 50 x 2,1 mm, 2,7 mm) at 30 °C with a elution gradient from 5% to 95% of B over 13 min., a being H_2O (0,1% FA) and B CH_3CN (0,1% FA). Flow rate was 0,4 mL min^{-1}.
MW-assisted reactions were performed in Synthos 3000 instrument from Anton Paar, equipped with a 4 x 24MG5 rotor and an IR probe as external control of the temperature. 0,3–3 mL glass vials sealed with a dedicated PEEK screw-cup together with a reliable PTFE seal were used for all reactions.
In synthesis of all derivatives the setting of temperature was always maintained to 180 °C in each experiment, except for 5a–i where temperature was always maintained to 125 °C.
The diastereoisomeric ratio has been determined by a GC-MS instrument (Shimadzu QP2010) equipped with a Quadrex 007-5 MS column, 30 m, coated with a 0,25 mm film, helium as a carrier gas, injector temperature of 270 °C, and oven temperature program: 50 °C hold 3 min, ramp 14 °C min^{-1} to 280 °C and held for 1 min. The enantiomeric ratio has been evaluated by using a Focus GC-FID Thermo Scientific instrument (FID=flame ionization detector) equipped with a chiral column Megadex DET Beta, 30 m, coated with a 0,25 mm film, helium as a carrier gas, injector temperature 250 °C, and oven temperature program: 50 °C, ramp 2 °C min^{-1} to 200 °C and hold for 1 min. Stock solution at a concentration of 1 mg mL^{-1} were prepared in UHPLC‑MS-grade methanol. Before analysis, the solution was diluted 1:1000 v/v in a vial to obtain a concentration of 1 µg mL^{-1}.

11.1 SYNTHESIS OF THE HYDROXYLAMINES

11.1.1 Synthesis of the *N*-Methylhydroxylamine hydrochloride 1a

To a solution of nitromethane (50g) and NH_4Cl (30g) in water (400ml), zinc dust was added during 2-3h under continuous stirring, maintaining the temperature within a range of 0-15°C.

The mixture was then filtered out, and the filtrate was neutralized by hydrochloric acid and evaporated on a water bath until the residue solidified under cooling. The residue was dissolved in ethanol, thereupon the solution was separated out of the ammonium chloride precipitate, and ether was added. The settled precipitate was filtered out and twice recrystallized from ethanol with the addition of ether (1:1). White solid, 89% yield, m.p.=86-87°C.
^{1}H-NMR (DMSO-d_6) δ (300MHz): 2.0 (s, 1H, OH), 2.86 (s, 3H, CH_3), 8.45 (s, 2H, NH_2).
^{13}C-NMR (DMSO-d_6) δ (75 MHz): 33.8.
ESI(+)-MS calcd for CH_6NO [M+H] : found 48.0441; calcd 48.0444.

11.1.2 Synthesis of the *N*-*tert*-butylhydroxylamine hydrochloride 1b

2-methyl-2-nitropropane (9,27g, 90 mmol) was dissolved in ethanol and the solution was cooled to 0°C in an ice-bath. Zinc dust (5,89g, 90 mmmol) was added. Glacial acetic acid (10,8 g, 180 mmol) was then added dropwise at such a rate that the temperature maintained below 10°C with vigorous stirring. Ice bath was removed and the reaction mixture was stirred for 3h at room temperature. The zinc dust was filtered through a Buchner funnel and the solvent was removed in vacuo. The residue was dissolved in diethyl ether dry $HCl_{(g)}$ saturated and the solid white obtained was filtered. The crude was recrystallized from ethanol. White solid, 79% yield, m.p.=178-179°C.
^{1}H-NMR (DMSO-d_6) δ (300MHz): 1.4 (s, 9H, CH_3), 7.8–8.5 (br, 2H, NHOH).
^{13}C-NMR (DMSO-d_6) δ (75 MHz): 26.0, 50.0.
ESI(+)-MS calcd for $C_4H_{12}NO$ [M+H]: found 90.0911; calcd 90.0913.

11.1.3 Synthesis of the *N*-benzylhydroxylamine hydrochloride 1c

In a 2-L, three-necked, round-bottomed flask equipped with a 500 mL pressure-equalizing dropping funnel, a thermometer, and a magnetic stirring bar were placed Na2WO4·2H_2O (2,94 g, 10mmol), Bn_2NH (98,5 g, 0,5 mol) and MeOH (0,5 L). The flask was cooled with an ice bath to 0–5 °C (internal temperature) and 30% aq H_2O_2 (170 mL, 1,5 mol) was added dropwise over a period of ca. 2 h. During the period of addition, the reaction mixture should be carefully kept at a temperature below 5 °C. The cooling bath was removed 2 h after the end of the addition of H_2O_2, and the mixture was stirred for 18 h at r.t. The contents of the flask were first transferred to a 5 L beaker and then crushed ice (3 kg) was next added to the mixture with vigorous stirring. The white precipitate of nitrone intermedium was filtered, washed with ice water until the filtrate gave a negative result with peroxide test (ca. 0,5 L of H_2O was needed). The wet crude nitrone was then placed in a 2-L round-bottomed flask containing aq 20% HCl (1 L). The flask was fitted to a rotary evaporator keeping the water bath temperature at 70 °C with slow rotation under atmospheric pressure. After 30 min, the pressure was carefully reduced to 100 mmHg while benzaldehyde was distilled off with H_2O. When the total volume remained 130 mL, the flask was removed from the rotary evaporator. The semi-solid mixture was washed with toluene (3×100 mL) and then concentrated in vacuo (the last trace of H_2O could be removed by adding toluene and distilling).
The residue was dried overnight in a vacuum desiccator to afford pale yellow solid of crude BnNHOH·HCl (62 g) which was purified by recrystallization from hot MeOH (120 mL) and

then Et_2O (350 mL) to yield the pure desired product. White crystal solid, 72% yield, m.p.=110-111°C.
^{1}H-NMR (D_2O) δ (300MHz): 11.9 (br, 2 H), 11.0 (br, 1 H), 7.57–7.37 (m, 5 H), 4.31 (s, 2 H).
^{13}C-NMR (D_2O) δ (75 MHz): 131.6, 130.3, 129.1, 128.0, 62.2.
ESI(+)-MS calcd for $C_7H_{10}NO$ $[M+H]^+$: found 154.0757; calcd 154.0757.

11.1.4 Synthesis of the *N*-phenylhydroxylamine 1d

In a 2-litre beaker, equipped with a thermometer and mechanical stirrer, place 25 g of ammonium chloride, 800 ml of water and 50g (41,6 ml, 0,41 mol) of redistilled nitrobenzene. Stir the mixture vigorously, and add 59 g (0,83 mol) of zinc powder of 90 per cent purity during about 15 minutes; the rate of addition should be such that the temperature rapidly rises to 60-65°C and remains in this range until all the zinc has been added. Continue the stirring for a further 15 minutes, by which time the reduction is complete as is shown by the fact that the temperature commences to fall. Filter the warm reaction mixture at the pump to remove the zinc oxide, and wash it with 100 ml of hot water. Place the filtrate in a conical flask, saturate it with common salt (about 300 g) and cool in an ice bath for at least 1 hour to ensure maximum crystallization of the desired product. Filter the pale yellow crystals of phenylhydroxylamine with suction and drain well. The crude product is washed by hexane and is recrystallized from cyclohexane to yielded a white crystal solid. The substances deteriorates upon storage and is therefore used immediately for a secondary preparation. For a Short time it is possible to storage in a freezer to -20°C in a brown glass bottle. (70%, m.p.=80-81°C).
^{1}H-NMR ($CDCl_3$) δ (500MHz): 7.31-7.28 (t, J = 7.5 Hz, 2H), 7.02-6.97 (m, 3H).
^{13}C NMR (125 MHz, $CDCL_3$): δ 149.8, 129.2, 122.5, 114.8.
ESI(+)-MS calcd for C_6H_8NO [M+H] : found 110.0596; calcd 110.0600.

11.1.5 Synthesis of the *N*-(3-chlorophenyl)-hydroxylamine hydrochloride 1e

In a 100 ml flask, equipped with a thermometer and magnetic stirrer, place 0,51 g of ammonium chloride, 18 ml of water and 1g (0,64 mmol) of 3-chloro-nitrobenzene. The flask was cooled with an ice bath to 0–5 °C and stir the mixture vigorously, and add 1,50 g (0,23 mmol) of zinc powder of 90 per cent purity during about 15 minutes; the rate of addition should be such that the temperature rapidly rises to 14-16°C and remains in this range until all the zinc has been added. Continue the stirring for a further 15 minutes, by which time the reduction is complete as is shown by the fact that the temperature commences to fall. Filter the warm reaction mixture at the pump to remove the zinc oxide, and wash it with 100 ml of hot water. The filtrate is extracted with ethyl acetate (3x10 ml) and the organic phase is dried on sodium sulfate anhydrous. The organic phase is evaporate and the crude is placed in a solution of diethyl ether dry $HCl_{(g)}$ saturated and the solid white obtained was filtered. The crude was recrystallized from ethanol to obtain a white solid. (70%, m.p.=56-57°C).
^{1}H-NMR ($CDCl_3$) δ (300MHz): 2.0 (s, 1H, OH), 7.31-7.55 (m, 4H, Ar), 8.01 (s, 2H, NH_2).
^{13}C NMR (75 MHz, $CDCL_3$): δ 119.3, 122.6, 128.8, 130.1, 134.3, 139.4.
ESI(+)-MS calcd for C_6H_7ClNO [M+H] : found 144.0214; calcd 144.0211.

11.2 SYNTHESIS OF THE KETONITRONES

General procedure for synthesis of nitrones 4a–o and 5a–i

The selected ketone (0,5 g) and appropriate hydroxylamine derivative (2 eq. or 3 eq. for *N*-phenylhydroxylamine) were grinded in a mortar, placed in apposite vessel and mixed in a vortex. The mixture was transferred to a microwave oven and was irradiated with the opportune power. After the appropriate time the crude oil was recrystallized with ethyl acetate (4a–o) or cyclohexane (5a–i).

(*E*)-*N*-Methyl-*C*-isatinyl nitrone 4a. Yellow solid, 97% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.28 (s, 3H, CH_3), 6.92 (d, J =7.74 Hz, 1H, Ar), 7.06 (t, J = 7.60 Hz, 1H, Ar), 7.38 (t, J = 7.60 Hz, 1H, Ar), 8.15 (d, J = 7.74 Hz, 1H, Ar), 10.90 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 50.1, 109.1, 117.6, 121.3, 123.2, 130.9, 133.1, 138.9, 160.8.
ESI(+)-MS calcd for $C_9H_9N_2O_2$ [M + H] 177.0664, found: 177.0655.

(*E*)-*N*-Benzyl-*C*-isatinyl nitrone 4b. Orange solid, 95% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.89 (s, 2H, CH_2Bn), 6.91 (d, J =7.78 Hz, 1H, Ar), 7.10 (dt, J = 0.91, 7.65 Hz, 1H, Ar), 7.25–7.54 (m, 6H, Ar), 8.12 (d, J = 7.65 Hz, 1H, Ar), 11.01 (s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 64.3, 109.8, 118.1, 122.04, 123.9, 128.5, 129.6, 129.1, 131.8, 133.4, 134.2, 139.6, 161.2.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{13}N_2O_2$ 253.0977, found: 253.0977.

(*E*)-*N*-Phenyl-*C*-isatinyl nitrone 4c. Orange red solid, 92% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.85 (d, J = 7.80 Hz, 1H, Ar), 7.15 (t, J = 7.65 Hz, 1H, Ar), 7.40 (t, J = 7.80 Hz, 1H, Ar), 7.45–7.65 (m, 5H, Ar), 8.27 (d, J = 7.65 Hz, 1H, Ar), 10.78 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 109.7, 118.4, 121.7, 123.9, 124.1, 128.7, 130.1, 132.2, 134.4, 140.9, 146.3, 159.9.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{11}N_2O_2$ 239.0821, found: 239.0817.

(*E*)-*N*-3-Cl-phenyl-*C*-isatinyl nitrone 4d (the most abundant). Orange solid, 87% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.35 (d, J = 7.71, 1H, Ar), 6.79 (t, J = 7.71, 1H, Ar), 6.85–7.05 (m, 2H, Ar), 7.07–7.18 (m, 1H, Ar), 7.27–7.44 (m, 2H, Ar), 7.45–7.56 (m, 1H, Ar), 11.04 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 111.6, 115.6, 116.1, 117.1, 121.9, 124.6, 125.4, 131.4, 134.0, 134.6, 146.0, 150.8, 155.6, 163.3.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{10}ClN_2O_2$ 273.0431, found: 273.0422; [M + H] + 2 calcd for $C_{14}H_{10}ClN_2O_2$ 275.0401, found: 275.0382.

(*E*)-*N*-Methyl-*C*-(1-methyl)-isatinyl nitrone 4e. Yellow solid, 90% yield.
^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27 (s, 3H, CH_3), 4.38 (s, 3H, CH_3), 6.82 (d, J = 7.83 Hz, 1H, Ar), 7.09 (t, J = 7.65 Hz, 1H, Ar), 7.38 (t, J = 7.83 Hz, 1H, Ar), 8.30 (d, J = 7.65 Hz, 1H, Ar).
^{13}C NMR (75 MHz, $CDCl_3$): δ 26.0, 51.2, 107.6, 118.3, 123.0, 123.0, 124.8, 131.3, 141.2, 160.9.

ESI(+)-MS: m/z [M + H] calcd for $C_{10}H_{11}N_2O_2$ 191.0821, found: 191.0815.

(*E*)-*N*-Benzyl-*C*-(1-methyl)-isatinyl nitrone 4f. Yellow solid, 92% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.25 (s, 3H, CH_3), 5.92 (s, 2H, CH_2), 7.08 (t, J = 8.26 Hz, 2H, Ar), 7.37–7.50 (m, 6H, Ar), 8.15 (d, J = 7.34 Hz, 1H, Ar).
^{13}C-NMR (75 MHz DMSO-d_6): δ 26.6, 65.2, 109.2, 117.8, 123.1, 124.0, 129.0, 129.0, 129.59, 132.2, 134.6, 141.5, 160.4.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{15}N_2O_2$ 267.1134, found: 267.1126.

(*E*)-*N*-Phenyl-*C*-(1-methyl)-isatinyl nitrone 4g. Orange solid, 90% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.11 (s, 3H, CH_3), 7.08–7.16 (m, 2H, Ar), 7.46–7.58 (m, 6H, Ar), 8.30 (d, J = 7.48 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.5, 110.7, 115.4, 116.5, 117.5, 122.8, 125.1, 125.5, 131.9, 134.4, 135.1, 148.5, 152.2, 155.3, 162.4.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{13}N_2O_2$ 253.0977, found: 253.0975.

(*E*)-*N*-3-Cl-phenyl-*C*-(1-methyl)-isatinyl nitrone 4h (the most abundant). Yellow solid, 88% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.21 (s, 3H, CH_3), 6.37–6.40 (m, 1H, Ar), 6.85–7.12 (m, 4H, Ar), 7.32–7.65 (m, 3H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.6, 110.7, 115.4, 116.5, 117.5, 122.8, 125.1, 125.5, 131.9, 134.4, 135.1, 148.5, 152.2, 155.3, 162.4.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{12}ClN_2O_2$ 287.0587, found: 287.0577; [M + H] + 2 calcd for $C_{15}H_{12}ClN_2O_2$ 289.0558, found: 289.0557.

(*E*)-*N*-Methyl-*C*-(5-Br)-isatinyl nitrone 4i. Orange solid, 95% yield.
^{1}H -NMR (300 MHz, DMSO-d_6): δ 4.26 (s, 3H, CH_3), 6.83 (d, J = 8.24 Hz, 1H, Ar), 7.50 (dd, J = 1.92 Hz, 8.24, 1H, Ar), 8.18 (d, J = 1.92 Hz, 1H, Ar), 11.02 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 51.4, 112.0, 113.7, 120.4, 126.0, 133.5, 134.0, 139.0, 161.4.
ESI(+)-MS: m/z [M + H] calcd for $C_9H_8BrN_2O_2$ 254.9769, found: 254.9762; [M + H] + 2 calcd for $C_9H_8BrN_2O_2$ 256.9749, found: 256.9746.

(*E*)-*N*-Benzyl-*C*-(5-Br)-isatinyl nitrone 4j. Orange solid, 93% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): d 5.89 (s, 2H, CH_2), 6.87 (d, J ¼ 7.95 Hz, 1H, Ar), 7.26–7.63 (m, 6H, Ar), 8.21 (s, 1H, Ar), 11.16 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): d 65.2, 112.2, 113.9, 120.5, 126.2, 129.0, 129.0, 129.5, 133.3, 134.4, 134.4, 139.2, 161.3.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{12}BrN_2O_2$ 331.0082, found: 333.0071; [M + H] + 2 calcd for $C_{15}H_{12}BrN_2O_2$ 333.0062, found: 333.0053.

(*E*)-*N*-Phenyl-*C*-(5-Br)-isatinyl nitrone 4k. Orange solid, 92% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.86 (dd, J = 2.93 Hz, 8.25 Hz, 1H, Ar), 7.40–7.77 (m, 6H, Ar), 8.37 (d, J = 1.83 Hz, 1H, Ar), 10.92 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 116.9, 118.4, 125.6, 129.1, 131.2, 134.0, 135.7, 135.8, 139.6,

145.3, 151.4, 164.8.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{10}BrN_2O_2$ 316.9932, found: 316.9919; [M + H] + 2 calcd for $C_{14}H_{10}BrN_2O_2$ 318.9905, found: 318.9899.

(*E*)-*N*-Methyl-*C*-(5-NO_2)-isatinyl nitrone 4l. Yellow solid, 95% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.28 (s, 3H, CH_3), 7.02 (d, J = 8.73 Hz, 1H, Ar), 8.23 (d, J = 8.73 Hz, 1H, Ar), 8.75 (s, 1H, Ar), 11.53 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 51.8, 110.2, 118.7, 118.7, 128.0, 133.3, 142.4, 145.2, 161.9.
ESI(+)-MS: m/z [M + H] calcd for $C_9H_8N_3O_4$ 222.0515, found: 222.0503.

(*E*)-*N*-Benzyl-*C*-(5-NO_2)-isatinyl nitrone 4m. Yellow solid, 92% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.91 (s, 2H, CH_2), 7.07 (d, J = 8.73 Hz, 1H, Ar), 7.31–7.58 (m, 5H, Ar), 8.28 (dd, J = 2.43 Hz, 8.73 Hz, 1H, Ar), 8.84 (d, J = 2.43 Hz, 1H, Ar), 11.70 (s, 1H, NH) ^{13}C-NMR (75 MHz, DMSO-d_6): δ 65.6, 110.4, 118.8, 119.0, 128.3, 129.1, 129.1, 129.6, 133.2, 134.1, 142.6, 145.4, 161.9.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{12}N_3O_4$ 298.0828, found: 298.0816.

(*E*)-*N*-Phenyl-*C*-(5-NO_2)-isatinyl nitrone 4n. Dark yellow solid, 92% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.06 (d, J = 8.79 Hz, 1H, Ar), 7.46–7.69 (m, 5H, Ar), 8.33 (dd, J = 2.37 Hz, 8.79 Hz, 1H, Ar), 9.00 (d, J = 1.83 Hz, 1H, Ar), 11.47 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 110.3, 119.2, 124.4, 128.8, 129.8, 131.2, 134.2, 142.4, 146.5, 146.7, 160.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{10}N_3O_4$ 284.0671, found: 284.0662.

(*E*)-*N*-Methyl-*C*-(5,7-Cl)-isatinyl nitrone 4o. Orange solid, 80% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.25 (s, 3H, CH_3), 7.47 (d, J = 2.01 Hz, 1H, Ar), 7.91 (d, J = 2.01 Hz, 1H, Ar), 11.37 (s, 1H, NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 51.8, 115.0, 121.0, 121.8, 126.6, 130.2, 133.5, 136.2, 161.4.
ESI(+)-MS: m/z [M + H] calcd for $C_9H_7Cl_2N_2O_2$ 244.9885, found: 244.9878; [M + H] + 2 calcd for $C_9H_7Cl_2N_2O_2$ 246.9855, found: 246.9849; [M + H] + 4 calcd for $C_9H_7Cl_2N_2O_2$ 248.9826, found: 248.9820.

(*Z*)-*N*-Methyl-*C*-indanyl nitrone 5a. White solid, 82% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): d 2.90–3.05 (m, 2H, CH_2), 3.11–3.22 (m, 2H, CH_2), 3.81 (s, 3H, CH_3), 7.29–7.44 (m, 3H, Ar), 8.84 (d, J = 7.81 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): d 28.7, 29.4, 49.6, 124.5, 127.0, 131.0, 134.4, 147.8, 149.7.
ESI(+)-MS: m/z [M + H] calcd for $C_{10}H_{12}NO$ 162.0919, found: 162.0912.

(*Z*)-*N*-Benzyl-*C*-indanyl nitrone 5b. White solid, 85% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.91–3.09 (m, 2H, CH_2), 3.10–3.23 (m, 2H, CH_2), 5.11 (s, 2H, CH_2Bn), 7.21–7.45 (m, 7H, Ar), 7.51 (d, J = 7.16 Hz, 1H, Ar), 8.92 (d, J = 7.63 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 29.0, 29.2, 66.6, 124.5, 127.1, 127.2, 128.2, 128.3, 128.9, 131.1, 133.4, 134.8, 147.7, 149.1.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{16}NO$ 238.1232, found: 238.1230.

(*Z*)-*N*-Methyl-*C*-(2-Me)-indanyl nitrone 5c. White solid, 61% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.28 (d, J = 6.99 Hz, 3H, CH_3), 2.70 (d, J = 16.5 Hz, 1H, HCH_2), 3.20–3.50 (m, 2H, CH + HCH_2), 3.85 (s, 3H, CH_3), 7.22–7.48 (m, 3H, Ar), 8.83 (d, J = 7.53 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 19.7, 36.4, 38.4, 48.9, 124.7, 127.1, 127.5, 131.1, 133.5, 145.9, 153.8.
ESI(+)-MS: m/z [M + H] calcd for $C_{11}H_{14}NO$ 176.1075, found: 176.1068.

(*Z*)-*N*-Methyl-*C*-(3-Me)-indanyl nitrone 5d. White solid, 78% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.38 (d, 3H, J = 7.02 Hz, CH_3), 2.53 (dd, 1H, J = 2.79 Hz, 17.76 Hz, HCH_2), 3.24 (dd, 1H, J = 7.29 Hz, 17.76 Hz, HCH_2), 3.40–3.58 (m, 1H, CH), 3.78 (s, 3H, CH_3), 7.28–7.51 (m, 3H, Ar), 8.83 (d, J = 7.68 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 21.7, 35.9, 38.8, 49.8, 123.5, 126.9, 127.3, 131.2, 133.8, 152.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{11}H_{14}NO$ 176.1075, found: 176.1071.

(*Z*)-*N*-Methyl-*C*-(2-Pr)-indanyl nitrone 5e. White solid, 20% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.90 (t, 3H, J = 6.47 Hz, CH_3), 1.25–155 (m, 4H, CH_2), 2.90–3.42 (m, 3H, CH + CH_2), 3.83 (s, 3H, CH_3), 7.18–7.49 (m, 3H, Ar), 8.79 (d, J ¼ 7.51 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.8, 20.0, 35.7, 35.9, 41.6, 48.8, 124.7, 127.0, 127.5, 131.3, 134.0, 134.5, 146.7.
ESI(+)-MS: m/z [M + H] calcd for $C_{13}H_{18}NO$ 204.1388, found: 204.1384.

(*Z*)-*N*-Methyl-*C*-(5-Br)-indanyl nitrone 5f. White solid, 88% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.0 (dd, J = 5.55 Hz, 12.03 Hz, 2H, CH_2), 3.14 (dd, J = 5.55 Hz, 12.51 Hz, 2H, CH_2), 3.81 (s, 3H, CH_3), 7.29–7.44 (m, 3H, Ar), 8.84 (d, J = 7.81 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 28.6, 29.5, 49.9, 125.1, 127.8, 128.0, 130.4, 133.6, 148.1, 149.4.
ESI(+)-MS: m/z [M + H] calcd for $C_{10}H_{11}BrNO$ 240.0024, found: 240.0020; [M + H] + 2 calcd for $C_{10}H_{11}BrNO$ 242.0004, found: 242.0000.

(*Z*)-*N*-Methyl-*C*-(5-F)-indanyl nitrone 5g. White solid, 83% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.01 (dd, J = 5.43 Hz, 11.73 Hz, 2H, CH_2), 3.14 (dd, J = 5.55 Hz, 11.97 Hz, 2H, CH_2), 6.80–7.12 (m, 2H, Ar), 8.86 (dd, J = 5.79 Hz, 8.64 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 28.8 (d, J_{CF} = 2.28), 29.9, 49.6, 111.9 (d, J^{2}_{CF} = 23.02), 114.3 (d, J^{2}_{CF} = 22.86), 128.8 (d, J^{3}_{CF} = 9.03), 130.9 (d, J^{4}_{CF} = 2.02), 148.1, 150.5 (d, J^{3}_{CF} = 8.95), 164.43 (d, J^{1}_{CF} = 250.96).
ESI(+)-MS: m/z [M + H] calcd for $C_{10}H_{11}FNO$ 180.0825, found: 180.0818.

(*Z*)-*N*-Methyl-*C*-(4-Br-7-OH)-indanyl nitrone 5h. Yellow solid, 67% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.86–2.95 (m, 2H, CH_2), 2.96–3.30 (m, 2H, CH_2), 3.70 (s, 3H, CH_3), 6.60 (d, 1H, J = 8.79 Hz, Ar), 7.37 (d, J = 8.79 Hz, 1H, Ar), 14.96 (s, 1H, OH).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 30.2, 47.8, 107.2, 118.5, 121.2, 138.0, 149.6, 155.4, 157.9.
ESI(+)-MS: m/z [M + H] calcd for $C_{10}H_{11}BrNO_2$ 255.9973, found: 255.9964; [M + H] + 2 calcd for $C_{10}H_{11}BrNO_2$ 255.9953, found: 255.9945.

(*Z*)-*N*-Benzyl-*C*-(4-Br-7-OH)-indanyl nitrone 5i. Pale yellow solid, 78% yield.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.97 (s, 4H, CH_2), 5.04 (s, 2H, CH_2), 6.62 (d, 1H, J = 8.79 Hz, Ar), 7.35–7.50 (m, 6H, Ar), 15.02 (s, 1H, OH).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 29.8, 30.2, 64.6, 107.1, 118.6, 121.3, 127.9, 128.8, 129.1, 132.4, 138.0, 149.6, 155.5, 158.1.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{15}BrNO_2$ 332.0286, found: 332.0276; [M + H] + 2 calcd for $C_{16}H_{15}BrNO_2$ 334.0266, found 334.0259.

11.3 THEORETICAL CALCULATIONS OF THE ELECTROPHILICITY OF THE KETONE REAGENTS.

All of the calculations were performed using the Gaussian09 program.[365]
Molecular geometries were optimized with the B3LYP functional[366] using added D3 version of Grimme's dispersion[367] with Becke-Johnson damping[368] in conjunction with Def2SVP basis set a doucle-ζ quality set with polarization functions on both hydrogens as well as non-hydrogen atoms.[369] This combination of functional and basis has been recently used successfully[370] particularly because the Grimme-D3 dispersion corrections have been specifically parametrized for Ahlrichs' basis sets. Analytical second derivatives of the energy were calculated to classify the nature of the minima, to determine the harmonic vibrational frequencies, and to provide zero-point vibrational energy corrections. The thermal and entropic contributions to the free energies were also obtained from the vibrational frequency calculations, using the unscaled frequencies.

11.3.1 Reactivity Indices

The electronic chemical potential μ, that is the negative of electronegativity χ, is associated with the charge transfer ability of the system in its ground state geometry and is the index pointing to the direction of the electronic flux during the cycloaddition. It can be approximated, using Koopmans' theorem, to the half value of the sum of the one-electron energies of HOMO and LUMO orbitals (Eq. 1).[363]

$$\mu = \frac{\varepsilon_{HOMO} + \varepsilon_{LUMO}}{2} \quad (1)$$

The chemical hardness η (approximated to be the difference between LUMO and HOMO energies)
is a measure of the stability of a system, the system having the maximum hardness being the most
stable (Eq. 2).[364]

$$\eta = \varepsilon_{HOMO} - \varepsilon_{LUMO} \quad (2)$$

The chemical softness parameter *S* is strictly related to the chemical hardness and it is due to the

inverse of 2η (Eq. 3).

$$S = \frac{1}{2\eta} \quad (3)$$

The global electrophilicity power ω is a useful descriptor of reactivity that allows a quantitative classification of the global electrophilic character of a molecule,[365] and it is extremely useful for evaluating both Diels-Alder reactions4 and 1,3-dipolar cycloaddition reactions.[367] The ω index measures the stabilization in energy when the system acquires an additional electronic charge ΔN from the environment and it is defined as illustrated in Eq. 4.[368]

$$\omega = \frac{\mu^2}{2\eta} \quad (4)$$

The maximum amount of electronic charge that the electrophile system may accept is given by:

$$\Delta N_{max} = -\frac{\mu}{\eta} \quad (5)$$

Hydroxylamine	E_0	G
1a	-170,831800	-170,856995
1b	-401,663482	-401,697409
1c	-362,407258	-362,438264
1d	-821,887369	-821,920554
1e	-476,816677	-476,851397
1f	-566,761050	-566,796116
1g	-1035,986147	-1036,030943
1h	-858,205142	-858,242438
1i	-837,943001	-837,986422

Table 11.1 *Calculated (B3LYP-D3BJ/def2SVP) absolute energies (hartrees) of hydroxylamines 1a-h.*

Ketones	E_0	G
2a	-512,612135	-512,645056
2b	-551,874436	-551,909245
2c	-3085,892123	-3085,928301
2d	-716,960109	-716,997196
2e	-611,780175	-611,814329
2f	-627,027817	-627,064097
2g	-849,400063	-849,440167
2h	-909,248800	-909,286713
2i	-740,286602	-740,326443
2j	-3085,893544	-3085,929692
2k	-716,957737	-716,994951

Table 11.2 *Calculated (B3LYP-D3BJ/def2SVP) absolute energies (hartrees) of ketones 2a-k.*

Ketones	E_0	G
3a	-422,592006	-422,624855
3b	-461,857110	-461,891515
3c	-461,856218	-461,891239
3d	-540,381406	-540,420124
3e	-2995,874151	-2995,910267
3f	-521,760373	-521,794436
3g	-461,859289	-461,892840
3h	-561,026211	-561,060984
3i	-576,273753	-576,310638
3j	-759,380596	-759,417464
3k	-626,939844	-626,976975
3l	-537,006034	-537,042206
3m	-521,749455	-521,783194
3n	-819,234663	-819,272436
3o	-666,207730	-666,245644
3p	-748,191259	-747,962896

Table 11.3 *Calculated (B3LYP-D3BJ/def2SVP) absolute energies (hartrees) of ketones 3a-o.*

Product	HOMO	LUMO	μ	η	ω	μ (eV)	η (eV)	ω (eV)	N (eV)
1a	-0,23462	0,05814	-0,0882	0,2928	0,0133	-2,40	7,97	0,36	4,78
1b	-0,23775	-0,01351	-0,1256	0,2242	0,0352	-3,42	6,10	0,96	4,69
1c	-0,21083	-0,00549	-0,1082	0,2053	0,0285	-2,94	5,59	0,78	5,43
1d	-0,21973	-0,01606	-0,1179	0,2037	0,0341	-3,21	5,54	0,93	5.18
1e	-0,23371	0,05973	-0,0892	0,2743	0,0135	-2,38	7,89	0,38	4.81
1f	-0,19350	-0,00684	-0,1002	0,1867	0,0269	-2,73	5,08	0,73	5,90
1g	-0,23941	-0,07976	-0,1596	0,1597	0,0798	-4,34	4,34	2,17	4,65
1h	-0,24104	-0,05289	-0,1470	0,1882	0,0574	-4,00	5,12	1,56	4,60
1i	-0,24357	-0,03613	-0,1399	0,2074	0,0471	-3,81	5,64	1,28	4,54
2a	-0,24758	-0,10323	-0,1754	0,1444	0,1066	-4,77	3,93	2,90	4,43
2b	-0,24069	-0,10142	-0,1711	0,1393	0,1050	-4,65	3,79	2,86	4,61
2c	-0,24571	-0,11208	-0,1789	0,1336	0,1197	-4,87	3,64	3,26	4,48
2d	-0,27356	-0,12618	-0,1999	0,1474	0,1355	-5,44	4,01	3,69	3.72
2e	-0,25447	-0,10569	-0,1801	0,1488	0,1090	-4,90	4,05	2,97	4,24
2f	-0,24017	-0,09217	-0,1662	0,1480	0,0933	-4,52	4,03	2,54	4,63
2g	-0,26389	-0,11692	-0,1904	0,1470	0,1233	-5,18	4,00	3,36	3,98
2h	-0,26432	-0,12211	-0,1932	0,1422	0,1313	-5,26	3,87	3,57	3,97
2i	-0,25906	-0,10967	-0,1844	0,1494	0,1138	-5,02	4,07	3,10	4,11
2j	-0,25423	-0,11018	-0,1822	0,1441	0,1152	-4,96	3,92	3,14	4,25
2k	-0,2708	-0,13673	-0,2038	0,1341	0,1548	-5,54	3,65	4,21	3,79
3a	-0,24658	-0,05999	-0,1533	0,1866	0,0630	-4,17	5,08	1,71	4,45
3b	-0,24539	-0,06035	-0,1529	0,1850	0,0631	-4,16	5,04	1,72	4,49
3c	-0,24544	-0,05994	-0,1527	0,1855	0,0628	-4,15	5,05	1,71	4,48
3d	-0,24495	-0,06003	-0,1525	0,1849	0,0629	-4,15	5,03	1,71	4,50
3e	-0,25333	-0,0692	-0,1613	0,1841	0,0706	-4,39	5,01	1.92	4,27
3f	-0,2508	-0,06159	-0,1562	0,1892	0,0645	-4,25	5,15	1.54	4,34
3g	-0,23462	0,05814	-0,0882	0,2928	0,0133	-2,40	7,97	0,36	4,78

3h	-0,24681	-0,05989	-0,1534	0,1869	0,0629	-4,17	5,09	1,71	4,45
3i	-0,25267	-0,06828	-0,1605	0,1844	0,0698	-4,37	5,02	1,90	4,29
3j	-0,23329	-0,04896	-0,1411	0,1843	0,0540	-3,84	5,02	1,47	4,82
3k	-0,25847	-0,0745	-0,1665	0,1840	0,0753	-4,53	5,01	2,05	4,13
3l	-0,26706	-0,1117	-0,1894	0,1554	0,1154	-5,15	4,23	3,14	3,90
3m	-0,23607	-0.04971	-0,1429	0,1864	0,0548	-3,89	5,07	1,49	4,74
3n	-0,25445	-0,07353	-0,1640	0,1809	0,0743	-4,46	4,92	2,02	4,24
3o	-0,26668	-0,07873	-0,1727	0,1880	0,0793	-4,70	5,11	2,16	3,91
3p	-0,26685	-0,10979	-0,1883	0,1571	0,1129	-5,12	4,27	3,07	3,90
3q	-0,23462	0,05814	-0,0882	0,2928	0,0133	-2,40	7,97	0,36	4,78

Table 11.4 *Calculated (B3LYP-D3BJ/def2SVP) reactivity indices.*

11.4 ANTIPROLIFERATIVE ACTIVITY EVALUATION OF NITRONES

MG63 and TE85 human osteosarcoma cell lines were maintained in DMEM, while K562 human chronic myeloid leukemia cell line in RPMI 1640. Culture medium was supplemented with 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 U/mL) and glutamine (2 mM); incubation was at 37 °C in a 5% CO_2 atmosphere. Compounds were solubilized in DMSO at 40 mM, kept at -80°C in the dark and diluted in complete medium immediately before use. The osteosarcoma cells were seeded at 4000 cells/well in 96-well plate 6 hours before the treatment. The leukemia cells were seeded at 20.000 cells/mL. Cells were treated with the test compounds at concentrations ranging from 3 to 200 μM. Untreated cells were placed in every plate as negative control. After 3 days of culture, the inhibitory effect on cell proliferation was analysed by addition of 25 μL MTT (thiazollyl blue) staining solution, in which metabolically active cells convert the yellow tetrazolium salt to purple formazan crystals providing a quantitative determination of viable cells. Two hours later, formazan crystals were solubilized in 100 μL of lysing buffer (50% DMF + 20% SDS, pH 4.7) for 16 hours, whereupon the spectrophotometric absorbance at 570 nm was measured. Half maximal inhibitory concentration (IC_{50}) was calculated using Scientist software. Three independent experiments were performed in triplicate.

11.5 ANTIOXIDANT EVALUATION OF NITRONES

The free radical scavenging activity against DPPH was determined at five different concentrations according to a previous work.26 Thus, EtOH solutions containing known amounts of the compounds (**4b**, **4c**, **4f, 4l**, **4m** and **5a**, **5b**, **5g**) and of DPPH were prepared, and the decrease in the absorbance of DPPH was measured at 517 nm by using a UV-Vis spectrophotometer (Varian Cary 50 Scan), after a period of 10 min since preparation. Experiments were carried out in triplicate and BHT (butylated hydroxytoluene) was used as the reference antioxidant.

11.6 SYNTHESIS OF THE VINYLNUCLEOBASES

11.6.1 General procedures for pyrimidine nucleobases and vinyladenine nucleobase.

The appropriate pyrimidine nucleobase and/or adenine (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 a trace of $(NH_4)_2SO_4$ until a clear solution was formed. Then, the solution was concentrated in vacuo. The residue was suspended in vinyl acetate (25,0 mL) and $Hg(OAc)_2$ (96 mg, 0,3 mmol), trimethylsilyl trifluoromethanesulfonate (245 mg, 1,1 mmol), and hydroquinone (0,1 g), the latter as a polymerization inhibitor, were added under N_2. The mixture was refluxed for 4h. When the reaction was finished, the mixture was filtered through neutral activated alumina and was washed with EtOAc. The solvents were removed at reduced pressure and the crude product was purified by flash chromatography with $CHCl_3$–MeOH (92,5:7,5) as eluent.

1-Vinyluracil 6. White solid, 93% yield, mp 178–180 °C.
^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.90 (dd, 1 H, 2'-CH*cis*, Jgem = 1.55, J*cis* = 9.31 Hz), 5.36 (dd, 1 H, 2'-CH*trans*, Jgem = 1.55, J*trans* = 16.03 Hz), 5.73 (d, 1 H, 5-CH, J = 7.77 Hz), 7.09 (dd, 1 H, 1'-CH,
J*cis* = 9.31, J*trans* = 16.03 Hz), 8.01 (d, 1 H, 6-CH, J = 7.77 Hz), 11.52 (br s, 1H , NH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 100.8, 102.7, 129.3, 139.6, 149.3, 162.9.
MS: *m*/*z* = 139 $[M + H]^+$, 96. HRMS: *m*/*z* calcd for $C_6H_6N_2O_2$: 138.12412; found: 138.12415.

1-Vinylthymine 7. White solid, 95% yield, mp 205–207 °C.
1H-NMR (300 MHz, DMSO-d_6): δ = 1.84 (d, 3 H, CH_3, J = 1.44), 4.84 (dd, 1 H, 2'-CH*cis*, Jgem = 1.95, J*cis* = 9.27 Hz), 5.33 (dd, 1 H, 2'-CH*trans*, Jgem = 1.95, J*trans* = 16.09 Hz), 7.10 (dd, 1 H, 1'-CH, J*cis* = 9.27, J*trans* = 16.09 Hz), 7.90 (d, 1 H, 6-CH, J = 1.44 Hz), 11.51 (br s, 1 H, NH).
13C-NMR (75 MHz, DMSO-d_6): δ = 11.9, 99.7, 110.61, 129.1, 134.9, 149.3, 163.6.
MS: *m*/*z* = 153 $[M + H]^+$, 110, 82. HRMS: *m*/*z* calcd for $C_7H_8N_2O_2$: 152.15070; found: 152.15073.

1-Vinyl-5-fluoro-uracil 8. White solid, 89% yield, mp 136–137 °C.
^{1}H-NMR (400 MHz, Chloroform-d) δ 7.52 (d, J = 5.8 Hz, 1H, F-uracil vinyl-CH), 7.12 (ddd, J = 15.9, 9.1, 1.8 Hz, 1H, vinyl-CH), 5.11-4.79 (m, 2H, vinyl-CH_2).
^{13}C-NMR (101 MHz, Chloroform-d) δ 156.1 (d, J = 27.3 Hz), 147.3, 141.1 (d, J = 241.8 Hz), 129.3, 123.1 (d, J = 34.2 Hz), 101.3.
HRMS (ESI) calcd for $C_6FH_6N_2O_2^+$ $[M+H]^+$ 157.0408; found 157.0414.

1-Vinylcytosine 9. White solid, 85% yield, mp 215–217 °C.
^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.78 (dd, 1 H, 2'-CH*cis*, Jgem = 1.33, J*cis* = 9.03 Hz), 5.23 (dd, 1 H, 2'-CH*trans*, Jgem = 1.33, J*trans* = 16.17 Hz), 5.84 (d, 1 H, 5-CH, J = 7.35 Hz), 7.24 (dd, 1 H, 1'-CH, J*cis* = 9.03, J*trans* = 16.17 Hz), 7.48 (br s, 2 H, NH_2), 7.93 (d, 1 H, 6-CH, J = 7.35 Hz).
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 95.5; 99.3; 131.6; 139.8; 153.9; 165.6.

MS: *m*/*z* = 138 $[M + H]^+$, 95, 70. HRMS: *m*/*z* calcd for $C_6H_7N_3O$: 137.13940; found: 137.13936.

9-Vinyladenine 10. White solid, 92% yield, mp 200–202 °C.
^{1}H-NMR (300 MHz, DMSO-d_6): δ = 5.16 (dd, 1 H, 2'-CH*cis*, Jgem = 0.81, J*cis* = 9.30 Hz), 6.11 (dd, 1 H, 2'-CH*trans*, Jgem = 0.81, J*trans* = 16.00 Hz), 7.33 (dd, 1 H, 1'-CH, J*cis* = 9.30, J*trans* = 16.00 Hz), 7.44 (br s, 2 H, NH_2), 8.25 (s, 1 H, 2-CH), 8.53 (s, 1 H, 8-CH).
^{13}C-NMR (75 MHz, DMSO-d_6): δ = 103.2, 119.1, 127.2, 138.6, 148.6, 153.2, 156.0.
MS: *m*/*z* = 162 $[M + H]^+$, 145, 136. HRMS: *m/z* calcd for $C_7H_7N_5$: 161.16418; found: 161.16421.

11.6.2 Synthesis of the 9-Vinylguanine 11.

2-(*N*-Acetyl)-6-(*O*-diphenylcarbamoyl) guanine.
Guanine (10,0 g, 66,2 mmol) and dry N,N-dimethylacetamide (85 ml) were placed in a 250 ml flask fitted with a reflux condenser under dry nitrogen. Acetic anhydride (20 ml, 211,6 mmol) was added to this suspension. The solution was warmed to 160°C. When the reaction was completed, it was cooled and the white solid obtained (2,9-diacetylguanine) was filtered and washed with EtOH.
To a suspention of 2,9-diacetylguanine (10g, 42,5 mmol), DIPEA (15 ml) and dry pyridine (200 ml), diphenylcarbamoyl chloride (10,88g, 46,9 mmol) was added. The reaction was stirred for 4h (TLC: CH_2Cl_2/MeOH 9:1 v/v). Then H_2O (20 ml) was added and the mixture was stirred for 10 min. The solvent were removed in vacuo. The residue was placed in a solution of H_2O/EtOH (1:1 v/v) and heated to 70°C. After 3,5h the suspension was cooled and filtered.
White solid, 91% yield, mp. 153-155°C.
^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.18 (s, 3H, CH_3CO), 7.26-7.56 (m, 10H, $N(C_6H_5)_2$), 8.46 (s, 1H, 8-CH).

2-(*N*-Acetyl)-6-(*O*-diphenylcarbamoyl)-9-vinyl-guanine.
The protected guanine nucleobase (4,4 mmol) was added to a suspension of $Hg(OAc)_2$ (96 mg, 0,3 mmol) in vinyl acetate (25,0 ml). Then, trimethylsilyltrifluoromethanesulfonate (245 mg, 1,1 mmol) and hydroquinone (0,1 g), the latter as a polymerisation inhibitor, were added. The mixture was refluxed for the 21h. When the reaction was over, the mixture was filtered through neutral activated alumina and was washed with 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.
Yellow solid, 82% yield, mp. 187-188°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.53 (s, 3H, CH_3), 5.19 (dd, 1H, 2'-CH_{cis}, J_{cis}=9.3, J_{gem}=1.18), 5.91 (dd, 1H, 2'-CH_{trans}, J_{trans}=16.0, J_{gem}=1.8), 7.10 (dd, 1H, 1'-CH, J_{trans}=16, J_{cis}=9.3), 7.20-7.55 (m, 10H, $N(C_6H_5)_2$), 8.12 (s, 1H, 8-CH) 8.25 (brs, 1H, 2-NH).

9-Vinylguanine 11.
In a solution of protected vinylguanine (2,5g, 6,0mmol) and MeOH (60 ml) ammonia was bubbled for 10 min. and then the suspension was warmed to 60°C for 1h. When the reaction was completed (TLC: $CHCl_3$/MeOH 9:1 v/v), the solvent was removed under reduced pressure. The residue was suspended in CH_2Cl_2 and stirred for 20 min.

White solid, 95% yield, mp. >300°C.
1H-NMR (300 MHz, DMSO-d_6): δ = 5.05 (d, 1 H, 2'-CH*cis*, *Jcis* = 9.39 Hz), 5.87 (d, 1 H, 2'-CH*trans*, *Jtrans* = 16.12 Hz), 6.70 (br s, 2 H, NH2), 7.08 (dd, 1 H, 1'-CH, *Jcis* = 9.39, *Jtrans* = 16.12 Hz), 8.12 (s, 1 H, 8-CH), 10.74 (br s, 1 H, 1-NH).
13C-NMR (75 MHz, DMSO-d_6): δ = 102.79, 115.64, 126.76, 134.70, 154.04, 156.78.
MS: *m*/*z* = 178 $[M + H]^+$, 161, 150. HRMS: *m*/*z* calcd for $C_7H_7N_5O$: 177.16358; found: 177.16354.

11.7 SYNTHESIS OF THE INDANYL AND ISATINYL SPIRO-ISOXAZOLIDINES *N,O*-NUCLEOSIDES

The opportune nitrone (2 eq.) and opportune vinylnucleobase(0.05 g) were grinded in a mortar, placed in apposite vessel and mixed in a vortex. The mixture was transferred to a microwave oven and was irradiated with the opportune power and temperature (when required). After the appropriate time the crude oil was purified by flash chromatography with hexane:AcOEt 7.75:2.25 v/v for 20-22 or $CHCl_3$/MeOH 9.50:0.50 v/v for all the other crudes.

***exo*-5'-Thyminyl-2'-methyl-spiro-[indane-3,3'-isoaxazolidine] 12.** White solid, 77% yield.
1H-NMR (400 MHz, $CDCl_3$): δ 1.76–1.90 (m, 2H, CH_2), 1.94 (d, J ¼ 1.08 Hz, 1H, CH_3), 2.38 (s, 3H, CH_3), 2.51–2.67 (m, 2H, CH_2), 2.79–3.05 (m, 2H, CH_2), 6.20 (dd, J ¼ 5.68 Hz, 7.36 Hz, 1H, CH), 7.12–7.35 (m, 4H, Ar), 7.79 (s, 1H, 6-CHThy), 9.39 (sb, 1H, NHThy).
13C-NMR (101 MHz, $CDCl_3$): δ 12.8, 29.2, 30.2, 36.1, 51.6, 77.9, 82.2, 111.1, 123.6, 125.2, 126.9, 129.0, 135.9, 139.8, 144.3, 150.7, 164.2.
ESI(+)-MS: m/z [M + H] calcd for $C_{17}H_{20}N_3O_3$ 314.1505, found: 314.1497.

exo-5'-Uracil-2'-methyl-spiro-[indane-3,3'-isoaxazolidine] 13. White solid, 77% yield.
1H-NMR (400 MHz, $CDCl_3$): δ 1.78–1.95 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 2.52–2.72 (m, 2H, CH_2), 2.79–3.10 (m, 2H, CH_2), 5.77 (d, J = 8.02 Hz, 1H, 5-CHUra), 6.19 (dd, J = 5.38 Hz, 7.24 Hz, 1H, CH), 7.10–7.40 (m, 4H, Ar), 8.05 (d, J = 8.02 Hz, 1H, 6-CHUra), 9.53 (sb, 1H, NHUra).
13C-NMR (101 MHz, $CDCl_3$): δ 29.4, 30.1, 35.9, 78.0, 82.6, 102.6, 123.6, 125.1, 127.0, 129.1, 139.5, 140.3, 144.3, 150.6, 163.7.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{18}N_3O_3$ 300.1348, found: 300.1337.

***exo*-5'-(5-fluoro)Uracil-2'-methy-spiro-[indane-3,3'-isoxazolidine] 14.** White solid, 74% yield.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.79–1.89 (m, 1H, CH_2), 2.40 (s, 3H, CH_3), 2.52–2.65 (m, 2H, CH_2), 2.80–2.96 (m, 2H, CH_2), 2.96–3.05 (m, 1H, CH_2), 6.17 (dd, J = 6.71 Hz, J = 1.18 Hz 1H, 5-CH), 7.15–7.26 (m, 4H, Ar), 8.10 (d, J = 6.24 Hz, 1H, 6-CHFUra), 9.80 (sb, 1H, NHUra).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 29.35, 30.18, 36.04, 51.93, 78.05, 82.99, 123.65, 124.61, 125.26, 127.18, 129.23, 139.54, 141.98, 144.25, 149.26, 157.17.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{17}FN_3O_3$ 318.1254, found 318. 1248

***exo*-5'-Cytosinyl-2'-methyl-spiro-[indane-3,3'-isoaxazolidine] 15.** White solid, 75% yield.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.79–1.96 (m, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.51–2.70 (m, 2H, CH_2), 2.78–3.13 (m, 2H, CH_2), 5.80 (d, J = 8.09 Hz, 1H, 5-CHCyt), 6.22 (dd, J = 5.39 Hz, 7.26 Hz, 1H, CH), 7.09–7.43 (m, 4H, Ar), 8.06 (d, J = 8.01 Hz, 1H, 6-CHCyt), 9.96 (sb, 2H, NH_2Cyt).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 29.32, 30.16, 36.01, 51.87, 78.02, 83.15, 122.60, 124.65, 126.26, 127.19, 129.29, 139.62, 142.01, 144.23, 149.23, 157.18.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{19}N_4O_2$ 299.1508, found 299.1500.

***exo*-5'-Adeninyl-2'-methyl-spiro-[indane-3,3'-isoaxazolidine]16.** White solid, 76% yield.
^{1}H-NMR (500 MHz, DMSO-d6): 1.82–2.01 (m, 2H, CH_2), 2.52 (s, 3H, CH_3), 2.54–2.75 (m, 2H, CH_2), 2.79–3.16 (m, 2H, CH_2), 6.50 (dd, J = 5.42 Hz, 7.36 Hz, 1H, CH), 7.12–7.53 (m, 4H, Ar), 8.12 (s, 1H, CHAde), 10.02 (sb, 2H, NH_2Ade).
^{13}C-NMR (125 MHz, DMSO-d6): δ 29.35, 30.34, 36.72, 51.94, 78.43, 83.55, 122.78, 124.98, 127.56, 129.11, 129.46, 139.78, 142.61, 144.78, 149.98, 157.98, 160,02.
ESI(+)-MS: m/z [M + H] calcd for $C_{17}H_{19}N_6O$ 323.1620, found 323.1615.

***exo*-5'-Thyminyl-2'-methyl-spiro-[indoline-3,3'-isoxazolidine]-1-methyl-2-one 18.** Red solid, 85% yield.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.02 (d, J = 1.21, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.64 (dd, J = 4.31 Hz, 14.03 Hz, 1H, HCH_2), 3.22 (s, 3H, CH_3), 3.28 (dd, J = 7.48 Hz, 14.03 Hz, 1H, HCH_2), 6.44 (dd, J = 4.31 Hz, 7.48 Hz, 1H, CH), 6.86 (d, J = 7.85 Hz, 1H, Ar), 7.09–7.14 (m, 1H, Ar), 7.24–7.29 (m, 1H, Ar), 7.36–7.41 (m, 1H, Ar), 7.77 (d, J = 1.21 Hz, 1H, 6-CHThy), 8.65 (sb, 1H, NHThy).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 12.8, 26.0, 38,0, 47.6, 72.3, 83.4, 108.6, 110.7, 123.3, 123.3, 124.1, 130.5, 135.3, 144.2, 150.2, 163.8, 173.9.
ESI(+)-MS: m/z [M + H] calcd for $C_{17}H_{19}N_4O_4$ 343.1406, found: 343.1403.

***exo*-5'-Uracil-2'-benzyl-spiro-[indoline-3,3'-isoxazolidine]-2-one 19.** Yellow solid, 84% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.76–289 (m, 1H, HCH_2), 3.41–3.58 (m, 1H, HCH_2), 5.05–5.14 (m, 2H, CH_2Bn), 5.59 (dd, J = 2.20 Hz, 7.95 Hz, 1H, CH), 6.68 (d, J =7.68 Hz, 1H, 5-CHUra), 6.75–7.92 (m, 9H, Ar), 7.97 (d, J = 7.68, 1H, 6-CHUra), 10.23 (sb, 1H, NH), 11.05 (sb, 1H, NHUra).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 61,4, 72.6, 101.2, 109.9, 122.0, 125.3, 127.1, 127.3, 128.1, 128.4, 128.9, 129.5, 130.2, 142.8, 143.4, 151.2, 163.1, 168.5, 180.2.
ESI(+)-MS: m/z [M + H] calcd for $C_{21}H_{19}NaN_4O_4$ 413.1226, found: 413.1219.

***exo*-5'-Uracil-2'-phenyl-spiro-[indoline-3,3'-isoxazolidine]-2-one 20.** Orange solid, 80% yield.
^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.98–3.11 (m, 1H, HCH_2), 3.25–3.33 (m, 1H, HCH_2), 5.79 (d, J = 8.07 Hz, 1H, 5-CHUra), 6.24 (dd, J = 5.31 Hz, 7.14 Hz, 1H, CH), 6.65–7.66 (m, 8H, Ar), 7.98 (d, J = 8.28 Hz, 1H, Ar), 8.55 (d, J = 8.07 Hz, 1H, 6-CHUra), 10.43 (sb, 1H, NH), 10.89 (sb, 1H, NHUra).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 46.8, 72.9, 80.9, 102.6, 110.5, 118.1, 124.4, 126.1, 128.8,

128.9, 128.2, 130.9, 132.7, 141.8, 145.6, 151.4, 163.4, 175.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{20}H_{17}N_4O_4$ 377.1250, found: 377.1237.

***exo*-5'-Thyminyl-2'-phenyl-spiro-[indoline-3,3'-isoxazolidine]-2-one 21.** Orange solid, 80% yield. ^{1}H-NMR (300 MHz, DMSOd$_6$): δ 1.89 (s, 3H, CH_3) 2.99–3.14 (m, 1H, HCH_2), 3.16–3.32 (m, 1H, HCH_2), 6.67–7.58 (m, 10H, Ar + CH), 8.53 (s, 1H, 6-CHThy), 10.46 (sb, 1H, NH), 11.48 (sb, 1H, NHThy).
^{13}C-NMR (75 MHz, DMSO-d$_6$): δ 12.8, 45.0, 73.5, 81.8, 100.3, 110.8, 117.3, 118.0, 122.8, 124.6, 128.8, 128.9, 130.9, 135.7, 142.8, 146.4, 151.1, 164.0, 175.8.
ESI(+)-MS: m/z [M + H] calcd for $C_{21}H_{19}N_4O_4$ 391.1406, found: 391.1402.

exo-50-Uracil-20-methyl-spiro-[indoline-3,30-isoxazolidine]-1-methyl-2-one 22. Orange solid, 85% yield.
^{1}H-NMR (300 MHz, DMSO-d$_6$): δ 2.42 (s, 3H, CH_3), 2.82 (dd, J = 4.86 Hz, 13.98 Hz, 1H, HCH_2), 3.05–3.22 (m, 4H, CH_3 + HCH_2), 6.34 (dd, J = 4.86 Hz, 7.47 Hz, 1H, CH), 7.00–7.21 (m, 2H, Ar), 7.38–7.55 (m, 2H, Ar), 7.98 (d, J = 8.16, 1H, 5-CHUra), 8.03 (d, J = 8.16 Hz, 1H, 6-CHUra), 11.46 (sb, 1H, NHUra).
^{13}C-NMR (75 MHz, DMSO-d$_6$): δ 26.3, 38,1, 46.3, 72.5, 82.6, 108.9, 109.5, 123.2, 124.9, 129.9, 130.7, 140.1, 144.7, 151.0, 163.7, 173.9.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{16}N_4O_4$ 329.1250, found: 329.1242.

***exo*-5'-Thyminyl-2'-methyl-spiro-[5-bromo-indoline-3,3'-isoxazolidine]-2-one 23.** Yellow solid, 84% yield.
1H-NMR (500 MHz, DMSO-d$_6$): δ 1.92 (s, 3H, CH_3), 2.97 (dd, J = 6.04 Hz, 13.73 Hz, 1H, HCH_2), 3.46 (dd, J = 7.14 Hz, 13.73 Hz, 1H, HCH_2), 3.54 (s, 3H, NCH_3), 6.40 (dd, J = 6.04 Hz, 7.14 Hz, 1H, CH), 6.85 (d, J = 8.23 Hz, 1H, Ar), 7.18–7.56 (m, 2H, Ar), 7.72–7.81 (m, 2H, Ar + 6-CHThy), 10.78 (sb, 1H, NH), 11.38 (sb, 1H, NHThy).
13C-NMR (125 MHz, DMSO-d$_6$): δ 16.7, 33.3, 42.4, 74.2, 86.3, 114.1, 116.3, 118.1, 129.9, 130.9, 137.2, 140.0, 146.5, 154.9, 168.2, 179.3.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{16}BrN_4O_4$ 407.0355, found: 407.0337; [M + H] + 2 calcd for $C_{16}H_{16}BrN_4O_4$ 409.0334, found: 409.0322.

***exo*-5'-Thyminyl-2'-benzyl-spiro-[5-bromo-indoline-3,3'-isoxazolidine]-2-one 24.** Yellow solid, 83% yield.
^{1}H-NMR (500 MHz, DMSO-d$_6$): δ 1.82 (s, 3H, CH_3), 2.8–3,2 (m, 2H, HCH_2), 4.35 (s, 2H, CH_2Bn), 6.84 (dd, J ¼ 6.24 Hz, 8.85 Hz, 1H, CH), 6.70–8.71 (m, 8H, Ar), 8.98 (d, J ¼ 5.42 Hz, 1H, 6-CHThy), 11.10 (sb, 1H, NH), 11.36 (sb, 1H, NHThy).
^{13}C-NMR (125 MHz, DMSO-d$_6$): δ 16.3, 33.3, 39.5, 67.0, 76.2, 113.6, 116.6, 117.7, 126.4, 132.1,
132.5, 132.9, 133.5, 137.8, 142.5, 144.4, 146.6, 149.4, 155.6, 167.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{22}H_{20}BrN_4O_4$ 483.0668, found: 483.0666; [M + H] + 2 calcd for $C_{22}H_{20}BrN_4O_4$ 485.0647, found: 485.0637.

***exo*-5'-Thyminyl-2'-benzyl-spiro-[5-nitro-indoline-3,3'-isoxazolidine]-2-one 25.** Orange solid, 81% yield.
1H-NMR (300 MHz, DMSO-d_6): δ 1.79 (s, 3H, CH_3), 3.54 (dd, J ¼ 7.03 Hz, 14.22 Hz, 1H, HCH_2), 3.93–4.19 (m, 3H, HCH_2 + CH_2Bn), 5.48 (dd, J ¼ 7.03 Hz, 9.72 Hz, 1H, CH), 6.92 (d, J ¼ 8.52 Hz, 1H, Ar), 7.20–7.78 (m, 6H, Ar), 8.15 (dd J ¼ 2.35 Hz, 8.70, 1H, Ar), 8.23 (d, J ¼ 2.19 Hz, 1H, 6-CHThy), 11.03 (sb, 1H, NH), 11.12 (sb, 1H, NHThy).
13C-NMR (75 MHz, DMSO-d6): δ 12.6, 22.5, 29.4, 58.7, 71.2, 108.8, 109.9, 120.5, 126.4, 127.1, 128.7, 132.0, 132.8, 137.8, 142.8, 145.0, 148.9, 151.5, 163.7, 180.3.
ESI(+)-MS: m/z [M + H] calcd for $C_{22}H_{20}N_5O_6$ 450.1414, found: 450.1403.

11.8 SYNTHESIS OF THE ALDONITRONES

The selected aldehyde (500 mg, 1 eq) and the hydroxylamine hydrochloride (1 eq) were placed in a apposite Pyrex vessel and mixed in a vortex. The mixture was transferred to an microwave oven and irradiated with a 600W power. After the appropriate time hot EtOAc was added to crude oil, by adding of a few drops of hexane if necessary, isolating the pure nitrone as solid, after filtration under vacuum and washing with cold dry EtOAc. The purification of compounds 28a–z was realized by flash chromatography ($CHCl_3$/MeOH 98.75/1.25 v:v).

(*Z*)-*N*-methyl-*C*-phenyl nitrone 28a. White solid, 92% yield, m.p. 81-82°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 3H, CH_3), 7.28-7.55 (m, 4H, Ar), 8.18-8.35 (m, 2H, Ar + =CH).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 54.4, 128.5, 130.5, 131.2, 133.7, 135.3.
ESI-HRMS calcd for $[C_8H_9NO + H]^+$ 136.0757, found 136.0764.

(*Z*)-*N*-methyl-*C*-(2-chloro)phenyl nitrone 28b. White solid, 92% yield, m.p. 71-72°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.93 (s, 3H, CH_3) 7.29–7.41 (m, 3H, Ar), 7.86 (s, 1H, =CH), 9.30 (dd, 1H, J=2.1, 7.7 Hz, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 55.13, 126.92, 128.13, 128.80, 129.28, 130.68, 130.90, 132.5.
ESI-HRMS calcd for $[C_8H_8ClNO + H]^+$ 170.0367, found 170.0358.

(*Z*)-*N*-methyl-*C*-(3-chloro)phenyl nitrone 28c. White solid, 91% yield, m.p. 43-44°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.89 (s, 3H, CH_3), 7.30-7.48 (m, 3H, Ar), 9.97 (d, 1H, J= 6.9 Hz, Ar), 8.36 (s, 1H, =CH).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 54.58, 126.37, 127.82, 129.63, 130.27, 131.98, 133.78, 134.46.
ESI-HRMS calcd for $[C_8H_8ClNO + H]^+$ 170.0367, found 170.0370.

(*Z*)-*N*-methyl-*C*-(4-cyano)phenyl nitrone 28d. White solid, 80% yield, m.p. 184-185°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.91 (s, 3H, CH_3), 7.43 (s, 1H, =CH), 7.67 (d, 2H, J=8.6, Ar), 8.26 (d, 2H, J=8.26, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 55.09, 124.76, 128.13, 128.92, 129.32, 130.90.
ESI-HRMS calcd for $[C_9H_8N_2O + H]^+$ 161.0709, found 161.0716.

(*Z*)-*N*-methyl-*C*-(2-fluoro)phenyl nitrone 28e. Pale yellow solid, 91% yield, m.p. 48–49°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.92 (s, 3H, CH_3), 7.08 (ddd, J =1.59, 8.22, 10.98 Hz, 1H, Ar), 7.18–7.26 (m, 1H, Ar), 7.33–7.44 (m, 1H, Ar), 7.67 (s, 1H, =CH), 9.23 (td, J = 1.59, 7.65 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 55.0, 114.5 (d, J= 1.20 Hz), 114.8, 124.4 (d, J = 3.01 Hz), 127.4 (d, J= 7.22 Hz), 128.6, 131.7 (d, J = 9.01 Hz), 159.9 (d, J_{CF} =253.12 Hz).
ESI-HRMS calcd for $[C_8H_8FNO+H]^+$ 154.0663 found 154.0662.

(*Z*)-*N*-methyl-*C*-(4-fluoro)phenyl nitrone 28f. White solid, 92% yield, m.p. 106-107°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H, CH_3), 7.41 (m, 3H, Ar), 8.15 (s, 1H, =CH), 8.21 (s, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 23.8, 108.6, 121.3, 135.0, 145.7, 173.2
ESI-HRMS calcd for $[C_8H_8FNO+H]^+$ 154.0663 found 154.0670.

(*Z*)-*N*-methyl-*C*-(4-methyl)phenyl nitrone 28g. White solid, 91% yield, m.p. 117-118°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 3H, CH_3), 4.20 (s, 3H, CH_3), 7.30 (s, 1H, Ar), 7.41 (m, 3H, Ar), 8.11 (s, 1H, =CH).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 24.6, 51.3, 126.5, 126.6, 127.8, 128.6, 128.8, 129.0, 138.5.
ESI-HRMS calcd for $[C_9H_{11}NO+H]^+$ 150.0913 found 150.0918.

(*Z*)-*N*-methyl-*C*-(4-methoxy)phenyl nitrone 28h. White solid, 91% yield, m.p. 71-72°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 6.90-7.30 (m, 4H, Ar), 8.10 (m, 1H, =CH).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 24.8, 51.9, 126.9, 127.3, 128.5, 128.9, 129.2, 129.3, 138.8.
ESI-HRMS calcd for $[C_9H_{11}NO_2+H]^+$ 166.0863 found 166.0882.

(*Z*)-*N*-methyl-*C*-4(*N*,*N*-dimethyl)aminophenyl nitrone 28i. White solid, 92% yield, m.p. 114-116°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.02 (s, 6H, $N(CH_3)_2$), 3.77 (s, 3H, CH_3), 6.66 (d, 2H, J= 9.0 Hz, Ar), 7.17 (s, 1H, =CH), 8.10 (d, 2H, J= 9.1 Hz, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 41.3, 59.2, 111.8, 119.9, 129.6, 135.0, 151.1.
ESI-HRMS calcd for $[C_{10}H_{14}N_2O+H]^+$ 179.1179 found 179.1195.

(*Z*)-*N*-benzyl-*C*-phenyl nitrone 28j. White solid, 94% yield, m.p. 80-81°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.06 (s, 2H, CH_2), 7.26-7.52 (m, 9H, Ar), 8.18-8.23 (m, 2H, Ar + =CH).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 71.1, 128.4, 128.5, 128.9, 129.2, 130.3, 130.4, 133.1, 134.2.
ESI-HRMS calcd for $[C_{14}H_{13}NO+H]^+$ 212.1070 found 212.1075.

(*Z*)-*N*-benzyl-*C*-(2-chloro)phenyl nitrone 28k. White solid, 95% yield, m.p. 86-87°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.09 (s, 2H, CH_2) 7.28–7.52 (m, 8H, Ar), 7.93 (s, 1H, =CH), 9.30 (s, 1H, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 71.90, 126.90, 128.09, 128.76, 128.84, 128.91, 129.12, 129.20, 129.77, 130.86, 132.71, 133.23.

ESI-HRMS calcd for $[C_{14}H_{12}ClNO+H]^+$ 246.0680, found 246.0695.

(*Z*)-*N*-benzyl-*C*-(4-cyano)phenyl nitrone 28l. Pale yellow solid, 83% yield, m.p. 141-142°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.09 (s, 2H, CH_2), 7.55–7.40 (m, 6H, Ar + =CH), 7.71 (d, 2H, J=8.6 Hz, Ar), 8.32 (d, 2H, J=8.6 Hz,).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 71.87, 112.85, 118.59, 128.05, 128.50, 129.08, 129.30, 132.16, 132.58, 132.77, 134.32.
ESI-HRMS calcd for $[C_{15}H_{12}N_2O+H]^+$ 237.1022, found 237.1030.

(*Z*)-*N*-benzyl-*C*-(2-fluoro)phenyl nitrone 28m. White solid, 93% yield, m.p. 84-85°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.07 (s, 2H, CH_2) 7.02–7.51 (m, 8H, Ar), 7.75 (s, 1H, =CH), 9.24 (td, 1H, J=1.8, 7.8, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 71.68, 114.47 (d, *J*=21.3 Hz), 119.00 (d, *J*=8.5 Hz), 124.31 (d, *J*=3.2 Hz), 126.37 (d, *J*=9.6 Hz), 128.63, 128.84, 128.90, 129.11, 131.64 (d, *J*=8.5 Hz), 133.26, 159.01 (d, *J*=252.9 Hz).
ESI-HRMS calcd for $[C_{14}H_{12}FNO+H]^+$ 230.0976, found 230.0985.

(*Z*)-*N*-benzyl-*C*-(4-methyl)phenyl nitrone 28n. White solid, 90% yield, m.p. 117-118°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.53 (s, 3H, CH_3), 5.01 (s, 2H, CH_2) 6.76–6.88 (m, 4H, Ar), 7.30-7.46 (m, 6H, Ar + =CH).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 55.0, 113.2, 123.0, 127.9, 128.2, 128.8, 129.9, 132.8, 134.0, 160.7
ESI-HRMS calcd for $[C_{15}H_{15}NO_2+H]^+$ 226.1226, found 226.1234.

(*Z*)-*N*-benzyl-*C*-(4-methoxy)phenyl nitrone 28o. White solid, 93% yield, m.p. 96-97°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.82 (s, 3H, CH_3), 5.04 (d, 2H, J=14.0 Hz, CH_2) 6.89–6.95 (m, 4H, Ar), 7.37-7.43 (m, 6H, Ar + =CH).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 55.2, 113.7, 123.3, 128.3, 128.8, 129.1, 130.5, 133.3, 133.9, 161.0
ESI-HRMS calcd for $[C_{15}H_{15}NO_2+H]^+$ 242.1176, found 242.1185.

(*Z*)-*N*-benzyl-*C*-4(*N*,*N*-dimethyl)aminophenyl nitrone 28p. White solid, 94% yield, m.p. 121-122°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.02 (s, 6H, $N(CH_3)_2$), 5.08 (s, 2H, CH_2) 6.72–6.85 (m, 4H, Ar), 7.86-7.95 (m, 6H, Ar + =CH).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 41.3, 54.2, 111.8, 119.9, 125.7, 127.7, 128.6, 129.6, 134.5, 151.1.
ESI-HRMS calcd for $[C_{16}H_{18}N_2O+H]^+$ 255.1492, found 255.1490.

(*Z*)-*N*-Phenyl-*C*-phenyl nitrone 28q. White solid, 93% yield, m.p. 110-111°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.46–7.51 (m, 6H, Ar), 7.77-7.79 (2, 2H, Ar), 7.92 (s, 1H, =CH), 8.39-8.41 (s, 2H, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 121.7, 128.6, 128.9, 129.1, 129.9, 130.9, 134.5, 149.0.
ESI-HRMS calcd for $[C_{13}H_{11}NO+H]^+$ 198.0913, found 198.0922.

(*Z*)-*N*-Phenyl-*C*-(2-chloro)phenyl nitrone 28r. Whitish solid, 97% yield, m.p. 49–50°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.31–7.55 (m, 6H, Ar), 7.68–7.85 (m, 2H, Ar), 8.42 (s, 1H, =CH), 9.52 (dd, J = 2.10, 7.92 Hz, 1H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 121.8, 127.2, 128.4, 129.2, 129.5, 130.2, 130.4, 131.5, 133.6, 149.5.
ESI-HRMS calcd for $[C_{13}H_{10}ClNO +H]^+$ 232.0523 found 232.0525.

(*Z*)-*N*-Phenyl-*C*-(2-fluoro)phenyl nitrone 28s. White solid, 95% yield, m.p. 125-126°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.28–7.60 (m, 8H, Ar), 7.69–7.88 (m, 2H, Ar), 8.45 (s, 1H, =CH), 9.54 (dd, J = 2.13, 8.15 Hz, 2H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 114.6, 115.5, 124.3, 125.4, 128.7, 130.3, 130.4, 131.1, 144.5, 159.1, 174.2.
ESI-HRMS calcd for $[C_{13}H_{10}FNO +H]^+$ 216.0825 found 216.0831.

(*Z*)-*N*-Phenyl-*C*-(4-methyl)phenyl nitrone 28t. White solid, 93% yield, m.p. 85-86°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.41 (s, 3H, CH_3), 7.28 (d, 2H, J=8.1 Hz, Ar), 7.46 (d, 3H, J=7.9 Hz, Ar), 7.76 (dd, J=8.1 Hz, 1.6 Hz 2H, Ar), 7.89 (s, 1H, =CH), 8.30 (d, J = 8.20 Hz, 2H, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 56.2, 114.8, 115.8, 125.3, 126.4, 128.8, 131.2, 131.9, 144.9, 174.2.
ESI-HRMS calcd for $[C_{14}H_{13}NO +H]^+$ 212.1070 found 212.1081.

(*Z*)-*N*-Phenyl-*C*-(4-methoxy)phenyl nitrone 28u. White solid, 92% yield, m.p. 85-86°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.87 (s, 3H, CH_3), 6.99 (d, 2H, J=9.0 Hz, Ar), 7.45 (m, 3H, Ar), 7.76 (d, J=8.0 Hz, 2H, Ar), 7.85 (s, 1H, =CH), 8.40 (d, J = 8.90 Hz, 2H, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 57.2, 115.0, 115.5, 125.8, 126.8, 129.5, 132.2, 133.1, 145.9, 174.8.
ESI-HRMS calcd for $[C_{14}H_{13}NO_2 +H]^+$ 228.1019 found 228.1026.

(*Z*)-*N*-Phenyl-*C*-(4-cyano)phenyl nitrone 28v. Brownish solid, yield 82%, m.p. 149–150°C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.47–7.54 (m, 3H, Ar), 7.73–7.81 (m, 4H, Ar), 8.01 (s, 1H, =CH), 8.48 (d, J =8.28 Hz, 2H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 121.7, 121.7, 128.8, 129.3, 129.4, 130.6, 132.3, 132.3, 134.5, 148.9.
ESI-HRMS calcd for $[C_{14}H_{10}N_2O+H]^+$ 223.0866 found 223.0867.

(*Z*)-*N*-Phenyl-*C*-4(*N*,*N*-dimethyl)aminophenyl nitrone 28z. White solid, 96% yield, m.p. 137-138°C.
^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.05 (s, 6H, $N(CH_3)_2$), 6.73 (d, 2H, J=9.1Hz, Ar) 7.41 (dt, 3H, J=7.2 Hz, 26.2 Hz, Ar), 7.76 (s, 3H, CH_3), 7.78 (s, 1H, =CH), 7.79 (s, 1H, Ar), 8.33 (d, 2H, J= 8.9 Hz, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 44.2, 54.1, 110.8, 118.9, 123.7, 127.9, 128.2, 129.1, 134.2, 151.8.
ESI-HRMS calcd for $[C_{15}H_{16}NO_2 +H]^+$ 241.1335, found 241.11344.

11.9 SYNTHESIS OF THE ISOXAZOLIDIN ESTERS AS PRECURSORS FOR ISOXAZOLIDIN-*Gem*-BISPHOSPHONIC ACIDS

The selected nitrone 28a-k (500 mg, 1 eq) and the selected vinyl compound 27a-c (1 eq) were placed in a apposite Pyrex vessel and mixed in a vortex. The mixture was transferred to an microwave oven and irradiated with a 750W power. After the appropriate time the reaction crude is placed in a flask for further reaction.

ethyl 2-benzyl-3-phenylisoxazolidine-5-carboxylate 29. Colorless oil, 98% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.31 (t, 3H, J=7.2 Hz, CH_3), 2.65 (ddd, 1H, J=4.8, 8.7, 12.6 Hz, CH_2), 2.99 (ddd, 1H, J=7.8, 9.3, 12.6 Hz, CH_2), 3.78 (d, 1H, J=13.8 Hz, CH_2Ph), 4.01 (d, 1H, J=13.8 Hz, CH_2Ph), 4.25 (q, 2H, J=7.2 Hz, CH_2), 4.25 (dd, 1H, J=7.8, 8.7 Hz, CH), 4.63 (dd, 1H, J=4.8, 9.3 Hz, CH), 7.22—7.50 (m, 10H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ: 14.1, 40.0, 60.4, 61.6, 78.2, 79.4, 126.0, 127.0, 127.9, 128.1, 128.5, 128.8, 137.6, 137.7, 170.8.
ESI(+)-MS: m/z [M + H] calcd for $C_{19}H_{22}NO_3$ 312.1594, found 312.1596.

ethyl 2-benzyl-3-(2-chlorophenyl)isoxazolidine-5-carboxylate 30. Colorless oil, 98% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.32 (t, 3H, J=7.2 Hz, CH_3), 2.64 (ddd, 1H, J=4.7, 8.4, 12.7 Hz, CH_2), 2.98 (ddd, 1H, J=7.7, 9.2, 12.5 Hz, CH_2), 3.77 (d, 1H, J=13.6 Hz, CH_2Ph), 4.05 (d, 1H, J=13.9 Hz, CH_2Ph), 4.24 (q, 2H, J=7.1 Hz, CH_2), 4.24 (dd, 1H, J=7.9, 8.5 Hz, CH), 4.63 (dd, 1H, J=4.7, 9.1 Hz, CH), 7.21—7.51 (m, 9H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ: 14.1, 39.5, 60.4, 61.6, 73.1, 79.4, 126.9, 127.0, 127.4, 127.9, 128.5, 128.9, 129.5, 133.4, 137.7, 139.9, 170.8.
ESI(+)-MS: m/z [M + H] calcd for $C_{19}H_{21}ClNO_3$ 346.1204, found 346.1206.

ethyl 2-benzyl-3-(2-fluorophenyl)isoxazolidine-5-carboxylate 31. Colorless oil, 97% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.34 (t, 3H, J=7.2 Hz, CH_3), 2.65 (ddd, 1H, J=4.6, 8.5, 12.8 Hz, CH_2), 2.97 (ddd, 1H, J=7.6, 9.3, 12.6 Hz, CH_2), 3.78 (d, 1H, J=13.8 Hz, CH_2Ph), 4.07 (d, 1H, J=13.7 Hz, CH_2Ph), 4.27 (q, 2H, J=7.3 Hz, CH_2), 4.25 (dd, 1H, J=7.6, 8.6 Hz, CH), 4.63 (dd, 1H, J=4.7, 9.2 Hz, CH), 7.23—7.52 (m, 9H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ: 14.2, 39.4, 60.7, 61.3, 73.5, 79.8, 126.7, 127.8, 127.6, 127.8, 128.4, 128.5, 129.6, 133.7, 137.8, 139.7, 170.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{19}H_{21}FNO_3$ 330.1500, found 330.1503.

methyl 2-(2-benzyl-3-phenylisoxazolidin-5-yl)acetate 32. Pale yellow oil, 95% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.02 (s, 3H, CH_3), 2.83 (dt, 1H, J=8.1, 12.9 Hz, CH), 3.77 (d, 1H, J=14.7 Hz, CH), 3.83 (t, 1H, J=8.1 Hz, CH_2), 3.88 (m, 1H, CH_2), 4.00 (d, 1H, J=14.7 Hz, CH_2Ph), 4.11 (d, 1H, J=11.4 Hz,CH_2Ph), 4.53 (d, 1H, J=11.1 Hz, CH_2CO), 4.38—4.47 (m, 1H, CH_2CO), 7.17—7.45 (m, 10H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): 36.9, 39.2, 51.9, 60.7, 73.8, 78.5, 125.9, 127.0, 127.9, 128.1, 128.5, 128.6, 137.7, 138.2, 171.5.
ESI(+)-MS: m/z [M + H] Calcd for $C_{19}H_{22}NO_3$: 312.1594, found 312.1596.

methyl 2-(2-benzyl-3-(2-chlorophenyl)isoxazolidin-5-yl)acetate 33. Pale yellow oil, 96% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.04 (s, 3H, CH_3), 2.85 (dt, 1H, J=8.3, 12.6 Hz, CH), 3.79 (d, 1H, J=14.6 Hz, CH), 3.83 (t, 1H, J=8.2 Hz, CH_2), 3.88 (m, 1H, CH_2), 4.02 (d, 1H, J=14.5 Hz, CH_2Ph), 4.13 (d, 1H, J=11.7 Hz,CH_2Ph), 4.55 (d, 1H, J=11.0 Hz, CH_2CO), 4.36—4.47 (m, 1H, CH_2CO), 7.15—7.46 (m, 9H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): 36.9, 39.2, 51.9, 60.7, 73.8, 78.5, 125.9, 127.0, 127.9, 128.1, 128.5,128.4, 128.6, 137.7,137.9, 138.2, 171.5.
ESI(+)-MS: m/z [M + H] Calcd for $C_{19}H_{21}ClNO_3$: 346.1204, found 346.1205.

methyl 2-(2-benzyl-3-(2-fluorophenyl)isoxazolidin-5-yl)acetate 34. Colorless oil, 97% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.05 (s, 3H, CH_3), 2.86 (dt, 1H, J=8.2, 12.4 Hz, CH), 3.76 (d, 1H, J=14.3 Hz, CH), 3.83 (t, 1H, J=8.1 Hz, CH_2), 3.89 (m, 1H, CH_2), 4.02 (d, 1H, J=14.6 Hz, CH_2Ph), 4.13 (d, 1H, J=11.6 Hz,CH_2Ph), 4.55 (d, 1H, J=11.2 Hz, CH_2CO), 4.36—4.47 (m, 1H, CH_2CO), 7.15—7.45 (m, 9H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): 36.4, 39.1, 51.7, 60.2, 73.4, 78.6, 125.7, 127.3, 127.8, 128.5, 128.6, 128.8, 128.9, 137.7, 137.9, 138.2, 171.5.
ESI(+)-MS: m/z [M + H] Calcd for $C_{19}H_{21}FNO_3$: 330.1500, found 330.1503.

methyl 3-(2-benzyl-3-phenylisoxazolidin-5-yl)propanoate 35. Colorless oil, 97% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.06 (s, 3H, CH_3), 2.85 (dt, 1H, J=8.2, 12.4 Hz, CH), 3.75 (d, 1H, J=14.3 Hz, CH), 3.80 (m, 1H, CH_2), 3.87 (t, 1H, J=8.1 Hz, CH_2), 3.88 (m, 2H,CH_2), 4.05 (d, 1H, J=14.6 Hz, CH_2Ph), 4.18 (d, 1H, J=11.6 Hz,CH_2Ph), 4.57 (d, 1H, J=11.2 Hz, CH_2CO), 4.36—4.47 (m, 1H, CH_2CO), 7.15—7.45 (m, 10H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): 36.4, 39.1, 51.7, 52.4 60.2, 73.4, 78.6, 125.7, 127.3, 127.8, 128.5, 128.6, 128.8, 128.9, 137.7, 171.5.
ESI(+)-MS: m/z [M + H] Calcd for $C_{20}H_{24}NO_3$: 326.1751, found 326.1761.

methyl 3-(2-benzyl-3-(2-chlorophenyl)isoxazolidin-5-yl)propanoate 36. Pale yellow oil, 96% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.05 (s, 3H, CH_3), 2.86 (dt, 1H, J=8.2, 12.4 Hz, CH), 3.76 (d, 1H, J=14.3 Hz, CH), 3.80 (m, 1H, CH_2), 3.83 (t, 1H, J=8.1 Hz, CH_2), 3.85 (m, 1H, CH_2), 3.88 (m, 2H, CH_2), 4.02 (d, 1H, J=14.6 Hz, CH_2Ph), 4.13 (d, 1H, J=11.6 Hz,CH_2Ph), 4.36—4.47 (d, 1H, J=11.2 Hz, CH_2CO), 4.55 (m, 1H, CH_2CO), 7.15—7.45 (m, 9 H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): 36.7, 39.4, 51.9, 52.8 60.5, 73.8, 78.7, 125.7, 127.5, 127.8, 128.5, 128.6, 128.7, 128.9, 137.8, 171.5.
ESI(+)-MS: m/z [M + H] Calcd for $C_{20}H_{23}ClNO_3$: 360.1361, found 360.1362.

methyl 3-(2-benzyl-3-(2-fluorophenyl)isoxazolidin-5-yl)propanoate 37. Pale yellow oil, 95% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.08 (s, 3H, CH_3), 2.89 (dt, 1H, J=8.2, 12.4 Hz, CH), 3.77 (d, 1H, J=14.3 Hz, CH), 3.80 (m, 1H, CH_2), 3.85 (t, 1H, J=8.1 Hz, CH_2), 3.88 (m, 1H, CH_2), 3.89 (m, 2H,CH_2), 4.08 (d, 1H, J=14.6 Hz, CH_2Ph), 4.13 (d, 1H, J=11.6 Hz,CH_2Ph), 4.36—4.47 (d, 1H, J=11.2 Hz, CH_2CO), 4.59 (m, 1H, CH_2CO), 7.15—7.45 (m, 9 H, Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): 36.2, 39.0, 51.4, 52.2 60.5, 73.2, 78.6, 125.1, 127.9, 127.2, 128.3, 128.6, 128.7, 128.9, 137.6, 171.8.
ESI(+)-MS: m/z [M + H] Calcd for $C_{20}H_{23}FNO_3$: 344.1646, found 344.1642.

ethyl 2-methyl-3-phenylisoxazolidine-5-carboxylate 38. Colorless oil, 97% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.31 (t, 3H, J=7.2 Hz, CH_3), 2.58—2.68 (m, 1H, CH), 2.96 (ddd, 1H, J=7.2, 9.3, 12.9 Hz, CH_2), 2.61 (s, 3H, CH_3), 3.75—3.86 (m, 1H, CH), 4.26 (q, 2H, J=7.2 Hz, CH_2), 4.62 (dd, 1H, J=5.7, 9.3 Hz, CH_2), 7.27—7.42 (m, 5H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.55, 43.30, 43.96, 62.28, 71.16, 74.38, 123.55, 125.88, 127.10, 129.43, 170.4.
ESI(+)-MS: m/z [M + H] calcd for $C_{13}H_{18}NO_3$ 236.1281, found 236.1284.

ethyl 2-methyl-3-(p-tolyl)isoxazolidine-5-carboxylate 39. Colorless oil, 94% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.35 (t, 3H, J=7.5 Hz, CH_3), 2.34 (s, 3H, CH_3), 2.57—2.69 (m, 1H, CH_2), 2.97 (ddd, 1H, J=7.5, 9.6, 13.2 Hz, CH_2), 2.63 (s, 3H, CH_3), 3.71—3.84 (m, 1H, CH), 4.24 (q, 2H, J=7.4 Hz, CH_2), 4.68 (dd, 1H, J=5.8, 9.8 Hz, CH), 7.27—7.45 (m, 4H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.54, 21.32 43.36, 43.98, 62.47, 71.69, 74.53, 123.59, 125.78, 127.34, 129.63, 175.43.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{20}NO_3$ 250.1428, found 250.1427.

ethyl 3-(2-fluorophenyl)-2-methylisoxazolidine-5-carboxylate 40. Colorless oil, 98% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.31 (t, 3H, J=7.1 Hz, CH_3), 2.64 (ddd, 1H, J=4.7, 8.5, 12.6 Hz, CH_2), 2.99 (ddd, 1H, J=7.8, 9.3, 12.6 Hz, CH_2), 3.78 (d, 1H, J=13.5 Hz, CH_2Ph), 4.08 (d, 1H, J=13.8 Hz, CH_2Ph), 4.26 (q, 2H, J=7.2 Hz, CH_2), 4.25 (dd, 1H, J=7.8, 8.4 Hz, CH), 4.62 (dd, 1H, J=4.6, 9.1 Hz, CH), 7.22—7.54 (m, 9H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ: 14.0, 39.2, 60.7, 61.8, 73.2, 79.8, 126.9, 127.1, 127.5, 127.9, 128.3, 128.7, 129.8, 133.6, 137.8, 139.9, 170.5.
ESI(+)-MS: m/z [M + H] calcd for $C_{19}H_{21}FNO_3$ 330.1500, found 330.1503.

ethyl 3-(4-fluorophenyl)-2-methylisoxazolidine-5-carboxylate 41. Colorless oil, 97% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.36 (t, 3H, J=7.5 Hz, CH_3), 2.59—2.69 (m, 1H, CH_2), 2.98 (ddd, 1H, J=7.5, 9.5, 13.0 Hz, CH_2), 2.63 (s, 3H, CH_3), 3.72—3.85 (m, 1H, CH), 4.24 (q, 2H, J=7.5 Hz, CH_2), 4.67 (dd, 1H, J=5.8, 9.8 Hz, CH), 7.27—7.45 (m, 4H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.53, 43.32, 43.97, 62.27, 71.19, 74.33, 123.57, 125.89, 127.20, 129.53, 175.4.
ESI(+)-MS: m/z [M + H] calcd for $C_{13}H_{17}FNO_3$ 254.1187, found 254.1185.

ethyl 3-(2-chlorophenyl)-2-methylisoxazolidine-5-carboxylate 42. Colorless oil, 97% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.32 (t, 3H, J=7.2 Hz, CH_3), 2.80 (s, 3H, CH_3), 2.86 (m, 1H, CH_2), 2.97 (ddd, 1H, J=7.2, 9.3, 12.9 Hz, CH_2), 3.05—3.25 (m, 1H, CH), 4.26 (q, 2H, J=7.2 Hz, CH_2), 4.60 (dd, 1H, J=5.7, 9.3 Hz, CH), 7.17—7.42 (m, 5H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.77, 38.08, 41.30, 44.63, 64.28, 76.71, 128.76, 128.85, 131.43, 131.59, 133.65, 133.75, 170.3.
ESI(+)-MS: m/z [M + H] calcd for $C_{13}H_{17}ClNO_3$ 270.0891, found 270.0892.

ethyl 3-(3-chlorophenyl)-2-methylisoxazolidine-5-carboxylate 43. Colorless oil, 98% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.33 (t, 3H, J=7.1 Hz, CH_3), 2.80 (s, 3H, CH_3), 2.87 (m, 1H, CH_2), 2.97 (ddd, 1H, J=7.3, 9.4, 12.2 Hz, CH_2), 3.05—3.25 (m, 1H, CH), 4.26 (q, 2H, J=7.2 Hz, CH_2), 4.60 (dd, 1H, J=5.7, 9.4 Hz, CH), 7.17—7.42 (m, 4H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.75, 38.02, 41.40, 44.61, 64.26, 76.73, 128.75, 128.87, 131.44, 131.57, 133.68, 133.74, 170.8.
ESI(+)-MS: m/z [M + H] calcd for $C_{13}H_{17}ClNO_3$ 270.0891, found 270.0896.

methyl 2-(3-(2-chlorophenyl)-2-methylisoxazolidin-5-yl)acetate 44. Pale yellow oil, 98% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.04 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 2.86 (dt, 1H, J=8.3, 12.6 Hz, CH), 3.76 (d, 1H, J=14.6 Hz, CH), 3.75 (m, 1H, CH_2), 3.80 (t, 1H, J=8.2 Hz, CH_2), 4.58 (d, 1H, J=11.0 Hz, CH_2CO), 4.34—4.48 (m, 1H, CH_2CO), 7.15—7.46 (m, 4H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): 36.5, 39.2, 51.5, 60.5, 73.8, 78.5, 127.9, 128.5, 128.6, 137.7, 171.5.
ESI(+)-MS: m/z [M + H] Calcd for $C_{13}H_{17}ClNO_3$: 270.0891, found 370.895.

methyl 3-(3-(2-chlorophenyl)-2-methylisoxazolidin-5-yl)propanoate 45. Pale yellow oil, 97% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.04 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 2.86 (dt, 1H, J=8.3, 12.6 Hz, CH), 3.76 (d, 1H, J=14.6 Hz, CH), 3.80 (m, 1H, CH_2), 3.80 (t, 1H, J=8.2 Hz, CH_2), 3.82 (m, 2H, CH_2), 4.58 (d, 1H, J=11.0 Hz, CH_2CO), 4.34—4.48 (m, 1H, CH_2CO), 7.15—7.46 (m, 4H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): 36.2, 39.3, 51.8, 55.5, 60.4, 73.9, 78.6, 127.9, 128.5, 128.6, 128.8, 129.6, 137.7, 171.5.
ESI(+)-MS: m/z [M + H] Calcd for $C_{14}H_{19}ClNO_3$: 284.1048, found 284.1050.

11.10 SYNTHESIS OF THE ISOXAZOLIDIN-GEM-BISPHOSPHONIC ACIDS

In a two-necked Flask is placed the cycloadduct 29-45 (0,61 mmol) in Ethanol (10 ml) and 2 ml of the aqueous NaOH 3M is added. The reaction is stirred at room temperature for 12h. The mixture is evaporated under vacuum and water is placed. pH is lowered to value 4 with a solution of HCl (9:1 v/v) and AcOet is added for extraction (3 x 3 ml). The combined organic layer is filtered, dried over Na_2SO_4 anhydrous and evaporated under vacuum. To this crude, under N_2 atmosphere, are added the dry CH_2Cl_2 (10 ml) and Oxalyl Chloride (0,74 mmol). The reaction is stirred at room temperature for 3h. The mixture is evaporated under vacuum. To the crude are added dry THF (10 ml) and tris(trimethulsilyl)phosphite (12,3 mmol). The reaction is stirred for 12h. The crude is evaporated under vacuum. In the flask is placed MeOH (10 ml) and the mixture is stirred for further 3h. In the mixture is added Et_2O to give the solid precipitate corresponding at the desired product.

((2-benzyl-3-phenylisoxazolidin-5-yl)(hydroxy)methylene)diphosphonic acid 46. White Solid. 87% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.22 (m, 1H, CH_2), 2.80 (m, 1H, CH_2), 3.11 (dd, J=12.53, 8.32 Hz, 1H, CH), 3.87 (d, 2H, J=13.83 Hz, CH_2), 4.56 (m, 1H, CH), 6.37 (br s, 5H, OH), 7.02-7.35 (m, 8H, Ar), 7.52-7.68 (m, 2H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.3, 60.4, 63.1, 72.4, 102.3, 105.2, 115.5, 124.6, 127.7, 127.9, 129.8, 137.4.
ESI(+)-MS: m/z [M + H] Calcd for $C_{17}H_{22}NO_8P_2$: 430.0815, found 430.0818.

((2-benzyl-3-(2-chlorophenyl)isoxazolidin-5-yl)(hydroxy)methylene)diphosphonic acid 47. White Solid. 86% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.24 (m, 1H, CH_2), 2.82 (m, 1H, CH_2), 3.12 (dd, J=12.53, 8.32 Hz, 1H, CH), 3.86 (d, 2H, J=13.83 Hz, CH_2), 4.55 (m, 1H, CH), 6.35 (br s, 5H, OH), 7.03-7.31 (m, 8H, Ar), 7.54-7.66 (m, 1H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.1, 60.5, 63.2, 72.3, 102.6, 105.6, 115.4, 124.2, 127.2, 127.4, 127.8, 128.6, 129.8, 137.8, 160.9.
ESI(+)-MS: m/z [M + H] Calcd for $C_{17}H_{21}ClNO_8P_2$: 464.0425, found 462.0428.

((2-benzyl-3-(2-fluorophenyl)isoxazolidin-5-yl)(hydroxy)methylene)diphosphonic acid 48. White Solid. 88% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.25 (m, 1H, CH_2), 2.83 (m, 1H, CH_2), 3.10 (dd, J=12.55, 8.31 Hz, 1H, CH), 3.89 (d, 2H, J=13.86Hz, CH_2), 4.55 (m, 1H, CH), 6.35 (br s, 5H, OH), 7.05-7.32 (m, 7H, Ar), 7.35-7.40 (d, 1H, J=7.17, Ar), 7.55-7.65 (m, 1H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.0, 60.7, 63.0, 72.0, 102.9, 105.8, 115.3, 124.1, 127.0,127.5, 127.9, 128.5, 129.7, 137.7, 160.6.
ESI(+)-MS: m/z [M + H] Calcd for $C_{17}H_{21}FNO_8P_2$: 448.0721, found 448.0723.

(2-(2-benzyl-3-phenylisoxazolidin-5-yl)-1-hydroxyethane-1,1-diyl)diphosphonic acid 49. White Solid. 89% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.15 (m, 1H, CH_2), 2.81 (m, 1H, CH_2), 3.05 (d, 2H, J= 6.70 CH_2) 3.20 (dd, J=12.53, 8.32 Hz, 1H, CH), 3.85 (d, 2H, J=13.83 Hz, CH_2), 4.50 (m, 1H, CH), 6.40 (br s, 5H, OH), 7.04-7.38 (m, 8H, Ar), 7.51-7.69 (m, 2H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.3, 60.4, 63.1, 68.7, 72.4, 102.3, 105.2, 115.5, 124.6, 127.7, 127.9, 129.8, 129.9, 137.4.
ESI(+)-MS: m/z [M + H] Calcd for $C_{18}H_{24}NO_8P_2$: 444.0972, found 444.0974.

(2-(2-benzyl-3-(2-chlorophenyl)isoxazolidin-5-yl)-1-hydroxyethane-1,1-diyl)diphosphonic acid 50. White Solid. 87% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.12 (m, 1H, CH_2), 2.85 (m, 1H, CH_2), 3.09 (d, 2H, J= 6.78 CH_2) 3.26 (dd, J=12.00, 8.02 Hz, 1H, CH), 3.88 (d, 2H, J=13.00 Hz, CH_2), 4.59 (m, 1H, CH), 6.48 (br s, 5H, OH), 7.08-7.42 (m, 9H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.8, 60.9, 63.4, 68.8, 72.6, 102.1, 105.3, 115.5, 124.7, 127.7, 127.9, 128.4, 128.6, 129.8, 129.9, 137.9.
ESI(+)-MS: m/z [M + H] Calcd for $C_{18}H_{23}ClNO_8P_2$: 478.0582, found 478.0583.

(2-(2-benzyl-3-(2-fluorophenyl)isoxazolidin-5-yl)-1-hydroxyethane-1,1-diyl)diphosphonic acid 51. White Solid. 85% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.10 (m, 1H, CH_2), 2.86 (m, 1H, CH_2), 3.09 (d, 2H, J= 6.80 CH_2) 3.28 (dd, J=12.05, 8.05 Hz, 1H, CH), 3.89 (d, 2H, J=13.02 Hz, CH_2), 4.56 (m, 1H, CH), 6.46 (br s, 5H, OH), 7.04-7.34 (m, 7H, Ar), 7.32-7.45 (d, 1H, J=7.18, Ar), 7.54-7.66 (m, 1H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.4, 60.1, 63.3, 68.9, 72.4, 102.5, 105.6, 115.4, 124.2, 127.7, 127.9, 128.3, 128.5, 129.8, 129.5, 137.8.
ESI(+)-MS: m/z [M + H] Calcd for $C_{18}H_{23}FNO_8P_2$: 462.0877, found 462.0879.

(3-(2-benzyl-3-phenylisoxazolidin-5-yl)-1-hydroxypropane-1,1-diyl)diphosphonic acid 52. White Solid. 88% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.15 (m, 1H, CH_2), 2.81 (m, 1H, CH_2), 3.05 (td, 2H, J= 8.15, 6.73 CH_2) 3.20 (dd, J=12.53, 8.32 Hz, 1H, CH), 3.42 (t, 2H, J=8.16, CH_2) 3.85 (d, 2H, J=13.83 Hz, CH_2), 4.50 (m, 1H, CH), 6.40 (br s, 5H, OH), 7.04-7.38 (m, 8H, Ar), 7.51-7.69 (m, 2H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.3, 60.4, 62.8, 63.1, 68.7, 72.4, 102.3, 105.2, 115.5, 124.6, 127.7, 127.9, 129.8, 129.9, 137.4.
ESI(+)-MS: m/z [M + H] Calcd for $C_{19}H_{26}NO_8P_2$: 458.1128, found 458.1126.

(3-(2-benzyl-3-(2-chlorophenyl)isoxazolidin-5-yl)-1-hydroxypropane-1,1-diyl)diphosphonic acid 53. White Solid. 89% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.14 (m, 1H, CH_2), 2.75 (m, 1H, CH_2), 3.12 (td, 2H, J= 8.15, 6.73 CH_2) 3.20 (dd, J=12.40, 8.12 Hz, 1H, CH), 3.42 (t, 2H, J=8.13, CH_2) 3.85 (d, 2H, J=13.12 Hz, CH_2), 4.50 (m, 1H, CH), 6.45 (br s, 5H, OH), 7.24-7.45 (m, 9H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 42.3, 61.4, 63.8, 64.1, 68.8, 72.6, 103.4, 105.5, 116.5, 125.6, 127.6, 127.8, 128.5, 128.8, 129.9, 129.6, 137.2.
ESI(+)-MS: m/z [M + H] Calcd for $C_{19}H_{25}ClNO_8P_2$: 492.0738, found 492.0735.

(3-(2-benzyl-3-(2-fluorophenyl)isoxazolidin-5-yl)-1-hydroxypropane-1,1-diyl)diphosphonic acid 54. White Solid. 85% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.13 (m, 1H, CH_2), 2.74 (m, 1H, CH_2), 3.14 (td, 2H, J= 8.14, 6.72 CH_2) 3.21 (dd, J=12.42, 8.10 Hz, 1H, CH), 3.42 (t, 2H, J=8.10, CH_2) 3.80 (d, 2H, J=13.10 Hz, CH_2), 4.52 (m, 1H, CH), 6.42 (br s, 5H, OH), 7.01-7.35 (m, 7H, Ar), 7.34-7.45 (d, 1H, J=7.16, Ar), 7.52-7.68 (m, 1H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 42.2, 61.5, 63.7, 64.4, 68.7, 72.6, 103.6, 105.7, 116.8, 125.8, 127.4, 127.6, 128.3, 128.4, 129.6, 129.8, 137.6.
ESI(+)-MS: m/z [M + H] Calcd for $C_{19}H_{25}FNO_8P_2$: 476.1036, found 476.1038.

(hydroxy(2-methyl-3-phenylisoxazolidin-5-yl)methylene)diphosphonic acid 55. White Solid. 84% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.17 (m, 1H, CH_2), 2.48 (s, 3H, J=13.42 Hz, CH_3), 2.75 (m, 1H, CH_2), 3.02 (dd, J=12.16, 8.25 Hz, 1H, CH), 4.45 (m, 1H, CH), 6.39 (br s, 5H, OH), 7.20-7.51 (m, 5H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 30.6, 43.3, 60.4, 63.1, 72.4, 124.6, 127.7, 127.9, 137.4.
ESI(+)-MS: m/z [M + H] Calcd for $C_{11}H_{18}NO_8P_2$: 354.0502, found 354.0506.

(hydroxy(2-methyl-3-(2-tolyl)isoxazolidin-5-yl)methylene)diphosphonic acid 56. White Solid. 86% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.26 (s, 3H, CH_3), 2.25 (m, 1H, CH_2), 2.53 (s, 3H, J=12.98 Hz, CH_3), 2.75 (m, 1H, CH_2), 3.02 (dd, J=12.60, 8.01 Hz, 1H, CH), 4.45 (m, 1H, CH), 6.39 (br s, 5H, OH), 7.20-7.51 (m, 4H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 25.9, 30.8, 43.9, 60.8, 63.5, 72.4, 124.2, 127.4, 127.6, 137.8.
ESI(+)-MS: m/z [M + H] Calcd for $C_{12}H_{20}NO_8P_2$: 368.0659, found 368.0655.

((3-(2-fluorophenyl)-2-methylisoxazolidin-5-yl)(hydroxy)methylene)diphosphonic acid 57. White Solid. 83% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.15 (m, 1H, CH_2), 2.49 (s, 3H, J=11.99 Hz, CH_3), 2.75 (m, 1H, CH_2), 3.06 (dd, J=12.20, 8.16 Hz, 1H, CH), 4.39 (m, 1H, CH), 6.42 (br s, 5H, OH), 7.10-7.28 (m, 2H, Ar), 7.36-7.42 (d, 1H, J=7.20, Ar), 7.58-7.69 (m, 1H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 30.6, 43.3, 60.4, 63.1, 124.6, 127.7, 127.9, 128.6, 128.9, 137.4.
ESI(+)-MS: m/z [M + H] Calcd for $C_{11}H_{17}FNO_8P_2$: 372.0408, found 372.0410.

((3-(4-fluorophenyl)-2-methylisoxazolidin-5-yl)(hydroxy)methylene)diphosphonic acid 58. White Solid. 84% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.10 (m, 1H, CH_2), 2.35 (s, 3H, J=13.42 Hz, CH_3), 2.70 (m, 1H, CH_2), 3.20 (dd, J=12.16, 8.25 Hz, 1H, CH), 4.46 (m, 1H, CH), 6.52 (br s, 5H, OH), 7.20-7.45 (m, 4H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.5, 60.6, 63.7, 72.3, 124.2, 127.4, 127.7, 127.9, 137.4.
ESI(+)-MS: m/z [M + H] Calcd for $C_{11}H_{18}FNO_8P_2$: 372.0408, found 372.0406.

((3-(2-chlorophenyl)-2-methylisoxazolidin-5-yl)(hydroxy)methylene)diphosphonic acid 59. White Solid. 86% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.18 (m, 1H, CH_2), 2.45 (s, 3H, J=11.99 Hz, CH_3), 2.78 (m, 1H, CH_2), 3.09 (dd, J=12.20, 8.16 Hz, 1H, CH), 4.46 (m, 1H, CH), 6.48 (br s, 5H, OH), 7.11-7.29 (m, 2H, Ar), 7.38-7.45 (d, 1H, J=7.20, Ar), 7.54-7.67 (m, 1H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 30.4, 43.1, 60.6, 63.4, 124.3, 127.8, 127.9, 128.7, 128.9, 137.8.
ESI(+)-MS: m/z [M + H] Calcd for $C_{11}H_{17}ClNO_8P_2$: 388.0112, found 388.0115.

((3-(3-chlorophenyl)-2-methylisoxazolidin-5-yl)(hydroxy)methylene)diphosphonic acid 60. White Solid. 87% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.15(m, 1H, CH_2), 2.46 (s, 3H, J=12.07 Hz, CH_3), 2.65 (m, 1H, CH_2), 3.11 (dd, J=12.25, 8.12 Hz, 1H, CH), 4.46 (m, 1H, CH), 6.49 (br s, 5H, OH), 7.09-7.35 (m, 3H, Ar), 7.35-7.44 (s, 1H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 29.3, 44.1, 61.6, 64.4, 124.1, 127.6, 127.9, 128.5, 128.9, 129.6, 137.2.
ESI(+)-MS: m/z [M + H] Calcd for $C_{11}H_{17}ClNO_8P_2$: 388.0112, found 388.0115.

(2-(3-(2-chlorophenyl)-2-methylisoxazolidin-5-yl)-1-hydroxyethane-1,1-diyl)diphosphonic acid 61. White Solid. 85% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.15 (m, 1H, CH_2), 2.48 (s, 3H, J=12.09 Hz, CH_3), 2.84 (m,

1H, CH_2), 3.02 (d, 2H, J= 6.52 CH_2) 3.24 (dd, 1H, J=11.80, 8.20 Hz, CH), 4.68 (m, 1H, CH), 6.52 (br s, 5H, OH), 7.04-7.56 (m, 4H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 43.8, 60.9, 63.4, 68.8, 72.6, 102.1, 105.3, 115.5, 124.7, 127.9, 129.8, 137.9.
ESI(+)-MS: m/z [M + H] Calcd for $C_{12}H_{19}ClNO_8P_2$: 402.0269, found 402.0266.

(3-(3-(2-chlorophenyl)-2-methylisoxazolidin-5-yl)-1-hydroxypropane-1,1-diyl)diphosphonic acid 62. White Solid. 86% yield.
^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.10 (m, 1H, CH_2), 2.45 (s, 3H, J=12.06 Hz, CH_3), 2.72 (m, 1H, CH_2), 3.10 (td, 2H, J= 8.10, 6.70 CH_2) 3.22 (dd, J=12.41, 8.11 Hz, 1H, CH), 3.40 (t, 2H, J=8.13, CH_2), 4.52 (m, 1H, CH), 6.43 (br s, 5H, OH), 7.24-7.47 (m, 4H, Ar).
^{13}C-NMR (75 MHz, DMSO-d_6): 42.3, 61.4, 62.4, 63.8, 64.1, 68.8, 103.4, 105.5, 116.5, 127.8, 128.5, 128.8, 137.2.
ESI(+)-MS: m/z [M + H] Calcd for $C_{13}H_{21}ClNO_8P_2$: 416.0425, found 416.0428.

11.11 SYNTHESIS OF THE AZIDES

11.11.1 Synthesis of the Benzyl azide 46.

Benzyl Bromide (2,0 ml, 16,84 mmol,1 eq) was dissolved in DMSO (40 ml). Sodium azide (1.64 g, 25,26 mmol, 1,5 eq) was added as a solid and the reaction was stirred overnight at ambient temperature. Water (75ml) was added slowly before extracting the product into diethyl ether (3 x 150 ml). The combined diethyl ether layers were washed with brine (2 x 150 ml), dried over sodium sulfate and the solvent removed to leave a clear oil.
Colorless oil, 73% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.33 (s, 2H, CH_2), 7.24-7.41 (m, 5H, Ar) ppm.
^{13}C-NMR (75 MHz, $CDCl_3$): δ 54.84, 128.25, 128.34, 128.87, 135.40
ESI(+)-MS: m/z [M + H] calcd for $C_7H_8N_3$ 134.0718, found 134.0716.

11.11.2 Synthesis of the Octyl azide 47.

Octyl Bromide (8,0 ml, 4,63 mmol, 1 eq) was dissolved in DMSO (160 ml). Sodium azide (4.52 g, 6,95 mmol, 1,5 eq) and $FeCl_3$ hexahydrate (2.49 g, 9,26 mmol, 0,2 eq) were added and the reaction was stirred overnight at ambient temperature. Water (75ml) was added slowly before extracting the product into diethyl ether (3 x 150 ml). The combined diethyl ether layers were washed with brine (2 x 150 ml), dried over sodium sulfate and the solvent removed to leave a clear oil.
Colorless oil, 95% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, J = 7.0 Hz, 3H, CH_3), 1.25– 1.42 (m, 10H, CH_2), 1.60 (quin, J = 7.3 Hz, 2H, CH_2–C–N_3), 3.25 (t, J = 7.0 Hz, 2H, CH2–N3).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 14.0, 22.6, 26.7, 28.8, 29.1, 31.7, 51.4.
ESI(+)-MS: m/z [M + H] calcd for $C_8H_{18}N_3$ 156.1501, found 156.1504.

11.11.3 Sunthesis of the Phenyl azide 48.

Aniline (5,11 g, 54,9 mmol) is dissolved in a suitable volume of degaseous water containing 2,5-3 equivalents of hydrochloridric acid by the application of hea if necessary, ad the solution is cooled in ice when the amine hydrochloride usually crystallises. The temperature is maintained at 0-5°C and an aqueous solution of sodium nitrite (7,60 g, 110 mmol) is added portionwise until, after allowing 3-4 minutes for reaction, the solution gives an immediate positive test for excess of nitrous acid with an external indicator – moist potassium iodide- starch paper. the precipitated amine hydrochlide, if any, dissolves during the diazotization to give a clear solution of the highly soluble diazonium salt. The excess to acid maintains the proper condition of acidity required to stabilisce the diazonium salt and hence to minimize secondary reactions, e.g. the interaction of some of the diazonium salt with unchanged amine to form a diazoamino compound, a reaction which occurs readly in neutral solution. After completion of the reaction, NaN_3 (8,92 g, 137 mmol) was added (the salient of nitrogen gas is observed) and the mixture was stirred slowly for 20 minutes at room temperature. The mixture was washed with distilled degaseuous wather and diethyl ether. The residue was extracted with diethyl ethr (3 x 30 ml) and the combined organic layer was washed with 5% aqueous solution of NaOH (12 ml) and then it was dried over Na_2SO_4. The solvent was evaporate at room pressure to afford the Phenyl azide.
Brown oil, 91% yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.34 (t, 2H, J = 7.6 Hz, Ar), 7.13 (t, 2H, J = 7.4 Hz, Ar), 7.02 (d, 1H, J = 7.4 Hz, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 142.1, 131.5, 126.5, 120.7.
ESI(+)-MS: m/z [M + H] calcd for $C_6H_6N_3$ 120.0562, found 120.0560.

11.12 SYNTHESIS OF THE ω-NITROSTYRENES

Equip a 1500 ml three-necked flask with a thermometer, mechanical stirrer and a dropping funnel. Place 1 mol of nitromethane, 1 mol of purified benzaldehyde derivative and 200 ml of methanol in the flask and cool it with a mixture of ice and salt to about -10°C. Dissolve 1.05 mol of sodium hydroxide in 40-50 ml of water, cool and dilute to 100 ml with ice and water; place this cold solution in the dropping funnel. Add the sodium hydroxide solution, with vigorous stirring, to the nitromethane mixture at such a rate that the temperature is held at 10-15 °C. Introduce the first few ml cautiously since, after a short induction period, the temperature may rise to 30 °C or higher; check the rise in temperature, if necessary, by adding a little crushed ice to to the reaction mixture. A bulky white precipitate forms; if the mixture becomes so thick that stirring is difficult, add about 10 ml of methanol. After standing for about 15 minutes, add 700 ml of ice-water containing crushed ice; the temperature should be below 5°C. Run the resulting cold solution immediately from a dropping funnel and with stirring into 500 ml of 4 M hydrochloridric acid contained in a 3 litre flask; adjust the rate of addition so that the stream just fails to break into drops. A pale yellow crystalline precipitate separates almost as soon as the alkaline solution mixes with the acid. The solid settles to the bottom of the vessel when the stirrer is stopped. Decant most of the cloudy liquid layer, filter the residue by suction and wash it

with water until free from chlorides. Transfer the solid to a breaker immersed in hot water; two layers form and on cooling again, the lower layer of nitrostyrene derivative solidifies; pour off the upper water layer. Dissolve the crude in 85 ml of hot ethanol to recrystallize the product.

(*E*)-ω-nitrostyrene 49. Yellow solid, 85% yield, m.p. 56-57°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.41–7.48 (m, 2H, Ar), 7.48–7.52 (m, 1H, Ar), 7.52–7.56 (m, 2H, Ar), 7.56–7.64 (dd, 1H, J = 13.7, 1.1 Hz, CH), 7.95–8.05 (dd, 1H, J = 13.7, 1.8 Hz, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 129.29, 129.53, 130.17, 132.30, 137.22, 139.23.
ESI(+)-MS: m/z [M + H] calcd for $C_8H_8NO_2$ 150.0555, found 150.0558.

(*E*)-ω-nitro-(2-chloro)styrene 50. Yellow solid, 87% yield, m.p. 51-52°C.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.68 (d, 1H, J = 18.5 Hz, CH), 7.28 (dd, 1H, J = 9.0 Hz, J = 7.5 Hz, Ar), 7.60 (dd, 1H, J = 10 Hz, J = 8.0 Hz, Ar), 7.64 (d, 1H, J = 8.5 Hz, Ar), 7.75 (d, 1H, J = 8.0 Hz, Ar,), 8.32 (d, 1H, J = 18.5 Hz, CH).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 127.7, 128.6, 128.7, 130.9, 133.0, 135.2, 136.2, 138.9;
ESI(+)-MS: m/z [M + H] calcd for $C_8H_7ClNO_2$ 184.0165, found 184.0168.

(*E*)-ω-nitro-(3-chloro)styrene 51. Yellow solid, 87% yield, m.p. 51-52°C.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.93 (d, 1H, J = 13.7 Hz, CH), 7.56 (d, 1H, J = 13.7 Hz, CH), 7.54 – 7.37 (m, 4H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 127.22, 128.75, 130.65, 131.85, 131.96, 135.49, 137.41, 138.12.
ESI(+)-MS: m/z [M + H] calcd for $C_8H_7ClNO_2$ 184.0165, found 184.0169.

(*E*)-ω-nitro-(4-chloro)styrene 52. Yellow solid, 88% yield, m.p. 113-114°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.38–7.45 (m, 2H, Ar), 7.45–7.53 (m, 2H, Ar), 7.53–7.64 (d, 1H, *J* = 13.7 Hz, CH), 7.90–8.01 (d, 1H, *J* = 13.7 Hz, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 76.88, 77.20, 77.51, 128.65, 129.87, 130.42, 137.53, 137.85,. 138.43.
ESI(+)-MS: m/z [M + H] calcd for $C_8H_7ClNO_2$ 184.0167, found 184.0169.

(*E*)-ω-nitro-(4-methyl)styrene 53. Yellow solid, 86% yield, m.p. 106-107°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.32–2.56 (s, 3H, CH_3), 7.17–7.37 (m, 2H, Ar), 7.37–7.52 (m, 2H, Ar), 7.52–7.68 (d, 1H, *J* = 13.7 Hz, CH), 7.92–8.07 (d, 1H, *J* = 13.6 Hz, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 21.84, 127.41, 129.36, 130.30, 136.41, 139.35, 143.29.
ESI(+)-MS: m/z [M + H] calcd for $C_9H_{10}NO_2$ 164.0712, found 164.0715.

(*E*)-ω-nitro-(4-Methoxy)styrene 54. Yellow solid, 89% yield, m.p. 87-88°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.52–4.21 (s, 3H, CH_3), 6.83–7.00 (d, 2H, *J* = 8.8 Hz, Ar), 7.43–7.47 (d, 1H, *J* = 2.0 Hz, Ar), 7.47–7.50 (m, 1H, Ar), 7.50 – 7.54 (s, 1H, CH), 7.85 – 8.01 (d, 1H, *J* = 13.6 Hz, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 55.59, 114.97, 122.54, 131.28, 135.00, 139.13, 163.02.
ESI(+)-MS: m/z [M + H] calcd for $C_9H_{10}NO_3$ 180.0661, found 180.0663.

(*E*)-ω-nitro-(2-nitro)styrene 55. Yellow solid, 80% yield, m.p. 107-108°C.
^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.42 (d, 1H, *J*=13.5, CH), 7.60 (dd, 1H, *J* = 7.5, 1.3, Ar), 7.68 (td, 1H, *J* = 7.9, 1.5, Ar), 7.74 (td, 1H, *J* = 7.5, 1.0, Ar), 8.21 (dd, 1H, *J* = 8.1, 1.3, Ar), 8.53 (d, 1H, *J* = 13.5, CH).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 125.7, 126.4, 129.6, 132.0, 134.2, 135.4, 139.9, 148.3.
ESI(+)-MS: m/z [M + H] calcd for $C_8H_7N_2O_4$ 195.0406, found 195.0408.

(*E*)-ω-nitro-(4-nitro)styrene 56. Yellow solid, 81% yield, m.p. 112-113°C.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.62 (d, 1H, J = 18 Hz, CH), 7.42 (d, 2H, J = 9.0 Hz, Ar); δ 7.78 (d, 2H, J = 9.5 Hz, Ar), 8.18 (d, 1H, J = 18.0 Hz, CH).
^{13}C-NMR (75 MHz, $CDCl_3$): δ 124.2, 129.6, 135.9, 136.0, 139.7, 149.3.
ESI(+)-MS: m/z [M + H] calcd for $C_8H_7N_2O_4$ 195.0406, found 195.0409.

11.13 SYNTHESIS OF THE ENAMINONES

To a solution of carbonyl compound (2 mmol) in CH_2Cl_2 (4 mL) or THF (4 ml), $Er(OTf)_3$ (20 mmol, 1%) and appropriate amine (2.2 mmol) were added and the resulting solution was stirred at r.t. When the starting material had disappeared or there was not change in the starting material/product ratio (GC-MS), the crude were poured into a sat. solution of Na_2CO_3 (5 mL), and extracted with CH_2Cl_2 (2 x 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Subsequently the mixture was poured into a solution of dilute HCl (4:1), then neutralized with a sature solution of Na_2CO_3, and extracted with CH_2Cl_2 (2 x 5 mL). The organic layer was dried over anhyd Na_2SO_4 and concentrated under reduced pressure. Products were then purified by recrystallization with ethyl acetate.

(*Z*)-4-(hexylamino)pent-3-en-2-one 57. Yellow oil. 97% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, 3H, *J* = 5.4 Hz, CH_3), 1.10–1.80 (m, 8H, 4×CH_2), 1.91 (s, 3H, CH_3C=C), 1.99 (s, 3 H, CH_3C=O), 3.22 (q, 2H, *J* = 6.6 Hz, CH_2N), 4.95 (s, 1H, CH=C), 10.85 (br s, 1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 13.89, 18.68, 22.41, 26.42, 28.60, 29.98, 31.36, 42.94, 94.88, 163.03, 194.46.
ESI(+)-MS: m/z [M + H] calcd for $C_{11}H_{22}NO$ 184.1696, found 184.1701.

(*Z*)-4-(phenylamino)pent-3-en-2-one 58. Yellow solid. 95% Yield, m.p. 47-48°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.99 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 5.18 (s,1H, CH), 7.11 (d, 2H, J=8.0 Hz, Ar), 7.20 (t, 1H, J=7.5 Hz, Ar), 7.35 (t, 2H, J=7.5 Hz, Ar), 12.4 (br s,1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 19.6, 29.0, 97.4, 124.6, 125.4, 128.9, 138.6, 160.0, 196.0.
ESI(+)-MS: m/z [M + H] calcd for $C_{11}H_{14}NO$ 176.1070, found 176.11075.

(*Z*)-4-(benzylamino)pent-3-en-2-one 59. Yellow oil. 98% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.90 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 4.44 (d, 2H, J = 6.4 Hz, CH_2), 5.04 (s, 1H, CH), 7.25 (m, 3H, Ar), 7.33 (t, 2H, J = 7.5 Hz, Ar), 11.16 (s, 1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 18.71, 28.70, 46.55, 95.76, 126.54, 127.26, 128.65, 137.87,

163.04, 195.21.
ESI(+)-MS: m/z [M + H] calcd for $C_{12}H_{16}NO$ 190.1226, found 190.1228.

(*Z*)-4-(isopropylamino)pent-3-en-2-one 60. Yellow oil. 87% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.26 (d, 6H, J = 7.0 Hz, CH_3 x 2), 1.94 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 3.72 (m, 1H, CH), 4.87 (s, 1H, CH), 10.80 (br s, 1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 21.30, 24.03, 29.82, 45.42, 49.65, 53.68, 197.84.
ESI(+)-MS: m/z [M + H] calcd for $C_8H_{16}NO$ 142.1226, found 142.1229.

(*Z*)-4-(vinylamino)pent-3-en-2-one 61. Yellow solid. 96% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.97 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.72 (m, 1H, CH), 5.20 (m, 1H, CH), 6.42 (m, 2H, CH_2), 10.82 (br s, 1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 18.92, 27.54, 91.32, 97.85, 131.24, 147.85, 190.10.
ESI(+)-MS: m/z [M + H] calcd for $C_7H_{12}NO$ 126.0913, found 126.0918.

(*Z*)-1-phenyl-3-(phenylamino)but-2-en-1-one 62. Yellow solid. 93% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.14 (s, 3H, CH_3), 5.89 (s, 1H, CH), 7.29 – 7.13 (m, 3H, Ar), 7.50 – 7.31 (m, 5H, Ar), 7.97 – 7.87 (m, 2H, Ar), 13.10 (s, 1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 20.41, 94.22, 124.75, 125.74, 127.03, 128.24, 129.12, 130.85, 138.62, 139.99, 162.16, 188.65.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{16}NO$ 238.1226, found 238.1228.

(*Z*)-1-phenyl-3-(benzylamino)but-2-en-1-one 63. Yellow solid. 97% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.07 (s, 3H, CH_3), 4.54 (d, 2H, J = 6.3 Hz, CH_2), 5.75 (s, 1H, CH), 7.51 – 7.16 (m, 8H, Ar), 7.98 – 7.75 (m, 2H, Ar), 11.75 (s, 1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 19.49, 47.03, 92.60, 126.85, 126.92, 127.52, 128.14, 128.86, 130.51, 137.72, 140.27, 164.84, 188.08.
ESI(+)-MS: m/z [M + H] calcd for $C_{17}H_{18}NO$ 252.1383, found 252.1386.

(*Z*)-ethyl 3-(hexylamino)but-2-enoate 64. Yellow oil. Quantitative Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, 3H, *J* = 5.4 Hz, CH_3), 1.20–1.65 (m, 11H, OCH_2CH_3, 4xCH_2), 1.91 (s, 3H, CH_3C=C), 3.19 (q, 2H, *J* = 6.5 Hz, CH_2N), 4.08 (q, 2H, *J* = 6.9 Hz, CH_2O), 4.43 (s, 1H, CH=C), 8.55 (br s, 1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 13.93, 14.59, 19.31, 22.45, 26.47, 30.36, 31.45, 43.00, 58.14, 81.66, 161.88, 170.60.
ESI(+)-MS: m/z [M + H] calcd for $C_{12}H_{24}NO_2$ 214.1802, found 214.1805.

(*Z*)-ethyl 3-(phenylamino)but-2-enoate 65. Yellow oil. 71% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.29 (t, 3H, J = 7.1 Hz, CH_3), 2.00 (s, 3H, CH_3), 4.16 (q, J = 7.1 Hz, 2H, CH_2), 4.69 (s, 1H, CH), 7.11-7.05 (m, 2H, Ar), 7.19- 7.12 (m, 1H, Ar), 7.36-7.28 (m, 2H, Ar), 10.38 (s, 1H, NH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.22, 19.74, 61.43, 83.20, 122.43, 129.51, 129.65, 138.72, 161.25, 161.90.
ESI(+)-MS: m/z [M + H] calcd for $C_{12}H_{16}NO_2$ 206.11176, found 206.1179.

11.14 SYNTHESIS OF THE 1,2,3-TRIAZOLES

In a 10 ml one-necked flask equipped with magnetic stir bar, is placed $Er(OTf)_3$ (20 mol %) and dissolved in a solution (5 ml) composed by methyl pyridium triflate and water 5:1 v/v. Subsequently are introducted dipolarophile 49-65 (1 eq) and azide 46-48 (2,5 eq). The flask is equipped with a bubble condenser and the reaction is conducted at 100°C for appropriate time.
The reaction system are extracted with CH_2Cl_2 (3 x 3 ml) and the combined layers are dried over Na_2SO_4 anhydrous, filtered and evaporate to give the corresponding product.

1-benzyl-5-phenyl-1,2,3-triazole 66. White Solid. 85% Yield. m.p. 72-73°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.54 (s, 2H, CH_2), 7.07 (m, 2H, Ar), 7.19 – 7.35 (m, 5H, Ar), 7.36 – 7.46 (m, 3H, Ar), 7.73 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 51.69, 126.80, 127.04, 128.03, 128.71, 128.78, 128.85, 129.40, 133.16, 135.41, 138.05.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{14}N_3$ 236.1182, found 236.1187.

1-benzyl-5-(2-Chloro)phenyl-1,2,3-triazole 67. White Solid. 85% Yield. m.p. 58-59°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.44 (s, 2H, CH_2), 6.95 (dd, 2H, J = 7.5, 1.7 Hz, Ar), 7.01 (dd, 1H, J = 7.6, 1.6 Hz, Ar), 7.16–7.28 (m, 4H, Ar), 7.38–7.41 (m, 1H, Ar), 7.49 (dd, J = 8.1, 1.0 Hz, 1H, Ar), 7.71 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 52.5, 126.4, 126.9, 127.7, 128.2, 128.6, 129.9, 131.2, 132.0, 134.3, 134.4, 134.81, 134.83,.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{13}ClN_3$ 270.0793, found 270.0795.

1-benzyl-5-(3-Chloro)phenyl-1,2,3-triazole 68. Colorless Oil. 86% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.51 (s, 2H, CH_2), 7.11 – 7.18 (m, 4H, Ar), 7.16 – 7.26 (m, 2H, Ar), 7.28 – 7.34 (m, 3H, Ar), 7.72 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 51.82, 116.06, 122.94, 127.05, 127.08, 128.21, 128.83, 129.64, 130.85, 133.36, 135.29, 137.11, 163.30.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{13}ClN_3$ 270.0793, found 270.0797.

1-benzyl-5-(4-Chloro)phenyl-1,2,3-triazole 69. Colorless Oil. 85% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.52 (s, 2H, CH_2), 7.01 – 7.16 (m, 4H, Ar), 7.17 – 7.26 (m, 2H, Ar), 7.25 – 7.34 (m, 3H, Ar), 7.71 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 51.81, 116.08, 122.91, 127.03, 128.21, 128.83, 130.85, 133.36, 135.29, 137.11, 163.30.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{13}ClN_3$ 270.0793, found 270.0797.

1-benzyl-5-(4-Methyl)phenyl-1,2,3-triazole 70. Yellow Oil. 87% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.38 (s, 3H, CH_3), 5.52 (s, 2H, CH_2), 7.08 (dd, J = 7.2, 2.2 Hz, 2H, Ar), 7.13 (d, 2H, J = 8.1 Hz, Ar), 7.21 (d, 2H, J = 8.0 Hz, Ar), 7.24 – 7.31 (m, 3H, Ar), 7.70 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 21.21, 51.60,123.79, 127.03, 128.00, 128.65, 128.71, 129.55, 133.02, 135.56, 138.14, 139.55.

ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{16}N_3$ 250.1339, found 250.1342.

1-benzyl-5-(4-Methoxy)phenyl-1,2,3-triazole 71. Yellow Oil. 88% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.83 (s, 3H, CH_3), 5.52 (s, 2H, CH_2), 6.88 – 6.99 (m, 2H, Ar), 7.02 – 7.12 (m, 2H, Ar), 7.17 (m, 2H, Ar), 7.22 – 7.33 (m, 3H, Ar), 7.68 (d, J = 1.2 Hz, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 51.54, 55.27, 114.31, 118.81, 126.98, 127.99, 128.70, 130.11, 132.91, 135.55, 137.90, 160.40.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{16}N_3O$ 266.1288, found 266.1290.

1-benzyl-5-(2-nitro)phenyl-1,2,3-triazole 72. Yellow Solid. 81% Yield. m.p. 149-151°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.42 (s, 2H, CH_2), 7.02 (dd, 1H, J = 7.6, 1.3 Hz, Ar), 7.17–7.22 (m, 5H, Ar), 7.51–7.56 (m, 1H, CH), 7.60–7.67 (m, 2H, Ar), 8.05 - 8.12 (m, 1H, Ar).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 52.6, 122.3, 124.9, 127.7, 128.4, 128.7, 131.1, 133.1, 133.2, 133.4, 133.7, 134.2, 148.4.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{13}N_4O_2$ 281.1033, found 281.1035.

1-benzyl-5-(4-nitro)phenyl-1,2,3-triazole 73. Yellow Solid. 82% Yield. m.p. 98-99°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.61 (s, 2H, CH_2), 7.03–7.10 (m, 2H, Ar), 7.28–7.30 (m, 3H, Ar), 7.43 (d, J = 8.8 Hz, 2H, Ar), 7.81 (s, 1H, CH), 8.25 (d, J = 8.7 Hz, 2H, Ar).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 52.5, 124.1, 126.9, 127.6, 128.6, 129.0, 129.7, 131.2, 134.1, 134.6, 148.3.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{13}N_4O_2$ 281.1033, found 281.1037.

1-octyl-5-phenyl-1,2,3-triazole 74. Yellow Oil. 83% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.83 (t, 3H, J = 6.6 Hz, CH_3), 1.17-1.24 (m, 10H, 5xCH_2), 1.75-1.85 (m, 2H, CH_2), 4.32 (t, 2H, J = 7.2 Hz, CH_2), 7.35-7.38 (m, 2H, Ar), 7.46-7.48 (m, 3H, Ar),7.65 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.24, 22.76, 26.58, 29.02, 29.15, 30.25, 31.85, 48.48, 127.52, 128.91, 129.26, 129.58, 133.15, 137.86.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{24}N_3$ 258.1965, found 258.1963.

1-octyl-5-(2-Chloro)phenyl-1,2,3-triazole 75. Yellow Oil. 84% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.84 (t, 3H, J = 6.4 Hz, CH_3), 1.18-1.26 (m, 10H, 5xCH_2), 1.74-1.83 (m, 2H, CH_2), 4.35 (t, 2H, J = 7.0 Hz, CH_2), 7.34-7.38 (m, 2H, Ar), 7.46-7.48 (m, 2H, Ar), 7.68 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.22, 22.74, 26.56, 29.04, 29.18, 30.25, 31.86, 48.49, 127.53, 128.92, 128.95, 129.26, 129.35, 129.58, 133.16, 137.89.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{23}ClN_3$ 292.1575, found 292.1577.

1-octyl-5-(3-Chloro)phenyl-1,2,3-triazole 76. Yellow Oil. 83% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.83 (t, 3H, J = 6.7 Hz, CH_3), 1.19-1.28 (m, 10H, 5xCH_2), 1.74-1.83 (m, 2H, CH_2), 4.35 (t, 2H, J = 7.1 Hz, CH_2), 7.33 (s, 1H, Ar), 7.46-7.49 (m, 3H, Ar), 7.69 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.24, 22.77, 26.57, 29.06, 29.19, 30.23, 31.85, 48.48, 127.52,

128.91, 128.93, 129.23, 129.33, 129.57, 133.15, 137.88.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{23}ClN_3$ 292.1575, found 292.1578.

1-octyl-5-(4-Chloro)phenyl-1,2,3-triazole 77. Yellow Oil. 81% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.81 (t, 3H, J = 6.5 Hz, CH_3), 1.20-1.28 (m, 10H, 5xCH_2), 1.76-1.85 (m, 2H, CH_2), 4.35 (t, 2H, J = 7.3 Hz, CH_2), 7.33-7.44 (m, 2H, Ar), 7.46-7.50 (m, 2H, Ar), 7.70 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.24, 22.76, 26.56, 29.05, 29.18, 30.22, 31.84, 48.49, 127.51, 128.92, 129.24, 129.57, 133.17, 137.84.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{23}ClN_3$ 292.1575, found 292.1576.

1-octyl-5-(4-Methyl)phenyl-1,2,3-triazole 78. Yellow Oil. 80% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.84 (t, 3H, J = 6.5 Hz, CH_3), 1.21-1.29 (m, 10H, 5xCH_2), 1.77-1.88 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 4.36 (t, 2H, J = 7.3 Hz, CH_2), 7.31-7.45 (m, 2H, Ar), 7.46-7.50 (m, 2H, Ar), 7.70 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.28, 21.23, 22.76, 26.59, 29.09, 29.19, 30.25, 31.87, 48.49, 127.52, 128.95, 129.26, 129.58, 133.13, 137.87.
ESI(+)-MS: m/z [M + H] calcd for $C_{17}H_{26}N_3$ 272.2121, found 272.2125.

1-octyl-5-(4-Methoxy)phenyl-1,2,3-triazole 79. Yellow Oil. 82% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.82 (t, 3H, J = 6.5 Hz, CH_3), 1.23-1.32 (m, 10H, 5xCH_2), 1.77-1.88 (m, 2H, CH_2), 3.82 (s, 3H, CH_3), 4.38 (t, 2H, J = 7.4 Hz, CH_2), 7.33-7.48 (m, 2H, Ar), 7.48-7.53 (m, 2H, Ar), 7.72 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.29, 21.25, 22.79, 26.56, 29.05, 29.14, 30.23, 31.88, 48.45, 55.23, 127.56, 128.93, 129.24, 129.59, 133.12, 137.87.
ESI(+)-MS: m/z [M + H] calcd for $C_{17}H_{26}N_3O$ 288.2070, found 288.2072.

1-octyl-5-(2-nitro)phenyl-1,2,3-triazole 80. Yellow Oil. 79% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.83 (t, 3H, J = 6.2 Hz, CH_3), 1.26-1.36 (m, 10H, 5xCH_2), 1.79-1.90 (m, 2H, CH_2), 4.35 (t, 2H, J = 7.1 Hz, CH_2), 7.34–7.40 (m, 2H, Ar), 7.42–7.46 (m, 2H, Ar), 7.78 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.28, 21.28, 22.79, 26.56, 29.09, 29.13, 30.20, 31.84, 48.45, 55.28, 127.48, 129.69, 133.27, 137.54.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{23}N_4O_2$ 303.1816, found 303.1817.

1-octyl-5-(4-nitro)phenyl-1,2,3-triazole 81. Yellow Oil. 78% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.85 (t, 3H, J = 6.5 Hz, CH_3), 1.26-1.36 (m, 10H, 5xCH_2), 1.78-1.89 (m, 2H, CH_2), 4.38 (t, 2H, J = 7.4 Hz, CH_2), 7.08 - 7.18 (m, 2H, Ar), 7.44-7.49 (m, 2H, Ar), 7.75 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 14.24, 21.22, 22.75, 26.54, 29.08, 29.16, 30.22, 31.87, 48.43, 55.22, 127.46, 128.97, 129.43, 129.67, 133.24, 137.89.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{23}N_4O_2$ 303.1816, found 303.1819.

1,5-diphenyl-1,2,3-triazole 82. Yellow Solid. 84% Yield. m.p. 113-114°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.32 – 7.39 (m, 5H, Ar), 7.23 (m, 2H, Ar), 7.41 – 7.48 (m, 3H, Ar), 7.87 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 125.19, 126.77, 128.58, 128.83, 129.20, 129.34, 133.40, 136.61, 137.70.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{12}N_3$ 222.1026, found 222.1029.

1-Phenyl-5-(2-Chloro)phenyl-1,2,3-triazole 83. Yellow Solid. 86% Yield. m.p. 98-99°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.09 - 7.19 (m, 3H, Ar), 7.35 - 7.39 (m, 3H, Ar), 7.43-7.45 (m, 3H, Ar), 7.93 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 116.4, 124.5, 125.2, 128.6, 128.8, 129.2, 129.3, 130.9, 131.5, 133.4, 134.8, 159.4.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{11}ClN_3$ 256.0641, found 256.0642.

1-Phenyl-5-(3-Chloro)phenyl-1,2,3-triazole 84. Yellow Solid. 85% Yield. m.p. 86-87°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.15−7.19 (m, 1H, Ar), 7.29−7.34 (m, 2H, Ar), 7.35−7.40 (m, 3H, Ar), 7.47− 7.55 (m, 3H, Ar), 8.01 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 125.4, 126.9, 128.3, 129.1, 129.4, 129.7, 130.1, 133.0, 134.4, 136.2, 137.0.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{11}ClN_3$ 256.0641, found 256.0644.

1-Phenyl-5-(4-Chloro)phenyl-1,2,3-triazole 85. Yellow Solid. 86% Yield. m.p. 123-124°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.13 – 7.20 (m, 2H, Ar), 7.34 (m, 4H, Ar), 7.42 – 7.50 (m, 3H, Ar), 7.86 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 125.18, 129.18, 129.42, 129.48, 129.78, 133.40, 135.45, 136.33, 136.63.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{11}ClN_3$ 256.0642, found 256.0638.

1-Phenyl-5-(4-Methyl)phenyl-1,2,3-triazole 86. Yellow Solid. 86% Yield. m.p. 148-149°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.35 (s, 3H, CH_3), 7.12 (m, 4H, Ar), 7.33 – 7.47 (m, 5H, Ar), 7.83 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 21.21, 123.71, 125.14, 128.37, 129.06, 129.24, 129.48, 133.11, 136.64, 137.71, 139.27.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{14}N_3$ 236.1182, found 236.1187.

1-Phenyl-5-(4-Methoxy)phenyl-1,2,3-triazole 87. White Solid. 87% Yield. m.p. 97-98°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.80 (s, 3H, CH_3), 6.83 – 6.88 (m, 2H, Ar), 7.12 – 7.19 (m, 2H, Ar), 7.34 – 7.40 (m, 2H, Ar), 7.40 – 7.46 (m, 3H, Ar), 7.80 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 55.21, 114.23, 118.79, 125.11, 129.03, 129.23, 129.82, 132.81, 136.62, 137.48, 160.15.
ESI(+)-MS: m/z [M + H] calcd for $C_{15}H_{14}N_3O$ 252.1137, found 252.1136.

1-Phenyl-5-(2-nitro)phenyl-1,2,3-triazole 88. Yellow Solid. 78% Yield. m.p. 125-126°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.08 - 7.17 (m, 3H, Ar), 7.33 - 7.38 (m, 3H, Ar), 7.44-7.47 (m,

3H, Ar), 7.94 (s, 1H, CH).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 116.4, 124.5, 125.6, 128.9, 129.1, 129.2, 129.6, 130.9, 131.8, 133.9, 134.9, 159.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{11}N_4O_2$ 267.0877, found 267.0874.

1-Phenyl-5-(4-nitro)phenyl-1,2,3-triazole 89. Yellow Solid. 76% Yield. m.p. 144-145°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.32–7.38 (m, 2H, Ar), 7.40–7.45 (m, 2H, Ar), 7.43–7.51 (m, 3H, Ar), 7.97 (s, 1H, CH), 8.19–8.22 (m, 2H, Ar).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 147.7, 135.5, 134.2, 133.1, 129.8, 129.6, 129.3, 125.4, 124.4, 124.1.
ESI(+)-MS: m/z [M + H] calcd for $C_{14}H_{11}N_4O_2$ 267.0877, found 267.0878.

1-(1-benzyl-5-methyl-1,2,3-triazol-4-yl)ethanone 90. White Solid. 87% Yield. m.p. 148-149°C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.45 – 2.50 (m, 3H, CH_3), 2.64 – 2.72 (m, 3H, CH_3), 5.51 (s, 2H, CH_2), 7.17 (m, 2H, Ar), 7.26 – 7.44 (m, 3H, Ar).
^{13}C-NMR (101 MHz, $CDCl_3$): δ 9.02, 27.55, 51.52, 127.11, 128.46, 128.98, 133.85, 136.74, 143.83, 194.20.
ESI(+)-MS: m/z [M + H] calcd for $C_{12}H_{14}N_3O$ 216.1131, found 216.1134.

(1-benzyl-5-methyl-1,2,3-triazol-4-yl)(phenyl)methanone 91. Yellow Oil. 82% Yield.
^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.55 (s, 3H, CH_3), 5.57 (s, 2H, CH_2), 7.21-7.22 (m, 2H, Ar), 7.33-7.38 (m, 3H, Ar), 7.48-7.51 (m, 2H, Ar), 7.57-7.60 (m, 1H, CH), 8.33-8.34 (m, 2H, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 9.6, 51.8, 127.3, 128.2, 128.6, 129.1, 130.6, 132.8, 134.0, 137.4, 139.3, 143.8, 187.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{17}H_{16}N_3O$ 278.1288, found 278.1286.

1-(1-octyl-5-methyl-1,2,3-triazol-4-yl)ethanone 92. Yellow Oil. 84% Yield.
^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.82 (t, 3H, J = 6.4 Hz, CH_3), 1.16-1.25 (m, 10H, 5xCH_2), 1.75-1.85 (m, 2H, CH_2), 2.65 (s, 3H, CH_3), 3.02, (s, 3H, CH_3), 4.32 (t, 2H, J = 7.4 Hz, CH_2).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 10.82, 14.25, 22.75, 26.56, 29.01, 29.16, 30.26, 31.86, 48.48, 56.32, 138.84, 143.43, 187.77.
ESI(+)-MS: m/z [M + H] calcd for $C_{13}H_{24}N_3O$ 238.1914, found 238.1916.

(1-octyl-5-methyl-1,2,3-triazol-4-yl)(phenyl)methanone 93. Yellow Oil. 80% Yield.
^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.82 (t, 3H, J = 6.4 Hz, CH_3), 1.16-1.25 (m, 10H, 5xCH_2), 1.75-1.85 (m, 2H, CH_2), 2.65 (s, 3H, CH_3), 4.32 (t, 2H, J = 7.4 Hz, CH_2), 7.47-7.50 (m, 2H, Ar), 7.56-7.59 (m, 1H, Ar), 8.32-8.33 (m, 2H, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 10.82, 14.25, 22.75, 26.56, 29.01, 29.16, 30.26, 31.86, 48.48, 128.21, 130.62, 132.75, 137.55, 138.84, 143.43, 187.77.
ESI(+)-MS: m/z [M + H] calcd for $C_{18}H_{26}N_3O$ 300.2070, found 300.2072.

1-(1-phenyl-5-methyl-1,2,3-triazol-4-yl)ethanone 94. Yellow Oil. 85% Yield.
^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.68 (s, 3H, CH_3), 3.02, (s, 3H, CH_3), 7.50-7.64 (m, 3H, Ar),

8.39-8.45 (m, 2H, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 10.6, 46.23, 125.4, 128.3, 130.1, 132.9, 137.4, 143.5, 187.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{11}H_{12}N_3O$ 202.0975, found 202.0974.

(1-phenyl-5-methyl-1,2,3-triazol-4-yl)(phenyl)methanone 95. Yellow Oil. 83% Yield.
^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.68 (s, 3H, CH_3), 7.49-7.61 (m, 8H, Ar), 8.38-8.39 (m, 2H, Ar).
^{13}C-NMR (125 MHz, $CDCl_3$): δ 10.6, 125.4, 128.3, 129.7, 130.1, 130.6, 132.9, 135.4, 137.4, 139.9, 143.5, 187.6.
ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{14}N_3O$ 264.1131, found 264.1135.

11.15 SYNTHESIS OF THE METHYLPYRIDINIUM TRIFLATE ([mPy][OTf]) USED AS IONIC LIQUID.

The IL [mPy][OTf] was prepared by halide-free direct synthesis by placing dry pyridine (0,29 mol) in a two-necked flask to which methyltrifluoromethanesulfonate (0,30 mol) was added dropwise.
The mixture was reacted at 80°C for 12 h. The crude product was washed with dichloromethane and dried under vacuum to yield 68,04 g (96%) of pure IL. The synthesis of [mPy][OTf] was confirmed by comparison of the spectra with literature reports.[371]

11.16 SYNTHESIS OF THE CATALYST (5S)-2,2,3-TRIMETHYL-5-THIOBENZYLMETHYL-4-IMIDAZOLIDINONE (96).

To an aqueous solution of $MeNH_2$ (2m, 60 mL) was added (S)-benzyl-l-cysteine methyl ester hydrochloride (10 g, 40 mmol), and the solution was stirred at RT for 10 h. The crude solution was treated with a saturated aqueous solution of $NaHCO_3$ (50 mL), and the free amine was extracted with $CHCl_3$ (40 ml x 3), dried (Na_2SO_4), filtered, and concentrated. To this residue was added MeOH (100 mL), acetone (16,6 mL, 220 mmol), and p-TSA (93,2 mg, 0,48 mmol). The solution was heated to reflux for 12 h, cooled to RT, and then concentrated in vacuo. The residue was washed with hexane (30 mL x 3), and yielded 8,36 g (80% overall) of (*5S*)-2,2,3-trimethyl-5-thiobenzylmethyl-4-imidazolidinone (96) as a yellow oil.
^{1}H-NMR (300 MHz, $CDCl_3$, 25°C, TMS): δ= 1.3 (s, 3H, CH_3), 1.4 (s, 3H, CH_3), 2.2 (bs, 1H, N-H), 2.8 (s, 3H, N-CH_3), 2.8 (dd, 1H, $^{3}J_{(H,H)}$=4.2, $^{2}J_{(H,H)}$=14.1 Hz; HCH_2S), 2.9 (dd, 1H, $^{3}J_{(H,H)}$=5.9, $^{2}J_{(H,H)}$=14.1 Hz; HCH_2S), 3.6 (dd, 1H, $^{3}J_{(H,H)}$=4.2, $^{3}J_{(H,H)}$=5.9 Hz, 5-H), 3.7 (d, 1H, $^{2}J_{(H,H)}$=13.4 Hz; HCH_2Bn), 3.8 (d, 1H, $^{2}J_{(H,H)}$=13.4 Hz, HCH_2Bn), 7.2‑7.4 ppm (m, 5H, Ar).
^{13}C-NMR (75 MHz, $CDCl_3$, 25°C, TMS): δ =25.2, 25.3, 27.0, 33.1, 36.8, 57.8, 75.6, 127.0, 128.5, 128.9, 138.2, 172.4 ppm;
HRMS (ESI): m/z: calcd for $[C_{14}H_{20}ON_2S+H]^+$: 265.1369, found 265.1368.

11.17 ENANTIOSELECTIVE DIELS-ALDER REACTIONS CATALYZED BY ORGANOCATALYST 96.

In a representative procedure catalyst 96 (0,036 mmol, 6 mol%) was dissolved in a mixture of [mPy][OTf]/HCl 0,01M 3:1 v/v. The dienophile (0,6 mmol) and the diene (0,4 mmol) were added sequentially. After the appropriate time, the reaction mixture was extracted with ether (2 ml x 3). The ether layer was dried by Na_2SO_4, filtered, evaporated under reduced pressure, and purified by flash chromatography.

***endo*-Bicyclo[2.2.2]oct-2-en-5-carboxaldehyde (entry 1, Table 10.2).** Colorless oil, 98% yield.
^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.27 (m, 1H), 1.35 (tt, 1H), 1.55 (m, 1H), 1.65 (m, 2H), 1.72 (m, 1H), 2.55 (br m, 1H), 2.63 (br m, 1H), 2.95 (br m, 1H), 6.10 (t, 1H), 6.32 (t, 1H), 9.44 (d, 1H).
^{13}C-NMR (125 MHz, $CDCl_3$) δ 24.7, 25.1, 26.6, 29.2, 30.6, 50.8, 130.6, 136.0, 203.6.
HRMS (ESI): m/z: calcd for $[C_9H_{13}O]^+$: 137.0966, found 137.0968.

(*1S,6R*)-6-formylcyclohex-2-en-1-yl acetate (entry 2, Table 10.2). Colorless oil, 93% yield.
^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.81 (m, 1H), 1.91 (m, 1H), 2.01 (m, 1H), 2.06, (m, 1H), 2.21 (s, 3H, CH_3), 3.10 (m, 1H), 4.65 (m, 1H), 5.59 (m, 2H, 2x CH), 9.72 (d, 1H, J=3.02 Hz).
^{13}C-NMR (125 MHz, $CDCl_3$) δ 19.63, 21.13, 24.75, 56.78, 74.06, 122.94, 134.76, 170.28, 204.12.
HRMS (ESI): m/z: calcd for $[C_9H_{13}O_3]^+$: 169.0865, found 169.0867.

(*R*)-4-Methylcyclohex-3-enecarbaldehyde (entry 3, Table 10.2). Colorless oil, 97% yield.
^{1}H-NMR (400MHz, $CDCl_3$) δ 1.65 (s, 3H, CH_3), 1.69 (m, 1H, CHH), 1.97 (m, 1H, CHH), 2.00 (br, 2H, CH_2), 2.21 (br, 2H, CH_2), 2.44 (m, 1H, CH), 5.41 (br, 1H, CH=C), 9.70 (s, 1H, CHO).
^{13}C-NMR (101MHz, $CDCl_3$) δ 22.01, 23.14, 24.62, 30.12, 46.32, 119.93, 133.92, 204.12.
HRMS (ESI): m/z: calcd for $[C_8H_{13}O]^+$: 125.0966, found 125.0969.

***endo*-(*1S,2S,4S*)-Bicyclo[2.2.1]hept-5-ene-2-carbaldehyde. (entry 5, Table 10.2).** Colorless Oil. 95% Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.32 (d, 1H, J = 8.1 Hz), 1.40-1.52 (m, 2H), 1.91 (ddd, 1H, J = 12.0, 9.0, 3.6 Hz), 2.90 (m, 1H), 2.98 (brs, 1H), 3.24 (brs, 1H), 6.00 (dd, 1H, J = 5.7, 2.7 Hz), 6.22 (dd, 1H, J = 5.7, 2.7 Hz,), 9.42 (d, 1H, J = 2.7 Hz,).
^{13}C-NMR (101 MHz, $CDCl_3$) δ 27.6, 42.7, 45.0, 49.6, 52.2, 131.8, 138.1, 205.2.
HRMS (ESI): m/z: calcd for $[C_8H_{11}O]^+$ 123.0810, found 123.0812.

endo-3-Methylbicyclo[2.2.1]hex-5-ene-2-carboxaldehyde. (entry 6, Table 10.2). Colorless Oil. 85% Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.18 (d, 3H, J = 6.9 Hz,), 1.44-1.51 (m, 1H), 1.55-1.60 (m, 1H), 1.77-1.87 (m, 1H), 2.34 (dd, 1H, J = 3.2, 4.3 Hz,), 2.56 (br, 1H), 3.13 (br, 1H), 6.05 (dd, 1H, J = 2.8, 5.6 Hz,), 6.29 (dd, 1H, J = 3.0, 5.8 Hz,), 9.37 (d, 1H, J = 3.2 Hz,).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 18.21, 34.35, 44.83, 48.03, 49.36, 52.61, 135.94, 204.12.
HRMS (ESI): m/z: calcd for $[C_9H_{13}O]^+$ 137.0966, found 137.0968.

endo-3-Phenylbicyclo[2.2.1]hex-5-ene-2-carboxaldehyde. (entry 7, Table 10.2). Colorless Oil. 90% Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.59-1.65 (m, 1H), 1.79-1.84 (m, 1H), 2.98 (ddd, 1H, J = 2.3, 3.5, 4.9 Hz,), 3.09 (dd, 1H, J = 1.6, 4.9 Hz,), 3.14 (br, 1H), 6.17 (dd, 1H, J = 2.8, 5.8 Hz,), 3.34 (br, 1H), 6.17 (dd, 1H, J = 2.8, 5.8 Hz,), 6.42 (dd, 1H, J = 3.3, 5.8 Hz,), 7.13-7.34 (m, 5H), 9.60 (d, 1H, J = 2.3 Hz,).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 42.46, 44.72, 46.28, 46.58, 52.53, 125.84, 126.82, 128.34, 133.12, 135.98, 204,15.
HRMS (ESI): m/z: calcd for $[C_{14}H_{15}O]^+$ 199.1123, found 199.1125.

11.17 ENANTIOSELECTIVE 1,3-DIPOLAR CYCLOADDITION REACTIONS CATALYZED BY ORGANOCATALYST 96.

In a representative procedure catalyst 96 (4,44 mmol, 20 mol%) was dissolved in a mixture of [mPy][OTf] 5:1 v/v (5 ml). In the same flask was introduced 1 eq of HCl 6N (0,074 ml). The dipolarophile (2,22 mmol, 3 eq) and the 1,3-dipole (0,74 mmol, 1 eq) were added sequentially. The reaction system was disponed to -20°C and, after the appropriate time, the reaction mixture was extracted with CH_2Cl_2 (2 ml x 3). The CH_2Cl_2 layer was dried by Na_2SO_4, filtered, evaporated under reduced pressure, and purified by flash chromatography.

***Endo*-2,5-dimethyl-4-formyl-3-phenylisoxazolidine (Entries 1-7, Table 10.4; entries 1-4, Table 10.5).** Colorless Oil, 85%, Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.50 (d, 3H, *J* = 6.3 Hz, CH_3), 2.60 (s, 3H, CH_3), 3.09 (m, 1H, CH), 3.83 (br s, 1H, CH), 4.54 (dq, 1H, *J* = 6.0, 12.3 Hz, CH), 7.26–7.39 (m, 5H, Ar), 9.74 (d, *J* = 2.5 Hz, 1H, CHO).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 21.9, 43.6, 66.3, 72.2, 73.5, 127.8, 128.5, 129.1, 137.8, 198.6.
HRMS (ESI): m/z: calcd for $[C_{12}H_{16}NO_2]^+$ 206.1176, found 206.1178.

***Endo*-2,5-dimethyl-4-formyl-3-(2-Chloro)phenylisoxazolidine. (Entries 1, Table 10.6).** Colorless Oil, 86%, Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.52 (d, 3H, *J* = 6.2 Hz, CH_3), 2.61 (s, 3H, CH_3), 3.09 (m, 1H, CH), 3.84 (br s, 1H, CH), 4.55 (dq, 1H, *J* = 6.1, 12.4 Hz, CH), 7.25–7.38 (m, 4H, Ar), 9.74 (d, *J* = 2.3 Hz, 1H, CHO).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 21.8, 43.7, 66.3, 72.3, 73.6, 127.9, 128.6, 129.2, 137.9, 198.7.
HRMS (ESI): m/z: calcd for $[C_{12}H_{15}ClNO_2]^+$ 240.0786, found 240.0783.

***Endo*-2,5-dimethyl-4-formyl-3-(3-Chloro)phenylisoxazolidine. (Entries 2, Table 10.6).** Colorless Oil, 84%, Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.54 (d, 3H, *J* = 6.3 Hz, CH_3), 2.62 (s, 3H, CH_3), 3.07 (m, 1H, CH), 3.82 (br s, 1H, CH), 4.57 (dq, 1H, *J* = 6.3, 12.6 Hz, CH), 7.26–7.39 (m, 4H, Ar), 9.75 (d, *J* = 2.4 Hz, 1H, CHO).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 21.8, 43.8, 66.2, 72.2, 73.7, 127.8, 128.5, 129.4, 137.6, 198.4.
HRMS (ESI): m/z: calcd for $[C_{12}H_{15}ClNO_2]^+$ 240.0786, found 240.0785.

***Endo*-2,5-dimethyl-4-formyl-3-(4-Chloro)phenylisoxazolidine. (Entries 3, Table 10.6).** Colorless Oil, 86%, Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.55 (d, 3H, *J* = 6.2 Hz, CH_3), 2.59 (s, 3H, CH_3), 3.02 (ddd, 1H, *J* = 8.0, 5.5, 2.3 Hz, CH), 3.82-4.01 (m, 1H, CH), 4.51 (dq, 1H, *Jd* = 5.9, *Jq* = 6.1 Hz, CH), 7.25-7.33 (m, 4H, Ar), 9.74 (d, 1H, *J* = 2.3 Hz, CHO).
^{13}C-NMR (101 MHz, $CDCl_3$) δ 72.2, 73.1, 73.5, 129.1, 129.3, 129.5, 129.6, 134.3, 136.7, 198.3.
HRMS (ESI): m/z: calcd for $[C_{12}H_{15}ClNO_2]^+$ 240.0786, found 240.0789.

***Endo*-2,5-dimethyl-4-formyl-3-(2-Fluoro)phenylisoxazolidine. (Entries 4, Table 10.6).** Colorless Oil, 82%, Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.53 (d, 3H, *J* = 6.2 Hz, CH_3), 2.62 (s, 3H, CH_3), 3.07 (m, 1H, CH), 3.83 (br s, 1H, CH), 4.56 (dq, 1H, *J* = 6.3, 12.6 Hz, CH), 7.25–7.38 (m, 4H, Ar), 9.74 (d, *J* = 2.4 Hz, 1H, CHO).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 21.6, 43.4, 66.2, 72.0, 73.0, 127.2, 128.3, 129.6, 137.4, 198.9.
HRMS (ESI): m/z: calcd for $[C_{12}H_{15}FNO_2]^+$ 224.1081, found 224.1081.

***Endo*-2,5-dimethyl-4-formyl-3-(4-Fluoro)phenylisoxazolidine. (Entries 5, Table 10.6).** Colorless Oil, 84%, Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.54 (d, 3H, *J* = 6.4 Hz, CH_3), 2.58 (s, 3H, CH_3), 3.03 (ddd, 1H, *J* = 8.1, 5.6, 2.4 Hz, CH), 3.84-4.02 (m, 1H, CH), 4.52 (dq, 1H, *Jd* = 5.8, *Jq* = 6.3 Hz, CH), 7.25-7.34 (m, 4H, Ar), 9.74 (d, 1H, *J* = 2.3 Hz, CHO).
^{13}C-NMR (101 MHz, $CDCl_3$) δ 72.3, 73.2, 73.4, 129.3, 129.5, 129.7, 129.8, 134.4, 136.5, 198.6.
HRMS (ESI): m/z: calcd for $[C_{12}H_{15}FNO_2]^+$ 224.1081, found 224.1083.

***Endo*-2,5-dimethyl-4-formyl-3-(4-Methyl)phenylisoxazolidine. (Entries 6, Table 10.6).** Colorless Oil, 87%, Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.51 (d, 3H, *J* = 6.3 Hz, CH_3), 2.34 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 3.09 (ddd, 1H, *J* = 8.4, 5.4, 2.5 Hz, CH), 3.78 (bs, 1H, CH), 4.53 (dq, 1H, *Jd* = 5.9, *Jq* = 6.3 Hz, CH), 7.12-7.26 (m, 4H, Ar), 9.74 (d, 1H, *J* = 2.5 Hz, CHO).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 21.6, 43.7, 72.2, 73.6, 127.5, 128.0, 129.6, 130.0, 134.5, 138.3, 198.7.
HRMS (ESI): m/z: calcd for $[C_{13}H_{18}NO_2]^+$ 220.1332, found 220.1335.

***Endo*-2-Allyl-4-formyl-3-phenyl-5-methylisoxazolidine. (Entries 7, Table 10.6).** Colorless Oil, 80%, Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.50 (d, *J* = 6.0 Hz, 3H, CH_3), 3.09 (ddd, *J* = 2.5, 5.8, 8.0 Hz, 1H, CHCHO), 3.31 (dd, *J* = 6.6, 14.3 Hz, 1H, CH_2=$CHCH_2N$), 3.46 (dd, *J* = 5.5, 14.3 Hz, 1H, CH_2=$CHCH_2N$), 4.10 (d, *J* = 7.7 Hz, 1H, CH), 4.51 (dq, *J* = 6.0, 6.0 Hz, 1H, CH), 5.84–5.98 (m, 1H, CH_2=$CHCH_2$), 5.06–5.28 (m, 2H, CH_2), 7.14–7.24 (m, 5H, Ar), 9.77 (d, *J* = 2.2 Hz, 1H, CHO).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 21.3, 59.1, 71.3, 71.9, 73.7, 118.1, 127.8, 128.4, 129.1, 133.9, 138.6, 198.7.
HRMS (ESI): m/z: calcd for $[C_{14}H_{18}NO_2]^+$ 232.1339, found 232.1137.

***Endo*-2-Benzyl-4-formyl-3-phenyl-5-methylisoxazolidine. (Entries 8, Table 10.6).** Colorless Oil, 82%, Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.52 (d, 3H, *J* = 6.2 Hz, CH_3), 3.15 (m, 1H, CH), 3.84 (d, 1H, *J* = 14.3 Hz, CH_2), 4.02 (d, 1H, *J* = 14.4 Hz, CH_2), 4.21 (d, 1H, *J* = 7.8 Hz, CH), 4.57 (dq, 1H, *J* = 6.1, 12.2 Hz, CH), 7.24–7.58 (m, 10H, Ar), 9.81 (d, 1H, *J* = 2.4 Hz, CHO).
^{13}C-NMR (101 MHz, $CDCl_3$) δ 21.21, 59.5, 71.1, 71.5, 73.4, 98.5, 127.1, 127.5, 128.2, 128.3, 128.6, 129.0, 137.3, 138.4.
HRMS (ESI): m/z: calcd for $[C_{18}H_{20}NO_2]^+$ 282.1489, found 282.1488.

***Endo*-2-Benzyl-4-formyl-3-(4-chloro)phenyl-5-methylisoxazolidine. (Entries 9, Table 10.6).** Colorless Oil, 84%, Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.50 (d, 3H, *J* = 6.0 Hz, CH_3), 3.06 (ddd, 1H, *J* = 7.4, 5.5, 2.2 Hz, CH), 3.84 (d, 1H, *J* = 14.3 Hz, CH_2), 3.97 (d, 1H, *J* = 14.0 Hz, CH_2), 4.16 (d, 1H, *J* = 7.7 Hz, CH), 4.55 (m, 1H, CH), 7.24–7.38 (m, 9H, Ar), 9.79 (d, *J* = 2.2 Hz, 1H, CHO),
^{13}C-NMR (75 MHz, $CDCl_3$) δ 198.6, 137.5, 137.2, 134.1, 129.8, 129.6, 129.4, 129.1, 128.8, 128.6, 127.6, 21.3.
HRMS (ESI): m/z: calcd for $[C_{18}H_{19}ClNO_2]^+$ 316.1099, found 316.1095.

***Endo*-2-Benzyl-4-formyl-3-(4-methoxy)phenyl-5-methylisoxazolidine. (Entries 10, Table 10.6).** Colorless Oil, 85%, Yield.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.50 (d, 3H, *J* = 6.3 Hz, CH_3), 3.08 (ddd, 1H, *J* = 8.0, 5.5, 2.5 Hz, CH), 3.76 (d, 1H, *J* = 14.6 Hz, CH_2), 3.80 (s, 3H, OCH_3), 3.99 (d, 1H, *J* = 14.3 Hz, CH_2), 4.06 (d, 1H, *J* = 8.2 Hz, CH), 4.52 (m, 1H, CH), 6.87-6.91 (m, 2H, Ar), 7.23–7.38 (m, 7H, Ar), 9.76 (d, *J* = 2.5 Hz, 1H, CHO).
^{13}C-NMR (75 MHz, $CDCl_3$) δ 21.5, 55.6, 59.5, 71.2, 71.8, 73.6, 114.6, 128.5, 128.7, 129.1, 130.1, 137.4, 137.7, 159.8, 199.1.
HRMS (ESI): m/z: calcd for $[C_{19}H_{22}NO_3]^+$ 312.1594, found 312.1596.

***Endo*-4-formyl-3-(4-chloro)phenyl-2-methylisoxazolidine. (Entries 11, Table 10.6).** Colorless Oil, 83%, Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.58 (s, 3H, CH_3), 3.45 (m, 1H, CH), 3.76 (d, *J* = 14.4 Hz, 1H, CH_2), 3.99 (d, *J* = 14.3 Hz, 1H, CH_2), 4.07 (d, 1H, *J* = 7.0 Hz, CH), 7.25-7.33 (m, 4H, Ar), 9.74 (d, 1H, *J* = 2.5 Hz, CHO).
^{13}C-NMR (101 MHz, $CDCl_3$) δ 21.9, 43.6, 66.3, 73.5, 127.8, 128.5, 129.1, 137.8, 198.6.
HRMS (ESI): m/z: calcd for $[C_{11}H_{13}ClNO_2]^+$ 226.0629, found 226.00627.

***Endo*-2-Benzyl-4-formyl-3-phenylisoxazolidine. (Entries 12, Table 10.6).** Colorless Oil, 80%, Yield.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.44 (m, 1H, CH), 3.78 (d, *J* = 14.2 Hz, 1H, CH_2), 3.99 (d, *J* = 14.2 Hz, 1H, CH_2), 4.07 (d, 1H, *J* = 7.1 Hz, CH), 4.27–4.30 (m, 2H, CH_2), 7.27–7.51 (m, 10H, Ar), 9.80 (d, *J* = 2.1 Hz, 1H, CHO).
^{13}C-NMR (101 MHz, $CDCl_3$) δ 59.6, 64.3, 65.8, 70.6, 127.3, 127.8, 128.2, 128.3, 128.6, 128.9, 137.1, 138.1, 198.4.
HRMS (ESI): m/z: calcd for $[C_{17}H_{18}NO_2]^+$ 268.1332, found 268.1335.

***Endo*-2-Benzyl-4-formyl-3-(4-Methyl)phenylisoxazolidine. (Entries 13, Table 10.6).** Colorless Oil, 82%, Yield.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H, CH_3), 3.38–3.46 (m, 1H, CH), 3.75 (d, 1H, *J* = 14.0 Hz, CH_2), 3.97–4.02 (m, 2H, CH and CH_2), 4.24–4.28 (m, 2H, CH_2), 7.19–7.47 (m, 7H, Ar), 9.77 (d, *J* = 2.2 Hz, 1H, CHO).

^{13}C-NMR (75 MHz, $CDCl_3$) δ 21.6, 59.9, 64.6, 66.2, 70.9, 127.5, 128.0, 128.5, 128.9, 129.9, 135.0, 137.5, 138.4, 199.1.

HRMS (ESI): m/z: calcd for $[C_{18}H_{20}NO_2]^+$ 282.1489, found 282.1492.

***Endo*-2-Benzyl-4-formyl-3-(4-Methoxy)phenylisoxazolidine. (Entries 14, Table 10.6).** Colorless Oil, 84%, Yield.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.40 (m, 1H, CH), 3.73 (d, 1H, *J* = 14.2 Hz, CH_2), 3.82 (s, 3H, OCH_3), 3.96–4.00 (m, 2H, CH and CH_2), 4.22–4.28 (m, 2H, CH_2), 6.94 (d, 2H, *J* = 8.7 Hz, Ar), 7.26–7.42 (m, 7H, Ar), 9.77 (d, *J* = 2.1 Hz, 1H, CHO).

^{13}C-NMR (101 MHz, $CDCl_3$) δ 55.2, 59.4, 64.1, 65.8, 70.3, 114.3, 127.2, 128.2, 128.6, 129.0, 129.6, 137.3, 159.6, 198.9.

HRMS (ESI): m/z: calcd for $[C_{18}H_{20}NO_3]^+$ 298.1438, found 298.1440.

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APPENDIX A: PUBBLICATIONS

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CHEMCATCHEM Full Papers

Efficient Organocatalyst Supported on a Simple Ionic Liquid as a Recoverable System for the Asymmetric Diels–Alder Reaction in the Presence of Water

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The synthesis, characterization, and evaluation of a new highly efficient organocatalyst, namely, (5*S*)-2,2,3-trimethyl-5-thiobenzylmethyl-4-imidazolidinone hydrochloride, has been achieved. The catalyst possesses important structural features that should increase the catalytic efficiency and solubility in polar media. The application of the ionic-liquid-supported imidazolidinone catalyst in enantioselective Diels–Alder reactions was investigated. The Diels–Alder reactions of several dienes and dienophiles proceeded efficiently in the presence of the catalyst to provide the desired products in moderate to good yields and from good to excellent enantioselectivities. The conformation study confirms that in the transition state the *Re* face is shielded completely by the phenyl ring and an approach on the less hindered *Si* face is preferred. Particularly remarkable is the fact that the entire ionic liquid/HCl 0.01 M/catalyst system can be recovered and reused in up to six runs without an appreciable loss of catalytic activity.

Introduction

Asymmetric organocatalysis is an attractive branch of chemistry and, in particular, the development of new chiral catalysts integrated with nontraditional solvents to form highly recyclable systems is one of the most innovative fields of chemistry to access a wide variety of chiral compounds under eco-friendly conditions.[1] Asymmetric Diels–Alder reactions provide a simple and direct access to a variety of important chiral cyclic compounds, and the elaboration of several efficient and reliable enantioselective catalytic methods is realized using metal-free organocatalysts.[2] In this regard, we have developed a new chiral organocatalyst and an efficient, recyclable system that promotes Diels–Alder reactions without the use of metal complexes. The immobilization of the organocatalyst in the polar ionic liquid (IL)/H_2O system facilitates the separation and reuse of the mixture, which promotes economic and environmental benefits.

Initially, MacMillan[3] studied iminium ion organocatalysis in Diels–Alder reactions through a typical mechanism based on LUMO-lowering activation. MacMillan's imidazolidinone catalyst is highly efficient in terms of yield and selectivity; however, it still has some drawbacks such as high catalyst loading and no possibility of recycling. In this context, several attempts have been made to recycle the catalyst in recent years.[4] In a previous study[4a] we demonstrated that the use of methylpyridinium ILs, such as methylpyridinium triflate ([mPy][OTf]), as a solvent in the Diels–Alder reactions of α,β-unsaturated aldehydes with both cyclic and acyclic dienes lead to the corresponding *endo*-cycloadducts with excellent diastereoselectivities and enantioselectivities. Higher selectivity, better yields, and shorter reaction times were observed in comparison with reactions performed in classical organic solvents.

Herein, we report the new highly efficient organocatalyst (5*S*)-2,2,3-trimethyl-5-thiobenzylmethyl-4-imidazolidinone (**3**) in combination with ILs. The new organocatalyst **3** bears an S atom that increases the solubility and enhances the interactions with the IL; in addition, intramolecular interactions caused by the S atom ensure the rigidity of the intermediate iminium ion and provide a high enantiofacial selectivity. Additional advantages are the ease of the synthetic procedure, good recovery of products, and recyclability of the whole IL/organocatalyst system.

Results and Discussion

Compound **3** was prepared in excellent chemical yield from benzylcysteine methyl ester hydrochloride (**1**) by adding methylamine in aqueous solution followed by treatment with ace-

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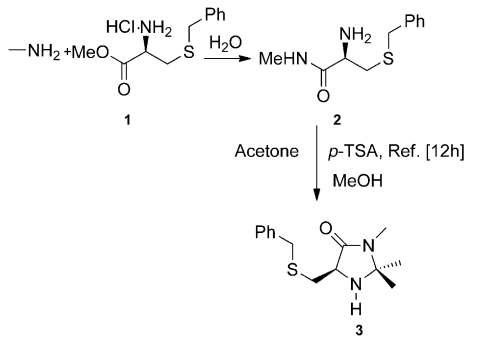
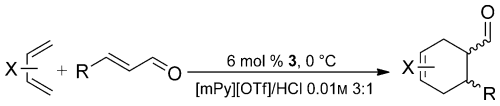


Scheme 1. Synthesis of **3**.

tone in methanol and *p*-toluenesulfonic acid (*p*-TSA; Scheme 1).

With this product in hand and with the experience of previous work, we developed a highly efficient IL/HCl 0.01 M/catalyst system to promote Diels–Alder reactions of a broad range of dienes and dienophiles under mild and recyclable conditions, which afforded the corresponding cycle in a high yield with good to high enantioselectivity (Scheme 2).



Scheme 2. General procedure for Diels–Alder reactions.

Initially, the catalytic ability of **3** in pyridinium-derived ILs and HCl 0.01 M was examined in a model Diels–Alder reaction between cyclohexadiene **4** and acrolein **5** (Table 1). The cycloaddition is performed conveniently at 0 °C using 6 mol% catalyst and is completed after 16 h with the formation of **6** with an excellent *endo/exo* ratio of 98:2% and an enantiomeric ratio (*er*) of 95:5%. The catalytic cycle is illustrated in Scheme 3.

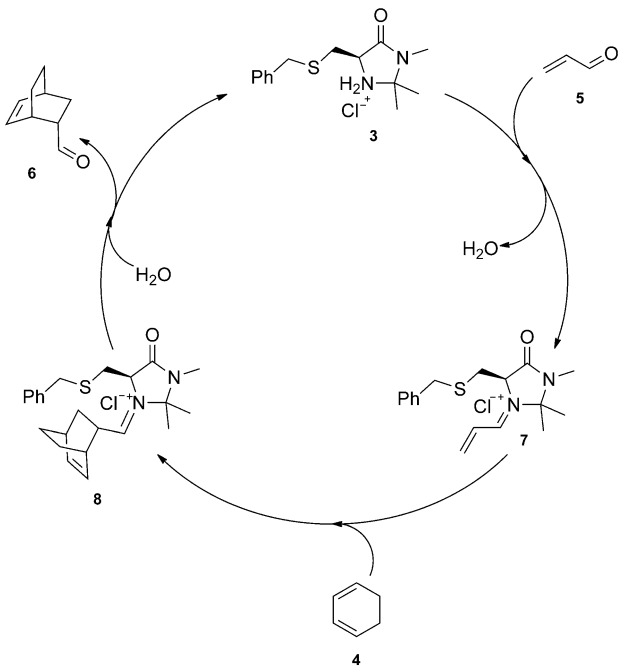
In agreement with MacMillan's model, it is expected that the *E* isomer will be formed preferentially, which prevents interactions between the olefin and the methyl substituent in the geminal position. Under these circumstances, the benzyl group on the catalyst framework acts as a shield on the *Re* face of the dienophile and promotes the approach of the diene by the less hindered *Si* face.

To explore the scope of the reaction, we applied the optimized reaction conditions to various substrates. The Diels–Alder reaction that involves α,β-unsaturated aldehydes and various dienes proceeded efficiently in the system [mPy][OTf]/HCl 0.01 M/catalyst to lead to the cycloadduct in good yields with good to excellent enantioselectivities (Table 2).

Table 1. Screening of reaction time and catalyst loading.

Entry	*t* [h]	Catalyst [mol%]	Yield[a] [%]	*endo/exo*[b]	*endo er*[b]
1	10	3	70	98:2	92:8
2	10	6	75	98:2	95:5
3	10	10	80	98:2	95:5
4	16	6	98	98:2	95:5

[a] Isolated yield. [b] Determined by GC analysis.



Scheme 3. Catalytic cycle for the cycloaddition process.

With the success of the reactions described above, we studied the recyclability of the IL/HCl 0.01 M/catalyst polar phase used in the Diels–Alder reaction. The recovery and recycling of the entire catalytic system can be exemplified as shown below. After the reaction, the polar phase was extracted three times by adding Et_2O (5 mL per time) directly into the separatory funnel; the extracted crude was purified by routine silica gel column chromatography, and catalyst **3** remained dissolved in the IL/water mixture.

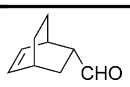
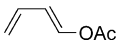
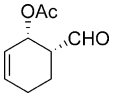
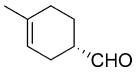
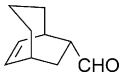
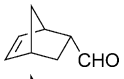
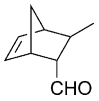
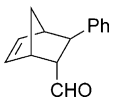
Therefore, IL-supported **3** can be reused directly in further reactions. As shown in the histogram, only a slight decrease in catalytic activity was observed if IL-supported **3** was recovered and reused in further reactions (Figure 1).

Theoretical calculations

The asymmetric induction observed in all cases that involve catalyst **3** is consistent with the conformation of the iminium ion **7**.

The conformational preferences of the iminium intermediate were studied in detail. We considered the three dihedral angles α, β, and γ illustrated in Figure 2.[5] For each dihedral angle, three staggered conformations were studied, and both the *E* and *Z* configurations of the iminium moiety were involved. Thus, a total of 54 conformers were considered of which 10 were eliminated because they led to a collision of atoms. The resulting 44 conformers were optimized fully at the CPCM = H_2O/M06-2X/6-31G(d) level.[6] A total of 42 energy minima that have different dihedral angles were obtained. In

Table 2. [mPy][OTf]-mediated Diels–Alder reaction of various dienes and dienophiles catalyzed by **3**.

Entry	Diene	R	Product (**6a–g**)	Yield [%]	*endo/exo*	*endo er*
1		H		98	98:2	95:5
2		H		93	94:6	97:3
3		H		97	–	96:4
4		H		90	95:5	99:1
5		H		95	86:14	94:6
6		Me		85	75:25	95:5
7		Ph		90	82:18	83:17

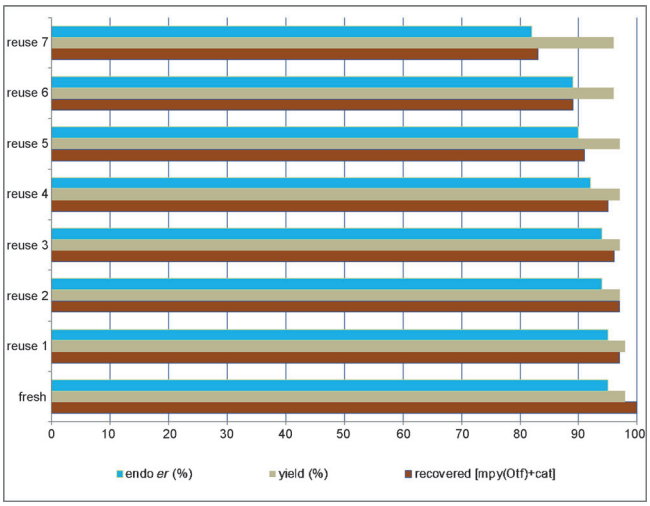


Figure 1. Histogram of recovery and reuse of the IL/HCl 0.01 M/catalyst system.

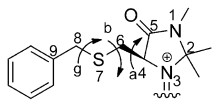


Figure 2. Dihedral angles of **7**.

general, *Z* isomers are less stable than the corresponding *E* conformers in agreement with previous results reported by Houk et al.[6] that demonstrated a high steric hindrance between the two methyl groups at C2 of the imidazolidinone ring and the substituent attached to N1. The seven energy minima below a difference of 2.0 kcal mol^{-1} with respect to the global minimum that accounts for 91.6% of all the conformations are listed in Table 3.[7] As a result of the large number of conformations studied, calculations that involve higher levels of theory were not performed for all of them. Full optimizations at the CPCM = H_2O/M06-2X/6-311+G (d,p) level of theory were performed for the above-mentioned seven energy minima. The relative energy values are also listed in Table 3.

No substantial changes were observed between calculations at the 2ξ and 3ξ levels, and the observed dihedral angles are almost identical in all cases. Calculations at the highest level provided four conformations within a difference of 1.0 kcal mol^{-1} (Figure 3). Of these conformations, three ((*E*)-c06, (*E*)-c07, and (*E*)-c02), which include the global minimum (*E*)-c06, present a stabilizing C–H···S interaction between one of the methyl groups at position C2 of the imidazolidinone ring and the methylthio group at position C5. The S–H distance was in the range of 2.91–2.95 Å. The differences between these conformers correspond to the rotation of dihedral angles β and γ, which corresponds to different orientations of the S–benzyl group. C–H···S interactions have been reported experimentally[8] and studied theoretically.[9]

In particular, theoretical calculations at the MP2/6-311+G(d,p) level[10] found an optimum S–HC(sp^3) distance of 3.095 Å. Notably, the global minimum of iminium ion **7** ((*E*)-c06) places the phenyl ring directly above the double bond to shield the *Re* face of the dienophile and promote approach of the diene by the less hindered *Si* face.

Therefore, the S atom plays a major role in the high enantioselectivity observed experimentally by fixing a partial conformation (through the above-mentioned C–H···S interaction)

Table 3. Dihedral angles [°] and relative energies [kcal mol^{-1}] of the more stable conformers of iminium intermediate **7**.

Conformer	ΔG (2ξ level)[a] α	β	γ	ΔΔG	ΔG (3ξ level)[b] α	β	γ	ΔΔG
E-c06	−55.8	−97.1	−53.5	1.6	−54.7	−97.6	−55.4	0.0
E-c07	−66.8	79.0	164.0	1.3	−67.0	78.0	163.9	0.2
E-c21	71.5	119.5	−56.3	0.4	71.6	115.9	−56.6	0.5
E-c02	−58.3	93.8	60.7	0.0	−67.3	89.4	63.1	0.6
E-c24	64.4	−84.2	−32.9	1.5	65.5	−85.0	−33.4	1.4
E-c26	64.8	49.5	47.3	1.6	65.8	50.1	47.7	1.7
E-c04	−59.5	−93.8	−169.6	1.7	−59.5	−93.6	−168.9	1.6

[a] CPCM = H_2O/M06-2X/6-31G(d). [b] CPCM = H_2O/M06-2X/6-311+G(d,p).

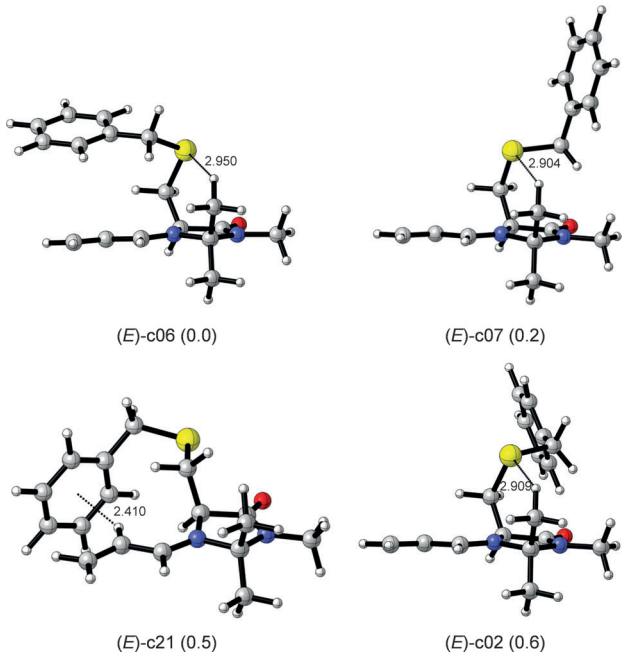


Figure 3. Most stable conformers for **7**. (Structures optimized at the CPCM = H_2O/M06-2X/6-311+G(d,p) level; relative energies given in kcal mol^{-1}).

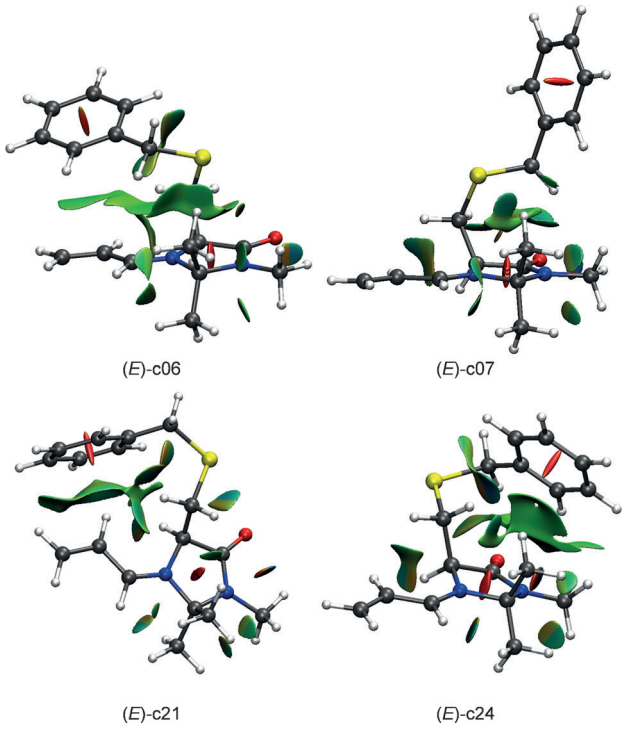


Figure 5. NCI analysis showing NCI isosurfaces for conformers (*E*)-c06, (*E*)-c07, (*E*)-c21, and (*E*)-c24. Color-coded according to $(\lambda_2)\rho$, the eigenvalue of the density Hessian, to indicate attraction (green–blue) or repulsion (red). The cutoff ($\rho = 0.2$ a.u.) was chosen to isolate purely noncovalent interactions.

that facilitates the orientation of the phenyl ring to hinder one of the diasterofaces of the iminium intermediate **7** completely.

Conformer (*E*)-c21 presents a C–H···π interaction between the phenyl ring and a vinylic proton (Figure 3). This sort of stabilizing interaction has been reported in the case of MacMillan's catalyst, for which the global minimum exhibits a stabilizing C–H···π interaction between the methyl group at C2 and the phenyl ring of the benzyl group at C5.[1,6a] The same interaction has also been found for one of the more stable conformers of iminium ion **7** ((*E*)-c24). These interactions are illustrated in Figure 4 for both the iminium ion of MacMillan's catalyst (2.42 Å) and conformer (*E*)-c24 of the iminium ion of **3** (2.84 Å). The NCI (non-covalent interaction) analyses performed for conformers (*E*)-c06, (*E*)-c07, (*E*)-c21, and (*E*)-c24, which show three different types of interactions, are illustrated in Figure 5.

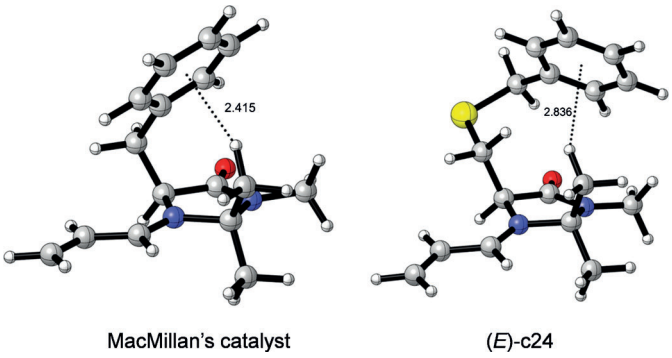


Figure 4. C–H···π interactions for optimized structures (CPCM = H_2O/M06-2X/6-311+G(d,p)) of the most stable conformer of the iminium ion of MacMillan's catalyst and conformer (*E*)-c24 of the iminium ion of catalyst **3**.

To evidence the stabilizing interactions, the M06-2X/6-311+G(d,p) wave functions were used for further NCI analysis, a semiquantitative visualization index based on the electron density and its derivatives that enables the identification of noncovalent interactions.[11] Visualization of favorable and unfavorable interactions was performed with NCI Plot Program[12] and VMD software.[13]

Both (*E*)-c06 and (*E*)-c07 conformers show a reduced density gradient (RDG) green surface that confirms the C–H···S noncovalent stabilizing interactions. In addition, the global minimum (*E*)-c06 shows a green surface that corresponds to a stabilizing interaction between the phenyl ring and the double bond. This interaction, difficult to identify by a mere visual inspection of the model, can be identified as a π–π interaction and it stabilizes the (*E*)-c06 conformer.

Conformers (*E*)-c21 and (*E*)-c24 show a typical RDG surface that corresponds to a C–H···π interaction in which a green conical form with a vertex that points to the center of the aromatic ring can be appreciated. As stated above, the C–H···π interaction takes place between the aromatic ring and the vinylic hydrogen atom for conformer (*E*)-c21 and the methyl group at C2 for conformer (*E*)-c24.

We used the most stable conformation of the catalyst (*E*)-c06 to locate the preferred transition structures for *endo* and *exo* approaches of cyclopentadiene by both *Re* and *Si* faces. The geometrical features and relative energies [kcal mol^{-1}] of the four transition structures are illustrated in Figure 6.

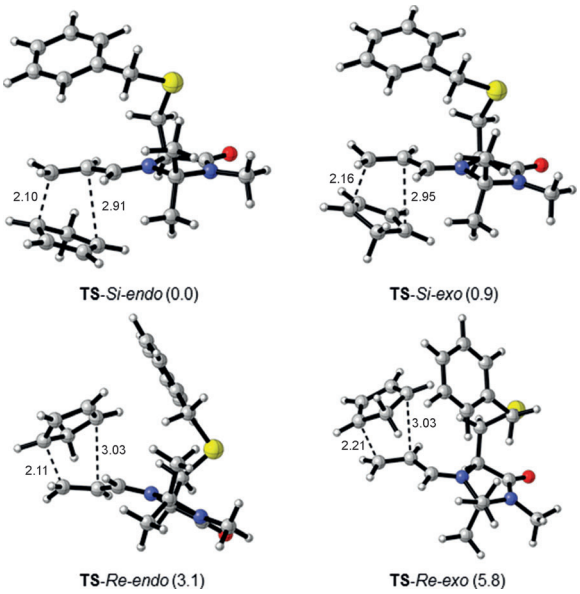


Figure 6. Transition structures for the reaction between (*E*)-c06 and cyclopentadiene. (Structures optimized at the CPCM = H2O/M06-2X/6-311+G(d,p) level; relative energies given in kcal mol^{-1}).

The energy difference between TS-*Si-endo* and TS-*Si-exo* is 0.84 kcal mol^{-1}, which justifies the formation of *endo/exo* mixtures in the case of the reaction with cyclopentadiene. A difference of more than 3 kcal mol^{-1} between the *Re* and *Si* approaches in the case of *endo* adducts (more than 5.0 kcal mol^{-1} for *exo* adducts) justifies the excellent enantioselectivity observed. The bond-formation lengths are in agreement with a highly asynchronous reaction as expected for a reaction performed in a highly polar medium.

Conclusions

We have described and characterized a new chiral organocatalyst as an efficient, economical, and practical method for enantioselective Diels–Alder reactions by using a reaction medium composed of an ionic liquid (IL), HCl 0.01 M, and organocatalyst, which leads to the desired products in good yields with good to excellent enantioselectivities. Theoretical calculations confirmed the presence of C–H···π interactions similar to those found for MacMillan's catalyst. However, important C–H···S stabilizing interactions are also evidenced by both DFT calculations and NCI analysis, which justifies the high stability of conformer (*E*)-c06. In this conformer the phenyl ring is placed over the unsaturated system to render the *Si* face as the less hindered one for the attack of the dienophile. The calculated relative energies between transition structures are in good agreement with both the formation of mixtures of *endo/exo* adducts in some cases and the excellent enantioselectivity observed experimentally. Notably, the complete polar system can be recovered readily and recycled for further transformations at least six times with the retention of its catalysis and enantioselectivity. In addition, only 6 mol% of catalyst and a slight excess of donor aldehyde (1.5 equiv.) are required, and no organic solvent is necessary except during the final purification step. These remarkable advantages make this approach effective and practical in the synthesis of fine chemicals.

Experimental Section

General methods

Commercial starting materials were used without further purification. Solvents were distilled before use. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz respectively in $CDCl_3$ using tetramethylsilane (TMS) as internal standard (Bruker ACP 300 MHz). Chemical shifts are given in ppm and coupling constants in Hz. The diastereoisomeric ratio has been determined by a GC–MS instrument (Shimadzu QP2010) equipped with a Quadrex 007–5 MS column, 30 m, coated with a 0.25 mm film, helium as a carrier gas, injector temperature of 270 °C, and oven temperature program: 50 °C hold 3 min, ramp 14 °C min^{-1} to 280 °C and held for 1 min. The enantiomeric ratio has been evaluated by using a Focus GC–FID Thermo Scientific instrument (FID = flame ionization detector) equipped with a chiral column Megadex DET Beta, 30 m, coated with a 0.25 mm film, helium as a carrier gas, injector temperature 250 °C, and oven temperature program: 50 °C, ramp 2 °C min^{-1} to 200 °C and hold for 1 min. ESI-HRMS spectra were recorded by using a Thermo Scientific Q Exactive (Thermo Fisher, Milan, Italy) mass spectrometer using ESI with positive polarities at 70 000 resolving power (defined as full width at half maximum (FWHM) *m/z* 200), IT (injection time) = 30 ms, and AGC (automatic gain control) target = 1 000 000, for selected-ion monitoring (SIM) by infusion at flow of 10 μL min^{-1}. Source conditions were: spray voltage 3.5 kV, sheath gas: 15 arbitrary units, auxiliary gas: 0 arbitrary units, sweep gas: 0 arbitrary units. Heater temperature: 30 °C, cap temperature: 320 °C. S-Lens RF level: 50. The instrument was calibrated using the recommended Thermo Scientific Calibration solution at the beginning of the analysis. Detection of the target compound was based on the theoretical exact mass. Data were evaluated by using Xcalibur 2.2.SP1 (Thermo Fisher Scientific, Bremen, Germany): the mass accuracy, calculated directly from Xcalibur, is defined with the formula Δ [ppm] = [(theoretical mass − measured mass)/theoretical mass] × 1 000 000. Stock solution at a concentration of 1 mg mL^{-1} were prepared in UHPLC–MS-grade methanol. Before analysis, the solution was diluted 1:1000 v/v in a vial to obtain a concentration of 1 μg mL^{-1}

Synthesis of methylpyridinium triflate ([mPy][OTf])

The IL [mPy][OTf] was prepared by halide-free direct synthesis by placing dry pyridine (0.29 mol) in a two-necked flask to which methyltrifluoromethanesulfonate (0.30 mol) was added dropwise. The mixture was reacted at 80 °C for 12 h. The crude product was washed with dichloromethane and dried under vacuum to yield 68.04 g (96%) of pure IL. The synthesis of [mPy][OTf] was confirmed by comparison of the spectra with literature reports.[14]

Synthesis of catalyst 3

To an aqueous solution of $MeNH_2$ (2 M, 60 mL) was added (*S*)-benzyl-L-cysteine methyl ester hydrochloride (10 g, 40 mmol), and the solution was stirred at RT for 10 h. The crude solution was treated with a saturated aqueous solution of $NaHCO_3$ (50 mL), and the free amine was extracted with $CHCl_3$ (40 mL × 3), dried (Na_2SO_4), filtered, and concentrated. To this residue was added

MeOH (100 mL), acetone (16.6 mL, 220 mmol), and *p*-TSA (93.2 mg, 0.48 mmol). The solution was heated to reflux for 12 h, cooled to RT, and then concentrated in vacuo. The residue was washed with hexane (30 mL×3), and yielded 8.36 g (80% overall) of (5*S*)-2,2,3-trimethyl-5-thiobenzylmethyl-4-imidazolidinone (**3**) as a yellow oil. ^{1}H NMR (300 MHz, [D]$CHCl_3$, 25 °C, TMS): δ = 1.3 (s, 3H; CH_3), 1.4 (s, 3H; CH_3), 2.2 (bs, 1H; N–H), 2.8 (s, 3H; N–CH_3), 2.8 (dd, 1H; ^3J(H,H) = 4.2, ^2J(H,H) = 14.1 Hz; HCH_2S), 2.9 (dd, 1H, ^3J(H,H) = 5.9, ^2J(H,H) = 14.1 Hz; HCH_2S), 3.6 (dd, 1H, ^3J(H,H) = 4.2, ^3J(H,H) = 5.9 Hz; 5-H), 3.7 (d, 1H, ^2J(H,H) = 13.4 Hz; HCH_2Bn), 3.8 (d, 1H, ^2J(H,H) = 13.4 Hz, HCH_2Bn), 7.2–7.4 ppm (m, 5H, Ar); ^{13}C NMR (75 MHz, [D]$CHCl_3$, 25 °C, TMS): δ = 25.2, 25.3, 27.0, 33.1, 36.8, 57.8, 75.6, 127.0, 128.5, 128.9, 138.2, 172.4 ppm; HRMS (ESI): *m/z*: calcd for $[C_{14}H_{20}ON_2S+H]^+$: 265.1369; found 265.1368.

General procedure for Diels–Alder reactions (6a–g)

In a representative procedure catalyst **3** (0.036 mmol, 6 mol%) was dissolved in a mixture of [mPy][OTf]/HCl 0.01 M 3:1 v/v. The dienophile (0.6 mmol) and the diene (0.4 mmol) were added sequentially. After the appropriate time, the reaction mixture was extracted with ether (2 mL×3). The ether layer was dried by Na_2SO_4, filtered, evaporated under reduced pressure, and purified by flash chromatography. ^{1}H and ^{13}C NMR data were consistent with values reported previously.[3,15]

Acknowledgements

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Keywords: conformation analysis · enantioselectivity · ionic liquids · organocatalysis · supported catalysts

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REVIEW

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Chemical approaches to inhibitors of isoprenoid biosynthesis: targeting farnesyl and geranylgeranyl pyrophosphate synthases

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Post-translational lipid modifications farnesylation and geranylgeranylation of proteins (protein prenylation) have been identified to mediate critical events in cancer, cardiovascular disorders, malaria and bone disorders like osteoporosis. To date eight compounds are commercialized for the treatment of bone disorders, and there are considerable efforts to develop selective small molecules that inhibit protein prenylation. This review summarizes the approaches currently employed to synthesize new inhibitors of isoprenoid biosynthesis. Bisphosphonates are mainly prepared through reaction of carboxylic acids with phosphorus reagents, Michael addition to tetraethylvinylidenebisphosphonate and alkylation of tetralkylmethyl bisphosphonate. Approaches to non-bisphosphonate derivatives include a variety of methodologies depending on the structure of the target compound.

Introduction

Isoprenoids (also known as terpenoids) are considered the most ancient and diverse class of natural products.[1] They have been found in sediments from 2.5 billion years ago[2] and more than 40.000 representatives have been found in all kingdoms of life.[3] They participate in a great variety of basic biological functions in plants[4,5] (*e.g.* growth regulation, pigments) and mammals[6–8] (*e.g.* steroids metabolism, cellular signaling, antioxidants), and have been used in the food, pharmaceutical, chemical and biofuel industries.[9,10]

Isoprenoids are biosynthesized ubiquitously in eubacteria, archaebacteria and eukaryotes by the consecutive condensation of the five-carbon monomer isopentenyl diphosphate (IPP) to its isomer dimethylallyl pyrophosphate (DMAPP) (Fig. 1).[11,12] Whereas

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Loredana Maiuolo (b. Cosenza, Italy) graduated in Chemistry at the University of Calabria (1995) where received her Ph.D (1999). She carried out two post-doctoral positions in Organic Chemistry at the University of Calabria (1999–2004). In 2005 she won a permanent position as Researcher in Organic Chemistry at University of Calabria. In 2014 she spent six months at University of Zaragoza as visiting professor. Her main fields of research include: Asymmetric Synthesis of Biologically Active Molecules by 1,3-Dipolar Cycloaddition; Synthesis of Organic Compounds in non-conventional Solvents; Lewis Acids Catalysts and Organocatalysis; Synthesis of Deuterated Compounds as Standard in Food Chemistry.

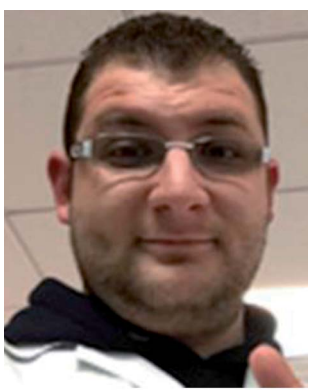
in mammals and yeast IPP is synthesized in the cytosol and endoplasmic reticulum from acetyl-CoA through mevalonic acid (mevalonate pathway), in higher plants and other microorganisms IPP is synthesized in the plastids by the condensation of pyruvate with glyceraldehyde-3-phosphate through 1-deoxyxylulose-5-phosphate (DXP) – also called methylerythritol (MEP pathway). Two consecutive condensations of IPP and DMAPP catalyzed by farnesyl pyrophosphate synthase (FPPS) provide geranyl diphosphate (GPP) and farnesyl diphosphate (FPP). The former is the precursor of monoterpenes and the latter of sesquiterpenes, triterpenes and sterols (*via* squalene biosynthesis) as well as other important secondary metabolites like ubiquinones and dolichols. An additional condensation of FPP with IPP, catalyzed by the enzyme geranylgeranyl pyrophosphate synthase (GGPPS), furnishes geranylgeranyl pyrophosphate (GGPP) precursor of di- and tetraterpenes and carotenoids (Fig. 1).

Given the importance of the metabolites accessible from isoprenoid biosynthesis, the enzymes involved in the process are excellent drug targets.[13,14] The non-mevalonate pathway (MEP pathway) is not present in mammalian systems; consequently the enzymes involved in MEP pathway are attractive drug targets[15] for the development of herbicides, antimicrobial drugs and fighting against pathogenic microorganisms like *P. falciparum* (malaria),[16,17] *T. cruzi* (Chagas disease)[18,19] and *M. tuberculosis*.[20] Enzymes in the MEP pathway, IspG and IspH, are anti-infective drug targets[21] and HMG-CoA reductase involved in the synthesis of IPP is the primary target of hypocholesterolemic drug therapy.[22]

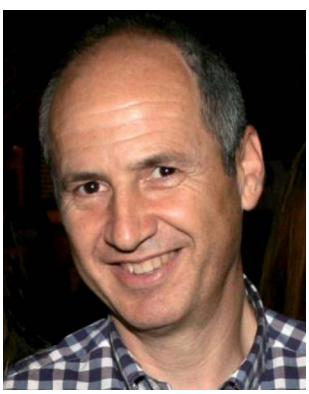
Protein prenylation, in particular farnesylation and geranylgeranylation, is one of the essential post-translational protein modification in the eukaryote.[23] Therefore inhibition and/or modulation of the enzymes FPPS and GGPPS will affect not only to essential secondary metabolites derived from isoprenoid biosynthesis[24] but also to the functionality of prenylated proteins.[25] FPPS has been identified as a target for a series of drugs acting as anticancer, antimicrobial and antiparasitic

Ignacio Delso (b. Bilbao, Spain) coursed Chemistry at the University of Zaragoza (2003) and in 2009 he won his Ph.D. In 2004 he spent six months at the University of Florence (Prof. Andrea Goti). In 2008 he carried out a second pre-doctoral stay (three months) at the IGQO, CSIC in Madrid, Spain (Dr Agatha Bastida). In 2008 he won a permanent position as Specialized Research Technician of the CSIC and since then he is the responsible of the NMR Service at CEQMA. His main research interest include: Asymmetric Synthesis. Chemical Biology. NMR and Computational Biochemistry.

Antonio De Nino (b. Capistrano (VV), Italy) received the degree in Chemistry at University of Calabria (1989). From 1993 to 2003, he was researcher in Organic Chemistry at the Chemistry Department of University of Calabria. Since 2003 he is Associate Professor of Organic Chemistry at the same University. His research activities are mainly devoted to study: Asymmetric Synthesis of Biologically Active Molecules by 1,3-Dipolar Cycloaddition; Synthesis of Organic Compounds in non-conventional Solvents; Lewis Acids Catalysts and Organocatalysis; Determination and Characterization of Microcomponents in Food and Natural Products; Reactivity of Enaminones Dianions with Electrophiles.



Vincenzo Algieri (b. Acri (CS), Italy) graduated in Chemistry (2014) at the University of Calabria. Currently, he is PhD student in the University of Calabria under the supervision of Prof. Antonio De Nino. He spent a year of his doctorate (2016) at University of Zaragoza in research group of prof. Pedro Merino. His main research interests are: Synthesis and Characterization of Heterocyclic Compounds at Potential Biological Activity, Enantioselective Organocatalysis and Solvent Free 1,3-Dipolar Cycloadditions.



Tomas Tejero (b. Zaragoza, Spain) coursed Chemistry at the University of Zaragoza (1980) where he received his Ph.D. (1985). In 1984 he became Assistant Professor and in 1985 he spent a year in the University Pierre et Marie Curie (Paris) under the supervision of Prof. J. Normant. In 1986 he returned to Zaragoza and in 1987 he was promoted to Senior Lecturer. In 2012 he was appointed Full Professor in the Department of Organic Chemistry at the University of Zaragoza. He is particularly interested in enantioselective processes and in new spectroscopic techniques to the field of the Asymmetric Synthesis.

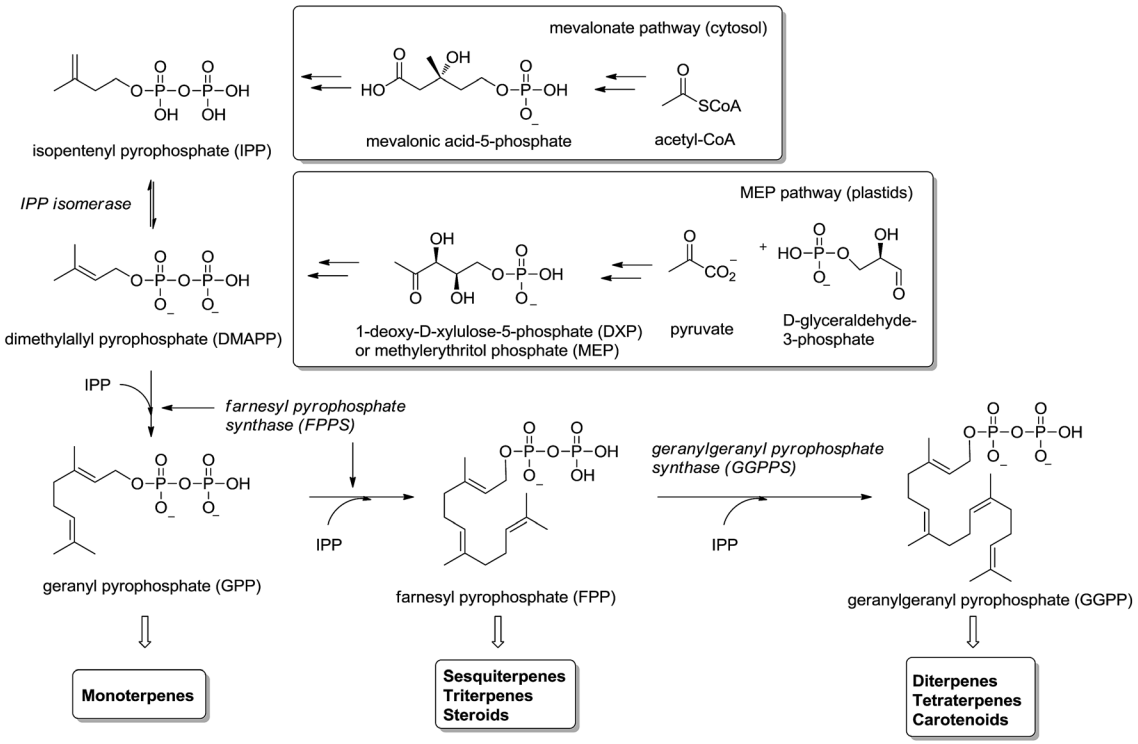


Fig. 1 Biosynthesis of isoprenoids. FPPS and GGPPS are essential enzymes.

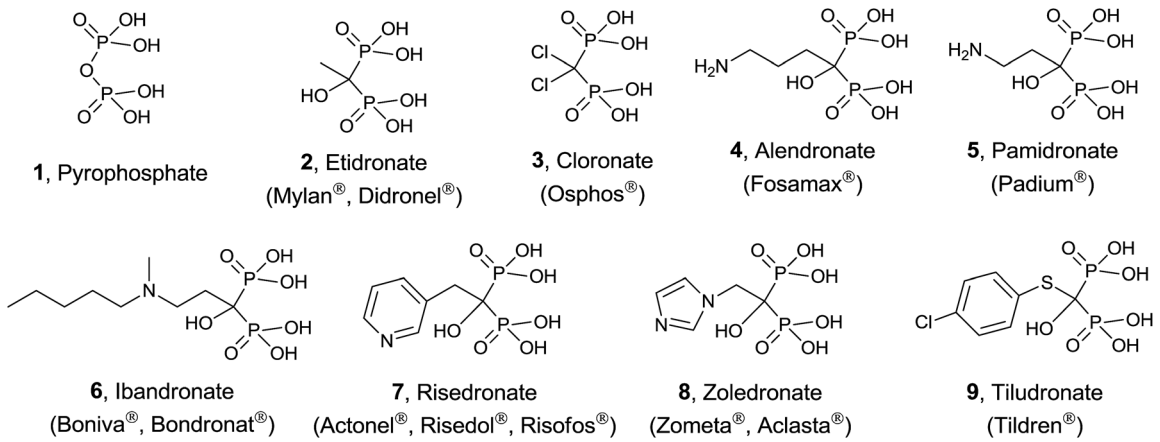


Fig. 2 FDA-approved bisphosphonates clinically used for the treatment of bone disorders. Compounds are presented in their protonated forms.

agents.[26] In particular, human FPPS is a drug target for cancer,[27,28] osteoporosis and related diseases,[29–31] Paget's disease,[32,33] metastatic diseases,[34] and cardiovascular disorders.[35] Inhibition of human GGPPS has been reported as a new route to bone antiresorption[36] and the same enzyme from *Plasmodium* has been identified as a target against malaria.[37] The multiple sequence alignment of FPPS and GGPPS found in different organisms[38,39] makes that the same potential inhibitor should be tested against both enzymes from diverse biological sources. The main group of inhibitors is constituted by bisphosphonates (BPs),[40] structural analogues of DMAPP considered chemically stable analogues of inorganic pyrophosphate. Bisphosphonates, known from more than 40 years[41] are being used clinically in the treatment of osteoporosis and malignant bone diseases (Fig. 2).[42]

Simple bisphosphonates correspond to analogues of pyrophosphate **1** with therapeutic properties in which the bridging oxygen atom has been replaced by a methylene group that can incorporate non-nitrogenated substituents. Typical examples are etidronate **2** and cloronate **3** and their mechanism of action consists of being incorporated to non-hydrolyzable analogues of ATP, inducing osteoclast apoptosis.[43]

On the other hand, nitrogen-containing bisphosphonates (*e.g.* alendronate **4**, pamidronate **5**, ibandronate **6**, risedronate **7**, zoledronate **8**) showed to be more than 10 000 times more active than non-nitrogenated derivatives. These analogues have

a diverse mechanism of action causing (i) disruption of normal function of essential signaling proteins[44] and (ii) accumulation of IPP which is incorporated into a toxic nucleotide metabolite.[45] More recently, a variety of non-nitrogenated bisphosphonates like tiludronate **9** and others bearing arylsulfonium and phosphonium groups showed cytotoxicity against cancer, inhibition of *Tp*FPPS and stimulation of T-cells in the human immune system revealing that the presence of a nitrogen atom is not strictly necessary. In addition to bisphosphonates, other inhibitors including quinoline and salicylic acid derivatives, have been reported. These non-bisphosphonate inhibitors bind to an allosteric site on FPPS identified by X-ray crystallography.[46]

The goal of this review is to highlight synthetic methodologies directed to the preparation of FPPS and GPPS inhibitors. Several reviews have been reported elsewhere on the design of inhibitors of isoprenoid synthase enzymes[13,14,47] and their mechanism of action.[45,48–51] The reader is directed to consult those reviews for details on therapeutic effects. Since the main focus of this survey is discussion on chemical approaches, references to biological activities of the inhibitors is only shortly reviewed here. No patents are considered in this review since they have been recently surveyed.[52,53] The review is organized on the basis of the structure of the inhibitor, *i.e.* bisphosphonates and non-bisphosphonates and then by the synthetic methodologies employed for their synthesis.

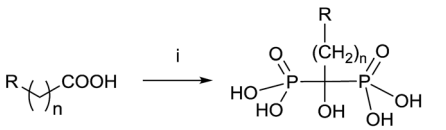
Bisphosphonates

Reaction of carboxylic acids with phosphorous reagents

The most general and attractive approach for preparing 1,1-bisphosphonates is the reaction of a carboxylic acid, easily accessible by conventional methods, with an inexpensive phosphorous reagent like phosphorous trichloride. Due to high therapeutic interest of 1,1-bisphosphonates, the first reports regarding their synthesis were published as patents and no clear experimental details were given, the reaction being difficult to scale up and lacking of reproducibility. Indeed, no mechanism of the reaction had been determined and a great variability was observed in reactants ratio, temperature, reaction time and work-up. In 1995, Kieczykowski and Jobson, from Merck and Co., Inc., reported a general procedure for reacting a carboxylic acid with phosphorous acid and phosphorous trichloride in the presence of methanesulfonic acid (Scheme 1).[54]

Since then, this method has been the most widely used for the synthesis of 1-hydroxy-1,1-bisphosphonates although in some particular case it has been reported contamination of the product with the methanesulfonate salt, that can be avoided by performing the reaction without solvent.[55] The procedure is amenable of being used with several substrates including those bearing an amino functionality.[36,56] For instance, alendronate **4** and pamidronate **5**, have been prepared by this route[57] and served as precursors of substituted analogues like compounds **13** (Scheme 2).[58,59] Similarly, conjugates **14** have been prepared by condensation of **5** with the corresponding carboxylic acid.[60] Other conjugates include incorporation to nucleosides[61] and oligonucleotides.[62] Pamidronate **5** prepared by this route has been further employed for the synthesis of ^{211}At-labeled amidobisphosphonates.[63]

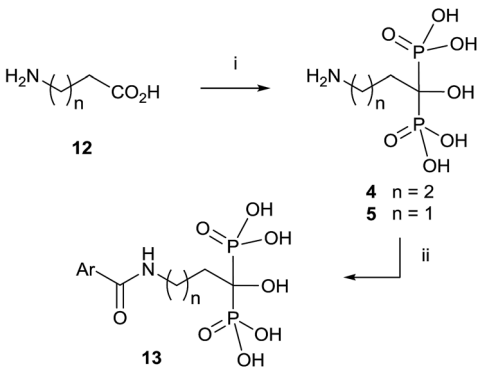
Alendronate **4** can also be obtained in a straightforward manner from pyrrolidone (Scheme 3).[64] Hydrolysis of **15** in



10 **11**

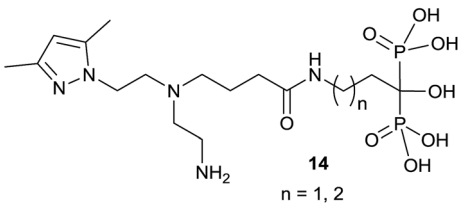
R	n	yield (%)	R	n	yield (%)
NH_2	2	57	3-imidazoyl	1	38
NH_2	3	89	3-pyridyl	1	26
NH_2	4	78	4-$H_2NC_6H_4$	3	30
NH_2	5	89			
Me	10	95			
Cl	1	31			

Scheme 1 Reagents and conditions: (i) H_3PO_3 (1 equiv.), $MeSO_3H$ (1 equiv.), PCl_3 (2 equiv.), 65 °C, 16–20 h; then H_2O, reflux; then pH = 4, 50% NaOH.



12 **13**

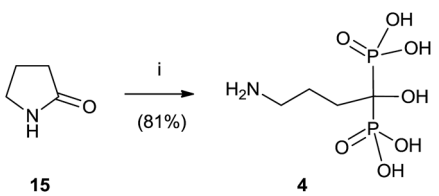
Ar: Ph, 2,3-diOMeC$_6$H$_3$, 3,4-CH$_2$O$_2$C$_6$H$_3$, 4-MeC$_6$H$_4$, 3-MeOC$_6$H$_4$, 2-MeC$_6$H$_4$, 4-MeOC$_6$H$_4$



14

n = 1, 2

Scheme 2 Reagents and conditions: (i) H_3PO_3, PCl_3. (ii) ArCOCl, NaOH.



15 **4**

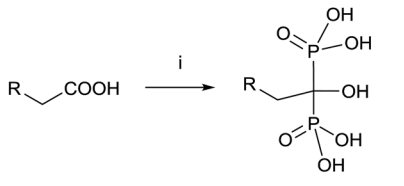
Scheme 3 Reagents and conditions: (i) H_3PO_3, PCl_3. (ii) ArCOCl, NaOH.

aqueous methanesulfonic acid followed by reaction with phosphorous trichloride and hydrolysis with water furnished compound **4**.

Good yields in the synthesis of heterocyclic (risedronate **7**, zoledronate **8**) and aminoalkyl bisphosphonates were obtained in just 20 min using microwaves and sulpholene as a solvent in the first stage of the reaction (Scheme 4).[65] A chiral analogue of risedronate **7** was prepared starting from 3-benzyloxybenzaldehyde **18** which was transformed into carboxylic acid **22**. Reaction of **22** with phosphorous acid and phosphorous chloride afforded analogue **23** (Scheme 5).[66] The complex formed from *Hs*FPPS and racemic **23** revealed that only *R*-isomer was present in the active site revealing a high enantiospecificity of the enzyme.

Oldfield and co-workers have demonstrated that the methodology originally reported by Kieczykowski and Jobson[54] allows preparing a huge number of different compounds, including aromatic, aliphatic, sulfur-containing and nitrogen-containing heterocyclic derivatives.[67–71] The exact protocol involved a hydrolysis, after the reaction of the carboxylic acid with phosphorous acid and phosphorous trichloride, and pH adjustment to 4.3 with a 50% NaOH solution, followed by recrystallization from water.[67]

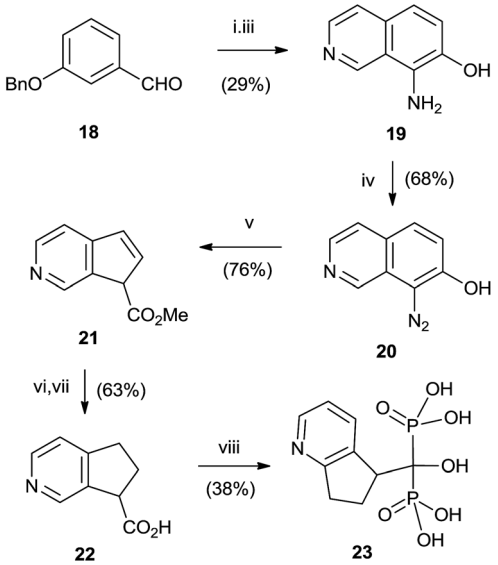
A modified procedure was reported by the same group for the synthesis of pyridinium-1-yl bisphosphonates **27**. The corresponding carboxylic acids **26** prepared through a cross-coupling reaction followed by *N*-alkylation with bromoacetic acid, were made to react with 5 equiv. of phosphorous acid and 5 equiv. of phosphorous trichloride in toluene without methanesulphonic acid. After treating the mixture with 6 N HCl and reflux for 1 h, addition of 2-propanol precipitated the 1,1-bisphosphonates which were further recrystallized from ethanol/water (Scheme 6).[72]



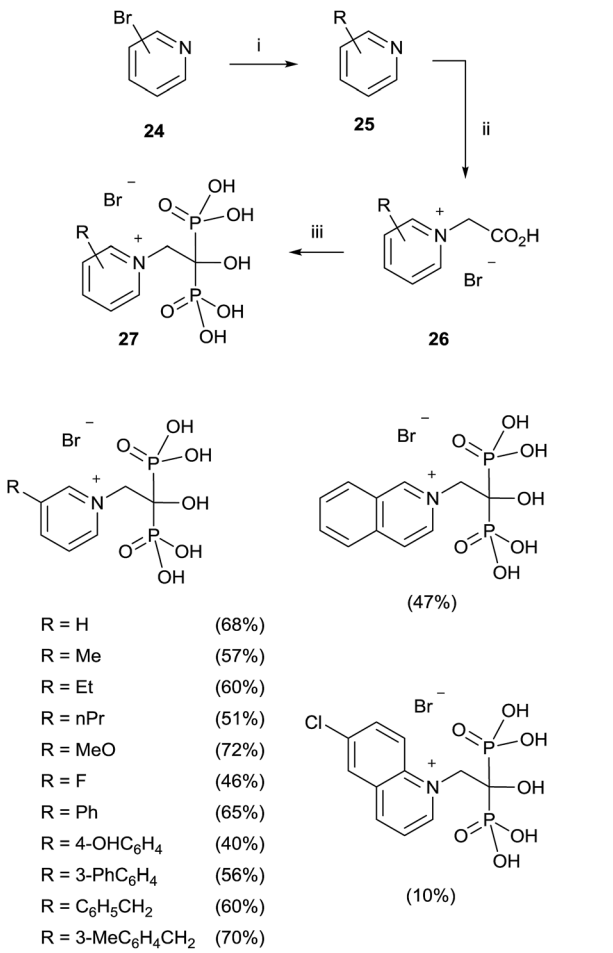
16 **4,5,7,8,17**

compound	R	yield (%)
8	3-imidazoyl	74
7	3-pyridyl	70
5	aminomethyl	64
4	aminoethyl	41
17	aminobutyl	53

Scheme 4 Reagents and conditions: (i) H_3PO_3 (3 equiv.), PCl_3 (4 equiv.), sulpholene, 65 °C, 3–7 min, MWI 200–400 W max.; then H_2O, MWI, 450–500 W max., 10 min, 150 °C.



Scheme 5 Reagents and conditions: (i) aminoacetaldehyde dimethylacetal, toluene, 6 h, 110 °C; then TFA, $BF_3{\cdot}Et_2O$, <10 °C, 4 days; then Et_2O, NH_4OH to pH 9. (ii) Sulfolane, nitronium tetrafluoroborate, rt, 6 h. (iii) 10% Pd/C, H_2, EtOH. (iv) MeOH·HCl, *t*-BuONO 0 °C to rt, 4 h. (v) $NaHCO_3$, MeOH, hv, 0 °C, 3 h. (vi) 10% Pd/C, MeOH, rt, 5 h. (vii) NaOH, 58 °C, 4 h. (viii) H_3PO_3, PCl_3, toluene, 110 °C, 4 h; then HCl, 100 °C, overnight.



R = H	(68%)
R = Me	(57%)
R = Et	(60%)
R = nPr	(51%)
R = MeO	(72%)
R = F	(46%)
R = Ph	(65%)
R = 4-OHC_6H_4	(40%)
R = 3-PhC_6H_4	(56%)
R = $C_6H_5CH_2$	(60%)
R = 3-$MeC_6H_4CH_2$	(70%)

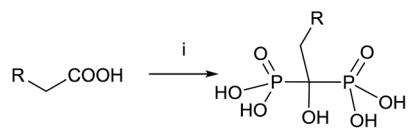
Scheme 6 Reagents and conditions: (i) R–$B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, H_2O, 10 h, reflux. (ii) $BrCH_2CO_2H$, pyridine, EtOAc, 2 days, rt. (iii) H_3PO_3 (5 equiv.), PCl_3 (5 equiv.), toluene, 80 °C, 5 h; then 6 N HCl, 1 h, reflux.

This protocol was also applied to the synthesis of aliphatic and aromatic derivatives with or without heteroatoms, demonstrating a great tolerance with respect to the chemical nature of the substrates.[73] Labelled pamidronic acid-$^{13}C_3$,^{15}N, alendronic acid-^{15}N, zoledronic acid-$^{15}N_2$ and risedronic acid-^{15}N were also synthesized by using those conditions.[74] Pyridinium fluorescently-labeled conjugates of risedronate **7** were synthesized using an epoxide linker which was bonded to the pyridyl nitrogen. The conjugates were used for fluorescence imaging of *Bacillus subtilis*.[75]

Despite this synthetic activity that demonstrate the utility of the carboxylic acid-approach, various inconsistent procedures appeared in patents concerning the synthesis of the most clinically used zoledronic and risedronic acids which has been the matter of some controversy regarding the reagents and the ratio between them to be used. Dedicated reviews have been reported with the aim of clarifying the situation including the mechanism[76] of the reaction.[77,78] In 2011, Keglevich and co-workers finally established that the best conditions correspond to the use phosphorous trichloride in 1 : 3.2 molar ratio in methanesulfonic acid.[79] Alternatively, it has also been reported the use of benzenesulfonic acid as a solvent.[80] When the reaction is carried out in the presence of paraformaldehyde, bisphosphonic acids can be obtained without the addition of water,[81] the use of microwaves enhancing the yield of the reaction.[82,83] By using their conditions, Keglevich and co-workers reported the synthesis of etidronate **2**, alendronate **4**, pamidronate **5**, ibandronate **6**, risedronate and **7** zoledronate acid **8** (Scheme 7).[84] The exact treatment of the reaction comprised the use of only 3.2 eq. of phosphorous trichloride as the P-reagent in methanesulfonic acid as a solvent (without addition of phosphorous acid). In fact, it had been reported the use of that solvent for large-scale preparations.[85] Time and temperature were adjusted in each case for the first stage of the reaction at which time water was added and the resulting mixture was heated at 105 °C for 5 h. This was followed by hydrolysis with 10 N NaOH and pH adjustment to 1.8–2.0. Notably, the different dronic acids prepared required different purification conditions. Whereas **2**, **4** and **6** were purified by direct precipitation in water, **5** was purified by digestion in MeOH followed by precipitation in water, **7** was purified by washing with water and **8** was purified by recrystallization from HCl. Further work in different solvents[86] allowed establishing the best conditions for doing the reaction.[83]

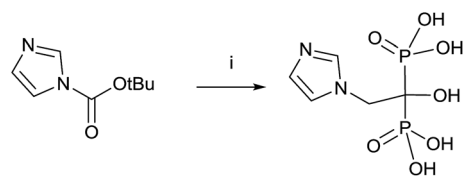
A variation using phosphorous oxychloride has allowed a one-pot multigram synthesis of zoledronic acid **8** in high yield (Scheme 8).[87] The procedure has been carried out with 200 g of compound **28**, easy accessible from imidazole.[88] The use of phosphorous oxychloride has also been successfully used for the synthesis of aledronate **4** and risedronic acid **7**.[89] On the other hand, phosphorous trichloride was the preferred reagent in the synthesis of benzidronate.[90]

Nitriles **29** can also be used as an alternative to carboxylic acids and the reaction can be performed under similar

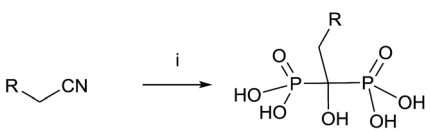


compound	R	time, T	yield (%)
2	Me	24 h, 75 °C	36
4	H_2N	24 h, 75 °C	57
5	H_2N	12 h, 75 °C	57
6	N	12 h, 75 °C	46
7	N	3 h, 80 °C	74
8	N N	3 h, 80 °C	53

Scheme 7 Reagents and conditions: (i) PCl_3 (3.2 equiv.), $MeSO_3H$, see scheme for time and temperature; then H_2O, 4–5 h, 105 °C; then 50% aq. NaOH to pH = 1.8–2.0.



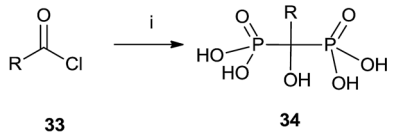
Scheme 8 Reagents and conditions: (i) H_3PO_3, 4-chlorobenzene, 25 °C, 15 min; then CH_3SO_3H, 30 min, 70 °C; then $POCl_3$, 95 °C, 24 h; then H_2O, 6 h, 90 °C; then 30% NaOH, pH = 4.1.



compound	R	yield (%)
7	3-pyridyl	85
30	2-pyridyl	71
31	4-pyridyl	75
6	N	65
32	H N	64

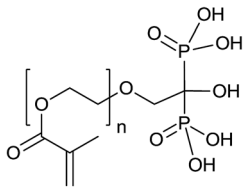
Scheme 9 Reagents and conditions: (i) CH_3SO_3H, 8 h, 100 °C; then PCl_3, 70 °C, 5 h; then H_2O, 15 h, 98 °C.

conditions but without the presence of phosphorous acid (Scheme 9).[91] Risedronate 7 prepared by this route was further labeled with ^{99}Tc by forming the corresponding complex.[92]



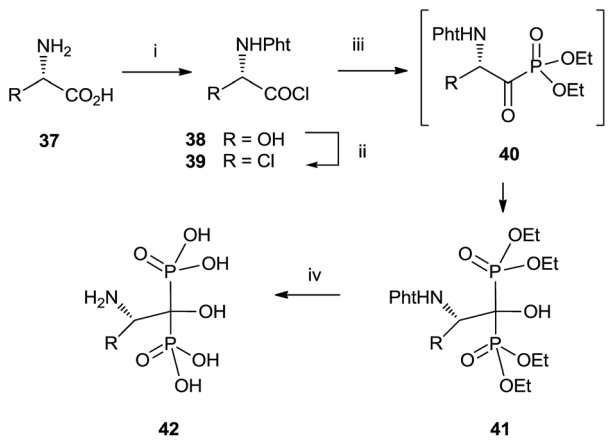
33 34

R	solvent	T (°C)	yield (%)
Me	none	25	98
C_5H_{11}	none	25	97
$C_{11}H_{23}$	none	25	97
$C_6H_5CH_2$	none	25	90
C_6H_5	none	25	91
4-$MeOC_6H_4$	THF	25	90
4-$NO_2C_6H_4$	THF	-70	85
3-pyridyl	THF	25	61



35 n = 5
36 n = 8

Scheme 10 Reagents and conditions: (i) $P(OSiMe_3)_3$, 0 °C then rt; then MeOH, 1 h, 25 °C.



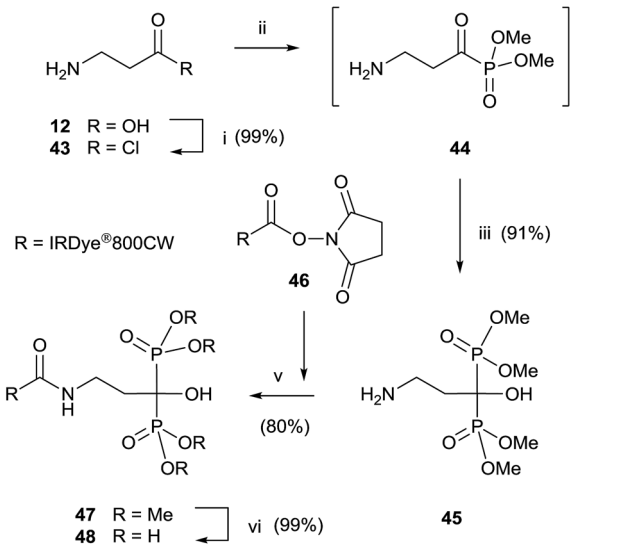
yield (%)

amino acid 37	38	39	41	42
phenylalanine	88	88	30	50
leucine	81	90	25	90
tryptophan	95	93	50	88
methionine	92	85	44	95
aspartic	55	88	18	40
glutamic	83	89	48	99
lysine	80	80	18	90
ornithine	26	92	15	82
valine	81	85	18	90

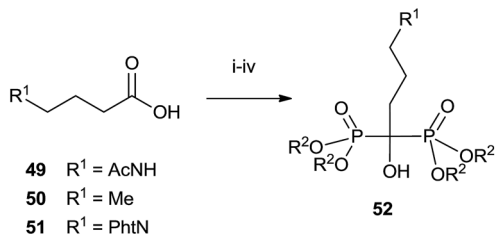
Scheme 11 Reagents and conditions: (i) *N*-(ethoxycarbonyl)phthalimide, $NaHCO_3$, 0 °C, 5 min. (ii) $SOCl_2$, CH_2Cl_2, 5 h, reflux. (iii) Triethyl phosphite, toluene, 1 h, 0 °C; then diethylphosphite, Et_3N, 1–3 h, 0–5 °C. (iv) 6 N HCl, reflux, overnight; then 5 N NaOH, pH = 4.4.

Reaction of acylphosphonates with dialkyl phosphites

In some instances, the direct reaction between a carboxylic acid and phosphorus trichloride takes place with low yields or fails completely as in the case of sterically hindered α-aminoacids. A discussion comparing this route with that developed at the Merck company has been reported.[93] An alternative is the use of the acyl chloride as starting material. The Arbuzov-type reaction[94] with a di-, trialkyl phosphite provides an α-ketophosphonate (acylphosphonates) capable of reacting with another molecule of di-, trialkyl phosphite to give the corresponding 1-hydroxy-1,1-bisphosphonate. The first report on this approach



Scheme 12 Reagents and conditions: (i) $SOCl_2$, CH_2Cl_2, 1 h, reflux. (ii) Trimethyl phosphite, toluene, 30 min, 0 °C. (iii) Dimethylphosphite, Et_3N, 30 min, 0–5 °C. (iv) DMSO, *N*-methylmorpholine, 4 h, rt. (v) Me_3SiBr, 18 h, rt; then 4 : 1 MeOH–H_2O, 30 min, rt.



compound	R^1	R^2	method	yield (%)
52a	AcNH	Et	A	0
52a	AcNH	Et	B	0
52a	AcNH	Et	C	22
52b	Me	Me	A	quant.
52a	AcNH	Me	A	70
52c	PhtN	Me	A	12

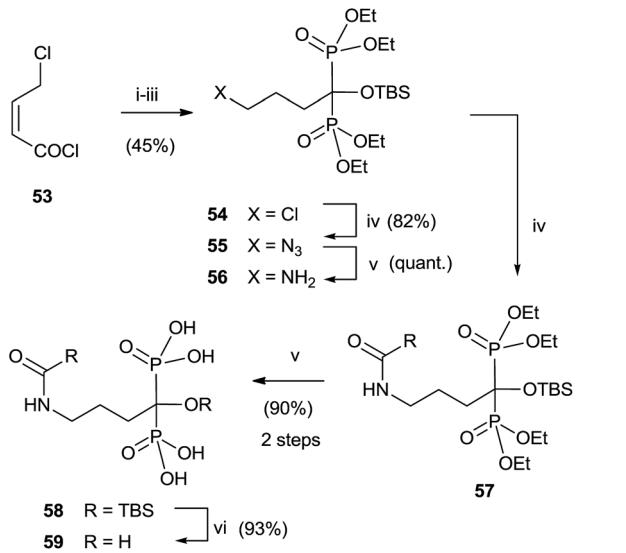
Scheme 13 Reagents and conditions: (i) $(COCl)_2$, CH_2Cl_2, DMF (cat.), 3 h, rt. (ii) $(R^2O)_3P$, 1 h, rt. (iii) $(R^2O)_3P$, TMSBr, 1 h, rt. (iv) Method A: aq. 6 M HCl, 17 h, reflux; method B: $MeSO_3H$, 17 h, reflux; method C: 48% HBr, 17 h, reflux.

described the use of tris(trimethylsilyl)phosphite as the only phosphorylating reagent (Scheme 10).[95] The reaction proceeds under mild conditions rendering the process compatible with labile substrates[96] including bile acids.[97] Acrylic ester bisphosphonates **35**,**36** with numerous potential applications in biomedicine are also accessible through this approach.[98]

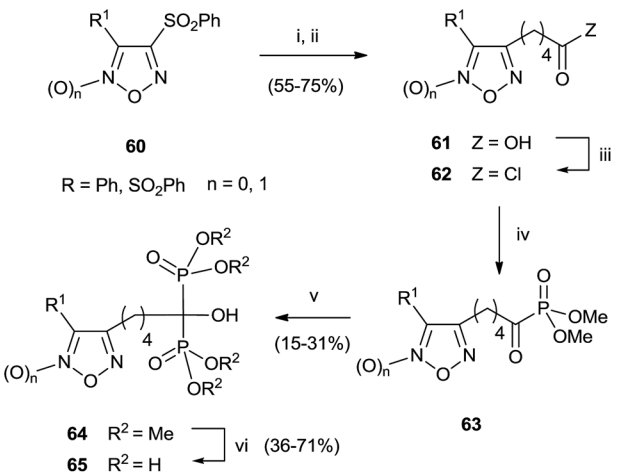
The direct use of trialkyl phosphites is also possible. A series of α-amino acid derived bisphosphonates **42** has been prepared in good yield by using as starting materials *N*-phthalimido-protected amino acids and using sequentially tri- and diethyl phosphite as P-reagents (Scheme 11).[99] Although the addition of phosphites is consecutive, the reaction is carried out in a one-pot procedure without isolating the intermediate ketophosphonate **40**.

Final deprotection of the amino group was made with 6 N HCl. The use of hydrazine is also possible and better yields are obtained. By using this variation, alendronate **4**, pamidronate **5**, and neridronate have been prepared as mono- and diesters, which were soluble in water at physiological pH.[100] The use of tri- and dimethylphosphite led to shorter reaction times. Conjugation of a fluorophore to methyester-protected pamidronate **45**, prepared from β-aminoacid **12**, was performed in DMSO in the presence of *N*-methylmorpholine (Scheme 12). Compound **48** was used as a contrast agent in image-guided surgery of large animals.[101]

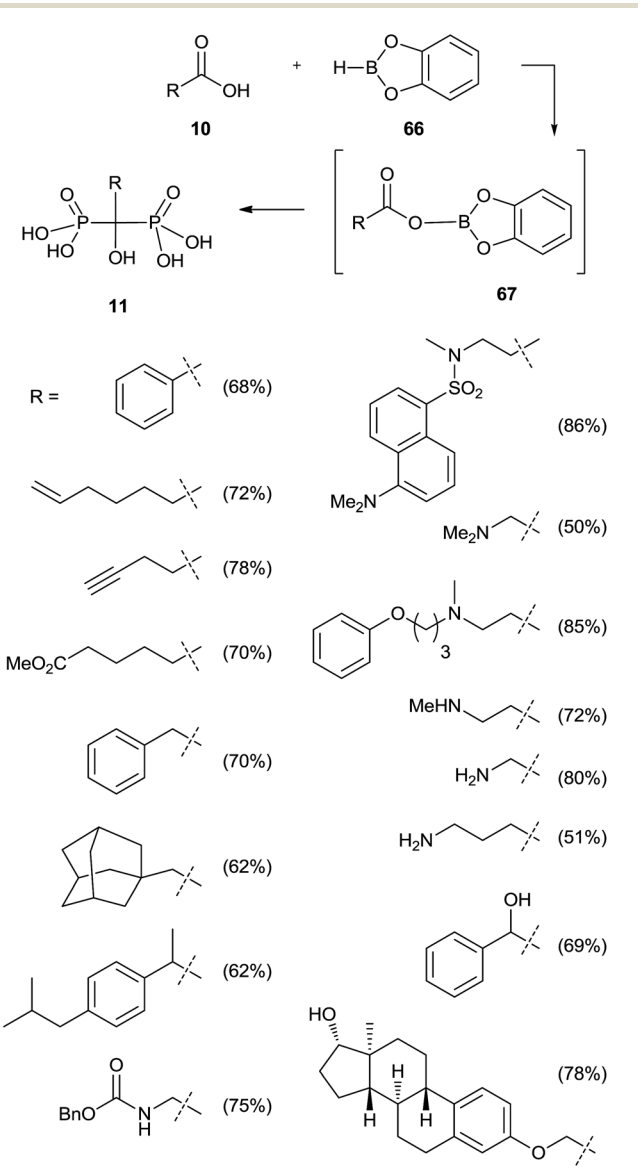
The use of dimethylphosphite also afforded better results in the synthesis of alendronate **4** reported by Seki in which both P-



Scheme 14 Reagents and conditions: (i) $P(OEt)_3$, 0 °C. (ii) $HOP(OEt)_2$, DMAP, CH_2Cl_2, room temp, 1 h. (iii) TBSCl, 15 h. (iv) NaN_3, DMF, 75 °C, 2 h. (v) Pd–C, 50 psi of H_2, AcOEt, 15 h. (vi) RCOCl, Et_3N, CH_2Cl_2 or RCO_2H, EDC, DIPEA, MeCN. (v) TMSI, MeCN and then MeOH. (vi) TBAF, THF.



Scheme 15 Reagents and conditions: (i) 1,5-pentanediol, NaOH 50% w/w, THF. (ii) Jones reagent, acetone, 0 °C to rt. (iii) $SOCl_2$. (iv) $P(OMe)_3$, dry THF, 0 °C to rt. (v) $HPO(OMe)_2$, Et_2NH, dry THF, 0 °C to rt; (vi) TMSBr, CH_2Cl_2; then MeOH, 0 °C to rt.



Scheme 16 Reagents and conditions: (i) THF, rt. (ii) $P(OSiMe_3)_3$, 16 h, 25 °C; then MeOH, 2 h.

reagents were compared. Differences were found in the final hydrolysis of the phosphate esters. Ethyl esters showed a lower reactivity towards hydrolysis (Scheme 13).[102]

Analogues of alendronic acid **4** have been prepared from the common precursor **53** through a practical one-pot, three step methodology. The protection of the 1-hydroxy group was necessary for avoiding mixtures in the acylation step (Scheme 14).[103]

A series of bisphosphonates bearing either the nitrogen-containing NO-donor furoxan (1,2,5-oxadiazole 2-oxide) or the related furazan (1,2,5-oxadiazole) systems in the lateral chain has been prepared by using trimethylphosphite as P-reagent (Scheme 15).[104]

Activation of the carboxylic acid as a dioxaborolane can be an alternative to the acid chloride. This approach requires the use of tris(trimethylsilyl)phosphite since dialkylphosphites showed no reaction (Scheme 16).[105] Also in this case a poor reactivity was observed for *N*-hydroxysuccinimide esters towards methyl or phenyl bis(trimethylsilyl)phosphites, in good agreement with previously reported results.[100]

Michael addition to tetraethylvinylidenebisphosphonate

Tetraethyl vinylidenebisphosphonate **68** is an easily available Michael acceptor and electron-poor dipolarophile/dienophile that results an excellent synthetic intermediate for the synthesis of 1,1-bisphosphonates (Scheme 17). The synthesis of **68** and its application in the preparation of bisphosphonates has been recently reviewed in 2014 by J. B. Rodriguez.[106] The reader is referred to this excellent compilation for the syntheses of bisphosphonates starting from **68** reported until 2013. Here we survey only those reported after publication of the Rodriguez's review.

The Cu-catalyzed 1,4-conjugate addition of boronic acids and indoles to **68** afforded 1,1-bisphosphonates lacking the *gem*-OH group (Scheme 18).[107] Whereas the reactions with boronic acids proceeded smoothly in toluene, the addition of indoles can be carried out in polar solvents like 1,2-dichloroethane or water with sodium dodecyl sulfate under micellar conditions.

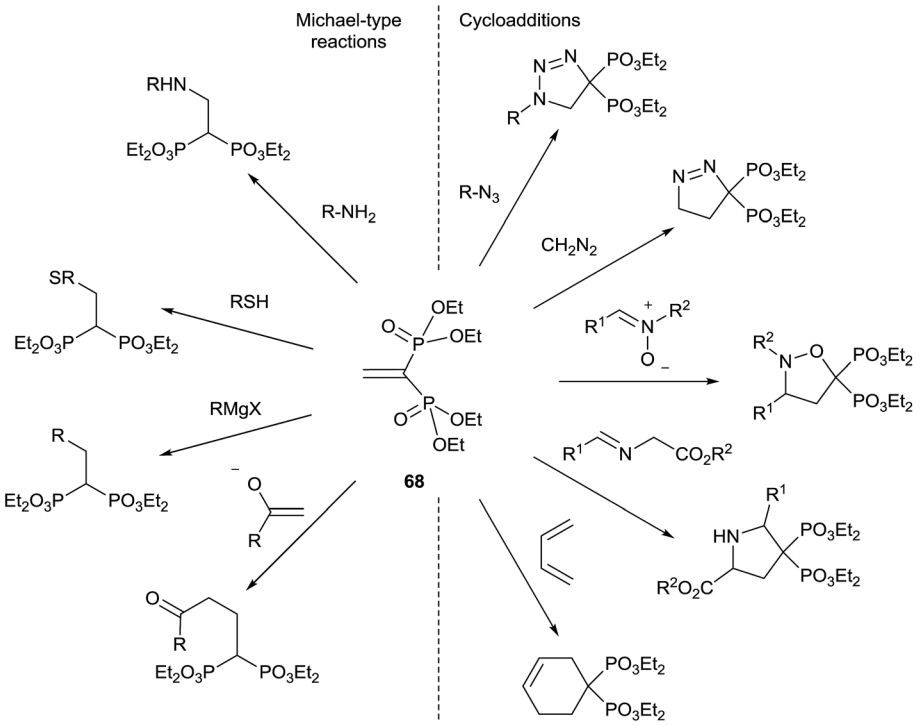
A series of spiro[indole-pyrrolizine], spiro[indole-indolizine], and spiro[indole-pyrrolidine] *gem*-bisphosphonates were synthesized through multicomponent reactions between isatins **74**, amino acids **75** and **68** in the presence of montmorillonite (Scheme 19).[108] Acyclic aminoacids can also be used.

The cycloaddition of aromatic nitrones **77** with **68** furnished spiro(isoxazolino)bisphosphonates **78** (Scheme 20).

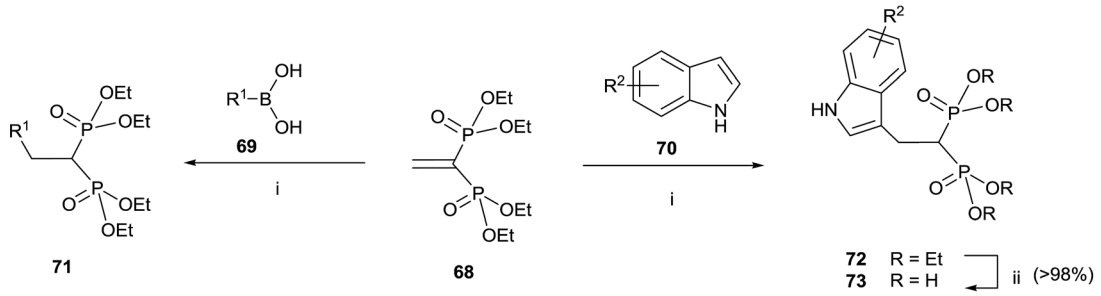
The reaction, carried out in the absence of solvent and under activation with microwaves takes place in several minutes with good yields.[109] In all these cases the bisphosphonates prepared by this route lack the 1-hydroxyl group but consist of interesting structurally constrained analogues.

Alkylation of tetralkylmethyl bisphosphonate

Alkylation of tetraethyl bisphosphonate **79** is an expeditious way of preparing bisphosphonates lacking the 1-hydroxy groups. However, precise reaction conditions must be used in order to avoid elimination reactions.[110] Alkylation of **79** with farnesyl and geranyl bromides **80** and **81** using sodium hydride as a base provided bisphosphonates **82a,b**. Further hydrolysis furnished free bisphosphonates **83a,b** (Scheme 21).[111]

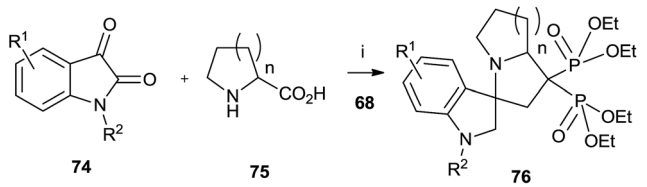


Scheme 17 Synthetic utility of tetraethyl vinylidenebisphosphonate **68**.[106]



R^1	yield (%)	R^1	yield (%)	R^2	yield (%)
Me	56	3-F-4-$CHOC_6H_3$	71	1-Me	67
iPr	26	2-naphthyl	91	1,2-diMe	>98
$C_6H_5CH_2$	46	4-methyl-1-naphthyl	>98	5-MeO	98
4-$tBuC_6H_4$	77	2-methoxy-1-naphthyl	78	5-MeO-6-CF_3	44
4-$MeOC_6H_4$	83	2-ethoxy-1-naphthyl	66	6-Br-7-Me	84
2,3-di$MeOC_6H_3$	96	6-hydroxy-2-naphthyl	69	5-COOH	50
3,4-di$MeOC_6H_3$	79	6-methoxy-2-naphthyl	91	5-COOMe	48
4-$Me_2NC_6H_4$	74	4-Br-1-naphthyl	83	2-Ph	77
4-$NO_2C_6H_4$	59	3-thienyl	>98	2-(4-C_6H_4)	38
4-BrC_6H_4	59	3-benzothienyl	53		
2-MeC_6H_4	69	4-indolyl	83		

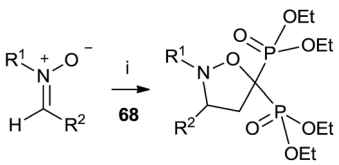
Scheme 18 Reagents and conditions: (i) $Cu(OTf)_2$ (10 mol%), toluene, 18 h, 70 °C. (ii) TMSBr, 18 h, rt; then water, 4 h, rt.



R^1	R^2	n	t (h)	dr	yield (%)
H	H	1	0.5	100:14	80
4-F	H	1	0.5	100:6	92
4-Cl	H	1	0.5	100:22	95
4-Br	H	1	0.5	100:20	93
3-Cl	H	1	2	100:0	57
4-Me	H	1	1	100:24	45
4-Me	Bn	1	2	100:17	47
H	Bn	1	2	100:18	44
H	H	2	2	100:46	65
4-F	H	2	2	1000:8	45
4-Cl	H	2	2	100:0	40
4-Br	H	2	2	100:4	45
4-Me	Bn	2	2	100:5	40
H	Bn	2	2	100:8	35

Scheme 19 Reagents and conditions: (i) montmorillonite, MeCN, 0.5–2 h, 80 °C. (ii) TMSBr, 18 h, rt; then water, 4 h, rt.

On the other hand, using potassium hydride an undesired elimination reaction was observed.[110] By oxidizing the allylic position of the terminal trisubstituted double bond in **80**, it was possible to introduce additional substituents at the end of the isoprenoid unit.[112] Further alkylation of compound **80** furnished, after hydrolysis, fluorescent



R^1	R^2	t (min)	yield (%)
Me	Ph	10	75
Me	2-ClC_6H_4	12	77
Me	3-pyridyl	13	3
Me	2-furyl	15	68
Bn	4-OHC_6H_4	14	74
Bn	Ph	18	83
Bn	2-ClC_6H_4	18	85
Bn	2-FC_6H_4	20	86

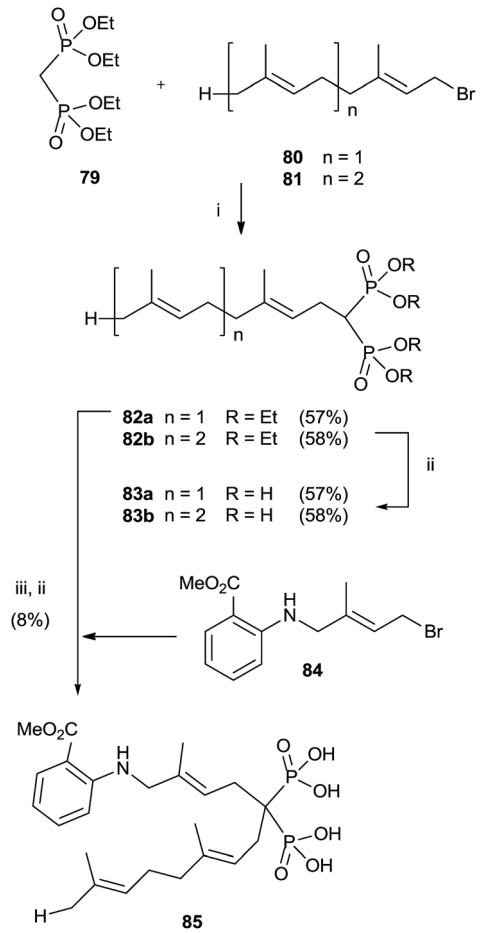
Scheme 20 Reagents and conditions: (i) neat, microwave irradiation 200 W.

anthranilate analogue **85** that showed some inhibition in geranylgeranylation.[113]

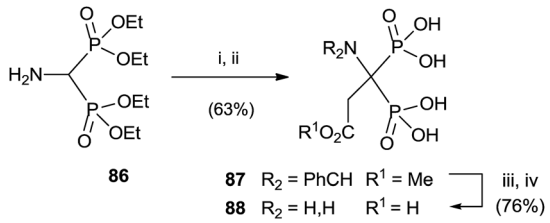
Aminobisphosphonate **86** was alkylated, after protection of the amino group, with methyl 2-bromo acetate. Potassium carbonate in the presence of triethylammonium bromide was used for deprotonating **86**. Further deprotection yielded the target bisphosphonate (Scheme 22).[114]

Other methods

Treatment of *N*-farnesyl lactams with an excess of base and diethyl phosphorochloridite furnished bisphosphonates **90** in



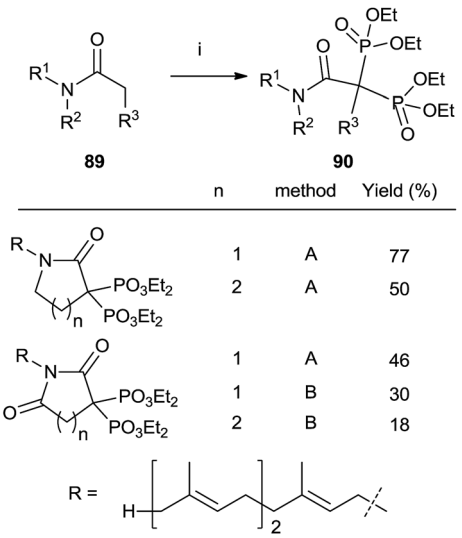
Scheme 21 Reagents and conditions: (i) NaH, THF, 0 °C, 1 h; then, 1 day, rt. (ii) TMSBr, collidine, 1 day, rt; then 0.5 M NaOH 16 days, rt. (iii) **84**, NaH, THF, 15-crown-5, overnight, rt.



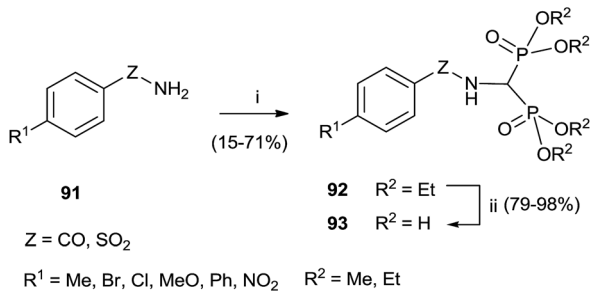
Scheme 22 Reagents and conditions: (i) *m*-ClC_6H_4CHO, $MgSO_4$, CH_2Cl_2. (ii) $BrCH_2CO_2Me$, Bu_4NBr, K_2CO_3, MeCN. (iii) 1 N HCl, MeCN; then NaOH. (iv) 6 N HCl.

good chemical yields. The methodology can be extended to other carbonyl compounds including amides and lactones although in the case of sterically hindered substrates the expected bisphosphonate is not obtained and other byproducts are formed. Whereas lactams can be phosphorylated with either LDA or LHMDS as a base, the former is preferred for amides and lactones (Scheme 23).[115]

Carboxyamide and sulfonamide bisphosphonates are accessible by treating the appropriate amide with trialkyl orthoformate and dialkylphosphites. Subsequent deprotection



Scheme 23 Reagents and conditions: (i) method A: LDA (2.2 equiv.); then $ClP(OEt)_2$ (2.3 eq.); then H_2O_2 (10 eq.). Method B: LHMDS; then $ClP(OEt)_2$; then LHMDS; then $ClP(OEt)_2$; then H_2O_2 (20 eq.).



Scheme 24 Reagents and conditions: (i) $HC(OR)_3$, $HP(OR)_2$, 150 °C. (ii) BBr_3, toluene, MeOH, reflux.

under typical conditions furnished bisphosphonates **90** (Scheme 24).[116]

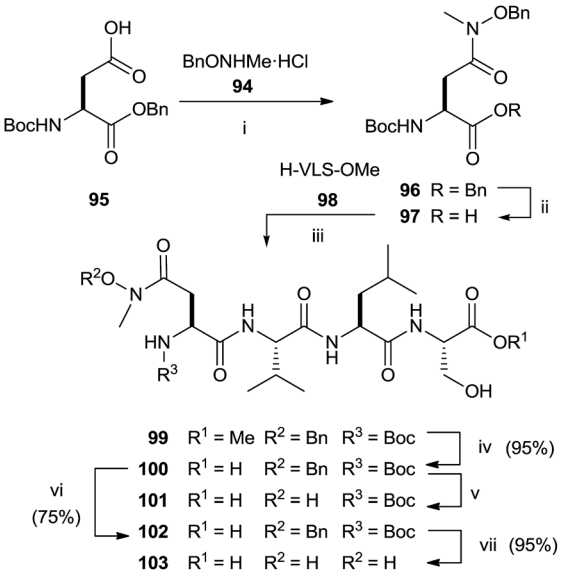
Non-bisphosphonate derivatives

Non-bisphosphonate antiresorptive agents are represented by molecules with variable dimensions and functional groups.

The presence of a hydroxamate moiety induces a major attitude in terms of metal chelation.[117] Thus, the hydroxamic group is expected to improve the ionic and/or metal chelation interactions with the active site of FPT (Farnesyl Protein Transferase).

The synthesis of *N*-methyl substituted hydroxamic acid **103** was carried out starting from *N*-methyl-*O*-benzyl-hydroxylamine hydrochloride **94** and aspartic acid derivative **95** (Scheme 25).[118] Deprotection of **96** and successive coupling of resulting **97** with tripeptide H-Val-Leu-Ser-OMe **98** furnished tetrapeptide **99**.

Basic hydrolysis with NaOH of the ester group in **99** provided **100** with 20% of the free aspartic acid derivative resulting from cleavage of hydroxamic function. The use of sodium carbonate in 2 : 1 MeOH–water avoided the formation of byproducts maintaining unchanged the chirality. Further deprotection



Scheme 25 Reagents and conditions: (i) CD1, iPr_2NEt. (ii) 1 N NaOH, MeOH, (iii) EDC, HOBT, iPr_2NEt, HVLS-OMe, (iv) Na_2CO_3 (1.0), 2 : 1 MeOH, H_2O. (v) H_2, 10% Pd/C, MeOH. (vi) Anhydrous HCl/dioxane, EtOAc. (vii) H_2, 10% Pd/C, MeOH.

steps gave provided **103** in good yields. In the same work, the authors prepared hydroxamic bisubstrate analogs **114**, **116**, **118** and **120** by introducing a full farnesyl group on the hydroxamic portion to improve the inhibition of FPT (Scheme 26).

The synthesis was carried out by a multistep sequence utilizing farnesyl bromide **104** as starting material which was transformed into **105** in moderate yield. Acylation of **105** required 2 eq. of the 3-carbomethoxy-propionyl chloride and 3 eq. of DIPEA. Saponification of the resulting **107** furnished intermediate **108** which was coupled with various tripeptides to

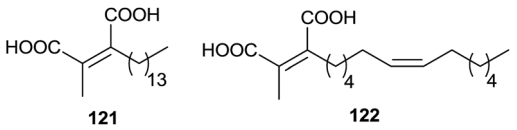


Fig. 3 Chaetomellic acids.

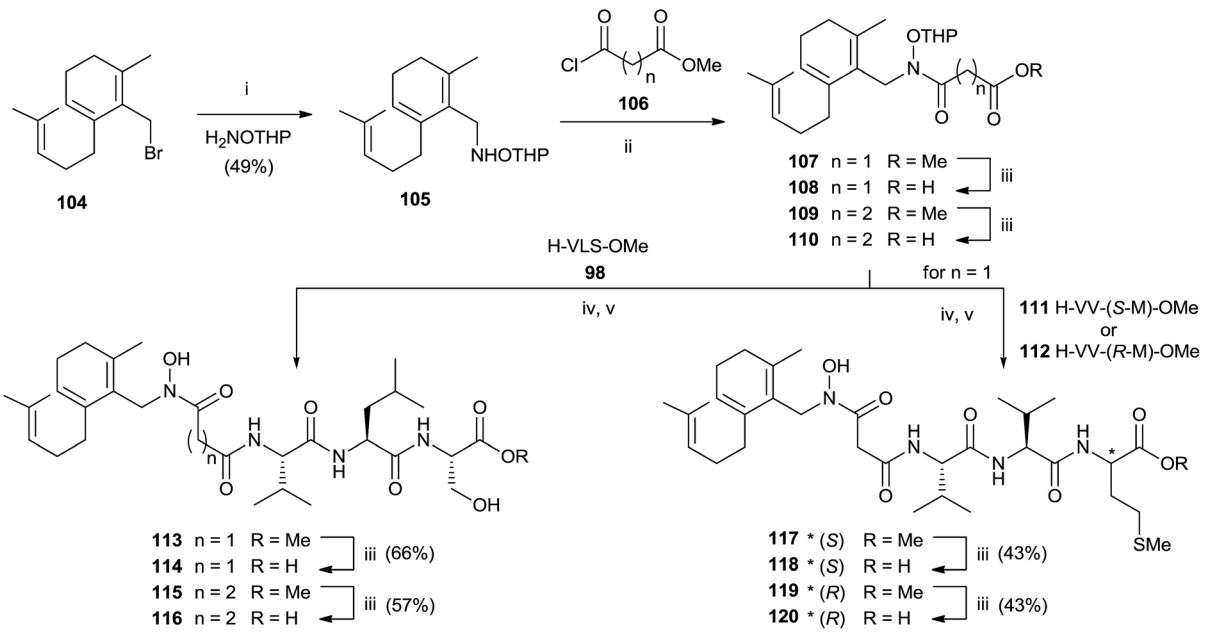
give bisubstrates **114**, **116**, **118** and **120** after opportune removal of protective groups. A critical step in the synthesis was the deprotection of THP group in the presence of farnesyl chain. The problem was solved by using *p*-TsOH although the yields were modest.

In 1993, the chaetomellic acids A and B **121** and **122**, classified as alkyl *cis*-dicarboxylates, were discovered to be potent inhibitors of FPTase because of analogy with the active site of FPP (Fig. 3).[119,120]

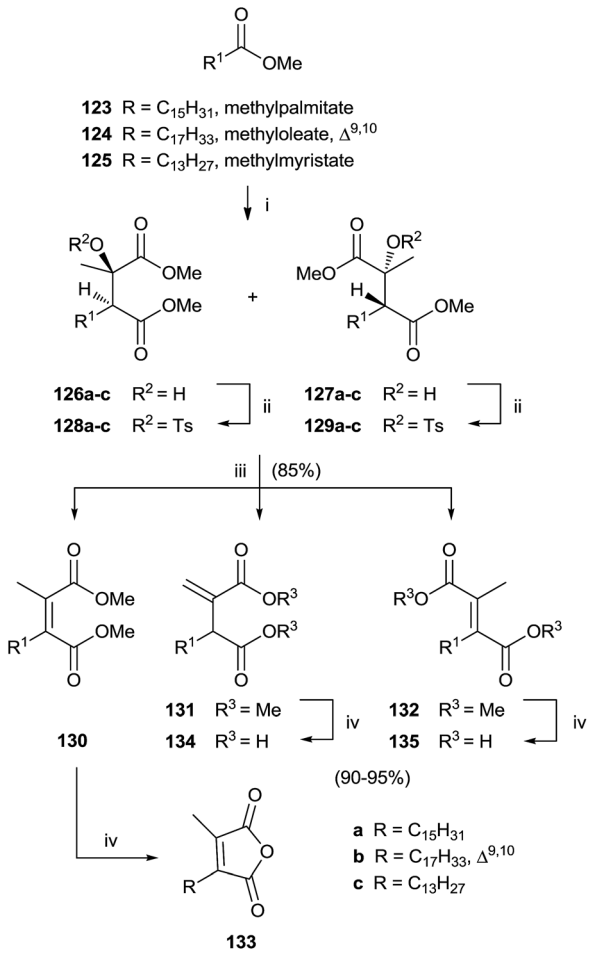
Singh and collaborators carried out the synthesis of various chaetomellic acids derivatives through a sequence of three steps.[121,122] Starting from fatty acid esters **123–125**, the reaction with methylpyruvate in the presence of LDA at −78 °C furnished a 1 : 1 diastereomeric mixture of aldol products **126** and **127** (Scheme 27). A β-elimination reaction of aldol substrates – opportunely protected with a tosyl group produced the tetra-substituted olefins **130–132** *via anti* or *syn* periplanar elimination. In the final step, the hydrolysis of ester derivatives by refluxing with a NaOH solution gave chaetomellic acid analogs **133–135** in moderate yield.

Tucker *et al.* showed that a large arylthio or aryloxy group adjacent to the cyano function provided compounds with high activity against GGPP and FPT enzymes.[123,124]

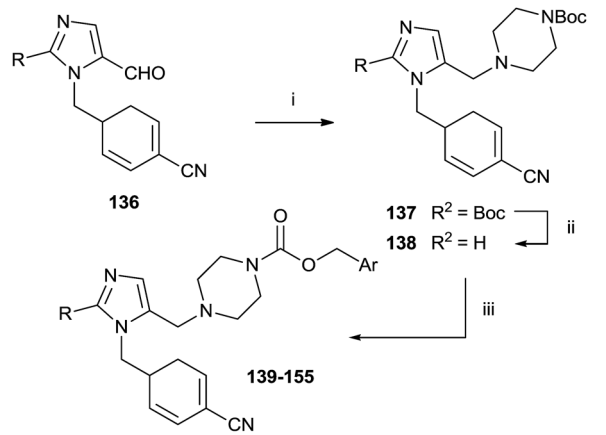
An approach to the synthesis of inhibitors **139–155** was developed by initial reductive amination of aldehydes **136** with *N*-Boc-piperazine, titanium iso-propoxide and $NaBH_3CN$ in THF–EtOH (Scheme 28).



Scheme 26 Reagents and conditions: (i) NH_2OTHP, THF. (ii) iPr_2NEt, THF. (iii) 1 N NaOH, MeOH. (iv) EDC, HOBT, iPr_2NEt. (v) *p*TsOH, MeOH.



Scheme 27 Reagents and conditions: (i) LDA, methylpyruvate, THF, −78 °C. (ii) *p*-Toluenesulfonic anhydride, CH_2Cl_2, C_5H_5N, 2,6-di-*tert*-butyl-4-methylpyridine, 40 °C. (iii) DBU, toluene, reflux. (iv) a: 1 N NaOH, MeOH, THF, H_2O, 80 °C. b: 4 N HCl.



compound	R	Ar
139	H	2-MeC_6H_4
140	H	3-MeC_6H_4
141	H	2-$MeOC_6H_4$
142	H	2-$EtOC_6H_4$
143	H	2- ClC_6H_4
144	H	2-$(MeOCH_2)C_6H_4$
145	H	2-$CF_3OC_6H_4$
146	H	2-$CF_3CH_2C_6H_4$
147	H	2-$MeSO_2C_6H_4$

compound	R	Ar
148	H	2-MeOpyridin-3-yl
149	H	2-EtOpyridin-3-yl
150	H	2,3-$(OCH_2O)C_6H_3$
151	H	2-$CF_3OC_6H_4$
152	H	3,4-$(OCH_2O)C_6H_3$
153	Me	Ph
154	Me	2-EtOPh
155	Me	2-CF_3OPh

Scheme 28 Reagents and conditions: (i) $Ti(OPr)_4$, $NaBH_3CN$, *N*-Boc-piperazine/THF–EtOH. (ii) TFA/CH_2Cl_2. (iii) Benzyl-(*p*-nitrophenyl) carbonate, DIEA/DMF, 80 °C.

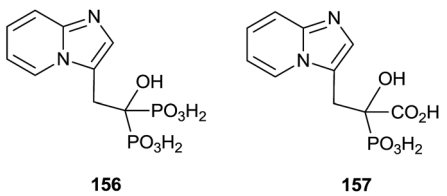


Fig. 4 Minodronic acid and analog.

The resulting compounds **137** were deprotected by TFA furnishing the free amines **138** in good yields. The reaction of **138** with appropriate *p*-nitrophenyl carbonate yielded target compounds **139–155** in good yields.

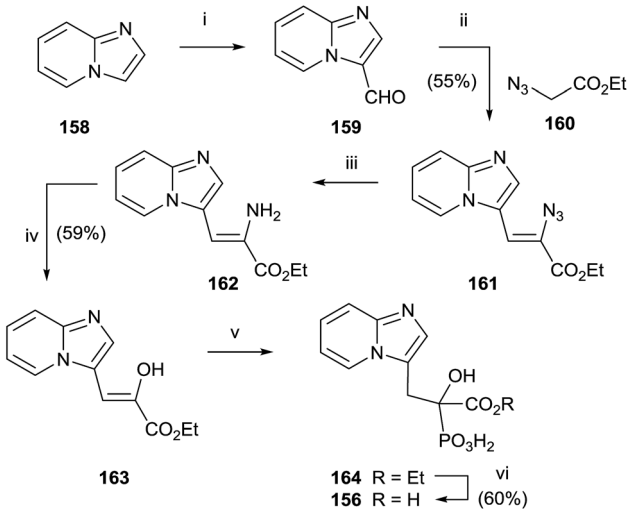
Phosphonocarboxylate (PC), analogues of *N*-BP, characterized by a carboxylic and a phosphonic group on the same carbon, exhibit a chiral structure in contrast to the respective bisphosphonates, increasing the possibility of stereospecificity in their biological activity.[125–128]

Minodronic acid **156** (Fig. 4) was the first bisphosphonate developed and approved for osteoporosis treatment in Japan and today is available in a number of countries worldwide.

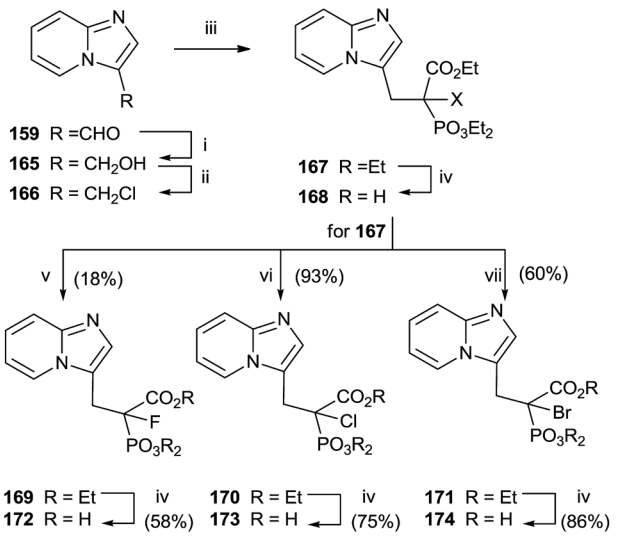
McKenna and co-workers reported, in 2010, the synthesis of the analogue **157** starting from imidazo[1,2-*a*]pyridine **158** (Scheme 29).[129] A Vilsmeier–Haack formylation of **158** furnished aldehyde **159** which was transformed into **160** and then the dehydroaminoester **162**. Hydrolysis of **162** and further addition of diethyl phosphite to the resulting **163** furnished protected bisphosphonate **164** that was conveniently deprotected into **156**. Resolution of **156** enantiomers by chiral HPLC furnished the (+)-**156** isomer, which revealed a potent inhibitory activity of RGGT.

Replacement of the geminal hydroxyl moiety of PC with a halogen atom allowed to obtaining three α-halo derivatives **172–174** with potential biological activity against RGGT.[130] The choice of this substitution had a significant impact to studying the role of the heterocyclic base respect to inhibition of enzyme activity. Starting from **159**, a multi-step approach was carried out to synthesize common precursor **167**. Halogenation with Selectfluor, *N*-chlorosuccinimide or *N*-bromosuccinimide provided haloderivatives **169–171**. Further hydrolysis of the ester groups afforded the free acids **172–174** (Scheme 30).

Phosphonocarboxylates can also be approached by routes commonly used for preparing bisphosphonates such as Arbuzov–Michaelis reaction of trialkyl phosphite with α-bromoesters,[131] reaction of diethyl phosphite with α-ketoesters,[129] and reaction of enolates and chlorodialkyl phosphites.[132] In



Scheme 29 Reagents and conditions: (i) Vilsmeier reagent, 140 °C. (ii) EtONa/EtOH, from −30 °C to rt, 4 h. (iii) H_2/10% Pd/C, MeOH, 2.5 h, rt. (iv) AcOH/H_2O (7/1 v/v), 1.5 h, 0 °C. (v) $(EtO)_2P(O)H$, 70 °C, 21 h. (vi) 6 N HCl, 6 h, reflux, (v) and (vi) combined.



Scheme 30 Reagents and conditions: (i) $NaBH_4$, MeOH, reflux. (ii) $SOCl_2$, reflux, (iii) triethyl phosphonoacetate, NaH, DMF, THF, 0 °C to rt. (iv) 12 M HCl, reflux. (v) Selectfluor, NaH, THF. (vi) *N*-Chlorosuccinimide, NaH, THF. (vii) *N*-Bromosuccinimide, NaH, THF.

addition, the insertion of carboxylic function can be performed by using lithium alkylphosphonate and diethyl carbonate[133] or CO_2.[134] Another methods include alkylation of trialkyl phosphonoacetate[135] and functionalization of trialkyl 2-phosphonoacrylate *via* Michael-type addition.[136]

Recently, Coxon and colleagues used various approaches among those described to synthesize phosphonocarboxylates **175–178** showing some structural diversity (Fig. 5).[137]

Interestingly, the exchange of hydroxyl group with an alkyl chain of different length increased in the hydrophobicity enhancing the activity against GGPPS and FPPS. The synthesis of derivatives **181** was carried starting from α-alkyl

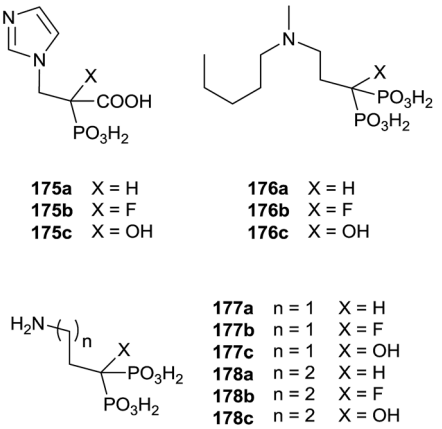
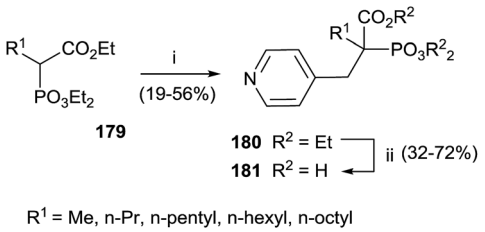


Fig. 5 Phophonocarboxylates.



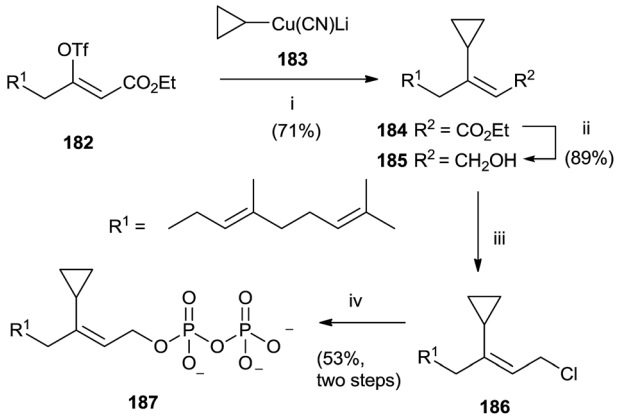
Scheme 31 Reagents and conditions: (i) picolyl chloride, NaH, DMF/THF. (ii) 12 M HCl, reflux.

phosphonoacetate **179** through an Arbuzov reaction and subsequent alkylation with picolyl chloride (Scheme 31). The corresponding free acids **181** were obtained upon hydrolysis with a 12 M solution of HCl at reflux.

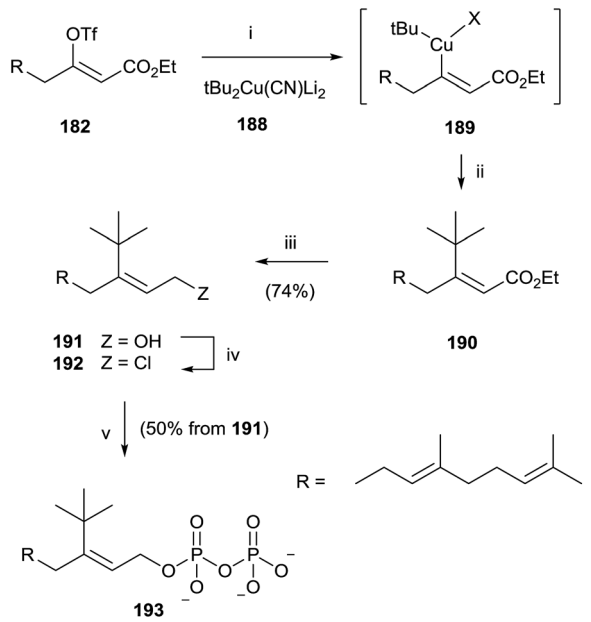
A category of FPP analogues is constituted from inhibitors with a chain that mimic farnesyl diphosphate or farnesyl group in position C-3. Gibbs *et al.* have synthesized 3-cyclopropyl-3-desmethyl FPP (3-cpFPP) **187** and 3-*tert*-butyl-3-desmethyl FPP (3-*t*bFPP), **193** as potential irreversible inactivators of FTPase.[138] The synthesis of **187** proceeded from **182**. Coupling with cyclopropyl cyanocuprate **183** gave **184** in 71% yield. Reduction of the ester group with DIBALH produced the corresponding alcohol **185** that was chlorinated and immediately treated with tris(tetrabutyl ammonium) hydrogen diphosphate to give **187** (Scheme 32).

Similarly, the synthesis of **193** was carried out through an initial reaction between **182** and the *t*-butyl cyanocuprate **188**. Further steps of reduction, chlorination and pyrophosphorylation furnished **191** (Scheme 33).

Gibbs and co-workers developed a stereoselective synthesis of *cis*-isoprenoid analogues such as **199** using the vinyl triflate method (Scheme 34).[139,140] The fundamental step was the stereoselective preparation of triflate derivative **196** from the enolate of β-ketoester **194**. The choice of solvent was found to have a significant impact on the trend of the reaction, not only in terms of yield, but also in the stereoselectivity. In fact, the use of DME instead of THF resulted in a loss of stereocontrol, while DMF as reaction solvent promoted only the stereoisomer



Scheme 32 Reagents and conditions: (i) cyclopropyl cianocuprate, Et_2O, −78 °C. (ii) DIBALH, PhMe, −78 °C. (iii) NCS, Me_2S, CH_2Cl_2. (iv) $(Bu_4N)_3HP_2O_7$, CH_3CN.



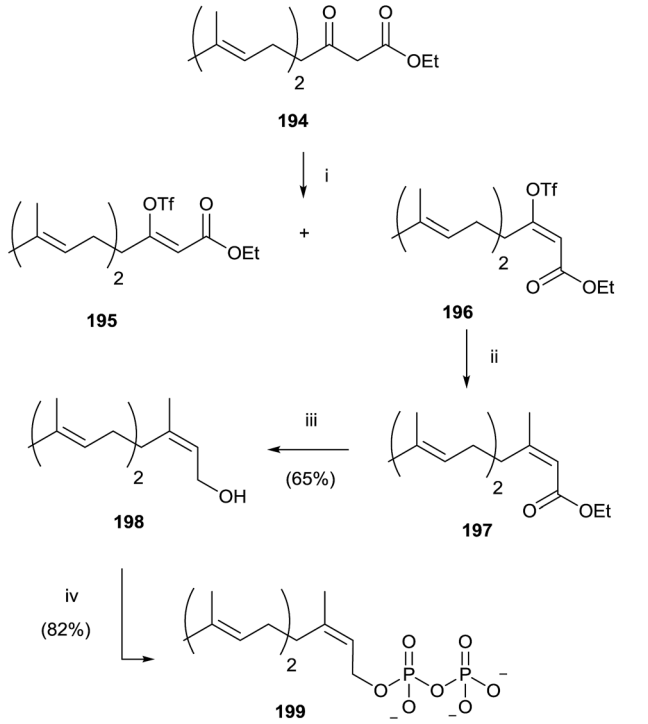
Scheme 33 Reagents and conditions: (i) $tBu_2Cu(CN)Li_2$, −78 °C. (ii) Reductive elimination. (iii) DIBALH, PhMe, −78 °C. (iv) NCS, Me_2S, CH_2Cl_2. (v) $(Bu_4N)_3HP_2O_7$, CH_3CN.

196 in excellent yield (93%). Coupling of **196** with tetramethyltin furnished the ester **197** that was reduced with DIBALH to the alcohol **198.** Bromination and pyrophosphorylation gave **199.**

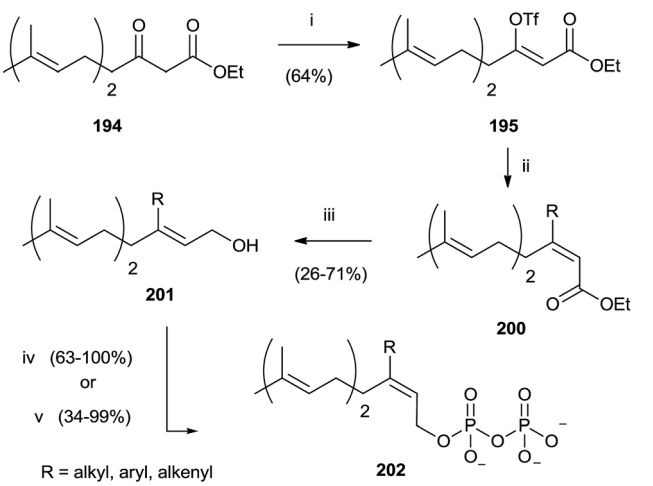
Compound **194** was employed to synthesize FPP analogues **202** with an alkyl or haloaryl chain in C-3 (Scheme 35).[141]

Grignard reagents were made to react with triflate **195** in the presence of copper cyanide with yields from poor to high (39–91%). Further elaboration of intermediates **200** furnished pyrophosphates **202.**

The presence of a sulfur atom as thiodiphosphate seems to promote the (*S*)-alkyl thiodiphosphates regioselectivity. Therefore, (*S*)-alkyl isopentenyl and allylic thiodiphosphates **204** and **206–209** were obtained by the procedure illustrated



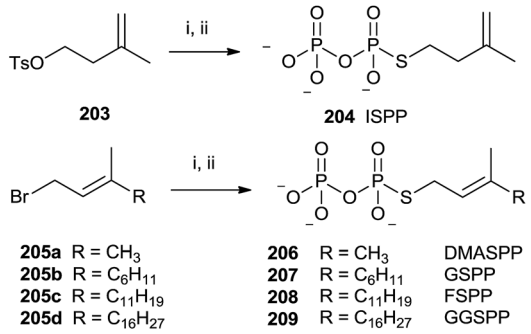
Scheme 34 Reagents and conditions: (i) $(Me_3Si)_2NK$, solvent, −78 °C, $PhN(SO_2CF_3)_2$. (ii) Me_4Sn, CuI, $Pd(AsPh_3)_2$, NMP, 100 °C. (iii) DIBALH, toluene, −78 °C. (iv) a: NBS, CH_2Cl_2, Me_2S; b: $(Bu_4N)_3HP_2O_7$, CH_3CN.



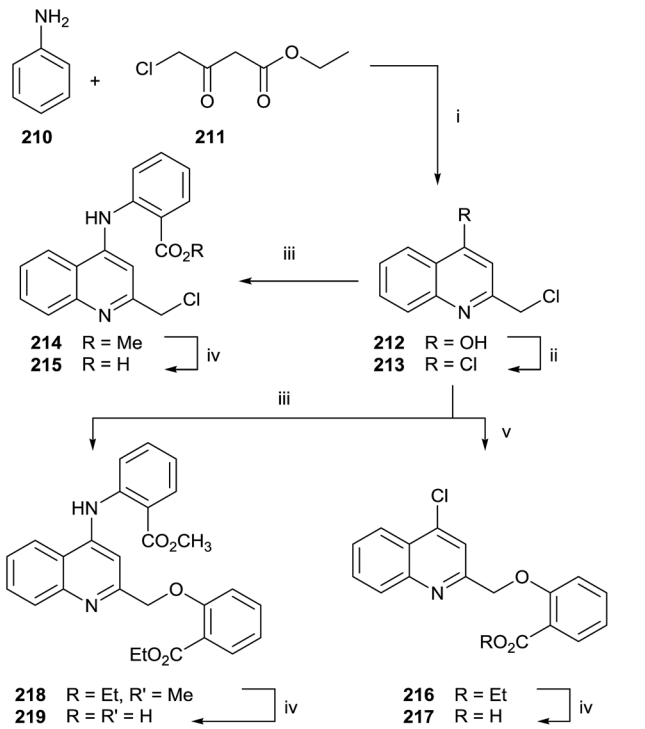
Scheme 35 Reagents and conditions: (i) $(Me_3Si)_2NK$, THF, $PhN(SO_2CF_3)_2$. (ii) RMgX, CuCN, ether. (iii) DIBALH, toluene. (iv) a: NBS, CH_2Cl_2, Me_2S; b: $(Bu_4N)_3HP_2O_7$, CH_3CN.

in Scheme 36.[142] The reaction proceeded through slow addition of the isoprenoid derivatives **203** and **205a–d** to an acetonitrile solution containing tris(tetra-*n*-butylammonium) thio-pyrophosphate (SPP_i). The residue was passed through an ion-exchange column, replacing the tetra-*n*-butylammonium cation with ammonium in order to purify the final product by cellulose chromatography (57–89% yield).

Quinolines and salicylic derivatives have also been shown to be inhibitors of FFPS. In particular, the combination of



Scheme 36 Reagents and conditions: (i) Tris-(tetra-*n*-butylammonium)thiopyro-phosphate, CH_3CN. (ii) Dowex AG 50W-X8 (NH_4^+ form).

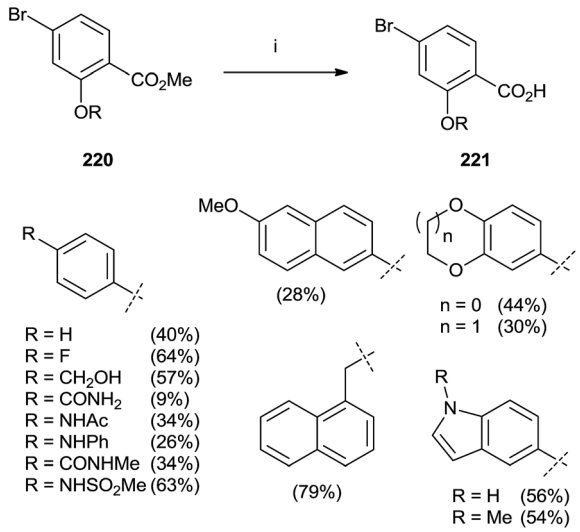


Scheme 37 Reagents and conditions: (i) PPA, 130 °C, 1 h. (ii) $POCl_3$, 100 °C, 3 h. (iii) Methyl 2-aminobenzoate, C_2H_5OH, conc. HCl, 5 h. (iv) THF/MeOH, LiOH, 3 h. (v) Ethyl-2-hydroxybenzoate, K_2CO_3, CH_3CN, 90 °C, 5 h.

quinolines with zoledronate seems to amplify the inhibition effect respect to the individual inhibitor. Therefore, a series of quinoline derivatives was synthesized starting from aniline **210** and ethyl 4-chloroacetoacetate **211** in PPA at 130 °C (Scheme 37).[143]

The crude mixture was directly chlorinated by $POCl_3$ at 100 °C. The resulting intermediate **213** was used without further purification in the following reaction with methyl 2-aminobenzoate **214** or ethyl 2-hydroxybenzoate **216**. Different reaction routes allowed obtaining three different quinoline analogues **215**, **217** and **219**.

In 2015, Marzinzik *et al.* synthesized a library of salicylic acid derivatives exploiting the presence of a bromine atom in the



Scheme 38 Reagents and conditions: (i) 1: boronic acid, $Pd(PPh_3)Cl_2$, Na_2CO_3, DME/EtOH, H_2O, MW (110 °C), 10 min. (ii) LiOH, MeOH/THF, MW (110 °C), 12 min.

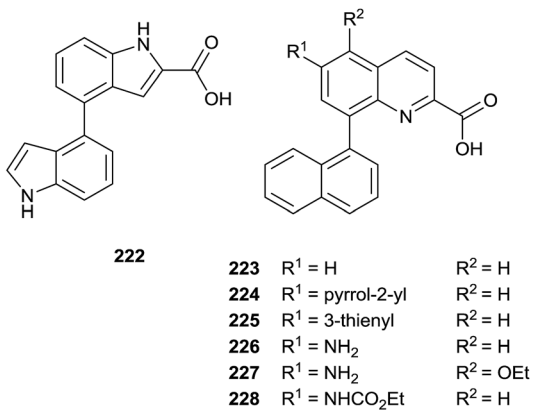


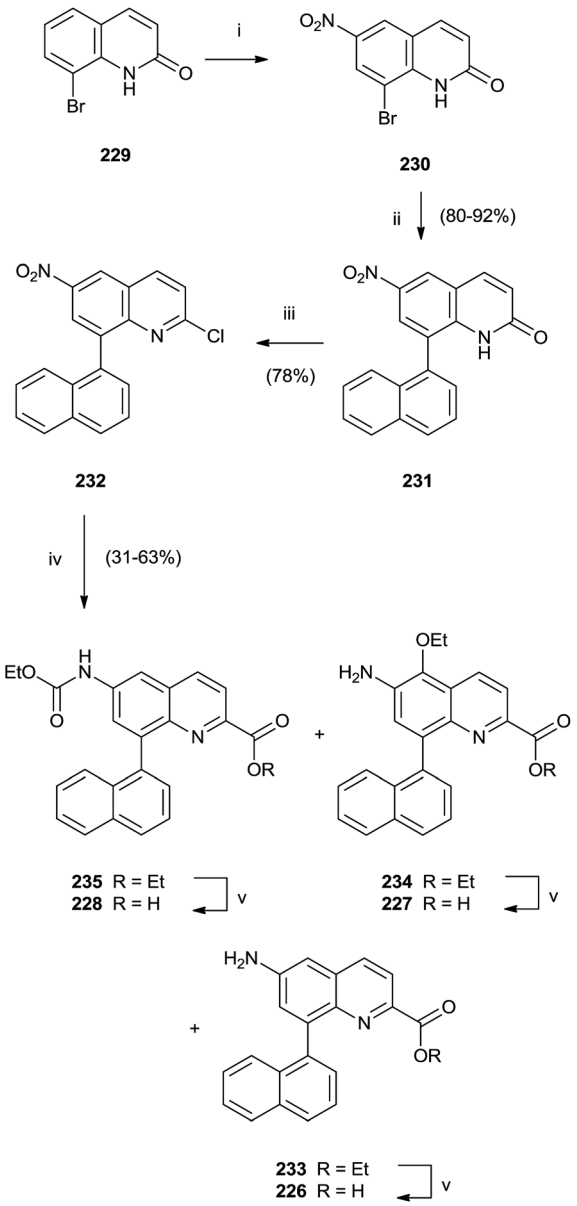
Fig. 6 Quinoline substrates.

para position of the carboxylic acid in the phenyl ring.[144] Compound **220** represented the starting point to synthesize a variety of salicylic acid analogues through an initial reaction with boronic acid by microwave irradiation. The final step with LiOH in MeOH/THF was promoted by the use of microwaves yielding substrates **221** (Scheme 38).

The same authors prepared a variety quinoline substrates **222–228** *via* different synthetic strategies (Fig. 6).

In particular, a unique method allowed the simultaneous synthesis of three active quinoline compounds **226–228** starting from nitration of 8-bromoquinoline-2(1*H*)-one **229** (Scheme 39).

Suzuki coupling of the crude of the nitro-derivatives **230** with naphthyl boronate gave **231** that was converted in **232** with $POCl_3$ in the presence of *N*,*N*-dimethylaniline and Et_4NCl. Palladium-catalyzed carbonylation and reduction of nitro group of **232** produced the intermediate esters **233–235** in moderate yield that were hydrolyzed with LiOH to perform quinolines **226–228**.



Scheme 39 Reagents and conditions: (i) fuming HNO_3, TFA, from 0 °C to rt. (ii) K_2CO_3, boronic acid, $[(C_6H_5)_3P]_2PdCl_2$, DMF/H_2O, 80–92 °C. (iii) $POCl_3$, Et_4NCl, *N*,*N*-dimethylaniline, CH_3CN. (iv) CO, $[(C_6H_5)_3P]_2PdCl_2$, Et_3N, EtOH, 110 °C. (v) LiOH, H_2O/dioxane.

Concluding remarks

Historically, bisphosphonates are benchmark drugs for the treatment of a variety of bone disorders including osteoporosis and bone metastasis. Their inhibitory activity of the isoprenoid biosynthesis resulted in other important applications as modulators of the metabolism of several protozoa parasites thus also being potential therapeutic agents for the treatment of trypanosomiasis (Chagas disease), leishmaniasis, toxoplasmosis and malaria. Bisphosphonates could also be useful in the treatment of other diseases such as breast cancer, myeloma multiple and progeria. Both clinical success in bone disorders and expectative for other diseases prompted the enormous synthetic activity directed to the preparation of bisphosphonates and more recently, nitrogen-containing bisphosphonates which are demonstrated better biological properties. In general, the reaction of carboxylic acids with phosphorous reagents like $POCl_3$ is the preferred approach to bisphophonates. The reaction, however, is very sensitive to steric hindrance and in such cases an Arbuzov-type reaction results more advisable. More recently, the use of tetraethylvinylidenebisphosphonate as the source of phosphorylated part of the molecule has facilitated enormously the access to a variety of bisphophonates. Moreover, the use of such a reagent presented a high tolerance to a variety of functional groups. For the particular case of bisphosphonates lacking the hydroxyl group the alkylation of tetralkylmethyl bisphosphonate is preferred; however, elimination reactions are common undesired lateral processes that, on the other hand, can be eliminated by using precise reaction conditions.

The presence of two phosphate units, in addition to difficult manipulation and purification, limits the oral bioavailability and contribute to undesired side-effects. In this respect, novel bisphosphonate analogues that selectively target FPPS and GGPPS enzymes might provide notable advantages over the currently used drugs and this is now the subject of intense investigation in medicinal chemistry and chemical biology. Achieving this target implies to have at disposition a series of synthetic strategies that allow not only the preparation of the target compound but also structural variations of the parent compound that provide lead compounds for further studies for treatment of the several diseases related to isoprenoid biosynthesis.

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REVIEW ARTICLE

Microwave-Assisted 1,3-Dipolar Cyclo-addition: Recent Advances In Synthesis of Isoxazolidines

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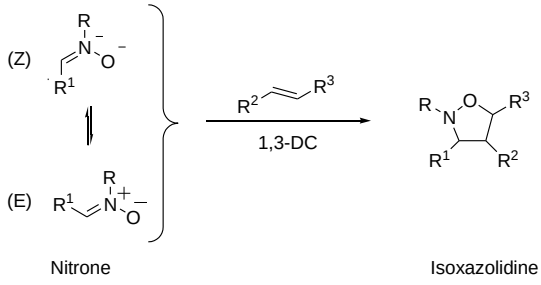
Abstract: The use of microwave irradiation in organic chemistry and, in particular in 1,3-dipolar cycloaddition has recently gained much attention and great progress has been accomplished in this area in the last years. In general, the modern synthetic chemistry is benefited by microwave irradiation that provides an environmentally benign approach, reduced reaction times and a few formation of by-products, increasing the yield. This review aims to collect recent developments on the synthesis of isoxazolidine compounds by microwave-assisted 1,3-dipolar cycloaddition, considering the absence of a recent review about this specific reaction method. Owing to the large extent of available literature, we have decided to restrict our review to the developments in the last six years, making a selection of the literature from 2010 to 2016. The discussion of several examples to construct isoxazolidines by irradiation of microwave will allow to focus on the advances in the development of microwave-assisted 1,3-dipolar cycloadditions, obtaining a broader vision of the real effects of this reaction methodology on yields, regio- and stereoselectivity of the reaction.

Keywords: Dipolar cycloaddition, isoxazolidines, microwave-assisted cycloaddition, nitrones, dipole, dipolarophile, cycloaddition stereochemistry.

1. INTRODUCTION

The reaction of 1,3-dipolar cycloaddition (1,3-DC) is a valid approach to construct a variety of five-membered heterocyclic compounds. In particular, nitrone cycloaddition to olefins is one of the most versatile method for the construction of isoxazolidines where the dipolarophiles are usually alkenes, whereas dipoles are represented by suitable nitrones (scheme **1**) [1-3].



Scheme 1. 1,3-Dipolar cycloaddition of nitrones to olefins.

Three types of selectivity must be considered in 1,3-dipolar cycloadditions: regioselectivity, diastereoselectivity and enantioselectivity.

Their prediction is realized through a consideration of steric and electronic factors, but most significantly through the frontier molecular orbital (FMO) theory [4-5].

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Both steric and electronic effects control the regio selectivity and, generally, in the cycloadditions of electron-rich or electron-neutral alkenes with nitrones, the 5-substituted isomer is obtained with respect to 4-sustituted isomer.

In cycloadditions of nitrones with an alkene the nitrone can approach in an *endo* or an *exo* mode (or *trans*/*cis* mode if the nitrone undergoes *Z*/*E*-interconversion) giving rise to two different diastereomers. The *endo*/*exo* selectivity in the 1,3-dipolar cycloaddition reaction is primarily controlled by the structure of the substrates or by a catalyst. Finally, the only factor present for control of the enantioselectivity is the chiral catalyst.

The classical reaction conditions provide thermal intermolecular or intramolecular 1,3-dipolar cycloadditions (*i.e.* toluene at reflux) but the formation of side products can often be obtained. In the last years, the microwave activation has been successfully applied to 1,3-dipolar cycloadditions, obtaining reactions cleaner, faster and with higher selectivity than conventional method. Moreover, the use of solvent-free conditions furnishes an environmental friendly methodology.

2. MICROWAVE-ASSISTED 1,3-DIPOLAR CYCLOADDITION

The beneficial effects of microwave irradiation are finding an increased role in process chemistry, especially in cases when usual methods require drastic reaction conditions or prolonged times. This non-conventional energy source is able to reduce chemical reaction time and the formation of by-products, increasing the yields [6-7]. In the last years,

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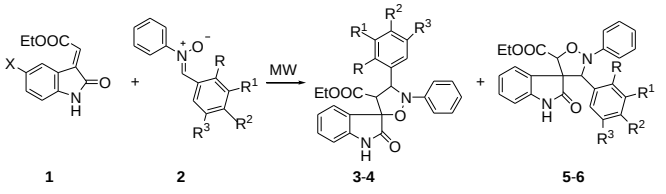
cycloaddition reactions have greatly benefited from the use of focused microwave-irradiation, by promoting the reactions at elevated temperatures in few minutes and thus avoiding many of the critical issues under conventional heating conditions. This chiefly includes the circumventing of the use of elevated reaction temperatures for lengthy times, thus avoiding polymerizations, decomposition of rather sensitive reagents *etc*. [8]. Therefore, all these problems have been solved by the rapid heating induced by microwave [9, 10].

However, the results cannot be explained solely by the effect of rapid heating but it is possible to invoke a specific radiation effect, called "the microwave effect" [11, 12]. Generally, the microwave irradiations are able to modify chemo-, regio- or stereoselectivity of the reaction. However, in literature, few examples of changes in 1,3-dipolar cycloaddition reactions are described because of the selectivity of concerted processes is prevalently due to frontier orbital interactions [13-15]. Solvent effect is really important in microwave-assisted 1,3-dipolar cycloaddition. In fact, the use of polar solvents in cycloaddition reactions seems to produce "hot spots", especially at the interface, affecting the performance of the reaction [16, 17]. Solvent-free conditions allow several evident advantages: i) the radiation is absorbed directly by the reagents, amplifying the effect of microwave; (ii) the reactivity is enhanced because of increased concentration; (iii)v work-up procedures are simplified; (iv) solid support can be used efficiently, *etc* [18-20].

2.1. Spiro-Isoxazolidines

The biological importance of compounds containing spiro carbon at C-3 position of indoline or oxindole skeleton has been emphasized in the literature [21-22].

More recently, the regioselective synthesis of spiro-indoline-isoxazolidines was achieved by the microwave-assisted cycloaddition between ethyl (5-fluoro-2-oxo-1,2-dihydro-3(*H*)-indol-3-ylidene)-acetate/ethyl(2-oxo-1,2-dihydro-3(*H*)-indol-3-ylidene)-acetate (**1**) and substituted α,*N*-diphenylnitrones (**2**) (scheme **2**) [23].



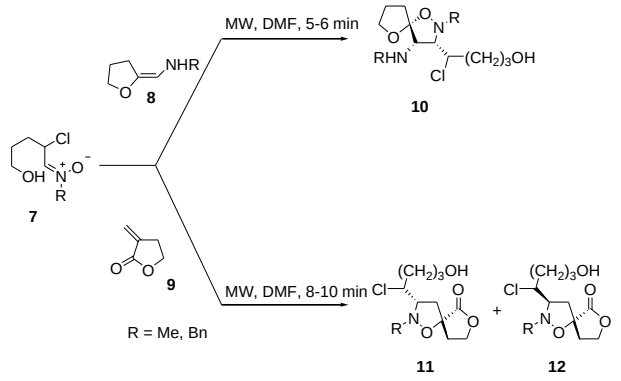
X = F, H; R = H, OMe; R^1 = H, Me, OMe, F, Cl, Br, NO_2;

R^2 = H, Me, CN, CF_3, OMe, OEt, F, Cl, Br, NMe_2, NO_2; R^3 = H, OMe

Scheme 2. Synthesis of spiro-indoline-isoxazolidines.

An initial multi-step approach was conducted to synthesize the precursors **1**, which were reacted with α,*N*-diphenylnitrones **2** under microwave irradiation (850 W) and solvent-free conditions, isolating two series of spiro-indoline-isoxazolidines (**3-4** and **5-6**). The regioselectivity of reaction was totally reversed for substrates with a fluorine atom in C-5 position of isatine ring, because of a probable exocyclic double bond shifting in (5-fluoro-2-oxo-1,2-dihydro-3(H)-indol-3-ylidene)-acetate/ethyl (2-oxo-1,2-dihy-dro-3(H)-indol-3-ylidene)-acetate (**1**) in two different ways. It is possible that the high electronegativity of fluorine atom decreases the electronic density of aromatic ring, favoring the conjugation of nitrogen lone pair toward aromatic group. In this way, the π-electrons polarize towards C-3 respect to C-10; differently the polarizability of the π-electrons favors C-10 position when a less electronegative hydrogen atom replaces fluorine atom in C-5. NMR studies allowed the assignment of the absolute configuration of the three newly formed stereocenters in (*R*) C-3', (*R*) C-5' and (*S*) C-4' for **3** and **4** while (*R*) C-3', (*R*) C-5' and (*R*) C-4' for **5** and **6**. All substrates were checked as anti-inflammatory against human umbilical vein endothelial cells (HUVECs), observing significant inhibitory activity.

In 2012, the formation of 5-spiro isoxazolidines **10**, **11** and **12** was performed using α-chloronitrones (**7**) as dipole and α-*N*-methyl/phenyl furan derivatives (**8**) or α-methylene-γ-butyrolactone (**9**) as dipolarophiles under irradiation of domestic microwave oven for a specific time (5-10 minutes) (scheme **3**) [24].

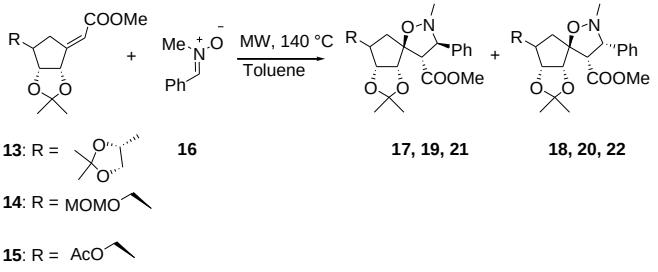


Scheme 3. Microwave-assisted synthesis of novel spiro-isoxazolidines.

Excellent regio- and diastereoselectivity towards 5-isomer *via* an *exo*-approach of *Z*-nitrone were observed in reaction with α-*N*-methyl/phenyl furan derivatives, while good diastereofacial selectivity was obtained in reaction with α-methylene-γ-butyrolactone, in which a mixture of diastereisomers was evidenced in the ratio approximatively 75:25 in favor of *exo*-cycloadducts. In subsequent year, the work was extended to the synthesis of various 5-spiro isoxazolidines deriving from α-chloronitrones and α-*N*-substituted furan derivatives by varying substituent groups of starting materials, with the further purpose of transforming them into corresponding 1,3-aminoalcohols [25].

In 2016, *gulo* and *ribo* furano-*exo*-glycals **13**-**15** and nitrone **16** were used to synthesize spiro sugar-isoxazolidines (Scheme **4**) [26].

Concerning *E*-configured *gulo* derivative **13**-*E*, cycloaddition with **16** in toluene under microwaves for 2 hours led to an inseparable mixture of C-3 isomers, thus preventing the use of these adducts for further developments.



Scheme 4. Microwave-assisted synthesis of spiro sugar-isoxazolidines.

Starting from **13**-*Z*, two diastereoisomers **17** and **18** were obtained as pure material in high yields and good selectivity [27-29]. *Exo*-glycal **14**-*Z* bearing a methoxymethyl protecting group was also chosen and two cycloadducts **19** and **20** were obtained after cycloaddition with nitrone **16** (yield: 94%). However, attempts at selective deprotection of methoxymethyl group proved unsuccessful. To side-step this problem, the reaction was performed on *exo* glycal **15**-*Z* with an acetate protection. Cycloaddition reaction with nitrone **16** was performed in toluene at 140 °C under microwave activation giving a set of two separable cycloadducts **21** and **22** in 87% overall yield.

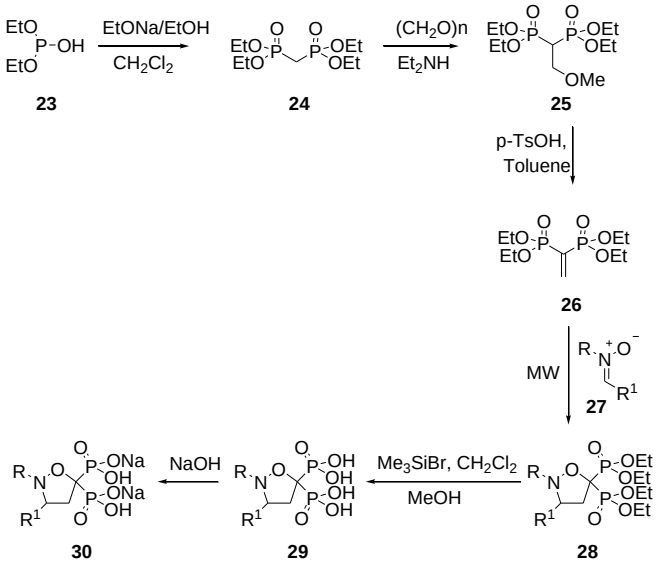
Spiro sugar-isoxazolidines obtained by 1,3-dipolar cycloaddition of activated *exo*-glycals and nitrones were efficiently functionalized at two sites, *i.e.* C-4 and C-7, with arginine, arginine mimetics and guanidylated appendages to using as polyfunctional building block for peptidomimetics design.

2.2. Bisphosphonated Isoxazolidines

Geminal bisphosphonates (BPs) are structural and stable analogues of naturally occurring pyrophosphates and constitute an important class of pharmacologically active molecules used in the treatment of bone diseases as osteoporosis, Paget's disease and tumor bone diseases [30-33].

The studies on the inhibitory potency of nitrogen cycles containing bisphosphonates indicate that the presence of two geminal phosphonate groups is determinant for interaction with the molecular target. In addition, a basic nitrogen in the heterocyclic side chain affects potency. Therefore, a recent proposal considered the synthesis of a new class of bisphosphonates having in *gem* position an isoxazolidine ring that simultaneously holds the required basic nitrogen and an oxygen atom in place of the hydroxy group, acting as third hook [34].

The key step in the synthesis involved the formation of dipolarophile tetraethylvinylidene-1,1-bisphosphonate **26** that was prepared in high yields with a three step-reaction, starting from diethyl posphite (**23**) and sodium ethoxide (Scheme **5**).



R = Me, Bn; R^1 = Ph, *o*-Cl-C6H4, 3-Pyridyl, 2-Furyl, *p*-OH-C_6H_4, *o*-F-C_6H_4

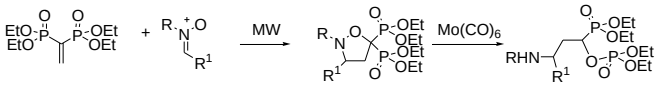
Scheme 5. Synthetic process to obtain bisphosphonate isoxazolidines **28** and their sodium salts **30**.

The final cycloaddition reaction of nitrones **27** with bisphosphonate vinyl derivative (**26**) came as result of several attempts to optimize the reaction protocol that preferred free-solvent MW irradiation to conventional conditions (toluene or dichlorometane at reflux) or presence of Lewis acids as catalyst.

A low MW power (200W), a short reaction time (10-20 min) and a slight excess of nitrone were required to complete the cycloaddition, isolating bisphosphonate isoxazolidines **28** in high yields. The regiochemistry of the reaction, detected by decoupling ^{1}H NMR experiments, followed the usual pattern with exclusive formation of the 5-bisphosphonated isomer over the 4-derivative. Accordingly, the cycloaddition takes place between the *Z*-nitrone and the vinylidene bisphosphonate, with attack of the *N*-oxygen atom on the germinal carbon of the vinylidene group. Considering that bisphosphonates are calcium-regulating agents used in the form of the sodium salt, the synthetized bisphosphonate esters (**28**) were hydrolyzed and the resulting acids **29** were transformed into the corresponding disodium salts **30** by reaction with two equivalent of aqueous sodium hydroxide, to exalt their biological activity.

In 2014, an application of isoxazolidine-substituted bisphosphonates was proposed through their transformation into molecules containing *gem*-phosphonate-phosphate group [35]. The literature reports that *gem*-phosphonate-phosphate derivatives shows an interesting biological activity in the treatment of sclerosis and diseases associated to the poor production of apoliprotein E [36].

Therefore, a set of bisphosphonates bearing a substituted isoxazoldine ring in germinal position (**32**) was prepared by eco-friendly regioselective cycloaddition (solvent-free condition, MW irradiation). Successively, the isoxazolidine ring was opened through cleavage of the *N*-*O* bond, producing *gem*-phosphonate-phosphate substrates **33** (Scheme **6**).



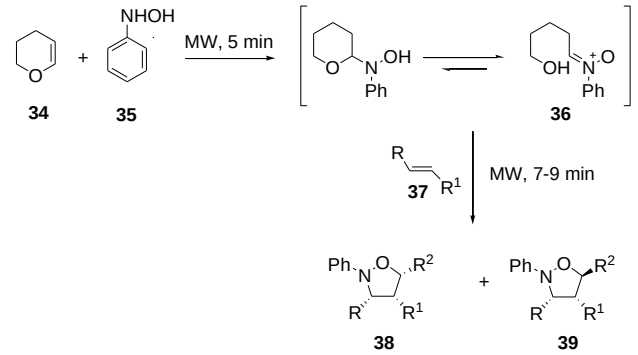
R = Me, Bn; R^1 = Ph, 2-Cl-C_6H_4, 4-Cl-C_6H_4,Naphthyl, 2-F-C_6H_4

Scheme 6. Synthesis and ring opening of bisphosphonate isoxazolidines **32**.

2.3. Fused Isoxazolidines

In 2012, solvent-free microwave method was used to perform a green synthesis of heterocycloadducts, starting from dihydropyran derived nitrone **36**, prepared *in situ* by treating 2,3-dihydro-4*H*-pyran (**34**) with *N*-phenylhydroxilamine (**35**) [37].

A set of cyclic and acyclic alkenes **37** was reacted with non-isolable nitrone **36** in absence of solvents and by MW irradiation, achieving prevalently bicyclic *cis*-isoxazolidines **38** with high yields and moderate diastereofacial control (Scheme **7**).

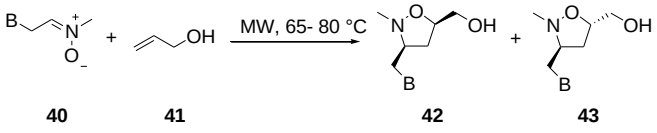


Scheme 7. Strategy to synthesize novel isoxazolidine derivatives **38** and **39**.

The thesis of an *exo*-approach of *Z*-nitrone for the formation of major product was supported as well as in the transition state, because of the 4,5-fused pyrrolidindione, the isoxazolidine ring adopts an envelope conformation with nitrogen atom directed out of the envelope (minor conformation) or inside (major configuration). In all enantiomers the configuration of H-3, H-4 and H-5 are *cis* as well as in the case of the two examples of cycloaddition with acyclic nitrones. Moreover, a useful application of the obtained isoxazolidines **38** and **39** was represented by their subsequent conversion to 1,3-aminoalcohols by Zn powder in acetic acid under microwave irradiation.

2.4. Homonucleosides

Based on the idea of replacing a furanose ring with a isoxazolidine moiety and introducing a methylene bond between the sugar mimetic ring and the nucleobase, MW-induced 1,3-dipolar cycloadditions between nitrones of nucleobases **40** and allyl alcohols **41** were conducted to afford homonucleosides analogs **42** and **43** (scheme **8**) [38].

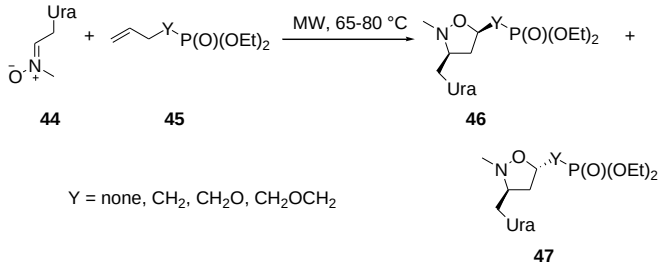


Scheme 8. Synthesis of homonucleosides **42** and **43** by microwave irradiation.

Excellent regioselectivity towards the 5-substituted isoxazolidines was observed, while moderate to good diastereofacial selectivity in favor of the *cis* isomer **42** was obtained, as confirmed by 2D NMR spectral data. The comparison of the results with those obtained by thermal condition highlighted the remarkable reduction of reaction time (from 15h to 2.5h).

Moreover, under same reaction conditions, uracil-derived nitrone **44** was reacted with vinyl-, allyl-, vinyloxymethyl- and allyloxymethylphosphonates **45** to form a mixture of two diastereoisomers of 5-phosphonated homonucleosides **46** and **47** at greater abundance to *cis* isomer (**46**) than *trans* isomer (**47**) (scheme **9**).

The *cis* and *trans* configuration of the all diastereoisomers were assigned through ^{1}H and ^{13}C NMR spectra.

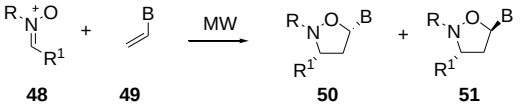


Scheme 9. Formation of 5-phosphonated homonucleosides **46** and **47**.

2.5. 3'-Substituted-4'Aza-2',3'-Dideoxynucleosides

N-vinylnucleobases may represent important starting materials for the synthesis of *N*,*O*-nucleoside analogues [39-42].

Therefore, exploiting the protocol for the direct vinylation of nucleobases through the reaction between transient protected nucleobases and vinyl acetate [43], the generation of a class of 3'-substituted-4'aza-2',3'-dideoxynucleosides **50** and **51** was obtained by reacting unprotected vinylnucleobases **49** and suitable nitrones **48** in the absence of solvent and /or catalyst using MW irradiation (scheme **10**) [44].



Scheme 10. Synthesis of *N*, *O*-nucleosides *via* cycloaddition of nitrones **48** and vinylnucleobases **49**.

The cycloadducts are formed in good yield and with a remarkable *cis–trans* selectivity, in some cases higher than 99:1 (de 98%). The stereoselectivity of the reaction may be predicted taking into account the possible geometries of approach of the two reacting species. The obtainment of the *cis* adduct **50** is explained by invoking either an *exo*-approach of the alkene to the (*Z*) nitrone isomer or an *endo* approach to the (*E*) nitrone isomer. The corresponding opposite parallel may be done in the case of *trans*-cycloadducts **51**. Considering that *N*-tert-butyl or *N*-aryl nitrones exist almost exclusively as (*Z*)-isomers, the approach for this cycloaddition was predominantly of (*Z*)-*exo* type. In contrast, with *N*-methyl, *N*-phenyl, and *N*-benzyl nitrones it was possible the formation under microwave conditions of small quantities of (*E*) isomers by possible *E*/*Z* interconversion, giving account for the formation of minor amounts of *trans*-cycloadducts.

Variable-temperature NMR spectra showed the presence of the sole (*Z*)-isomer of nitrone, even after heating at 80 °C for 24 h or after MW-irradiation for 10 min, thus excluding an *E*/*Z* isomerisation. Consequently, the formation of minor amounts of *trans*-cycloadduct may be explained by the oc-

currence of a second reaction pathway for the diene-dienophile approach, that is, the (*Z*)-*endo*. This latter reaction channel is not active in the case of bulky *N*-substituents due to a disfavored transition state (TS 1) if compared with the approach of smaller *N*-alkyl nitrone derivatives to the dienophile (TS 2) (Fig. **1**).

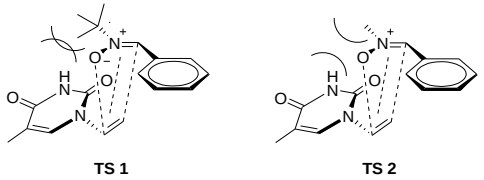
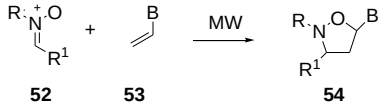


Fig. (1). Model transition states for the diene-dienophile approach.

Most of the N,O-nucleoside derivatives prepared according to this procedure were evaluated by in vitro assays for their antiproliferative activity against human lymphoblastoid cell lines (LCL), Ji-Joye cells, an EBV-positive Burkitt cell line and Jurkat cells, a human T-cell lymphoblast-like cell line with good capacity to inhibit the cancer cell growth at a relatively low concentration.

Successively, in 2014, a small library of 3'-substituted-4'aza-2',3'-dideoxynucleosides **54** were synthesized through same reaction conditions by investigation of variations in the four canonical quadrants according to specific absorption rate (SAR) tests [45] designed to prove the minimal structural requirements for antiproliferative activity in the NCI 60 panel of human cancers (scheme **11**) [46].



B = Thy, 5-FUra

R = Me , *t*-butyl, Bn

R^1 = Ph, 2-Cl-C_6H_4, 2-F-C_6H_4, 4-Cl-C_6H_4, 3-pyridyl, 1-naphthyl

Scheme 11. Synthesis of *N*, *O*-nucleosides by microwave-induced 1,3-dipolar cycloaddition.

SE quadrant was varied with thymine or fluorouracil as nucleobases, while NW and SW quadrants were modified with various R and R^1 substituents, respectively (Fig. **2**).

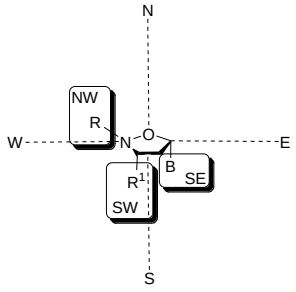
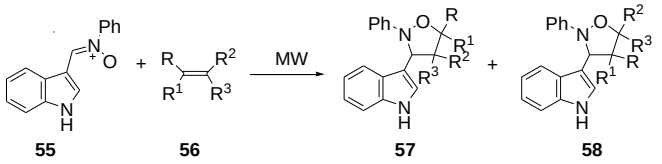


Fig. (2). Representation of isoxazolidines in quadrants for changes of B, R and R^1.

The structural changes planned by SAR tests were crossed with physicochemical data related to solubility and permeability [47].

The combination of these data with *in vitro* test of all isolated substrates confirmed that thymine, *N*-benzyl substituents and aromatic rings were the optimal combine for biological activity against different lines of ovarian and colon carcinoma.

Pyridinyl isoxazolidines are substrates with a pyridyl group in the C-3 position of isoxazolidine ring, which have exhibited good anticancer activities [48-50]. Recently, a substitution of pyridyl group with an indole ring was proposed and 3-indolyl-isoxazolidines (**57** and **58)** were synthesized under solvent-free microwave irradiation conditions in stereoselective manner with high yields. The cycloadditions were performed between *C*-(3-indolyl)-*N*-phenylnitrone **55** and mono-substituted, disubstituted and cyclic dipolarophiles **56** at 150 W and 100 °C in a short time (scheme 12) [51].



R = H, Me, C(O)-N(Ph)-C(O); R^1 = H; R^2 = H, Me, C(O)-N(Ph)-C(O)

R_3 = Ph, CO_2CH_3, pyridyl, CN, $CONH_2$, $COCH_3$

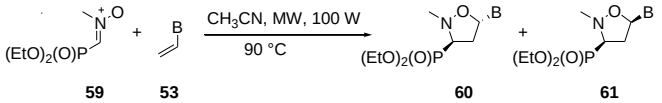
Scheme 12. Synthesis of substituted 3-indolyl-isoxazolines.

All isoxazolidine analogues **57** and **58** were obtained in high yield and good stereoselection.

2.6. Phosphonated *N,O*-Nucleosides

Truncated phosphonated carbocyclic 2'-oxa-3'-aza nucleosides (TPCOANs) are a class of *N,O*-nucleosides characterized by a phosphonate group directly linked to the *C*4'-position of the isoxazolidine moiety, which mime the first monophosphate group of natural nucleosides.

Recently, a one-step procedure was suggested for the formation of TPCOANs [52].



B = Thy, 5F-Ura

Scheme 13. Synthesis of truncated phosphonated carbocyclic 2'-oxa-3'-aza nucleosides.

Vinylnucleobases **53** as dipolarophile and phosphonate nitrones **59** as dipole were employed in 1,3-dipolar cycloaddition (scheme **13**) and, by careful choice of solvent and reaction conditions (acetonitrile, MW, 100W, 90 °C) it was possible to isolate final phosphonated cycloadducts **60** and **61** with good yields and high distereomeric selectivity towards *trans*-isomer (**60**), as confirmed by 2D NOE NMR experiments.

3. CONCLUSION

Microwave-assisted cycloaddition reactions give rapid access to fused multi-cyclic and heterocyclic skeletons in a seemingly graceful and exceedingly selective single-step operation. The driving force for all recent reported developments in the isoxazolidine synthesis by 1,3-dipolar cycload-

dition reactions has been cleaner reactions, faster and with higher selectivity than conventional method.

In conclusion, 1,3-dipolar cycloadditions induced by MW irradiation are highly attractive reactions for the synthesis of heterocycles and other heteroatom-containing molecules.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

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Nitrones and nucleobase-containing spiro-isoxazolidines derived from isatin and indanone: solvent-free microwave-assisted stereoselective synthesis and theoretical calculations†

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The spiro-oxindoles have found wide application because of their antiviral properties. However, in the literature few examples of synthesis of their precursors, oxindole-nitrones, are reported. In this paper, we initially present a rapid and efficient synthetic approach to ketonitrones by solvent-free microwave-assisted reaction between isatin or indanone derivatives and various hydroxylamines. The synthetic protocol is facile, clean, fast, high-yielding and stereoselective. Then, we explored the possibility to synthesize nucleobase-containing spiro-isoxazolidines with isatin and indanone nuclei by solvent-free MW-assisted 1,3-dipolar cycloaddition, obtaining good results in yields (74–85%), and regio- and diastereoselectivity. Theoretical calculations were done to analyze the difference of reactivity of isatin and indanone derivatives with hydroxylamines.

Introduction

The spiro-oxindole scaffold is the characteristic structural core of numerous alkaloids and unnatural biologically active compounds.[1] Inspired by this important moiety, a number of methods to synthesize isatin and oxindole derivatives were realized during the last few years. In fact, the biological importance of compounds containing spiro-carbon at the C-3 position of the indoline or oxindole skeleton has recently been emphasized in the literature.[2,3] In particular, spiro-oxindoles and spiro-isoxazolidines possess antiviral activity for various human diseases, inhibiting poxvirus,[4] ectromelia,[5] rhinovirus,[6] HIV-1 (ref. 7) and, more recently, as inhibitors of MDM2/p53 interaction.[8,9]

The 1,3-dipolar cycloaddition between a ketonitrone and a vinylic substrate may be considered a common procedure for the preparation of these compounds.[10–12] Although a broad range of methodologies to synthesis of aldonitrones is available today,[13–20] in contrast the preparation of ketonitrones is not always accomplished by simple condensation reaction.[21–23] A thorough search of the relevant literature yielded only few articles related to synthetic procedures of isatin and oxindole nitrone.[24,25] In particular, a methodology regards the preparation of N-substituted isatin nitrones *via* a multi-step reaction sequence.[26]

Recently, the synthesis of (*Z*)-*N*-aryl oxindole nitrones was performed through a *N*-arylation of 3-(hydroxyimino) indolin-2-ones with diaryliodonium salts, demonstrating the current interest in having efficient routes towards those sort of nitrones.[27] On the contrary, to our knowledge, methodologies of indanone nitrones are not present in literature.

As a result, the development of a simple and convenient method for the synthesis of isatinyl and indanyl nitrones realized from ketones and hydroxylamines through mild conditions could be highly desirable.

In recent years, the use of microwave technology in organic chemistry, allows to prepare organic compounds very fastly, with high purity and better yield respect to other conventional methods.[28,29]

Recently, we described a green approach to synthesize aldo- and ketonitrones by solvent-free condensation of alkyl- or aryl-hydroxylamines hydrochlorides with aromatic aldehydes and ketones under microwave irradiation, bypassing the critical results obtained with ketonitrones.[30]

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Then, in present work, we firstly describe a facile synthesis of isatinyl and indanyl nitrones *via* microwave-assisted reaction between ketones and hydroxylamines under solvent-free conditions. In our approach, we observed very short reaction times and a reduced formation of by-products, isolating the expected products in high yields and excellent stereoselectivity. Successively, we report for the first time a regio- and diastereomeric synthesis of nucleobase-containing spiro-isoxazolidines with an indoline or an indane ring by MW-assisted 1,3-dipolar cycloaddition, obtained in yields between 74–85% and *via* solvent-free procedure.

Results and discussion

To find the optimal conditions for the synthesis of nitrones, we chose *N*-methylhydroxylamine hydrochloride **1a** and 1-isatin **2a** and indanone **3a** as ketones. The methodology consists of the co-grinding of the ketone and hydroxylamine in a mortar, followed by transfer of the mixture in a sealed vessel and further mixing by a vortex; finally the mixture is placed in a microwave oven. The optimization study is collected in Table 1.

The best conditions for both ketones (entries 5 and 11) were those corresponding to an irradiation of 600 W ($T = 180$ °C) for 10 min for ketone **2a** and an irradiation of 400 W ($T = 180$ °C) for 30 min for ketone **3a**. The corresponding products **4a** and **5a** were obtained in 97% and 82% chemical yield, respectively. In both cases, nitrones were obtained as single isomers, *E*-**4a** and *Z*-**5a** respectively, as confirmed by NOESY experiments. When the reaction was carried out in EtOH/H_2O as a solvent and in the presence of 2 equivalent of sodium acetate as reported in literature on similar substrates,[31] compounds **4a** and **5a** were obtained in 70% and 78% chemical yield, respectively, in 24 hours (entries 7 and 12). Moreover, the presence of sodium acetate in reaction mixture does not significantly change the trend of the reaction (entry 6) and therefore we chose not to use it.

In an effort to expand the scope of the reaction, we explored coupling of substituted ketones with *N*-methyl hydroxylamine hydrochloride (**1a**) *N*-benzyl hydroxylamine hydrochloride (**1b**),[32] *N*-phenyl hydroxylamine (**1c**),[33] *N*-3-chloro-phenyl hydroxylamine (**1d**) and *N*-*t*-butylhydroxylamine hydrochloride (**1e**) (Table 2).

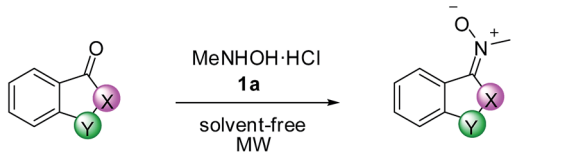
The reaction works well with various isatin ketones **2** and we noticed that the presence of an electron-withdrawing group (Br or NO_2) on C-5 of phenyl ring of isatin does not significantly alters their performance (entries 8–13). Methylation of nitrogen does not reduce the reactivity (entries 4–7). Phenylhydroxylamine **1c** was used in further excess (ratio ketone/phenylhydroxylamine 1 : 3) because of its known degradability at temperature higher than 50 °C.[34] Attempts to reduce by-products by lowering power of MW (200 or 400 W) or by reducing the maxim temperature were unnecessary. In all cases the *E*-isomer was the only obtained with the exceptions of compounds **4d** and **4h** for which small quantities of *Z* isomers (ratio *E*/*Z*: 78 : 22 and 75 : 25 for **4d** and **4h**, respectively) were obtained. In general, ketones **3** present lower reactivity with respect to isatin ketones **2**. In all cases, the *Z*-isomer was the only obtained. When an alkyl group is present on the 2-C or 3-C of the carbon backbone of indanone ring (entries 17 and 18), the yield drops presumably due to a minor electrophilicity of carbonyl group. The propyl group introduces additional steric hindrance, and the yield drops to 20% (entry 19).

The presence of Br or F in C-5 of indanone ring (entries 20 and 21) does not produce significant differences of reactivity respect to unsubstituted indanone. On the other hand, the reaction is very sensitive to the size of the cycloalkyl ring (tetralone *versus* indanone). Tetralone derivatives showed very little reactivity and longer reaction times than other substrates (entries 25–27) are required for obtaining just traces of products.

Moreover, resonance effect in C-4 or in C-7 of isatine and indanone ring (entries 14, 22 and 23) causes major reaction times and/or minor yields because the electrophilicity of the acceptor carbonyl group is reduced. Unfortunately, the large size of *t*-butyl group of **1e** dramatically influences both reactions of isatin **2a** and indanone **3a**, not leading to the formation of any product (entries 15 and 24). Attempts to carry out the reactions using the conditions previously applied in entries 7 and 12 of Table 1 confirmed the reaction trend, revealing that these reactions are sensitive to steric effects.

As a follow-up work, some nucleobase-containing spiro-isoxazolidines with an indoline or an indane ring were synthesized by MW-assisted 1,3-dipolar cycloaddition between nitrones as dipole and vinylnucleobases as dipolarophile. It is generally been recognized that the incorporating of different bioactive scaffolds into one molecule is the powerful strategy to

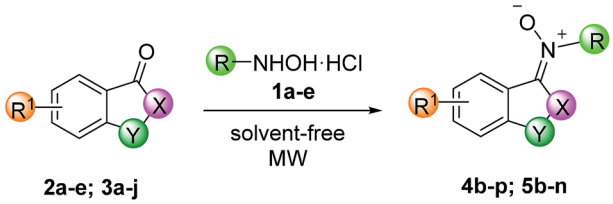
Table 1 Optimization of isatinyl and indanyl nitrones synthesis[a]



Entry	Ketone[b]	MW (W)	T (°C)	Time (min)	Product	Yield (%)
1[c]	**2a**	200	180	10	**4a**	33
2[c]	**2a**	400	180	10	**4a**	42
3	**2a**	400	180	10	**4a**	53
4	**2a**	400	180	20	**4a**	61
5	**2a**	600	180	10	**4a**	97
6[d]	**2a**	600	180	10	**4a**	80
7[e]	**2a**	—	rt	1440	**4a**	70
8	**3a**	600	180	10	**5a**	37
9	**3a**	600	180	15	**5a**	51
10	**3a**	400	180	10	**5a**	25
11	**3a**	400	180	30	**5a**	82
12[d]	**3a**	—	rt	1440	**5a**	78

[a] Reaction conditions: 2.0 eq. of **1a** were used unless otherwise indicated. [b] 1.0 eq. was used. [c] 1.0 eq. of **1a** was used. [d] 1.0 eq. of NaOAc was added. [e] 1 : 1 EtOH/water was used as a solvent and 2.0 eq. of NaOAc were added.

Table 2 Synthesis of nitrones 4 and 5



Entry	Hydroxylamine	R	Ketone	X	Y	R^1	Time (min)	Product	Yield (%)
1	**1b**	Bn	**2a**	C=O	NH	H	12	**4b**	95
2	**1c**	Ph[a]	**2a**	C=O	NH	H	10	**4c**	92
3	**1d**	3-Cl-Ph	**2a**	C=O	NH	H	10	**4d**	87
4	**1a**	Me	**2b**	C=O	NMe	H	10	**4e**	90
5	**1b**	Bn	**2b**	C=O	NMe	H	12	**4f**	92
6	**1c**	Ph[a]	**2b**	C=O	NMe	H	10	**4g**	90
7	**1d**	3-Cl-Ph	**2b**	C=O	NMe	H	10	**4h**	88
8	**1a**	Me	**2c**	C=O	NH	5-Br	10	**4i**	95
9	**1b**	Bn	**2c**	C=O	NH	5-Br	10	**4j**	93
10	**1c**	Ph[a]	**2c**	C=O	NH	5-Br	10	**4k**	92
11	**1a**	Me	**2d**	C=O	NH	5-NO_2	10	**4l**	95
12	**1b**	Bn	**2d**	C=O	NH	5-NO_2	13	**4m**	92
13	**1c**	Ph[a]	**2d**	C=O	NH	5-NO_2	10	**4n**	92
14	**1a**	Me	**2e**	C=O	NH	5,7-Cl	25	**4o**	80
15	**1e**	*t*-Bu	**2a**	C=O	NH	H	60	**4p**	—
16	**1b**	Bn	**3a**	CH_2	CH_2	H	30	**5b**	85
17	**1a**	Me	**3b**	CHMe	CH_2	H	30	**5c**	61
18	**1a**	Me	**3c**	CH_2	CHMe	H	35	**5d**	78
19	**1a**	Me	**3d**	CH^nPr	CH_2	H	32	**5e**	20
20	**1a**	Me	**3e**	CH_2	CH_2	5-Br	30	**5f**	88
21	**1a**	Me	**3f**	CH_2	CH_2	5-F	28	**5g**	83
22	**1a**	Me	**3g**	CH_2	CH_2	4-Br-7-OH	60	**5h**	67
23	**1b**	Bn	**3g**	CH_2	CH_2	4-Br-7-OH	48	**5i**	78
24	**1e**	*t*-Bu	**3a**	CH_2	CH_2	H	60	**5j**	—
25	**1a**	Me	**3h**	CH_2	CH_2CH_2	H	50	**5k**	Traces
26	**1b**	Bn	**3h**	CH_2	CH_2CH_2	H	50	**5l**	Traces
27	**1a**	Me	**3i**	CH_2	CH_2CH_2	7-F	45	**5m**	Traces
28	**1a**	Me	**3j**	CH_2	CH_2CH_2	6-OMe	55	**5n**	—

[a] Ketone/phenylhydroxylamine ratio 1 : 3.

construct substrates with structural novelty and biological potential.

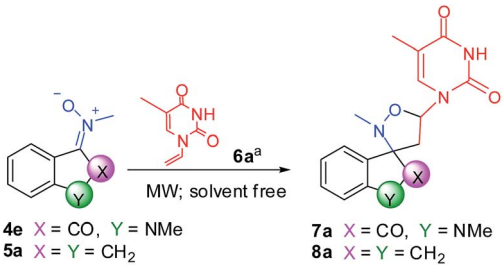
Considering that, some spiroindoline–isoxazolidines have shown different degrees of anticancer activity mainly based on the isoxazolidine ring fused at C-3 position of oxindole backbone,[35–37] we hypothesized that the simultaneous presence of an spiro carbon, an oxindole-like ring, an isoxazolidine portion and a nucleobase might generate novel molecular entities with amplified anticancer activity as, for example, inhibitors of MDM2/p53 interaction. Moreover, to our knowledge, in literature spiro-isoxazolidines with nucleobases and indoline or indane ring on their backbone have been never synthesized.

As is well known, the typical insertion of a nucleobase on a sugar or isoxazolidine scaffold consists of the method of Vorbrüggen,[38] but for a long time now, we have developed a simple method by solvent-free MW-assisted 1,3-dipolar cycloaddition between nitrones as dipole and vinylnucleobases[39] as dipolarophile.[40]

The formation reactions between *N*-1-vinylthymine **6a** and nitrones **4e** and **5a**, respectively, were taken as examples for optimization of reaction conditions. The target conjugates were prepared following the synthetic procedure similar to ketonitrones previously prepared, consisting of the co-grinding of the ketonitrone and vinylnucleobase in a mortar, followed by transfer of the mixture in an appropriate vessel and further mixing of the solids in a vortex without use of solvents; finally the solid mixture was placed in a microwave oven. The results are summarized in Table 3.

We prevalently changed the MW power that, based upon our experience, turns out to be the key parameter in MW-assisted cycloadditions, by keeping the nitrone/vinylnucleobase ratio to 2 : 1, respectively. The optimized conditions for the formation of **7a** are listed in entry 3 (Table 3). On the contrary, the indanone nucleus obliged us to reduce maintained temperature (T = 125 °C) to avoid the formation of a lot quantity of by-products (entries 5–10, Table 3). Despite everything, reaction

Table 3 Optimization of synthesis of nucleobase-containing spiro-isoxazolidines **7a** and **8a**



Entry	Nitrone[b]	MW (W)	T (°C)	Solvent	Time (min)	Product	Yield (%)
1	**4e**	650	180	—	30	**7a**	59
2	**4e**	750	180	—	20	**7a**	62
3	**4e**	850	180	—	10	**7a**	85
4	**4e**	—	110	Toluene	4320	**7a**	25
5	**5a**	650	180	—	10	**8a**	22
6	**5a**	650	80	—	30	**8a**	34
7	**5a**	650	125	—	30	**8a**	38
8	**5a**	750	80	—	10	**8a**	41
9	**5a**	750	100	—	30	**8a**	61
10	**5a**	750	125	—	30	**8a**	77
11	**5a**	—	110	Toluene	4320	**8a**	20

[a] 1.0 eq. of **6a** were used. [b] 2.0 eq. was used.

times were major respect to isatin derivatives and yields were good (entry 10, Table 3). Finally, we compared our results with those obtained from classical conditions of 1,3-dipolar cycloaddition, using toluene and reflux (entries 4 and 11, Table 3). It is possible to highlight that reaction times had to be extended to three days, obtaining very poor yields and many by-products.

Then, the reaction conditions of **7a** and **8a** were expanded to the formation of some substituted nucleobase-containing spiro-isoxazolidines, using various our nitrones and some vinyl-nucleobases (*N*-1-vinylthymine **6a** or *N*-1-vinyluracil **6b**). The results are collected in Table 4. All cycloadducts were isolated in good yields and short reaction times (8–30 minutes). The effect of substituents on indoline ring was also investigated, observing slightly yields in presence of bromo or nitro group as substituents because of formation of by-products (entries 5–7, Table 4). All cycloaddition reactions were highly regioselective and goodly diasteroselective, furnishing only one regioisomer (5-substituted) and prevalent *exo*-adduct (Table 4), as confirmed through NOESY experiments. In particular, we observed a specific correlation between the proton on C-6 of thymine or uracil and the proton on C-4 of aromatic ring, only possible in the *exo* approach of cycloaddition.

Theoretical calculations

In order to correlate the reactivity of ketones with hydroxylamines we evaluated the electrophilicity of both reagents by calculating ω reactivity index. The ω index establishes an absolute scale of electrophilicity in the sense that the hierarchy of electrophilicity is built up from the electronic structure of molecules. The electrophilicity hierarchy may be systematically rationalized on the basis of substituent effects. As a result, electron-withdrawing groups lead to electrophilic activation, and electron-releasing groups lead to electrophilic deactivation. Accordingly, by establishing a scale in which electrophiles and nucleophiles are present, couples of reagents (ketones and hydroxylamines in our case) presenting large differences of electrophilicity (*i.e.* $\Delta\omega > 2.0$) will be the more reactive.

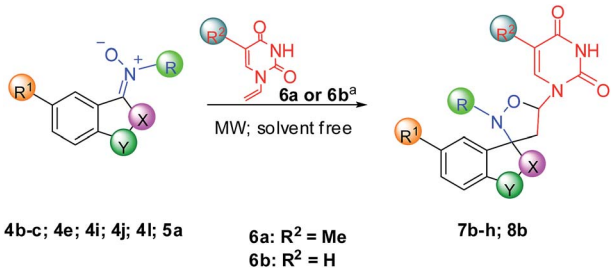
Calculations were carried out at b3lyp-d3bj/def2svp level of theory according to the procedure described elsewhere. We considered the experimentally studied ketones **2a–e** and **3a–j** but also ketones **2f–k**, **3k–3p** were calculated in order to predict an extended reactivity. The results are illustrated in Fig. 1.

Among the ketones the most electrophilic one is predicted to be **2d**, which has been studied experimentally. Among the hydroxylamines the most nucleophilic one resulted **1a**, also experimentally studied. Consequently, the reaction between **2d** and **1a** (Table 2, entry 11) is the most favoured as, indeed, is experimentally demonstrated (high yield and only 10 min of reaction). In general, isatin derivatives are the most reactive with values of $\Delta\omega > 2$ with some exception. The lower reactivity predicted ($\Delta\omega < 2$) for indanyl and tetralonyl derivatives is experimentally confirmed by the longer reaction times required and lower yields obtained. In fact, the absence of product obtained for the reaction between ketone **3j** and hydroxylamine **1a** correlates with a low difference in electrophilicity ($\Delta\omega = 1.11$).

Experimental

Commercial starting materials were used without further purification. Reactions were monitored by TLC using silica plates 60-F264, commercially available from Merk. ^{1}H and ^{13}C NMR

Table 4 MW-assisted synthesis of nucleobase-containing spiro-isoxazolidines



Entry	Nitrone[b,c]	R	R^1	R^2	Time (min)	7,8	Yield (%)	*exo*/*endo* ratio
1	**4b**	Bn	H	H	12	**7b**	85	83 : 17
2	**4c**	Ph	H	H	8	**7c**	84	73 : 27
3	**4c**	Ph	H	Me	8	**7d**	85	72 : 28
4	**4e**[d]	Me	H	H	10	**7e**	84	82 : 18
5	**4i**	Me	Br	Me	10	**7f**	77	86 : 14
6	**4j**	Bn	Br	Me	12	**7g**	75	88 : 12
7	**4l**	Bn	NO_2	Me	12	**7h**	74	75 : 25
8	**5a**[e]	Me	H	H	30	**8b**	76	78 : 22

[a] 1.0 eq. of **6a** or **6b** were used. [b] 2.0 eq. was used. [c] X = CO; Y = NH unless otherwise indicated. [d] Y = NMe. [e] X = Y = CH_2.

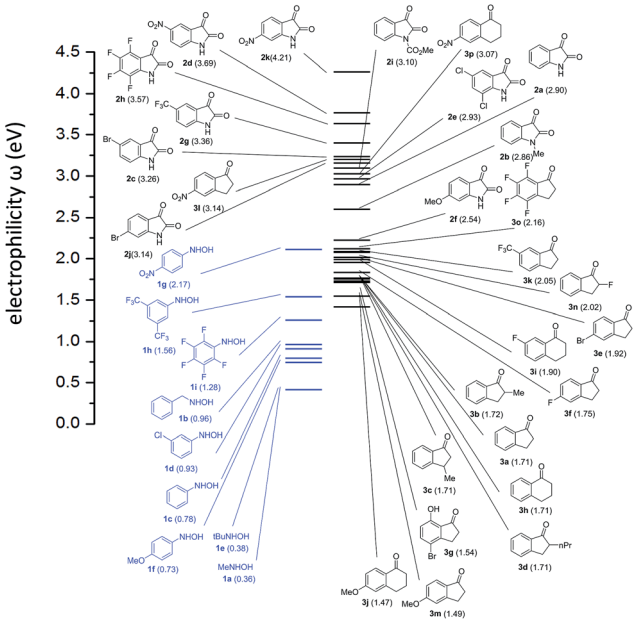


Fig. 1 Electrophilicity (ω in eV) scale for ketones and hydroxylamines.

spectra were recorded at 300, 400 and 500 MHz and 75, 100 and 125 MHz, respectively, in $CDCl_3$ and DMSO-d_6 using tetramethylsilane (TMS) as internal standard (Bruker ACP 300 MHz, 400 MHz and 500 MHz). Chemical shifts are given in parts per million and coupling constants in Hertz. The stereochemistry were established by NOESY experiments.

LC-MS analysis were carried using an Agilent 6540 UHD Accurate - Mass Q-TOF LC-MS (Agilent, Santa Clara, CA) fitted with a electrospray ionisation source (Dual AJS ESI) operating in positive ion mode. Chromatographic separation was achieved using a C18 RP analytical column (Poroshell 120, SB-C18, 50 × 2.1 mm, 2.7 μm) at 30 °C with a elution gradient from 5% to 95% of B over 13 min., a being H_2O (0.1% FA) and B CH_3CN (0.1% FA). Flow rate was 0.4 mL min^{-1}.

MW-assisted reactions were performed in Synthos 3000 instrument from Anton Paar, equipped with a 4 × 24MG5 rotor and an IR probe as external control of the temperature. 0.3–3 mL glass vials sealed with a dedicated PEEK screw-cup together with a reliable PTFE seal were used for all reactions. In synthesis of all derivatives the setting of temperature was always maintained to 180 °C in each experiment, except for **5a–i** where temperature was always maintained to 125 °C.

General procedure for synthesis of nitrones 4a–o and 5a–i

The selected ketone (0.5 g) and appropriate hydroxylamine derivative (2 eq. or 3 eq. for *N*-phenylhydroxylamine) were grinded in a mortar, placed in apposite vessel and mixed in a vortex. The mixture was transferred to a microwave oven and was irradiated with the opportune power. After the appropriate time the crude oil was recrystallized with ethyl acetate (**4a–o**) or cyclohexane (**5a–i**).

(*E*)-*N*-Methyl-*C*-isatinyl nitrone 4a. Yellow solid, 97% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.28 (s, 3H, CH_3), 6.92 (d, J = 7.74 Hz, 1H, Ar), 7.06 (t, J = 7.60 Hz, 1H, Ar), 7.38 (t, J = 7.60 Hz, 1H, Ar), 8.15 (d, J = 7.74 Hz, 1H, Ar), 10.90 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 50.1, 109.1, 117.6, 121.3, 123.2, 130.9, 133.1, 138.9, 160.8. ESI(+)-MS calcd for $C_9H_9N_2O_2$ [M + H] 177.0664, found: 177.0655.

(*E*)-*N*-Benzyl-*C*-isatinyl nitrone 4b. Orange solid, 95% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.89 (s, 2H, CH_{2Bn}), 6.91 (d, J = 7.78 Hz, 1H, Ar), 7.10 (dt, J = 0.91, 7.65 Hz, 1H, Ar), 7.25–7.54 (m, 6H, Ar), 8.12 (d, J = 7.65 Hz, 1H, Ar), 11.01 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 64.3, 109.8, 118.1, 122.04, 123.9,

128.5, 129.6, 129.1, 131.8, 133.4, 134.2, 139.6, 161.2. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{15}H_{13}N_2O_2$ 253.0977, found: 253.0977.

(*E*)-*N*-Phenyl-*C*-isatinyl nitrone 4c. Orange red solid, 92% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.85 (d, J = 7.80 Hz, 1H, Ar), 7.15 (t, J = 7.65 Hz, 1H, Ar), 7.40 (t, J = 7.80 Hz, 1H, Ar), 7.45–7.65 (m, 5H, Ar), 8.27 (d, J = 7.65 Hz, 1H, Ar), 10.78 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 109.7, 118.4, 121.7, 123.9, 124.1, 128.7, 130.1, 132.2, 134.4, 140.9, 146.3, 159.9. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{14}H_{11}N_2O_2$ 239.0821, found: 239.0817.

(*E*)-*N*-3-Cl-phenyl-*C*-isatinyl nitrone 4d (the most abundant). Orange solid, 87% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.35 (d, J = 7.71, 1H, Ar), 6.79 (t, J = 7.71, 1H, Ar), 6.85–7.05 (m, 2H, Ar), 7.07–7.18 (m, 1H, Ar), 7.27–7.44 (m, 2H, Ar), 7.45–7.56 (m, 1H, Ar), 11.04 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 111.6, 115.6, 116.1, 117.1, 121.9, 124.6, 125.4, 131.4, 134.0, 134.6, 146.0, 150.8, 155.6, 163.3. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{14}H_{10}ClN_2O_2$ 273.0431, found: 273.0422; [M + H] + 2 calcd for $C_{14}H_{10}ClN_2O_2$ 275.0401, found: 275.0382.

(*E*)-*N*-Methyl-*C*-(1-methyl)-isatinyl nitrone 4e. Yellow solid, 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27 (s, 3H, CH_3), 4.38 (s, 3H, CH_3), 6.82 (d, J = 7.83 Hz, 1H, Ar), 7.09 (t, J = 7.65 Hz, 1H, Ar), 7.38 (t, J = 7.83 Hz, 1H, Ar), 8.30 (d, J = 7.65 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.0, 51.2, 107.6, 118.3, 123.0, 123.0, 124.8, 131.3, 141.2, 160.9. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{10}H_{11}N_2O_2$ 191.0821, found: 191.0815.

(*E*)-*N*-Benzyl-*C*-(1-methyl)-isatinyl nitrone 4f. Yellow solid, 92% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.25 (s, 3H, CH_3), 5.92 (s, 2H, CH_2), 7.08 (t, J = 8.26 Hz, 2H, Ar), 7.37–7.50 (m, 6H, Ar), 8.15 (d, J = 7.34 Hz, 1H, Ar), ^{13}C NMR (75 MHz DMSO-d_6): δ 26.6, 65.2, 109.2, 117.8, 123.1, 124.0, 129.0, 129.0, 129.59, 132.2, 134.6, 141.5, 160.4. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{16}H_{15}N_2O_2$ 267.1134, found: 267.1126.

(*E*)-*N*-Phenyl-*C*-(1-methyl)-isatinyl nitrone 4g. Orange solid, 90% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.11 (s, 3H, CH_3), 7.08–7.16 (m, 2H, Ar), 7.46–7.58 (m, 6H, Ar), 8.30 (d, J = 7.48 Hz, 1H, Ar), ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.5, 110.7, 115.4, 116.5, 117.5, 122.8, 125.1, 125.5, 131.9, 134.4, 135.1, 148.5, 152.2, 155.3, 162.4. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{15}H_{13}N_2O_2$ 253.0977, found: 253.0975.

(*E*)-*N*-3-Cl-phenyl-*C*-(1-methyl)-isatinyl nitrone 4h (the most abundant). Yellow solid, 88% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.21 (s, 3H, CH_3), 6.37–6.40 (m, 1H, Ar), 6.85–7.12 (m, 4H, Ar), 7.32–7.65 (m, 3H, Ar), ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.6, 110.7, 115.4, 116.5, 117.5, 122.8, 125.1, 125.5, 131.9, 134.4, 135.1, 148.5, 152.2, 155.3, 162.4. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{15}H_{12}ClN_2O_2$ 287.0587, found: 287.0577; [M + H] + 2 calcd for $C_{15}H_{12}ClN_2O_2$ 289.0558, found: 289.0557.

(*E*)-*N*-Methyl-*C*-(5-Br)-isatinyl nitrone 4i. Orange solid, 95% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.26 (s, 3H, CH_3), 6.83 (d, J = 8.24 Hz, 1H, Ar), 7.50 (dd, J = 1.92 Hz, 8.24, 1H, Ar), 8.18 (d, J = 1.92 Hz, 1H, Ar), 11.02 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.4, 112.0, 113.7, 120.4, 126.0, 133.5, 134.0, 139.0, 161.4. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_9H_8BrN_2O_2$ 254.9769, found: 254.9762; [M + H] + 2 calcd for $C_9H_8BrN_2O_2$ 256.9749, found: 256.9746.

(*E*)-*N*-Benzyl-*C*-(5-Br)-isatinyl nitrone 4j. Orange solid, 93% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.89 (s, 2H, CH_2), 6.87 (d, J = 7.95 Hz, 1H, Ar), 7.26–7.63 (m, 6H, Ar), 8.21 (s, 1H, Ar), 11.16 (s, 1H, NH) ^{13}C NMR (75 MHz, DMSO-d_6): δ 65.2, 112.2, 113.9, 120.5, 126.2, 129.0, 129.0, 129.5, 133.3, 134.4, 134.4, 139.2, 161.3. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{15}H_{12}BrN_2O_2$ 331.0082, found: 333.0071; [M + H] + 2 calcd for $C_{15}H_{12}BrN_2O_2$ 333.0062, found: 333.0053.

(*E*)-*N*-Phenyl-*C*-(5-Br)-isatinyl nitrone 4k. Orange solid, 92% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.86 (dd, J = 2.93 Hz, 8.25 Hz, 1H, Ar), 7.40–7.77 (m, 6H, Ar), 8.37 (d, J = 1.83 Hz, 1H, Ar), 10.92 (s, 1H, NH) ^{13}C NMR (75 MHz, DMSO-d_6): δ 116.9, 118.4, 125.6, 129.1, 131.2, 134.0, 135.7, 135.8, 139.6, 145.3, 151.4, 164.8. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{14}H_{10}BrN_2O_2$ 316.9932, found: 316.9919; [M + H] + 2 calcd for $C_{14}H_{10}BrN_2O_2$ 318.9905, found: 318.9899.

(*E*)-*N*-Methyl-*C*-(5-NO_2)-isatinyl nitrone 4l. Yellow solid, 95% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.28 (s, 3H, CH_3), 7.02 (d, J = 8.73 Hz, 1H, Ar), 8.23 (d, J = 8.73 Hz, 1H, Ar), 8.75 (s, 1H, Ar), 11.53 (s, 1H, NH) ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.8, 110.2, 118.7, 118.7, 128.0, 133.3, 142.4, 145.2, 161.9. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_9H_8N_3O_4$ 222.0515, found: 222.0503.

(*E*)-*N*-Benzyl-*C*-(5-NO_2)-isatinyl nitrone 4m. Yellow solid, 92% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.91 (s, 2H, CH_2), 7.07 (d, J = 8.73 Hz, 1H, Ar), 7.31–7.58 (m, 5H, Ar), 8.28 (dd, J = 2.43 Hz, 8.73 Hz, 1H, Ar), 8.84 (d, J = 2.43 Hz, 1H, Ar), 11.70 (s, 1H, NH) ^{13}C NMR (75 MHz, DMSO-d_6): δ 65.6, 110.4, 118.8, 119.0, 128.3, 129.1, 129.1, 129.6, 133.2, 134.1, 142.6, 145.4, 161.9. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{15}H_{12}N_3O_4$ 298.0828, found: 298.0816.

(*E*)-*N*-Phenyl-*C*-(5-NO_2)-isatinyl nitrone 4n. Dark yellow solid, 92% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.06 (d, J = 8.79 Hz, 1H, Ar), 7.46–7.69 (m, 5H, Ar), 8.33 (dd, J = 2.37 Hz, 8.79 Hz, 1H, Ar), 9.00 (d, J = 1.83 Hz, 1H, Ar), 11.47 (s, 1H, NH) ^{13}C NMR (75 MHz, DMSO-d_6): δ 110.3, 119.2, 124.4, 128.8, 129.8, 131.2, 134.2, 142.4, 146.5, 146.7, 160.6. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{14}H_{10}N_3O_4$ 284.0671, found: 284.0662.

(*E*)-*N*-Methyl-*C*-(5,7-Cl)-isatinyl nitrone 4o. Orange solid, 80% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.25 (s, 3H, CH_3), 7.47 (d, J = 2.01 Hz, 1H, Ar), 7.91 (d, J = 2.01 Hz, 1H, Ar), 11.37 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.8, 115.0, 121.0, 121.8, 126.6, 130.2, 133.5, 136.2, 161.4. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_9H_7Cl_2N_2O_2$ 244.9885, found: 244.9878; [M + H] + 2 calcd for $C_9H_7Cl_2N_2O_2$ 246.9855, found: 246.9849; [M + H] + 4 calcd for $C_9H_7Cl_2N_2O_2$ 248.9826, found: 248.9820.

(*Z*)-*N*-Methyl-*C*-indanyl nitrone 5a. White solid, 82% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.90–3.05 (m, 2H, CH_2), 3.11–3.22 (m, 2H, CH_2), 3.81 (s, 3H, CH_3), 7.29–7.44 (m, 3H, Ar), 8.84 (d, J = 7.81 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7, 29.4, 49.6, 124.5, 127.0, 131.0, 134.4, 147.8, 149.7. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{10}H_{12}NO$ 162.0919, found: 162.0912.

(*Z*)-*N*-Benzyl-*C*-indanyl nitrone 5b. White solid, 85% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.91–3.09 (m, 2H, CH_2), 3.10–3.23 (m, 2H, CH_2), 5.11 (s, 2H, CH_{2Bn}), 7.21–7.45 (m, 7H, Ar), 7.51 (d, J = 7.16 Hz, 1H, Ar), 8.92 (d, J = 7.63 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.0, 29.2, 66.6, 124.5, 127.1, 127.2, 128.2, 128.3, 128.9, 131.1, 133.4, 134.8, 147.7, 149.1. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{16}H_{16}NO$ 238.1232, found: 238.1230.

(*Z*)-*N*-Methyl-*C*-(2-Me)-indanyl nitrone 5c. White solid, 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (d, J = 6.99 Hz, 3H, CH_3), 2.70 (d, J = 16.5 Hz, 1H, H_{CH_2}), 3.20–3.50 (m, 2H, CH + H_{CH_2}), 3.85 (s, 3H, CH_3), 7.22–7.48 (m, 3H, Ar), 8.83 (d, J = 7.53 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.7, 36.4, 38.4, 48.9, 124.7, 127.1, 127.5, 131.1, 133.5, 145.9, 153.8. ESI(+)-MS: m/z [M + H] calcd for $C_{11}H_{14}NO$ 176.1075, found: 176.1068.

(*Z*)-*N*-Methyl-*C*-(3-Me)-indanyl nitrone 5d. White solid, 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (d, 3H, J = 7.02 Hz, CH_3), 2.53 (dd, 1H, J = 2.79 Hz, 17.76 Hz, H_{CH_2}), 3.24 (dd, 1H, J = 7.29 Hz, 17.76 Hz, H_{CH_2}), 3.40–3.58 (m, 1H, CH), 3.78 (s, 3H, CH_3), 7.28–7.51 (m, 3H, Ar), 8.83 (d, J = 7.68 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.7, 35.9, 38.8, 49.8, 123.5, 126.9, 127.3, 131.2, 133.8, 152.6. ESI(+)-MS: m/z [M + H] calcd for $C_{11}H_{14}NO$ 176.1075, found: 176.1071.

(*Z*)-*N*-Methyl-*C*-(2-Pr)-indanyl nitrone 5e. White solid, 20% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, 3H, J = 6.47 Hz, CH_3), 1.25–155 (m, 4H, CH_2), 2.90–3.42 (m, 3H, CH + CH_2), 3.83 (s, 3H, CH_3), 7.18–7.49 (m, 3H, Ar), 8.79 (d, J = 7.51 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.8, 20.0, 35.7, 35.9, 41.6, 48.8, 124.7, 127.0, 127.5, 131.3, 134.0, 134.5, 146.7. ESI(+)-MS: m/z [M + H] calcd for $C_{13}H_{18}NO$ 204.1388, found: 204.1384.

(*Z*)-*N*-Methyl-*C*-(5-Br)-indanyl nitrone 5f. White solid, 88% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.0 (dd, J = 5.55 Hz, 12.03 Hz, 2H, CH_2), 3.14 (dd, J = 5.55 Hz, 12.51 Hz, 2H, CH_2), 3.81 (s, 3H, CH_3), 7.29–7.44 (m, 3H, Ar), 8.84 (d, J = 7.81 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.6, 29.5, 49.9, 125.1, 127.8, 128.0, 130.4, 133.6, 148.1, 149.4. ESI(+)-MS: m/z [M + H] calcd for $C_{10}H_{11}BrNO$ 240.0024, found: 240.0020; [M + H] + 2 calcd for $C_{10}H_{11}BrNO$ 242.0004, found: 242.0000.

(*Z*)-*N*-Methyl-*C*-(5-F)-indanyl nitrone 5g. White solid, 83% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01 (dd, J = 5.43 Hz, 11.73 Hz, 2H, CH_2), 3.14 (dd, J = 5.55 Hz, 11.97 Hz, 2H, CH_2), 6.80–7.12 (m, 2H, Ar), 8.86 (dd, J = 5.79 Hz, 8.64 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.8 (d, J_{CF} = 2.28), 29.9, 49.6, 111.9 (d, J^2_{CF} = 23.02), 114.3 (d, J^2_{CF} = 22.86), 128.8 (d, J^3_{CF} = 9.03), 130.9 (d, J^4_{CF} = 2.02), 148.1, 150.5 (d, J^3_{CF} = 8.95), 164.43 (d, J^1_{CF} = 250.96). ESI(+)-MS: m/z [M + H] calcd for $C_{10}H_{11}FNO$ 180.0825, found: 180.0818.

(*Z*)-*N*-Methyl-*C*-(4-Br-7-OH)-indanyl nitrone 5h. Yellow solid, 67% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.86–2.95 (m, 2H, CH_2), 2.96–3.30 (m, 2H, CH_2), 3.70 (s, 3H, CH_3), 6.60 (d, 1H, J = 8.79 Hz, Ar), 7.37 (d, J = 8.79 Hz, 1H, Ar), 14.96 (s, 1H, OH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.2, 47.8, 107.2, 118.5, 121.2, 138.0, 149.6, 155.4, 157.9. ESI(+)-MS: m/z [M + H] calcd for $C_{10}H_{11}BrNO_2$ 255.9973, found: 255.9964; [M + H] + 2 calcd for $C_{10}H_{11}BrNO_2$ 255.9953, found: 255.9945.

(*Z*)-*N*-Benzyl-*C*-(4-Br-7-OH)-indanyl nitrone 5i. Pale yellow solid, 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.97 (s, 4H, CH_2), 5.04 (s, 2H, CH_2), 6.62 (d, 1H, J = 8.79 Hz, Ar), 7.35–7.50 (m, 6H, Ar), 15.02 (s, 1H, OH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.8, 30.2, 64.6, 107.1, 118.6, 121.3, 127.9, 128.8, 129.1, 132.4, 138.0, 149.6, 155.5, 158.1. ESI(+)-MS: m/z [M + H] calcd for $C_{16}H_{15}BrNO_2$ 332.0286, found: 332.0276; [M + H] + 2 calcd for $C_{16}H_{15}BrNO_2$ 334.0266, found 334.0259:

General procedure for synthesis of nucleobase-containing spiro-isoxazolidines 7a–h and 8a–b

The opportune nitrone (2 eq.) and *N*-1-vinylthymine **6a** or *N*-1-vinyluracil **6b** (0.05 g) were grinded in a mortar, placed in apposite vessel and mixed in a vortex. The mixture was transferred to a microwave oven and was irradiated with the opportune power and temperature (when required). After the appropriate time the crude oil was purified by flash chromatography with hexane : AcOEt 7.75 : 2.25 v/v for **7c–e** or $CHCl_3$/MeOH 9.75 : 0.25 v/v for all the other crudes.

***exo*-5′-Thyminyl-2′-methyl-spiro-[indoline-3,3′-isoxazolidine]-1-methyl-2-one 7a.** Red solid, 85% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.02 (d, J = 1.21, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.64 (dd, J = 4.31 Hz, 14.03 Hz, 1H, H_{CH_2}), 3.22 (s, 3H, CH_3), 3.28 (dd, J = 7.48 Hz, 14.03 Hz, 1H, H_{CH_2}), 6.44 (dd, J = 4.31 Hz, 7.48 Hz, 1H, CH), 6.86 (d, J = 7.85 Hz, 1H, Ar), 7.09–7.14 (m, 1H, Ar), 7.24–7.29 (m, 1H, Ar), 7.36–7.41 (m, 1H, Ar), 7.77 (d, J = 1.21 Hz, 1H, 6-CH_{Thy}), 8.65 (s_b, 1H, NH_{Thy}). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.8, 26.0, 38,0, 47.6, 72.3, 83.4, 108.6, 110.7, 123.3, 123.3, 124.1, 130.5, 135.3, 144.2, 150.2, 163.8, 173.9. ESI(+)-MS: m/z [M + H] calcd for $C_{17}H_{19}N_4O_4$ 343.1406, found: 343.1403.

***exo*-5′-Uracil-2′-benzyl-spiro-[indoline-3,3′-isoxazolidine]-2-one 7b.** Yellow solid, 85% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.76–289 (m, 1H, H_{CH_2}), 3.41–3.58 (m, 1H, H_{CH_2}), 5.05–5.14 (m, 2H, CH_{2Bn}), 5.59 (dd, J = 2.20 Hz, 7.95 Hz, 1H, CH), 6.68 (d, J = 7.68 Hz, 1H, 5-CH_{Ura}), 6.75–7.92 (m, 9H, Ar), 7.97 (d, J = 7.68, 1H, 6-CH_{Ura}), 10.23 (s_b, 1H, NH), 11.05 (s_b, 1H, NH_{Ura}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 61,4, 72.6, 101.2, 109.9, 122.0, 125.3, 127.1, 127.3, 128.1, 128.4, 128.9, 129.5, 130.2, 142.8, 143.4, 151.2, 163.1, 168.5, 180.2. ESI(+)-MS: m/z [M + H] calcd for $C_{21}H_{19}NaN_4O_4$ 413.1226, found: 413.1219.

***exo*-5′-Uracil-2′-phenyl-spiro-[indoline-3,3′-isoxazolidine]-2-one 7c.** Orange solid, 84% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.98–3.11 (m, 1H, H_{CH_2}), 3.25–3.33 (m, 1H, H_{CH_2}), 5.79 (d, J = 8.07 Hz, 1H, 5-CH_{Ura}), 6.24 (dd, J = 5.31 Hz, 7.14 Hz, 1H, CH), 6.65–7.66 (m, 8H, Ar), 7.98 (d, J = 8.28 Hz, 1H, Ar), 8.55 (d, J = 8.07 Hz, 1H, 6-CH_{Ura}), 10.43 (s_b, 1H, NH), 10.89 (s_b, 1H, NH_{Ura}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.8, 72.9, 80.9, 102.6, 110.5, 118.1, 124.4, 126.1, 128.8, 128.9, 128.2, 130.9, 132.7, 141.8, 145.6, 151.4, 163.4, 175.6. ESI(+)-MS: m/z [M + H] calcd for $C_{20}H_{17}N_4O_4$ 377.1250, found: 377.1237.

***exo*-5′-Thyminyl-2′-phenyl-spiro-[indoline-3,3′-isoxazolidine]-2-one 7d.** Orange solid, 85% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.89 (s, 3H, CH_3) 2.99–3.14 (m, 1H, H_{CH_2}), 3.16–3.32 (m, 1H, H_{CH_2}), 6.67–7.58 (m, 10H, Ar + CH), 8.53 (s, 1H, 6-CH_{Thy}), 10.46 (s_b, 1H, NH), 11.48 (s_b, 1H, NH_{Thy}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.8, 45.0, 73.5, 81.8, 100.3, 110.8, 117.3, 118.0, 122.8, 124.6, 128.8, 128.9, 130.9, 135.7, 142.8, 146.4, 151.1, 164.0, 175.8. ESI(+)-MS: m/z [M + H] calcd for $C_{21}H_{19}N_4O_4$ 391.1406, found: 391.1402.

***exo*-5′-Uracil-2′-methyl-spiro-[indoline-3,3′-isoxazolidine]-1-methyl-2-one 7e.** Orange solid, 84% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.42 (s, 3H, CH_3), 2.82 (dd, J = 4.86 Hz, 13.98 Hz, 1H, H_{CH_2}), 3.05–3.22 (m, 4H, CH_3 + H_{CH_2}), 6.34 (dd, J = 4.86 Hz, 7.47 Hz, 1H, CH), 7.00–7.21 (m, 2H, Ar), 7.38–7.55 (m, 2H, Ar), 7.98 (d, J = 8.16, 1H, 5-CH_{Ura}), 8.03 (d, J = 8.16 Hz, 1H, 6-CH_{Ura}),

11.46 (s_b, 1H, NH_{Ura}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.3, 38,1, 46.3, 72.5, 82.6, 108.9, 109.5, 123.2, 124.9, 129.9, 130.7, 140.1, 144.7, 151.0, 163.7, 173.9. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{16}H_{16}N_4O_4$ 329.1250, found: 329.1242.

***exo*-5′-Thyminyl-2′-methyl-spiro-[5-bromo-indoline-3,3′-isoxazolidine]-2-one 7f.** Yellow solid, 77% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.92 (s, 3H, CH_3), 2.97 (dd, J = 6.04 Hz, 13.73 Hz, 1H, H_{CH_2}), 3.46 (dd, J = 7.14 Hz, 13.73 Hz, 1H, H_{CH_2}), 3.54 (s, 3H, NCH_3), 6.40 (dd, J = 6.04 Hz, 7.14 Hz, 1H, CH), 6.85 (d, J = 8.23 Hz, 1H, Ar), 7.18–7.56 (m, 2H, Ar), 7.72–7.81 (m, 2H, Ar + 6-CH_{Thy}), 10.78 (s_b, 1H, NH), 11.38 (s_b, 1H, NH_{Thy}) ^{13}C NMR (125 MHz, DMSO-d_6): δ 16.7, 33.3, 42.4, 74.2, 86.3, 114.1, 116.3, 118.1, 129.9, 130.9, 137.2, 140.0, 146.5, 154.9, 168.2, 179.3. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{16}H_{16}BrN_4O_4$ 407.0355, found: 407.0337; [M + H] + 2 calcd for $C_{16}H_{16}BrN_4O_4$ 409.0334, found: 409.0322.

***exo*-5′-Thyminyl-2′-benzyl-spiro-[5-bromo-indoline-3,3′-isoxazolidine]-2-one 7g.** Yellow solid, 75% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.82 (s, 3H, CH_3), 2.8–3,2 (m, 2H, H_{CH_2}), 4.35 (s, 2H, CH_{2Bn}), 6.84 (dd, J = 6.24 Hz, 8.85 Hz, 1H, CH), 6.70–8.71 (m, 8H, Ar), 8.98 (d, J = 5.42 Hz, 1H, 6-CH_{Thy}), 11.10 (s_b, 1H, NH), 11.36 (s_b, 1H, NH_{Thy}) ^{13}C NMR (125 MHz, DMSO-d_6): δ 16.3, 33.3, 39.5, 67.0, 76.2, 113.6, 116.6, 117.7, 126.4, 132.1, 132.5, 132.9, 133.5, 137.8, 142.5, 144.4, 146.6, 149.4, 155.6, 167.6. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{22}H_{20}BrN_4O_4$ 483.0668, found: 483.0666; [M + H] + 2 calcd for $C_{22}H_{20}BrN_4O_4$ 485.0647, found: 485.0637.

***exo*-5′-Thyminyl-2′-benzyl-spiro-[5-nitro-indoline-3,3′-isoxazolidine]-2-one 7h.** Orange solid, 74% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.79 (s, 3H, CH_3), 3.54 (dd, J = 7.03 Hz, 14.22 Hz, 1H, H_{CH_2}), 3.93–4.19 (m, 3H, H_{CH_2} + CH_{2Bn}), 5.48 (dd, J = 7.03 Hz, 9.72 Hz, 1H, CH), 6.92 (d, J = 8.52 Hz, 1H, Ar), 7.20–7.78 (m, 6H, Ar), 8.15 (dd J = 2.35 Hz, 8.70, 1H, Ar), 8.23 (d, J = 2.19 Hz, 1H, 6-CH_{Thy}), 11.03 (s_b, 1H, NH), 11.12 (s_b, 1H, NH_{Thy}) ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.6, 22.5, 29.4, 58.7, 71.2, 108.8, 109.9, 120.5, 126.4, 127.1, 128.7, 132.0, 132.8, 137.8, 142.8, 145.0, 148.9, 151.5, 163.7, 180.3. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{22}H_{20}N_5O_6$ 450.1414, found: 450.1403.

***exo*-5′-Thyminyl-2′-methyl-spiro-[indane-3,3′-isoaxazolidine] 8a.** White solid, 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.76–1.90 (m, 2H, CH_2), 1.94 (d, J = 1.08 Hz, 1H, CH_3), 2.38 (s, 3H, CH_3), 2.51–2.67 (m, 2H, CH_2), 2.79–3.05 (m, 2H, CH_2), 6.20 (dd, J = 5.68 Hz, 7.36 Hz, 1H, CH), 7.12–7.35 (m, 4H, Ar), 7.79 (s, 1H, 6-CH_{Thy}), 9.39 (s_b, 1H, NH_{Thy}). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.8, 29.2, 30.2, 36.1, 51.6, 77.9, 82.2, 111.1, 123.6, 125.2, 126.9, 129.0, 135.9, 139.8, 144.3, 150.7, 164.2. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{17}H_{20}N_3O_3$ 314.1505, found: 314.1497.

***exo*-5′-Uracil-2′-methyl-spiro-[indane-3,3′-isoaxazolidine] 8b.** White solid, 76% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.78–1.95 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 2.52–2.72 (m, 2H, CH_2), 2.79–3.10 (m, 2H, CH_2), 5.77 (d, J = 8.02 Hz, 1H, 5-CH_{Ura}), 6.19 (dd, J = 5.38 Hz, 7.24 Hz, 1H, CH), 7.10–7.40 (m, 4H, Ar), 8.05 (d, J = 8.02 Hz, 1H, 6-CH_{Ura}), 9.53 (s_b, 1H, NH_{Ura}). ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.4, 30.1, 35.9, 78.0, 82.6, 102.6, 123.6, 125.1, 127.0, 129.1, 139.5, 140.3, 144.3, 150.6, 163.7. ESI(+)-MS: *m*/*z* [M + H] calcd for $C_{16}H_{18}N_3O_3$ 300.1348, found: 300.1337.

Conclusions

In summary, we have initially developed an environmentally benign and fast method by microwave irradiation and solvent-free conditions for the synthesis of significant number of substituted isatinyl and indanyl nitrones as important precursors of spiro-isoxazolidines. Then, we applied a MW-assisted 1,3-dipolar cycloaddition to some previously synthesized keto-nitrones as dipole and vinylthymine or vinyluracil as dipolarophile, obtaining nucleobase-containing spiro-isoxazolidines with indoline or indane ring in good yields, excellent regioisomery and high diastereoisomery. The importance of this application is surely the direct insertion of a nucleobase on spiroisoxazolidines with indoline or indane scaffold that in our opinion represents a great chemical novelty. Finally, we have also enriched this work, studying by theoretical calculations the effect of various substituents about the reactivity of the carbonyl groups with different hydroxylamines in synthesis of nitrones.

Conflicts of interest

There are no conflicts to declare.

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Antiproliferative activity of novel isatinyl/indanyl nitrones (INs) as potential spin trapping agents of free radical intermediates†

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A series of ketonitrones derived from isatin and indanone (INs) were synthesized and evaluated for their antiproliferative activities against several human cancer cell lines. Then, the antioxidant properties of these substrates were measured by the DPPH test to report their biological activity in terms of their *spin trapping* action. In particular, one substrate has showed very high biological and scavenging activity, probably due to the strong correlation between its spin trapping activity and structure.

Introduction

Small organic molecules have proven to be invaluable tools for investigating biological systems, but there is still much to learn from their use.[1–3]

Nitrones are small molecules that have the general chemical formula X–CH═NO–Y. Their structural nature endows them their "spin-trap" ability for trapping free radical intermediates (R˙), forming stable radical adducts (X–CHR═NO˙–Y).[4] In fact, studies of their spin trapping activity involved the reaction of nitrones with reactive free radicals such as hydroxyl (˙OH), lipid alkoxy (˙OL) or lipid hydroxyperoxyl (˙OOL) radicals, observing the formation of more stable radicals that can be classified and quantified by electron spin resonance (ESR) or electron paramagnetic resonance (EPR) spectroscopy.[5–7]

Considering a) that biological systems may actively produce reactive oxygen species (ROS) and reactive nitrogen species (RNS), b) that specific oxidation products are produced from reactions between biological molecules and ROS or RNS, c) that ROS and RNS play an important role in many pathological diseases, nitrones and, in particular, PBN-nitrones, where X is a phenyl group and Y is a *tert*-butyl group, were recently considered as therapeutics due to their widespread anti-cancer activity.[8,9] The mechanistic basis of their anti-cancer action is not known. It is probable that their ability to scavenge radical intermediates that are produced during disease processes, including cancer, is the basis for their anti-cancer activity.[4] Moreover, their action on important membrane enzymes and as anti-inflammatory agents seems to contribute to the enhancement of their antioxidant activity.

Isatin and oxindole derivatives have found wide application in medicinal chemistry, for example as potential anticonvulsants,[10] cyclin-dependent kinase 2-inhibitors[11] or inhibitors of poxvirus,[12] ectomelia,[13] rhinovirus,[14] HIV-1[15] and corona-virus,[16] the latter responsible for severe acute respiratory syndrome (SARS). Moreover, the insertion of an oxindole nucleus in spiro compounds has recently attracted attention because of their prevalence in various natural products and biologically active molecules.[17] In fact, the key to their activity seems to be the combination of a spiro carbon and a variously substituted oxindole core. Therefore, the oxindole moiety could be considered as a useful tool in drug discovery.

It is generally recognized that incorporating different bioactive scaffolds into one molecule is a powerful strategy to construct substrates with structural novelty and biological potential.[18] In an attempt to generate novel molecular entities with amplified spin trapping and antiproliferative activity on cancer cells, we synthesized some isatin and indanone ketonitrones, combining the simultaneous presence of an oxindole-like ring and a nitrone portion.

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Results and discussion

In the literature, a few examples of synthetic procedures for nitrones of oxindole derivatives have been reported thus far.[19–22] Recently, we have realized an environmentally-friendly approach to synthesize aldo- and ketonitrones by solvent-free condensation of alkyl- or arylhydroxylamine hydrochlorides with aromatic aldehydes under microwave irradiation.[23]

Herein, we report the practical and stereoselective synthesis of isatinyl and indanyl nitrones (INs) obtained from substituted hydroxylamines **1a–c** and isatin derivatives **2a–c** or indanones **3a–b**, as shown in Table 1. Then, we investigated the antiproliferative activity of these substrates on MG63 and TE85 human osteosarcoma cell lines and the K562 human erythroleukemic cell line; next we sought to correlate it with their antioxidant properties measured by the DPPH assay.

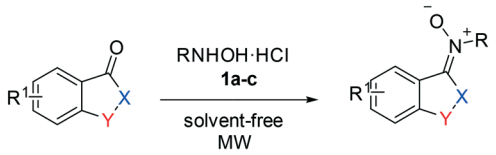
The target compounds **4a–e** and **5a–c** were prepared by following our previously reported methodology[23] which consists of co-grinding the ketone and hydroxylamine in a mortar, followed by transferring the mixture without the use of solvent in an appropriate vessel and further mixing of the solids in a vortex mixer. Finally, the mixture is placed in a microwave oven without the use of solvent and is irradiated at 600 W or 400 W for variable time periods in the order of minutes.

The variously substituted products **4a–e** and **5a–c** were obtained in high yields (83–95%). The low reactivity of the indanone ring prompted us to reduce the MW power and to increase the reaction time. In all cases, nitrones were obtained as single isomers, (*E*) **4a–e** and (*Z*) **5a–c**, respectively, as confirmed by NOESY experiments. In particular, for nitrone **4b**, the formation of only a single product, the *E*-isomer, is observed with respect to *Z*/*E* mixtures obtained by other synthetic methodologies.[24]

In an attempt to understand the molecular characteristics and the effects produced by the modifications introduced into our nitrones on tumour cell proliferation, the nitrones prepared according to this procedure were evaluated for their antiproliferative activity against human osteosarcoma (MG63 and TE85)[25] and chronic myeloid leukemia (K562)[26] cell lines. Cells were cultured for three days in the absence or in the presence of increasing concentration of nitrones and then their viability was evaluated by analyzing the activity of the oxidative metabolism by the 3-(4,5- dimethylthiazolyil-2)-2,5-diphenyltetrazolium bromide (MTT) assay.[27] In Fig. 1, the proliferation of the treated cells was expressed as percentage compared to untreated control cells.

The data indicated that not all the nitrones had antiproliferative activity, since compounds **5a–c** and **4b** were completely inactive. Compounds **4c–e** inhibited the

Table 1 Synthesis of isatinyl and indanyl nitrones



2a-c; 3a-b **4a-e; 5a-c**

Entry	R	R^1	Y	X	MW (W)	Time (min)	Product	Yield (%)
1	Me	5-NO_2	NH	CO	600	10	**4a**	95
2[a]	Ph	H	NH	CO	600	10	**4b**	92
3	Bn	5-NO_2	NH	CO	600	13	**4c**	92
4	Bn	H	NH	CO	600	12	**4d**	95
5	Bn	H	NMe	CO	600	12	**4e**	92
6	Me	H	CH_2	CH_2	400	30	**5a**	82
7	Me	5-F	CH_2	CH_2	400	28	**5b**	83
8	Bn	H	CH_2	CH_2	400	30	**5c**	85

[a] A ketone/alkylhydroxylamine ratio of 1 : 2 for all substrates except for entry 2 with a ratio of 1 : 3.

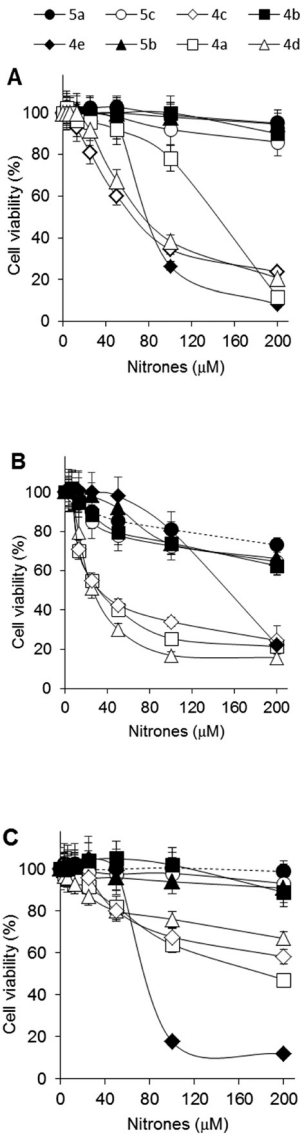


Fig. 1 Effect of nitrones **4a–e** and **5a–c** on cell proliferation. MG63 (A), TE85 (B) and K562 (C) cells were incubated for three days in the presence of the indicated compounds. Viable cells were measured by the MTT test and reported as % relative to untreated control. The arithmetic mean value ± standard deviation of three experiments performed in triplicate is shown.

proliferation of osteosarcoma MG63 cells compared to untreated cells (Fig. 1A, IC_{50} about 77 μM).

In Fig. 1B, the antiproliferative activities of nitrones **4a** and **4c–d** on TE85 cells (IC_{50} about 32 μM) are shown. The active compounds are less effective in inhibiting the proliferation of K562 cells, with the exception of nitrone **4e** with an IC_{50} of about 78 μM (Fig. 1C). Together, these results have shown that the indanyl derivatives do not possess antiproliferative activity on the tumour cells analyzed, as well as the derivative **4b** with the phenyl substituent. By contrast, isatinyl nitrones containing methyl or benzyl groups possess antiproliferative activity.

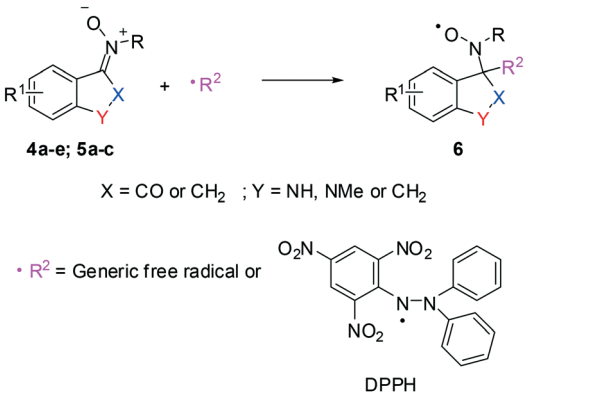
The *in vitro* antioxidant activity of these ketooxindole nitrones (**4a–e** and **5a–c**) was evaluated by using the stable organic free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity assay.[28–31]

In Scheme 1, the reaction mechanism for the spin-trapping action of nitrones **4a–e** and **5a–c** with a generic free radical (including the DPPH radical) is illustrated, according to the literature.[32] It can be noted from the scheme below that the reaction produces the highly stable nitroxyl radical **6**.[33,34]

When DPPH reacts with a radical scavenger, its maximum absorbance decreases. A freshly prepared DPPH solution displayed a deep purple colour with the absorption maximum at 517 nm. The ethanolic solution of DPPH was added to the solution of the synthesized compounds in ethanol. After 10 min of incubation in the dark, the absorbance was measured by using absolute ethanol as blank. BHT (butylated hydroxytoluene) was used as reference. In the presence of an antioxidant, the DPPH absorbance decreases. The antioxidant activity was calculated as radical scavenging activity (RSA%) as expressed in eqn (1):

$$\mathrm{RSA\%} = [(A_0 - A_i)/A_0] \times 100 \quad (1)$$

where A_0 and A_i are the DPPH absorbance at 517 nm in the absence and presence of the synthesized compounds, respectively. The RSA% for ketonitrones **4a–e** and **5a–c** at five different concentrations (*i.e.*, 1.15, 2.45, 4.00, 6.25 and 9.10 × 10^{-6} mol L^{-1}) of the tested compounds with DPPH at 517 nm is reported in Table 2.



Scheme 1 Reaction mechanism of nitrones **4a–e** and **5a–c** with free radicals.

As can be seen in Table 2, all the compounds exhibited antioxidant activity. The substances can be divided into three main groups. The first group is composed of two substances (**4c** and **4e**) with higher values of antioxidant activity than others. In particular, the highest values were observed for **4e**, for which the RSA% is equal to 60% at a concentration of 2.45 × 10^{-6} mol L^{-1}.

Four substances compose the second group with intermediate antioxidant activity (**4a–b**, **5a** and **5c**). For all of these compounds, the RSA% was higher than 60% at a concentration equal to 6.25 × 10^{-6} mol L^{-1}. In the third group, only two compounds with lower antioxidant activity were included: **4d** and **5b**. For these last two compounds, the RSA% was 80% at a concentration equal to 9.10 × 10^{-6} mol L^{-1}. As can be seen in Table 2 and Fig. 2, all nitrones **4a–e** and **5a–c** exhibited a radical scavenging activity significantly higher than that of the reference (BHT). We have not investigated concentrations higher than 9.10 × 10^{-6} mol L^{-1} as the RSA% trend reaches a plateau.

The RSA% of nitrones **4a–e** and **5a–c** may be compared with the value of archetypal (*Z*) α-phenyl-*N*-*tert*-butyl nitrone (PBN) reported in the literature.[35] Its RSA% is 1.4 ± 0.9 at a concentration of 0.5 × 10^{-3} mol L^{-1} calculated through the DPPH test, demonstrating antioxidant properties significantly lower with respect to those of our nitrones.

For all the tested compounds, the EC_{50} values were also calculated by using the GraphPad Prism 5.01 program, as reported in the literature (Table 2).[36] The EC_{50} is the antioxidant concentration required to obtain 50% radical inhibition. As can be seen in Table 2 the EC_{50} values of all eight synthesized nitrones are higher than that of the reference substance (BHT).

The antioxidant properties of INs seem to agree quite well with the biological results, confirming the importance of the isatinyl ring. In particular, the presence of both aromatic systems and electron-donating groups (*e.g.* methyl group) improves the performance, as can particularly be seen for substrate **4e**.

Experimental

Commercial starting materials were used without further purification. Reactions were monitored by TLC using 60-F264 silica plates, commercially available from Merck. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, in $CDCl_3$ and DMSO-d_6 using tetramethylsilane (TMS) as an internal standard (Bruker ACP 300 MHz). Chemical shifts are given in parts per million and coupling constants in Hertz. The stereochemistry was established by NOESY experiments. Purity was verified by NMR and HPLC.

LC-MS analysis was carried out using an Agilent 6540 UHD Accurate – Mass Q-TOF LC-MS (Agilent, Santa Clara, CA) equipped with an electrospray ionisation source (Dual AJS ESI) operating in positive ion mode. Chromatographic

Table 2 Percentage of *in vitro* radical scavenging activity of INs

Compounds	Concentrations (10^{-6} mol L^{-1})					EC_{50}
	1.15	2.45	4.00	6.25	9.10	
4a	—	—	1.1 ± 0.5	72 ± 2	79 ± 2	1.2 ± 0.2
4b	1.1 ± 0.5	1.2 ± 0.5	1.2 ± 0.5	69 ± 2	80 ± 2	1.3 ± 0.2
4c	—	—	60 ± 1	67 ± 2	80 ± 2	1.0 ± 0.2
4d	1.1 ± 0.5	1.1 ± 0.5	1.2 ± 0.5	1.2 ± 0.5	78 ± 2	1.8 ± 0.1
4e	1.2 ± 0.5	60 ± 1	68 ± 2	70 ± 2	79 ± 2	0.6 ± 0.3
5a	—	—	1.1 ± 0.5	60 ± 1	78 ± 2	1.3 ± 0.2
5b	—	—	1.1 ± 0.5	1.2 ± 0.5	79 ± 2	1.8 ± 0.1
5c	1.1 ± 0.5	1.2 ± 0.5	1.3 ± 0.5	70 ± 2	79 ± 2	1.2 ± 0.2
BHT	—	4.6 ± 0.5	11.7 ± 0.5	23.0 ± 0.5	27.0 ± 0.5	3.8 ± 0.5

separation was achieved using a C18 RP analytical column (Poroshell 120, SB-C18, 50 × 2.1 mm, 2.7 μm) at 30 °C with an elution gradient from 5% to 95% of B over 13 min., A being H_2O (0.1% FA) and B CH_3CN (0.1% FA). The flow rate was 0.4 ml min^{-1}.

MW-assisted reactions were performed on a Synthos 3000 instrument from Anton Paar, equipped with a 4x24MG5 rotor and an IR probe as an external control of the temperature. 0.3–3 mL glass vials sealed with a dedicated PEEK screw-cup together with a reliable PTFE seal were used for all reactions. In the synthesis of all the derivatives, the temperature was always maintained at 180 °C in each experiment, except for **5a–c** where the temperature was always maintained at 125 °C.

General procedure for synthesis of nitrones 4a–e and 5a–c

The selected ketone (0.50 g) and appropriate hydroxylamine derivative (2 eq. or 3 eq. for *N*-phenylhydroxylamine) were ground in a mortar, placed in an appropriate vessel and mixed using a vortex mixer. The mixture was transferred to a microwave oven and irradiated with suitable power. After the appropriate time, the crude oil was recrystallized with ethyl acetate (**4a–e**) or cyclohexane (**5a–c**).

(*E*)-*N*-(5-Nitro-2-oxoindolin-3-ylidene)methanamine oxide 4a

Yellow solid, 95% yield, (0.52 g). ^{1}H NMR (300 MHz, DMSO-d_6): 4.28 (s, 3H, CH_3), 7.02 (d, J = 8.73 Hz, 1H, Ar), 8.23 (d, J = 8.73 Hz, 1H, Ar), 8.75 (s, 1H, Ar), 11.53 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 51.8, 110.2, 118.7, 118.7, 128.0, 133.3, 142.4, 145.2, 161.9. ESI (+)-MS: m/z [M + H] calcd for $C_9H_8N_3O_4$ 222.0515, found: 222.0503.

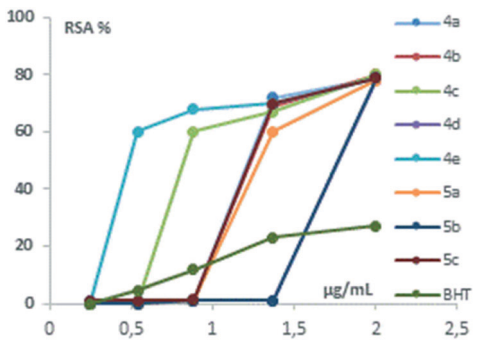


Fig. 2 Graphical presentation of *in vitro* DPPH radical scavenging activity of compounds relative to the standard antioxidant BHT.

(*E*)-*N*-(2-Oxoindolin-3-ylidene)aniline oxide 4b

Orange red solid, 92% yield, (0.74 g). ^{1}H NMR (300 MHz, DMSO-d_6): 6.85 (d, J = 7.80 Hz, 1H, Ar), 7.15 (t, J = 7.65 Hz, 1H, Ar), 7.40 (t, J = 7.80 Hz, 1H, Ar), 7.45–7.65 (m, 5H, Ar), 8.27 (d, J = 7.65 Hz, 1H, Ar), 10.78 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 109.7, 118.4, 121.7, 123.9, 124.1, 128.7, 130.1, 132.2, 134.4, 140.9, 146.3, 159.9. ESI (+)-MS: m/z [M + H] calcd for $C_{14}H_{11}N_2O_2$ 239.0821, found: 239.0817.

(*E*)-*N*-(5-Nitro-2-oxoindolin-3-ylidene)-1-phenylmethanamine oxide 4c

Yellow solid, 92% yield (0.64 g). ^{1}H NMR (300 MHz, DMSO-d_6): 5.91 (s, 2H, CH_2), 7.07 (d, J = 8.73 Hz, 1H, Ar), 7.31–7.58 (m, 5H, Ar), 8.28 (dd, J = 2.43 Hz, 8.73 Hz, 1H, Ar), 8.84 (d, J = 2.43 Hz, 1H, Ar), 11.70 (s, 1H, NH) ^{13}C NMR (75 MHz, DMSO-d_6): δ 65.6, 110.4, 118.8, 119.0, 128.3, 129.1, 129.1, 129.6, 133.2, 134.1, 142.6, 145.4, 161.9. ESI (+)-MS: m/z [M + H] calcd for $C_{15}H_{12}N_3O_4$ 298.0828, found: 298.0816.

(*E*)-*N*-(2-Oxoindolin-3-ylidene)-1-phenylmethanamine oxide 4d

Orange solid, 95% yield (0.80 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.89 (s, 2H, CH_2Bn), 6.91 (d, J = 7.78 Hz, 1H, Ar), 7.10 (dt, J = 0.91, 7.65 Hz, 1H, Ar), 7.25–7.54 (m, 6H, Ar), 8.12 (d, J = 7.65 Hz, 1H, Ar), 11.01 (s, 1H, NH), ^{13}C NMR (75 MHz, DMSO-d_6): δ 64.3, 109.8, 118.1, 122.04, 123.9, 128.5, 129.6, 129.1, 131.8, 133.4, 134.2, 139.6, 161.2. ESI (+)-MS: m/z [M + H] calcd for $C_{15}H_{13}N_2O_2$ 253.0977, found: 253.0977.

(*E*)-*N*-(1-Methyl-2-oxoindolin-3-ylidene)-1-phenylmethanamine oxide 4e

Yellow solid, 92% yield (0.76 g). ^{1}H NMR (300 MHz, DMSO-d_6): 3.25 (s, 3H, CH_3), 5.92 (s, 2H, CH_2), 7.08 (t, J = 8.26 Hz, 2H, Ar), 7.37–7.50 (m, 6H, Ar), 8.15 (d, J = 7.34 Hz, 1H, Ar), ^{13}C NMR (75 MHz DMSO-d_6): δ 26.6, 65.2, 109.2, 117.8, 123.1, 124.0, 129.0, 129.0, 129.59, 132.2, 134.6, 141.5, 160.4. ESI (+)-MS: m/z [M + H] calcd for $C_{16}H_{15}N_2O_2$ 267.1134, found: 267.1126.

(*Z*)-*N*-(2,3-Dihydro-1*H*-inden-1-ylidene)methanamine oxide 5a

White solid, 82% yield (0.50 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.90–3.05 (m, 2H, CH_2), 3.11–3.22 (m, 2H, CH_2), 3.81 (s, 3H, CH_3), 7.29–7.44 (m, 3H, Ar), 8.84 (d, J = 7.81 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7, 29.4, 49.6, 124.5, 127.0, 131.0, 134.4, 147.8, 149.7. ESI (+)-MS: m/z [M + H] calcd for $C_{10}H_{12}NO$ 162.0919, found: 162.0912.

(*Z*)-*N*-(5-Fluoro-2,3-dihydro-1*H*-inden-1-ylidene)methanamine oxide 5b

White solid, 83% yield (0.48 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01 (dd, J = 5.43 Hz, 11.73 Hz, 2H, CH_2), 3.14 (dd, J = 5.55 Hz, 11.97 Hz, 2H, CH_2), 6.80–7.12 (m, 2H, Ar), 8.86 (dd, J = 5.79 Hz, 8.64 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.8 (d, JCF = 2.28), 29.9, 49.6, 111.9 (d, J2CF = 23.02), 114.3 (d, J2CF = 22.86), 128.8 (d, J3CF = 9.03), 130.9 (d, J4CF = 2.02), 148.1, 150.5 (d, J3CF = 8.95), 164.43 (d, J1CF = 250.96). ESI (+)-MS: m/z [M + H] calcd for $C_{10}H_{11}FNO$ 180.0825, found: 180.0818.

(*Z*)-*N*-(2,3-Dihydro-1*H*-inden-1-ylidene)-1-phenylmethanamine oxide 5c

White solid, 85% yield (0.76 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.91–3.09 (m, 2H, CH_2), 3.10–3.23 (m, 2H, CH_2), 5.11 (s, 2H, CH_2Bn), 7.21–7.45 (m, 7H, Ar), 7.51 (d, J = 7.16 Hz, 1H, Ar), 8.92 (d, J = 7.63 Hz, 1H, Ar), ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.0, 29.2, 66.6, 124.5, 127.1, 127.2, 128.2, 128.3, 128.9, 131.1, 133.4, 134.8, 147.7, 149.1. ESI (+)-MS: m/z [M + H] calcd for $C_{16}H_{16}NO$ 238.1232, found: 238.1230.

Antiproliferative activity evaluation

Human cell lines were obtained from ATCC (Manassas, VA). MG63 (CRL-1427) and HOS TE85 (CRL-1543) osteosarcoma cells were maintained in DMEM, while K562 (CCL-243) chronic myeloid leukemia cells in RPMI 1640. The culture medium was supplemented with 10% fetal bovine serum, penicillin (100 U mL^{-1}), streptomycin (100 U mL^{-1}) and glutamine (2 mM); incubation was conducted at 37 °C in a 5% CO_2 atmosphere. Compounds were solubilized in 40 mM DMSO, kept at −80 °C in the dark and diluted in complete medium immediately before use. The osteosarcoma cells were seeded at 4000 cells per well in a 96-well plate 6 hours before the treatment. The leukemia cells were seeded at 20 000 cells per mL. Cells were treated with the test compounds at concentrations ranging from 3 to 200 μM. Untreated cells were placed in every plate as negative control. After 3 days of culture, the inhibitory effect on cell proliferation was analysed by addition of 25 μL MTT (thiazolyl blue) staining solution, in which metabolically active cells convert the yellow tetrazolium salt to purple formazan crystals providing a quantitative determination of viable cells. Two hours later, formazan crystals were solubilized in 100 μL of lysing buffer (50% DMF + 20% SDS, pH 4.7) for 16 hours, whereupon the spectrophotometric absorbance at 570 nm was measured. The half maximal inhibitory concentration (IC_{50}) was calculated using Scientist software. Three independent experiments were performed in triplicate.

Antioxidant evaluation

The free radical scavenging activity against DPPH was determined at five different concentrations according to a previous work.[28] Thus, EtOH solutions containing known amounts of the compounds (**4a–e** and **5a–c**) and of DPPH were prepared in a range of concentrations from 1.15×10^{-6} to 9.10×10^{-6} mol L^{-1} (Table 2). A decrease in the absorbance of DPPH was measured at 517 nm, against ethanol as blank, by using a UV-vis spectrophotometer (Varian Cary 50 Scan), after a period of 10 min since preparation. Experiments were carried out in triplicate and BHT (butylated hydroxytoluene) was used, in the same concentration range of the compounds, as the reference antioxidant.

Conclusions

In summary, a series of isatinyl/indanyl nitrones (INs) were synthesized and their antiproliferative activity on MG63, TE85 and K562 cells was determined. Derivatives with the isatin ring showed the highest activity, while the indanone nitrones were practically inactive. Then the antioxidant activities were evaluated through the DPPH test. The results indicated that all compounds are markedly more active than the reference compound BHT. Among them, nitrone **4e** exhibits the most potent antioxidant activity at low concentration and its *spin-trap* action matches perfectly with its antiproliferative effect on cancer cells. Collectively, the current study may provide a new insight into the treatment of degenerative diseases such as cancer.

Conflicts of interest

There are no conflicts to declare.

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