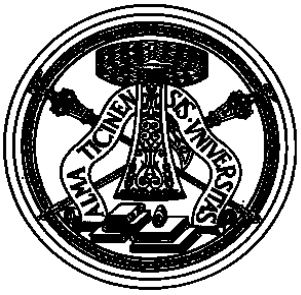

UNIVERSITÁ DEGLI STUDI DI PAVIA

DOTTORATO IN SCIENZE CHIMICHE E FARMACEUTICHE

XXXII CICLO

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Pericyclic Reactions for Antivirals

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SUMMARY

1. INTRODUCTION

Viral infections and malignancies are among the commonest causes of mortality in our society, and this motivates strong interest in the design and synthesis of new molecules that can effectively counteract these diseases. The discovery of several new series of nucleoside analogues with antiviral activity modified the classical way of thinking about these structures as antiviral agents. Nucleoside analogs are a major class of chemotherapeutic agents.[1] Currently, more than 30 commercially available antiviral and antitumor nucleosides are available (Figura 1). Among nucleoside analogs, non-natural L-enantiomers have been particularly interesting. The most notable example is lamivudine, which has been studied for its important role in the treatment of human immunodeficiency virus and hepatitis B virus infections.[2] The reason for their high therapeutic applicability is that, when biologically active, L-nucleosides have proven to possess favorable toxicological, chemical, and biochemical features, such as potency, low toxicity, and high metabolic stability. These characteristics have been the key factors in their success as drugs.

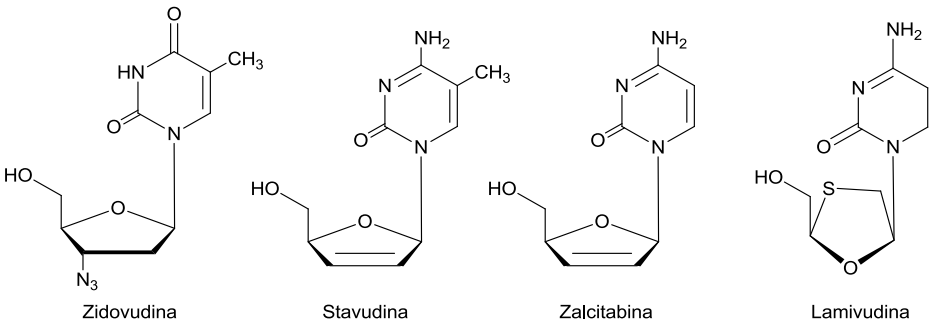


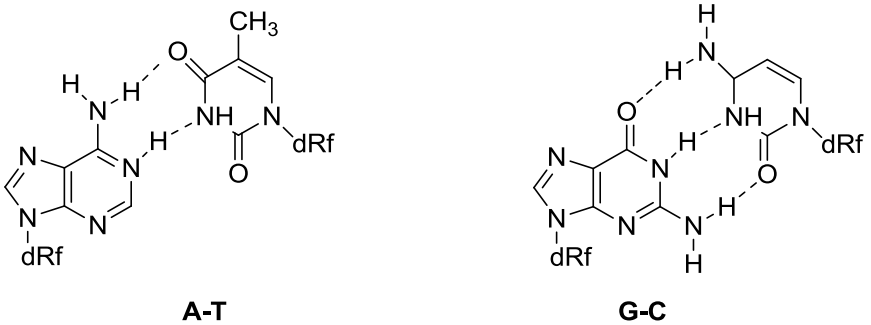
Figure 1 : Nucleoside analogues

1.1 NUCLEOSIDE IN CHEMICAL BIOLOGY

The structure of duplex DNA is based on the complementary Watson-Crick hydrogen bonding patterns of A-T and G-C pairs (Figure 2). Replacement of the natural nucleobases by different surrogates has become very popular in recent years. The original aim just to investigate the structure and function of nucleic acids (NA) was extended toward three main goals:

1. Formation of stabilized duplexes.
2. Design of universal nucleobases not discriminating between the complementary bases.
3. Extension of the genetic alphabet.

The employment of small organic molecules (nucleoside and nucleotide analogues) in studying enzyme mechanisms (mainly polymerases or enzymes of nucleic acid metabolism) is another important area of application. Because of the increased stability and modified properties, nucleosides have been extensively studied and many promising examples of their use in chemical biology were reported.[3] The Nucleosides can in principle target any enzymes of the nucleic acid metabolism. Purine nucleoside analogues can inhibit a wide range of enzymes that use purine-based cofactors (i.e., kinases, oxidoreductases, GPCR, and tubulin, etc.) and act as agonists or antagonists of purinoceptors. The major advantage of nucleosides is the stability toward cleavage of the nucleosidic bond due to the replacement of the nucleosidic C-N bond by the non hydrolyzable C-C bond. Therefore, they are expected to be resistant to enzymatic cleavage. Replacement of the N atom by C in the position of the nucleosidic bond often dramatically changes the properties of the heterocyclic moiety. Thus, for example, replacement of pyrimidine by pyridine or replacement of purine by pyrrolopyrimidine dramatically changes the tautomeric populations and acido basic properties of the heterocycles and functional groups (OH, NH_2).



dRf= 2'-deoxyribofuranosyl

Figura 2: Natural Watson-Crick Base Pair

Nucleoside analogs are commonly used in HIV/AIDS therapy and as a first line therapeutics for many tumor types. After entering through the membrane, nucleoside analogs are phosphorylated by intracellular nucleoside kinases into active 50-mono-, di-, and triphosphates. The drug resistance to nucleoside analogs refers to nucleoside transporter deficiency, reduced nucleoside kinase activity, overexpression of multidrug resistance proteins, or modifications in apoptotic pathways.125 A conceivable strategy to avoid resistance is to use a prephosphorylated nucleoside directly as a drug that can thus bypass intracellular phosphorylation. However, nucleoside triphosphates (NTPs) are very poorly internalized by cells. Attempts to get around this problem by masking the phosphate groups by turning them into phosphate esters have not been successful until recently.126 The fully substituted di- and triphosphate esters become unstable. Difficulties in the development of lipophilic NTP prodrugs arise due to hydrolytical instability of the pyrophosphate bond, while in the natural NTPs this bond is kinetically stable due to negative charges that slow down cleavage by nucleophiles.An alternative to the lipophilic prodrug approach could be in using drug delivery systems such as liposomes, nanogels, nanoparticles, and so on, which could potentially solve the problem of poor cell absorption of NTPs.

SiO_2 nanoparticles represent a simple and versatile method for the preparation of SiO_2-dNTP conjugates. Thus conjugates of antiviral phosphorylated nucleoside analogs may also possess potential antitumor activity.

1.2 NUCLEOSIDE ANALOGUES FEATURE

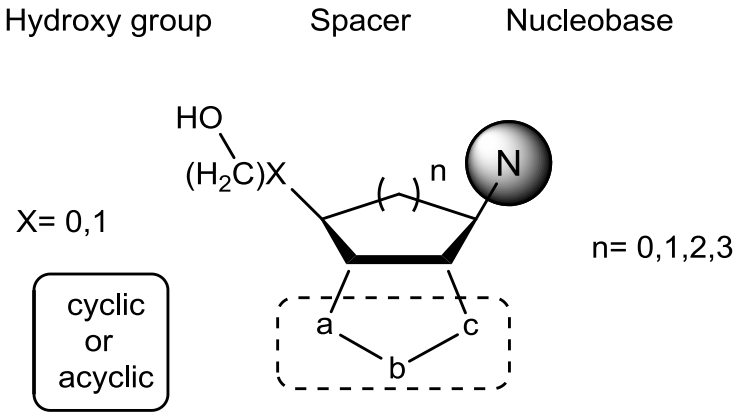


Figure 3: Nucleoside feature

Structurally, the typical feature of a nucleoside is the presence of a spacer between the nucleobase and the hydroxy functionality, where the spacer can be either an open-chain or a cyclic moiety: in the cyclic case, ring size modifications and the mono- or polycyclic nature of the spacer between the nucleobase and the hydroxy group affect both the conformational bias and the activities and functions of the nucleo-side analogues.[4] The carbocyclic moiety is normally represented by a cyclopentane ring, although four-, six- and seven-membered rings are often proposed as alternatives. A hydroxy group is normally present. This feature discriminates between the two families of classical nucleoside analogues (x = 1), bearing hydroxymethylene moieties, and nor-nucleoside analogues (x = 0), in which the hydroxy groups are directly linked to the carbocyclic rings. The nucleobases can be of the purine or pyrimidine type and a variety of functionalized structures, suitable for various types of functionalization.

Most effective antiviral agents act as prodrugs for their corresponding phosphorylated metabolites. The hydroxyl group on the side chain is recognized and phosphorylated by the kinases that produce the corresponding nucleotides. Modifications in this part of the nucleoside structure are often required. Recently, N–O chemistry, based on nitroso reagents, has been extensively applied for the synthesis of nucleosides as antivirals and antibiotics. In some cases, the N–O functionality serves as an isoster of the hydroxymethylene group [i.e., the hydroxylamine (HO–NH) moiety]. In others, the mild cleavage conditions usable for the N–O bond allow the stereoselective introduction of hydroxy and amino groups for further synthetic elaborations.

1.3 PERICYCLIC REACTION FOR ANTIVIRALS

Many synthetic strategies have been developed and summarized in excellent reviews.[5-6] Different approaches may be employed to classify these strategies:

- Connection of an appropriate functional group to an anomeric position of a preformed carbohydrate moiety, followed by a construction of the aglycon unit.
- Connection of an appropriate functional group to a preformed aglycon, followed by a construction of the carbohydrate moiety.
- Direct coupling of a preformed carbohydrate moiety with an aglycon.
- Modification of the existing C-nucleoside.
- Modular approaches.

The Pericyclic reactions, in particular cycloaddition reactions, represent the best approach in the construction of aglycons.

In organic chemistry, a pericyclic reaction is a type of organic reaction proceed through a concerted mechanism involving a single, cyclic transition state (Figura 4). In this transition state, a concerted rearrangement of the electrons takes place that causes σ and π-bonds to simultaneously break and form.

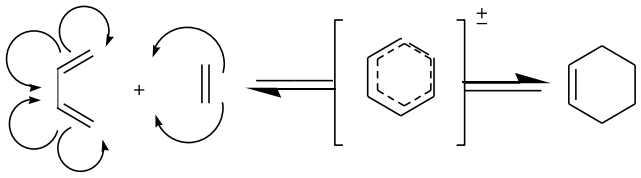


Figure 4. Cycloaddition model

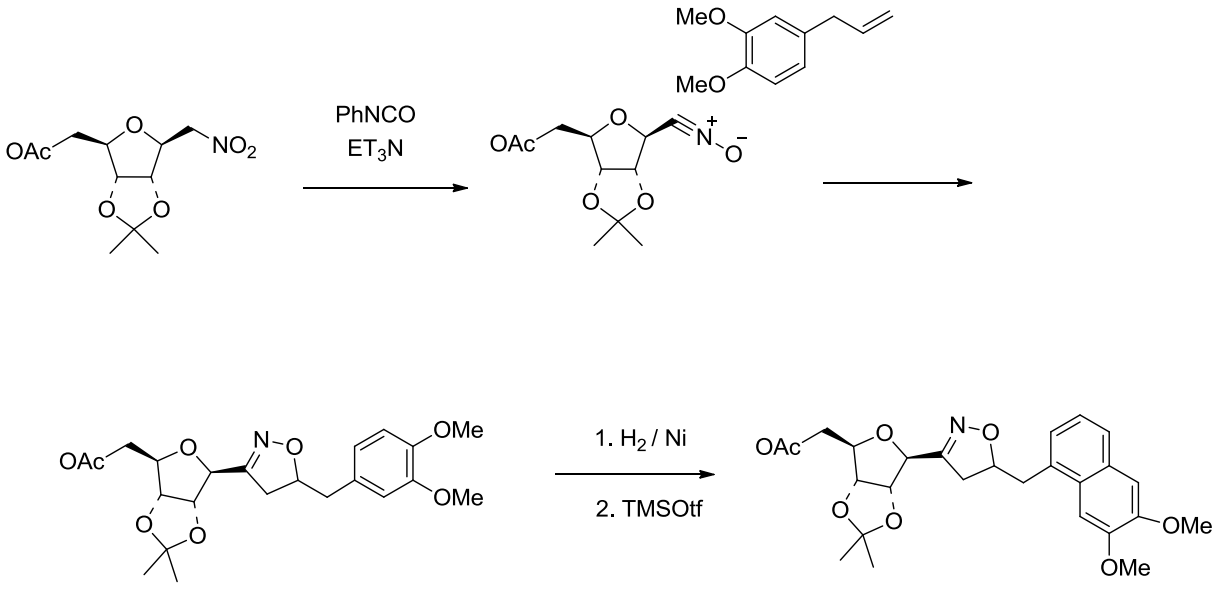
The pericyclic reactions are characterized by a continuous reorganization of electrons through cyclic transition structures with specific orbital alignments and often have characteristic and predictable stereochemistry.

In many cases, the reactions exhibit regioselectivity that can be directly related to the effect of orbital interactions or similarly, substituent effects on reactivity.

Several research groups have studied and synthesized molecules with antiviral activity through the use of pericyclic reactions.

The application of DA cycloaddition reactions to the synthesis of antiviral compounds also finds in the hetero version of these [4+2] processes a wide panorama of valuable reactions. It has been known for some time that aminosugar derivatives that inhibit glycoprotein processing have potential activity against HIV.

The first attempts were related to the total synthesis of showdomycin, where Diels-Alder cycloaddition reaction was used to construct the carbohydrate moiety with an appropriate functional group in the anomeric position. In the same year (1984), Kozikowski proposed a 1,3-dipolar cycloaddition reaction for the synthesis of artificial nucleoside analogues bearing an isoxazoline ring instead of ribose and for the total synthesis of blastomycinone. With this background, some years later, the same author reported the first example of 1,3-dipolar cycloaddition for the synthesis of dimethoxynaphthyl C-nucleoside.[7] The sequence commenced with the nitromethylene derivative, which was first converted into the nitrile oxide. Subsequent cycloaddition of the substituted allyl benzene afforded isoxazoline, whose reduction with nickel-Raney gave the corresponding hydroxy ketone that was cyclized to the aryl nucleoside on treatment with trimethylsilyl triflate (Scheme 1).



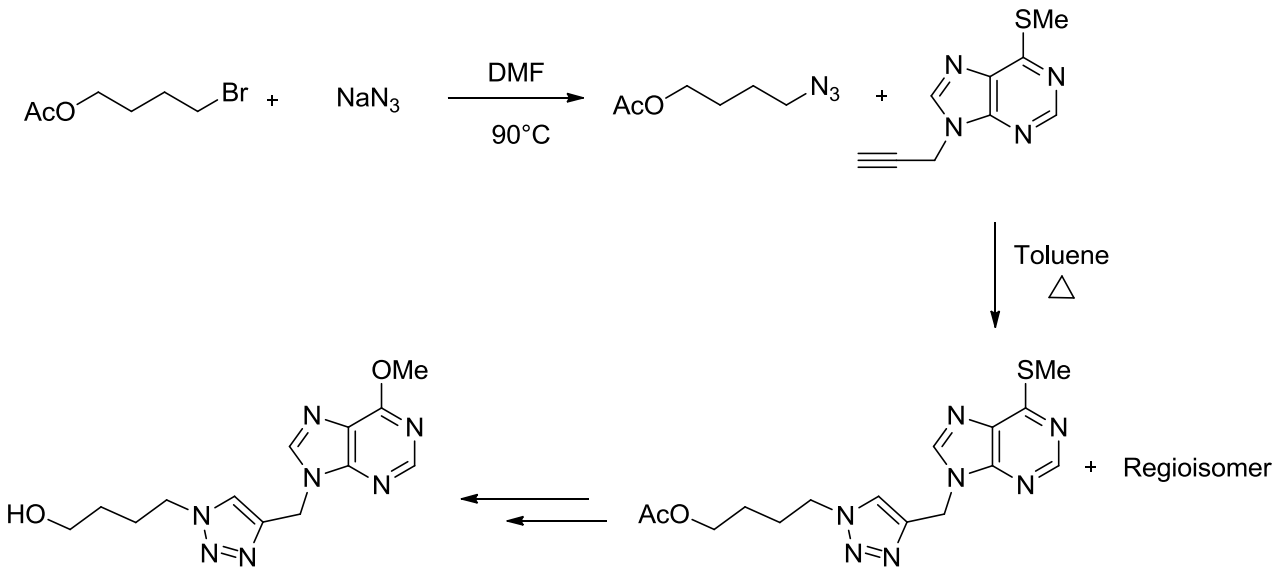
Scheme 1: 1,3-Dipolar Cycloaddition in Construction of Aglycon Unit.

1,3-Dipoles represent a valuable class of reacting intermediates frequently used in the synthesis of antiviral compounds. They can be used for both the introduction of suitable residues necessary to display the correct antiviral activity and as starting material for the generation of other reacting

species, such as the nitrosocarbonyl intermediates, which represent the key intermediates for the synthesis of the target compounds.

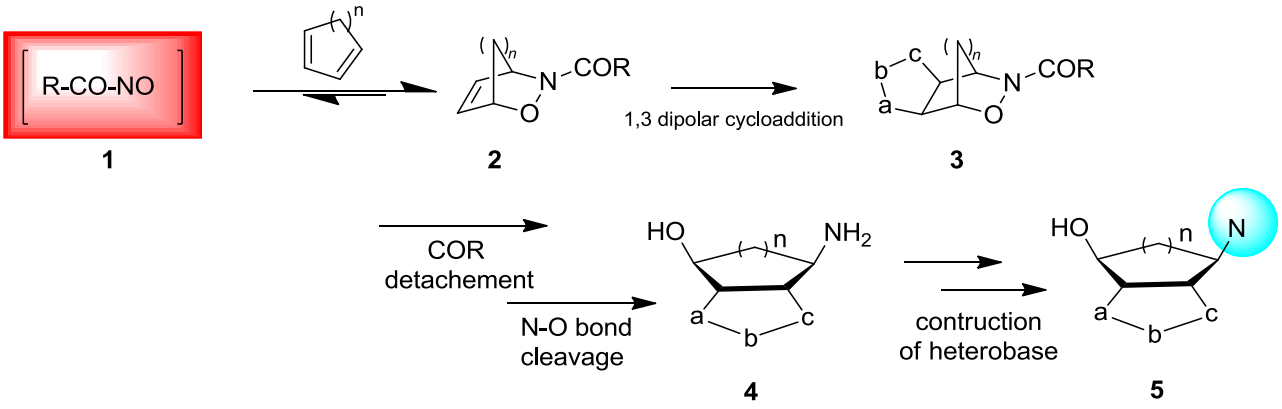
One of the most frequently used 1,3-dipoles is azide. The versatility of this 1,3-dipole is well known since its use in "click chemistry" as well as for the preparation of triazoles, valuable heterocycles in the synthesis of biologically active compounds. Organic azides belong to the propargyl-allenyl category of dipoles and since the discovery of triazole formation from phenyl azide and dimethyl acetylenedicarboxylate in 1893, they found large success in the construction of heterocyclic frameworks and core structures of natural and bioactive compounds.

A simple case is shown in scheme 2 where the treatment of 4-acetoxybutylbromide with sodium azide in anhydrous DMF gave the 4-acetoxybutylazide in high yield with respect to that reported for the preparation of the reported nucleoside analogues.



Scheme 2: Azido Strategy To Nucleoside Analogues

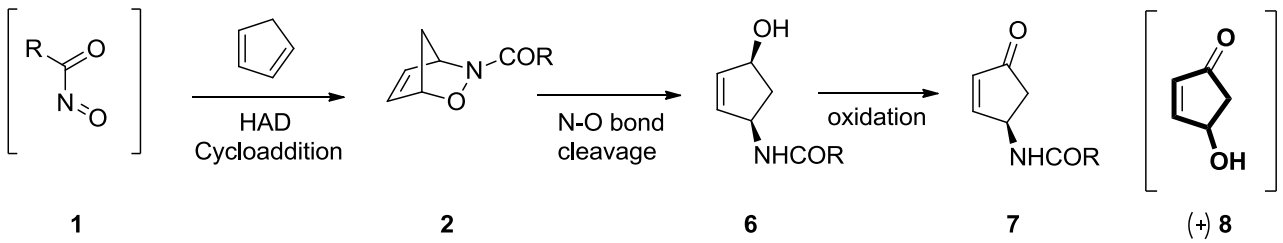
Another 1,3-dipole that found attention in the synthesis of nucleoside analogs is the *N*-acylnitrile oxides **1**. An innovative procedure for the synthesis of carbocyclic nucleosides, starting from cyclopentadiene and based on nitrosocarbonyl intermediates is reported in Scheme 3.[8]



Scheme 3: The Synthesis Of A New Class Of Carbocyclic Nucleosides

Intermediates **1**, generated by the mild oxidation of nitrile oxides with tertiary amine N-oxides, are efficiently trapped by cyclic dienes to afford the hetero-Diels–Alder (HDA) cycloadducts **2**. These are highly reactive dipolarophiles and have been employed for the synthesis of conformationally restricted carbocyclic moiety aminols **4** through amide hydrolysis and N–O bond cleavage of the cycloadducts **3**.[9] Aminols **4** served for the linear construction of purine and pyrimidine nucleosides **5**.

Nitrosocarbonyl compounds **1** have found wide applications in nucleoside synthesis because of their high reactivity in hetero-Diels–Alder (HDA) cycloaddition reactions with cyclopentadiene and dienes in general; moreover through the N-O cleavage we can obtained the 4-hydroxy-2-cyclopentenone. The chemistry of this scaffold has been extensively investigated and reviewed, as the hydroxycyclopentenone moiety often appears in natural products and biologically active compounds, or constitutes the privileged starting material for their synthesis (Scheme 4).



Scheme 4: Nitrosocarbonyl compound in the Synthesis Of Nucleosides Analogues

1.4 THE CHEMISTRY OF RACEMIC 4-HYDROXY-2-CYCLOPENTENONE

The 4-hydroxy-2-cyclopentenone moiety is a highly valued molecular scaffold recognized as one of the most versatile chiral intermediate used as a key synthon for the synthesis of natural products and pharmaceutical drugs.[10]

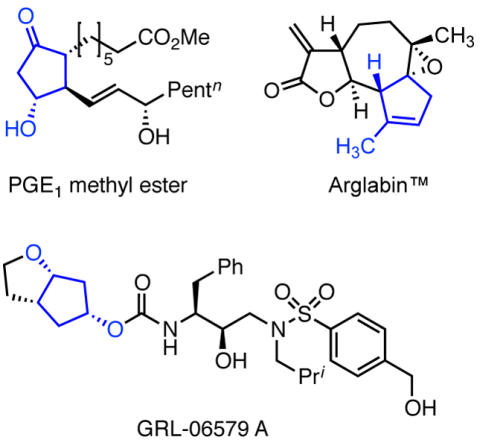
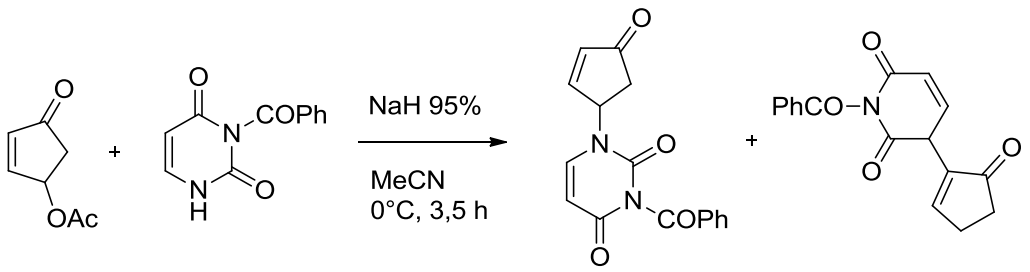


Figure 4 : Examples Of Natural Scaffold

Historically, the chemistry of 4-hydroxy-2-cyclopentenones **8** was developed and shaped by progress in the synthesis of prostaglandins, notably in the early 1980s, but over the years this core fragment has become a subunit of primary importance in a wide array of natural product syntheses.
Indeed, a variety of natural product structures incorporate this moiety directly examples include prostanoid, indenones, alkaloids (daphnipaxianines A–B, actumine and hypserpanine A families), terpenes and protease inhibitor GRL-06579 (Figure 4).
Consequently, a considerable number of synthetic approaches toward racemic and enantiopure derivatives thereof utilizing the chiral pool, chiral reagents, or catalysts have been developed.[11]
A majority of these transformations involve selectively changing the oxidation state of the corresponding 1,3-cis-diol or 1,3-diketone.
Racemic 4-oxocyclopent-2-en-1-yl acetate was recently employed in a concise synthesis of nucleoside analogues with pyrimidine and purine heterobases.[12]
The protocol, involving a typical nucleophilic substitution process with uracil, thymine, 6-chloropurine and some adenines.

One compound, belonging to the class of products **11** and bearing an uracil base, was highly active against HPV.[13](Scheme 5)



9 **10** **11a** **11b**

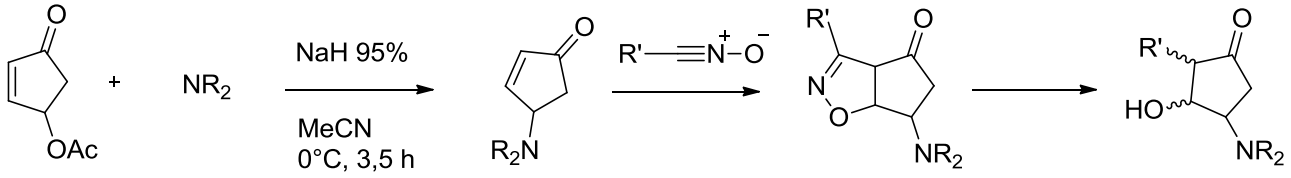
	R	11a	11b
a	H	75%	9%

Scheme 5: Synthesis Of Uracil Derivativatives

Please chek the formula of **11b** (no nitrogen)

The coupling reactions were carried out by the in situ generation of the conjugate bases of benzoyl-protected uracil base, obtained according to literature procedures,[14] by treatment with NaH (95 %) in dry acetonitrile. The regioisomeric adducts **11a** and **11b** were obtained in very good yields; 89:11 ratio.

The protocol is based on a typical nucleophilic substitution process and is not applicable to an enantioselective approach to the desired products. Later we will explore a new protocol to selectively obtain compounds **11b**.

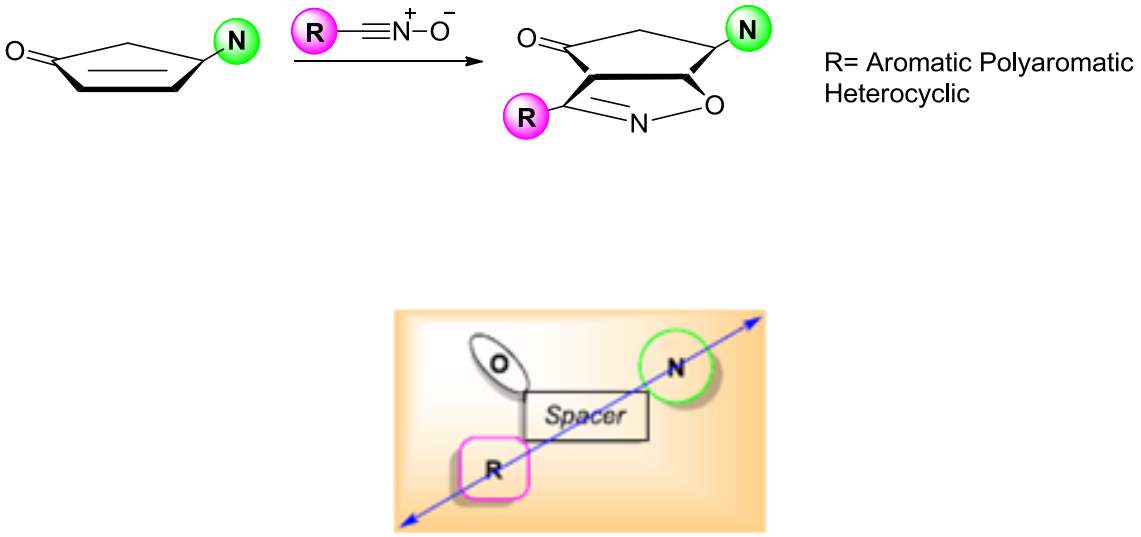


9 **12** **13** **14**

Scheme 6: Synthesis Of Nucleoside Analogues

Compound **9** (scheme 6) reacts with nitrile oxides to furnish cycloadducts **13**. Their synthetically elaboration afforded derivatives **14** through a simple N-O bond cleavage. These compounds are potential candidates for antiviral activity.

The cycloaddition of nitrile oxides to heterobase functionalized cyclopentenones can be used to afford derivatives where a variety of substituents can be inserted via the 1,3-dipole moiety.[15] (Scheme 7).



Scheme 7: Applications in Nucleoside Synthesis

In conclusion the use of nitrile oxides in the preparation of antiviral derivatives *via* 1,3-dipolar cycloaddition reactions represents a valuable method to achieve these targets.

1.5 NITROSOCARBONYL COMPOUNDS IN ORGANIC CHEMISTRY

Nitrosocarbonyl or acylnitroso compounds (Figure 5) attracted a great deal of attention in the area of organic synthesis during the last decades since highly functionalized molecules can be readily achieved starting from nitroso compounds through different reactions.

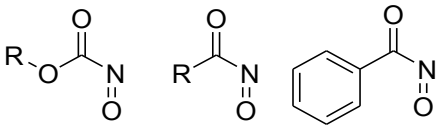


Figure 5 : example of acylnitroso compounds

The generation and trapping of these intermediates often represent the pivotal step in many organic syntheses, for example in literature it is reported the use of nitrosocarbonyl HDA reactions for the construction of carbocyclic nucleoside analogues and their use as antiviral agents. The synthesis and study of carbocyclic nucleosides represent an important area of therapeutic research and many methods have been developed that allow access to this class of molecules. There are no comprehensive studies on nitrosocarbonyl intermediates from the theoretical point of view. Sometimes in the literature the structures of these fleeting species appeared in the context of more general investigations on related compounds.

The first work reporting a nitrosocarbonyl structure was published by Wiberg and co-workers in 1992 where the structures, energies, and rotational profiles of heterobutadienes were studied according to DFT calculations. Among the structures reported, the nitrosocarbonyl O=C=N^+=O was studied without any reference to possible reactions as dienophile or enophile. The chemistry of simple nitroso compounds R=N^+=O offered a simplified ground on which to develop theoretical studies on the reactivity of these species. Houk and co-workers deeply investigated this area starting from the reactions of nitrosyl hydride with 1,3-butadiene in HDA cycloaddition reactions.[16] In 2002, Houk published DFT studies on the ene reaction of nitroso compounds with propene. In this paper the authors reported, for the first time, the possibility that the reaction involves a polarized diradical intermediate in a stepwise process.[17]

DFT calculations, and in general theoretical approaches to nitrosocarbonyl chemistry, can be further exploited to better understand the behavior of these intermediates. Some effort could be done in studying the reactivity in nitroso ene reaction as well as to understand all the factors that could affect and enhance their stability. The continuous updating of modern calculation methods will certainly offer new chances to clarify the intrinsic nature of nitrosocarbonyls, possibly widening

their field of application and making it suitable even in unusual conditions for this king of compounds such as in biological environments.

1.5.1 NITROSOCARBONYL PROPERTIES

The term "nitrosocarbonyl" appeared for the first time in 1897 in Berichte der Deutschen Chemischen Gesellschaft by Emil Fischer and dealing with the decomposition of theobromine.[18] Here it refers to an N-nitroso urea derivative where the NO group is attached to the nitrogen atom and not a carbonyl group. More properly, the name "nitrosocarbonyl" can be replaced with "acyl nitroso" compounds. From the IUPAC rules point of view, an "acyl nitroso" derivative is an organic molecule where the nitroso group is attached to a generic acyl group with a single bond.[19] However, in the review by Kirby dealing with the C-nitroso compounds, the name "nitrosocarbonyl" is used to describe the structure of these intermediates and, in particular, Kirby indicates as nitrosocarbonyl-alkanes and -arenes the way to describe compounds where the −CONO moiety is linked to an aliphatic or aromatic group. The most reactive nitroso compounds include those directly connected to an electron-withdrawing group and nitroso compounds reported are among the most reactive nitroso dienophiles used for example in HDA or ene reactions.

Acylnitroso species are highly reactive dienophiles, first detected spectroscopically in the gas phase in 1991 by neutralization-reionization mass spectrometry and then in solution in 2003 by time-resolved infrared spectroscopy. It is estimated that the lifetime of the acylnitroso species at infinite dilution in an organic solution is on the order of 1 ms. Arylnitroso compounds are, in comparison to acylnitroso analogues, quite stable, and in many cases, they can be isolated and stored.

Acylnitroso compounds can exist in either an s-cis or s-trans conformation along the carbonyl–nitrogen bond (Figure 6). It is evident from the data reported in the literature, the preference for a given acylnitroso species to exist as either conformer must be calculated on a case-by case basis.

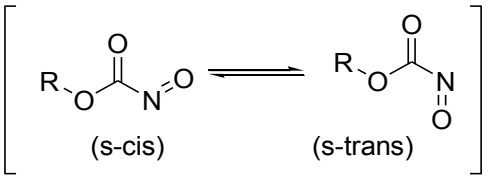


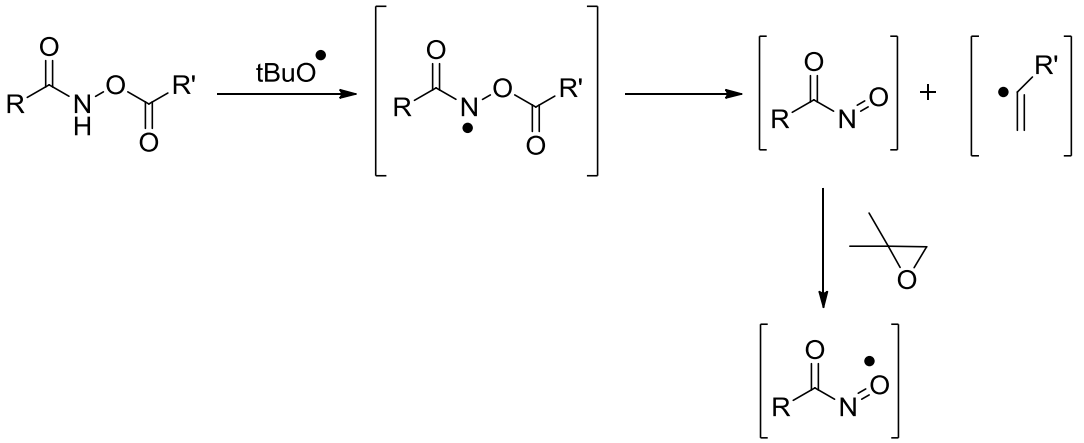
Figure 6: S-Cis And S-Trans Isomers Of Nitrosocarbonyl Compunds.

5.2 NITROSOCARBONYL DETECTION

In the "Tilden Lecture" in 1977, G. W. Kirby affirmed that "...the postulated intermediates, RCONO, have, so far, not been detected by physical methods." Since that time, the detection of these fleeting intermediates has remained an open question.

However, the attempts to find evidence of nitrosocarbonyls started immediately in the scientific community and the very first investigation was reported by Forrester and co-workers in 1978.

The authors treated the N-acyloxyamide [20] with di-tert-butyl peroxide, irradiating at 0−100 °C in the EPR cavity. No signals were detected, but when the lamp was masked and the solution heated at 100 °C a triplet of doublets slowly appeared. These spectra were attributed to radical nitrosocarbonyl species, as confirmed by addition of few drops of oxirane, when the spectrum was observed to intensify 5-fold (scheme 8). This and other experiments tried to indicate indirectly the presence of the nitrosocarbonyl species.[21]



Scheme 8: Radical Promoted Generation of Nitrosocarbonyl Intermediates

We have to wait until the year 2003 to find a definitive direct observation of a nitrosocarbonyl in solution by using time-resolved IR spectroscopy (TRIR). Toscano and co-workers had the intuition to use the 3,5-diphenyl-1,2,4-oxadiazole-4-oxid (Wieland heterocycle) to generate photochemically the nitrosocarbonyl benzene, in accordance with the protocol proposed by the Pavia group.[22] Laser photolysis (355 nm, 5 ns, 4 mJ) of a solution of the Wieland heterocycle produced the TRIR difference spectra shown in Figure 7.[23] When the nitrosocarbonyl is formed by photochemical cleavage of the heterocycle, the red line of the TRIR spectrum is shown; upon addition of diethylamine the signal of compound is progressively quenched to leave the amide represented by

the blue line. The stability of product was determined to be at least 180 μs. These results are the cornerstone and the ultimate evidence of the nitrosocarbonyl intermediates.

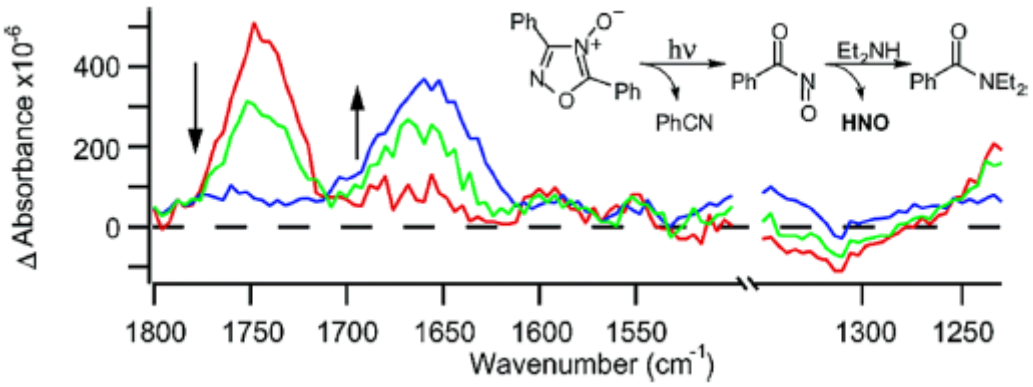
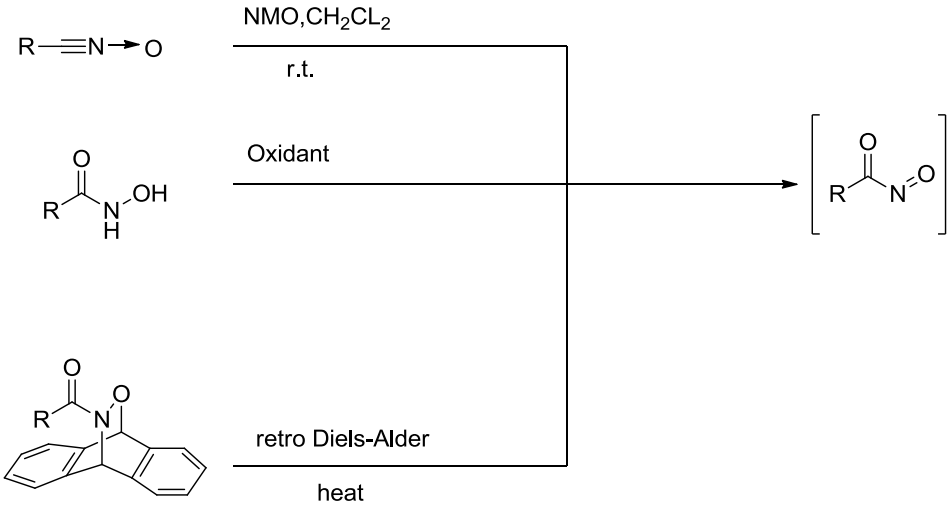


Figure 7: TRIR Spectrum Of Nitrosocarbonyl

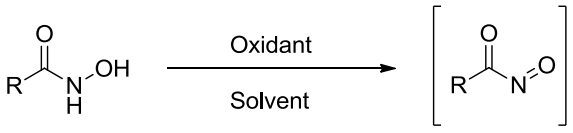
1.5.3 PREPARATION OF NITROSOCARBONYL SPECIES

The study on the generation of acylnitroso species was pioneered by Kirby in a review of this subject in 1977. The nitrosocarbonyl species are obtained from the oxidation of hydroxylamine derivatives, using organic and inorganic natural products. They can also be generated by oxidation of nitrile oxides and by cyclo-reversion from the corresponding cycloadducts (scheme 9).



Scheme 9: Generation of Acylnitroso Intermediate

The oxidation method employs organic oxidants such as periodate salts, Dess-Martin periodinane, NMO-nitrile oxides, Swern-Moffat reagent, and t-BuOOH as well as inorganic reagents such as PCC and hypochlorite. These reagents are often not compatible with oxidant sensitive functionalities. In order to overcome this problem, a strategy was devised to obtain a "masked" form of nitroso (scheme 10). Hence, acylnitroso species were generated from hydroxamic acid in situ and subsequently trapped by suitable molecules.



Oxidants:

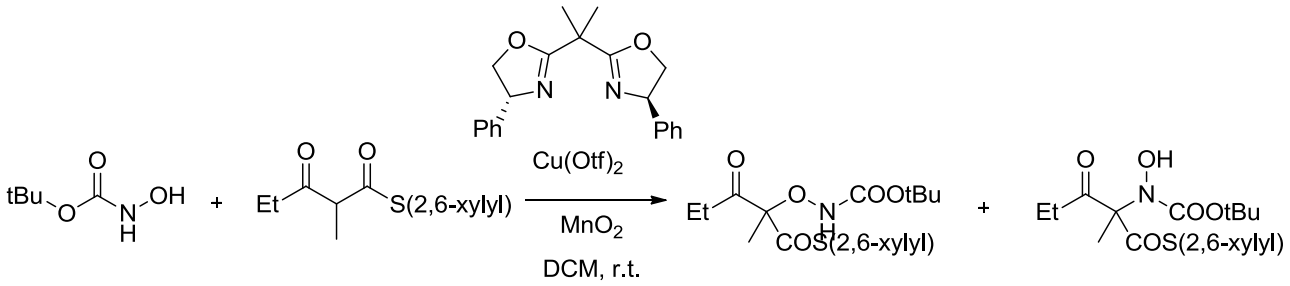
$NaIO_4$ (H_2O/CH_3OH)
$PhI(OAc)_2$ (CH_2Cl_2)
PhIO (CH_2Cl_2)
R_4NIO_4 (CH_2Cl_2)
DMSO/ $(COCl)_2$,(CH_2Cl_2)

Scheme 10: Generation Of Acylnitroso Intermediate From Hydroxamic Acid

1.5.4 NITROSOCARBONYL REACTIONS

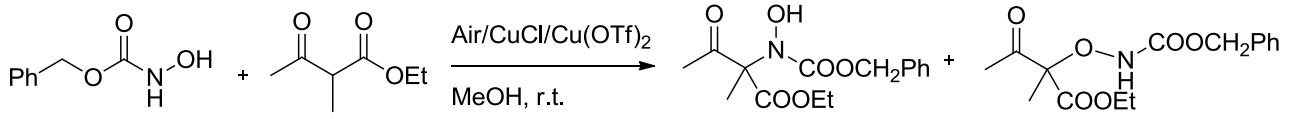
Nitrosocarbonyl intermediates can be used for multiple uses such as classical HAD, ene reaction where the nitrogen and oxygen atoms of the nitroso group are individually linked to a nucleophilic substrate. The first examples of this novel synthetic procedure were presented by the Yamamoto and Read de Alaniz groups in 2012 in two papers published nearly at the same time.

Yamamoto used the carbamate to generate the corresponding nitrosocarbonyl by using MnO_2 as a mild and efficient oxidant in the presence of a Cu(II) complex with the (R,R)-PhBox ligand. The nitrosocarbonyl was captured by an oxopentanoate derivative[24] to afford the O- and N-products in 73% and <1% yields (scheme 11).[25] The method was extended to a variety of nucleophiles; the yields were in the range 45−91% with excellent ee (78−99%).



Scheme 11: Catalyzed O- and N- Functionalization with Nitrosocarbonyl

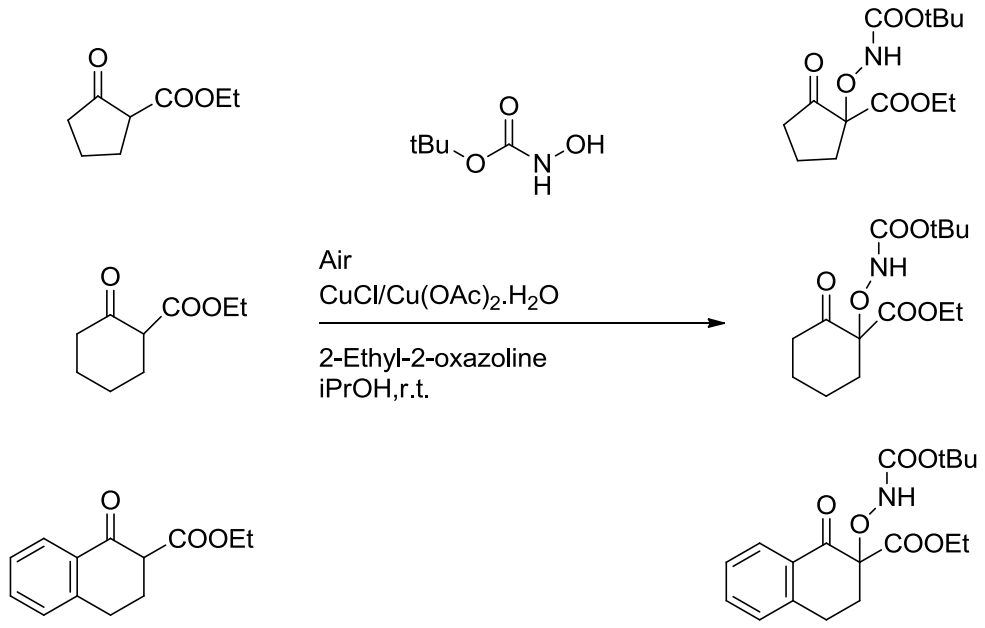
Read de Alanix reported on the copper-catalyzed α-amination of carbonyl compounds using nitrosoformate intermediates as an electrophilic source of nitrogen. Ethyl 2-methyl-3-oxobutanoate was allowed to react with the benzyl hydroxycarbamate under aerobic conditions in the presence of Cu(I)/Cu(II) as the catalysts in methanol as solvent (Scheme 12).



Scheme 12: Aerobic Generation of Nitrosocarbonyls

The new methodology harnesses the power of benzyl hydroxycarbamate as a viable electrophilic source of nitrogen in α-functionalization reactions.

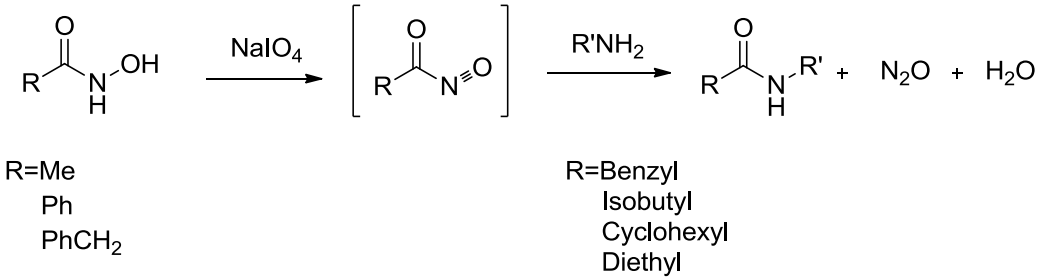
On pursuing the investigation on the ambient reactivity of nitrosocarbonyls as electrophiles, the Read de Alaniz group extended the studies to cyclic ketoesters. The nitrosocarbonyl was generated through aerobic conditions in the presence of Cu(I)/Cu(II) as the catalysts and 2-ethyl-2-oxazoline, getting very good results in terms of yields relative to products for the α-oxygenation as reported in scheme 13.



Scheme 13: Aerobic Oxygenation of Cyclic Ketoesters

Other reactions regard the generation of nitrosocarbonyl intermediates in the presence of nucleophiles, but contrary to the cases reported above, we refer to classical nitrogen nucleophiles, such as amines or their derivatives.

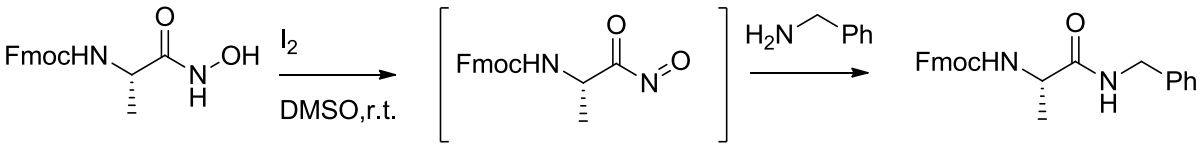
Oxidation of hydroxamic acids in the presence of primary or secondary amines generates nitric oxide (N_2O) and the corresponding *N*-alkyl-amides. King and co-workers in 1996 reported these reactions performed in water as solvent affording the amides in 16−65% yields and N_2O in 42−69% yields.



Scheme 14: Amides and N_2O Generation from Nitrosocarbonyls

The identification of N_2O suggested the intermediacy of HNO, and the method was proposed in view of the generation of this highly reactive species.

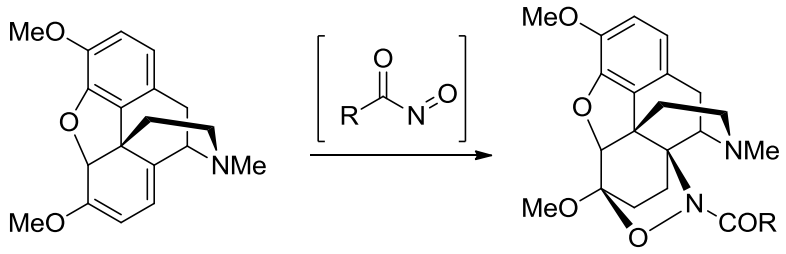
Years later, an efficient and straightforward approach for the coupling of N-protected hydroxamic acids with amino components in the presence of iodine was delineated. The iodine oxidation of hydroxamic acid in the presence of benzylamine (DMSO as solvent at room temperature) afforded in the amide in 95% yield (Scheme 15).[26] If the amine is an amino acid, the method nicely represents a protocol for new peptide bond formation.



Scheme 15: Iodine Oxidation of Hydroxamic Acid

1.5.5 NITROSOCARBONYL HETERO-DIELS–ALDER REACTIONS

The most common use of nitroso compounds is related to their ability to participate in [4+2] cycloaddition reactions. The first nitroso HDA reactions using aryl- and alkylnitroso compounds were reported by Wichterle[27] and Arbuzov[28] in 1947 and 1948, respectively. One of the earliest examples of a HDA reaction using an acylnitroso compound was reported by Kirby and Sweeny in 1973, where acylnitroso compounds were generated in the presence of thebaine to afford the corresponding cycloadducts selectively (Scheme 16).[29] The remarkable selectivity observed in acylnitroso HAD reactions provides access to 3,6-dihydro-1,2-oxazines and ultimately 1,4-amino alcohols.



Scheme 16: The cycloaddition reported by Kirby and Sweeny

The mechanism of the acylnitroso HDA reaction has been studied computationally by Leach and Houk: the n–p repulsion exhibited by the lone pair of electrons on the nitrogen atom, termed the "exo lone pair effect",[30,31] is responsible for this strong preference for the placement of the nitrogen substituent in an endo position.

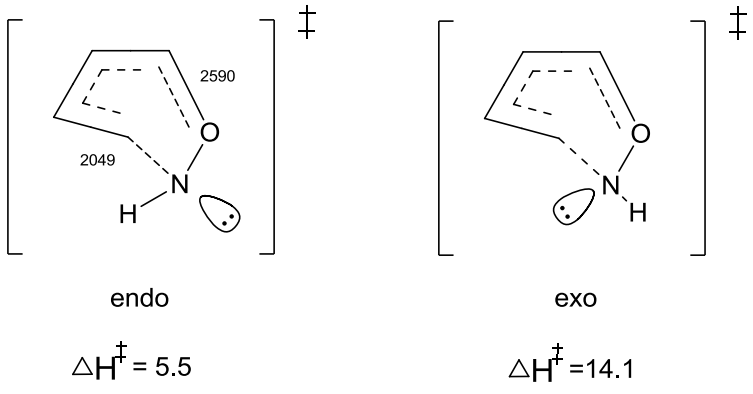
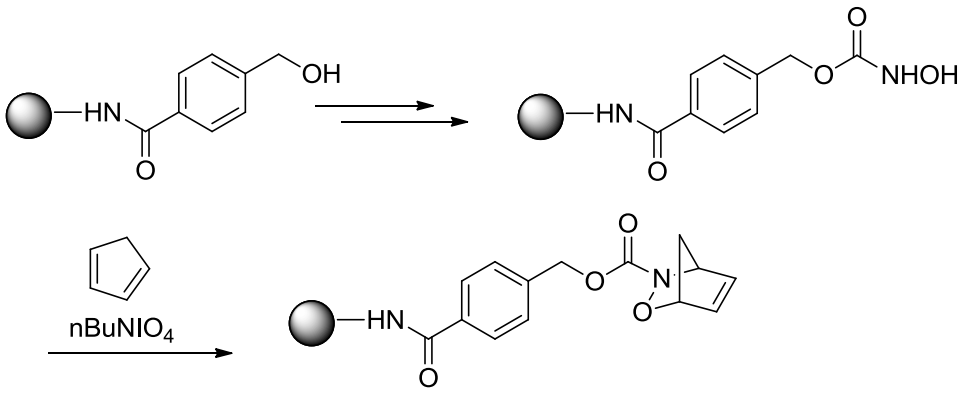


Figure 8 : Computed Energies For Transition States Of The Nitroso HAD Reaction

The combined preference for placement of the nitrogen substituent in an endo position with the CN bond in the transition state can explain the high regio- and stereoselectivities observed in acylnitroso HDA reactions. The regioselectivity in nitroso HDA reactions can be rationalized on the basis of frontier MO theory. Dienes with strongly electron donating or electron withdrawing substituents provide cycloadducts with higher regioselectivity then dienes with substituents that are only weakly electron donating or electron withdrawing.[32]

There are a number of reviews detailing the use of asymmetric nitroso HDA reactions in organic syntheses. Methods for performing asymmetric nitroso HAD reactions include the use of chiral nitroso dienophiles, chiral dienes, and, with mixed success, the use of chiral catalysis.

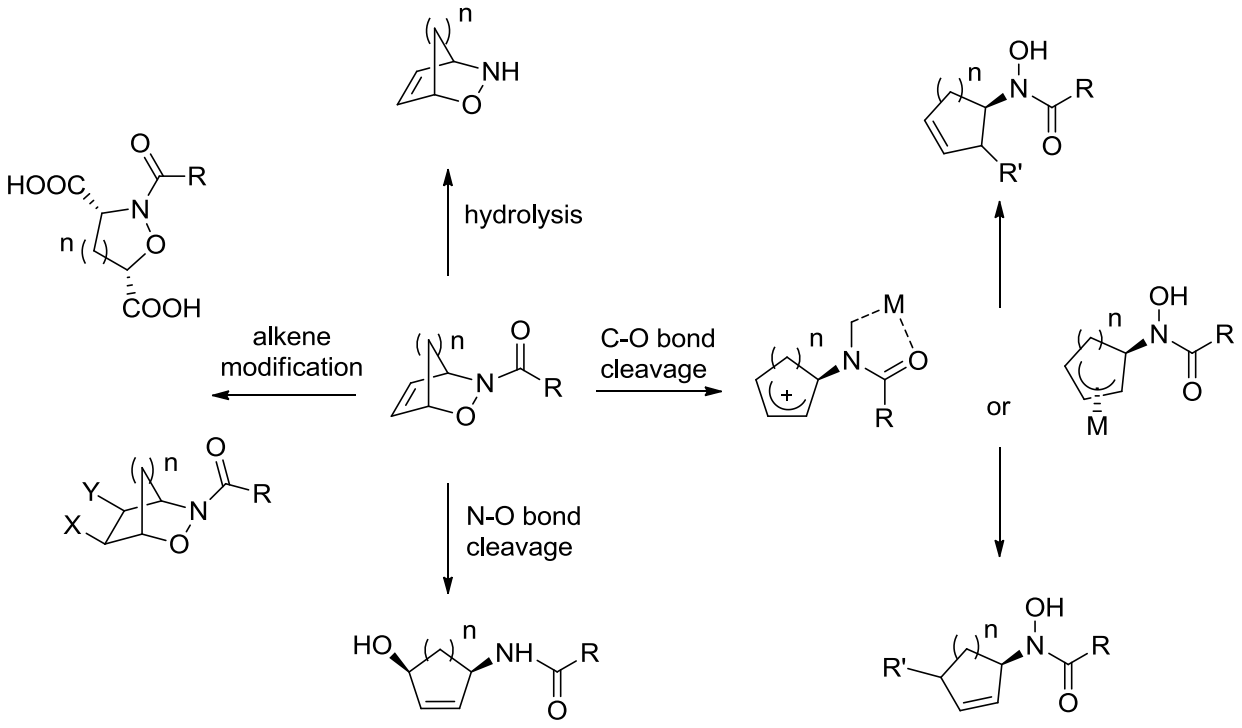
Although nitrosocarbonyl HDA reactions have been widely used in organic synthesis in solution, there have only been a few accounts of performing nitrosocarbonyl HAD reactions on a solid support. One example reported by Krchnak et al.[33] utilized Wang resin supported hydroxamic acids derived from alcohols (Scheme 17). The hydroxamic acid were oxidized using tetrabutylammonium periodate in the presence of dienes to yield cycloadducts.



Scheme 17: Nitrosocarbonyl HAD Reaction On A Solid Phase

1.5.6 CHEMISTRY OF 3,6-DIHYDRO-1,2-OXAZINES

Most of the utility of the acylnitroso HDA reaction in organic syntheses arises from the rich chemistry of the resulting cycloaddition products. The rapid construction of a wide variety of functional groups in one molecule allows access to a number of molecular scaffolds from simple bicyclic cycloadducts reported in the scheme 18. The structural modification of cycloadducts can be divided into one of the four main areas: cleavage of the N-acyl bond to yield oxazines, cleavage of the NO bond to yield amino alcohols, cleavage of the CO bond to yield compounds and alkene modification to afford compounds.

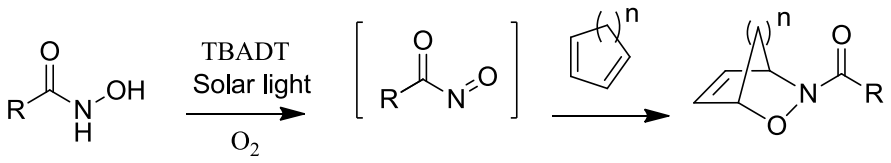


Scheme 18: Modification Of Bicyclic 3,6-Dihydro-1,2-Oxazines

Additionally, compounds such as oxazines have demonstrated the ability to undergo a number of rearrangements and other chemical reactions. The carbonyl group of cycloadducts is susceptible to hydrolysis under relatively mild conditions (R=alkyl or aryl).[34] This provides the basis for removing many of the chiral auxiliaries. The following section details various transformations of cycloadducts commonly utilized in synthetic organic applications.

In this thesis we propose an innovative method for the generation of nitrosocarbonyl species from hydroxamic acid. This protocol uses TBADT as a valuable photocatalyst for sunlight-induced

organic reactions. The reaction represents a photocatalytic oxidation of hydroxamic acid to generate fleeting nitrosocarbonyl intermediates. These highly reactive species were efficiently trapped with suitable reactive dienes, such as cyclopentadiene and 1,3-cyclohexadiene, to afford the desired Hetero Diels-Alder (HDA) cycloadducts in good yields (Scheme 19).



Scheme 19 : Photoredox Catalytic Approaches For Nitroso And Nitrosocarbonyl Generation

1.6 AN INNOVATIVE HYDROXAMIC ACID : PANOBINOSTAT

Between the different hydroxamic acids synthetized and tested, the synthesis of one in particular attracted our attention: Panobinostat marketed under the name Farydak.

The Farydak belongs to a novel class of compounds called deacetylase (DAC) inhibitors and was recently approved by the FDA and EMA to be used in combination with bortezomib and dexamethasone to treat patients with multiple myeloma who have received two prior regimens, including bortezomib and an IMiD.

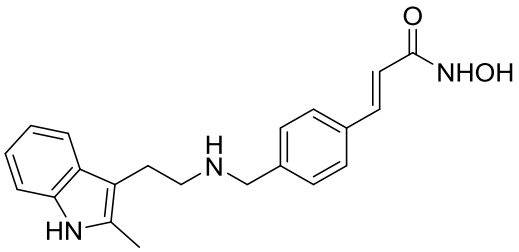


Figure 9: The Panobinostat structure

Panobinostat inhibits a broad range of DACs (Fig.10), which are also known as histone DACs (HDAC) because histones were the first known targets of DACs.[35] It is now known that DACs regulate the acetylation of approximately 1,750 proteins involved in diverse biologic processes, including DNA replication and repair, chromatin remodeling, gene transcription, cell-cycle progression, protein degradation, and cytoskeletal reorganization. Over expression of DACs has been observed in multiple myeloma and is associated with poor outcomes.

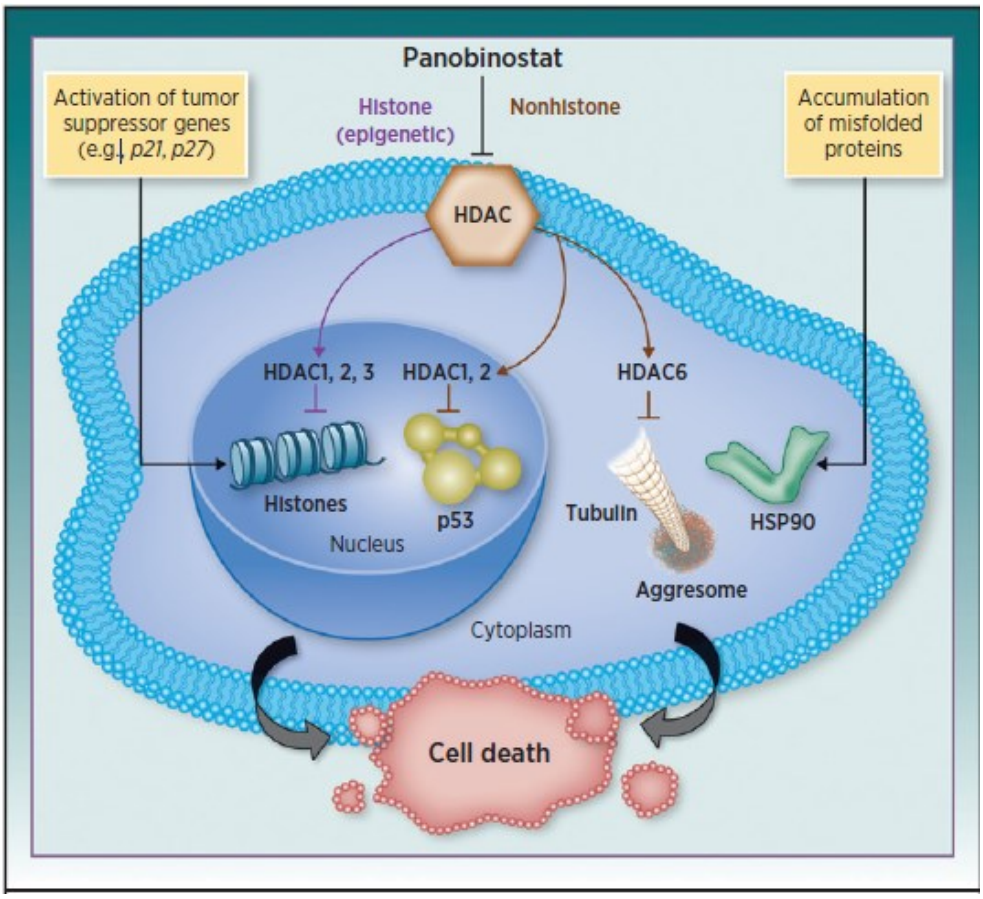


Figure 10: Mechanism Of Panobinostat
American Society of Hematology; 2014. Abstract nr 2120.

The importance of this molecule is highlighted by its physicochemical properties and structural features that are compatible with the inhibition of HDAC. The structure contains basic nitrogen functionality in the spacer region between the terminal aryl and the hydroxamic had the overall effect of improving the physicochemical properties of the compound. Incorporating the basic nitrogen into molecular spacer or a vinylic benzene group improved the metabolic stability.

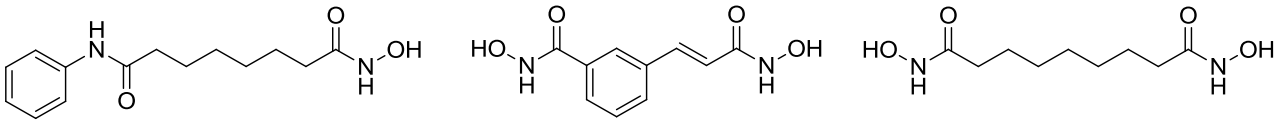


Figure 11: Chemical Structures of Know HDAC inhibitors

Other hydroxamic acid-containing compounds, known as SAHA and CBHA demonstrated inhibitory in vitro activity against HDACs, but there was no mention of any in vivo activity.[36] However, in 1999 a study had been published on a close related bishydroxamate compound, ABHA, for which in vivo antitumour activity had been demonstrated in a mouse xenograft model using human melanoma cells.[37] Based on these studies, ABHA was chosen as our positive control for a HDAC inhibition activiy study toward in vivo antitumor activity.

The hydroxamate group is a necessary feature for a potent HDAC inhibitor. Structurally similar compounds with a carboxylate or a carboxamide in place of the hydroxamate were a thousand fold less potent. The 1,2-trans olefin is a critical structural feature since the cis olefin and the saturated ethyl bridge resulted in much less potent compound. The 1,4-phenyl ring substituents (Figure 12) in the centre portion of our chemotype is optimal, but small ring substituents on the phenyl are also tolerated. Likewise, the one methylene between the 1,4-phenyl ring and the basic amine is also optimal, and small groups attached to the methylene were tolerated. The distance between the basic amine and the terminal aryl could vary over two to four methylene groups and small attached groups were tolerated.[38] A wide variation of substituents on the basic amine is permissible, but generally N-acylation and N-sulfonylation decreased HDAC inhibitory activity, whereas alkyl groups maintained or improved potency (Figure 12).

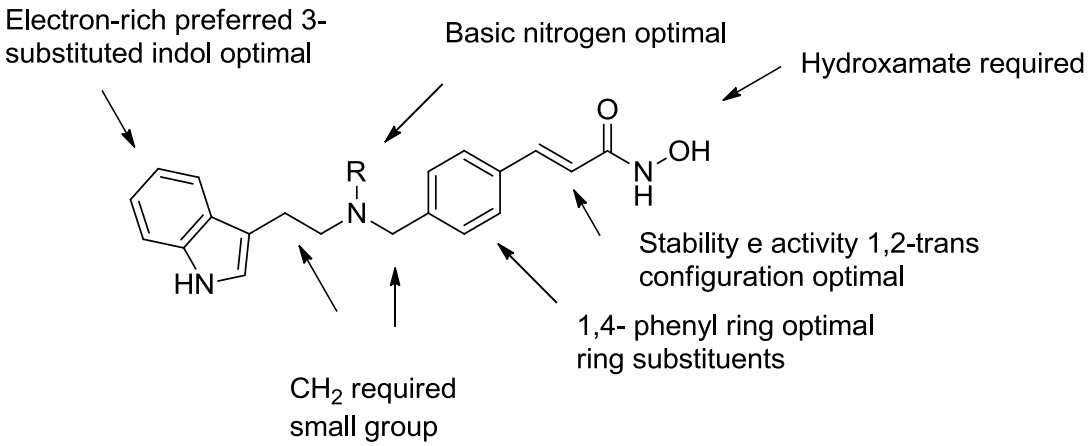
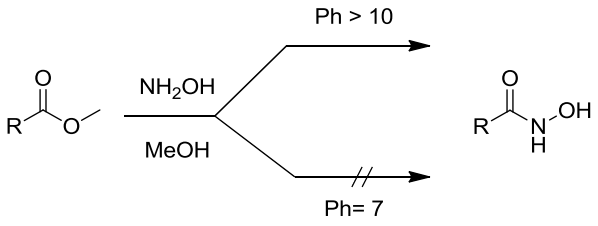


Figure 12: SAR Of The Cinnamoyl Hydroxamate Chemotype

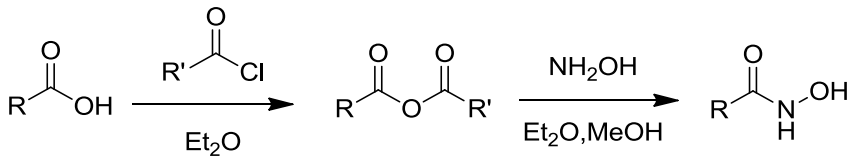
The economical way of making hydroxamic acid derivative is the reaction of hydroxylamine with acyl chlorides or esters.[39] The preparation of acyl chlorides is often tedious when other acid labile functional groups are present in the substrate. Moreover, it will be very difficult to prevent further acylation during the reaction with hydroxylamine. In this context, for our process, starting from the corresponding ester, the reaction of hydroxylamine does not proceed under neutral conditions (Scheme 20); it always needs an alkaline environmental (pH>10).[40] Hence, this method is not

compatible with several functional groups such as halides, carbonyls and other base-sensitive groups.



Scheme 20: Preparation of Hydroxamic Acids from esters

On the other hand, there is a simple one-step approach for the preparation of hydroxamic acids from carboxylic acid derivatives as shown in scheme 21.



Scheme 21: Preparation of Hydroxamic Acids from carboxylic acid

Several hydroxamic acids have been synthesized during this PhD thesis using the classical approaches previously mentioned, with particular focus to Panobinostat. Our approach to access the target molecule is depicted in scheme 22. We explored the key coupling, a reductive amination using a wide range of conditions such as: solvents, reductive agents and additives. Unfortunately, for patent reasons it is not possible to describe in details our approach and the reagents used.

However the reaction took place successfully, affording the desired product with a yield of 62%.

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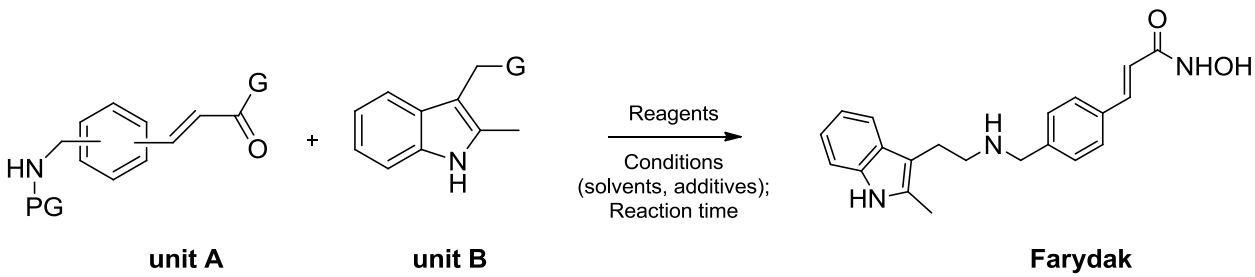
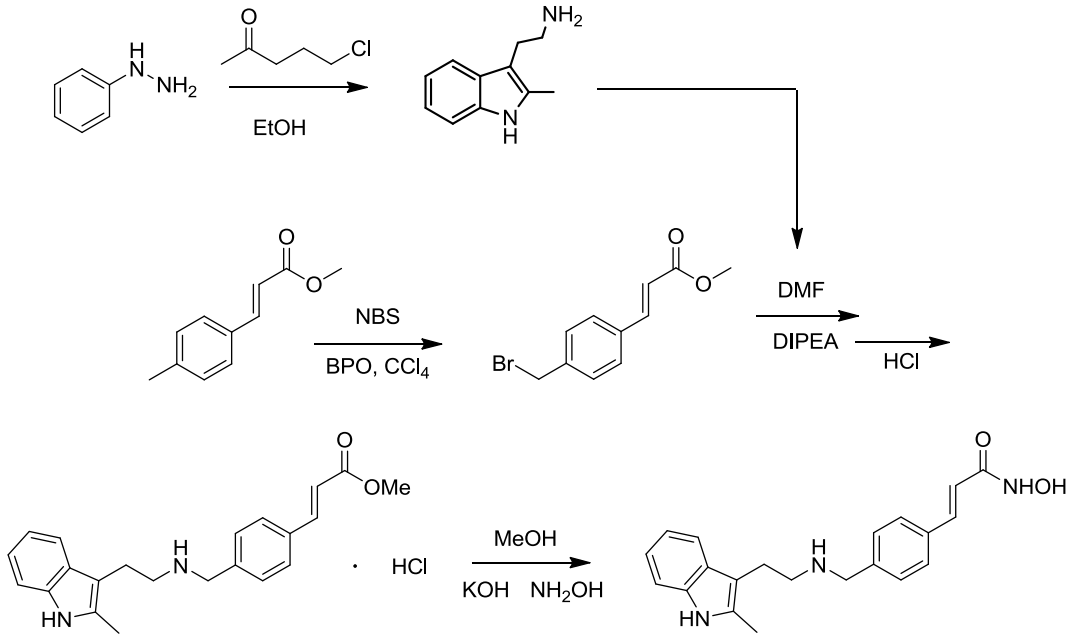


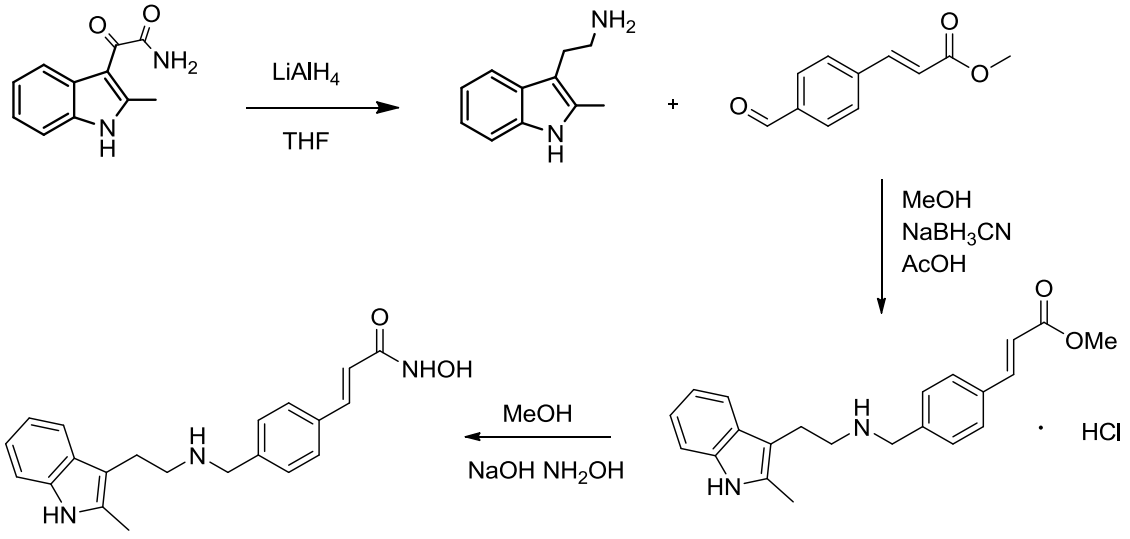
Scheme 22: Preparation Of Farydak

Liu Qian et al. ("Chinese Journal of Industrial Medicine", 2011,42 (10), 725-727) previously, disclosed a different method for the preparation of the same structure and the synthetic strategy is described in scheme 23 :



Scheme 23: Preparation Of Farydak I

The reaction makes large use of toxic materials, thus affecting the safety of the final product. A different approach has been proposed by Novartis [42] (Scheme 24), but the method use harsh conditions, and impurities not suitable for industrial production were formed.



Scheme 24: Preparation Of Farydak II

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2. AIM OF THE THESIS

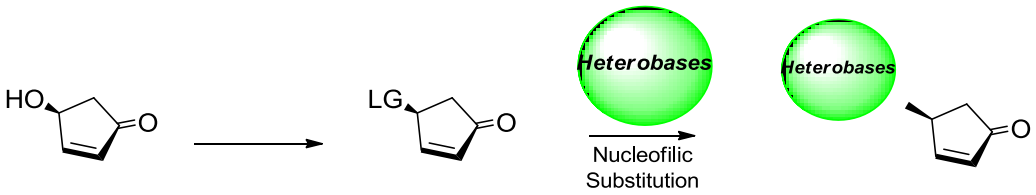
The synthesis of new nucleoside analogues is a valuable approach in viral chemotherapy showing a remarkable effect towards specific viral infections

The aim of this work is to synthetize new carbocyclic nucleosides by taking advantage of the chemistry of nitrosocarbonyl intermediates and nitrile oxides.

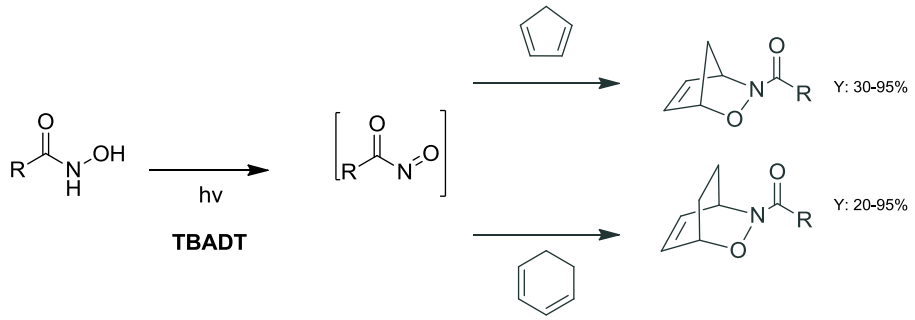
The thesis can be divided in three main topics:

a) First of all, were conducted synthetic studies of nucleoside analogues of racemic 4-hydroxy-2-cyclopentenone, a scaffold often found in natural products and biologically active compounds. This core can be easily functionalized with suitable leaving groups to perform nucleophilic substitution reaction or metal-catalyzed synthesis to access nucleoside analogues by insertion of several heterobases. The reaction can be also evaluated in its optically pure version.

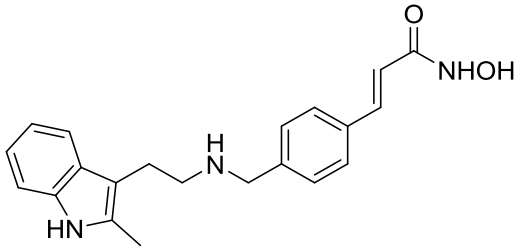
Docking studies guided the choice of the functional groups in order to increase or modify properly the biological activity. Apoptotic activities were evaluated for the most promising compounds.



b) In this part, nitrosocarbonyls were generated using a photochemical reaction method. The starting material, an hydroxamic acid or its corresponding salt, was tested in oxidant room of TBADT. The tetrabutylammonium decatungstate (TBADT) is an efficient and robust photocatalyst able to promote photoredox reactions, as well as hydrogen atom transfer processes, starting from different classes of organic substrates. The [4+2] cycloaddition of dienes with nitrosocompounds, namely the nitroso-Diels-Alder (NDA) reaction, is a versatile method to generate highly reactive acylnitroso species from hydroxamic acid derivatives. Since nitrosocarbonyl intermediates participate in a variety of organic reactions, the *in situ* formation of this highly reactive species using photoredox conditions furnished a general procedure for patterning surfaces bearing a range of properties.



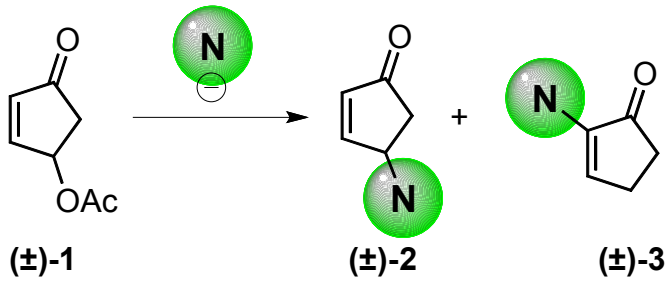
c) Finally, we proposed a new synthesis of Panobinostat, a polifunctionalized hydroxamic acid with important biological properties. Specifically, it is an orally administered drug for the treatment of patients with multiple myeloma, as reported above. In this context, for its relevant pharmacological role, it is essential to identify new step-economy and waste-economy approach to access this important compound.



3. NUCLEOSIDE ANALOGUES OF RACEMIC 4-HYDROXY-2-CYCLOPENTENONE

Racemic and enantiomerically pure 4-oxocyclopent-2-en-1-yl acetates **1** are valuable intermediates in organic synthesis, often employed in the short-cut preparation of nucleoside analogues with pyrimidine and purine heterobases.[1] The chemistry of these scaffolds have been extensively investigated and reviewed since the hydroxycyclopentenone moiety often appear in natural and bioactive products[2] or constitutes the privileged starting compound for their synthesis.

In a previous work, my research group investigated the use of acetate **1** as a substrate for the functionalization of cyclopentane ring with uracil, thymine, 6-chloropurine and some adenines in a fast-track giving nucleoside analogues.[3] It was found that the expected 4-heterosubstituted products could be obtained along with the isomeric 2-heterosubstituted compounds as minor components. The protocol for their synthesis stands on a simple nucleophilic substitution of the acetate group of the cyclopentenone scaffold **1** with the desired heterobase.



Scheme 1. Synthesis Of 4-Substituted- And 2-Substituted-Cyclopentenone Derivatives

On pursuing the investigations on the synthesis of nucleoside analogues based on the cyclopentenone scaffold, two elements were the focus of our subsequent researches. The first one regarded the selectivity of the substitution process: upon the addition of the 4-oxocyclopent-2-en-1-yl acetate **1** at 0 °C to solutions of the heterobase anions in anhydrous acetonitrile and leaving under stirring for 3 hours, mixtures of the two regioisomeric products **2** and **3** were obtained.

Being our aim to obtain single regioisomers, a modified protocol able to govern the selectivity outcome was studied. In particular, our focus was to obtain 2-substituted cyclopentenone derivatives that have previously displayed only modest antiviral activities. In order to improve their performances, a structure-activity relationship (SAR) was performed.

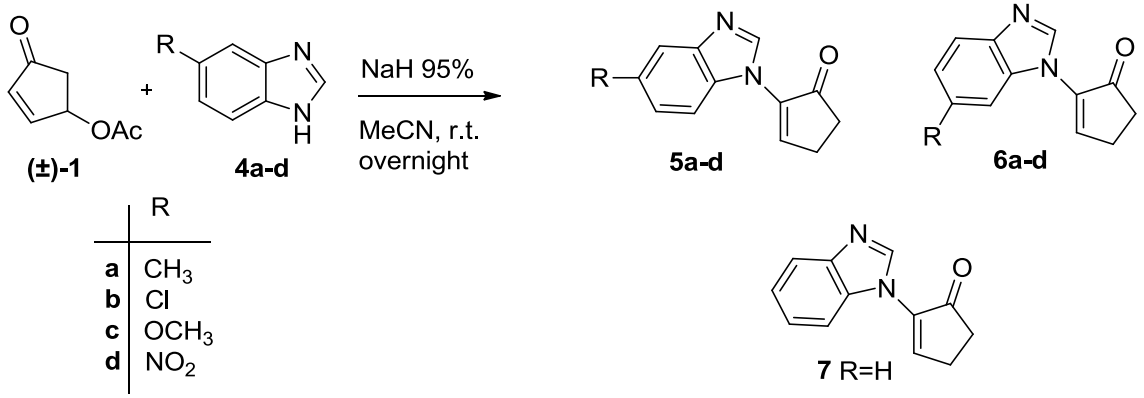
Our strategy consisted in the use of benzoimidazole derivatives, as simple heterocyclic models, bearing a variety of substituent pattern reactive enough as nucleophiles and suitable for locating structural changes or derivatization pathways. So, our aim has been to develop a step-by-step tuning

of both the heterocycle structure and of the cyclopentenone moiety in order to get the required information on the structure-activity relationship (SAR) for these molecules. Their biological evaluations corroborate our study.

3.1 SYNTHESIS OF 2-SUBSTITUTED-CYCLOPENTENONE DERIVATIVES

The racemic 4-oxocyclopent-2-en-1-yl acetate[4] **(±)-1** was obtained by acetylation reaction[5] of the corresponding 4-hydroxy-2-cyclopentenone according to known procedures.[6,7]

The coupling reactions were conducted by in situ generation of the conjugated bases of the commercially available substituted benzoimidazole derivatives **4a-d** with NaH 95% in dry acetonitrile (Scheme 2). The benzoimidazole anions generation required the use of a Schlenk tube apparatus under vacuum, stirring the suspension for 15 minutes at room temperature. Afterwards, the acetonitrile solution of the cyclopentenone acetate **1** was added and the reactions were left under stirring in inert atmosphere overnight at room temperature. Quenching was performed by pouring the mixtures in ice mixed with NaCl/NH_4Cl 1:3. Extraction with DCM allowed for the obtaining of organic phases that were dried over anhydrous Na_2SO_4. Residues were submitted to chromatographic separation to isolate and purification the products.



Scheme 2. Synthesis of 2-benzoimidazole-cyclopentenone derivatives 5 and 6.

In order to get evidence of electronic effects on the reaction regeselection, selected benzoimidazoles **4a-d**, functionalized with both electron-donor or electron-withdrawing substituents, were tested. Compound **7**, containing the substituent-free benzoimidazole ring, was also synthesized for comparison purposes. 2-Substituted cyclopentenone derivatives were selectively obtained, as a

regioisomeric mixtures of compounds **5a-d** and **6a-d**. Compounds **5a-d** are obtained from the addition of N1 nitrogen atom of benzoimidazole, while compounds **6a-d** of N3 one, since the aza-anion resonates between these two nitrogens. No 4-substituted cyclopentenone derivatives (type 2 of Scheme 1) were isolated or even detected in the crude reaction mixtures. The synthetic method leads selectively to the 2-substituted compounds (type 3 of Scheme 1). Compounds **5a-d** and **6a-d** were obtained as inseparable mixtures of regioisomers and every attempt to separated them with chromatographic techniques failed due to the low level of structural differentiation between the single pairs. Table 1 reports chemical yields, physical data and the relevant IR bands of the synthetized adducts. The yields are good in all the cases. They never reach quantitative values due to partial decomposition of the starting acetate **1**.It is known, in fact, that it is unstable upon standing at room temperature: decomposition starts in a couple of days to afford another unstable compound, the cyclopentadienenone (for mechanism, vide infra). From the structural point of view, the IR spectra clearly report the presence of the carbonyl C=O groups and the C=N bands referred to the imidazole moieties and the values are found at the expected wave numbers typical of these compounds.

Table 1. Chemical yields, physical data and relevant IR bands of compounds 5a-d, 6a-d and 7 .

Entry	**5/6**	Yield (%)	M.p. (°C)[a]	IR (cm^{-1})[b] $\delta_{C=O}$	$\delta_{C=N}$
1	**a**	53	205-210	1686	1607
2	**b**	60	215-219	1674	1595
3	**c**	51	210 (dec.)	1684	1607
4	**d**	67	194-200	1692	1590
5	**7**	70	>250	1678	1581

[a]From diisopropyl ether with few drops of ethanol. [b]Nujol mulls.

However, the structural assignment remained an open problem and was adequately solved even in the presence of mixtures of compounds by NMR experiments by collecting the ^{1}H NMR spectra in deuterated DMSO and analyzing the signals with the help of further experiments, such as COSY and NOESY and upon comparison with the single compound spectrum obtained from the reaction with benzoimidazole (Table 2).

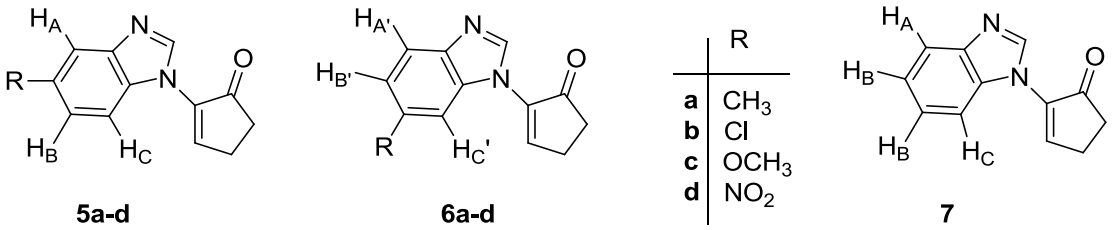


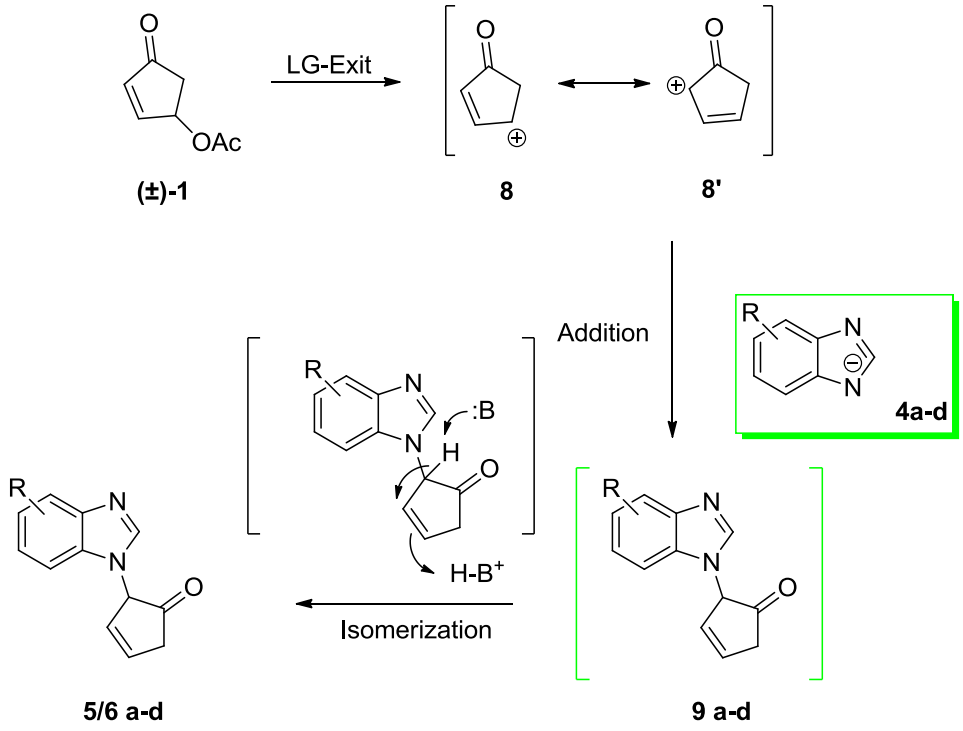
Table 2. ^{1}H NMR characterization of compounds **5a-d**, **6a-d** and **7**

Compounds **5a-d**, bearing the substituent **R** on the **C5** of the benzoimidazole ring, are the minor components of the mixtures where the ratios range from 1:1.2 for compounds **5/6a,c** to 1:2 for the nitro derivatives **5/6d** up to 1:4 for the chloro derivative **5/6b**. They showed the more deshielded chemical shifts of the CH=N of the benzoimidazole ring with respect to the regioisomers **6a-d**, while some order inversions are found for the signals corresponding to the olefinic =CH protons. The aromatic signals of the benzenic part of the heterocyclic moiety deserve the typical multiplicity relative to two protons with an ortho coupling, being one of them coupled with the third proton through a *meta* coupling constant (not always detectable).

Protons HC in adducts **5a-c** are more deshielded with respect to all the other signals presumably because of the proximity with the carbonyl group of the cyclopentenone moiety (free rotation around the NHET-C= bond) with a single exception of **5d**, where the nitro group displays deshielding effects on the other adjacent protons. The same happens for the protons HC' of compounds **6a,b,d** with the single exception of **5c**, where the methoxy group displays shielding effects on the other adjacent protons. The chemical shifts of the benzoimidazole **7** closely resemble those of the methyl derivatives and confirm that the HC proton is the most deshielded among the aromatic protons because of the proximity with the carbonyl group of the cyclopentenone moiety.

The attributions were corroborated through COSY and NOESY experiments performed on the methoxy derivatives **5/6c** that showed more separated signals with respect to the other compounds and similar intensities, thus allowing to perform efficiently these double resonance experiments. In particular in the NOESY experiment, the proton HC' of **6c** at 7.34 δ gives a NOE spot with the methoxy group at 3.89 δ as well as with the olefinic proton at 6.67 δ; this latter gives also a NOE spot with the CH=N proton at 8.67 δ. On the other hand, in the case of compound 5c, the doublet at 7.90 δ gives a single NOE spot with the olefinic proton at 6.59 δ; this latter also spatially interacts with the CH=N proton at 8.76 δ. On the basis of these observations all the correlations for the structure assignments for the other compounds derive consequently.

3.2 REACTION MECHANISM



Scheme 3. Synthesis of 2-benzoimidazole-cyclopentenone derivatives **5** and **6**: mechanism.

On the basis of the above investigations, a possible reaction mechanism can be suggested, as shown in scheme 3. The structures of compounds **5a-d, 6a-d** and **7** rely on a leaving group (LG)-elimination-addition-isomerization process that starts from the acetate **1**, affording the allyl cation **8**-**8'** that, under the reported experimental conditions, adds the benzoimidazole anions **4a-d** to give the not-isolable adducts **9a-d**. These latters undergo C=C double bond isomerization to the final products **5/6a-d**. The proposed mechanism has been previously demonstrated upon starting from enantiomerically enriched acetate **1** that could not afford any optically active products but just racemic compounds, as a definitive confirmation that the allyl cation of type **8** is involved in the product formation and that a SN_2 type nucleophilic substitution can be discarded among the possible reaction routes.[8]

Another key point about the synthetized compounds regards their thermal and photochemical stability. We have investigated this points taking into account previous observation[8] and in view of novel opportunities for a further functionalization of the products, useful for SAR studies that require structural changes. Compounds **5/6a-d** were heated at reflux in methanol for several hours and the stability of the compounds monitored by TLC. After 24h, compounds **5/6a-d** were found unchanged and their stability upon moderate heating was confirmed by ^{1}H NMR spectra that gave

the same signals as the original samples. On the other hand, when heated in the presence of bases (e.g. triethylamine) compounds **5/6a-d** undergo decomposition leaving detectable and isolable benzoimidazole residues and the cyclopentane moiety, reasonably as cyclopentadieneone, as previously suggested.[8]

We also exposed samples of the compounds **5/6a-d** to UV light at 254, 310 and 400 nm for a couple of hours to test their photostability. Even in these cases we noted the release of the benzoimidazole moieties that has been detected by NMR being the cyclopentene moiety degraded.

In summary, we can conclude that these structures are thermally (in the presence of bases) and photochemically labile and that, unless with the help of further structural modifications able to stabilize them, they hardly employed for further transformations.

3.3 ANTIVIRAL EVALUATION

In literature there are different nucleoside analogues expressing antiviral activity. Our purpose has been to evaluate the antiviral activity of the synthesized compunds that were sent to the NIAID (NIH, USA)[9] for in-vitro tests against a variety of viruses for a primary screening study. Products **5/6a-d** were tested against the Herpesviridae family, Varicella-Zoster virus, (VZV), from the Hepatic virus HBV, Respiratory Viruses such as Influenza A virus H1N1 (IV/H1N1), Adenovirus-5 (AD5), from the Togavidae family, Chikungunya virus (CV), from the Flaviridae group, Yellow Fever Virus (YFV), from the Bunyaviridae family, Punta Toro Virus (PTV), and from the Papovaviridae the Human Papilloma Virus (HPV).

Compounds **5/6a-d** were found inactive against VZV; although the CC50 is >150 (identical to Aciclovir used as reference compound), the EC50 was >150, enormously higher than the reference. Similarly, compounds **5/6a-d** were found inactive against HBV. Lamivudine, used as reference compound, has an EC50 = 0.041. On the other hand our compounds have a value >100. The primary antiviral data of compounds **5/6a-d** against IV/H1N1 and AD5 were compared with the relative reference compounds and show that compounds **5/6a,b,d** were found inactive while **5/6c**, that bear a methoxy substituent, showed a modest activity although the EC50 is more than 10 times that of Ribavirin. On the other hand, the activities against AD5 were disappointing. Table 3 gathers the EC50, CC50 and SI50 values (μg/mL) obtained in the in vitro tests against the reported viruses for the compound **5/6c**, only. A complete data table for all the compounds can be found in the SM.

Finally, the primary tests against YFV and CV demonstrated a total inefficacy of all the compounds against these viruses as well as of the tests against PTV and HPV.

Table 3. Primary antiviral activities of compounds **5c** and **6c** against IV/H1N1.

Entry	**Compound**	Drug Assay Name (Cytopathic eff.)	EC_{50}	CC_{50}	SI_{50}
		IV/H1N1[a]			
1	**5/6c**	Visual	15	>100	>6.7
		Neutral Red	25	>100	>4.0
		IV/H1N1[a]			
2	*Ribavirin*[b]	Visual	1.2	>320	>270
		Neutral Red	1.4	>320	>270

Drug conc. Range 0.1-100 µg/mL (vehicle, DMSO). [b]Control conc. Range 0.32-320 µg/mL. [c]Drug conc. Range 0.048-150 µM (vehicle, DMSO). [d]Control conc. Range 0.048-150 µM.

On the basis of these results we have focused our attention to the possible two conformers of the benzoimidazole-cyclopentenone structures and the methoxy derivatives for planning further derivatizations in order to strongly modify the structures at different positions of the entire skeleton, triggering a more effective biological activity. We have located the more stable optimized conformation of adduct **7** and those of the two methoxy derivatives **5c** and **6c** at the B3LYP(6-31G)(d) level.[10]

3.4 COMPUTATIONAL STUDIES

The two types of conformers have been identified as *syn* and *anti* on the basis of the relative position of the cyclopentenone carbonyl group and the H-C=N proton of the imidazole ring (Figure 1).

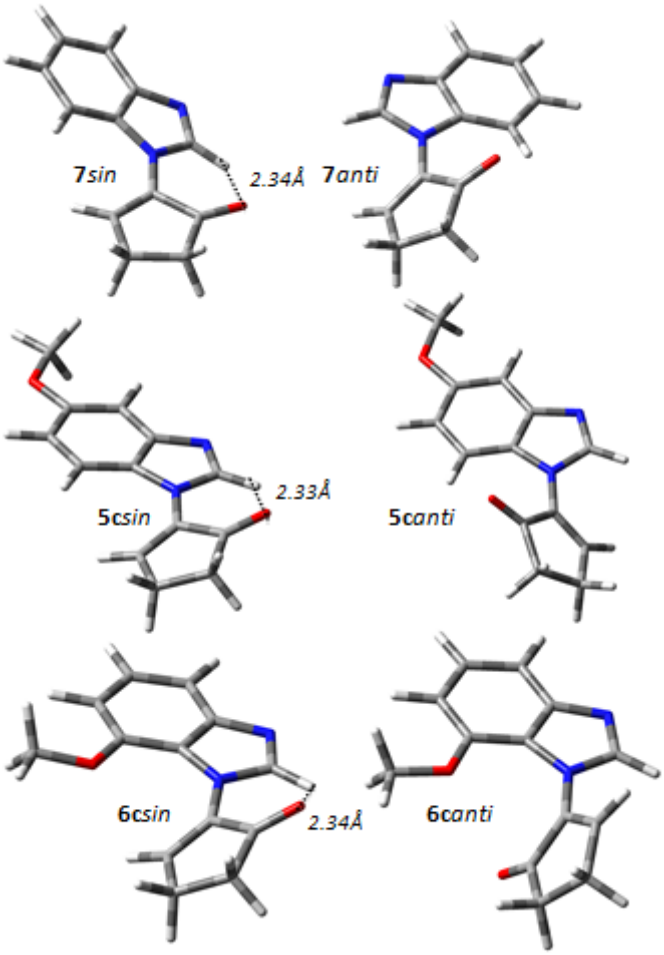


Figure 1. Optimized conformers of benzoimidazole-cyclopentenone **7** and methoxy derivatives **5c** and **6c**. Values in Å indicate the H-bonding distances.

Conformer **7***syn* was found more stable than **7***anti* by 1.91 kcal/mol and shows a stabilizing intramolecular H-bonding between the H-C=N proton of the imidazole ring and the cyclopentenone carbonyl group at a distance of 2.34Å. The intramolecular H-bonding keeps the benzoimidazole ring of the **7***syn* conformer slightly tilted by 26.4° with respect to the cyclopentenone average plane. Conformer **7***anti* does not gain any stabilizing effect through H-bonding and the two rings are tilted by 44.4°.

Moving to the methoxy derivatives that showed the most promising antiviral activities against the IV/H1N1, the comparison of the conformers offer a similar picture. Conformer *syn* **5c** is more stable than **5c** *anti* by 1.99 kcal/mol and stabilized by an intramolecular H-bonding (2.33Å) with the two sings on different planes (dihedral angle 25.4°). Conformer **6c** *sin* is more stable than **6c** *anti* by 1.62 kcal/mol and stabilized by an intramolecular H-bonding (2.34Å) with the two sings on different planes (dihedral angle 36.1°). The absence of a stabilizing intramolecular H-bonding in the comformers of type *anti* brings the two cyclic moieties of the molecules (cyclopentenone and methoxy-benzoimidazole) to be tilted by larger dihedral angles (up to 50°).

These conformational pictures somewhat suggest scope and limitations in the modification and/or derivatization of compounds **5/6** and **7**.

In conclusion, we have reported a selective approach to 2-heterosubstituted cyclopent-2-en-1-ones through a valuable protocol that avoids the presence of side regiosiomeric compounds.[11]

The preliminary biological in vitro tests revealed a general inactivity for most of the products with significant indication for some of them bearing specific substituents. These results immediately suggested possible structural changes able to trigger the relative biological activities.

3.5 EXPERIMENTAL SECTION

General: All melting points are uncorrected. Elemental analyses were done on a C. Erba 1106 elemental analyzer. IR spectra (Nujol mulls for solids) were recorded on an FT-IR Perkin-Elmer RX-1. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 400 and 300 in the specified deuterated solvents. Chemical shifts are expressed in ppm from internal tetramethylsilane (□). Column chromatography and tlc: silica gel 60 (0.063-0.200 mm) (Merck); eluent cyclohexane/ethyl acetate from 9:1 to 5:5. MPLC chromatographic separations were performed on a Biotage Flash Master Personal apparatus; eluent cyclohexane/ethyl acetate from 9:1 to 5:5.

Materials: Furfuryl alcohol, benzoimidazole and substituted benzoimidazoles were purchased from Sigma-Aldrich. All other reagents and solvents were purchased from Sigma-Aldrich and Alfa-Aesar and used without any further purification.

Synthesis of 2-(5-substituted-1H-benzo[d]imidazol-1-yl)cyclopent-2-en-1-ones (5a-d) and 2-(6-substituted-1H-benzo[d]imidazol-1-yl)cyclopent-2-en-1-ones (6a-d); (general procedure).

An excess of 5-substituted benzoimidazoles **4a-d** (1.5 equiv.) were dissolved in 8 mL of anhydrous acetonitrile and 1.7 equiv. NaH 95% were added portionwise under stirring in a Schlenk tube at room temperature, applying mechanical vacuum until complete evolution of hydrogen gas. After 15 minutes, 1 g (7.14 mmol) of the 4-hydroxy-2-cyclopentenone acetate **(±)-1** was dissolved in the minimum amount of anhydrous acetonitrile and added to the solution under stirring. The reactions are left under stirring at room temperature overnight. The reactions were quenched with ice mixed with NaCl/NH_4Cl 1:3 and the water phase extracted with DCM. The organic phases were dried over Na_2SO_4 and, upon evaporation of the solvent, the residues were submitted to chromatographic separation to isolate the purified products **5/6a-d**.

5/6a (0.8 g, 53%) as a straw yellow solid; [Found: C, 73.6; H, 5.7; N, 13.2%. $C_{13}H_{12}N_2O$ requires C, 73.56; H, 5.70; N, 13.20%]; R_f (100% MeOH) 0.59; δ_{max} (nujol) 1686, 1607 cm^{-1}; δ_H (300 MHz DMSO) 2.46 (3H, s, C<u>H</u>$_3$), 2.50 (3H, s, C<u>H</u>$_3$), 2.54 (2H, m, C<u>H</u>$_2$), 3.37 (2H, m, C<u>H</u>$_2$), 6.64 (1H, s, C<u>H</u>=), 6.67 (1H, s, C<u>H</u>=), 7.24 (1H+1H, t, *J* 7 Hz, arom.), 7.60 (1H, s, arom.), 7.66 (1H, d, *J* 9 Hz, arom.), 7.81 (1H, s, arom.), 7.86 (1H, d, *J* 9.0 Hz, arom.), 8.69 (1H, s, C<u>H</u>=N), 8.72 (1H, s, C<u>H</u>=N); □$_C$ (75.0 MHz, DMSO) 205.8, 205.7, 165.6, 165.5, 144.9, 142.7, 142.6, 142.2, 134.8, 133.6, 132.0, 129.8, 126.1, 125.5, 120.2, 119.9, 113.7, 113.6, 113.5, 113.2, 33.4, 28.1, 28.0, 21.3, 20.9.

5/6b (1.0 g, 60%) as a straw yellow solid; [Found: C, 62.0; H, 3.9; N, 12.0%. $C_{12}H_9N_2OCl$ requires C, 61.95; H, 3.90; N, 12.04%]; R_f (100% MeOH) 0.61; δ_{max} (nujol) 1674, 1618 cm^{-1}; δ_H (300 MHz DMSO) 2.55 (2H, m, C<u>H</u>$_2$), 3.34 (2H, m, C<u>H</u>$_2$), 6.67 (1H, s, C<u>H</u>=), 6.75 (1H, s, C<u>H</u>=), 7.45 (1H+1H, d, *J* 7 Hz, arom.), 7.82 (1H, d, *J* 9 Hz, arom.), 7.91 (1H, s, arom.), 8.11 (1H, s, arom.), 8.87 (1H, s, C<u>H</u>=N), 8.90 (1H, s, C<u>H</u>=N); δ_C (75.0 MHz, DMSO) 205.3, 164.7, 143.4, 142.9, 132.0, 129.1, 124.4, 124.1, 121.2, 119.6, 114.6, 114.4, 114.1, 113.2, 33.1, 27.6.

5/6c (0.8 g, 51%) as a light brown solid; [Found: C, 68.4; H, 5.3; N, 12.3%. $C_{13}H_{12}N_2O_2$ requires C, 68.41; H, 5.30; N, 12.27%]; R_f (100% MeOH) 0.60; δ_{max} (nujol) 1684, 1607 cm^{-1}; δ_H (300 MHz DMSO) 2.53 (2H, m, C<u>H</u>$_2$), 3.36 (2H, m, C<u>H</u>$_2$), 3.84 (3H, s, C<u>H</u>$_3$O), 3.89 (3H, s, C<u>H</u>$_3$O), 6.59 (1H, s, C<u>H</u>=), 6.67 (1H, s, C<u>H</u>=), 7.00 (1H, d, *J* 2 Hz, arom.), 7.02 (1H, d, *J* 2 Hz, arom.), 7.34 (1H, d, *J* 2 Hz, arom.), 7.36 (1H, d, *J* 2 Hz, arom.), 7.68 (1H, d, *J* 8 Hz, arom.), 7.90 (1H, d, *J* 8 Hz, arom.), 8.67 (1H, s, C<u>H</u>=N), 8.76 (1H, s, C<u>H</u>=N); δ_C (75.0 MHz, DMSO) 205.4, 205.3, 165.1, 165.0, 157.2, 156.4, 145.4, 142.8, 141.3, 138.3, 132.1, 125.6, 120.4, 113.6, 113.5, 113.1, 112.9, 112.5, 103.0, 987.2, 55.5, 55.2, 33.0, 27.7, 27.5.

5/6d (1.2 g, 67%) as a brown solid; [Found: C, 59.3; H, 3.7; N, 17.3%. $C_{12}H_9N_3O_3$ requires C, 59.26; H, 3.73; N, 17.28%]; R_f (100% MeOH) 0.58; δ_{max} (nujol) 1692, 1590 cm^{-1}; δ_H (300 MHz DMSO) 2.58 (2H, m, C<u>H</u>$_2$), 3.42 (2H, m, C<u>H</u>$_2$), 6.78 (1H, s, C<u>H</u>=), 6.80 (1H, s, C<u>H</u>=), 8.02 (1H, d, *J* 9 Hz, arom.), 8.27 (3H, s, arom.), 8.65 (1H, bs, arom.), 8.71 (1H, d, *J* 2 Hz, arom.), 9.10 (1H, s, C<u>H</u>=N), 9.17 (1H, s, C<u>H</u>=N); δ_C (75.0 MHz, DMSO) 205.7, 205.6, 165.0, 164.9, 148.8, 147.7, 146.4, 144.5, 144.2, 144.1, 135.9, 131.2, 120.9, 119.9, 119.6, 116.1, 116.06, 115.8, 114.3, 109.9, 33.7, 33.6, 28.1, 28.05.

Synthesis of 2-(1H-benzo[d]imidazol-1-yl)cyclopent-2-en-1-one (7)

An excess of benzoimidazole (1.5 equiv.) was dissolved in 8 mL of anhydrous acetonitrile and 1.7 equiv. NaH 95% were added portionwise under stirring in a Schlenk tube, applying mechanical vacuum until complete evolution of hydrogen gas. After 15 minutes, 1 g (7.14 mmol) of the 4-hydroxy-2-cyclopentenone acetate **(±)-1** was dissolved in the minimum amount of anhydrous acetonitrile and added to the solution under stirring. The reaction is left under stirring at room temperature overnight. The reaction was quenched with ice mixed with $NaCl/NH_4Cl$ 1:3 and the water phase extracted with DCM. The organic phases were dried over Na_2SO_4 and, upon evaporation of the solvent, the residue was submitted to chromatographic separation to isolate the purified product **7**.

7 (1.0 g, 70%) as a light brown solid; [Found: C, 72.7; H, 5.1; N, 14.1%. $C_{12}H_{10}N_2O$ requires C, 72.71; H, 5.09; N, 14.13%]; R_f (100% MeOH) 0.62; δ_{max} (nujol) 1678, 1581cm^{-1}; δ_H (300 MHz DMSO) 2.55 (2H, m, C$\underline{H}_2$), 3.39 (2H, m, C$\underline{H}_2$), 6.65 (1H, s, C$\underline{H}$=), 7.43 (1H+1H, m, arom.), 7.81 (1H, m, arom.), 8.03 (1H, m, arom.), 8.83 (1H, s, C$\underline{H}$=N); δ_C (75.0 MHz, DMSO) 205.3, 165.2, 144.2, 142.4, 131.4, 124.5, 123.8, 120.0, 113.5, 113.3, 33.0, 27.7.

Biological tests

Antiviral assays. The National Institute of Allergy and Infectious Diseases (NIAID) provides free and confidential services for suppliers, who are interested in submitting compounds to be evaluated for antiviral activity. Tested compounds were delivered in standard DMSO solutions.

Primary antiviral activities of compounds 5a-d and 6a-d against IV/H1N1 and AD5.
Products **5**/**6a-d** were tested against the Herpesviridae family, Varicella-Zoster virus, (VZV, virus strain, Ellen, cell line, HFF), from the Hepatic virus HBV (virus strain ayw; cell line 2.2.15), Respiratory Viruses such as Influenza A virus H1N1 (IV/H1N1, virus strain, InfluenzaA/California/7/2009; cell line, MDCK), Adenovirus-5 (AD5, Adenoid75, virus strain; cell line, HFF), from the Togavidae family, Chikungunya virus (CV, virus strain, S27/VR-64, cell line, Vero 76), from the Flaviridae group, Yellow Fever Virus (YFV, virus strain, 17D; cell line, Huh7), from the Bunyaviridae family, Punta Toro Virus (PTV, virus strain, Adames; cell line, Vero 76),

and from the Papovaviridae the Human Papilloma Virus (HPV, virus strain, HE611260.1; cell line, C-33A).

Table x. Primary antiviral activities of compounds **5a-d** and **6a-d** against IV/H1N1 and AD5.

Entry	**5/6**	Drug Assay Name (Cytopathic eff.)	EC_{50}	CC_{50}	SI_{50}
		IV/H1N1[a]			
1	**a**	Visual	37	>100	>2.7
		Neutral Red	>100	>100	0
2	**b**	Visual	>100	>100	0
		Neutral Red	>100	>100	0
3	**c**	Visual	15	>100	>6.7
		Neutral Red	25	>100	>4.0
4	**d**	Visual	32	>100	>3.1
		Neutral Red	28	>100	>3.6
		Ribavirin[b]			
5		Visual	1.2	>320	>270
		Neutral Red	1.4	>320	>270
		AD5[c]			
6	**a**	CellTiter-Glo	>150	>150	1
7	**b**	CellTiter-Glo	>150	>150	1
8	**c**	CellTiter-Glo	>150	>150	1
9	**d**	CellTiter-Glo	>30	118.61	<4
		Cidofovir[d]			
10		CellTiter-Glo	2.93	121.85	42

[a]Drug conc. Range 0.1-100 µg/mL (vehicle, DMSO). [b]Control conc. Range 0.32-320 µg/mL. [c]Drug conc. Range 0.048-150 µM (vehicle, DMSO). [d]Control conc. Range 0.048-150 µM.

3.6 REFERENCES

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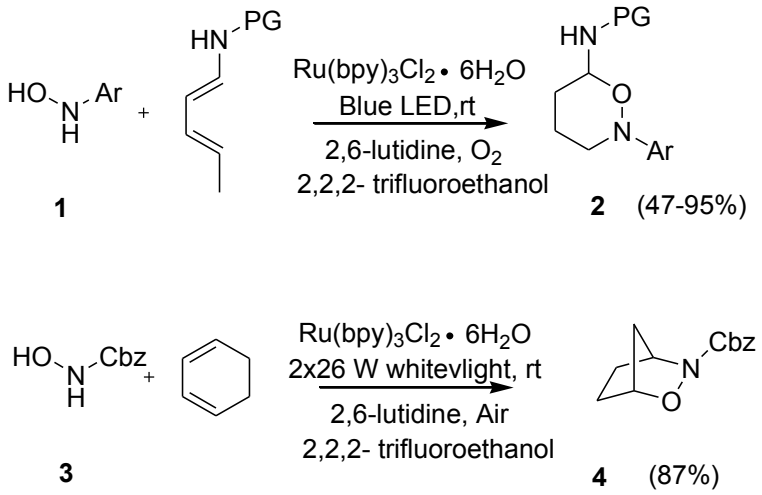
4. PHOTOREDOX CATALYTIC APPROACHES FOR NITROSO AND NITROSOCARBONYL GENERATION

An emerging tool in organic chemistry is represented by photoredox catalysis, a family of processes where a catalyst is responsible for light absorption and activation of organic molecules via an electron transfer step.[1] An ever-growing number of pioneering works carried out by different research groups determined in recent years the renaissance of this approach in organic synthesis, especially in the wake of the twelve principles of Green Chemistry.[2]

Thus, given the importance of nitroso compounds as dienophiles and enophiles, suitable for the construction of valuable C–O and C–N bonds through hetero-DA (HDA) and ene reactions, photoredox catalysis has been adopted as well for the development of mild and green methods for their generation. As an example, $Ru(bpy)_3Cl_2$ has been used as photoredox catalyst in the presence of O_2 (or air) to convert arylhydroxylamines **1**[3] or hydroxycarbamates **3**[4] to nitroso and nitrosocarbonyl intermediates, respectively (Scheme 1).



Scheme 1. G. Masson e J. Read de Alaniz approaches.

In view of the above and pushed by our interest in the development of novel approaches for the generation of nitrosocarbonyl intermediates, we explored the competence of a well-known photocatalyst, namely the decatungstate anion $[W_{10}O_{32}]^{4-}$,[5] in the generation of such species (Figure 1).

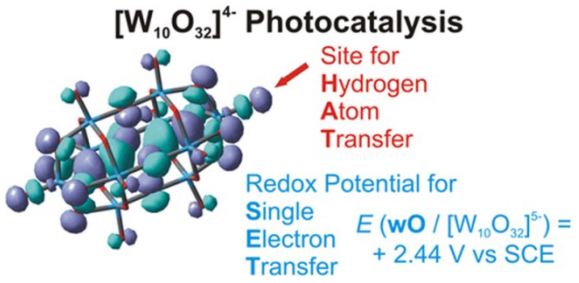
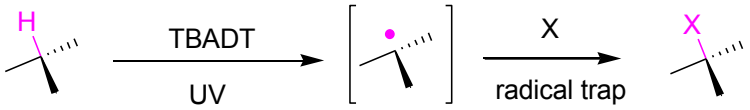


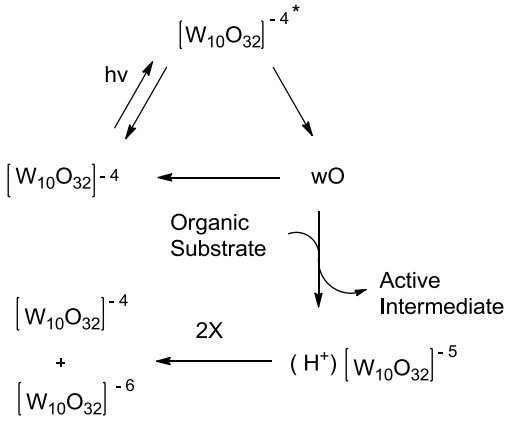
Figure 1. TBADT Photocatalysis

The latter were subsequently trapped in-situ by cyclic dienes in a [4+2] cycloaddition process. The TBADT is an efficient and robust photocatalyst able to promote photoredox reactions, as well as hydrogen atom transfer (scheme 2) processes to generate radical species, starting from different classes of organic substrates.



Scheme 2. Hydrogen Atom Transfer (HAT)

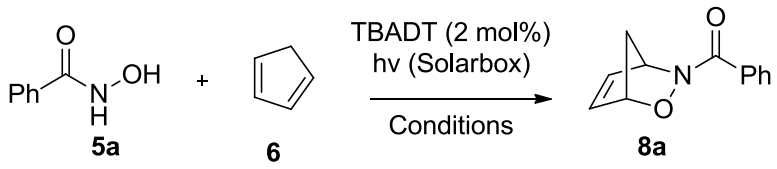
The choice of this photocatalyst is related to its remarkable properties in the excited state. In fact, upon absorption of a UV-B photon, the highly oxidizing excited state wO is ultimately formed, which shows a redox potential E(wO/$[W_{10}O_{32}]^{5-}$) around +2.4 V vs SCE (Scheme 3).[6] Remarkably, such a mighty excited state can be smoothly generated upon solar light irradiation as well, fully profiting of the potentialities of the so-called "window-ledge" chemistry.[7]



Scheme 3. Excitation and Ensuing Reactivity of the Decatungstate Anion $[W_{10}O_{32}]^{4-}$

We commenced our investigation by choosing a model reaction. In particular, the feasibility of the HDA reaction was tested by studying the conversion of benzohydroxamic acid **5a** to the cycloadduct **8a**, in the presence of cyclopentadiene. The reaction was carried out with tetrabutylammonium decatungstate as the photoredox catalyst and the solution was irradiated in a solar light simulator equipped with a Xenon lamp (light intensity: 500 $W \cdot m^{-2}$). Decatungstate is able to promote the oxidation of hydroxamic acids to nitrosocarbonyl intermediates in a very short time, with only 1h irradiation and reaction completion is guaranteed upon leaving the reaction in the dark for a residual time.

1. Optimization of reaction conditions for the HDA cycloaddition between benzohydroxamic acid and 1,3cyclopentadiene. [a]



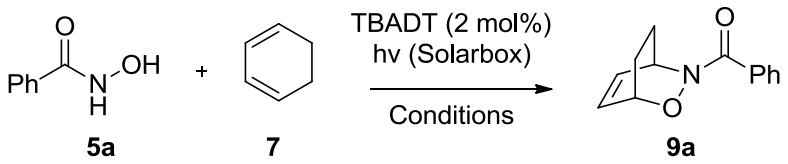
Entry	Conditions	Yield (%)
1	MeCN, N_2, 24h	n.d.
2	MeCN, air, 24h	13[b]
3	MeCN, air, 24h[c]	14
4	MeCN, air, 1+2h	30
5	MeCN, O_2, 1+2h	32
6	MeCN/H_2O 5:1,O_2, 1+2h	43
7	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	49
8[d]	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	44
9[e]	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	9
10[f]	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	6
11[g]	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	50

[a] Reaction conditions: **5a** (0.26 mmol), **6** or **7** (0.52 mmol) and TBADT ((n-Bu4N)4[W10O32], 2 mol%) in 10 mL of the chosen reaction medium. Reaction yields were determined via HPLC analysis. [b] Massive polymerization of **6** occurred. [c] TBADT, 4 mol%. [d] Reaction carried out by irradiation with a 18W LED with emission centered at 365 nm. [e] The reaction vessel was wrapped in aluminium foil (blank experiment). [f] Reaction carried out in the absence of TBADT (blank experiment). [g] **5a** was replaced by the corresponding potassium salt **5'a**. n.d.: not detected.

When a N_2-sparged solution of **5a** was irradiated, HDA cycloadduct **8a** was not formed (Table 1, entry 1), despite complete conversion (>95%) of benzohydroxamic acid was observed. However, when adopting an air-equilibrated acetonitrile solution of **5a**, the HDA product **8a** was obtained in 13% yield based on HPLC analysis (entry 2). The crude reaction mixture was mainly composed of dimers and/or polymers of **6**, along with fragments of ammonium salts deriving from the catalyst. An increased amount of the photocatalyst (up to 4 mol%) did not ameliorate the reaction yield (entry 3).

Due to the large amount of decomposition products formation, we hypothesized that prolonged irradiation could have a detrimental effect on the reaction. Indeed, when the reaction mixture was irradiated for 1 hour and then left under stirring at room temperature in the dark for two additional hours, cycloadduct **8a** was obtained in 30% yield, thus confirming our hypothesis (entry 4). The use of an oxygen-saturated solution did not improve further the reaction yield (entry 5). Conversely, when an aqueous reaction medium (MeCN/H_2O 5:1, entry 6) was used, compound **8a** was obtained in 43% yield. Finally, to investigate the effect of the acidity developed during the process, a mild base, i.e. $NaHCO_3$ (acting as an insoluble additive, 0.5 equiv.), was added to the buffer. HDA cycloadduct **8a** was formed in an improved yield of 49% (entry 7). Addition of 1 equiv. bicarbonate under the same conditions afforded the desired product in comparable yield (48% data not shown). On the other hand, when the reaction was performed by irradiating in the UV-A range with a 365 nm LED (18W power), a slightly diminished yield (44%, entry 8) was observed. In all cases, the observed conversion of **5a** was > 95%. Furthermore, blank experiments confirmed that light and photocatalyst were both necessary for the reaction to occur outcome(entries 9, 10). Interestingly, when replacing benzohydroxamic acid with the corresponding potassium salt (**5'a**) under optimized reaction conditions, the same yield of **8a** was observed (entry 11).

Table 2. Optimization of reaction conditions for the HDA cycloaddition between benzohydroxamic acid and 1,3cyclohexadiene. [a]



5a **7** **9a**

Entry	Conditions	Yield (%)
1	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	39
2	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	55
3[f]	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	41
4[g]	MeCN/H_2O 5:1,O_2, 1+2h $NaHCO_3$ (0.5 equiv)	63

[a] Reaction conditions: 5a (0.26 mmol), 6 or 7 (0.52 mmol) and TBADT ((n-Bu4N)4[W10O32], 2 mol%) in 10 mL of the chosen reaction medium. Reaction yields were determined via HPLC analysis. [f] Reaction carried out in the absence of TBADT (blank experiment). [g] **5a** was replaced by the corresponding potassium salt **5'a**. n.d.: not detected .

Afterwards, we extended our optimization to 1,3-cyclohexadiene (**7**) diene (table 2). When the reaction conditions reported in entry 7 (Table 1) were adopted, HDA cycloadduct **9a** was obtained in 39% yield (entry 1, Table 2). Notably, a further increase in the amount of $NaHCO_3$ (from 0.5 to 1 equiv. 0.5 in the Table) led to an improved formation of **9a** (55%, entry 2), with an almost negligible formation of tar.

Finally, **5a** could be conveniently substituted with **5'a**, affording **9a** in 41% with 0.5 equiv. of bicarbonate (entry 3) and, of relevance leading to **9a** in 63% yield with 1 equiv. of base (0.5 in the Table entry 4). Moreover, by tracking the reaction time profile for the formation of **7a** and **8a** under the optimized conditions, we confirmed that 1 hour was the time required for the reaction to occur (Figure 2).

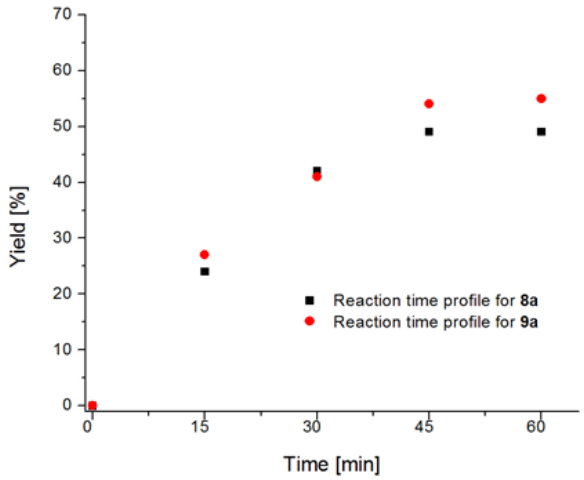
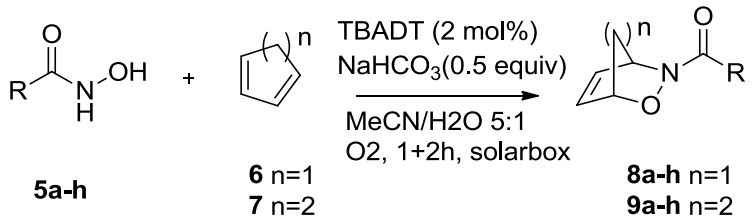


Figure 2. Reaction time profiles for the formation of products **8a** and **9a**

With the optimized conditions in hand, we started investigating the scope of the reaction by employing different hydroxamic acid derivatives.

As shown in Table 3, the reactions were performed on a representative set of hydroxamic acids bearing aromatic (**5a-d**; brominated compounds **5c**,**d** were synthesized by literature protocols),[8] heteroaromatic (**5e-f**, synthesized by literature protocols)[9] and aliphatic (**5g-h**) substituents. Reactions conducted on **5** proceeded smoothly affording the cyclopentadiene derivatives **8a-h** generally in good yields, ranging from 30 to 84%. Even better results were obtained by using 1,3-cyclohexadiene **7** as trapping agent, and products **9a-h** were isolated in yields ranging from 34 to 95%. Notably, while compounds **8b**,**d**,**g** and **9b**,**d**,**g** are known in the literature,[10-12] all the other products were here prepared for the first time and fully characterized.

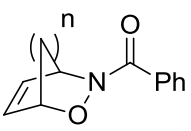
Table 3. TBADT-Mediated [4+2] Cycloaddition Between 1,3-Cycloalkenes (6 and 7) and Substituted Hydroxamic Acids 5a-j.[a]



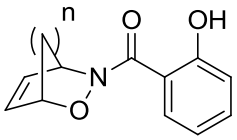
5a-h

6 n=1
7 n=2

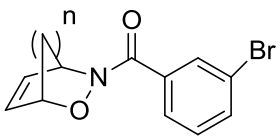
8a-h n=1
9a-h n=2



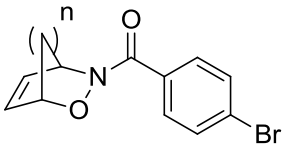
8a 49%
9a 55%



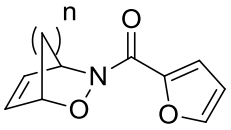
8b 30%
9b 34%



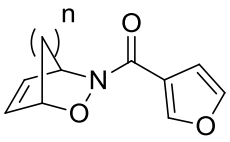
8c 55%
9c 48%



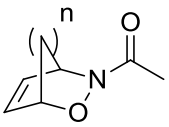
8d 65%
9d 56%



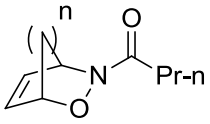
8e 44%
9e 37%



8f 49%
9f 46%



8g 84%
9g 95%

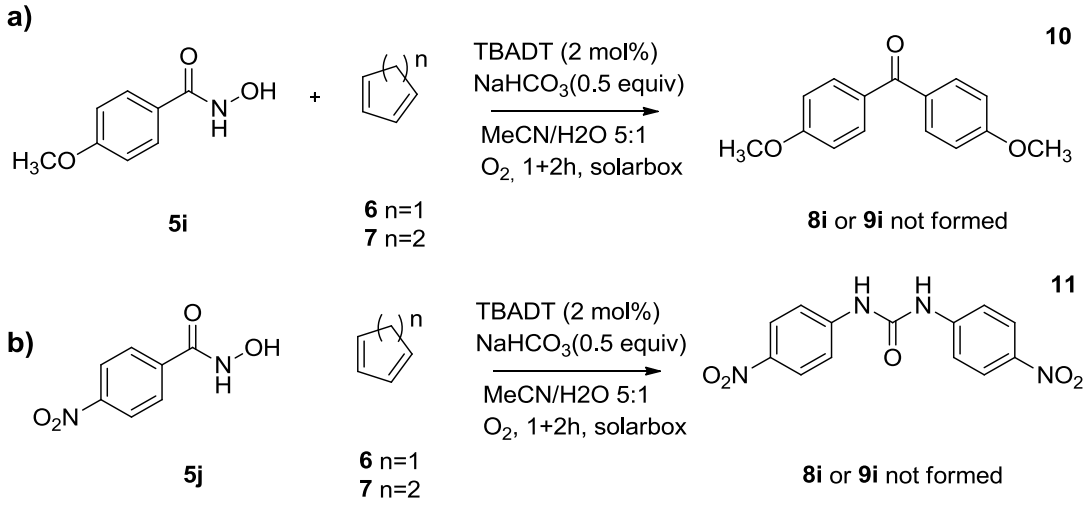


8h 33%
9h 53%

[a] Reaction conditions: **5a-h** (0.26 mmol), **6** or **7** (0.52 mmol) and TBADT ((n-Bu4N)4[$W_{10}O_{32}$], 2 mol%) in the presence of $NaHCO_3$ (0.5 equiv.) in 10 mL of MeCN/H_2O 5:1. Solutions were bubbled with oxygen (3 min), capped and then irradiated under simulated solar light (SolarBox equipped with a Xe lamp; 500 W m^{-2}). The obtained products were isolated via flash column chromatography.

In view of evaluating the scope of the protocol and to have additional insight on potential electronic effects, we further investigated the reactions of two substituted benzohydroxamic acids, bearing respectively electron-donating (-OMe, **5i**) and electron-withdrawing (-NO_2, **5j**) groups (scheme 4). The reactions were performed under identical conditions as in Table 3, but the expected products

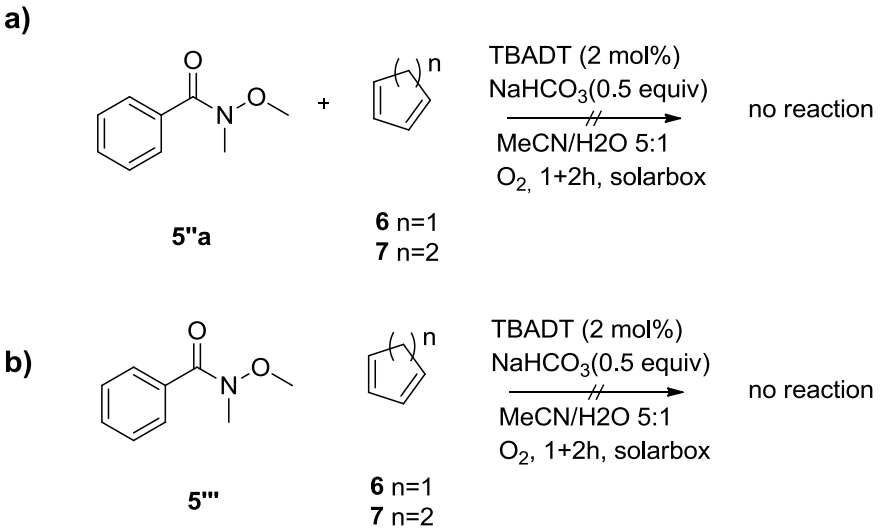
8i,**j** and **9i**,**j** were not isolated. However, the reaction mixtures revealed the presence of ketone **10** and the urea **11**, whose structures are closely related to the dimerization[13] and decomposition,[14] with rearrangement, of the original nitrosocarbonyl intermediates[15] **11i**,**j**. These results somewhat confirm the generation of the expected intermediates, that do not survive for long and enough time to be trapped by the diene in solution.



Scheme 4. HDA cycloaddition between substituted hydroxamic acid 5i,j and cyclopentadiene or 1,3-cyclohexadiene.

On the other hand, they dimerize and decompose leaving to the final stable products of these processes, *i.e.* compounds **10** and **11**.

As reported in Scheme 5 (check scheme for formulas of **5** and also **5'''a)**, we also performed dedicated control experiments to have some insights into the mechanism; in particular, di- (**5''a**) and mono-methylated (**5'''a**) benzohydroxamic acids were subjected to the reaction under optimized conditions. The reactions were performed either in the presence or absence of **6**. In all cases the product formation was not observed, with recovery of the unreacted starting materials.



Scheme 5. Control experiments.

In conclusion, the Decatungstate is able to promote the oxidation of hydroxamic acids to nitrosocarbonyl intermediates in a very short time, with only 1h irradiation and reaction completion is guaranteed upon leaving the reaction in the dark for a residual time. Trapping was efficiently obtained with reactive dienes to afford the desired HDA cycloadducts in fair to good yields. Optimism for future progress lies in the extension of the present methodology to the challenging ene reaction of nitrosocarbonyl intermediates applying this synthetic scheme for more complex molecule syntheses.

4.1 Laser Flash Photolysis (LFP) Experiments.

To shine light on the initial photocatalytic process, we performed dedicated laser flash photolysis (LFP) experiments. These were intended to study the evolution in time of the different species deriving from the decatungstate anion in the presence of increasing amounts of acetohydroxamic acid (**5g**). As already reported in the scheme 3 (section 4) upon absorption of a UV-photon, the decatungstate anion ultimately populates a reactive excited state named wO (with a lifetime around 50-60 ns in neat acetonitrile) that can interact with organic substrates present in solution to give the mono-reduced form $[W_{10}O_{32}]^{5-\cdot}$. Notably, both species, viz. wO and $[W_{10}O_{32}]^{5-}$, are deeply blue colored and their evolution in time can be easily tracked by monitoring the absorption of the solution at 780 nm.[16] Thus, upon laser excitation at 355 nm of a decatungstate solution in acetonitrile/water 5:1, it was possible to observe the decay of the wO species, which showed a lifetime of 57 ns.

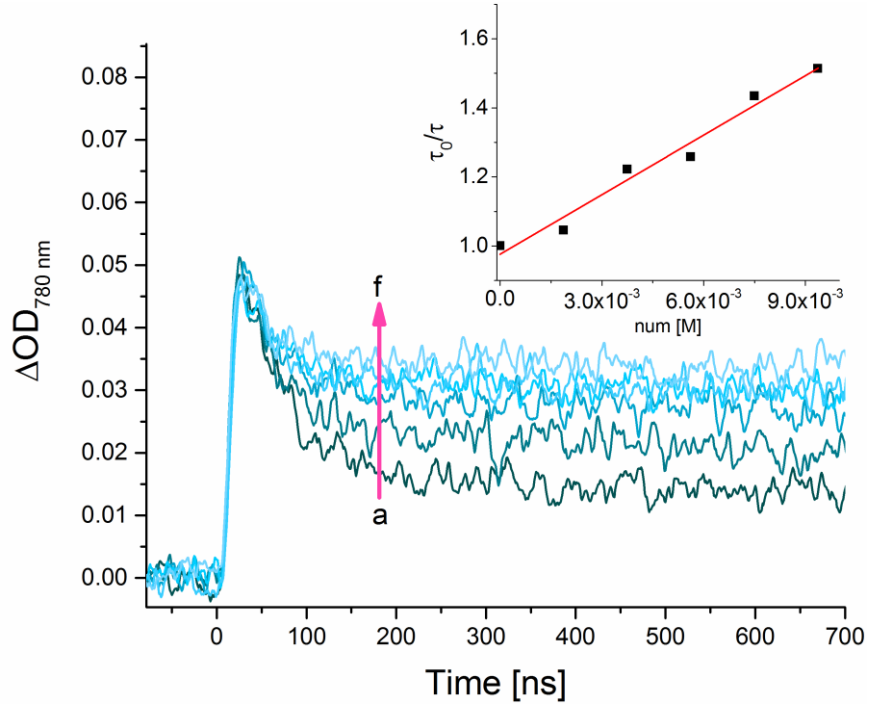
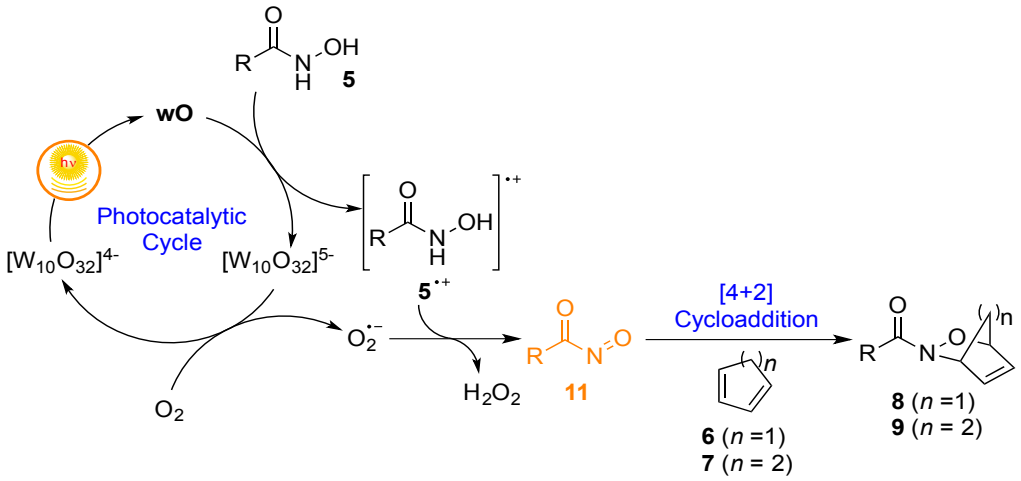


Figure 3. Nanosecond decays at 780 nm of the wO transient in the presence of increasing concentrations of acetohydroxamic acid **5g** in MeCN/H2O 5:1. (a) 0 M; (b) $1.87 \cdot 10^{-3}$ M; (c) $3.75 \cdot 10^{-3}$ M; (d) $5.62 \cdot 10^{-3}$ M; (e) $7.50 \cdot 10^{-3}$ M; (f) $9.37 \cdot 10^{-3}$ M. Insert: Stern-Volmer plot of the recorded decay.

Upon addition of increasing amounts of the quencher **5g** in the range from 0 to $9 \cdot 10^{-3}$ M, the lifetime decreased more and more, finally reaching a minimum value around 38 ns. On a longer timescale, a persistent and flat signal, attributed to the mono-reduced form $[W_{10}O_{32}]^{5-}$, is apparent and its intensity increases along with the amount of **5g** present in solution (Figure 3). Additionally, a Stern-Volmer plot allowed to determine the bimolecular quenching constant (kQ) to be $4.6 \cdot 10^{8}$ $M^{-1} \cdot s^{-1}$. This value indicates a very efficient quenching of wO by **5g**. On the other hand, literature reports indicate that alkenes can likewise quench wO, ultimately leading to the corresponding oxidized derivatives when oxygen is present in the reaction mixture.[17] Indeed, we cannot exclude that this pathway is operative also under our conditions, nevertheless it is not productive, since we never observed oxidation products deriving from **6** or **7**.

4.2 Reaction Mechanism

On the basis of the above investigations, a plausible reaction mechanism can be suggested, as shown in Scheme 6. Thus, upon irradiation with solar light, TBADT populates the highly



Scheme 6. Plausible reaction mechanism.

oxidizing species wO, whose redox potential (~ + 2.4 V vs SCE) is sufficient to trigger the mono-electronic oxidation of hydroxamic acids **5** (as an example, $E(5a^{\bullet+}/5a)$ = + 1.35 V vs SCE),[18] leading to the corresponding radical cations $\mathbf{5}^{\bullet+}$ and the reduced photocatalyst $[W_{10}O_{32}]^{5-}$. The latter is then restored by the oxygen dissolved in solution, which is an essential reaction partner as demonstrated by the process performed under inert atmosphere (see Table 1). On the other hand, $\mathbf{5}^{\bullet+}$ can undergo deprotonation, aided by the inorganic base present in the system, and the desired nitrosocarbonyl intermediates **11** are obtained upon reaction with superoxide anion (in turn formed in the photocatalyst restoration step). The final HDA adducts **8** or **9** result from the [4+2] cycloaddition of **11** with **6** or **7**. Indeed, the reaction worked satisfactorily also when adopting **5'a** as the substrate and a similar reaction pathway as that described for the neutral species **5a** can be proposed. It is worth noting that we can safely exclude the in situ formation of **5'a** from **5a** promoted by the week base $NaHCO_3$, being a pKa = 8.80 reported in the literature for **5a**.[18,19]

4.3 EXPERIMENTAL SECTION

Synthesis of TBADT

Tetrabutylammonium bromide (TBAB, 2.4 g) and sodium tungstate dehydrate (5.0 g) were dissolved each in 1 L of deionized water in two flasks and kept at 90 °C under vigorous stirring. Concentrated hydrochloric acid was added dropwise to both solutions in order to adjust the pH at 2. The TBAB solution was then added to the tungstate solution and a white suspension of TBADT formed immediately. The resulting solution was maintained at 90 °C for 30 minutes and then cooled, before filtration. The collected solid phase was washed with water and dried. The white solid, finely grounded, was suspended in dichloromethane (20 mL per gram of solid) and kept under stirring for 2 hours.

Pure TBADT was separated from yellow surnatant solution by filtration on a Bichner funnel. TBADT was obtained in 85-95% yield with purity >90% (UV analysis: ε_{323} = 1.35.104 dm3mol-1cm-1 in acetonitrile).

Laser flash photolysis experiments

Laser flash photolysis studies were performed by adopting the third harmonic of a Q-switched Nd:YAG laser (λ_{EXC} = 355 nm). $1.0 \cdot 10^{-4}$ M starting solutions of TBADT $(n\mathrm{Bu}_4)_4[\mathrm{W}_{10}\mathrm{O}_{32}]$ were employed, with an absorbance value of ~0.5 at 355 nm. The sample solution was placed in a 1x1 cm quartz cell and excited with single pulses (13 mJ, 1 Hz) delivered from the laser and analyzed with a pulsed Xe arc lamp. Lifetimes of the reactive transient **wO** were obtained by fitting the first order decay profiles recorded at 780 nm by using the following equation:

$$y = y_0 + \mathrm{A}\, e^{-\frac{x}{\tau}}$$

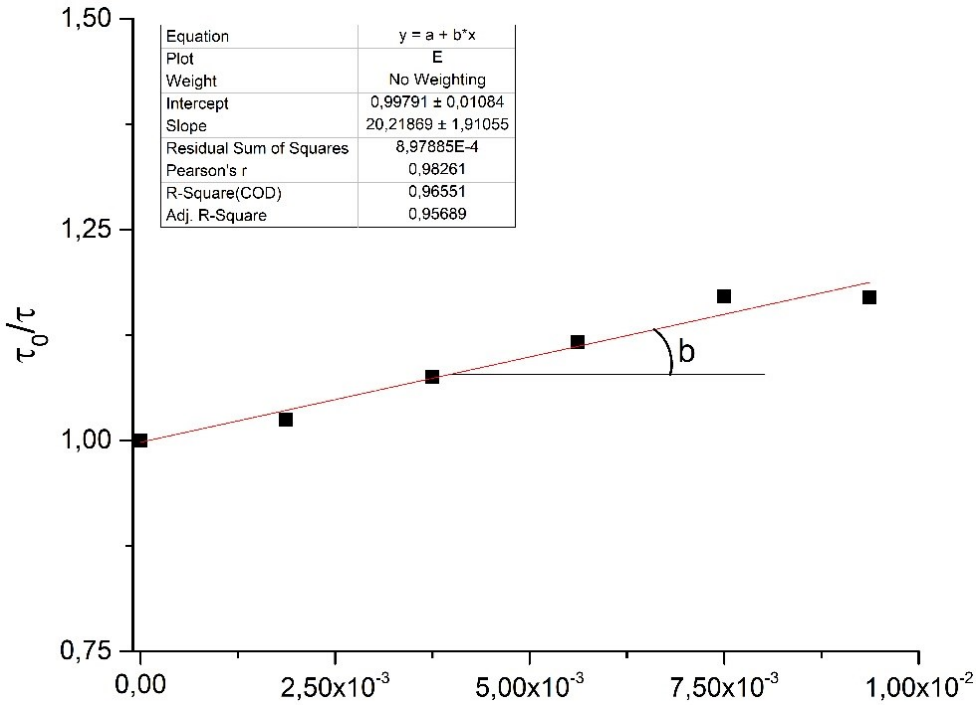
- **Experiments in MeCN/H_2O 5:1**

Starting solution: TBADT $1.0 \cdot 10^{-4}$ M in MeCN/H_2O 5:1.

Quenching solution: acetohydroxamic acid 0.75 M in MeCN/H_2O 5:1.

The Stern-Volmer plot was fitted with the following equation:

$$\frac{\tau_0}{\tau} = 1 + k_Q \tau_0 [Q]$$



$b = k_Q \tau_0$ $\tau_0 = 4.4 \cdot 10^{-8} s$ $k_Q = \frac{b}{\tau_0} = 4.6 \cdot 10^8 \, M^{-1} s^{-1}$

4.4 TYPICAL PROCEDURE FOR HDA CYCLOADDITION REACTIONS.

In a cylindrical flask (Ø 4cm x h 3 cm) 0.26 mmol of the chosen hydroxamic acid were dissolved in the acetonitrile (10 mL) or acetonitrile/water mixture (10+2 mL) along with 2% TBADT and 2 equivalents of suitable dienes (freshly distilled cyclopentadiene or 1,3-cyclohexadiene). When required, suitable amounts of $NaHCO_3$ were also added. The mixture was saturated with O_2 gas for three minutes in the cases reported. The sealed flask was then irradiated in a Solarbox 1500 (Xe lamp, 500 Wm^{-2}) for a proper reaction time and eventually left under stirring in the dark when requested. The mixture was filtered and the clear solution passed through LC-SAX SPE tubes (1 mL) for capturing cations and anions. The resulting solution was diluted to 25 mL in acetonitrile and analyzed in HPLC for the reaction yield determination. ^{1}H NMR spectra were also recorded to corroborate the structure assignments.

For all the newly synthesized compounds the full characterization was conducted and the relative data are reported in the SI.

Characterization of compounds 8c, 8e, 8f, 9c, 9e and 9f

Compound **8c**: oil. FT-IR: $\nu_{C=O}$ 1727, $\nu_{C=C}$ 1654 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 1.90 (d, 1H, CH_2), 2.15 (d, 1H, CH_2), 5.38 (s, 2H, CH-O and CH-N), 6.40 (d, 1H, CH=), 6.59 (bs, 1H, CH=), 7.42 (m, 1H, arom.), 6.63 (d, 1H, arom.), 7.72 (d, 1H, arom.), 7.94 (s, 1H, arom.). ^{13}C NMR (δ, $CDCl_3$): 29.2, 47.8, 76.7, 84.5, 127.0, 128.1, 131.3, 132.4, 134.0, 170.0. Elemental analysis: calcd. for $C_{12}H_{10}BrNO_2$ (MW = 278.99) C, 51.45; H, 3.60; N, 5.00. Found: C, 51.46; H, 3.59; N, 4.99.

Compound **8e**: oil. FT-IR: $\nu_{C=O}$ 1730, $\nu_{C=C}$ 1650 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 1.89 (d, 1H, CH_2), 2.11 (d, 1H, CH_2), 5.42 (s, 1H, CH-N), 5.60 (s, 1H, CH-O), 6.39 (m, 1H, CH=), 6.50 (m, 1H, fur.), 6.59 (m, 1H, CH=), 7.24 (m, 1H, fur.), 7.58 (s, 1H, fur.). ^{13}C NMR (δ, $CDCl_3$): 48.0, 63.8, 84.7, 111.5, 118.2, 132.7, 136.0, 145.3, 146.3, 160.2. Elemental analysis: calcd. for $C_{10}H_9NO_3$ (MW = 191.19) C, 62.82; H, 4.75; N, 7.33. Found: C, 62.83; H, 4.79; N, 7.32.

Compound **8f**: oil. FT-IR: $\nu_{C=O}$ 1734, $\nu_{C=C}$ 1684 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 1.89 (d, 1H, CH_2), 2.09 (d, 1H, CH_2), 5.40 (s, 1H, CH-N), 5.50 (s, 1H, CH-O), 6.39 (m, 1H, CH=), 6.65 (m, 1H, CH=), 6.85 (m, 1H, fur.), 7.39 (m, 1H, fur.), 8.09 (s, 1H, fur.). ^{13}C NMR (δ, $CDCl_3$): 48.0, 63.1, 84.8, 110.5, 132.6, 136.5, 142.0, 142.4, 147.4, 146.3, 169.9. Elemental analysis: calcd. for $C_{10}H_9NO_3$ (MW = 191.19) C, 62.82; H, 4.75; N, 7.33. Found: C, 62.83; H, 4.79; N, 7.32.

Compound **9c**: yellowish oil. FT-IR: $\nu_{C=O}$ 1729, $\nu_{C=C}$ 1653 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 1.63 (m, 2H, CH_2), 2.25 (d, 2H, CH_2), 4.83 (bs, 1H, CH-N), 5.30 (b, 1H, CH-O), 6.58 (m, 1H, CH=), 6.70 (bs, 1H, CH=), 7.28 (m, 2H, arom.), 7.58 (m, 2H, arom.), 7.83 (s, 1H, arom.). ^{13}C NMR (δ, $CDCl_3$): 23.4, 72.1, 77.1, 115.2, 127.1, 129.4, 131.7, 133.6, 164.0. Elemental analysis: calcd. for $C_{13}H_{12}BrNO_2$ (MW = 294.15) C, 53.08; H, 4.11; N, 4.76. Found: C, 53.06; H, 4.10; N, 4.79.

Compound **9e**: brown oil. FT-IR: $\nu_{C=O}$ 1766, $\nu_{C=C}$ 1683 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 1.57 (m, 2H, CH_2), 2.25 (m, 2H, CH_2), 4.91 (s, 1H, CH-N), 5.46 (s, 1H, CH-O), 6.58 (m, 1H, fur.), 6.60 (m, 1H, CH=), 6.71 (m, 1H, CH=), 7.17 (m, 1H, fur.), 7.54 (s, 1H, fur.). ^{13}C NMR (δ, $CDCl_3$): 20.8, 23.4, 72.1, 111.3, 112.3, 117.0, 117.8, 131.4, 145.0, 156.7, 180.2. Elemental analysis: calcd. for $C_{11}H_{11}NO_3$ (MW = 205.21) C, 64.38; H, 5.40; N, 6.83. Found: C, 64.37; H, 5.41; N, 6.82.

Compound **9f**: yellow oil. FT-IR: $\nu_{C=O}$ 1725, $\nu_{C=C}$ 1602 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$): 1.57 (m, 2H, CH_2), 2.26 (m, 2H, CH_2), 4.90 (bs, 1H, CH-N), 5.44 (s, 1H, CH-O), 6.56 (m, 1H, CH=), 6.72 (m, 1H, CH=), 6.87 (s, 1H, fur.), 7.39 (s, 1H, fur.), 8.04 (s, 1H, fur.). ^{13}C NMR (δ, $CDCl_3$): 20.8, 23.5, 72.1, 77.3, 111.0, 131.3, 133.0, 142.1, 146.9, 162.9, 187.6. Elemental analysis: calcd. for $C_{11}H_{11}NO_3$ (MW = 205.21) C, 64.38; H, 5.40; N, 6.83. Found: C, 64.36; H, 5.39; N, 6.82.

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5. PANOBINOSTAT: AN INNOVATIVE HYDROXAMIC ACID

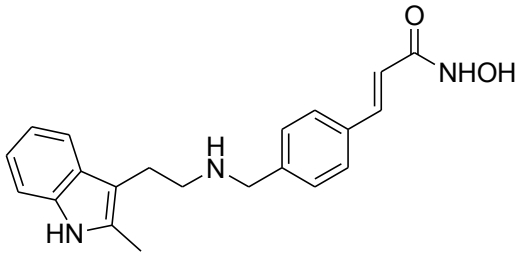


Figure 1. Panobinostat

Panobinostat (Farydak) is one of the most important hydroxamic acid functionalized with a heterocyclic ring . In particular, due to its drawback, as an hydroxamic acid, it can be involved in the generation of nitrosocarbonyl specie which can be trapped generating new molecules for new potentially medical applications. The US Food and Drug Administration (FDA) approved Panobinostat (Farydak; Novartis Pharmaceuticals), an orally administered inhibitor of histone deacetylase (HDAC), for the treatment of patients with multiple myeloma who have received at least two previous regimens, including bortezomib and an immunomodulatory agent.[1,2]

Panobinostat was approved under the FDA's accelerated approval program, which allows the use of a drug to treat a serious or life-threatening disease based on clinical data showing the drug has an effect on a surrogate end point likely to predict clinical benefit. The FDA approval was based on progression-free survival data. An improvement in survival or in disease-related symptoms has not yet been demonstrated for panobinostat, and confirmatory clinical trials must be conducted to verify and describe the drug's clinical benefit.[3]According to Richard Pazdur, MD, Director of the Office of Hematology and Oncology Products in the FDA's Center for Drug Evaluation and Research, Panobinostat is the formulation of Panobinostat) has a new mechanism of action that distinguishes it from prior drugs approved to treat multiple myeloma, making it a potentially attractive candidate agent. Farydak's approval is particularly important because it has been shown to slow the progression of multiple myeloma. Panobinostat's labeling includes a boxed warning to alert patients and healthcare professionals about the risk for severe diarrhea and severe and fatal cardiac events, including cardiac ischemic events, arrhythmias, and electrocardiogram changes associated with the use of Panobinostat. Consequently, Panobinostat was approved with a Risk Evaluation and Mitigation Strategy program, which includes a communication plan to inform and educate healthcare professionals about these risks.

5.1 MECHANISM OF ACTION

Multiple myeloma is a hematologic malignancy that accounts for approximately 1% of all neoplasms and 13% of hematologic malignancies[4]. It is characterized by proliferation of clonal plasma cells within the bone marrow and extramedullary sites that in most instances secrete a monoclonal protein. Typical clinical characteristics include hypercalcemia, renal insufficiency, anemia, and bone disease ("CRAB" features). Other manifestations of the disease include increased risk of infection and peripheral neuropathy[5]. It has been estimated there were 24,050 newcases of multiple myeloma and 11,090 deaths due to multiple myeloma in the United States in 2014.

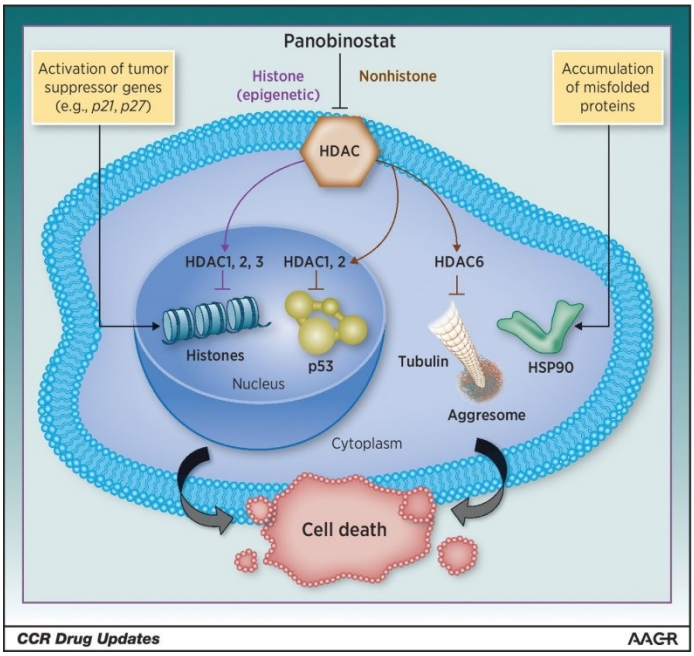
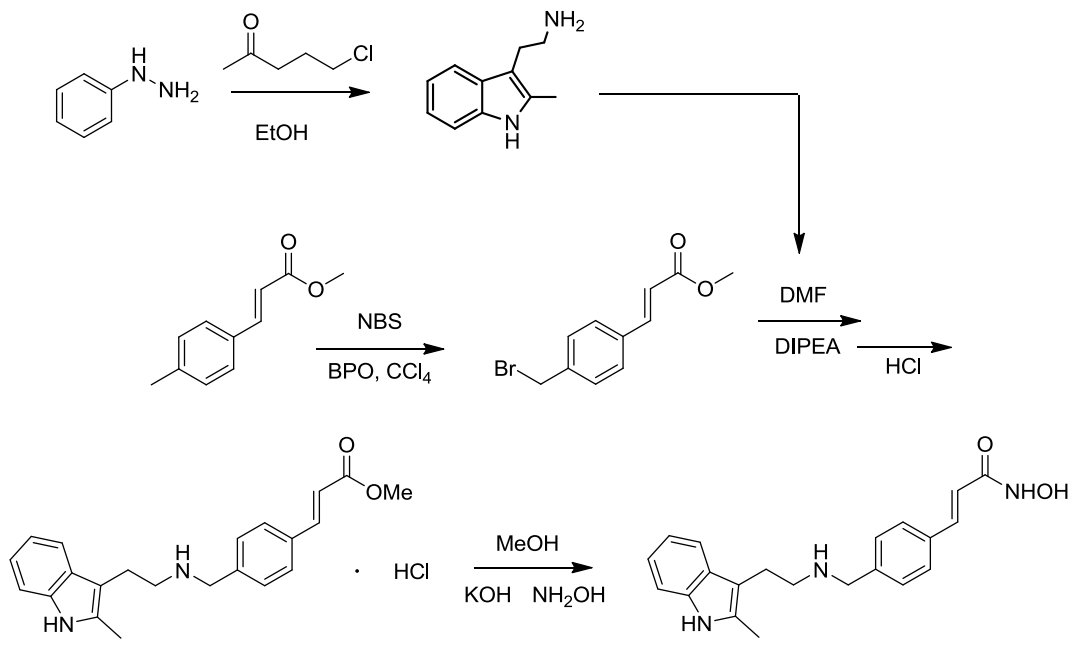


Figure 2. Mechanism Of Panobinostat American Society of Hematology; 2014. Abstract nr 2120

Panobinostat inhibits a broad range of DACs (Figura 2), which are also known as histone DACs (HDAC) because histones were the first known targets of DACs. It is now known that DACs regulate the acetylation of approximately 1,750 proteins involved in diverse biologic processes, including DNA replication and repair, chromatin remodeling, gene transcription, cell-cycle progression, protein degradation, and cytoskeletal reorganization [6]. Overexpression of DACs has been observed in multiple myeloma and is associated with poor outcomes[7]. Panobinostat is an inhibitor of all class I (HDACs 1, 2, 3 and 8), class II (HDACs 4, 5, 6, 7, 9, and 10), and class IV (HDAC 11) HDACs, with half maximal inhibitory concentrations in the nanomolar range for all

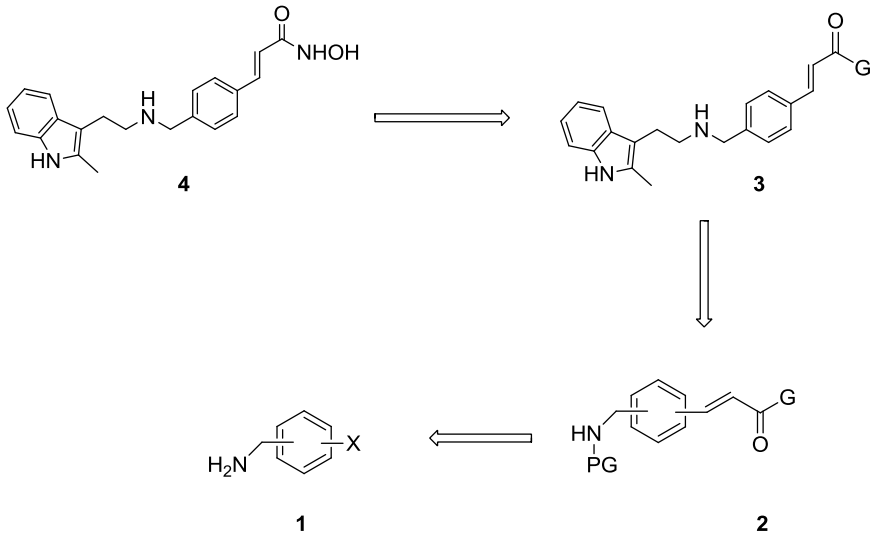
class I, II, and IV HDACs. The potency of panobinostat was 10-fold greater for all HDACs compared with vorinostat, another pan-DAC inhibitor that was investigated for the treatment of multiple myeloma, and panobinostat is among the most potent pan-DAC inhibitors in clinical development [8,9]. Panobinostat is thought to elicit antitumor activity primarily through epigenetic modulation of gene expression and inhibition of protein metabolism. Inhibition of class I HDACs, which target histones and transcription factors, may help reactivate epigenetically silenced tumor suppressor genes and modify gene expression via inhibition of signal transducer and activator of transcription t, and hypoxia-inducible factor. Panobinostat has also been shown to act synergistically with the PI bortezomib. This synergy can be explained in part via the effects of Panobinostat on protein degradation. Multiple myeloma cells have high levels of protein turnover and hence a susceptibility to PIs which inhibit metabolism and elimination of proteins generated within the cell and through this mechanism produce a proapoptotic signal. However, there is an alternative pathway of protein metabolism wherein if the proteasome cannot eliminate these proteins quickly enough, the proteins form aggregates known as aggresomes that are transported by microtubules to an autophagosome, where they are degraded by lysosomes. HDAC6 interaction with tubulin and the motor protein, dynein, is critical to the transport of these protein aggregates for degradation. Inhibition of HDAC6 leads to hyperacetylated microtubules and inefficient aggresome-mediated degradation. Bortezomib inhibits proteasome degradation of protein and induces aggresome formation; coadministration of bortezomib and panobinostat, and simultaneous inhibition of the proteasome and aggresome pathways, results in synergistic cytotoxicity [10]. In addition, in vitro and in vivo models of multiple myeloma have demonstrated that panobinostat in combination with bortezomib plus dexamethasone or the IMiD lenalidomide plus dexamethasone enabled dysregulation of additional genes that were not altered by doublet therapy alone . [11]



Scheme 1: Liu Qian strategy

The previous studies related to Panobinostat synthesis were performed by Prof. Qian and in particular by the Novartis R&D Institute, which is also the company that is the affiliate vendor of this pharmaceutical compound.

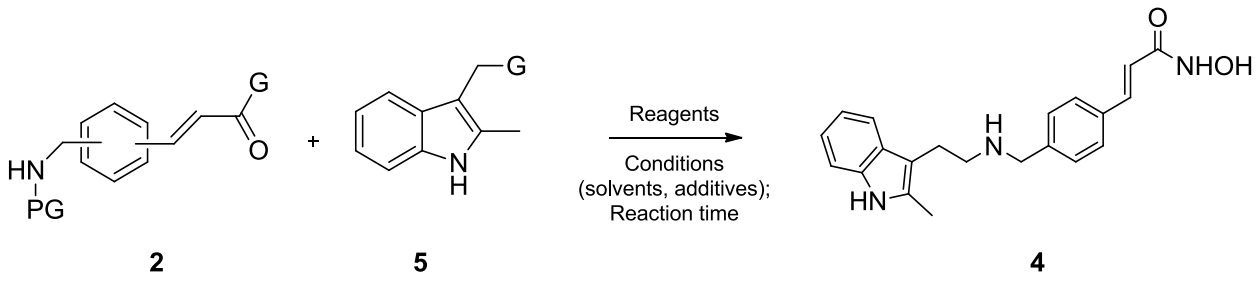
Inspired by these works, we proposed a new approach starting from the benzylamine **1** (Scheme 2).



Scheme 2. Retrosynthetic approach

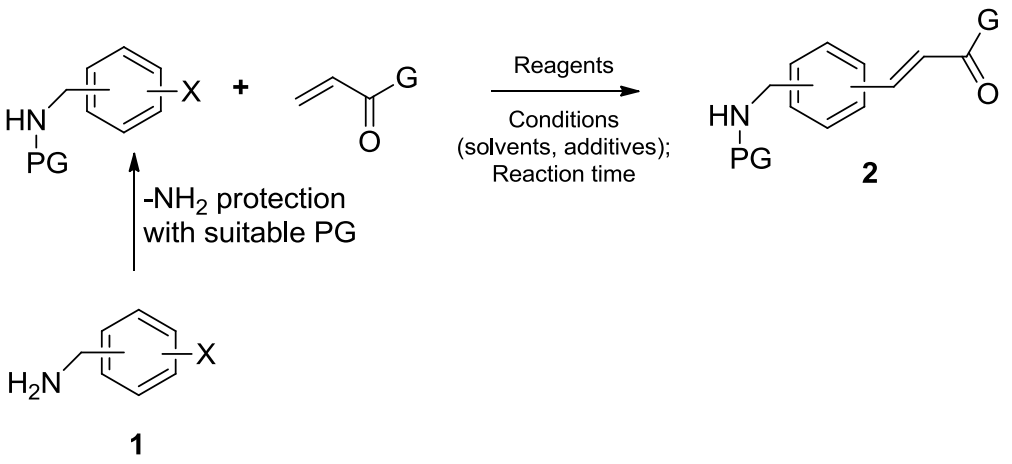
In this method, the benzyl amine reacts in the first step with an acrylate to give the compound which is the important building block for the key step of the whole process The subsequent coupling between **2** and **5** gave **3** then transformed into **4**.

It must be underlined that the reaction details are covered by submitted patent and some specific details are omitted.



Scheme 3. Panobinostat Synthesis

Compound **2** is not commercially available. Its backbone can be easily prepared with organometallic approaches, but it can be easily obtained from the corresponding amine through an Heck reaction (Scheme 4).



X= any halogen or other activating group such as OTf or ONf

Scheme 4: Synthesis of compound **2**

The Heck coupling was extensively explored using suitable aryl halides and acrylic substrates in different conditions (more than 50 different experimental conditions were explored in this part of thesis, due to high novelty aspect of this work detailed conditions were omitted because there are covered by submitted patent). In this work we focus our efforts on the catalyst and in particular on

its different loading in order to obtain a compound less toxic and more biocompatible for its use as a human drug.

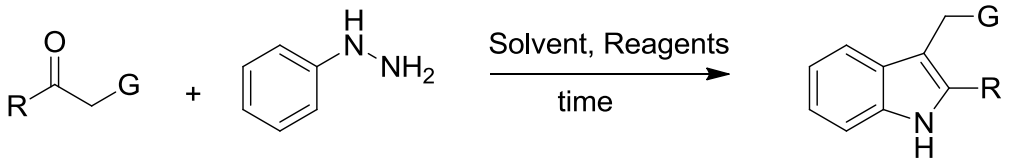
The experiments shown that using the same conditions in each entry changing only the catalyst loading, we obtained the desired product in 67% yield using only 1.0 % mol of catalyst. The experiments performed are summarized in the table below.

Temperature (°C)	Time	Catalyst	Yield%
100	12h	5.0 % mol	65%
100	12h	3.0 % mol	61%
100	12h	2.0 % mol	63%
100	12h	1.0 % mol	67%

Table 1. Effect of Catalyst on Heck reaction

These studies allowed us to obtain one of the two essential building blocks for the construction of the Panobinostat backbone.

The second component (compound **5**) can be synthetized through the classical Fisher reaction:

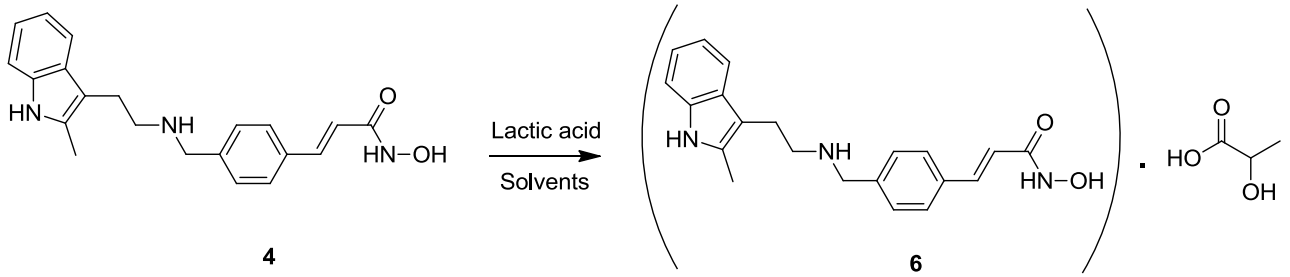


5

Scheme 5. Synthesis of compound 5

Fischer's synthesis was discovered in 1883 and is a chemical reaction that produces the indole aromatic heterocycle.[12] It uses phenylhydrazine and a carbonyl derivative (aldehyde or ketone) as reagents; the acid catalyst plays a key role in this reaction: Bronsted acids such as HCl, H_2SO_4 and p-toluenesulfonic acid have been used successfully. Lewis acids such as boron trifluoride, zinc chloride, iron chloride and aluminum chloride are also useful catalysts.

Once obtained the two reaction's partners **2** and **5**, we explored their key coupling affording **4**: a reductive amination using a wide range of conditions such as solvents, reductive agents and additives. The reaction took place successfully, affording the desired product with a yield of 62%.



Scheme 6. Synthesis of Panobinostat lactate

Panobinostat pharmaceutical formulation is made up from Panabinostat DL-lactate salt and marketed under the name "FARYDAK". For the different physical properties exhibited by salts and polymorphs affect important pharmaceutical parameters such as storage, stability, compressibility, biodisponibility, density and dissolution rates (important in determining biodisoponibility). Stability differences may result from changes in chemical reactivity e.g., differential hydrolysis or oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph), mechanical changes (e.g., tablets crumble on storage as a kinetically favored crystalline form converts to thermodynamically more stable crystalline form) or both (e.g., tablets of one polymorph are more susceptible to breakdown at high humidity conditions).

For these reasons, the last goal was to create the Panobinostat lactate in order to carry out final studies between the molecule we synthesized and the one on the market. The protocol used is also subject to patent censure, but as can be seen from the diagram, the attention in this reaction must be placed on the choice and quantity of solvents in order to obtain the sal . In this thesis we obtained the desired salt in 89% yield.

In conclusion, in this section, we proposed a new strategy for the synthesis of Panobinostat lactate: the protocol is inspired in terms of step by the Novartis approach, but substantial modifications led us to the creation of a new patent. Further studies will be conducted to determine the crystalline form of Panobinostat lactate. The structure investigation of this crystalline form was carried out using X-Ray diffraction studies on powders, in close collaboration with Prof. Lorenzo Malavasi Research team: a new polymorphic structure of this important drug but for secrecy reasons we cannot include the diffraction pattern of this formulation.

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6. CONCLUSION

In the search for effective, selective, and nontoxic antiviral agents, a variety of strategies have been exploited to design nucleoside analogs.

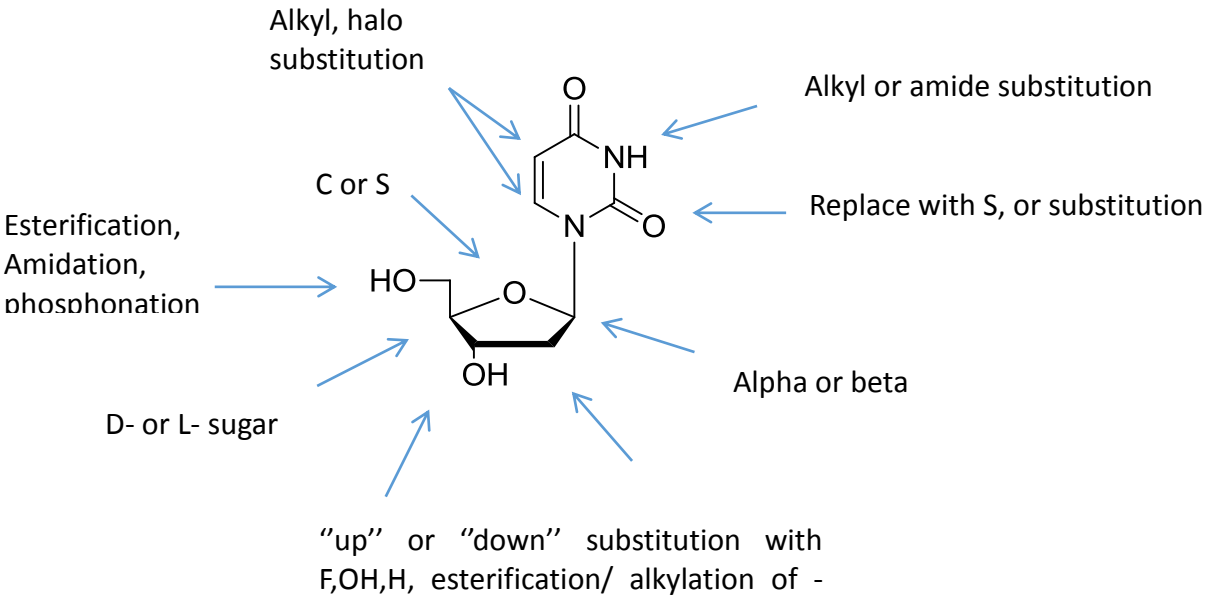


Figure 1. Nucleoside Analogues - Multimodal Therapeutic Agents

First of all, in this thesis we explored the chemistry of hydroxy-2-cyclopentenone core, as illustrated by elegant multi-step syntheses of complex molecular targets.

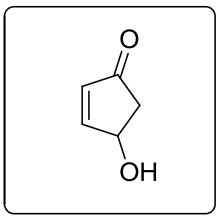
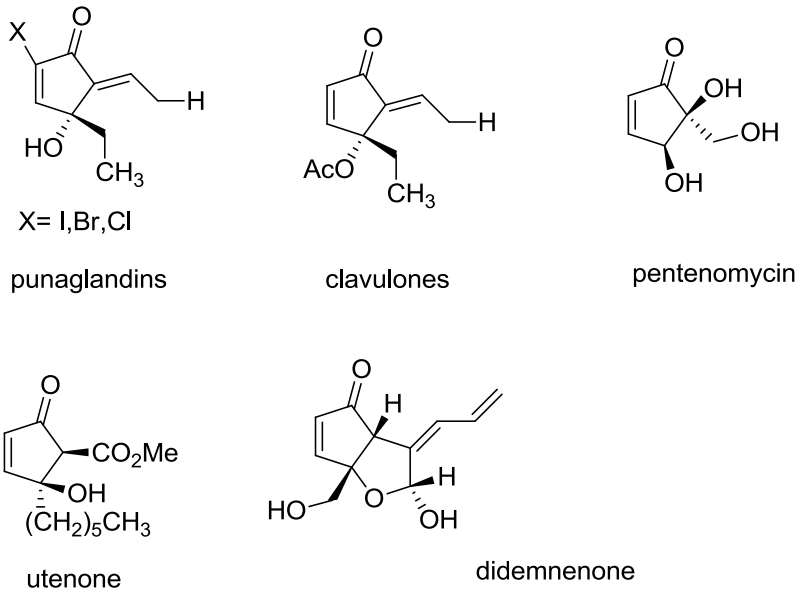
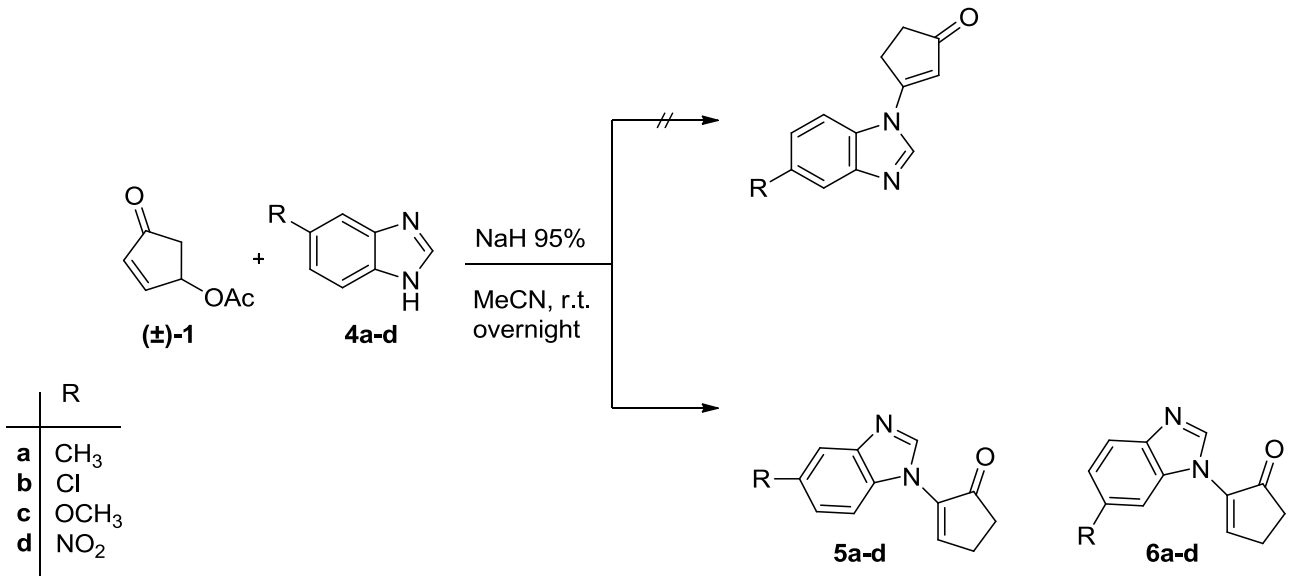


Figure 2. Hydroxy-2-cyclopentenone core



Scheme 1. Representative Panel Of Natural Products Containing The Hydroxy-2-cyclopentenone Fragment

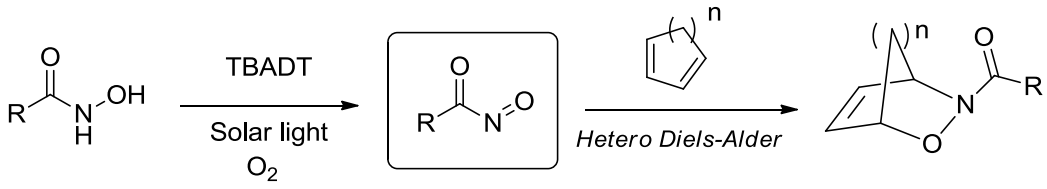
We have reported on a selective approach to 2-heterosubstituted cyclopent-2-en-1-ones through a valuable protocol that avoids the presence of side regiosiomeric compounds. The methodology has been applied to substituted benzoimidazole as model heterocyclic systems in view of future introduction of other heterocyclic scaffolds as well as suitable for further transformations as function of the tested biological activity as antivirals.



Scheme 2. Selective Approach To 2-Heterosubstituted Cyclopent-2-en-1-ones.

We demonstrated that the chemistry of nitrosocarbonyl species is very interesting. These transient intermediates can be used for designing of many precursors of natural products and many complex organic molecules. Hetero-Diels-Alder reactions as well as ene reactions of acylnitroso intermediates occupy the central position as an essential step in the chemical transformations.

Thus, we have here demonstrated the possibility to oxidize hydroxamic acids for the *in situ* generation of fleeting nitrosocarbonyl intermediates. Tetrabutylammonium decatungstate (TBADT) has emerged as a valuable photocatalyst for sunlight-induced organic reactions.



Scheme 2. Selective Approach to Cycloadducts Generation

Decatungstate is able to promote the oxidation of hydroxamic acids to nitrosocarbonyl intermediates in a very short time. The trapping was efficiently obtained with reactive dienes, such as cyclopentadiene and 1,3-cyclohexadiane, to afford the desired HDA cycloadducts in fair to good yields.

The feasibility of the HDA reaction was tested by studying the conversion of several hydroxamic acids.

Between the synthesized simple and complex hydroxamic acids, the protocol set up for the synthesis of **Panobinostat** has been of considerable interest. We elaborated substantial modifications to the synthesis protocol already known that were patented, as underlined before.

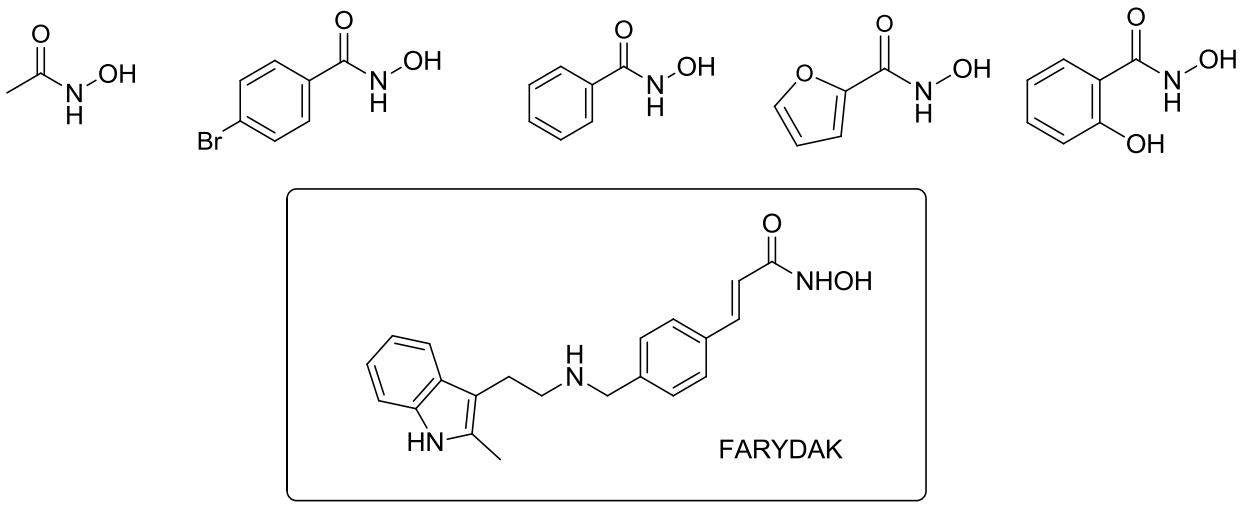
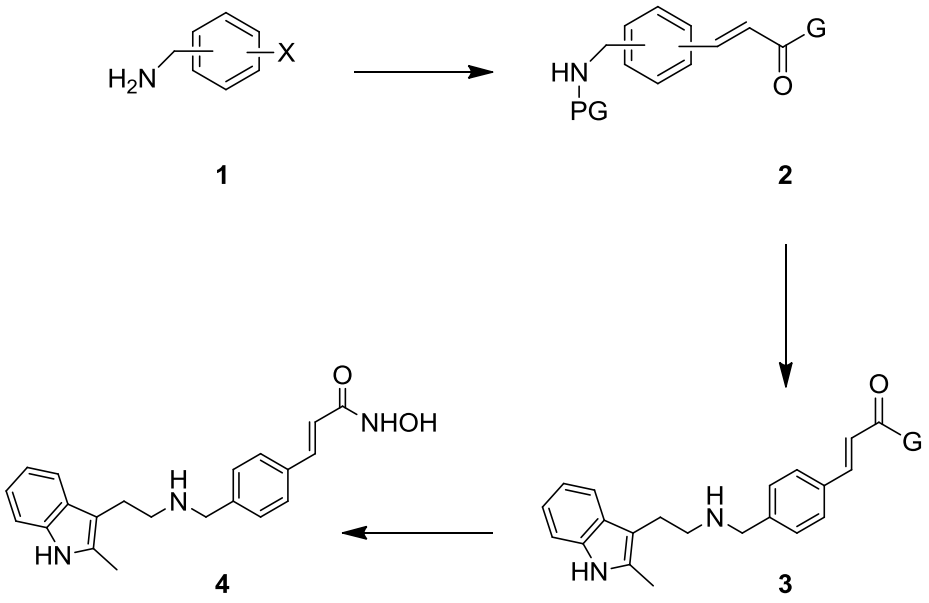


Figure 3. Tested Hydroxamic acids change the name of **Panobinostat**

We elaborated substantial modifications of synthesis protocol but we can't report the real scheme for patent reasons. Moreover, the Farydak is the first DAC inhibitor approved by the FDA for the treatment of relapsed multiple myeloma and has been submitted for approval to regulatory agencies globally.



Scheme 3. Selective Approach to Panobinostat Generation

7. AKNOWLEDGMENT

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