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Review

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Synthesis and biological activity of potential antiviral compounds through 1,3-dipolar cycloadditions. Part 2: nitrones, nitrile oxides and imines, and other 1,3-dipoles

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Dedicated to Prof. Paola Vita Finzi on the occasion of her 90th birthday

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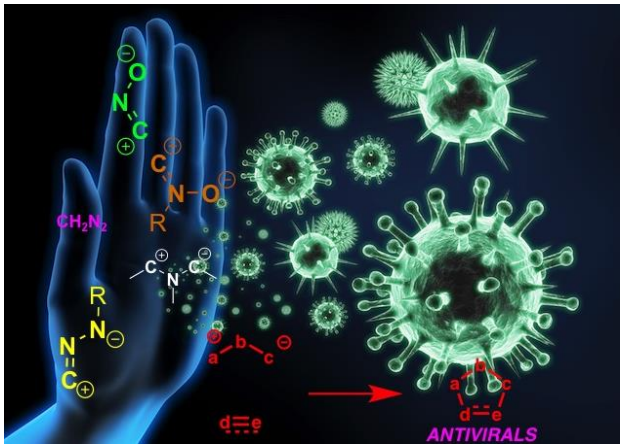
Abstract

Prominent in the current stage of drug development, antiviral compounds can be efficiently prepared through cycloaddition reactions. This review reports the use of 1,3-dipolar cycloadditions of nitrones, nitrile oxides and imines and other 1,3-dipoles in the light of their application for the preparation of synthons in the design and synthesis of compounds that were tested for their antiviral activities against a variety of viruses. Since nitrile oxides represent valuable precursors of nitrosocarbonyl intermediates, their use in the synthesis of antiviral compounds is reported. The products obtained from these pericyclic reaction approaches were tested for their activities in terms of blocking the virus replication and the relevant biological data are highlighted.



Keywords: Antivirals, 1,3-dipolar cycloadditions, nitrones, nitrile oxides, nitrile imines, nitrosocarbonyls, azomethine ylides, diazoalkanes, synthesis, biological evaluation, nucleosides

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1. Introduction

In a previous work, we dealt with the synthesis of antiviral compounds through the 1,3-dipolar cycloadditions, of azides.[1] We have also recalled to mind the most relevant aspects of the chemistry of the 1,3-dipoles and their role in the cycloaddition reactions leading to heterocyclic five-membered rings. In this second part of our work, we wish to expose the chemical uses of other 1,3-dipoles in the synthetic approach to antiviral compounds.

Nitrones (also called azomethine oxides) have a long and remarkable history.[2] The name *nitrone* derives from the contraction of the term *nitrogen ketone*, trying to make a positive comparison between the chemistry of these two functional moieties. The nitrone functionality indeed incorporates a modified imminium portion that, like the carbonyl group, is capable of undergoing nucleophilic attack. However, it is the ability of nitrones to serve as 4π-components in cycloaddition reactions in the presence of suitable dipolarophiles as 2π-partners that addresses the predominant attention on these valuable 1,3-dipoles.

Nitrones are easily accessible reactive intermediates, extremely useful for selective organic syntheses.[3] Various catalytic, selective, and mild methods for their preparation have been developed, the most recognized and applied being the condensation of *N*-monosubstituted hydroxylamines on suitable carbonyl derivatives. The unique properties of nitrones are reflected in the originating products. The reactions where nitrones are employed are extremely important because selective carbon-carbon bond formations can be carried out diastereo- and enantioselectively. These processes can be performed also because of the configurational stability of nitrones and in some cases the chelation effect of oxygen. Several reviews on nitrones have been published, in which the rich chemistry of these intermediates has been detailed.[4-11]

Nitrile oxides and imines are also used in the synthesis of antiviral compounds. From their discovery in the last decade of the 19th century on, nitrile oxides received a wide popularity from 1950 onwards because of the variety of their reactions with various dipolarophiles for the construction of five-membered rings.[12] This reactivity made nitrile oxides increasingly important as valuable tools in organic synthesis and the chemical elaborations of their cycloadducts as well as the theoretical investigations on 1,3-dipolar cycloadditions enriched the understanding of the reaction mechanisms and the applications in organic chemistry.[13]

Nitrile oxides have an illustrious history and from the discovery of fulminic acid as early as 1800 and benzonitrile oxide in 1886 by Gabriel and Koppe, Werner, Buss, Wieland, Huisgen, Quilico and Grünanger contributed significantly to the development of this exciting chemistry, in particular when the generation by dehydrohalogenation of hydroxymoyl chlorides was introduced. Although nitrile imines have been observed

spectroscopically in solution and in low-temperature photochemical studies, these 1,3-dipoles can be isolated but in few cases. Hydrazonoyl halides are the most common precursors of nitrile imines, their first observation dating back to 1894. Similarly, these 1,3-dipoles became popular as synthons for pyrazole derivatives.[13]

Several articles and reviews have been published on the chemistry of nitrile oxides and imines where the wide chemistry of these intermediates has been detailed. The progress in the chemistry of these 1,3-dipoles include the preparation of a variety of heterocyclic compounds of natural origin as well as bioactive molecules and synthons for different molecular targets.[14,15] We also give here a brief overview about the use of nitrosocarbonyls in the synthesis of antiviral compounds due to the fact that nitrile oxides represent valuable precursors of these fleeting intermediates. The overview will be completed by some examples of compounds prepared through the chemistry of azomethine ylides and diazoalkanes.

The focus of this review (Part 2) is again the synthesis of antiviral compounds, pointing out the attention for those compounds having a demonstrated biological activity.

The entire literature examined covers the years from the 1990s, the period corresponding to the beginning of the use of 1,3-dipolar cycloadditions for the synthesis of biologically active molecules, through 2021. Literature is increasing a lot in this field, testifying the high interest in 1,3-dipolar cycloadditions as synthetic tools to antiviral compounds. For the 1,3-dipoles of this review, we count 77 relevant manuscripts.

Due to this, the scope is to give a clear, comprehensive, and, as much as possible, exhaustive picture of the methodologies that chemists can apply for future developments in this area. The main limitation lies in the choice of 1,3-dipoles that can be used in the context.

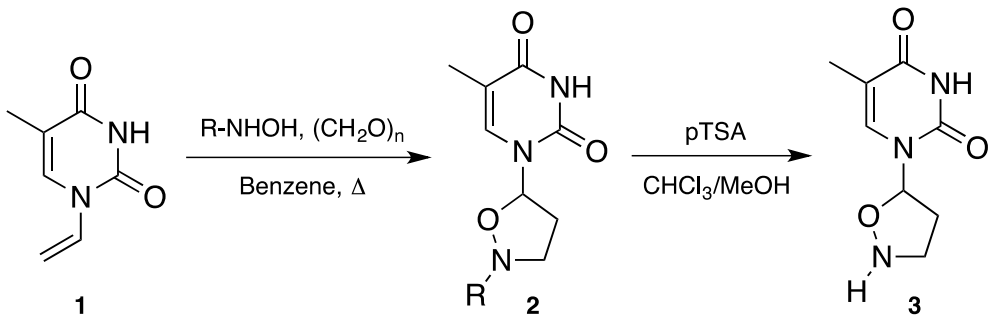
This review completes the overview in the synthesis of antiviral compounds through 1,3-dipoles, marking the key role of unconventional catalyzed and uncatalyzed cycloaddition reactions as valuable tools to prepare new derivatives in a unique reaction pathway, even scalable in industrial processes.

2. Synthesis of antiviral compounds

Nitrones are highly useful synthetic tools through their ability to generate nitrogen- and oxygen-based functionalities, introducing stereoselectively chiral centers.[3] In this section we will discuss the application these 1,3-dipoles in an extensive synthesis of antiviral compounds. They were first prepared by Beckmann in 1890 (*Ber. Dtsch. Chem. Ges*. **1890**, *23*, 3331) and several methods are reported in literature, apt to fit the structural requirements in the planned syntheses. They constitute the best valuable entry to isoxazolidines; these heterocyclic rings in fact are a remarkable alternative to sugar moieties in nucleosides and they mimic the spacer of natural compounds. The facility of preparation of these 1,3-dipoles and the variety of substituents that can be placed on the 1,3-dipolar structures determine the success of these reactive species in the synthesis of new biologically active compounds.[4]

2.1 Synthesis of antiviral compounds through Nitrones

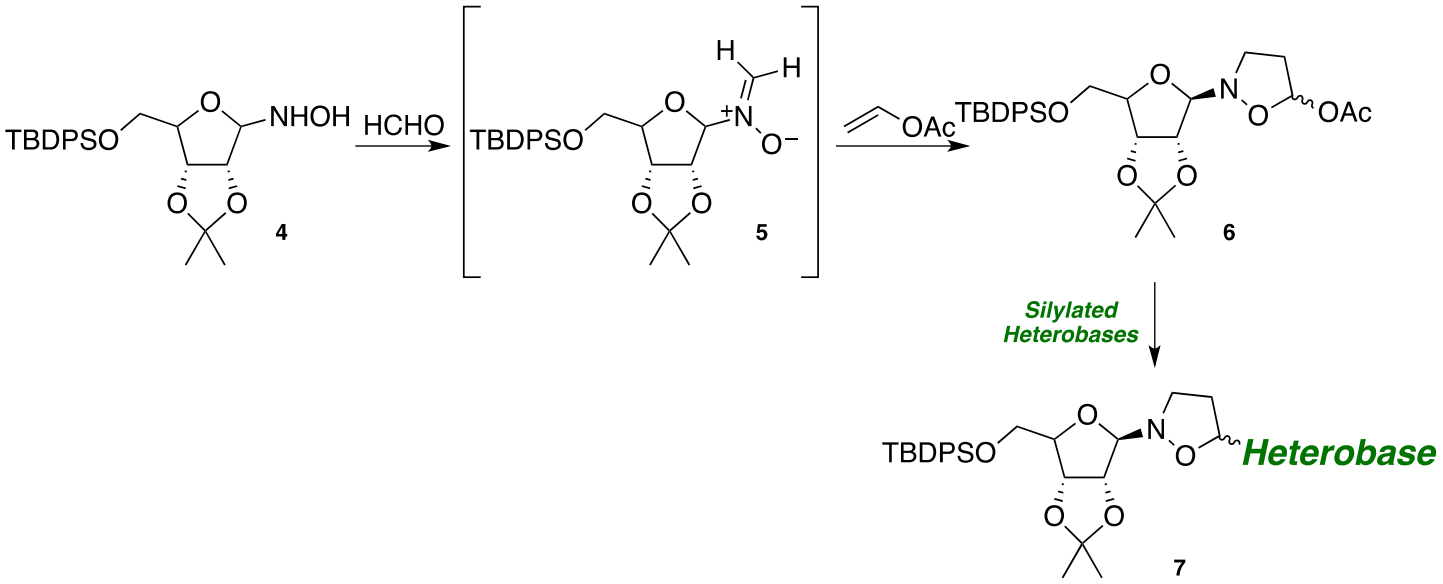
An example of the ease and simplicity of nitrone use for the preparation of isoxazolidine-based nucleosides is hereby given where vinyl-substituted thymine **1** is the dipolarophile that capture the *in situ* generated nitrone from a substituted hydroxylamine and paraformaldehyde to afford the intermediate **2** (Scheme 1). Detachment of the R group can be obtained by treatment with pTSA to give in very good yields (81%) the final adduct **3**.[16]



Scheme 1. Synthetic pathway through nitrone chemistry.

The method relies on the initial preparation of vinyl-derivatized heterobases according to well-established procedures[17] which can reacted in nitrone cycloadditions for the preparation of *N,O*-nucleosides. A variety of examples with a wide application potential in nucleoside synthesis have been reported.[18-20]

N,O-Nucleosides set a primary role to nitrones in their synthetic approach . An interesting example reports that the protected ribose hydroxylamine **4** can be easily transformed into the corresponding nitrone **5** that reacts in situ with vinyl acetate to afford the diastereoisomeric isoxazolidines **6** (Scheme 2). Vorbrüggen nucleosidation conducted with silylated heterobases led to the desired *N,O*-nucleosides **7** in very good yields which were subjected to biological tests.

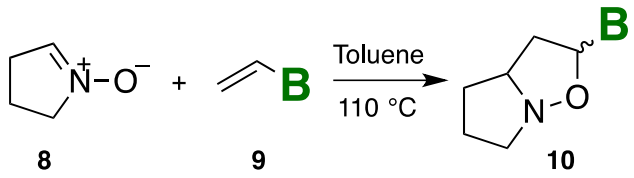


Scheme 2. Synthetic pathway to *N,O*-nucleosides **7**.

The cytotoxicity and the apoptotic activity of the obtained compounds indicated that, among the variety of "heterobases" introduced in **7**, the fluorouracil derivative, while presenting low levels of cytotoxicity (Trypan blue exclusion test) is, conversely, a good inductor of cell death by apoptosis on lymphoid and, less efficiently, monocytoid cells. More important, the compound seems to act as a strong potentiator of Fas-induced cell death, opening new perspectives in future investigations on its possible use as a therapeutic agent.[21] The same synthetic approach was applied for the preparation of 4'-α-C-branched *N,O*-nucleosides, based on the 1,3-dipolar cycloaddition of nitrones with vinyl acetate followed by coupling with silylated nucleobases. The obtained compounds were evaluated for their activity against the viruses HSV-1, HSV-2, and

HTLV-1. None of these derivatives reached 50% inhibition at the highest concentrations tested (*e.g.* 320 μM), indicating a lack of significant antiviral activity.[22] At variance, phosphonated carbocyclic 2'-oxa-3'-aza-nucleosides were synthesized in good yields by the above described 1,3-dipolar cycloaddition methodology. The cytotoxicity and the reverse transcriptase inhibitory activity of the obtained compounds were investigated. Phosphonated carbocyclic 2'-oxa-3'-aza-nucleosides, while showing low levels of cytotoxicity, exert a specific inhibitory activity on two different reverse transcriptases, which is comparable with that of AZT, opening new perspectives on their possible use as therapeutic agents, in antiretroviral and anti-HBV chemotherapy.[23]

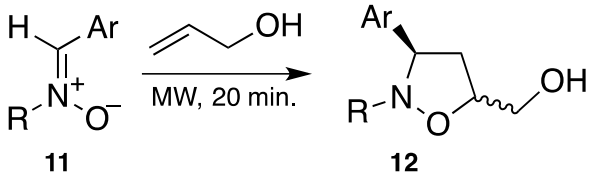
New conformationally locked bicyclic *N,O*-nucleosides analogues were prepared by using the 1,3-cycloaddition reaction of Δ1-pyrrolidine-1-oxide **8** with *N*1-vinylpyrimidine derivatives **9** to afford the analogues **10** in very good yields (Scheme 3). The synthesized modified nucleosides were preliminarily screened in a cell-based system (VERO cells) for their ability to inhibit HSV-1 replication. In addition, cytotoxicity was evaluated in parallel in an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium]-based assay.



Scheme 3. Synthesis of bicyclic *N,O*-nucleosides of type **10**.

The uracil derivative of type **10** displayed an interesting inhibition of HSV-1 replication in the concentration range from 100 μM (41% of cells killed) to 500 μM (83% of cells killed). For the other compounds, no significant toxicity was detected in the treatment in the MOLT-3 (human lymphoblastoid) and VERO (African Green Monkey) cell lines assayed (CC_{50} > 1000 in every case).[24]

Isoxazolidinyl polycyclic aromatic hydrocarbons were synthesized in good yields by 1,3-dipolar cycloaddition methodology promoted by microwave irradiation between nitrone **11** and allylic alcohol (Scheme 4). The obtained cycloadducts **12** were evaluated for their cytotoxicity and antiviral activity.



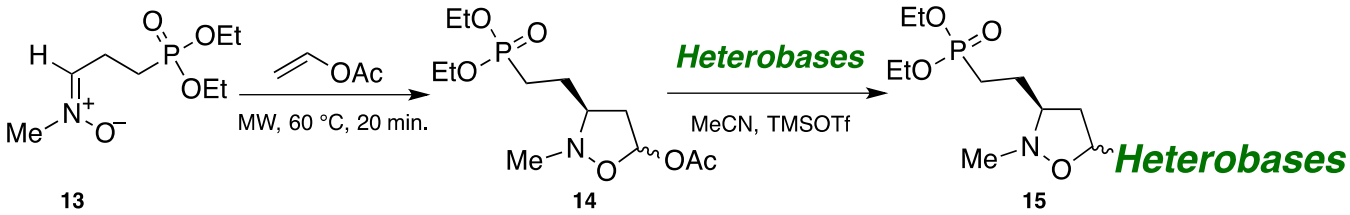
R = Me, Bn
Ar = 9-Phenanthryl, 1-pyrenyl

Scheme 4. Synthesis of *N,O*-nucleosides of type **12** from polyaromatic nitrones.

In particular, compounds bearing large aromatic substituents showed high levels of cytotoxicity on MOLT-3 leukemia cells, also exerting a remarkable enhancing activity on apoptosis caused by anti-Fas antibody addition. In addition, these compounds exhibited specific antiviral effects against the Punta Toro virus.[25]

A new phosphonated nitrone (**13**) was used for the synthesis of carbocyclic 2'-oxa-3'-aza-nucleosides in a 1,3-dipolar cycloaddition reaction of with vinyl acetate followed by coupling with silylated nucleobases

(Scheme 5). The obtained compounds **15** have been evaluated for their ability to inhibit the reverse transcriptase of avian myeloblastosis retrovirus; however no significant activity was observed. Nevertheless, the methodology appears to be very promising to introduce directly a phosphonated moiety on a nucleosidic scaffold.[26]

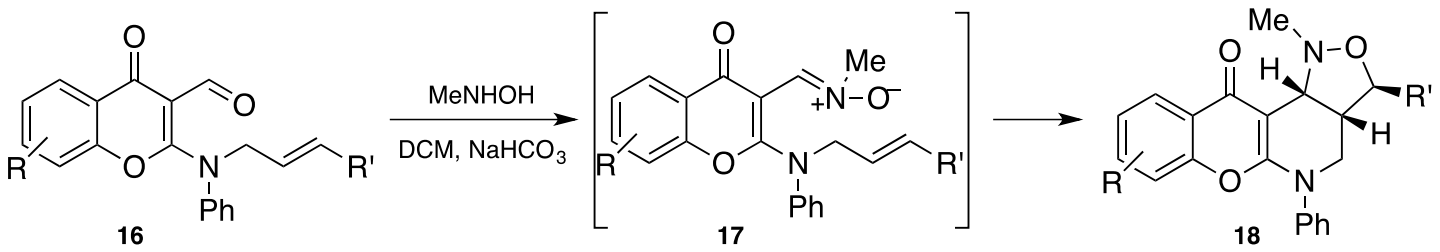


Scheme 5. Synthesis of phosphonated *N,O*-nucleosides **15**.

A new template of C-4'-truncated phosphonated nucleosides was obtained in good yields by the same research group according to two different routes, which exploit the reactivity of a phosphonated nitrone. The one-step procedure is based on the 1,3-dipolar cycloaddition of a phosphonated nitrone with vinyl nucleobases and leads to the unnatural nucleosides as the main adducts. On the other hand, the target β-anomers were obtained in high yield by a two-step procedure based on the 1,3-dipolar cycloaddition of a phosphonated nitrone with vinyl acetate followed by nucleosidation reaction. Preliminary biological assays showed that β-anomers are able to inhibit the reverse trancriptase of different retroviruses at concentrations in the nanomolar range, with a potency comparable with that of tenofovir.[27] On the other hand, truncated phosphonated C-1'-branched *N,O*-nucleosides were synthesized in good yields by 1,3-dipolar cycloaddition methodology, starting from *N*-methyl-*C*-(diethoxyphosphoryl)nitrone. The biological tests showed that β-anomers are able to inhibit HIV *in vitro* infection at concentrations in the micromolar range. Higher SI values with respect to AZT indicated that the compounds were endowed with low cytotoxicity.[28] All these research activities were furtherly developed[29] and summarized by the Catania and Messina research groups in a Mini-Review dealing with phosphonated *N,O*-nuclesides[30] and in a review on the use of isoxazolidines as scaffolds for the preparation of biologically active compounds.[31]

A general overview of the use of phosphonated nitrones for the synthesis of nucleoside analogues is given by Kokosza, Balzarini, and Piotrowska in a paper published on *Nucleosides, Nucleotides and Nucleic Acids* in 2014.[32] A series of 5-substituted 3-phosphonylated isoxazolidines were obtained via cycloaddition of *N*-methyl-*C*-(diethoxyphosphoryl)nitrone with *N*-heteroaromatic acrylamides. The products were evaluated for their antiviral activity against a broad range of DNA and RNA viruses, but were found inactive.

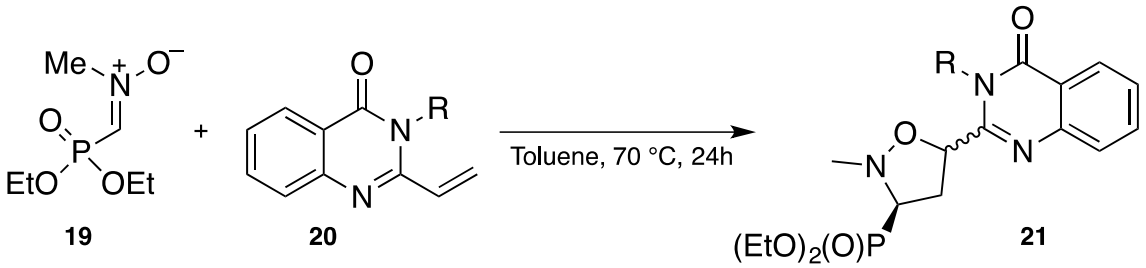
Chromone derivatives **16** are naturally occurring heterocyclic compounds possessing a wide spectrum of biological activities such as anti-inflammatory, antifungal, antimicrobial, antiviral, antitumor and anticancer. In addition, the *N* and *O* containing five-membered heterocycles, isoxazolidines, and isoxazoline derivatives have been shown to display useful anticancer and antiviral properties. Joining the two aspects, newly synthesized chromano-piperidine fused isoxazolidines **18** were prepared according to the procedure shown in Scheme 6 for *in-vitro* cytotoxic evaluation against different human cancer cell lines. The basis of an intramolecular 1,3-dipolar cycloadditions of *in-situ* generated nitrones **17** yields the desired chromano-piperidine fused isoxazolidines **18** in 80-92% yields. All the synthesized compounds were evaluated for their cytotoxic potential against various human cancer cell lines. Unfortunately, no data were given regarding the antiviral activity of these isoxazolidines.[33]



Scheme 6. Intramolecular nitrone cycloaddition reaction to compounds **18**.

Nevertheless, this synthetic pathway remains a nice example of intramolecular approach to isoxazolidines. The (3-diethoxyphosphoryl)-isoxazolidines, substituted at C5 with various quinazolinones, were synthesized by the 1,3-dipolar cycloaddition of *N*-methyl-*C*-(diethoxyphosphoryl)nitrone **19** with *N*3-substitued 2-vinyl-3*H*-quinazolin-4-ones **20** (Scheme 7). All the isoxazolidines **21** were assessed for antiviral activity against a broad range of DNA and RNA viruses. Some of the synthesized compounds showed weak activity (EC_{50} = 6.84−15.29 μM) toward Varicella Zoster virus (VZV) (TK+ strain), which was only one order of magnitude lower than that of acyclovir used as a reference drug. Those compounds that contained the benzyl substituents at *N*3 in the quinazolinone skeleton exhibited slight antiproliferative activity towards the tested immortalized cells with IC_{50} in the 21−102 μM range.[34]

Further developments led to investigate the cycloadditions of other *N*-substituted *C*-(diethoxyphosphoryl)nitrones to *N*-allylated quinazoline-2,4-diones functionalized at *N*3 with substituted benzoyl or benzyl groups. The synthesized isoxazolidine phosphonates were assessed for the antiviral activity against a broad range of DNA and RNA viruses. Some of the compounds exhibited the highest activity toward both TK^+ and TK^- VZV strains (mean EC_{50} values in the range of 3.0−8.7 μM).

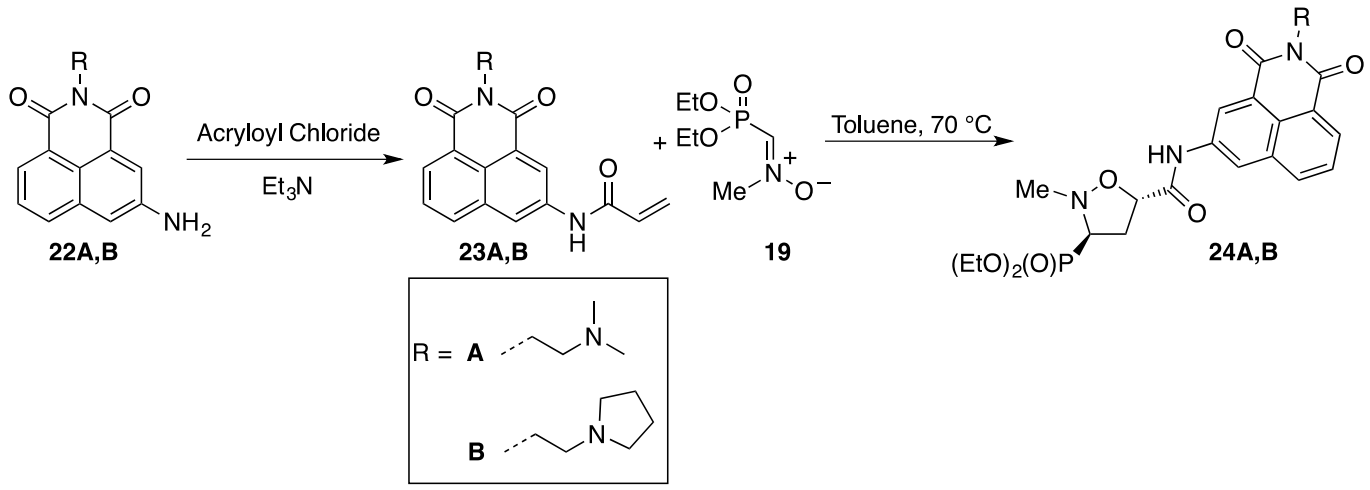


Scheme 7. Synthesis of (3-diethoxyphosphoryl)-isoxazolidines of type **21**.

The isoxazolidine phosphonates having benzyl substituents both at *N*3 of the quinazoline-2,4-dione skeleton and at *N*2 of the isoxazolidine ring displayed some anti-cytomegalovirus potency but at the same time showed significant cytostatic activity for human embryonic lung fibroblasts (used to carry out the antiviral assays) as well as for other cell lines (*i.e.* CEM, L1210, HeLa and HMEC-1).[35] Variably substituted quinazoline-2,4-diones were also used to prepare new isoxazolidines and these new products gave interesting results in terms of antiviral activities.[36] The same nitrone **19** was employed in a series of 1,3-dipolar cycloaddition reactions with allyl benzenes, variably substituted. A wide variety of isoxazaolidines was obtained but none of them showed a remarkable antiviral activity against a broad panel of DNA and RNA viruses.[37]

The same nitrone **19** was employed to prepare a new series of 5-arylcarbamoyl-2-methylisoxazolidin-3-yl-3-phosphonates of type **24A,B**, which were synthesized via the 1,3-dipolar cycloaddition reaction of the *N*-

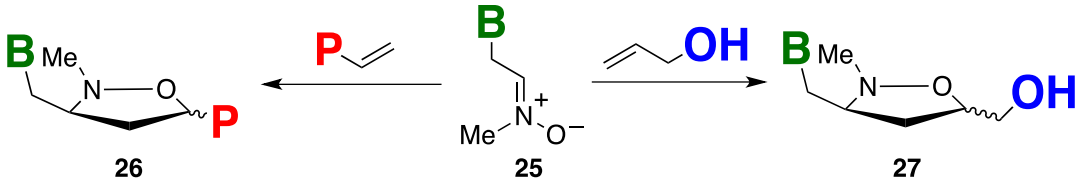
methyl-*C*-(diethoxyphosphoryl) nitrone **19** with *N*-substituted naphthalimide acrylamides **23A,B** prepared from the amine **22A,B** upon functionalization with acryloyl chloride in presence of triethylamine (Scheme 8).



Scheme 8. Synthesis of 5-arylcarbamoyl-2-methylisoxazolidin-3-yl-3-phosphonates **24A,B**.

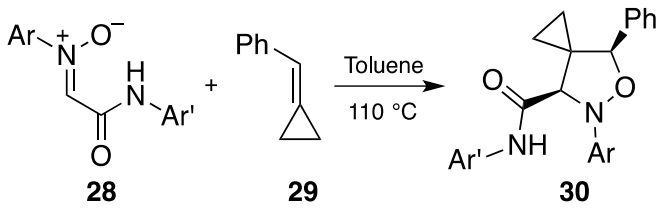
All *cis*- and *trans*-isoxazolidine phosphonates obtained were assessed for antiviral activity against a broad range of DNA and RNA viruses. Isoxazolidines *trans*-**24A** and *trans*-**24B** exhibited the highest activity (EC_{50} = 8.9 μM) toward cytomegalovirus. Compounds *cis*- and *trans*-**24A,B** were found potent against Herpes Simplex- (HSV) and Vaccinia viruses (EC_{50} 45-58 μM). The antiproliferative tests of all obtained isoxazolidines displayed promising activities toward the tested cancer cell lines with IC_{50} in the range 1.1−19 μM.[38]

Isoxazolidine analogues of homonucleos(t)ides were synthesized from nucleobase-derived nitrones **25** (uracil, 5-fluorouracil, 5-bromouracil, thymine, adenine) employing 1,3-dipolar cycloadditions with allyl alcohol as well as with alkenylphosphonates (allyl-, allyloxymethyl-, vinyloxymethyl- and vinylphosphonate) (Scheme 9). The methodology represents a nice example of an upside-down approach to isoxazolidines with respect to the examples hitherto reported. Besides the reactions with vinylphosphonate, the additions proceded regioselectively to produce mixtures of cis (major) and trans (minor) 3,5-disubstituted isoxazolidines **26** and **27**. Unfortunately, none of the tested compounds were endowed *in vitro* with antiviral activity against a variety of DNA and RNA viruses at subtoxic concentrations (up to 250 μM) nor exhibited antiproliferative activity towards L1210, CEM, and HeLa cells.[39]



Scheme 9. Synthetic approach to isoxazolidines **26** and **27**.

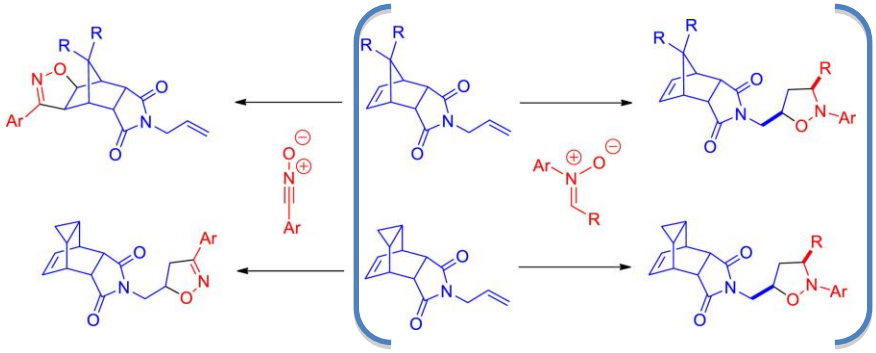
The 1,3-dipolar cycloaddition of benzylidenecyclopropane **29** with various aldonitrones of type **28** gave regioselectively the corresponding 4-spirocyclopropane isoxazolidines **30** in 70-86% yields. In the case of aldonitrones, bearing carbamoyl- or aryl-groups on the carbon atom, only the *cis*-isomer is formed (Scheme 10).



Scheme 10. Synthetic pathway to spiro-isoxazolidine **30**.

The compounds synthesized were tested for their virus-inhibiting activity against influenza virus in *in vitro* experiments. Some of the tested substances demonstrated a selectivity Index (SI) higher than 10. Analysis of the structure-activity relationship suggested that among the compounds tested, side groups on the benzene rings are important for anti-viral activity. In the case of isoxazolidine with two substituents (Ar = 4-MeOPh, Ar' = 4-MePh) the SI is 18. This increase in anti-viral activity was due to increased affinity to the target, as the values of toxicity did not change significantly.[40]

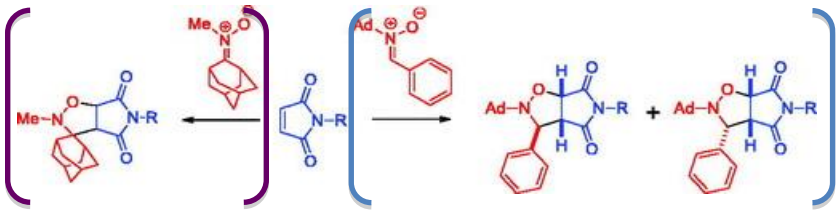
Cycloaddition reactions of *N*-allyl substituted polycyclic derivatives of isoindole-1,3-dione with nitrones were investigated and the authors demonstrate the reactions proceed regio- and stereoselectively on the C=C double bond of the *N*-allyl substituent affording the substituted isoxazolidines in good yields (Scheme 11).



Scheme 11. General synthetic pathways through nitrone and nitrile oxide 1,3-dipolar cycloaddition reactions.

The regioselectivity of the cycloaddition of this dipolarophiles with nitrile oxides depends on the structure of unsaturated hydrocarbon scaffold and the reaction selectively leads to adducts on endocyclic or the C=C double bond of the *N*-allyl substituent. The compounds studied here showed the absence or a moderate activity against the influenza virus. Indeed, all of them demonstrated selectivity indices lower than 10. Moderate activity was observed (IC_{50} = 183 μM; SI = 4) for the product, which has a chlorine atom in the para position of the phenyl ring of the oxazole moiety. Other substituents resulted in lowering of virus inhibiting activity.[41]

The same authors investigated the reactions between adamantane-derived aldo- and keto-nitrones in the presence of maleimides to prepare the corresponding isoxazolidines (Scheme 12). In the case of aldonitrones, reactions afforded diastereomeric mixtures. The ratio of isomers changed during the reaction process due to the reversibility of the cycloaddition reaction. The cytotoxic and virus-inhibiting activity against influenza virus was investigated for selected adducts with moderate toxicity but low antiviral activities.[42]

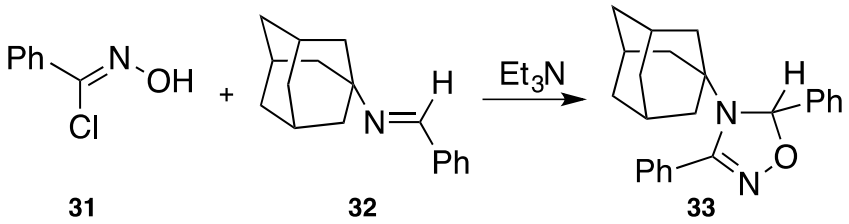


Scheme 12. Synthetic general pathways to maleinide-isoxazolidine derivatives.

2.2 Synthesis of antiviral compounds through nitrile oxides and imines

The synthesis of nucleoside analogues through nitrile oxides and imines is a recent topic in the field of antiviral compounds. The main limits in the synthesis of nucleosides are determined by their high instability and some difficulties for their preparation and for these reasons a few cases are reported. However, the correct choice of the proper substituent can solve selectivity problems and in many cases facilitate the access to biologically active molecules.

A first example of use of the chemistry of nitrile oxides was reported by Italian authors about the synthesis of adamantyl derivatives according to the protocol shown in Scheme 13. The 1,3-dipolar cycloaddition of the *in situ* generated benzonitrile oxide from the corresponding hydroxamoyl chloride **31** and triethylamine in the presence of the imine **32** afforded the cycloadduct **33** in good yields.

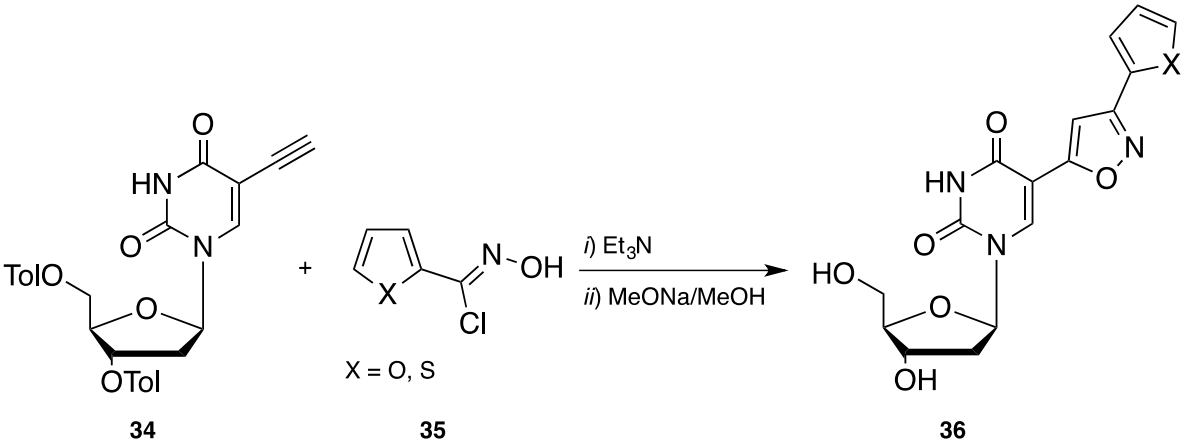


Scheme 13. Synthesis of adamantly 1,2,4-oxadiazole derivatives.

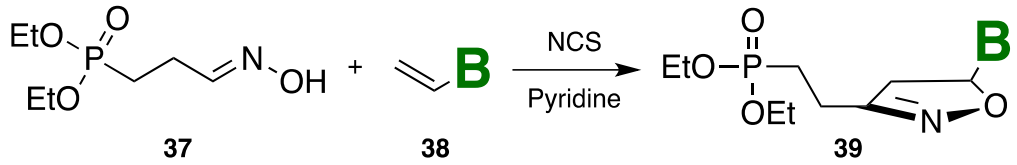
Compound **33** was tested against HIV and showed a reduction greater than 50% of viral cytopathic effects (63 μM). Furthermore, the introduction of a substituent on the phenyl ring at C-5 decreases the anti-HIV activity; in fact, all the compounds failed to counteract these effects of HIV since the cell growth of HIV-infected cells remained close to 40 or < 40% of that of uninfected untreated cultures. Values of cell growth of HIV-infected cells between 0-50% indicate the lack of any substantial anti-HIV activity.[43]

A series of novel 2'-deoxyuridines with a furyl or thienyl substituents in the 5-position were synthesized as potential anti-HSV-1 agents through the 1,3-dipolar cycloaddition of the hydroxamoyl chlorides **35** to the dipolarophile **34** (Scheme 14). The compounds **36** show moderate activities against HSV-1. These new compounds demonstrated poor affinity for the virus-specific enzyme thymidine kinase.[44]

The dihydroisoxazole nucleosides **39** as phosphonate derivatives were efficiently prepared via 1,3-dipolar cycloaddition reactions of nitrile oxides, *in situ* obtained from the corresponding oxime **37**, with vinyl nucleobases **38** for antiviral studies (Scheme 15).[45]

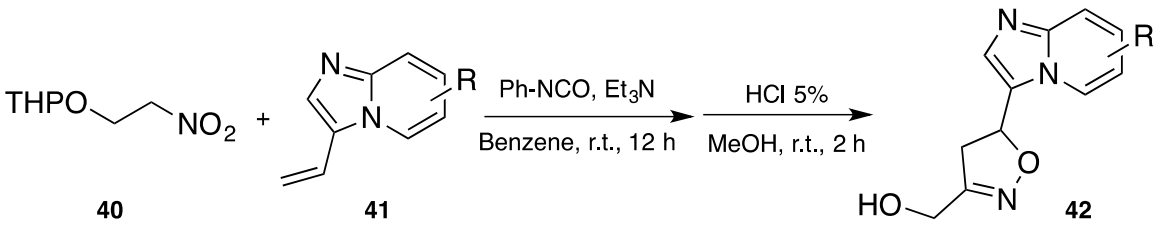


Scheme 14. Synthesis of 2'-deoxyuridines with a furyl or thienyl substituents.



Scheme 15. Synthesis of phosphonate isoxazolines **39**.

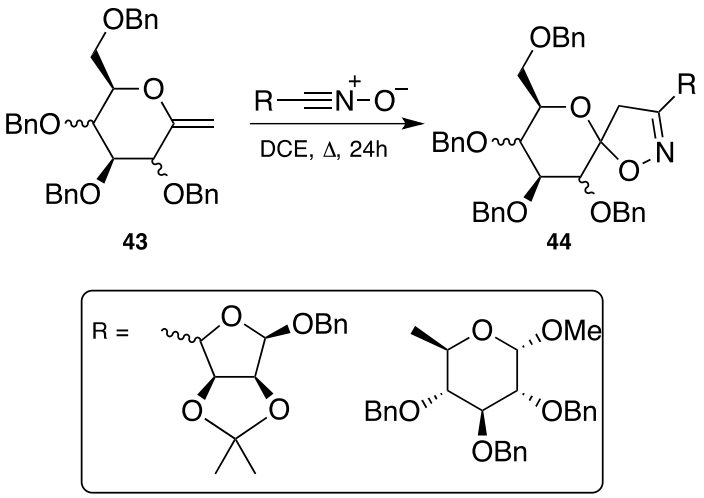
No biological data were provided by the authors who pursued their studies on isoxazoline synthesis this time by generating the nitrile oxide *in situ* from nitro-aliphatic compounds **40** by treatment with isocyanate (Scheme 16). Coupling with imidazopyridine derivatives **41** afforded, after deprotection, the desired compounds **42** in very good yields.



Scheme 16. Synthesis of imidazopyridine-functionalized isoxazolines **42**.

Only a weak anti-HIV-1 activity (EC_{50} = 59.5 μM) was observed for one of the synthesized products.[46]

The use of phosphonated nitrile oxides in 1,3-dipolar cycloaddition reactions with vinyl- or allyl-derivatized heterobases attracted the attention of various research groups aiming to reach a fast track towards nucleoside analogues. Various studies were conducted on this side and the products obtained were biologically evaluated against some viruses. However, the results were below the expectations although the methodology remain a valuable approach to phosphonated derivatives.[47]



Scheme 17. Synthesis of spiro-isoxazoline disaccharides.

A series of spiro-isoxazoline *C*-disaccharides was synthesized by the key step of 1,3-dipolar cycloaddition reactions of exo-glycals of type **43** and sugar-based nitrile oxides. The cycloaddition reactions were carried out stereoselectively and afforded α-isomers of type **44** exclusively except in the case of galactose (Scheme 17). The biological activities of the new disaccharides tested against the glycosidases (α-amylase, α-glucosidase, and β-glucosidase) and HIV-1 (replication in MT-4 cells) and BVDV (nose strain, replication in MDBK cells, a model system of HCV) revealed that the compounds had little inhibitory effects on both the viruses.[48]

Three new types of aryl diketo acid isosteres were designed and synthetized by the conversion of the biologically labile 1,3-diketo units into heteroaromatic motif such as isoxazole, isothiazole, or 1*H*-pyrazole. The aim was to improve the physicochemical property of ADK-based HIV-1 integrase inhibitors. The synthesis of the heteroaromatic carboxylic acids was established by employing phenyl diketoester or benzaldehyde as the starting material and 1,3-dipolar cycloaddition as the key reaction (Figure 1). The 3-benzyloxyphenyl-substituted isoxazole carboxylic acid displayed the best integrase inhibitory and antiviral activities, with *N*-hydroxyl amidation enhancing the *in vitro* and *in vivo* potency.[49]

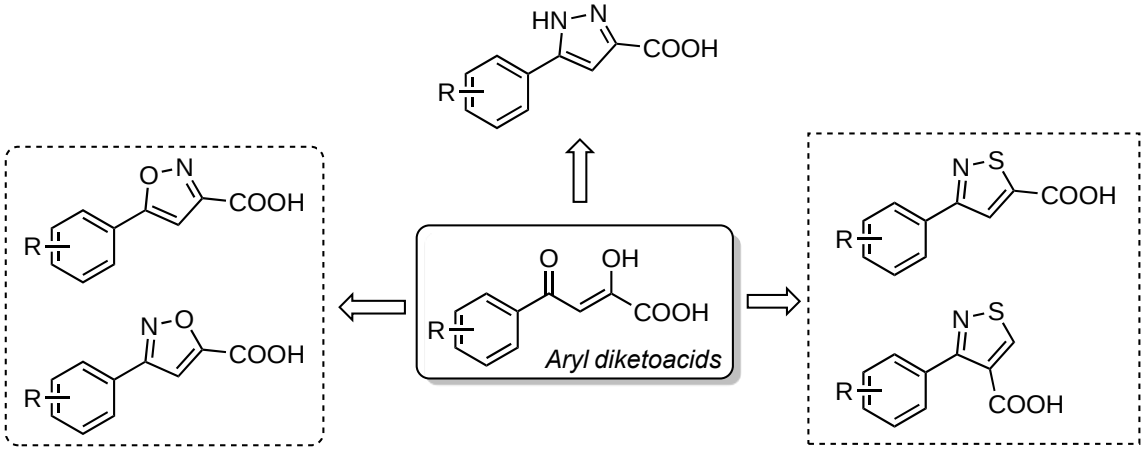
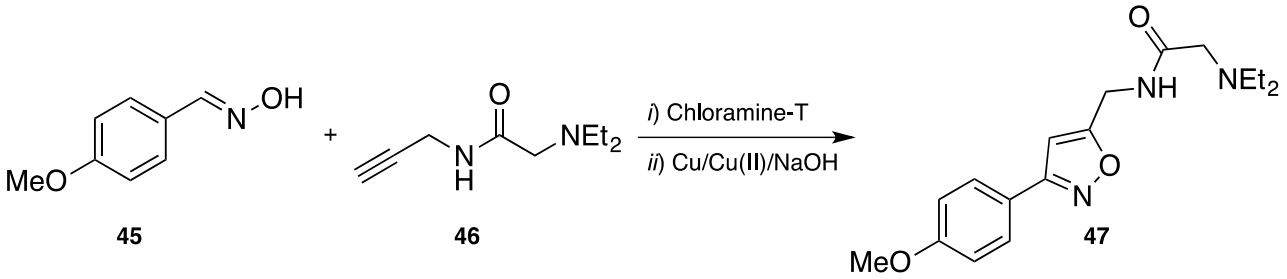


Figure 1. Synthesis of the heteroaromatic carboxylic acids from aryl diketo acids.

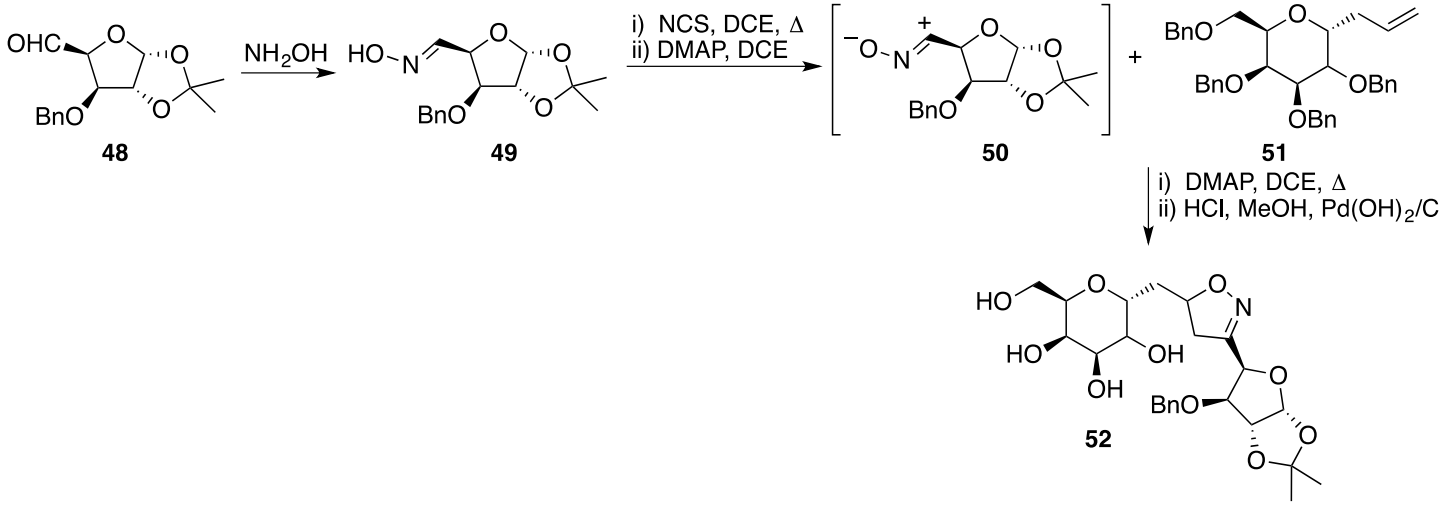
An antifiloviral screening system, based on a pseudotyping strategy, was developed on the basis of 1,3-dipolar cycloaddition reactions. The application is located in the discovery of a new group of small molecules

that selectively inhibit the Ebola and Marburg glycoprotein (GP)-mediated infection of human cells. Using Ebola Zaire GP-pseudotyped HIV particles bearing a luciferase reporter gene and 293T cells, a library of 237 small molecules was screened for inhibition of GP-mediated viral entry. From these assays, lead compound **47** (Scheme 18) was identified as a selective inhibitor of filoviral entry with an IC_{50} of 30 μM. To analyze functional group requirements for efficacy, a SAR analysis of this 3,5-disubstituted isoxazole was then conducted with 56 isoxazole and triazole derivatives prepared using "click" chemistry. The results revealed that while the isoxazole ring can be replaced by a triazole system, the 5-(diethylamino)acetamido substituent found in **47** is absolutely required for inhibition of viral-cell entry. Variation of the 3-aryl substituents provided a number of more potent antiviral agents with IC_{50} values ranging to 2.5 μM. Lead compound **47** and three of its derivatives were also found to block the Marburg glycoprotein (GP)-mediated infection of human cells.[50]



Scheme 18. Synthetic pathway to isoxazole **47**.

A series of novel isoxazoline linked pseudodisaccharide derivatives **52** were regiospecifically synthesized by the 1,3-dipolar cycloaddition reactions of α-allyl-*C*-glucopyranoside **51** and sugar-derived nitrile oxide **50** with good yields. The nitrile oxide was prepared starting from the aldehyde **48** upon conversion into the corresponding oxime **49** and chlorination with NCS followed by *in situ* generation of the 1,3-dipole in the presence of the suitable dipolarophile **51** (Scheme 19).



Scheme 19. Synthetic strategy to isoxazoline **52**.

Compound **52**, the structure of which was elucidated by NMR spectroscopy and MS spectrometry and confirmed by X-ray crystallography, showed interesting glycosidase inhibitory activity along with other

compounds, determined with hydrolytic reactions of glycosidases using acarbose as a control. However, compound **52** showed a moderate selective inhibition of β-glucosidase; the inhibition of **52** was 20% at the concentration of 2.7 µmol/mL. HIV-RT and antitumor activity were also preliminarily evaluated. Some of these types of cycloadducts exhibited potent inhibitory activity to HIV-RT.[51]

The association between glioblastoma (GBM) and human cytomegalovirus (HCMV) infection has been the intensively debated topic over the decades for developing new therapeutic options. Peroxides from natural and synthetic sources are known to serve as potential antiviral and anticancer agents. In this regard a concise and efficient strategy has been demonstrated to access a novel class of peroxides containing a spiro-isoxazoline to primarily investigate the biological activities.

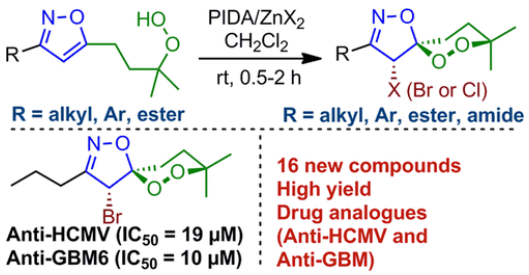


Figure 2. Synthetic strategy to peroxide-containing spiro-isoxazolines (Reproduced with permission.[52] Copyright 2019, ACS).

The synthetic compounds were evaluated for *in vitro* antiviral and antiproliferative activity against HCMV and glioblastoma cell line (GBM6), respectively. While the compound in Figure 2 showed moderate anti-CMV activity (IC_{50} = 19 µM), surprisingly, an independent biological assay for the same compound revealed its antiproliferative activity against the human glioblastoma cell line (GBM6) with an IC_{50} of 10 µM. Hence, the unification of an isoxazoline, obtained from nitrile oxide cycloaddition reaction, and peroxide heterocycles could be a potential direction to initiate the HCMV-GBM drug discovery program.[52]

Electrophilic fluorine-mediated dearomative spirocyclization has been developed to synthesize a range of fluoro-substituted spiro-isoxazoline ethers and lactones (Figure 3). The *in vitro* biological assays of synthesized compounds were probed for anti-viral activity against human cytomegalovirus (HCMV) and cytotoxicity against glioblastomas (GBM6) and triple negative breast cancer (MDA MB 231). Interestingly, the compounds, the structures of which are reported in Figure 4, showed significant activity against HCMV (IC_{50} = 10 µM), while other compounds differently substituted revealed the highest cytotoxicity with IC_{50} = 36 to 80 µM. The synthetic efficacy and biological relevance offer an opportunity to further drug-discovery/development of fluoro-spiro-isoxazolines as novel anti-viral and anti-cancer agents.[53]

Highly functionalized potential NA inhibitors, analogues of peramivir, were synthesized via a new and versatile method starting from a stereoselective 1,3-dipolar cycloaddition reaction between the nitrile oxide derived from 2-ethylbutanal and the commercially available and inexpensive cyclopentadiene and 1,3-cyclohexadiene, which afforded the isoxazolino-cyclopentene or cyclohexene intermediates, respectively (Figure 4). The subsequent reaction of the C=C bond in different conditions allowed the functionalization of the five (or six) membered carbon nucleus. Further functionalized derivatives displaying an amino and a hydroxyl group were achieved via the final opening of the isoxazoline ring.[54]

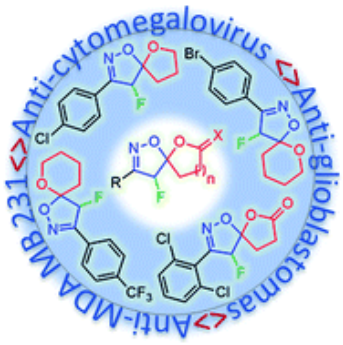


Figure 3. Structures of fluoro-substituted spiro-isoxazoline ethers and lactones (Reproduced with permission.[53] Copyright 2020, RSC).

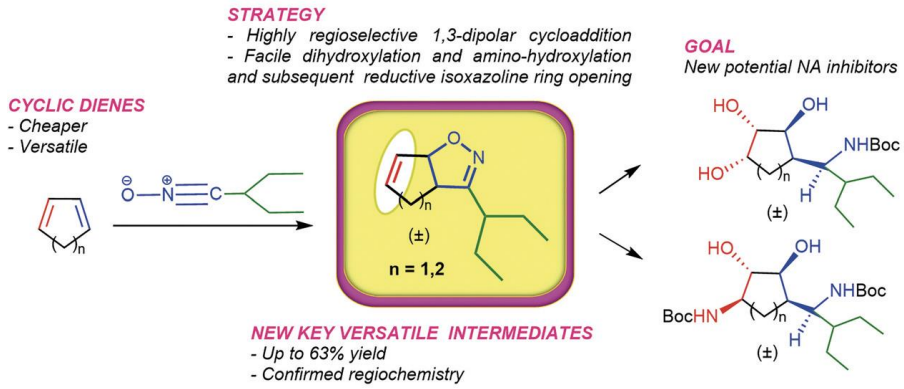
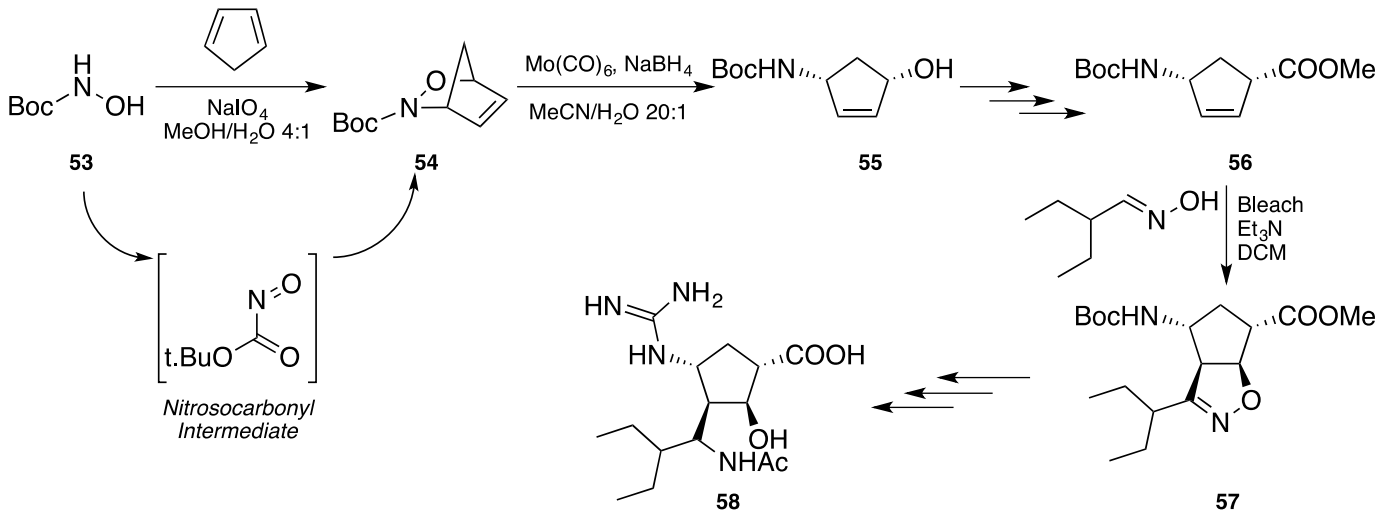


Figure 4. Synthetic strategy to NA-inhibitors via nitrile oxide cycloaddition reactions (Reproduced with permission.[54] Copyright 2020, Thieme Verlag).

Nitrile oxides are not only starting intermediates for conducting 1,3-dipolar cycloaddition reactions. Their structure can be modified, giving rise to new highly reactive intermediates with unique and significant synthetic potential.

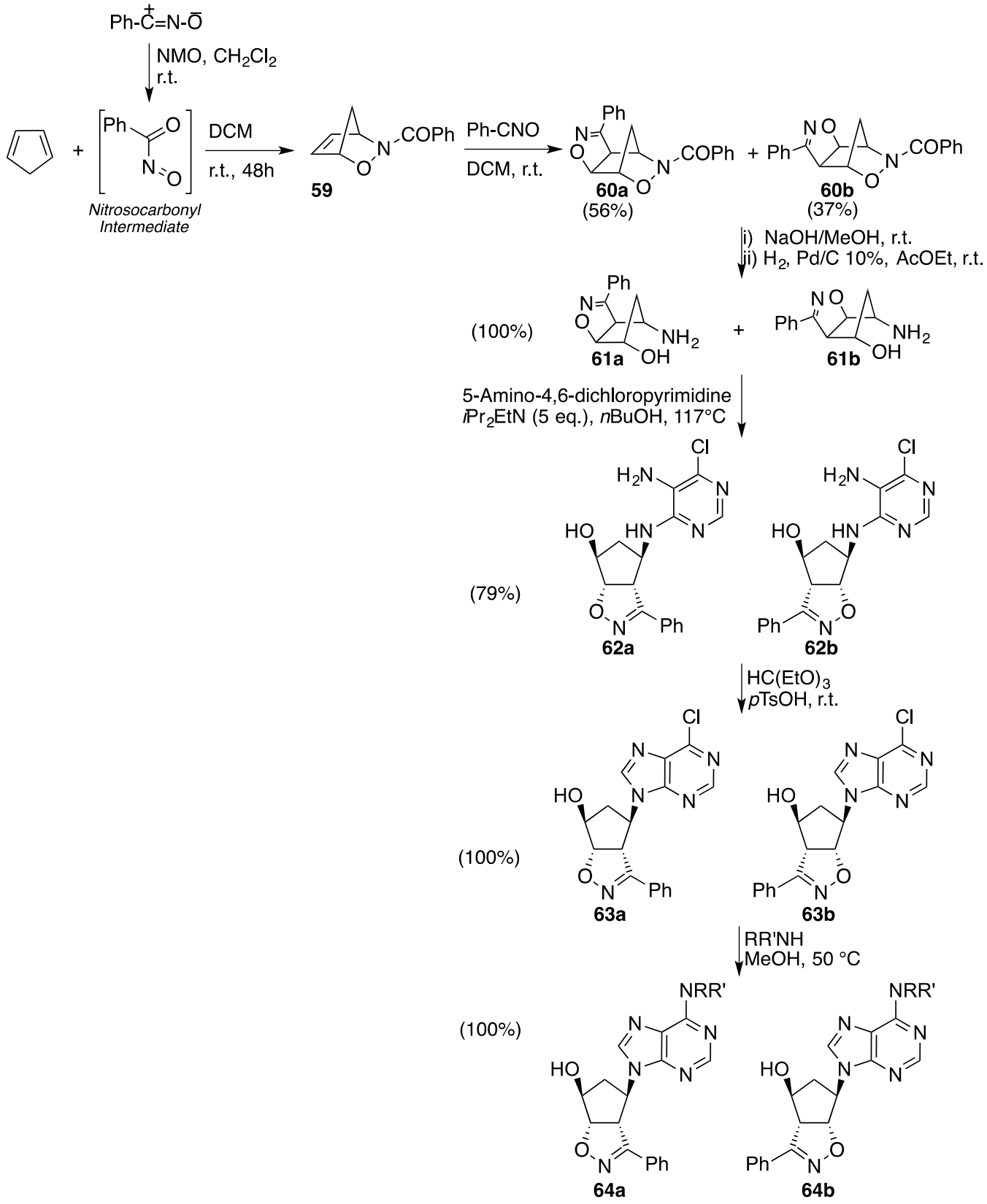
The following example introduces the use of nitrile oxides in the synthesis of analogues for the contrast to viral infections and as a synthetic tool that widen the view towards antiviral compounds. We introduce here the classical approach to *nitrosocarbonyl intermediates* derived from the periodate oxidation of hydroxylamine derivatives **53** (Scheme 20); in the presence of freshly distilled cyclopentadiene, the *in situ* generated nitrosocarbonyl intermediate undergoes HDA cycloaddition to afford the cycloadduct **54** which is synthetically elaborated into the ester **56**. This latter is the dipolarophile of choice in the 1,3-dipolar cycloaddition reaction with a suitable oxime leading to the cycloadduct **57** that is subsequently converted into the desired compound **58**.



Scheme 20. Synthetic strategy to (1*S*,2*S*,3*R*,4*R*)-3-((*R*)-1-acetamido-2-ethylbutyl)-4-guanidino-2-hydroxycyclopentane-1-carboxylic acid **58**.

The target compound serves to develop a neuraminidase inhibitor to treat infections due to influenza.[55] The example just reported is a typical case of carbocyclic nucleoside synthesis. The modification of some structures by fusion of a carbocyclic or heterocyclic rings to the carbasugar moiety increases the lipophilicity,[56] maintaining at the same time the rigidity imposed by the C2'–C3' double bond. Recently, carbocyclic nucleosides containing a fused isoxazoline ring and lacking a methylene group in the side chain of the carbocyclic unit were suggested as a new class of analogues.[57] Nucleosides lacking a methylene group in the side chain linked to the spacer have been reported and in some cases display reduced cytotoxicity. The synthetic route that was exploited is based on the HDA cycloaddition of dienes with transient acyl nitroso moieties,[58] affording "privileged structures" for the synthesis of carbocyclic nucleosides. The HDA cycloadduct *N*-benzoyl-2,3-oxazanorborn-5-ene **59** was obtained from freshly distilled cyclopentadiene that quantitatively trapped the nitrosocarbonyl benzene generated *in situ* in good yields through the mild oxidation of benzonitrile oxide (Ph-CNO, BNO) with a slight excess of *N*-methylmorpholine *N*-oxide (NMO) according to the reported procedure (Scheme 21).

The HDA cycloadduct **59** was found to be an excellent dipolarophile towards BNO addition, affording the two regioisomeric cycloadducts **60a,b** which were easily isolated in quantitative combined yield by chromatographic separation in a 3 : 2 regioisomeric ratio. A proper tuning of the subsequent synthetic steps had to be done for the scale-up necessary for the synthesis of nucleosides in the due amount for the biological tests.



Scheme 21. Nitrosocarbonyl approach to nucleoside analogues **64**.

The easy detachment of the benzoyl group from the cycloadducts **60a,b** was achieved through NaOH/MeOH treatment at room temperature for 24 h, affording the fairly stable hydroxylamines. The

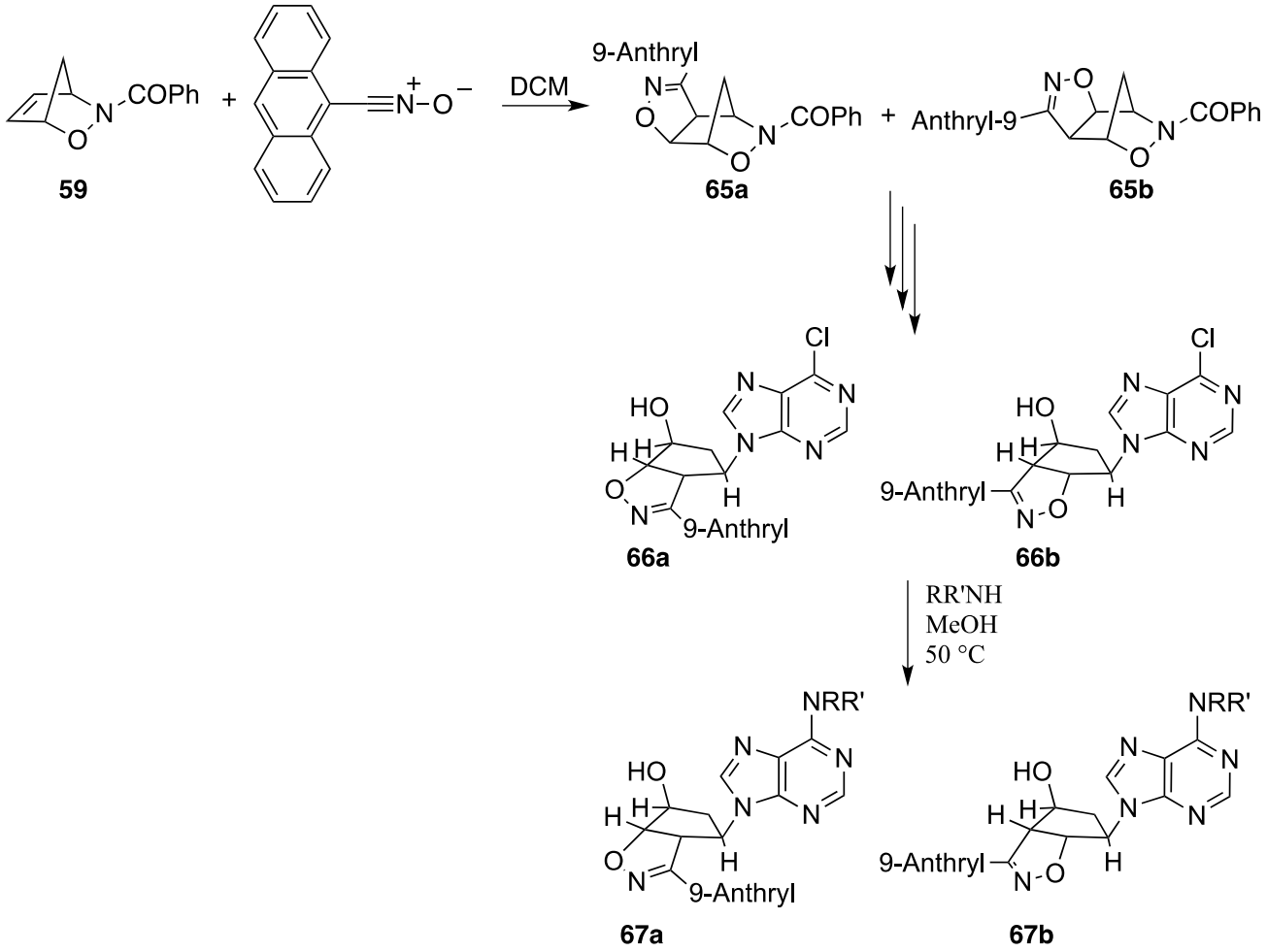
reaction was conducted by adding a slight excess of NaOH, added portionwise in 2−3 h, to a methanol solution of cycloadducts **60a,b**, stirred until complete disappearance of the starting materials. The hydrogenolytic cleavage of the N–O bond of the hydroxylamines was performed by the reaction with Pd/C 10% in ethyl acetate at room temperature for 3 h. Hence, the aminols **61a,b** were obtained and submitted to the transformations into the desired nor-nucleosides through the linear construction of the purine and pyrimidine rings. The synthesis of the purine-nucleosides requires the two steps. The first one is the condensation of the regioisomeric aminols **61a,b** with the 5-amino-4,6-dichloropyrimidine to give the pyrimidine derivatives of type **62a,b**.

This condensation is indeed the most difficult step in many reported syntheses, which attained only low to moderate yields. Better yields of **62a,b** were achieved by increasing the reaction temperature gradually up to the boiling point of the solvent and working in sealed tubes. The pyrimidine derivatives **62a,b** were obtained in 79% yields for both regioisomeric compounds. The conversion of the pyrimidine derivatives **62a,b** into the chloropurine compounds **63a,b**, was achieved by treatment with triethyl orthoformate and a catalytic amount of pTsOH. The final chlorine displacement method with selected amines was conducted upon heating MeOH solutions of **62a,b** at 50 °C in the presence of an excess NH_3 or some primary or secondary amines affording the adenine derivatives **64a,b** in more than 92% yields, which have been submitted to the biological tests. Compounds **64a,b** were evaluated for their inhibitory activity against a wide variety of viruses, including herpes simplex virus type 1, vaccinia virus, vesicular stomatitis virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus and respiratory syncytial virus. All synthesized compounds showed no specific antiviral effect but some differences between the regioisomeric nucleosides can be however noted. Regioisomers of type **b**, involving a phenyl group distal to the heterocyclic base, display better response than regioisomers of type **a**, which have proximal phenyl and heterocyclic base. Moreover, all compounds were evaluated for antiviral activity against HIV-1 (strain IIIB) and HIV-2 (strain Rob) in MT-4 cell culture and none of these compounds showed inhibitory activity at concentration up to 400 μg/mL. The modest antiviral activity showed by these compounds could be likely linked to the lack of substrate activity for cellular and/or viral nucleoside kinases, or alternatively, the lack of recognition of the compounds by the viral DNA or RNA polymerases.[59]

The same strategy described above was applied for the synthesis of isoxazolino-carbocyclic nor-nucleosides incorporating an anthracene moiety through nitrosocarbonyl intermediates chemistry. A variety of analogues were attained starting from the already reported stereodefined heterocyclic aminols through the linear construction of purine heterocyclic rings. The synthesis hinges on the *exo*-selective 1,3-dipolar cycloaddition of the stable anthracenenitrile oxide to the *N*-benzoyl-2,3-oxazanorborn-5-ene **59** and simple elaborations of the regioisomeric cycloadducts **65a,b** (Scheme 22). The synthetic route of Scheme 21 was here applied again and the chloropurine compounds **66a,b** were prepared.[60]

A selection of nucleoside primary and secondary amines derivatives **67a,b** were initially tested for their inhibitory activity against a variety of viruses, including Hepatitis B and C, Human Papilloma virus (HPV) as well as Influenza viruses of type A and B. Modest anti-viral activities were observed in Hepatitis assays while the activities in the cases of Influenza viruses were almost negligible. Good anti-viral activity was found for compound of type **67b** (R = H; R' = Et), with no cellular toxicity at the dose tested in the case of HPV.

The larger activity shown by those compounds, which bear aromatic residues, could suggest a possible mechanism based on DNA-intercalation. Some isoxazolidinyl polycyclic aromatic hydrocarbons were proposed as DNA-intercalating antitumor agents and molecular modeling studies on these structures confirm the degree of binding when a polycyclic aromatic residue is linked to an isoxazoline heterocyclic ring.[61]



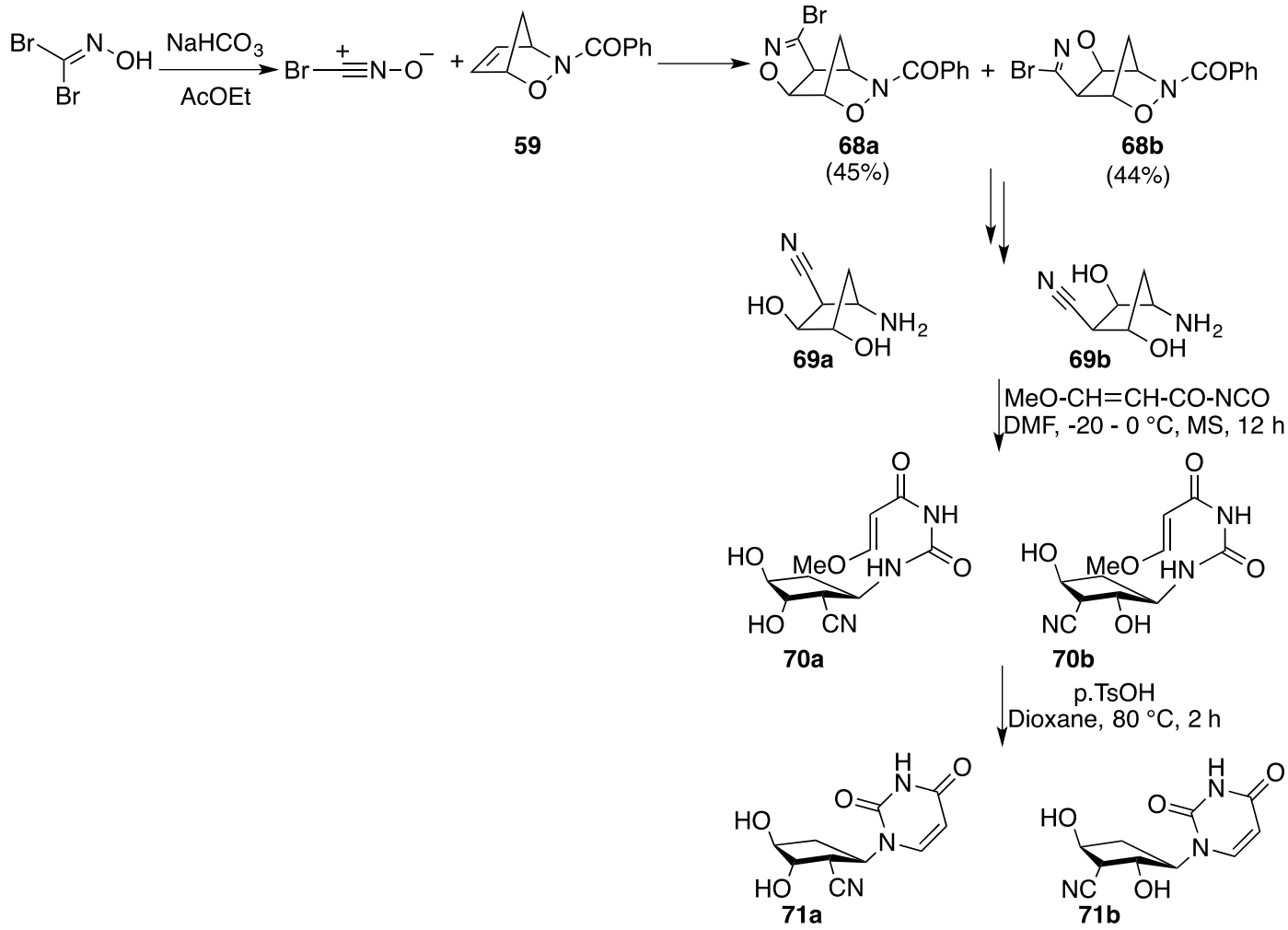
Scheme 22. Synthetic strategy to anthracene-substituted nucleoside analogs.

These investigations were completed by the synthesis of isoxazolino-carbocyclic anthracene nor-nucleosides, prepared through nitrosocarbonyl chemistry, containing uracil residues, tested for their inhibitory activity against some viruses, such as Herpes simplex viruses of type 1 and 2, Zoster virus and Hepatitis B and C. The activities were almost negligible in most of the cases. Furthermore, a remarkable antiviral activity was found for a specific compound with no cellular toxicity at 1-100 μM dose concentration in the case of HPV.[62]
The new nucleosides **71a,b** (Scheme 23) were found structurally similar to a potent antiviral compound, brivudin (BVDU), and molecular modeling as well as docking experiments allowed to select one of the two regioisomeric structures as promising candidate for antiviral tests, due to the nice level of binding with the thymidine kinase (TK), the enzyme involved in virus replication.[63]

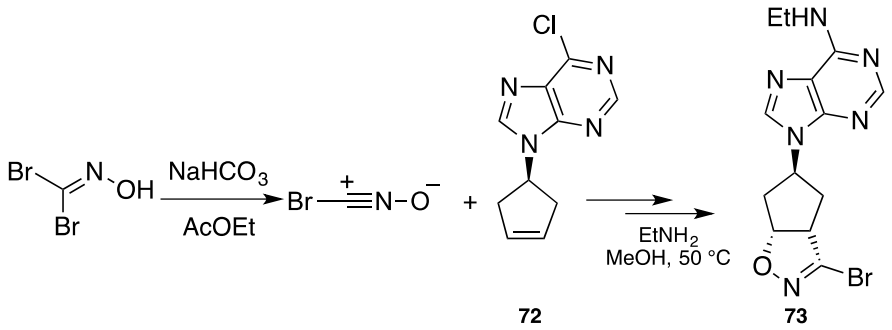
Their synthesis was carried on starting from the bromonitrile oxide, *in situ* generated from the corresponding stable dibromooxime, through the cycloaddition to the oxazanorbornene **59** and subsequent synthetic elaboration to the desired uracil derivatives. The same bromonitrile oxide was also used in the derivatization of cyclopentene-purine derivatives **72** (Scheme 24) affording the regioisomeric cycloadduct **73** that was tested against the respiratory virus influenza A H1N1 cell line MDCK (strain California 7/2009) with a EC_{50} = 0.80.[64]

The same strategy applied for the anthracene derivatives (see Scheme 22) has been applied for the synthesis of isoxazolino-carbocyclic nornucleosides incorporating a quinoline moiety through nitrosocarbonyl

intermediate chemistry. Adenine analogues were attained through the linear construction of purine heterocyclic rings.



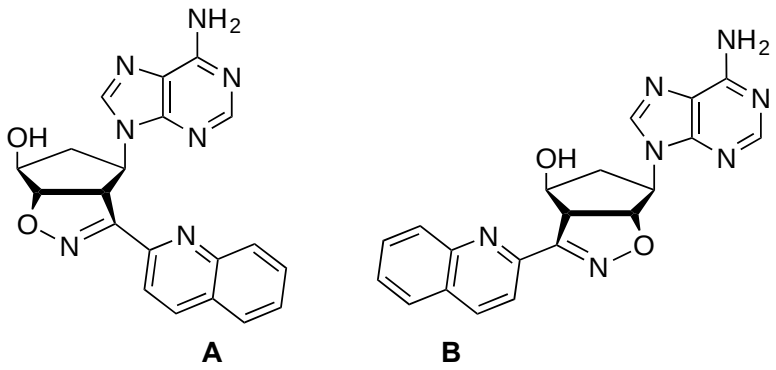
Scheme 23. Bromonitrile oxide strategy to nucleoside analogues.



Scheme 24. Use of bromonitrile oxide in the derivatization of cyclopentene derivatives.

The synthesis hinges on exo-selective 1,3-dipolar cycloaddition of quinolinenitrile oxide to the 2,3-oxazanorborn-5-enes and simple elaboration of the cycloadducts. The nucleoside derivatives were initially tested for their inhibitory activity against a variety of viruses, including HBV, PTV and Flu A virus H1N1. High antiviral activities were found for the compounds shown in Figure 5 in the case of Flu A H1N1.[65]

*Primary antiviral activity of derivatives **A** and **B** against Influenza virus A (FLU-A, H1N1)*



A **B**

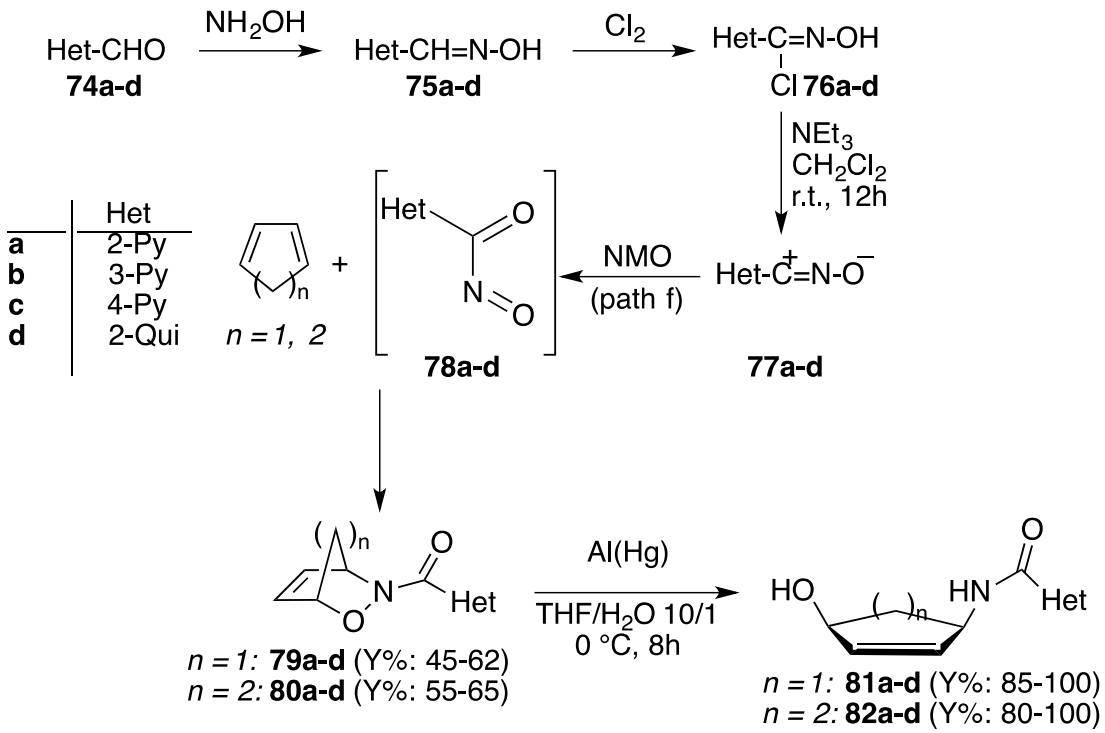
	EC_{50}	EC_{90}	CC_{50}	SI_{50}
A	1.33	>100	>100	>75
B	0.80	>100	68.80	86
Ribavirin	5.85	13.28	>100	>17

Figure 5. Antiviral activities of quinoline-substituted nucleoside analogues.

On pursuing the studies on antiviral compounds and in the search of novel small molecules with potential biological activity, some nucleoside analogues were prepared and found to be moderately active against human herpes and varicella viruses or strongly active against HPV as well as influenza A virus H1N1 by taking advantage of the synthetic protocol relied upon the nitrosocarbonyl chemistry derived from nitrile oxides. The methodology takes advantages of the chemistry of 1,3-dipoles, specifically the nitrile oxides, to generate other highly reactive species of remarkable synthetic value, the nitrosocarbonyl intermediates. In fact, pyridine and quinoline hydroxamoyl chlorides **76a-d** were prepared according to the literature procedures (Scheme 25). The selected aldehydes **74a-d** were converted in high yields into the corresponding oximes **75a-d** following the classical methods and from the latter the desired hydroxamoyl chlorides were obtained upon chlorination with chlorine gas.[66]

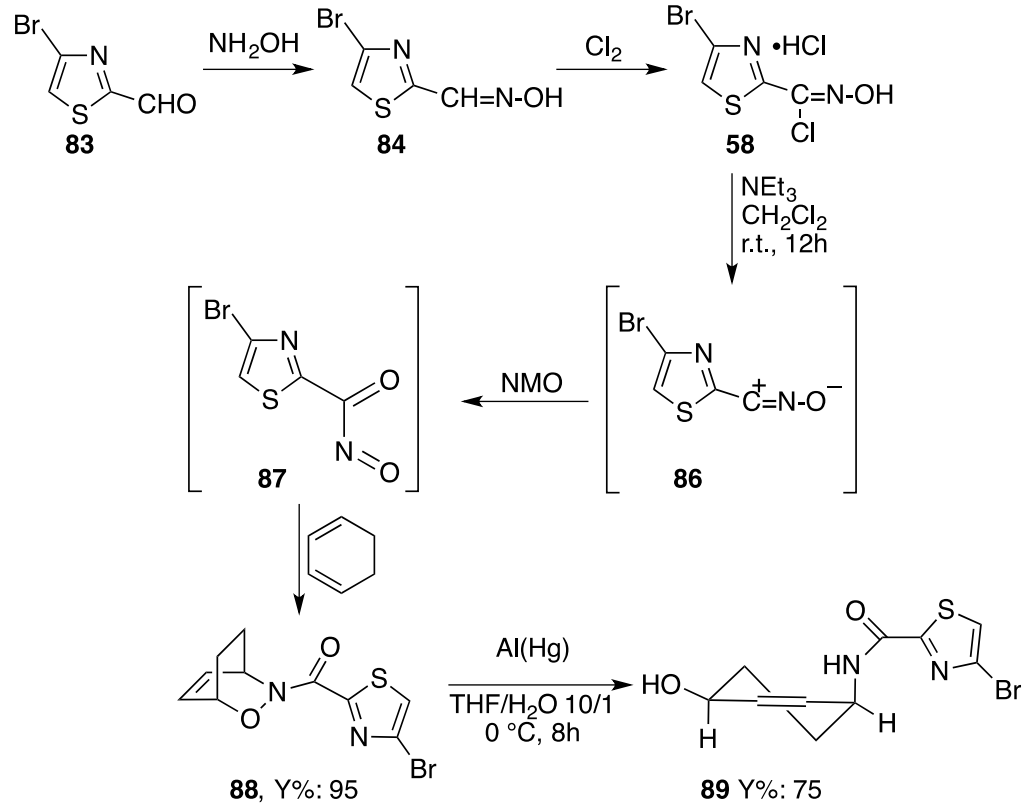
The *in situ* generation of the nitrile oxides **77a-d** is required in the present cases since none of the nitrile oxides at hand displayed any stability at room temperature, possibly in the solid state, for a long time. The mild oxidation of these 1,3-dipoles with NMO is conducted in one pot in the presence of the required trapping dienes (freshly distilled cyclopentadiene or 1,3-cyclohexadiene) to afford the nitrosocarbonyl HDA cycloadducts **79a-d** and **80a-d**. The derivatives **81a-d** and **82a-d**, obtained through N-O bond reductive cleavage, were tested against a variety of viruses but positive results were obtained only in the case of the respiratory influenza A H1N1 virus. The majority of the compounds were found to be inactive, but in a single case (**82d**), the EC_{50} values are just 10 times those of the reference compound.

Once more, the nitrosocarbonyl chemistry, originated from nitrile oxides, represent a valuable synthetic tool to achieve remarkably active targets to contrast HPV. The fleeting heterocyclic nitrosocarbonyl **87** derived from the corresponding nitrile oxide is involved in a short-cut synthesis of 4-bromo-*N*-[(1R*,4S*)-4-hydroxy-2-cyclohexen-1-yl]-2-thiazolecarboxamide **89** (Scheme 26). The synthetic strategy is based on HDA cycloaddition of the *in situ* generated nitrile oxide **86** to 1,3-cyclohexadiene followed by mild reductive cleavage of the N-O bond conducted on the cycloadduct **88**.



a, 2-pyridyl; **b,** 3-pyridyl; **c,** 4-pyridyl; **d,** 2-quinolyn.

Scheme 25. Nitrosoxcarbonyl strategy to nucleoside analogues.

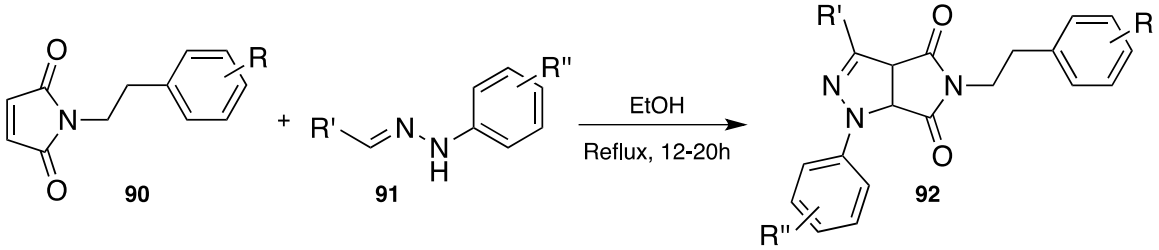


Scheme 26. Bromo-thiazole nucleoside analogue synthesis.

The new 2-thiazolecarboxamide derivative **89** is obtained in good yield and the results of the *in vitro* antiviral tests indicated that the product was active against HPV. These findings demonstrated that the synthetic methodology works well for the preparation of heterocyclic substituted novel compounds that displayed interesting and promising activity against viruses and in particular against the HPV. The results clearly showed that compound **89** was inefficient against Herpes, Hepatitis as well as Influenza viruses. An exception emerged against HPV-11. Compound **89** showed an EC_{50} value <1 along with a CC_{50} value >100 μM. The secondary screening demonstrated that compound **89** was found less effective in raft cultures than in primary assay in HEK 293 cells. The $EC_{50/90}$ values are higher than those of the reference compound U0126, as is the CC_{50} value. The $SI_{50/90}$ values are however close to the reference.[67]

Structurally related to nitrile oxides, nitrile imines share the common status of 1,3-dipoles, *in situ* generated in different ways.[13] Typically, a huge variety of hydrazonoyl halides have been described in the literature and these compounds are traditional precursors of nitrile imines upon dehydrohalogenation in the presence of organic bases. Alternatively, thermolysis or photolysis of tetrazoles offer the method to obtain "free" nitrile imines. Other methods involve oxathiadiazolones, 1,3,4-oxadiazolin-2-ones and sydnones as starting materials for the preparation of these 1,3-dipoles.[68]

Few examples are reported in recent literature; the search for new molecular constructs that resemble the critical two-metal binding pharmacophore and the halo-substituted phenyl functionality required for HIV-1 integrase inhibition represents an extremely interesting area of research within drug discovery. Modified 1-[2-(4-fluorophenyl)ethyl]-pyrrole-2,5-dione scaffolds **193** (Scheme 27) were used to design 35 novel compounds with improved biological activities against HIV-1. The synthetic pathway is represented by the 1,3-dipolar cycloaddition with a hydrazone **194** that, under the experimental condition applied, generates *in situ* the corresponding nitrile imine that adds to the dipolarophile to afford compounds **195**.



Scheme 27. Nitril imine-based antiviral compounds synthesis.

These new compounds show single-digit micromolar antiviral potencies against HIV-1 and low toxicity. Some of them had potent anti-HIV-1 activities (EC_{50} < 5 μM) and excellent therapeutic index (TI, CC_{50}/EC_{50} > 100).

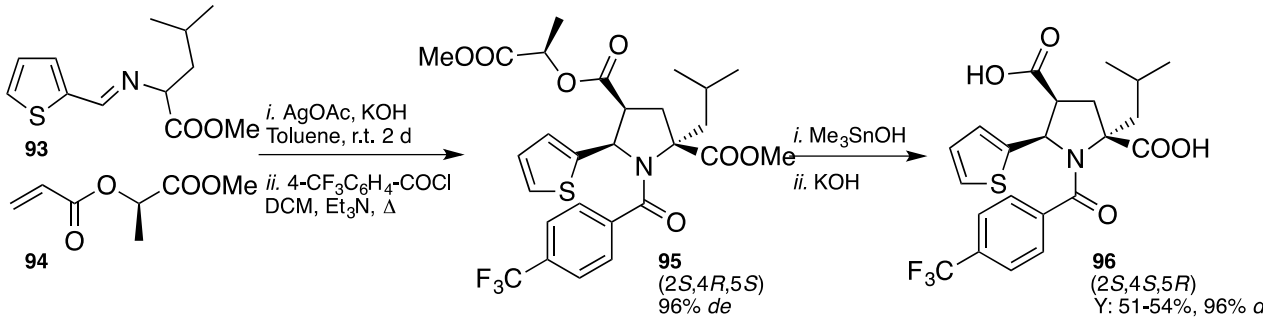
The structural class of agents presented may represent an attractive platform for developing anti-HIV-1 drugs.[69]

2.3 Synthesis of antiviral compounds through azomethine ylides

Azomethine ylides belong to the class of azomethinuim betaines without a double bond in the sextet structure but with internal octet stabilization.[70] A number of methods, including thermolysis or photolysis of readily prepared aziridines, dehydrohalogenation of immonium salts and proton abstraction from imine derivatives of α-amino acids represent a variety of pathway to generate azomethine ylides. Since they are unstable species, they are prepared *in situ* in low concentration and in dried solvents in the presence of suitable dipolarophiles. From the synthetic point of view, azomethine ylides have proven to be extraordinarily rich in their chemistry. The wide range of dipolarophiles able to trap these 1,3-dipoles offers the way to prepare mono- bi- and

tricyclic heterocycles. The main advantages of these reactions are the ready accessibility of reactants, the experimental convenience and high yields, control of stereo- and regiochemistry and minimum of competing side-reactions. Azomethine ylides also found application in the synthesis of molecules with potential antiviral activities.

A diastereoselective approach to thieno-substituted pyrrolidine derivatives has been proposed by Spanish authors, aiming to obtain Hepatitis C virus RNA-dependent RNA Polymerase inhibitors of type **95** and **96**. The strategy relies upon the 1,3-dipolar cycloaddition reaction of an azomethine ylide and lactate esters (Scheme 28). Excellent results were obtained from the synthetic point of view demonstrating the robustness of the protocol.



Scheme 28. Synthesis of Hepatitis C virus RNA-dependent RNA Polymerase inhibitors.

The synthesized compounds are known to have remarkable responses against the Hepatitis C virus.[71] The same authors extensively studied these approaches to biologically active prolines and in particular they developed the enantioselective 1,3-dipolar cycloaddition of azomethine ylides and alkenes in processes catalyzed by chiral phosphoramidite-silver(I) complexes. The monodentate phosphoramidite silver perchlorate complex proved to be a very efficient chiral catalyst for a wide range of 1,3-dipolar cycloaddition reactions between azomethine ylides and various dipolarophiles. This type of monodentate complexes can promote cycloadditions involving sterically hindered components. A valuable application of this methodology is the direct synthesis of the dicarboxylic acid, a very effective agent inhibitor of the HCV polymerase.[72]

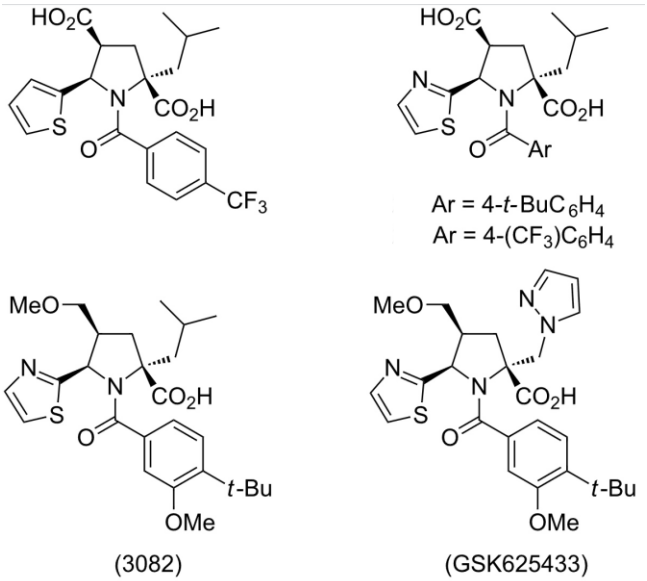
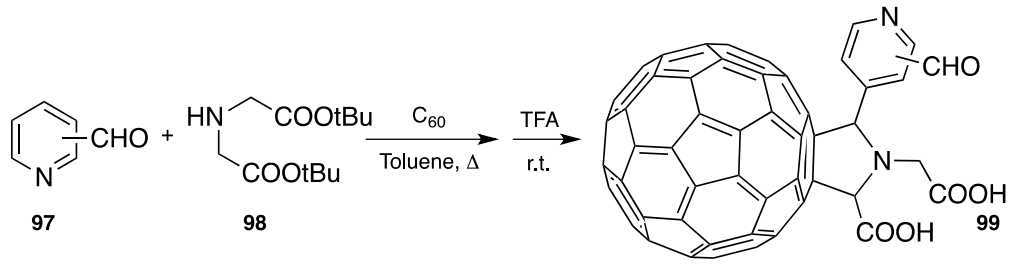


Figure 6. Four GSK inhibitors of HCV.

The synthesis of a GSK 2nd generation inhibitor of HCV (Figure 32) by enantioselective 1,3-dipolar cycloaddition between a leucine derived iminoester and tert-butyl acrylate as proposed by the Spanish research group. The comparison between silver(I) and gold(I) catalysts in this reaction was established by working with chiral phosphoramidites or with the chiral ligand BINAP. The best reaction conditions were used for the total synthesis of the HCV inhibitor by a four-step procedure affording this product in 99% ee and in 63% overall yield. The origin of the enantioselectivity of the chiral gold(I) catalyst was investigated through DFT calculations: the stabilizing coulombic interaction between the nitrogen atom of the thiazole moiety and one of the gold atoms was found to be crucial. In this work the complexity of the 1,3-dipolar cycloaddition reaction of azomethine ylides and acrylates was demonstrated. Many parameters take control of the reaction outcome and a small variation of these can cause a dramatic effect in the overall enantiodiscrimination of the process.[73]

On pursuing these studies, BINAP-$AgSbF_6$-catalyzed 1,3-dipolar cycloadditions between azomethine ylides and electrophilic alkenes were investigated and compared with analogous transformations mediated by other BINAP-silver(I) salt complexes. Maleimides and 1,2-bis(phenylsulfonyl)ethylene were found to be suitable dipolarophiles for obtaining very good enantioselectivities. Even better values in term of enantioselectivity were obtained by a multicomponent version of the reaction. Some very interesting applications of the disulfonylated cycloadducts in the total synthesis of cis-2,5-disubstituted-pyrrolidines, precursors of natural products or valuable intermediates in the synthesis of antiviral compounds, were discovered.[74]

All these research activities and the results obtained in the search of antiviral compounds for the combat of HCV were summarized in two review works. The more advantageous HCV inhibitors incorporating polysubstituted prolines or pyrrolidines were detailed in their synthetic approaches. The improvement of current treatments by combination of antiviral drugs is the driving force aiming to reduce the fast proliferation of this virus. The enhancement of efficiency in short periods of treatment is pivotal from the economical point of view and for the hope of all infected people. New protease or polymerase inhibitors have been recently developed in order to substitute the traditional highly toxic PEG-interferon α-2b/ribavirin tandem. The contribution in this field concerns the elaboration of the GSK's first and second generation polymerase inhibitors through enantioselective processes based on silver(I)- and gold(I)-catalyzed 1,3-dipolar cycloadditions of azomethine ylides.[75,76]



Scheme 29. Synthesis of pyridine/pyridinium-type fullerene derivatives.

Azomethile ylides were employed in the synthesis of a novel set of pyridine/pyridinium-fullerene derivatives. The products were assessed for human immuno deficiency virus-reverse transcriptase inhibition activities. Pyridine aldehydes **97** were allowed to react with suitable secondary amine derivative **98** in the presence of fullerene and, after deprotection of the ester groups, the desired product **99** was obtained (Scheme 29). It showed potent human immunodeficiency virus-reverse transcriptase inhibition without

cytotoxicity. The CC_{50} value of compound **99** is CC_{50} = 39.4 μM. These results suggest that the novel fullerene derivatives are capable of inhibiting HIV-RT without injuring living cells.[77]

A hepatitis C virus RNA polymerase inhibitor (GSK3082) and a series of analogues with structural diversity at the 5-position were prepared from a 2,2,4,5-tetrasubstituted pyrrolidine obtained with a well-defined stereochemistry from the 1,3-dipolar cycloaddition of the chiral imino ester derived from leucine tert-butyl ester and (*R*)-2,3-O-isopropylidene-glyceraldehyde with methyl acrylate (Figure 7).

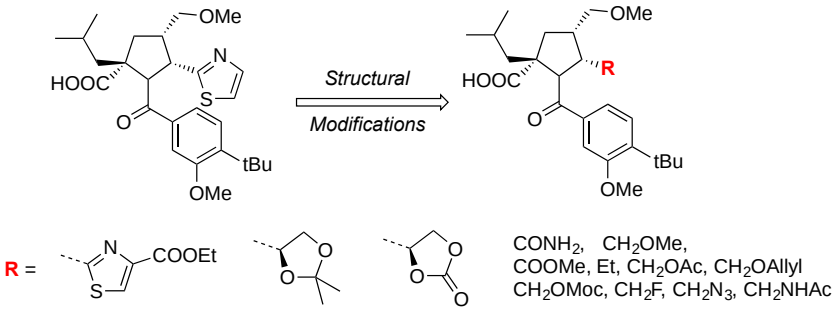


Figure 7. Structure of GSK3082 and a series of analogues.

Angularly and peri-fused tricyclic pyrrolidino pyrazolines were efficiently prepared by LiCl-catalyzed domino aza-Michael addition-1,3-dipolar cycloaddition reactions (Figure 8). The absolute stereochemistry is controlled in the aza-Michael addition step, nonaflyl azide serves as effective diazo transfer reagent to the formed enolate and the resulting diazo dipole engages in the 1,3-dipolar cycloaddition step. The resulting tricyclic pyrrolidino pyrazolines can be easily transformed to enantiomerically enriched non proteinogenic spirocyclic α,β,γ-triamino acids, angularly or peri-fused tricyclic β-prolines or pyrimidines. The activity of the tricyclic amino acid derivatives against the hepatitis C virus was determined.[78]

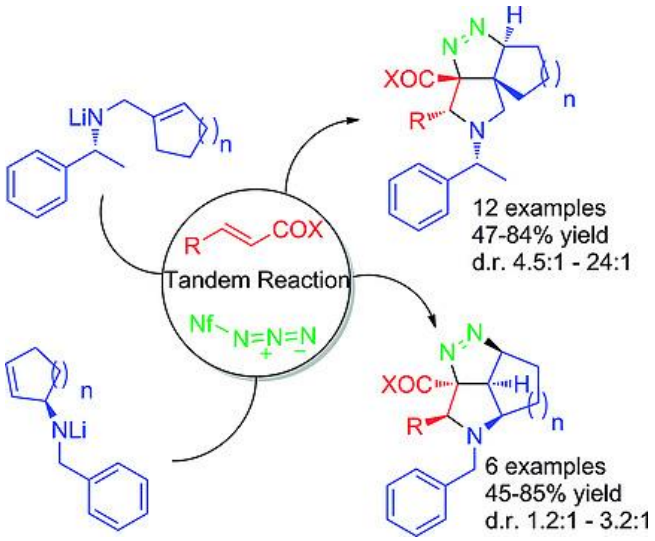


Figure 8. Synthetic strategy for angularly and peri-fused tricyclic pyrrolidino pyrazolines. (Reproduced with permission.[78] Copyright 2018, Wiley).

Remarkably, cyclohexane-annulated, but methyl-substituted tetrahydropyrimidine and both bicyclo[4.1.0]heptane-fused β-proline derivatives were active despite the fact, that they contain neither an

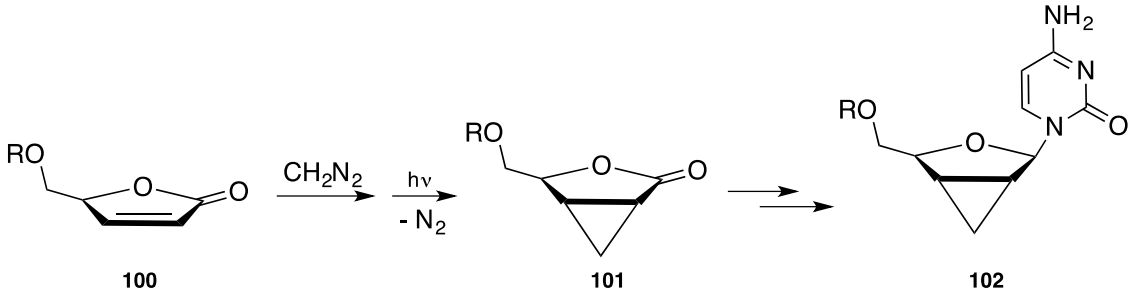
aryl substituent at C-3 nor a five membered carbocyclic ring. The compounds were not cytotoxic (CC_{50} >50 μM).

The chiral 2,2-dimethyl-1,3-dioxolane moiety provided by the glyceraldehyde was used as a synthetic equivalent for different substituents and functional groups and these transformations usually required mild reaction conditions and valuably simplified work-up procedures. The inhibitory activity of the resulting GSK3082 analogues was studied *in vitro* in a cell-based assay of the subgenomic HCV RNA replication system. Some of the prepared analogues showed good inhibitory activities with IC_{50} values in the nanomolar concentration range.[79]

2.4 Synthesis of antiviral compounds through diazoalkanes

The chemistry of aliphatic diazo compounds dates back more than 100 years and apart from the significance of diazoalkanes for the generation of carbenes, these compounds play a remarkable role in cycloaddition chemistry. Diazo group transfer or electrophilic diazoalkane substitutions are the methods that can be used in the synthesis of these reactive species leading to new applications in cycloaddition chemistry.[80]

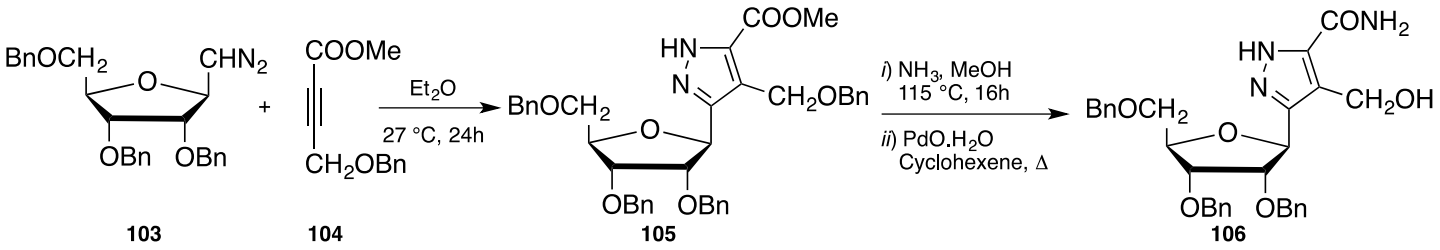
A six-step synthesis of 2',3'-dideoxy-2',3'-α-methanocytidine **102** from (5*S*)-benzoyloxymethyl-(5*H*)-furan-2-one has its key step in the stereoselective formation of (1*R*,4*S*,5*S*)-4-benzoyloxymethyl-3-oxabicyclo[3.1.0]hexan-2-one **101** via 1,3-dipolar cycloaddition of diazomethane to **100** followed by photoinduced elimination of nitrogen. Reduction to the corresponding lactol followed by acetylation yielded the desired intermediate that reacts with 2,4-bis(trimethylsilyl)cytosine and $EtAlCl_2$, followed by deprotection and chromatography giving **102** (Scheme 30), which exhibited only weak activity against the human immunodeficiency virus (HIV).[81]



Scheme 30. Synthesis of nucleoside **102** through diazomethane strategy.

The synthesis of 4-hydroxymethyl-3(5)-(β-D-rifuranosyl) pyrazole-5(3)-carboxamide **106** starts with the 1,3-dipolar cycloaddition reaction of the diazoalkane **103** and methyl 4-benzyloxy-2-butynoate (**104**, Scheme 31).

The cycloadduct **105** was then transformed into the target compound **106** through selective deprotection and conversion into the amide derivative. An analogous procedure was applied for the preparation of the truncated acyclic side chain of an acyclovir-like derivative. Both synthesized compounds did not show any *in vitro* antiviral activity against human immunodeficiency virus (HIV-1), sandfly fever, Punta Toro, Japanese encephalitis, yellow fever, Venezuelan equine encephalomyelitis, and vaccinia viruses. Both compounds were also non-toxic.[82]



Scheme 31. Synthesis of 4-hydroxymethyl-3(5)-(β-D-ribofuranosyl)pyrazole-5(3)-carboxamide **106**.

The 1,3-dipolar cycloaddition between diazomethane and Psilostachyin (PSH) was recently investigated through molecular electronic density theory (MEDT) in order to elucidate the chemo-, regio- and stereoselectivity. Reactivity indices, transition structures theory, IGM and ELF analysis were employed to reveal the mechanism of the reaction.

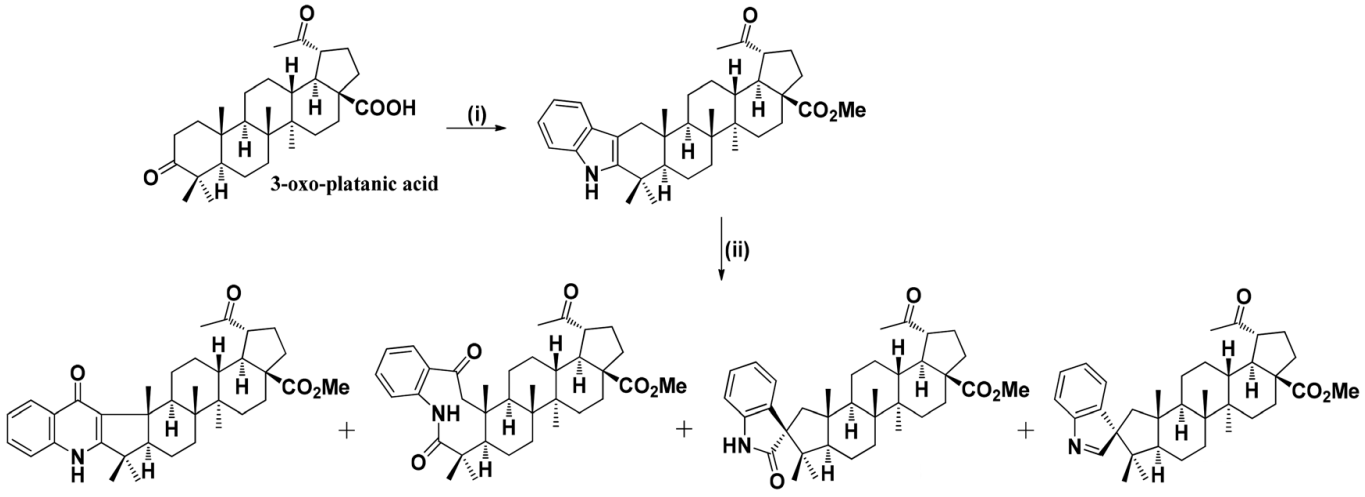
The addition of DZM to PSH takes place through a one-step mechanism and an asynchronous transition state. The addition of ether does not affect the chemo-, regio- and stereo-selectivity of the reaction. The obtained products were investigated for their potential inhibition against COVID-19-Mpro by performing molecular docking calculations.[83]

Recent literature does not report any other significative example of diazoalkane-synthetic approach to antivirals.

2.5 Synthesis of antiviral compounds through miscellaneous 1,3-dipoles

In this paragraph we summarize the synthesis of antiviral compounds conducted through different 1,3-dipoles in a very limited number of examples.

Nitrogen-containing heterocyclic triterpenoids have been prepared by the reaction of 2,3-indolotriterpenoids with ozone (O_3) and dimethyldioxirane. The oxidation of indolo-fused 28-oxo-allobetulinor methyl platanoate with O_3 led to a mixture of a quinolone as the major products and a nine-membered 2,3-seco-2-oxolactam and three different types of spiroindoles as byproducts (Scheme 32).



Scheme 32. Oxidation of 2,3-indoloplatanoate with O_3. (i) Indole ring construction via Fischer methodology. (ii) O_3, DCM.

The formation of quinolone and 2,3-seco-2-oxolactam derivatives could be explained by the standard 1,3-dipolar cycloaddition of O_3 to the C2(3)-double bond of the triterpene core similar to the products observed in the ozonolysis of indoles in the Witkop-Winterfeldt oxidation. The formation of spiroindoles was quite unexpected and could be explained through the 1,2-cycloaddition of O_3 to the C2(3)-double bond followed by intramolecular rearrangement of the 2,3-epoxy-intermediate.

The formation of only two isomeric triterpene spiroindolinones was achieved by the oxidation of 2,3-indolo-28-oxo-allobetulin with dimethyldioxirane, explained through the rearrangement of the 2,3-epoxy-intermediate. The 19β,28-epoxy-18α-olean-28-oxo-2-nor-2,3-4'(1*H*)-quinolone was found to be the most active compound against HPV-11 with EC_{50} 0.45 μM and SI_{50} 322 in a primary assay and SI_{90} < 10 against HPV-16 in a secondary assay.[84]

3. Conclusions

The ability of the 1,3-dipolar cycloaddition reactions to produce heterocycles extends its importance to many areas of the organic synthesis and the synthesis of antiviral compounds represents a topic in constant growing. The use of 1,3-dipole offers a unique way for the preparation of a variety of compounds with potential biological activity. It must be taken into account that heteroatom-containing cycloadducts may be transformed in other functionalized molecules cyclic or acyclic. Furthermore, the ability of 1,3-dipole to generate rings even containing stereocentres in one synthetic operation is a pivotal step when a valuable compound has to be prepared.

We have presented an updated review on the applications of 1,3-dipoles in the synthesis of antiviral compounds[85] having from low to relevant biological activities. With respect to the 1,3-dipoles structures described by Huisgen,[86,87] only a few of them were effectively employed in the synthesis reported in the last 30 years (Figure 9). In the first part of the work, we dealt with the azide chemistry.

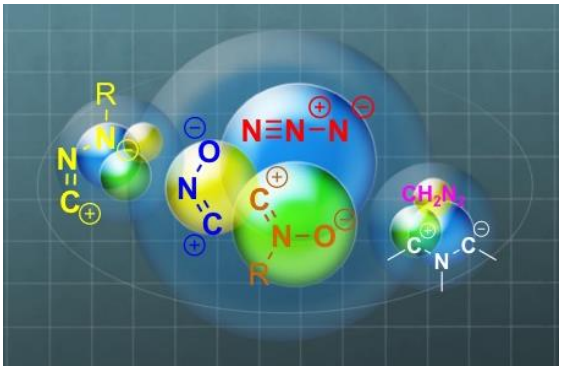


Figure 9. Structures of most relevant 1,3-dipoles used in antiviral compounds syntheses.

We completed this second part presenting the applications of nitrones; we wish to complete the report citing a couple of reviews that complete the overview on the use of these 1,3-dipoles to the synthesis of nucleoside analogues. The first one deals with the synthesis of antiviral compounds from non-carbohydrate precursors[88] while the second specifically applies the chemistry of sugars towards modified nucleosides.[89]

Nitrones behave differently with respect to azides[1] since their chemistry represents the main route towards isoxazolidines that are valuable heterocyclic scaffolds for the synthesis of *N,O*-nucleosides. The easy

way by which nitrones can be prepared offers a wide variety of solutions for the construction of nucleosides and analogues through variable synthetic strategies. In most cases the results are excellent both from the synthetic and the biological points of view. Here the role of linkers is completely run out since nitrone are unfit in this extent.

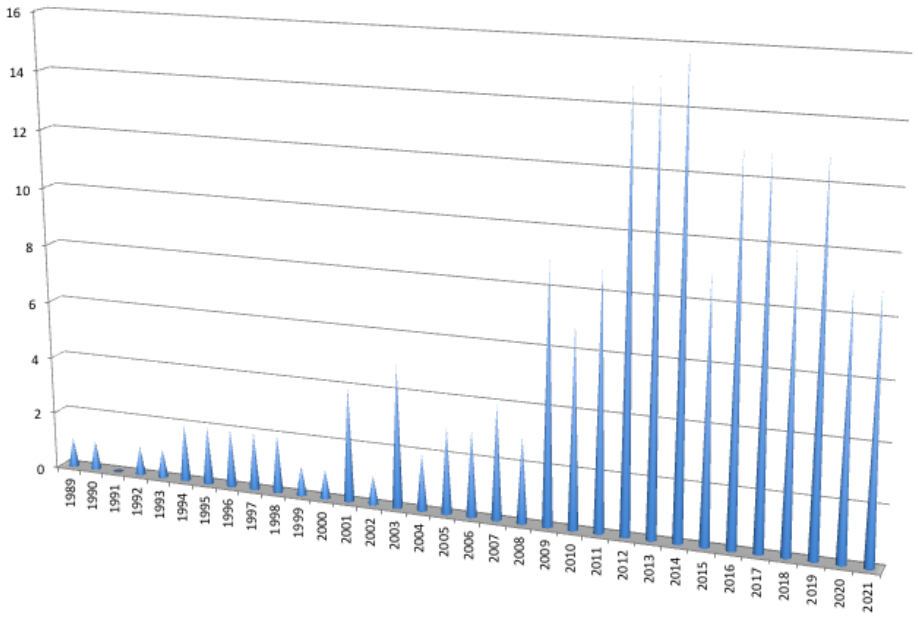


Figure 10. Distribution of 188 papers published from 1989 up to December 2021.

On the contrary, in few cases nitrile oxides seem to overlap the pathway of azides as linkers in antiviral compounds syntheses but their use as 1,3-dipoles for the construction of isoxazole (33rd in the Orange Book frequency list)[90] or isoxazoline rings remain the best examples of application of this type of chemistry. The value of these dipoles was also augmented by their mild oxidation to nitrosocarbonyl intermediates[58] that served as valuable tools for the stereoselective introduction of OH and NH_2 functionalities, used in the linear construction of nucleosides and other analogues as well as for the preparation of synthons via HDA cycloadditions suitable to be elaborated synthetically in biological active molecules.

Nitrilimines, azomethine ylides and diazoalkanes played a secondary role with some interesting examples that can be offered by the azomethine ylides because of their pivotal role in pyrrolidine construction; indeed, these heterocycles are on the 8th rank in the Orange Book, denoting their undoubted value in biomolecules.
This is the picture in the use of 1,3-dipolar cycloaddition reactions in the synthesis of antiviral compounds. Figure 10 shows the year-by-year distribution of 188 papers published from 1989 through December 2021.
There can be clearly noticed an increasing interest in 1,3-dipoles as valuable synthetic instruments for the preparation of antivirals from 2009 until today.

4. Perspectives and General Comments

Some comments can be now considered in conclusion of these works (Ref. 1 and present paper) dealing with the use of 1,3-dipolar cycloaddition reaction in the synthesis of antiviral compounds.

Infectious disease experts for years warned that a pathogen would one day bring the world to its knees. In 2020, their nightmare scenario became reality. Nowadays the COVID19 pandemic highlighted in stark relief, beyond the containment measures, the importance of the pharmaceutical industry. Big-Pharma concentrated their efforts on vaccines and antibodies as drugs for COVID19, incomprehensibly overlooking antiviral chemotherapeutics for the moment. It seems evident that to return to anything resembling normal, the world would need diagnostics, drugs, and vaccines. Although vaccines have already been developed and antibody-based treatments may well be available before a SARS-CoV2-specific antiviral molecule is approved, the work on viral lifecycle inhibitors is still worth pursuing. Vaccines may not be 100% effective in preventing infection; so anti-COVID19 drugs will be needed in next years. Moreover, SARS-CoV2 is the third coronavirus to afflict us since the beginning of the century, and there is no reason to think we will not see more coronavirus pandemics. Given the close conservation of coronavirus replication complex, success in identifying specific inhibitors could offer a strategy broadly active against such others threats. Targeting viral protease offers a robust strategy for the development of effective treatments for viral infections. New molecules identification would provide promising starting points for further therapeutically approach developments.

Thus, many drug developers dropped everything else to turn their attention to tackling the virus. The public suddenly became aware of complexity of drug manufacturing, fallibility of clinical trial design, and tricky nature of immunity. The problems related to availability and supply of medicinal treatments in different countries emerged as an unexpected limit in our global world. In this context, the creativity of the use of 1,3-dipolar cycloaddition reactions lies in the formulation of alternative synthetic routes and processes for the production of important antiviral drugs. These approaches hinge heavily on the combination of synthetic transformations and biological tests along with modeling studies.

The robustness and reliability of the synthetic approaches is a pivotal point and pillar of the cycloaddition strategy. With the COVID19 crisis, the realization of effective treatments for patients has yet to be developed. One of the main obstacles for developing new beneficial tools for human health is process complexity and length from conception, design and synthesis of new molecules for clinical practice. The use of reliable synthetic tools aspires to minimize the translational gap between design-synthesis of putative drugs and clinical testing results. Strongly targeted anti-SARS-CoV2 therapy and possible exclusion of toxic or off-target adverse effects, have been taken into deep consideration in all the phases of the research activities, every research activity must be as much translational as possible and as much attractive as possible for pharmaceutical industry.

Treatment of global disease outbreak is a public health heavy problem facing the world. The current potential pandemic underscores the great risk to public health and global economy. Planning and answering proportionately to such pandemics, public health officials need a systematic assessment of the socio-economic and health impact of the disease, interventions, and other mitigation efforts. Policy makers need to understand the intervention possibilities for limiting pandemic risk, assisting vulnerable populations. These interventions may include social distancing, prioritized governmental distribution of vaccines and antiviral medications, and pharmaceutical consumption in the private sector.[91] The aim of several studies on this ground is to evaluate the economic and social impact of typical interventions proposed by public health officials and preventive behavioral changes adopted by private citizens in the event of a flu-like epidemic. The methods applied are individual-based simulation model in specific geographical areas for addressing this critical problem. The economic costs include loss in productivity due to sickness and indirect cost incurred through disease avoidance and caring for dependents. The results show that an important factor responsible for preventing income loss is the modification of individual behavior. The conclusion is that the preventive behavior of the private citizens is a relevant factor in controlling the epidemic. Besides social distancing,

antiviral prophylactic or very-early-therapeutic use could be more convenient and less intrusive to the personal lifestyle.

Dissemination strategies must be addressed to 4 targets.

1. General Public, addressed with layman's terms to deliver the message: chemistry at health service, conveying the potential of the approach pursued in this project, the advantages of green chemistry and the potential of producing vital therapeutics.
2. Industrial Leaders with potential interest in driving innovation and economic exploitation of the project results that will be relayed by personal contacts, workshop participation and industrial associations.
3. Scientific Experts targeted through scientific communication, publications, and conference presentations.
4. Specific Scientific Publication on international journals, patents or reports will obviously complete the dissemination strategy.

Additionally, collaborations with institutions that may aid in the overall success of the proposed research will be sought and encouraged. A community research partnership is ideally part of a larger collaboration, including the interests of each partner and spans a wide range of activities focused on the development of new approaches to antiviral compounds. Disclosure of key findings during project execution and its completion is a crucial step in community-based research to further advancements in the described research field.

A distinguished chemist mentioned the Joys of Molecules[92] *and we are conscious that the first important predicted result will be the common joy to share new molecules synthesis and explore their activity. We expect that 1,3-dipolar cycloaddition reactions will produce new knowledge in the adopted procedures and acquisitions in biological investigations, offering the opportunity to communicate the Joys of Molecules to young people who, hopefully, will be engaged into this topic. Building of small libraries of compounds with a synthetic accessibility through various methods and cycloadditions in particular, employing many different starting building blocks and leading to a great variety of functionalities, are all valuable elements in a research project. Close to these, there is the willingness to join efforts for pursuing a synthetic work in service to the biological knowledge. This is cooperation, scientific coordination, straightforward targeting; we expect significant applications and improved knowledge in contrast of the pandemic emergency.*

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References

1. Faita, G.; Leusciatti, M.; Quadrelli, P. *ARKIVOC*, **2022**, *viii*, 19. https://doi.org/10.24820/ark.5550190.p011.794
2. Hamer, J.; Macaluso, A. *Chem. Rev.* **1964**, *64*, 473.
3. Tufariello, J. J. *Nitrones* in *1,3-Dipolar Cycloaddition Chemistry*, Vol. 2 By Padwa, Albert; Ed., 1984, 83.

4. Torssell, K. G. B. *Nitrile Oxides, Nitrones and Nitronates in Organic Synthesis: Novel Strategies in Synthesis;* VCH Publishers: New York, 1988.
5. Martin, J. N.; Jones, R. C. F. *Nitrones* in *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products* Padwa, A.; Pearson, W. H. Ed.; Wiley, J. and Sons, Inc.: Hoboken (NJ), 2003, p 1.
6. Cardona, F.; Goti, A. *Angew. Chem. Int. Ed.* **2005**, *44*, 7832. https://doi.org/10.1002/anie.200502640
7. Brandi, A.; Cardona, F.; Cicchi, S.; Cordero, F. M.; Goti, A. *Chem. Eur. J.* **2009**, *15*, 7808. https://doi.org/10.1002/chem.200900707
8. Xu, X.; Doyle, M. P. *Acc. Chem. Res.* **2014**, *47*, 1396. https://doi.org/10.1021/ar5000055
9. Gothelf, K. V.; Jorgensen, K. A. *Chem. Rev* **1998**, *98*, 863. https://doi.org/10.1021/cr970324e
10. Brandi, A.; Cardona, F.; Cicchi, S.; Cordero, F. M.; Goti, A. *Org. React.* **2017**, *94*, 1.
11. Murahashi, S.-I.; Imada, Y. *Chem. Rev.* **2019**, *119*, 4684. https://doi.org/10.1021/acs.chemrev.8b00476
12. Grundmann, G. Grünanger, P. *The Nitrile Oxides* Springer-Verlag: Heidelberg, 1971.
13. Caramella, P.; Grünanger, P. *Nitrile Oxides and Imines – 1,3-Dipolar Cycloaddition Chemistry* Wiley: New York, Padwa, A. Ed., 1984; Vol. 1, pp 291.
14. Jäger, V.; Colinas, P. A. *Nitrile Oxides* in *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products* Padwa, A.; Pearson, W. H. Ed.; Wiley: Hoboken (NJ): 2003, p 361.
15. Sharp, J. T. *Nitrile Ylides and Imines* in *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products* Padwa, A.; Pearson, W. H. Ed.; Wiley: Hoboken (NJ): 2003, p 473.
16. Leggio, A.; Liguori, A.; Procopio, A.; Siciliano, C.; Sindona, G. *Tetrahedron Lett*. **1996**, *37*, 1277. https://doi.org/10.1016/0040-4039(95)02373-9
17. Giglio, G.; Napoli, A.; Leggio, A.; Liguori, A.; Procopio, A.; Siciliano, C.; Sindona, G. *Synth. Commun.* **1996**, *26*, 4211. https://doi.org/10.1080/00397919608004659
18. Leggio, A.; Liguori, A.; Maiuolo, L.; Napoli, A.; Procopio, A.; Siciliano, C.; Sindona, G. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3097.
19. Colacino, E.; Converso, A.; Liguori, A.; Napoli, A.; Siciliano, C.; Sindona, G. *Tetrahedron* **2001**, *57*, 8551.
20. Colacino, E.; De Luca, G.; Liguori, A.; Napoli, A.; Siciliano, C.; Sindona, G. *Nucleosides, Nucleotides and Nucleic Acids* **2003**, *22*, 743. https://doi.org/10.1081/NCN-120022624
21. Chiacchio, U.; Corsaro, A.; Iannazzo, D.; Piperno, A.; Pistarà, V.; Rescifina, A.; Romeo, R.; Valveri, V.; Mastino, A.; Romeo, G. *J. Med. Chem*. **2003**, *46*, 3696. https://doi.org/10.1021/jm0308186
22. Chiacchio, U.; Genovese, F.; Iannazzo, D.; Piperno, A.; Quadrelli, P.; Corsaro, A.; Romeo, R.; Valveri, V.; Mastino, A. *Bioorg. Med. Chem*. **2004**, *12*, 3903. https://doi.org/10.1016/j.bmc.2004.04.041
23. Chiacchio, U.; Balestrieri, E.; Macchi, B.; Iannazzo, D.; Piperno, A.; Rescifina, A.; Romeo, R.; Saglimbeni, M.; Sciortino, M. T.; Valveri, V.; Mastino, A.; Romeo, G. *J. Med. Chem*. **2005**, *48*, 1389.

https://doi.org/10.1021/jm049399i
24. Procopio, A.; Alcaro, S.; De Nino, A.; Maiuolo, L.; Ortuso, F.; Sindona, G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 545. https://doi.org/10.1016/j.bmcl.2004.11.048
25. Rescifina, A.; Chiacchio, M. A.; Corsaro, A.; De Clercq, E.; Iannazzo, D.; Piperno, A.; Romeo, G.; Romeo, R.; Valveri, V. *J. Med. Chem*. **2006**, *49*, 709. https://doi.org/10.1021/jm050772b
26. Chiacchio, U.; Iannazzo, D.; Piperno, A.; Romeo, R.; Romeo, G.; Rescifina, A.; Saglimbeni, M. *Bioorg. Med. Chem*. **2006**, *14*, 955. https://doi.org/10.1016/j.bmc.2005.09.024
27. Piperno, A.; Giofré, S. V.; Iannazzo, D.; Romeo, R.; Chiacchio, U.; Rescifina, A.; Piotrowska, D. G. *J. Org. Chem*. **2010**, *75*, 2798. https://doi.org/10.1021/jo902485m
28. Romeo, R.; Carnovale, C.; Giofré, S. V.; Romeo, G.; Macchi, B.; Frezza, C.; Marino-Merlo, F.; Pistarà, V.; Chiacchio, U. *Bioorg. Med. Chem*. **2012**, *20*, 3652. https://doi.org/10.1016/j.bmc.2012.03.047
29. Giofré, S. V.; Romeo, R.; Garozzo, A.; Cicero, N.; Campisi, A.; Lanza, G.; Chiacchio, M. A. *ARKIVOC* **2015**, (*vii*), 253. https://doi.org/10.3998/ark.5550190.p009.353
30. Giofré, S. V.; Romeo, R.; Chiacchio, U.; Romeo, G.; Chiacchio, M. A. *Mini-Rev. Org. Chem*. **2015**, *12*, 249.
31. Chiacchio, M. A.; Giofré, S. V.; Romeo, R.; Romeo, G.; Chiacchio, U. *Curr. Org. Synth*. **2016**, *13*, 726. https://doi.org/10.2174/1570179412666150914195807
32. Kokosza, K.; Balzarini, J.; Piotrowska, D. G. *Nucleosides, Nucleotides and Nucleic Acids* **2014**, *33*, 552. https://doi.org/10.1080/15257770.2014.909046
33. Singh, S.; Chopra, A.; Singh, G.; Saxena, A. K.; Ishar, M. P. S. *J. Pharm. Res.* **2013**, *7*, 337. https://doi.org/10.1016/j.jopr.2013.04.018
34. Piotrowska, D. G.; Andrei, G.; Schols, D.; Snoeck, R.; Grabkowska-Druzyc, M. *Molecules* **2016**, *21*, 959. https://doi.org/10.3390/molecules21070959
35. Piotrowska, D. G.; Andrei, G.; Schols, D.; Snoeck, R.; Łysakowska, M. *Eur. J. Med. Chem.* **2017**, *126*, 84. https://doi.org/10.1016/j.ejmech.2016.10.002
36. Grabkowska-Druzyc, M.; Andrei, G.; Schols, D.; Snoeck, R.; Piotrowska, D. G. *Molecules* **2018**, *23*, 1889. https://doi.org/10.3390/molecules23081889
37. Piotrowska, D. G.; Wroblewski, A. E.; Balzarini, J.; Andrei, G.; Schols, D.; Snoeck, R.; Felczak, A.; Wronska, N.; Lisowska, K.; Glowacka, I. E. *Acta Pol. Pharm.-Drug Res.* **2017**, *74*, 1091.
38. Kokosza, K.; Andrei, G.; Schols, D.; Snoeck, R.; Piotrowska, D. G. *Bioorg. Med. Chem.* **2015**, *23*, 3135. https://doi.org/10.1016/j.bmc.2015.04.079
39. Piotrowska, D. G.; Balzarini, J.; Andrei, G.; Schols, D.; Snoeck, R.; Wroblewski, A. E.; Gotkowska, J. *Tetrahedron* **2016**, *72*, 8294. https://doi.org/10.1016/j.tet.2016.10.073
40. Sirotkina, V.; Efremova, M. M.; Novikov, A. S.; Zarubaev, V. V.; Orshanskaya, I. R.; Starova, G. L.; Kostikov, R. R.; Molchanov, A. P. *Tetrahedron* **2017**, *73*, 3025. https://doi.org/10.1016/j.tet.2017.04.014
41. Efremova, M. M.; Molchanov, A. P.; Novikov, A. S.; Starova, G. L.; Muryleva, A. A.; Slita, A. V.; Zarubaev, V. V. *Tetrahedron* **2020**, *76*, 131104.

https://doi.org/10.1016/j.tet.2020.131104
42. Molchanov, A. P.; Lukina, V. M.; Efremova, M. M.; Muryleva, A. A.; Slita, A. V.; Zarubaev, V. V. *Synth. Commun.* **2020**, *50*, 1367. https://doi.org/10.1080/00397911.2020.1738494
43. Chimirri, A.; Grasso, S.; Monforte, A. M.; Monforte, P.; Zappalà, M.; Carotti, A. *IL FARMACO* **1994**, *49*, 509.
44. Luyten, I.; Jie, L.; Van Aerschot, A.; Pannecouque, C.; Wigerinck, P.; Rozenski, J.; Hendrix, C.; Wang, C.; Wiebe, L.; Balzarini, J.; De Clercq, E.; Herdewijn, P. *Antiviral Chemistry & Chemotherapy* **1995**, *6*, 262. https://doi.org/10.1177/095632029500600409
45. Gi, H.-J.; Xiang, Y.; Schinazi, R. F.; Zhao, K. *J. Org. Chem*. **1997**, *62*, 88.
46. Pan, S.; Wang, G.; Schinazi, R. F.; Zhao, K. *Tetrahedron Lett*. **1998**, *39*, 8191.
47. Romeo, G.; Iannazzo, D.; Piperno, A.; Romeo, R.; Saglimbeni, M.; Chiacchio, M. A.; Balestrieri, E.; Macchi, B.; Mastino, A. *Bioorg. Med. Chem*. **2006**, *14*, 3818. https://doi.org/10.1016/j.bmc.2006.01.028
48. Zhang, P.-Z.; Li, X.-L.; Chen, H.; Li, Y.-L.; Wang, R. *Tetrahedron Lett*. **2007**, *48*, 7813. https://doi.org/10.1016/j.tetlet.2007.09.007
49. Zeng, L.-F.; Zhang, H.-S.; Wang, Y.-H.; Sanchez, T.; Zheng, Y.-T.; Neamati, N.; Long, Y.-Q. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4521. https://doi.org/10.1016/j.bmcl.2008.07.047
50. Yermolina, M. V.; Wang, J.; Caffrey, M.; Rong, L. L.; Wardrop, D. J. *J. Med. Chem.* **2011**, *54*, 765. https://doi.org/10.1021/jm1008715
51. Zhang, P.; Wei, C.; Wang, E.; Wanga, W.; Liu, M.; Yin, Q.; Chen, H.; Wang, K.; Li, X.; Zhang, J. *Carbohydrate Res.* **2012**, *351*, 7. https://doi.org/10.1016/j.carres.2011.11.025
52. Das, P.; Hasan, M. H.; Mitra, D.; Bollavarapu, R.; Valente, E. J.; Tandon, R.; Raucher, R.; Hamme, A. T. *J. Org. Chem.* **2019**, *84*, 6992. https://doi.org/10.1021/acs.joc.9b00746
53. Das, P.; Boone, S.; Mitra, D.; Turner, L.; Tandon, R.; Raucher, D.; Hamme, A. T. *RSC Adv.* **2020**, *10*, 30223. https://doi.org/10.1039/d0ra06148d
54. Bartolotta, R.; La Rosa, C.; Nava, D. *Synthesis* **2020**, *52*, 933. https://doi.org/10.1055/s-0039-1690039
55. Mineno, T.; Miller, M. J. *J. Org. Chem.* **2003**, *68*, 6591. https://doi.org/10.1021/jo034316b
56. Belmonte, L.; Barré, P.; de Bracco, M. M. E.; Ruibal-Ares, B. H. *Curr. Med. Chem*. **2003**, *10*, 303. https://doi.org/10.2174/0929867033368358
57. Quadrelli, P.; Scrocchi, R.; Caramella, P.; Rescifina, A.; Piperno, A. *Tetrahedron* **2004**, *60*, 3643. https://doi.org/10.1016/j.tet.2004.02.057
58. For a review on nitrosocarbonyl intermediates see: Memeo, M. G.; Quadrelli, P. *Chem. Rev.* **2017**, *117,* 2108. https://doi.org/10.1021/acs.chemrev.6b00684
59. Quadrelli, P.; Martinez Vazquez, N.; Scrocchi, R.; Corsaro, A.; Pistarà, V. *The Scientific World J.* **2014**, Article ID 492178, 12 pages. https://doi.org/10.1155/2014/492178
60. Moggio, Y.; Legnani, L.; Bovio, B.; Memeo, M. G.; Quadrelli, P. *Tetrahedron* **2012**, *68*, 1384.

https://doi.org/10.1016/j.tet.2011.12.047
61. Johnson, J. A.; Gangemi, J. D. *Antimicrob. Agents Chemother*. **1999**, *43*, 1198. https://doi.org/10.1128/AAC.43.5.1198
62. Memeo, M. G.; Lapolla, F.; Maga, G.; Quadrelli, P. *Tetrahedron Lett.* **2015**, *56*, 1986. https://doi.org/10.1016/j.tetlet.2015.02.114
63. Savion, M.; Memeo, M. G.; Bovio, B.; Grazioso, G.; Legnani, L.; Quadrelli, P. *Tetrahedron* **2012**, *68*, 1845. https://doi.org/10.1016/j.tet.2011.12.086
64. Scagnelli, L.; Memeo, M. G.; Carosso, S.; Bovio, B.; Quadrelli, P. *Eur. J. Org. Chem.* **2013**, 3835. https://doi.org/10.1002/ejoc.201300202.
65. Quadrelli, P.; Mella, M.; Legnani, L.; Al-Saad, D. *Eur. J. Org. Chem.* **2013**, 4655. https://doi.org/10.1002/ejoc.201300119
66. Al-Saad, D.; Memeo, M. G.; Quadrelli, P. *The Scientific World J.* **2014**, Article ID 472373, 10 pages. https://doi.org/10.1155/2014/472373
67. Al-Saad, D.; Memeo, M. G.; Quadrelli, P. *Lett. Org. Chem.* **2016**, *13*, 757.
68. Deepthi, A.; Acharjee, N.; Sruthi, S. L.; Meenakshy, C. B. *Tetrahedron* **2022**, *116*, 132812. https://doi.org/10.1016/j.tet.2022.132812
69. Liu, G.-N.; Luo, R.-H.; Zhou, Y.; Zhang, X.-J.; Li, J.; Yang, L.-M.; Zheng, Y.-T.; Liu, H. *Molecules* **2016**, *21*, 1198. https://doi.org/10.3390/molecules21091198
70. Lown, J. W. *Azomethine Ylides – 1,3-Dipolar Cycloaddition Chemistry* Padwa, A., Wiley: New York: 1984, Vol. 1, pp. 653.
71. Najera, C.; de Gracia Retamosa, M.; Sansano, J. M.; de Cozar, A.; Cossio, F. P. *Eur. J. Org. Chem.* **2007**, 5038. https://doi.org/10.1002/ejoc.200700267
72. Najera, C.; de Gracia Retamosa, M.; Martin-Rodriguez, M.; Sansano, J. M.; de Cozar, A.; Cossio, F. P. *Eur. J. Org. Chem.* **2009**, 5622. https://doi.org/10.1002/ejoc.200900774
73. Martin-Rodriguez, M.; Najera, C.; Sansano, J. M.; de Cozar, A.; Cossio, F. P. *Beilstein. J. Org. Chem.* **2011**, *7*, 988. https://doi.org/10.3762/bjoc.7.111
74. Mancebo-Aracil, J.; Martin-Rodriguez, M.; Najera, C.; Sansano, J. M.; Costa, P. R. R.; Crizanto de Lima, E. *Tetrahedron, Asymm.* **2012**, *23*, 1596. https://doi.org/10.1016/j.tetasy.2012.10.015
75. Najera, C.; Sansano, J. M. *Monat. Chem* **2011**, *142*, 659. https://doi.org/10.1007/s00706-011-0467-9
76. Najera, C.; Sansano, J. M. *L'Actualité Chimique* **2013**, *370*, 28.
77. Yasuno, T.; Ohe, T.; Takahashi, K.; Nakamura, S.; Mashino, T. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 3226. https://doi.org/10.1016/j.bmcl.2015.05.086
78. Just, D.; Hernandez-Guerra, D.; Kritsch, S.; Pohl, R.; Cisařova, I.; Jones, P. G.; Mackman, R.; Bahador, G.; Jahn, U. *Eur. J. Org. Chem*. **2018**, 5213. https://doi.org/10.1002/ejoc.201800585
79. Galvez, J. A.; Clavería-Gimeno, R.; Galano-Frutos, J. J.; Sancho, J.; Velazquez-Campoy, A.; Abian, O.; Díaz-de-Villegas, M. D. *Eur. J. Med. Chem.* **2019**, *171*, 401. https://doi.org/10.1016/j.ejmech.2019.03.019

80. Regitz, M. *Diazoalkanes – 1,3-Dipolar Cycloaddition Chemistry* Wiley: New York, Padwa, A. Ed., 1984, Vol. 1; pp 394.
81. Beard, A. R.; Butler, P. I.; Mann, J.; Partlett, N. K. *Carbohydrate Res*. **1990**, *205*, 87. https://doi.org/10.1016/0008-6215(90)80130-U
82. Sauer, D. R.; Schneller, S. W.; Gabrielsen, B. *Carbohydrate Res*. **1993**, *241*, 71. https://doi.org/10.1016/0008-6215(93)80096-W
83. Salah, M.; Belghiti, M. E.; Aitouna, A. O.; Zeroual, A.; Jorio, S.; El Alaoui Abdellaoui, H.; El Hadki, H.; Marakchi, K.; Komiha, N. *J. Mol. Graph. Modell.* **2021**, *102*, 107763. https://doi.org/10.1016/j.jmgm.2020.107763
84. Khusnutdinova, E. F.; Kazakova, O. B.; Lobov, A. N.; Kukovinets, O. S.; Suponitsky, K. Y.; Meyers, C. B.; Prichard, M. N. *Org. Biomol. Chem*. **2019**, *17*, 585. https://doi.org/10.1039/c8ob02624f
85. Najera, C.; Sansano, J. M. *Org. Biomol. Chem* **2009**, *7*, 4567. https://doi.org/10.1039/B913066G
86. Huisgen; R. *J. Org. Chem.* **1976**, *41*, 403. https://doi.org/10.1021/jo00865a001
87. Huisgen; R. *1,3-Dipolar Cycloaddition Chemistry* Padwa, A. Ed.; Wiley-Interscience; 1984, Vol. 1, p. 1.
88. Casu, F.; Chiacchio, M. A.; Romeo, R.; Gumina, G. *Curr. Org. Chem*. **2007**, *11*, 1017. https://doi.org/10.2174/138527207781058754
89. Hýrošov, E.; Fišera, L.; Medvecký, M.; Reissig, H.-U.; Al-Harrasi, A.; Kóóš, M. *ARKIVOC* **2009**, (*ix*), 122. https://doi.org/10.3998/ark.5550190.0010.909
90. Taylor, R. D.; MacCoss, M.; Lawson, A. D. G. *J. Med. Chem.* **2014**, *57*, 5845. https://doi.org/10.1021/jm4017625
91. Barrett, C.; Bisset, K.; Leidig, J.; Marathe, A. M. M. *Epidemics* **2011**, *3*, 19. https://doi.org/10.1016/j.epidem.2010.11.002
92. Nicolaou, K. C. *J.Org.Chem.* **2005**, *70,* 7007. https://doi.org/10.1021/jo050963m

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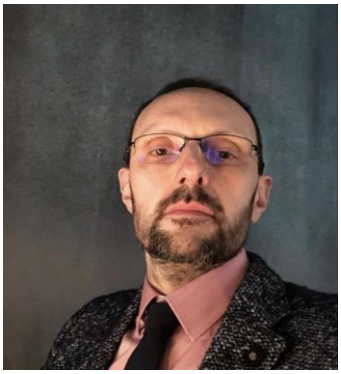

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