5-(3-Phosphonated *1H*-1,2,3-triazol-4-yl)isoxazolidines: synthesis, DFT studies and biological properties

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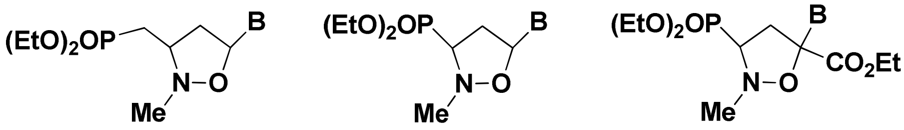
Abstract
5-Triazolyl-2-methylisoxazolidin-3-yl 3-phosphonates have been synthesized by 1,3-dipolar cycloaddition of *N*-methyl-*C*-diethoxyphosphorylnitrone and vinyl triazoles. The process showed a complete regioselectivity and a nearly exclusive *cis* stereoselectivity. M062X/6-31G(d,p) calculations rationalize the regio- and the stereochemical results. The formation of a hydrogen bond along a particular reaction channel significantly stabilizes both transition states and products related to *cis*-adducts. Biological tests indicate that the obtained compounds do not show relevant antiviral and anticancer activity.

Keywords: 1,3-Dipolar cycloaddition, click chemistry, DFT studies, phosphonated C-nucleosides, stereoselectivity

Introduction

In these last years, the nucleoside structure has been exploited as an efficient template for the development of new therapeutically useful compounds.[1-3] In this context, structural modifications on the sugar moiety of natural nucleosides and/or modifications of the heterocyclic base have been performed. The first option involved changes in the (2-deoxy)-D-ribofuranose moiety as the inversion of hydroxyl group configuration, their elimination leading to dideoxy- or dideoxy-dydehydro-nucleosides, their substitution/functionalization by various groups or cleavage of the sugar ring leading to acyclic nucleosides.[4] Other deeper structural modifications include the replacement of the oxygen atom by a methylene group, a sulfur or a nitrogen atom, or the additional insertion of a second heteroatom in the sugar moiety.[5-6]

In particular, *N,O*-nucleosides, characterized by the presence of an isoxazolidine system as mimetic of the ribose spacer, have been designed and shown to be endowed with antiviral and/or antitumoral activity.[7-13] PCOANS **1** have shown to be potent inhibitors of reverse trascriptase (RT) of different retroviruses;[14-17] truncated phosphonated azanucleosides **2** are able to inhibit HIV and HTLV-1 viruses at nM concentration;[8] truncated phosphonated *N,O*-psiconucleosides **3** inhibit HIV infection with low or absent cytotoxicity (Figure 1).[18]



1 B = Ty, 5-Fu, Cy **2** B = Ty, 5-Fu **3** B = Ty, 5-Fu, U, Cy

Figure 1. Phosphonated *N,O*-nucleosides.

Structural modifications concerning the purine or pyrimidine nucleobases have also been investigated[19-20] and shown to led to biologically interesting compounds. In particular, the use of unnatural heterocycles as nucleobases in the design of novel nucleoside analogues not only enhances the *in vivo* stability of the obtained compounds, but also confers novel mechanisms of action. In this context, the triazole heterocycle has been exploited as mimetic of the natural nucleobase. Triazole is considered an universal base, capable of forming base/pairs with all five nucleobases and this potentially confers an increased interacting ability of triazole nucleosides with their biological receptors.[21-22]

Recently, we have synthesized new series of 1,2,3-triazolyl-*N,O*-nucleosides: 3-hydroxymethyl-5-(*1H*)-1,2,3-triazol)isoxazolidines **4** inhibit the proliferation of follicular and anaplastic human thyroid cancer cell lines, with IC_{50} values ranging from 3.87 to 8.76 μM;[23]1,2,3-triazole-appended *N,O*-nucleosides **5** show a good anticancer activity especially on the U87MG human primary glioblastoma cell line;[24] *C*-5’-triazolyl *N,O*-nucleosides **6** inhibit cell proliferation of Vero, HEp-2, MDCK, HFF, BS-C-1 cell 50% (CC_{50}) at concentration between 5.0-40.0 μM;[25] *C*-nucleosides **7**, containing a 1,2,3-triazole ring linked to an isoxazolidine system show a significant antiproliferative effect in HepG2, HT-29 and SH-SY5Y cell lines (Figure 2).[26]

In continuation of these efforts, we have designed a novel series of phosphonated *C*-nucleosides **8**, featured by the presence of a 1,2,3-triazole ring linked to the isoxazolidine moiety by a C-C bond. The rationale of this choice lies on the major stability of these compounds, with respect to N-nucleosides, toward the enzymatic cleavage of the nucleosidic bond, due to the replacement of the C-N bond by the non hydrolyzable C-C bond.

The synthetic route is based on a 1,3-dipolar cycloaddition process:[27-29] the stereo- and regio-chemistry of the reaction has been assessed in terms of theoretical calculations. The obtained compounds have been tested for their antitumoral and their antiviral activities.

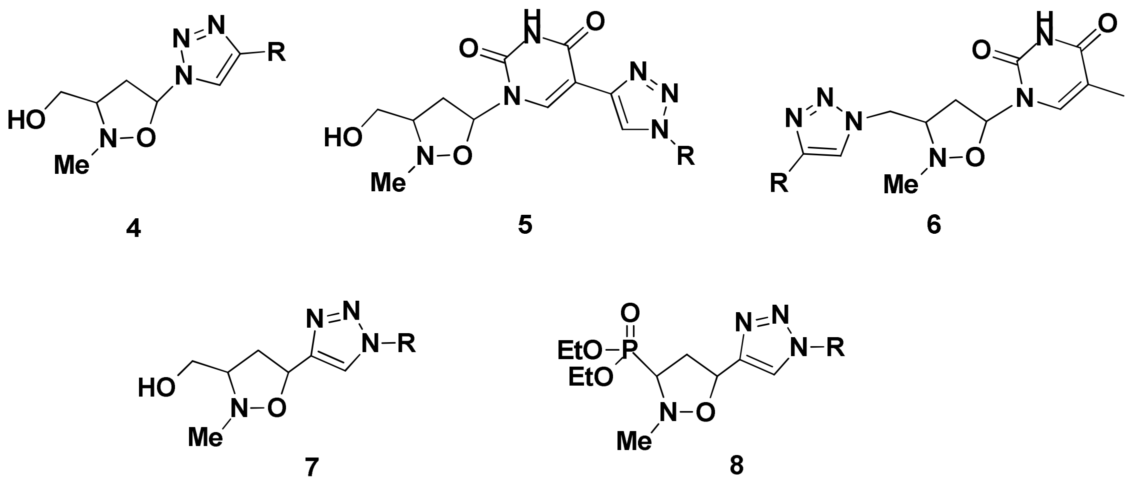
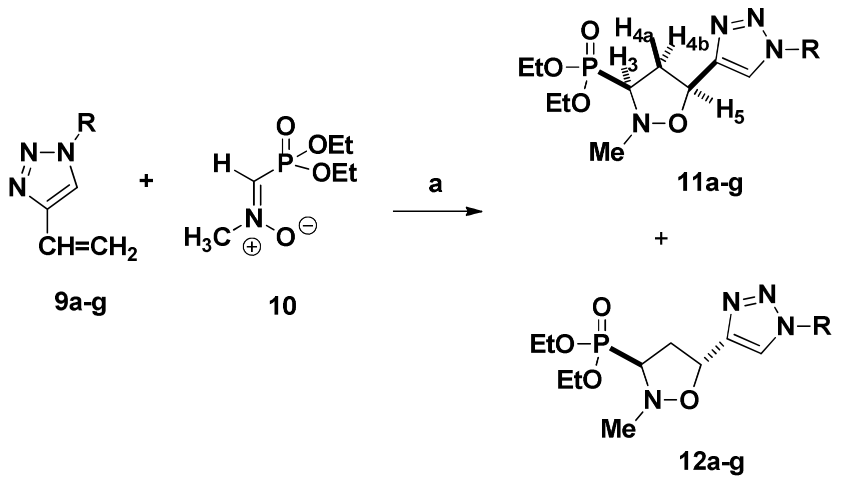


Figure 2. 1,2,3-Triazolyl-*N*,*O*-nucleosides.

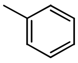
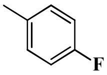
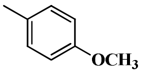
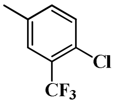
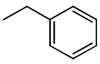
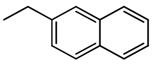
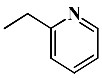
Results and Discussion

The synthesis of 3-phosphonated 5-(*1H*-1,2,3-triazol-4-yl)isoxazolidines **11a-g** and **12a-g** was performed by 1,3-dipolar cycloaddition (DC) involving the phosphonated nitrone **10**[18] and the corresponding 4-vinyl triazoles **9a-g**[26] (Scheme 1).



Scheme 1. Synthesis of 3-phosphonated 5-(*1H*-1,2,3-triazol-4-yl)isoxazolidines **11a-g** and **12a-g.** Reagents and conditions: a) CH_2Cl_2, MW, 100 W, 2 h, 90 °C.

Table 1. 3-phosphonated 5-(*1H*-1,2,3-triazol-4-yl)isoxazolidines **11a-g** and **12a-g** produced *via* 1,3-dipolar cycloaddition

R	Cycloaddition products	Ratio *cis:trans*[a]	Yield[b] %
	**11a** **12a**	99:1	93
	**11b** **12b**	99:1	94
	**11c** **12c**	99:1	93
	**11d** **12d**	99:1	93
	**11e** **12e**	99:1	93
	**11f** **12f**	99:1	95
	**11g** **12g**	99:1	96

[a]*Cis*/*trans* ratio determined by ^{1}H NMR analyses. [b]Isolated yield of **11a-g** after purification

The reaction was carried out under conventional heating or microwave irradiation. In particular, the reaction in refluxing toluene, ethanol, and acetonitrile for 24-48 h proceeded slowly and in moderate yields (10-20%), leading to a mixture of 5-substituted *cis* and *trans* adducts **11** and **12** respectively. When the reaction was performed in CH_2Cl_2 on microwave irradiation at 100 W for 2 h at 90 °C, an acceleration of the reaction time together with an increased yield was obtained (93-96%.). In all experiments, a 99:1 mixture of *cis* and *trans* cycloadducts was observed.

The regiochemistry of the adducts, as 3,5-substituted regioisomers, was readily deduced from ^{1}H NMR data. In each case, there was one proton signal at 5.30-5.15 ppm, resonating as doublet of doublets, which corresponds to the H_5 proton; the alternative 3,4-substituted regioisomers are not reported to show a resonance at this chemical shift value.

The relative stereochemistry of the main compounds **11a-g** was determined by 2D NOE experiments. In particular, the ^{1}H NMR of the adduct **11b**, chosen as model compound, shows a positive NOE effect observed for H_{4b} (the downfield resonance of protons at C-5, 2.97 ppm) upon irradiation of H_5 (δ 5.27 ppm); analogously, irradiation of H_3 (δ 3.23 ppm) in the same compound gives rise to the enhancement of the signal corresponding to H_{4b}. A NOE effect was also observed when H_{4b} was irradiated giving rise to an enhancement for the signals corresponding to H_3 and H_5 . These results are clearly indicative of a *cis* relationship of the $PO(OEt)_2$ and the triazole units.

The observed stereochemistry is in deep contrast with the precedent results reported for the cycloaddition reactions of this nitrone with various electron-rich, electron-poor and conjugative dipolarophiles, in which the *trans* adducts are the major stereoisomers.[8,30] Then, to rationalize the observed high regio- and stereoselectivity, a computational study at DFT level[31-34] was performed using the M062X/6-31G(d,p) calculations.[35]

Theoretical results

We selected the cycloaddition of **10** with **9a** as the reaction model for the theoretical investigation. Nitrone **10** can exist in the *Z* and *E* forms with an energetic preference for the *Z* isomer (ΔE=1.7 and ΔG_{298}=1.7 kcal mol^{-1}). Nevertheless, a not negligible nitrone amount (~6%) adopts the *E* configuration and hence its involvement in the cycloaddition cannot be excluded. Other configurations are due to possible rotating groups around the P-C bond for both *E* and *Z* nitrones; however, they lie at higher energy. For 1-phenyl-4-vinyl-*1H*-1,2,3-triazole **9a**, the *s-trans* and *s-cis* conformers are almost isoenergetic (ΔE=0.6 and ΔG_{298}=-0.2 kcal mol^{-1}); therefore they are expected to be equally present in the reaction mixture. Because of the presence of *E and Z* forms of the nitrone and the s-*cis and s-trans* triazole conformers, parallel models must be proposed to study the cycloaddition reaction.

Prediction of regioselectivity

The observed experimental trend towards the formation of 3,5-disubstituted derivatives, as exclusive adducts, has been rationalized according to four different approaches, based on the simple electronic structure of the reagents.[36-37]

Houk's rule. Considering the HOMO-LUMO relative position, the dominant electronic interaction involves the LUMO of the 1,3-dipole and the HOMO of the dipolarophile, i.e. an inverse-electron demand 1,3-dipolar cycloaddition reaction (Figure 3). According to Houk's rule, the regioselectivity is governed by the atoms that bear the largest HOMO and LUMO coefficient orbitals;[33] thus, in the present case, the theoretical formation of the 3,5 regioisomer is in agreement with the experimental findings.

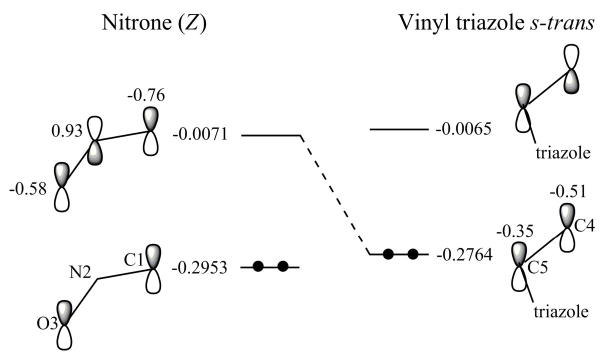


Figure 3. Energies (hartree) and coefficients of HOMO and LUMO, useful for the application of the Houk's rule (energies are not in scale).

Atomic charges. Another way to foresee the regiochemistry of 1,3-dipolar cycloaddition consists of using the charges obtained from Hirshfeld population analysis. The nitrone shows a negative charge on both C1 (~-0.08 e.u.) and O3 (~-0.26 e.u.) atoms (Figure 4), and also the carbons of the vinyl triazole carry negative charges (C5 ~ -0.05 and C4 ~ -0.10 e.u.). Hence, the cycloaddition will occur with the formation of the 3,5-regioisomer which involves the oxygen atom (the most negative end of the dipole) attached to C5 (less negative end of the vinyl-triazole, with respect to C4).

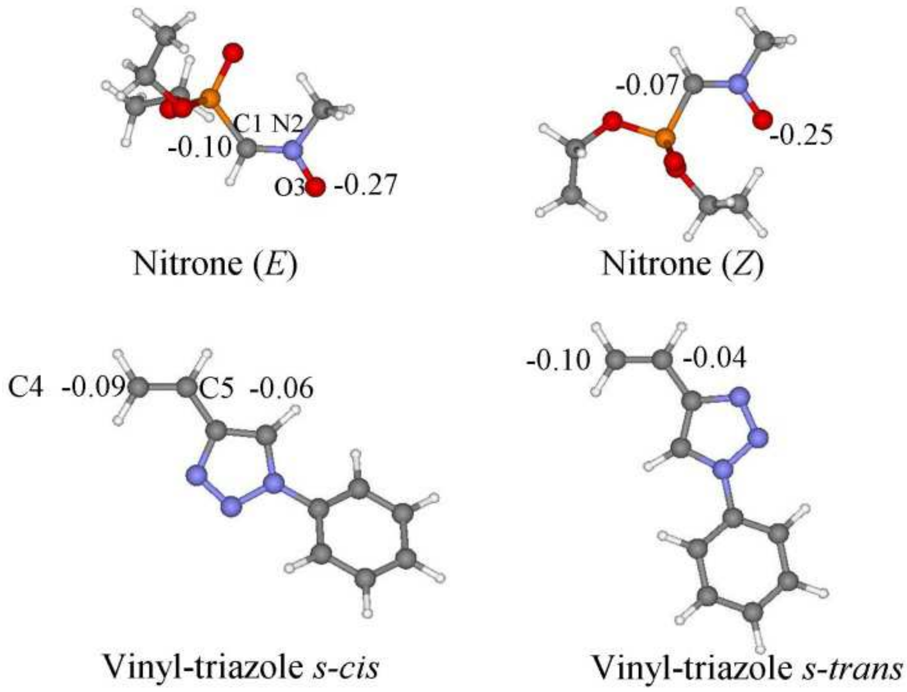


Figure 4. Hirshfeld charges (e.u.) on reactive atoms of reagent in the various conformations.

DFT reactivity indices. We have also analyzed the cycloaddition reaction using the global indexes, as defined in the context of DFT, which are useful tools to understand the reactivity of molecules in their ground states. Thus, the electronic chemical potential μ, the chemical hardness η, the chemical softness S, and the global electrophilicity power ω were calculated according to the previous reported formulas (Table 2).

The electronic chemical potential of the nitrone is more negative than that of the vinyl-triazole. Consequently, electron density transfer will take place from the vinyl-triazole to nitrone; i.e., a $HOMO_{dipolarophile}$-$LUMO_{dipole}$ interaction occurs. Table 2 reveals that the nitrone acts towards vinyl-triazole as an electrophile due to the larger value of its ω. Moreover, the electrophilicity differences between vinyl-triazole and nitrone ($\Delta\omega < 0.2$ eV) indicate a lower polar character for these cycloadditions, and their values are characteristic of nonpolar pericyclic reactions.[38-39]

Table 2. Frontier molecular orbital energies and global properties for nitrone **10** and vinyl-triazole **9a** calculated at the M062X/6-31G(d,p) level[a]

	HOMO	LUMO	μ	η	S	ω
Nitrone (*Z*)	-0.29531	-0.00706	-0.15118	0.14412	3.46921	2.16
Nitrone (*E*)	-0.29299	-0.00890	-0.15094	0.14204	3.52014	2.18
Vinyl-triazole *s-cis*	-0.27342	-0.00241	-0.13791	0.13550	3.68990	1.92
Vinyl-triazole *s-trans*	-0.27639	-0.00646	-0.14142	0.13496	3.70466	2.02

[a]HOMO, LUMO, μ, and η in hartree, S in hartree^{-1} and ω in eV.

Fukui functions and related local properties. The Fukui functions of an atom A, in a molecule with N-electrons, are calculated by finite difference method using the gross electronic population of the reactive site A in neutral ($P_A(N)$), cationic ($P_A(N-1)$) and anionic ($P_A(N+1)$) systems.

$f_A^+ = P_A(N+1) - P_A(N)$ Electrophilicity of atom A in molecule of N electrons

$f_A^- = P_A(N) - P_A(N-1)$ Nucleophilicity of atom A in molecule of N electrons

The related local softness, $s^{\pm}_A$, and the local philicity index, $\omega^{\pm}_A$, are easily obtained multiplying global quantities and condensed Fukui functions: $s^-_A = f^-_A S$, $s^+_A = f^+_A S$, $\omega^-_A = f^-_A S$, and $\omega^+_A = f^+_A S$

Table 3. M062X/6-31G(d,p) calculated Fukui functions (f^+ and f^-), local softness (s^+ and s^-) and local philicity power (ω^+ and ω^-) properties of the reactive sites in nitrone and vinyl-triazole based on Hirshfeld population. For reactive atom labeling see Figure 3

	atom	f^+	s^+	ω^+	f^-	s^-	ω^-
Nitrone (*Z*)	O3	0.144	0.501	0.012			
	C1	0.173	0.600	0.014			
Vinyl-triazole *s-trans*	C4				0.149	0.552	0.011
	C5				0.066	0.245	0.005

Being the present 1,3-dipolar cycloaddition an inverse electron demand type process, the nitrone undergoes a nucleophilic attack by the vinyl-triazole; thus, the local properties to be used are reported in Table 3. For the dipolarophile, C4 has the higher local nucleophilicity index, ω- with respect to C5. Therefore, C4 will be a preferred site for the electrophilic attack by the dipole. For the dipole carbon atom, higher f+ is found compared to the oxygen atom. Therefore, the C4 of the dipolarophile will be linked to the carbon atom of the dipole, following the favorable interaction between the highest nucleophilic and electrophilic sites of the reagents. These data, therefore, predict the formation of the 3,5-regioisomeric isoxazolidine in the cycloaddition reaction, in complete agreement with the experimental results.

The regioselectivity criteria of four center reactions is explained by Gazquez Mendez rule:[40] the interaction between two chemical species is more favored when the softness difference of two interacting atoms is minimum. Values of ΔS found for this 1,3-dipolar cycloaddition are:

$\Delta S(3,4)=(s^-_{C4} - s^+_{O3})^2+(s^-_{C5} - s^+_{C1})^2 = (0.552 - 0.501)^2+(0.245 - 0.600)^2 =0.002601+0.12605=0.12865$
$\Delta S(3,5)=(s^-_{C4} - s^+_{C1})^2 + (s^-_{C5} - s^+_{O3})^2 = (0.552 - 0.600)^2+(0.245 - 0.501)^2 =0.002304+0.06554=0.06784$

where atoms C4 and C5 of the nucleophile interact with atoms O3 and C1 of the electrophile to give rise to the preferred regioisomer and s^-_{C4}, s^+_{O3}, s^-_{C5}, and s^+_{C1} are the respective local softness of the reactive sites. Calculation of softness matching index ensures the simultaneous fulfillment of local HSAB concept at the two reacting termini. The reaction pathway involving lower value of ΔS will be the favored one. The ΔS(3,5) is smaller than ΔS(4,5). This suggests that 3,5-substituted isoxazolidine will be generated from the present cycloaddition reaction, in complete agreement with the experimental findings.

Stereochemistry

The reaction of nitrone **10** with alkenes usually gives rise to *trans* adducts preferentially; however, in the present case, the almost exclusive formation of the *cis* isomer occurs unexpectedly. To rationalize this behavior, quantum chemical calculations have been carried out to evaluate the activation and the formation energies for 3,5-regioisomers.

Calculations show that the reaction occurs throught labile intermediates (**I**) because of the formation of weak bonds between the nitrone and alkene with long distances for the newly forming C1- - -C4 and O3- - -C5 bonds (> 3 Å). The formation of intermediates occurs without any energy barrier and their shape depend on the initial structure of geometry optimization (Table 4 and Figures S1 and S2). It is evident that the relative orientation of nitrone and alkene substituents determine, even at this stage, some energetical preference (Table 4) and this issues will be better clarified in the transition state discussion, where steric constraints become more stringent.

The intermediate formation is an endoergonic process, mainly because of the reduction in entropy contents due to the degrees of freedom lost in the nitrone-alkene coupling ($-T\Delta S$: 10 - 15 kcal mol^{-1}). The next stage of reaction concerns the transition state (**TS**) that lies at high energy and hence it is the rate limiting step.

The *E*/*Z*-nitrone and *s-cis*/*s-trans* vinyl-triazole isomers can be combined in both *exo* or *endo* modes, giving rise to eight possible transition states (Table 4 and Figure 5). Furthermore, the compounds under analysis are prochiral molecules, thus transition states and final products, formed in the transformation, can adopt both *S* and *R* configurations, doubling the number of possible structures. Here, transition states and products of *S*,*S* and *S*,*R* compounds are reported, while the related *R*,*R* and *R*,*S* enantiomers are energetically the same and are not further considered.

Table 4. Relative Electronic and Gibbs energies (ΔE and $\Delta G°_{298}$, kcal mol^{-1}) for various conformations of intermediates, transition states, and kinetic products of the cycloaddition reaction

	Intermediate		TS		Kinetic product	
	ΔE	$\Delta G°_{298}$	ΔE	$\Delta G°_{298}$	ΔE	$\Delta G°_{298}$
(E) - endo – s-cis	-11.1	3.1	8.6	23.0	-36.2	-17.7
(E) – endo - s-trans	-11.8	3.7	12.1	26.6	-36.6	-18.1
(Z) - exo – s-cis	-12.1	3.6	9.9	24.2	-38.3	-18.7
(Z) - exo – s-trans	-13.1	2.4	3.6	20.9	-41.8	-23.6
(E) - exo – s-cis	-10.5	3.5	8.1	22.9	-35.5	-18.1
(E) - exo – s-trans	-7.7	5.1	10.3	24.5	-36.8	-18.4
(Z) - endo – s-cis	-6.9	3.2	9.7	23.5	-36.7	-18.9
(Z) - endo – s-trans	-2.5	7.8	14.2	28.2	-39.0	-21.7

The newly forming isoxazolidine ring has three substituents at C1, N2, and C5 atoms and, of course, the relative stability of the transition states depends on the interactions among these groups. The activation energy found for the eight transition states clearly defines a preferred conformation (*Z*) - *exo* –*s*- *trans* for the transition state, which leads to the formation of the *cis*-adduct. At about 5 and 2 kcal mol^{-1} higher in electronic and Gibbs energies, respectively, there are two reaction channels in which the nitrone in the (*E*) conformation leads to the formation of both *cis* and *trans*-adducts. Other reaction pathways lie at higher energy and cannot be reached even at 90 °C. The Boltzmann distribution, calculated at 90 °C for the three reaction channels, assuming a $\Delta\Delta$G of 2 kcal mol^{-1}, predicts a *cis*:*trans* ratio of 94:6, in reasonable agreement with the experimental trend.

The nitrone cycloaddition with monosubstituted conjugative dipolarophiles (such as vinyl triazoles), capable of secondary orbital interactions, proceeds through *endo* transition states.[41] According to this rule, one might expect that the preferred path involves an *endo* attack on the (*E*)-nitrone, and not an *exo* attack on the (*Z*)- nitrone. On the contrary, in our case, the cycloaddition takes place involving the (*Z*) nitrone with an *exo* transition state.

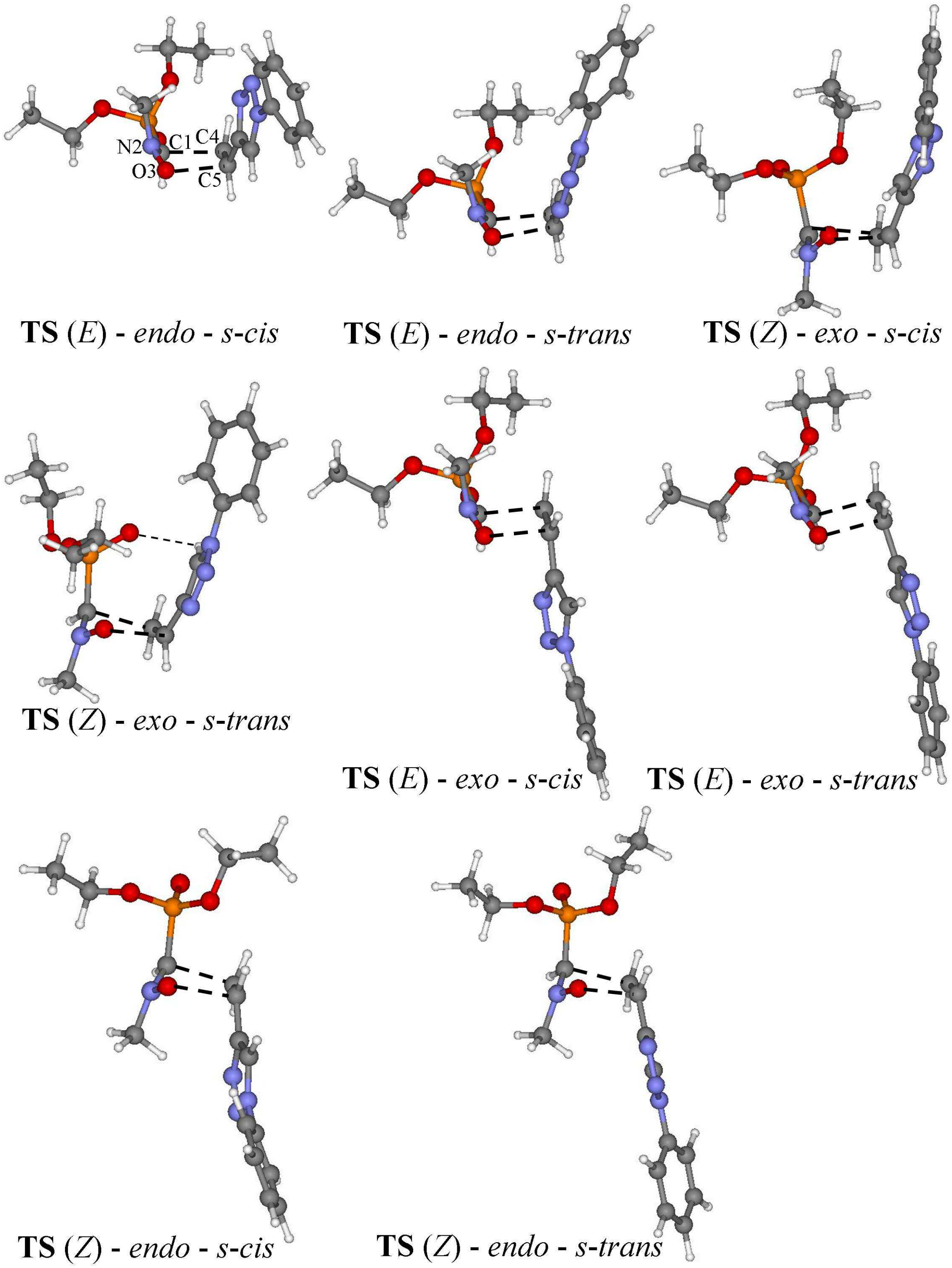


Figure 5. Molecular structures of the eight transition states for the formation of the *cis* and *trans*-adducts of the 3,5 regioisomer.

It is not easy to rationalize the computed activation energies on the basis of simple arguments. For example, if we assume that interactions between the three substituents at the isoxazolidine ring are steric in nature, the *(E) - endo – s-cis* and *(E) - endo – s-trans* attacks should be very high in energy, because the three substituents are on the same side of the ring. Actually, the *(E) - endo – s-trans* attack lies high in energy, but the *(E) - endo – s-cis* attack is energetically accessible. Similarly, it is not obvious that a change in *s-cis* / *s-trans* orientation of the (*Z*)-*exo* attack implies a $\Delta\Delta E$ and $\Delta\Delta G$ of 6.3 and 3.3 kcal mol^{-1}. However, for the *(Z) - exo – s-trans* process, the molecular structure reveals the formation of a weak intramolecular H-bond involving the oxygen atom of the P=O group and the hydrogen atom of the triazole ring (the P=O …….H triazole distance is 2.33 A). It is well known that the phosphoryl group is a good proton acceptor and forms strong hydrogen bonds with various substrates. Furthermore, it has been found that several 1,4-diaryl-1,2,3-triazole compounds can form C-H···X hydrogen bonding (X=electron-withdrawing atom, X = O, N, F, Cl, and Br).[42-44]

The bond distances of the five-membered rings are similar for all the transition states. In particular, the newly forming bonds C1--C4 and O3--C5 spread in a narrow range 2.104-2.191 Å, thus indicating that the reaction is essentially concerted. These bonds almost lie in the same plane, with ring puckering of few degrees (the C1C4C5O3 torsion angle deviate few degrees from zero). The nitrogen atom lies out of this plane, thus the newly forming isoxazolidine ring adopts an envelope conformation with the methyl in the axial position.

The eight kinetic evaluated products spread in a narrow energy range ($\Delta G°_{298}$ =-17.7 / -23.6 kcal mol^{-1}) and indicate an high exoergonic process. The *(Z) - exo – s-trans* product is the most stable and the molecular structure reveals the formation of the intramolecular H-bond involving the P=O as acceptor and the triazole ring as donor (P=O---H-C 2.147 Å), similarly to the H-bond observed in the related transition state. Therefore, the *(Z) - exo – s-trans* pathway, that leads to the formation of *cis*-adducts, is kinetically and thermodynamically preferred.

Biological results

N,O-Nucleosides **11a-g** were evaluated for their ability to inhibit the replication of a variety of DNA and RNA viruses, using the following cell-based assays: (a) Vero cell cultures: poliovirus 1, human echovirus 9, Coxsackievirus B4, adenovirus type 2, herpes simplex type 1 (HSV-1), herpes simplex type 2 (HSV-2); (b) human embryonic lung fibroblast cells (MRC-5): cytomegalovirus (CMV: VR-538); (c) African green monkey kidney cells (BS-C-1): varicella-zoster virus (VZV). Acyclovir was used as the reference compound. Unfortunately, no inhibitory activity against any virus was detected for the evaluated compounds.

The cytoxicity of the tested compounds towards uninfected host cells, defined as the minimum cytotoxic concentration (MCC) that causes a microscopically detectable alteration of normal cell morphology, was also assayed. Moreover, to determine if the compounds have any effect on cell proliferation, the cytostatic activity was evaluated by measuring the 50% cytostatic inhibitory concentration (CC_{50}), using the MTT test on Vero, HEp2 and HFF-1 cells. All the tested compounds except **11d** were not able to inhibit cell proliferation at 200µM concentrations.

Compound **11d** shows cell proliferation inhibitory activity with CC_{50} values at 62.5, 30 and 35 μM against Hep2, HFF-1 and Vero cells respectively.[19]

Conclusions

A novel series of phosphonated C-nucleosides, featured by the presence of a 1,2,3-triazole ring as a mimetic of natural nucleobases, linked to the isoxazolidine moiety by a C-C bond, has been synthesized. The very high regio- and stereo-selectivity of the reaction process, which involves a cycloaddition reaction of a phoshonated nitrone with vinyl 1,2,3-triazoles, has been rationalized on the basis of theoretical calculations. In particular, DFT calculations showed the inverse-electron demand nature of this 1,3-dipolar cycloaddition. The HOMO/LUMO energies, character and reactivity indices, charges on reactive sites, and Fukui functions of reagents support the formation of the 3,5-regioisomer in agreement with experimental results.

Activation and formation energies, calculated for eight different reaction pathways, explain the high stereoselectivity of the cycloaddition process. In particular, the formation of a hydrogen bond between the phosphoryl group and C-H of triazole ring, along the (Z) - *exo –s-trans* reaction channel, stabilizes both transition states and final products; the formation of *cis*-adducts is kinetically and thermodynamically favored.

Biological tests indicate that the obtained compounds do not show relevant antiviral and anticancer activity.

Experimental Section

General. Solvents and reagents were used as received from commercial sources. Melting points were determined with a Kofler apparatus. Elemental analyses were performed with a Perkin–Elmer elemental analyzer. NMR spectra (^{1}H NMR recorded at 500 MHz, ^{13}C NMR recorded at 126 MHz) were obtained with Varian instruments, and data are reported in ppm relative to tetramethylsilane. Thin-layer chromatographic separations were carried out on Merck silica gel 60-F254 precoated aluminum plates. Flash chromatography was carried out using Merck silica gel (200– 400 mesh). Preparative separations were carried out using an MPLC Büchi C-601 instrument using Merck silica gel 0.040–0.063 mm, and the eluting solvents were delivered by a pump at the flow rate of 3.5–7.0 mL/min. C-[(*tert*-Butyldiphenylsilyl)oxy]-*N*-methyl nitrone **10** was prepared according to described procedures.[8] Benzyl/alkyl and aromatic azides were synthesized according to literature procedures.[24]

General 1,3-dipolar cycloaddition procedure. A solution of **9a** (0.50 g, 2.92 mmol) and nitrone **10** (1.10 g, 3.50 mmol) in CH_2Cl_2 (5 mL) was put in a sealed tube and irradiated under microwave conditions at 150 W, 80 °C, for 2 h (CEM Discover Microwave reactor). The removal of the solvent *in vacuo* afforded a crude material which, after flash chromatography purification by using as eluent a mixture of cyclohexane/ethyl acetate 7:3, gave compound **11a**, as yellow oil, 93% yield. The 1H NMR spectrum shows the presence of *cis* and *trans* isomer respectively in 99:1.

Diethyl ((3*RS*,5*RS*)-2-methyl-5-(1-phenyl-1*H*-1,2,3-triazol-4-yl)isoxazolidin-3-yl)-phosphonate (11a). Yellow oil. ^{1}H-NMR (500MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.68 (dd, *J* 8.3, 0.9 Hz, 2H), 7.50 – 7.47 (m, 2H), 7.42 – 7.38 (m, 2H), 5.25 (t, *J* 7.5 Hz, H5, 1H), 4.26 – 4.15 (m, 4H), 3.20 (dd, *J*=11.8, 5.0 Hz, H4, 1H), 3.02 – 2.93 (m, 2H), 2.92 (s, 3H), 1.33 (dd, *J* 15.3, 7.2 Hz, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 146.62, 136.93, 129.73, 128.97, 120.68, 120.56, 71.65 (d, *J* 7.2 Hz), 71.48 (d, *J* 7.6 Hz), 64.37 (d, *J* 169.9 Hz), 63.25 (d, *J* 6.6 Hz), 62.58 (d, *J* 7.0 Hz), 46.50, 37.83, 16.63 (d, *J* 4.3 Hz), 16.52 (d, *J* 5.1 Hz). Anal calc for: $C_{16}H_{23}N_4O_4P$ Found: C, 52.52; H, 6.40; N, 15.35. Requires C, 52.46; H, 6.33; N, 15.29%

Diethyl ((3*RS*,5*RS*)-5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-2-methylisoxazolidin-3-yl)-phosphonate (11b). Yellow oil, 94% yield. ^{1}H-NMR (500MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.36 – 7.28 (m, 3H), 7.23 – 7.17 (m, 2H), 5.48 – 5.41 (m, 2H), 5.11 (t, *J* 7.5 Hz, 1H), 4.25 – 4.07 (m, 4H), 3.12 (t, *J* 7.8 Hz, 1H), 2.89 – 2.79 (m, 5H), 1.29 (q, *J* 7.0 Hz, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 146.08, 134.38, 129.09, 128.76, 128.13, 122.23, 71.64 (d, *J* 7.6 Hz), 71.41 (d, *J* 7.2 Hz), 64.26 (d, *J* 169.0 Hz), 63.12 (d, *J* 6.6 Hz), 62.40 (d, *J* 7.0 Hz), 54.17 (d, *J* 10.4 Hz), 46.32, 37.74, 16.55 (d, *J* 5.8 Hz), 16.44 (d, *J* 5.9 Hz). Anal calc for: $C_{17}H_{25}N_4$O4P Found: C, 53.74; H, 6.68; N, 14.80. Requires C, 53.68; H, 6.62; N, 14.73%.

Diethyl ((3*RS*,5*RS*)-5-(1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)-2-methylisoxazolidin-3-yl)-phosphonate (11c). Yellow oil. 93% yield. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.71 – 7.64 (m, 2H), 7.24 – 7.16 (m, 2H), 5.30 – 5.23 (m, 1H), 4.29 – 4.17 (m, 4H), 3.25 – 3.18 (m, 1H), 3.02 – 2.92 (m, 5H), 1.40 – 1.32 (m, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 162.64 (d, *J* 249.2 Hz), 146.89, 133.32, 122.86 (d, *J* 8.8 Hz), 120.78 (d, *J* 25.3 Hz), 116.96 (d, *J* 23.4 Hz), 71.70 (d, *J* 7.9 Hz), 71.52 (d, *J* 7.9 Hz), 64.45 (d, *J* 166.4 Hz), 63.33 (d, *J* 6.7 Hz), 62.66 (d, *J* 7.0 Hz), 46.58, 37.92, 16.71 (d, *J* 4.7 Hz), 16.60 (d, *J* 4.9 Hz).: C, 50.00; H, 5.77; N, 14.58; Anal calc for: $C_{16}H_{22}FN_4O_4P$ Found: C, 50.12; H, 5.83; N, 14.64. Requires C, 50.00; H, 5.77; N, 14.58%.

Diethyl ((3*RS*,5*RS*)-2-methyl-5-(1-(naphthalen-2-ylmethyl)-1*H*-1,2,3-triazol-4-yl)-isoxazolidin-3-yl)phosphonate (11d). Yellow oil, 93% yield. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.76 (m, 3H), 7.72 (s, 1H), 7.52 – 7.46 (m, 2H), 7.45 (s, 1H), 7.31 (dd, *J* 8.4, 1.7 Hz, 1H), 5.62 (d, *J* 2.7 Hz, 2H), 5.14 (t, *J* 7.5 Hz, 1H), 4.23 – 4.11 (m, 4H), 3.14 (t, *J* 7.8 Hz, 1H), 2.91 – 2.80 (m, 5H), 1.35 – 1.27 (m, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 146.21, 133.20, 131.71, 129.31, 127.38, 125.50, 122.35, 71.70 (d, *J* 7.6 Hz), 71.46 (d, *J* 8.3 Hz), 64.30 (d, *J* 168.7 Hz), 63.20 (d, *J* 6.5 Hz), 62.49 (d, *J* 7.0 Hz), 54.43, 46.39, 37.81, 16.58 (d, *J* 5.9 Hz), 16.47 (d, *J* 6.3 Hz). Anal calc for: $C_{21}H_{27}N_4O_4P$ Found: C, 58.71; H, 6.39; N, 13.09. Requires C, 58.71; H, 6.39; N, 13.09%.

Diethyl ((3*RS*,5*RS*)-5-(1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)-2-methylisoxazolidin-3-yl)phosphonate (11e). Yellow oil, 93% yield. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.58 (d, *J* 9.0 Hz, 2H), 6.98 (d, *J* 9.0 Hz, 2H), 5.25 (t, *J* 7.5 Hz, 1H), 4.26 – 4.14 (m, 4H), 3.83 (s, 3H), 3.21 (t, *J* 7.6 Hz, 1H), 3.05 – 2.86 (m, 5H), 1.40 – 1.29 (m, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 160.00, 146.38, 130.40, 122.45, 120.89, 114.87, 71.73 (d, *J* 7.5 Hz), 71.54 (d, *J* 7.7 Hz), 64.41 (d, *J* 169.5 Hz), 63.29 (d, *J* 6.6 Hz), 62.61 (d, *J* 6.9 Hz), 55.78, 46.52, 37.84, 29.75, 16.65 (d, *J* 5.2 Hz), 16.54 (d, *J* 5.3 Hz). Anal calc for: $C_{17}H_{25}N_4O_5P$ Found: C, 51.60; H, 6.42; N, 14.20. Requires C, 51.51; H, 6.33; N, 14.13%.

Diethyl ((3*RS*,5*RS*)-5-(1-(4-chloro-3-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)-2-methylisoxazolidin-3-yl)phosphonate (11f). Yellow oil, 95% yield. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.05 (d, *J* 2.4 Hz, 1H), 8.04 (s, 1H), 7.87 (dd, *J* 8.6, 2.4 Hz, 1H), 7.66 (d, *J* 8.7 Hz, 1H), 5.25 (t, *J* 7.5, 1H), 4.25 – 4.14 (m, 4H), 3.20 (t, *J* 8.3 Hz, 1H), 3.01 – 2.85 (m, 5H), 1.38 – 1.30 (m, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 147.52, 135.43, 133.25, 132.65, 130.08 (q, *J* 32.6 Hz), 124.38, 122.11 (d, *J* 273.8 Hz), 120.30, 119.75, 71.47 (d, *J* 24.0 Hz), 64.37 (d, *J* 168.7 Hz), 62.99 (d, *J* 80.1 Hz), 46.48, 37.89, 16.64 (d, *J* 5.4 Hz), 16.53 (d, *J* 5.6 Hz). Anal calc for: $C_{17}H_{21}ClF_3N_4O_4P$ Found: C, 43.61 H, 4.46; N, 12.06. Requires C, 43.55; H, 4.52; N, 11.95%.

Diethyl ((3*RS*,5*RS*)-2-methyl-5-(1-(pyridin-2-ylmethyl)-1*H*-1,2,3-triazol-4-yl)isoxazolidin-3-yl)phosphonate (11g). Yellow oil, 96% yield. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.63 – 8.52 (m, 1H), 7.70 (s, 1H), 7.67 (td, *J* 7.7, 1.8, 1H), 7.26 (dd, *J* 3.8, 2.7, 1H), 7.19 (d, *J* 7.7, 1H), 5.61 (s, 2H), 5.17 (t, *J* 7.5, H5, 1H), 4.23 – 4.15 (m, 4H), 3.17 (t, *J* 7.7, H4, 1H), 2.93 – 2.83 (m, 5H), 1.37 – 1.30 (m, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 154.26, 149.91, 146.28, 137.50 (d, *J* 23.5 Hz), 123.62 (d, *J* 16.0 Hz), 122.67 (d, *J* 13.4 Hz), 71.75 (d, *J* 7.6 Hz), 71.54 (d, *J* 6.5 Hz), 64.41 (d, *J* 170.9 Hz), 63.28 (d, *J* 6.6 Hz), 62.56 (d, *J* 6.9 Hz), 55.78, 46.48, 37.91, 16.67 (d, *J* 5.4 Hz), 16.56 (d, *J* 5.6 Hz). Anal calc for: $C_{16}H_{24}N_5O_4P$ Found: C, 50.30; H, 6.23; N, 18.31. Requires C, 50.39; H, 6.34; N, 18.36%.

Supporting Information

Preparation and analytical data of compounds **11a-g**. Calculation Methods and copies of ^{1}H and ^{13}C NMR spectra of all new compounds.

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