ChemPubSoc Europe
DOI: 10.1002/open.201900230
Open Access ChemistryOPEN Full Papers

Electrocyclic Ring-Opening of 1,2,4-Oxadiazole[4,5-*a*] piridinium Chloride: a New Route to 1,2,4-Oxadiazole Dienamino Compounds

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1,2,4-Oxadiazole[4,5-*a*]piridinium chloride adds nucleophiles to undergo electrocyclic ring opening affording 1,2,4-oxadiazole dienamino derivatives. These pyridinium salts represent a special class of Zincke salts that are prone to rearrange when treated with primary amines or in the presence of bicarbonate to give the pyridones. The pivotal tuning of the experimental conditions leads to a straightforward synthesis of valuable 1,2,4-oxadiazole dienamine derivatives. The mechanism is also discussed in the light of NMR experiments and theoretical calculations.

1. Introduction

1,2,4-Oxadiazoles are valuable heterocycles extensively studied both from the synthetic and applicative point of view by numerous research organic chemistry groups. The synthesis of these heterocycles can be accomplished through conventional and unconventional methodologies and several methods are reported in the literature.[1]

The great deal of interest in 1,2,4-oxadiazoles derives from their biological activities and the use in drug discovery often as hydrolysis-resisting bioisosteric replacements for amide or ester functionalities because of their electronic properties. Some derivatives can also act as a bioisostere of the carboxylic acid function in retinoid structures. Compounds containing the oxadiazole ring were found to be anti-inflammatory or antiviral agents, agonists of muscarinic receptors, peptidomimetics, antitumor agents.[1,2]

Many oxadiazole-based biologically active compounds share some specific structural features (Figure 1). Often, the oxadiazole rings are linked through a spacer (alkylic, aromatic, containing ether or ester functionalities, ect.) to a second heterocyclic moiety.[3] This is, for instance, the case of the linezolid-like 1,2,4-oxadiazole derivatives whose antibacterial activities were evaluated using Gram-positive and -negative pathogens.[4] Oxadiazole-based derivatives can find application in the treatment of type-II diabete[5] and as anticancer compounds.[6] A series of novel ether-linked bis(heterocyclic)

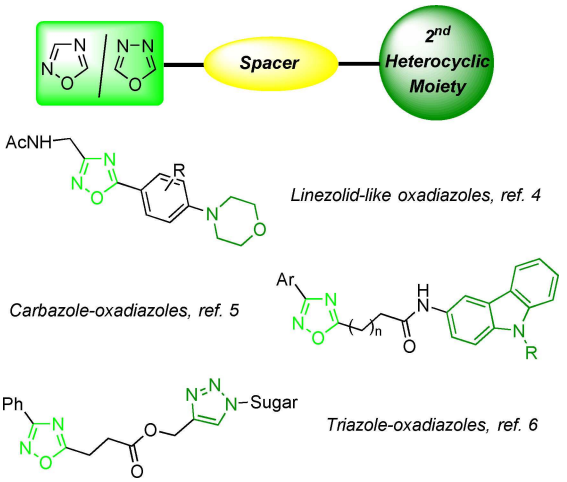

Figure 1. Structural features of some 1,2,4- and 1,3,4-oxadiazole compounds.

1,3,4-oxadiazoles[7] have been synthetized via a [3+2] cycloaddition reaction of nitrile oxides with allylic alcohol, exhibiting anti-inflammatory activities in carrageenan-induced edema (comparable to ibuprofen).[8] Similarly, bis-1,3,4-oxadiazoles, synthetized by oxidative cyclization of Schiff bases, were screened for *in vitro* antibacterial activities.[9]

Following up the interest relaying on 1,2,4-oxadiazole derivatives,[10] we reconsidered under a new light the approach to 1,2,4-oxadiazoles based on 1,3-dipolar cycloadditions of nitrile oxides. These 1,3-dipoles of type **2** are traditionally *in situ* generated from the corresponding hydroxymoyl chlorides of type **1** in the presence of bases, typically tertiary amines (Scheme 1).[11] When instead, nucleophilic aromatic bases, such as pyridine, are used the formation of the furoxan dimers **3**, is in competition with the formation of zwitterion **4**.

This intermediate is formed in a low stationary concentration, and is involved into the dimerization process that leads to dioxadiazines **5** or undergoes a further cycloaddition, leading to a stable bis-cycloadduct **7**.[12] Aside from reversion to the reactants, the zwitterion **4** can enter also an electrocyclic closure to the monocycloadduct **6**, in equilibrium with the

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/open.201900230

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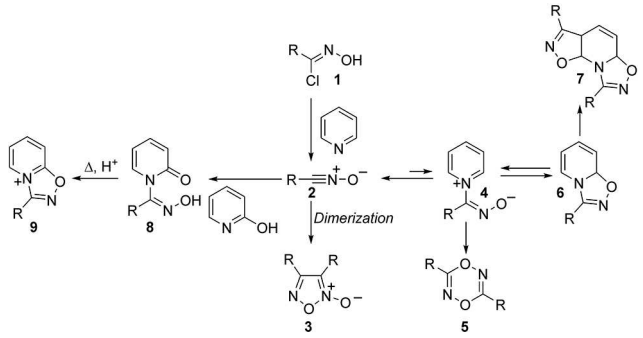
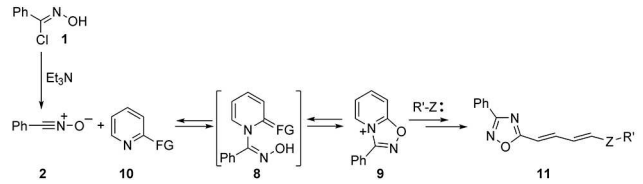


Scheme 1. Nitrile oxide formation from chloroximes **1** and pyridine and subsequent dimerization and cycloaddition processes.



Scheme 2. Nitrile oxide reactions with 2-substituted pyridines and reaction pathway to 5-substituted 1,2,4-oxadiazoles. FG, functional groups.

reactants and unstable towards cycloreversion. A further cycloaddition of nitrile oxide leads to the stable bis-cyclocadducts **7**.[13,14] By replacing pyridine with 2-hydroxypyridine, adduct **8** can be obtained and upon heating in acidic conditions a stable salt of type **9** is formed (Scheme 1).[12]

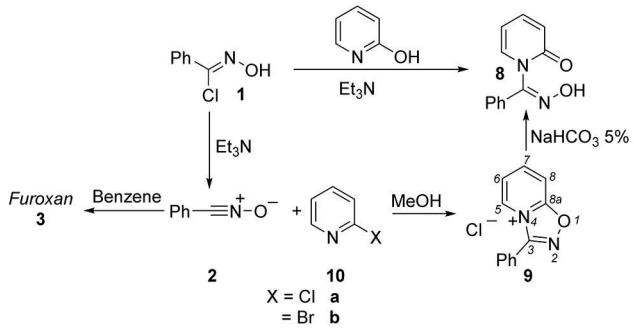
Expanding our investigation on the reactivity of nitrile oxides with 2-substituted pyridines, we envisioned the study of benzonitrile oxide (BNO) **2** (Scheme 2, R=Ph), identifying an unexpected route to 5-dienamino substituted 1,2,4-oxadiazoles when in the presence of a nucleophile of type R′-Z.

The scope of this methodology towards these derivatives is presented and investigated in the light of the mechanism proposed, with the aim to set up the protocol for obtaining single products with reliable and positive impacts on the synthetic ground.

2. Results

Supported by our previous findings (Scheme 1), we commenced to study the BNO cycloaddition reaction in the presence of variously functionalized pyridines (FG=halogen and amino groups).

Upon *in situ* generation of BNO **2** in a benzene solution with an excess of 2-chloro-pyridine **10a** (2–5 equiv.), the BNO dimer 3,4-diphenylfuroxan **3** was obtained in nearly quantitative yields (Scheme 3). When the same reaction is conducted in polar and protic solvents (*e.g.* MeOH) in the presence of the same excess pyridine **10a**, the furoxan formation is circumvented and, upon evaporation of the solvent, the salt **9** is obtained as solid compound in 71% yield. The synthesis can be carried on by



Scheme 3. Synthesis of the 1,2,4-oxadiazole[4,5-*a*]pyridinium salt.

using the 2-bromo-pyridine **10b** with similar results in terms of yields. The structure relies upon the corresponding analytical and spectroscopic data. Compound **9** is a solid colorless compound, having m.p. 197–8 °C (from chloroform), and shows at the ^{1}H NMR spectrum (DMSO) highly deshielded signals corresponding to the pyridine moiety; the proton H5 in the heterocycle is found at δ 9.26 as a doublet (J = 7 Hz) while the H7 is found at δ 8.93 as a double doublet (J = 7 and 8 Hz). Slightly shielded are the other protons H6 and H8, found at δ 8.12 and 8.82, respectively, coupled with the adjacent protons, as expected.

An indirect confirm of the reported structure of **9** came from the hydrolysis experiments. The salt **9** is stable for days in water solution but unstable in the presence of bases. When treated with 5% solution $NaHCO_3$, compound **9** gives rise to oxadiazole ring opening reaction and just after ten minutes the insoluble adduct **8** starts separating off the water solution. After one day the reaction is nearly quantitative and the adduct **8** was isolated in 93% yield, identical to the product obtained upon BNO generation in the presence of 2-hydroxypyridine.[12–14] The reaction can be speed up by using 5% NaOH solution; completion is reached after ½ hour (monitoring by TLC) and the final pyridine **8** can be obtained as a solid compound by addition of 5% HCl solution or bubbling CO_2 to neutralize the solution.

The salt **9** is stable in methanol solution as well as in other organic solvents. Once evaluated the instability of **9** in a basic environment, we then tested the chemical behavior in the presence of organic bases, such as ammonia and amines. To ensure a low concentration of free bases in solution (*vide infra*) and monitor the competitive formation of different compounds, the reactions were performed by adding triethylamine (2 equiv.) to an excess of amines hydrochlorides (1.5 equiv.) (Scheme 4). The *in situ*-formed amines smoothly react with **9** yielding unexpected products, the (1*E*,3*E*)-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-amines **11a–j**, isolated by simple evaporation of the alcoholic solvent, washing of the dichloromethane (DCM) organic solution with water and final evaporation of the dried organic phase. The products **11a–j** were recrystallized by proper solvents and obtained in general in nearly quantitative yields, with a few exceptions as reported in Table 1.

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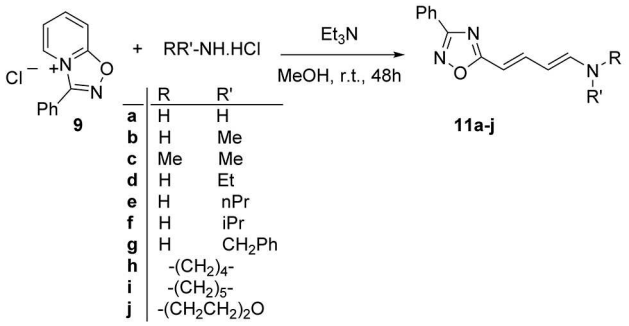
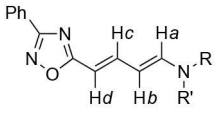


Scheme 4. Synthesis of the 4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-amines **11 a–j**.

Table 1. Yields, Mp.s (solvent) of compounds **11 a–j**.



Entry	11	R	R′	Yield (%)	m.p. (°C)	(Solvent)
1	**a**	H	H	72	>100 (dec.)	iPr_2O
2	**b**	H	Me	94	94–96	iPr_2O
3	**c**	Me	Me	98	99–101	EtOH
4	**d**	H	Et	66	>95 (dec.)	EtOH
5	**e**	H	nPr	90	82–84	Et_2O
6	**f**	H	iPr	89	88–89	iPr_2O
7	**g**	H	CH_2Ph	87	115–117	EtOH
8	**h**	$-(CH_2)_4-$		98	115–116	MeOH
9	**i**	$-(CH_2)_5-$		99	88–89	MeOH
10	**j**	$-(CH_2CH_2)_2O$		99	138–139	MeOH

The structures of compounds **11 a–j** are based upon the corresponding analytical and spectroscopic data. The most relevant and diagnostic feature in the ^{1}H NMR spectra is the sequence of four olefinic protons found in the range 5.00–8.70 δ. From all the reported compounds, the representative case of the morpholine derivative **11 j** is shown in the ^{1}H NMR spectrum (DMSO) of Figure 2. Typically, protons of type H*a* and H*b* are doublets quite deshielded, close to the aromatic protons region.

Conversely, the H*d* (doublets) and H*c* (triplets or double doublets) protons are found up-field below 6.50 δ. More details on the spectroscopic characterizations of compounds **11 a–j** can be found in the experimental section. The reactions can be also conducted with free amines although with lower yields and the reactions appear dirtier since the high concentration of the bases somewhat activates a competitive decomposition process. Concerning the stability of the synthesized compounds, products from primary amines are less stable both in the solid state and in solution and degrade to unspecified fragments. In particular, the ammonia derivative **11 a** decomposes rapidly in solution, *e.g.* during the acquisition of the NMR spectra, and undergoes rapid oxidation when left to open air.

In order to expand the synthetic scope of the reaction leading to dienamino heterocycles of type **11**, we also considered the use of heterocyclic amines, starting from the 2-amino-pyridines.

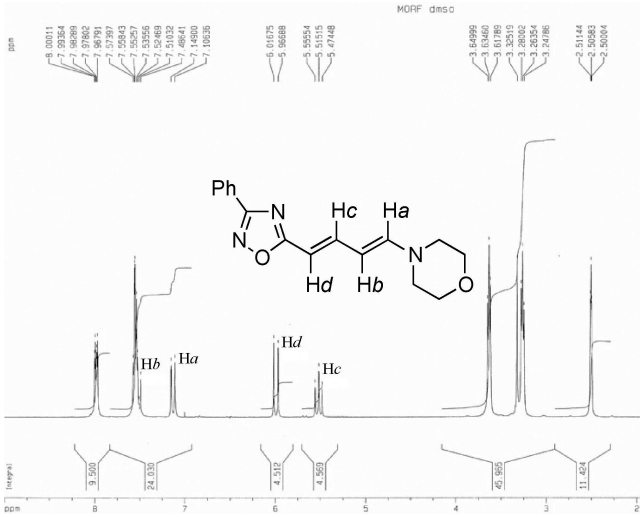


Figure 2. ^{1}H NMR spectrum (DMSO) of morpholino derivative **11 j**.

We have conducted the reaction between the pyridinium salt **9** and the 2-amino-pyridine or *N*-methylamino-pyridine (2.5 equiv.) under the previously described experimental conditions. After 2 days, the reaction mixture was washed with water and the dried organic phase was submitted to chromatographic separation to isolate the expected products of type **12 a,b** (Scheme 5).

Scheme 5. From salt **9**, the synthesis of the *N*-[(1*E*,3*E*)-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-yl]pyridin-2-amines **12 a,b**.

The products were isolated in 95% and 98% yields, respectively for **12 a** and **12 b** and their structures rely upon the corresponding analytical and spectroscopic data. In particular the ^{1}H NMR spectra showed the typical dienamine structure already observed in compounds **11** along with the aromatic signals attributable to the pyridine moiety. Figure 3 shows the ^{1}H NMR spectrum (DMSO) of the compound **12 a** with the main attributions. More insight on the mechanism and synthetic suitability came from the experiments performed directly between both 2-amino-pyridine and *N*-methylamino-pyridine and *in situ* generated BNO, in polar solvents (MeOH or $CHCl_3$) (Scheme 6). After a couple of days, the worked-up mixtures afforded the desired compounds.

Compounds **12 a,b** were isolate in 30% and 25% yield, respectively, along with the dioxadiazine **5** (8%) from the dimerization of BNO under basic conditions,[15,16] the 3,5-diphenyl-1,2,4-oxadiazole-4-oxide **13** (15%) whose formation is clearly attributable to the BNO cycloaddition to the corresponding amidoxime derived from aminopyridine addition to BNO[17]

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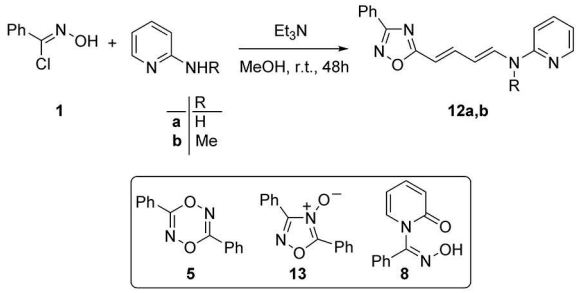


Scheme 6. Synthesis of the *N*-[(1*E*,3*E*)-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-yl]pyridin-2-amines **12 a,b** from 2-amino-pyridine or *N*-methylamino-pyridine and BNO.

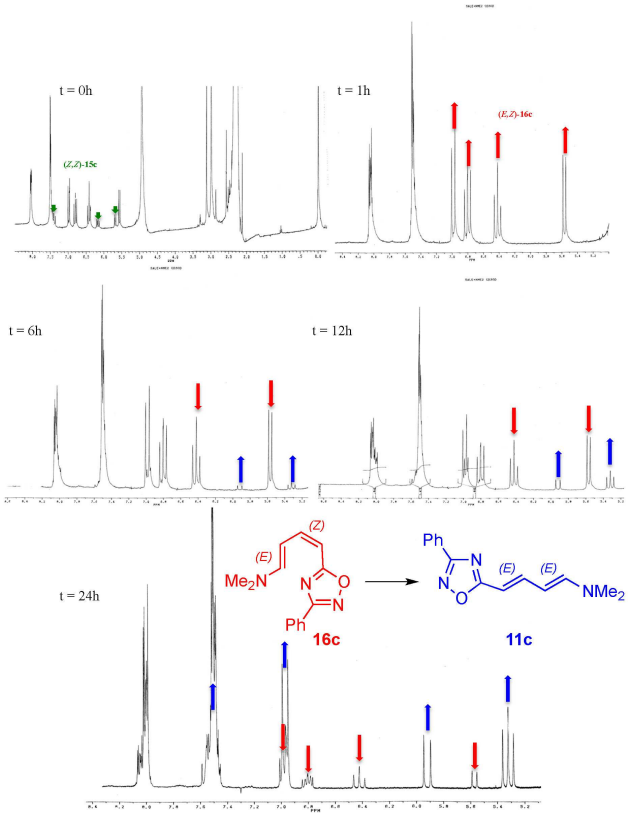


Figure 4. ^{1}H NMR spectra of the reaction between the pyridinium salt **9** and dimethylamine in CD_3OD at t = 0, 1 h, 6 h, 12 h and 24 h.

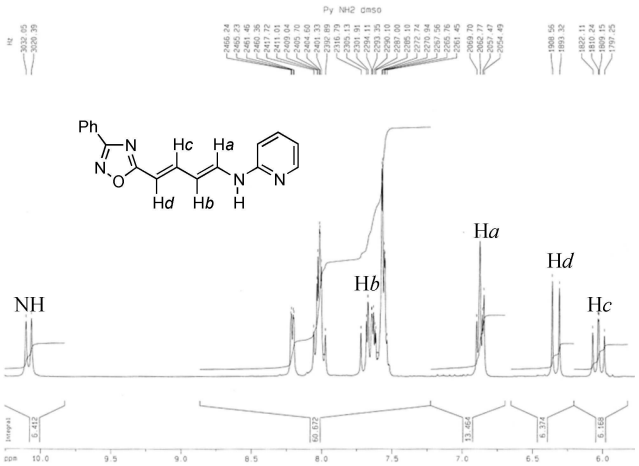


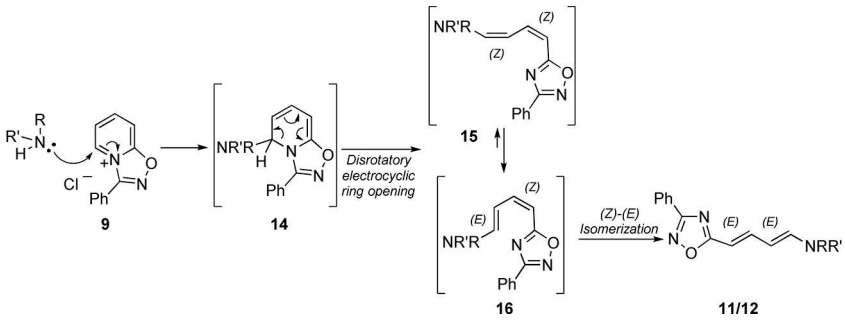
Figure 3. ^{1}H NMR spectrum (DMSO) of amino-pyridine derivative **12 a**.

and the pyridine adduct **8** (43 %) as a result of the addition of OH^- to the salt **9** that is the reaction intermediate of the whole pericyclic process (see also Scheme 3).

Scheme 7 shows the nucleophilic addition of the amines to the electrophilic carbon C5 of the pyridinium ring of **9**, that triggers the pivotal step represented by the disrotatory electrocyclic ring opening of the not isolable intermediate **14** leading to compounds **11/12**. Since the stereochemistry of the obtained products of type **11/12** does not correspond to the primary expected ones (dienamines **15** and **16** were not isolated), according to the pericyclic reaction selection rules, we decided to further investigate the ring-opening step.

We monitored the reaction following the formation and disappearance of the intermediates generated by the nucleophilic addition to the salt **9** as well as the final products by NMR technique, in CD_3OD as solvent. Here we report the case of the reaction of **9** with dimethylamine as representative of the behavior of all the amines used as reported in Scheme 4 and Table 1.

At the very beginning of the reaction (t = 0 h) the fast electrocyclic ring opening of **14** affords the compound (*E,Z*)-**16 c** as major component of the reaction mixture while the (*Z,Z*)-**15 c**, also detectable, remains the minor component and rapidly disappears (Figure 4). The structure attribution is based on the



Scheme 7. Electrocyclic ring opening mechanism in the reactions between the oxadiazole-pyridinum salt **9** with amines and isomerization of dienamino derivatives.

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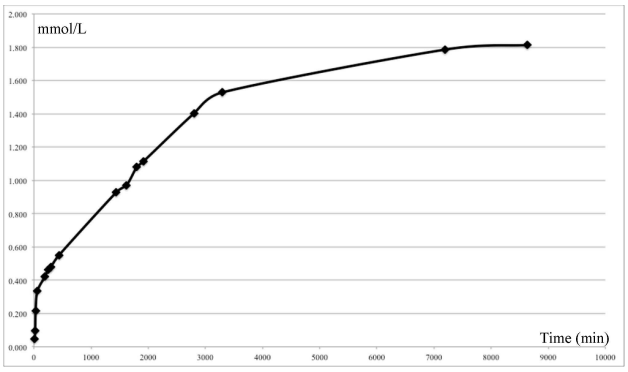

Figure 5. Formation of compound **11 j** with time performed from salt **9** in the presence of morpholine in MeCN as solvent at 25 °C.

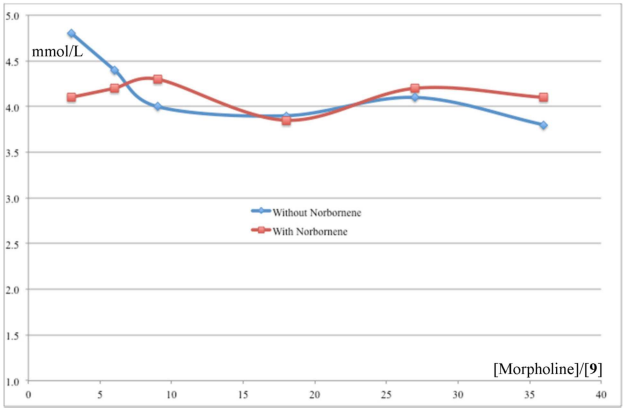

Figure 6. Reactions of the salt **9** with morpholine in $CHCl_3$ as solvent at 60 °C at 68 % of (***E,Z***)/(***E,E***) isomerization, with and without norbornene.

Table 2. Isomeric ratios and life times of intermediates from electrocyclic ring opening reactions of the salt **9** with amines.

Entry	RR′NH	Z,Z/E,Z[a]	Z,Z life time[b]	E,Z/E,E[a]	E,Z/E,E conv. time[b]
1	**a** R=R′=H	1/8	3 h	2/1	2 d
2	**b** R=H, R′=Me	1/6	2 h	2/1	2 d
3	**c** R=R′=Me	1/3	1 h	9/1	3 d
4	**d** R=H, R′=Et	1.6/1	12 h	10/1	3 d
5	**e** R=H, R′=nPr	1.3/1	8 h	8/1	2 d
6	**f** R=H, R′=iPr	1/1	8 h	8/1	2 d
7	**i** R=R′=–$(CH_2)_5$–	1/1	50 min.	10/1	2 d
8	**j** R=R′= –$(CH_2CH_2)_2O$–	1/1	40 min.	60/1	3 d

[a]. Isomeric ratio determined as an average of the heights of the olefinic signals in the ^1H NMR spectra recorded in CD_3OD: **9**, 10 mg; amines, 2 equivs. (if liquid, an excess if gas). [b]. Lifetime; time for complete disappearance signals of isomer at hand.

coupling constants clearly readable and measured from the NMR spectra. In particular, the intermediate (*Z,Z*)-**15 c** showed two *J* of 11 and 12 Hz consistent for a (*Z,Z*) configuration of the two C=C double bonds. On the other hand, the intermediate (*E,Z*)-**16 c** showed two *J* of 13 and 11 Hz consistent for a (*E,Z*) configuration.

After 1 h the (*E,Z*)-**16 c** is the only product observed still growing from the reactants. From this point on the reaction proceeds slowly and after 6 h a little amount of the final product, (*E,E*)-**11 c** is found. Compound (*E,E*)-**11 c** showed a typical C=C (*E,E*) configuration due to the *J* of 13 and 15 Hz; it slowly increases in the reaction mixture in 12 h time and overtakes the (*E,Z*)-**16 c** isomer only after 24 h. Reaction completion occurs after 4 days with complete disappearance of the salt **9** and full conversion in the final products. Table 2 gathers the data relative to analogous NMR experiments (CD_3OD) conducted for almost all the amines used for the synthesis of compounds **11**, showing different kinetics for the different nucleophiles.

Referring to entry 3, the Z/Z isomer is the minor component of the mixtures formed just after the electrocyclic ring opening. Isomer Z/Z life time ranges from few hours in the cases of aliphatic amines (entries 1–6) to 40–50 minutes in the cases of the cyclic ones (entries 7, 8), reasonably because of the steric demand of the piperidine or morpholine rings. The surviving E/Z isomer slowly converts into the E/E compound within the time of few days. Cyclic amines (pyrrolidine, pyperidine and mopholine) react faster at the beginning of the reactions that are speed up in methanol rather than in chloroform.

To monitor the kinetic of the process we followed the reaction of the salt **9** with amines by HPLC analyses and Figure 5 shows the formation of compound **11 j** with time performed in the presence of morpholine in acetonitrile (for analytical reasons).

A quantitative conversion requires 3.09 mmol/L of the product **11 j** and this theoretical value is nearly reached after 4 day (6000 min). In order to verify if the morpholine adduct **11 j** is the solely product of the reaction we have conducted a faster reaction in chloroform at 60 °C, also in the presence of norbornene as scavenger of the BNO.

Figure 6 reports two plots of reactions of **9** with morpholine at the 68 % of (*E,Z*)/(*E,E*) isomerization. The nearly identical pattern confirms that the morpholine addition occurs only at the position 5 of the pyridinium ring leading to the dienamino products of type **11** excluding any competitive cycloreversion reaction. As a consequence, Scheme 8 shows the allowed and forbidden paths for secondary amines. Addition to the carbon C8a of the pyridinium ring (red arrows) leading to **17**, does not occur at all.

When primary amines are used, the detection and in some cases the isolation of 2-amino-pyridines bearing the substituent corresponding to the primary amine could be explained by invoking a competitive process as shown in the blue box of Scheme 8: it leads to the undetectable and not-isolable intermediate **18** that evolves through base deprotonation to **19** and final fragmentation into the 2-substituted pyridine and BNO. The BNO presence in the reaction mixture is confirmed by the detection of dimerization products whose formation under basic conditions was already accounted through several reported mechanisms.[14–17] To confirm the previous observations, a few experiments were conducted on the 5-methyl-1,2,4-oxadiazole-[4,5-a]-pyridinium **21** (Scheme 9).

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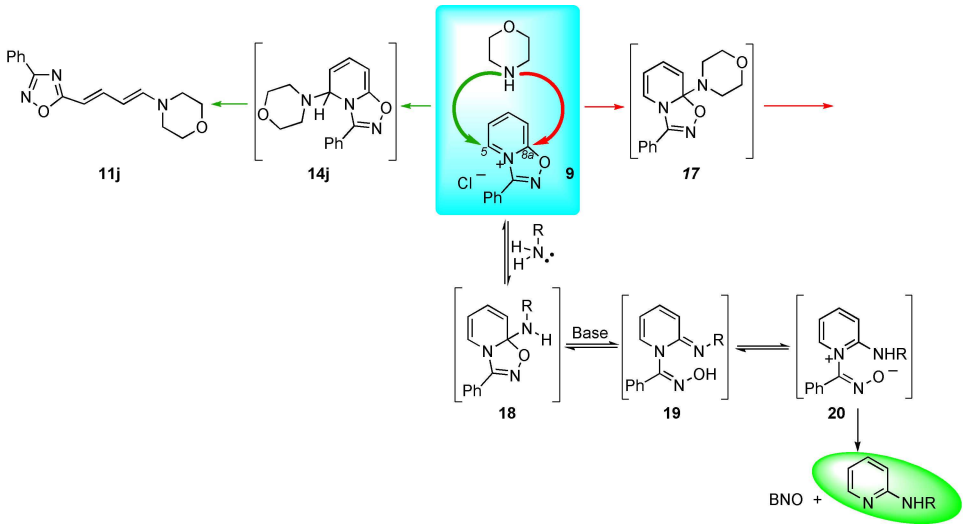
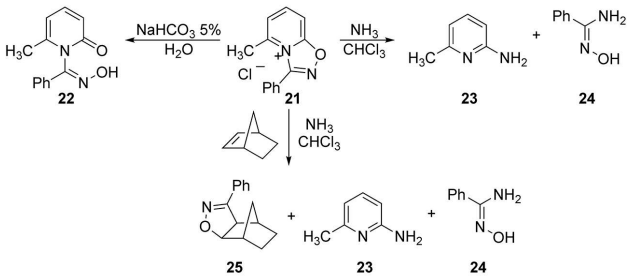


Scheme 8. Secondary and primary amines addition mechanisms to salt **9**.



Scheme 9. Reactions of the 5-methyl-1,2,4-oxadiazole-[4,5-a]-pyridinium chloride **21** with ammonia, bicarbonate and ammonia in the presence of norbornene.

The salt **21** was prepared according to the well-established procedure from 2-chloro-2-picoline and BNO. The structure of **21** is consistent with the corresponding analytical and spectroscopic data; in the ^{1}H NMR spectrum (DMSO), besides the typical aromatic signals, a singlet at δ 2.30 corresponds to the methyl group. The salt **21** smoothly reacts with $NaHCO_3$ 5% solution in water to afford quantitatively the pyridone **22** whose structure was confirmed by the spectroscopic analyses. When the salt **21** was treated with ammonia in chloroform as solvent at room temperature the addition of the base occurs on the C8a carbon atom leading to the 5-amino-2-picoline **23** (40%) and the benzamidoxime **24** (37%), found identical to known authentic samples as a product of the addition of NH_3 to BNO.[17,18] Moreover, when the salt **21** is allowed to react with ammonia in the presence of excess norbornene in chloroform solution at room temperature, besides the 5-amino-2-picoline **23** (30%) and the benzamidoxime **24** (5%), the BNO norbornene cycloadduct **25** was isolated in 49% yield. As clearly shown, the methyl on the C5 of the pyridinium ring forbids the nucleophilic addition at the same carbon atom and activates the cycloreversion pathway of the salt **21**.

3. Discussion

The results here reported clearly show the intriguing reactivity of the 1,2,4-oxadiazole-pyridinium salts of type **9**. These monocycloadducts obtained through a pseudo-pericyclic[19] addition of nitrile oxides to pyridine derivatives deserve a remarkable interest, not only from the mechanistic point of view but also synthetically. The ring-opening of Zincke salts dates back over a century[20] and the activation of pyridines as their pyridinium salts triggers the nucleophilic addition at the 2- and 5-positions leading to dienamino derivatives.[21] Being synthetically remarkable, in recent years this methodology found application in the synthesis of nitrogen heterocycles by the conversion of pyridinyl–anilines into indoles and a plausible mechanism was proposed to occur conceivably through an electrocyclic pathway.[22] The authors did not investigate the mechanism that was proposed to be simply ionic followed by alkene geometrical isomerization. The literature on this point only corroborates the nucleophilic addition step, ring opening and alkene isomerization.[23]

However we wish to summarize here through Scheme 10 the complex picture of the reactions involved that were accounted by the experimental results exposed in this work. The addition of amines to the flat pyridinium ion can occur on both sides of the heterocyclic ring leading to the not-isolable adduct **14**. Before discussing the disrotatory electrocyclic ring-opening, a conformational analysis of **14** was conducted through DFT calculations at the B3LYP/6-31G* level.[24] We have optimized at the lowest energy level the four possible conformers of adduct **14a** (R=R′=H): adducts **14*eq*** and **14*eq*′** are mirror images with the NH_2 group in the equatorial position at the C5 carbon atom in conformational equilibrium with the axial enantiomeric structures **14*ax*** and **14*ax*′**. The theoretical calculations demonstrated that only the axial conformer structures **14*ax*** and **14*ax*′** correspond to energy minima (see calculated structures in Scheme 10) and the equatorial conformers cannot be located not only at this level of theory but

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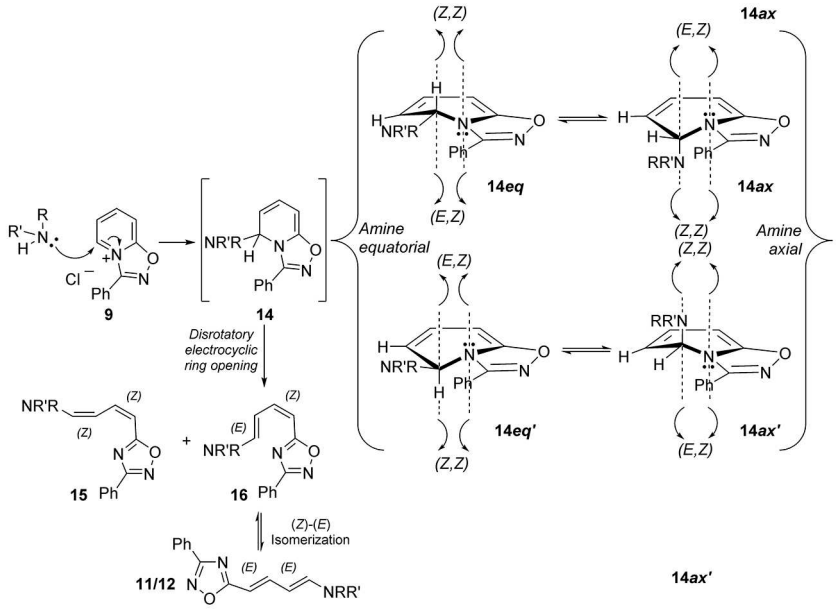

Scheme 10. Nucleophilic addition of amines to the salt **9** with disrotatory mechanism outcome from adduct **14**.

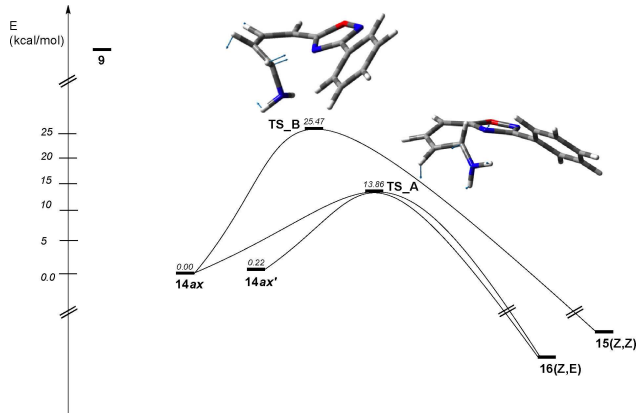

Figure 7. DFT calculated TSs for the electrocyclic ring-opening of compounds **14**. Numbers near the structure are relative energies in kcal/mol.

even higher or even introducing constraints during the optimization runs. Structures **14*ax*** and **14*ax*′** only differ by 0.22 kcal/mol. Adducts **14*ax*** and **14*ax*′** can undergo *disrotatory electrocyclic ring-opening* following the orientations indicated by the curly arrows; for each structure two pathway are possible, leading to either the (*Z,Z*)-**15 a** or (*E,Z*)-**16 a** intermediates.

According to the NMR investigations and Table 2 results, both the ring-opening orientation are allowed: the (*Z,Z*) path suffers of steric problems since this pathway locates the amine substituents inward the 6π array, presumably causing steric clashes with the oxadiazole ring. From the NMR experiments, the (*Z,Z*)/(*E,Z*) ratio ranges from 1/8 to 1/1 upon increasing the steric demand of the amines. Significantly, at the same concentration level of (*Z,Z*)/(*E,Z*) intermediates, the life time decreases upon the steric requirements of the bases (Table 2, entries 6–8). On the other hand, the (*E,Z*) path is preferred and **16 a** is more stable than **15 a** by 2.29 kcal/mol. The final step is the (*E,Z*)-(*E,E*) isomerization that occurs in a longer time scale (see Table 2) to afford the final dienamino derivative **11 a**. We have also located the two Transition Structures (TS) connecting the structures **14** with the intermediates (*Z,Z*)-**15 a** and (*E,Z*)-**16 a** (Scheme 11) (B3LYP 6-31+g(d,p) M06). **TS_A** can be reached from both **14*ax*** and **14*ax*′** and is found at 13.86 kcal/mol above. IRC data confirmed that **TS_A** connects **14*ax*** and **14*ax*′** with intermediate (*E,Z*)-**16 a**. The route to (*Z,Z*)-**15 a** passes at higher energy, 25.47 kcal/mol above **14*ax*** and **14*ax*′** through **TS_B** (Figure 7).

Regarding the (*Z,E*)-isomerization process, the literature reports some examples of this processes but nothing strictly related to the structures at hand.[22,25]

Scheme 12 shows in a simple and straightforward manner how compounds of type (*Z,Z*)-**15** can easily isomerize to (*E,Z*)-**16** through the zwitterion **15**′ and the conformer **15**′′ derived from rotation around the C–C bond as indicated by the blue arrow. Similarly occurs for the slow isomerization that converts **16** into the final products of type **11** or **12**.

4. Conclusions

Pyridinium salts of type **9** belong to the wide family of the Zincke salts.[26] In a recent perspective article, Vanderwal accounted for the use of pyridines for the preparation acyclic and heterocyclic compounds belonging to several classes of organic compounds through the valuable and century-old Zincke ring-opening reaction.[27] Moreover, Zincke reaction found applications in the synthesis of spin-lanbels TEMPO derivatives,[28] materials and nanomaterials,[29] preparation of ligands for Pd-catalyzed reactions,[30] up to the application in the drug synthesis.[31]

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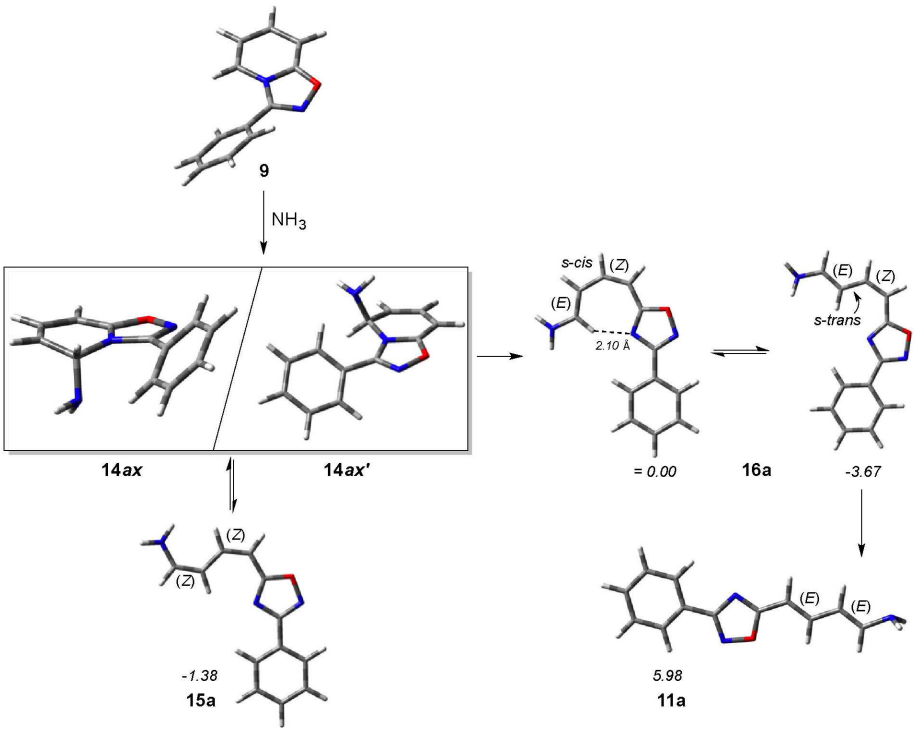
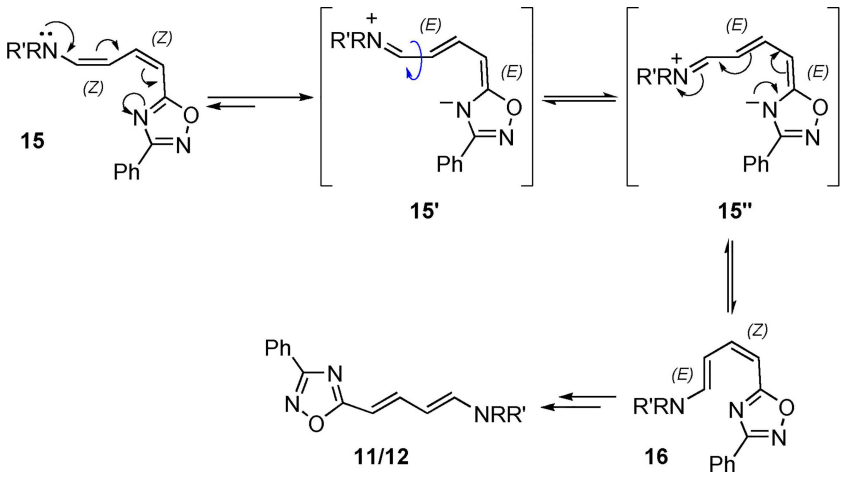


Scheme 11. DFT (B3LYP/6-31G*) calculated structures of key intermediates and products. Numbers near the structure are relative energies in kcal/mol. Hydrogen-bonding distance (dashed line) is expressed in Å.



Scheme 12. Isomerization mechanism of compounds **15**.

The 1,2,4-oxadiazole[4,5-*a*]piridinium chlorides **9** represent a special category of Zincke salts displaying a unique chemical behavior, interesting and valuable on both mechanistic and applicative points of view. First of all, these salts are easy to prepare in high yields and from a variety of halogen-substituted pyridines **10** (Scheme 13). Further substituents on the pyridine ring can decorate the diene moiety in final products of type **11**. Moreover, the nitrile oxides **2** can be also selected from a wide range of aromatic and aliphatic derivatives and easily generated from the corresponding hydroxymoyl chlorides **1** and in some case they are stable enough to simplify the reaction conditions.[32]

Finally the choice of the amines that trigger to electrocyclic ring-opening determines the structure of the diene-end moiety. For these reasons a number of elements can be properly tuned-up to design the desired structure of the final compounds of type **11**, thus expanding the synthetic scope of the reaction. In this view, the overall mechanism and chemical behavior of these pyridinium salts must be taken into account also for the synthetic design of the desired products.

At the end, Scheme 14 summarizes the reactivity of salts **9** and the connections with the various chemical species as we have reported in this work from the experiments conducted that disclosed the intriguing chemistry of these compounds,

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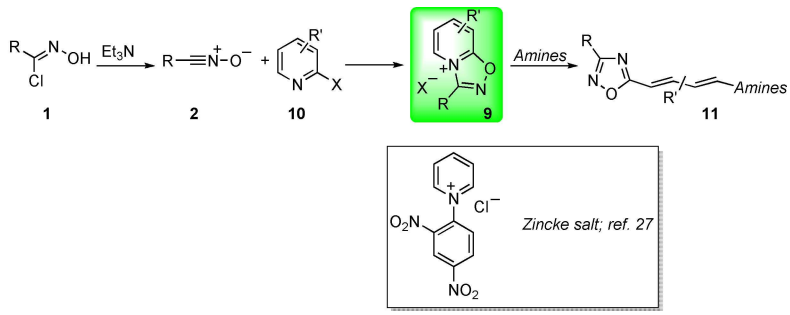
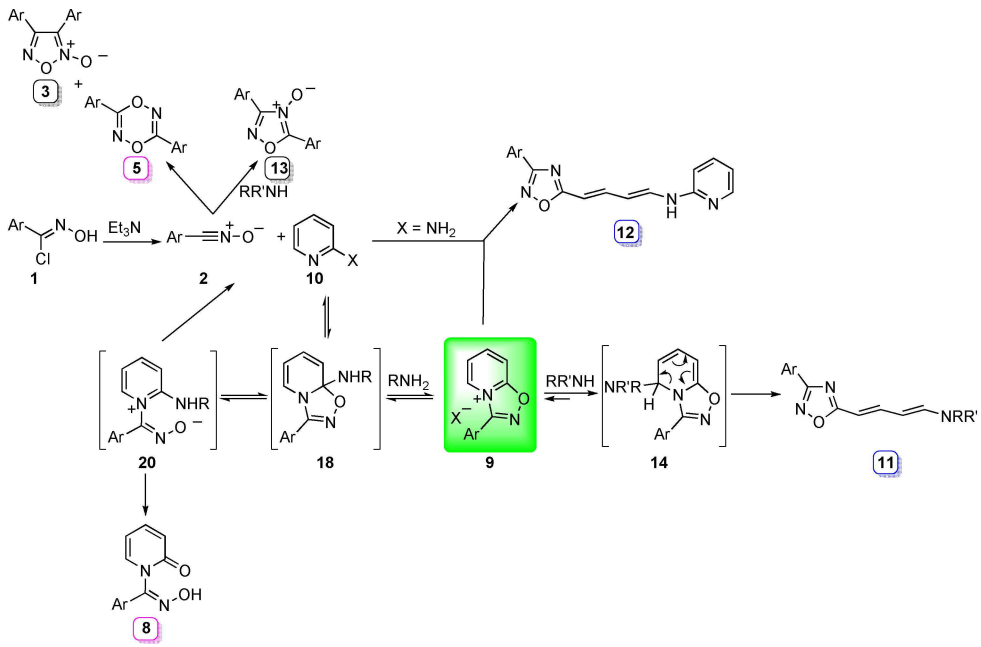
offering a chart to the different reaction pathways. Pyridinium salts **9** can be prepared either from halogen-substituted pyridine **10** or from 2-amino-pyridines. In this latter case dienamine products **12** are formed when the 2-amino-pyridine are used in excess. Through electrocyclic ring-opening triggered by amines or 2-amino-pyridines the dienamino compounds **11** and **12** are obtained. However, the salts **9** are prone to decompose when treated with primary amines or in the presence of bicarbonate to give the pyridone **8**. A cyclo-reversion process is also active leading to the pyridine and nitrile oxides that dimerize to the dioxadiazines **5** and the 1,2,4-oxadiazole-4-oxides **13** when in the presence of amines.

The best way to prepare the products of type **11** is definitively the separate preparation of the salt **9** and then submit the salt to treatment with amines as hydrochloride salts in the presence of triethylamine to ensure a low concentration of the free base and to gain high yields of the desired products. Slightly different is the behavior of 2-amino-pyridines that, being less nucleophilic, can be used without passing through their hydrochloride salts and compounds **12** can be easily isolated in optimum yields. The tuning of the experimental conditions is the key point to have a straightforward synthesis of valuable dienamine derivatives.

Samples of some of the synthetized compounds were sent to the NIAID (NIH, USA)[33] for *in-vitro* tests against a variety of viruses for a primary antiviral evaluation. Products **11h–j** and **12a** were tested against the Herpesviridae family, Varicella-Zoster virus, (VZV), from the Hepatic virus HBV, Respiratory Viruses such as Influenza A virus H1N1 (IV/H1N1), Adenovirus-5 (AD5), from the Togavidae family, Chikungunya virus (CV), from the Flaviridae group, Yellow Fever Virus (YFV), from the Bunyaviridae family, Punta Toro Virus (PTV), and from the Papovaviridae the Human Papilloma Virus (HPV). Compounds **11h–j** and **12a** were found inactive against all the viruses tested with slight differences. Other targets and planned investigations will promise further developments.



Scheme 13. Synthetic pathway to pyridinium salts **9** and dienamines **11**.



Scheme 14. Mechanistic chart: from pyridinium salts **9** to dienamine products **11** and **12** through electrocyclic ring-opening. Nitrile oxide dimerization processes to furoxans **3**, dioxadiazines, **5** and 1,2,4-oxadiazole-4-oxides **13** and decomposition pathway of salts **9** under basic conditions to pyridines **8**.

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Experimental Section

All melting points (Mp) are uncorrected. Elemental analyses were done on a elemental analyzer available at the Department. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer (solvents specified). Chemical shifts are expressed in ppm from internal tetramethylsilane (δ) and coupling constants (J) are in Hertz (Hz): b, broad; s, singlet; bs, broad singlet; d, doublet; t, triplet; m, multiplet. IR spectra (nujol mulls) were recorded on a spectrophotometer Perkin-Elmer RX-1 available at the Department and absorptions (ν) are in cm^{-1}. Column chromatography and tlc: silica gel H60 and GF_{254}, respectively; eluents: cyclohexane/ethyl acetate 9:1 to pure ethyl acetate. UV spectra were recorded on a Perkin-Elmer Lambda 16 UV Spectrophotometer in acetonitrile as solvent. HPLC analyses were carried out by means of a WATERS 1525 instrument, equipped with an UV2487 detector (λ = 254 nm) both controlled by Breeze software and a RP C-18 Intersil ODS-2 column: a mixture of H_2O/CH_3CN 60:40 was used as eluent. Quantitative determinations were conducted using the 5,5-dimethyl-3-phenyl-4,5-dihydroisoxazole as internal standard.

Starting and Reference Materials

2-Hydroxypyridine, 2-chloropyridine, 2-bromopyridine, 2-aminopyridine, 2-methylaminopyridine and 2-amino-6-methylpyridine were purchased from Sigma-Aldrich (Merck). Ammonia and all the primary and secondary amines used in this work were also purchased from Sigma-Aldrich (Merck). The corresponding hydrochloride salts were synthesized from standard procedures.

Benzhydroximoyl chloride was obtained by treatment of benzaldoxime with sodium hypochlorite.[32] Addition of a slight excess of Et_3N to a DCM solution of benzhydroximoyl chloride furnished *in situ* BNO.

Solvents and all the other reagents were purchased from Sigma-Aldrich (Merck) and used without any further purification with the single exception of triethylamine that was carefully distilled and used in all the reactions, when requested.

Synthesis of the 1,2,4-oxadiazole[4,5-a]piridinium Chloride 9

To a methanol solution (40 mL) of 5.0 g (32 mmol) of benzhydroxymoyl chloride **1**, 6.0 g (10 mL, 53 mmol) of 2-chlropyridine were added and the mixture was cooled at 0 °C with an ice-bath. Triethylamine (30 mmol, 4.8 mL) dissolved in 20 mL methanol was added dropwise under stirring and the solution turns from colourless to pale yellow. The reaction is left under stirring for 6 h; after this period of time, methanol is removed at reduced pressure and the residue is taken up with benzene to dissolve the pyridine excess. Upon filtration, the crude salt **9** is obtained along with the triethylamine hydrochloride. Purification of **9** is secured by dissolving the salt mixture with chloroform and leaving to crystallized the pyridinium salt **9** in colourless crystals. The product was submitted to complete characterization.

Similarly, the reaction can be performed by using 2-bromopyridine following the same experimental procedure and getting analogous results.

1,2,4-Oxadiazole[4,5-a]piridinium chloride ***9***, 5.29 g (71 %), colourless crystals from chloroform, m.p. 197–198 °C. IR: $\nu_{C=N}$ 1633 cm^{-1}. ^{1}H-NMR (DMSO) δ: 7.83 (t, 2H, *J* = 7 Hz, phenyl); 7.92 (t, 1H, *J* = 7 Hz, phenyl); 8.03 (d, 2H, *J* = 7 Hz, phenyl); 8.12 (t, 1H, *J* = 6 Hz, pyridine); 8.82 (d, 1H, *J* = 8 Hz, pyridine); 8.93 (dd, 1H, *J* = 7, 8 Hz, pyridine); 9.26 (d, 1H, *J* = 7 Hz, pyridine). ^{13}C-NMR (DMSO) δ: 110.4; 122.7; 125.2; 128.8; 130.0; 130.1; 130.7; 134.0; 147.6; 155.5, 161.2. Anal. Calcd for $C_{12}H_9N_2OCl$ (232.67): C, 61.95; H, 3.90; N, 12.04. Found: C, 61.96; H, 3.89; N, 12.06.

Synthesis of the 1-[(hydroxyimino)(phenyl)methyl] pyridin-2(1H)-one 8

To a solution of 46 mg (0.2 mmol) of the salt **9** in 2 mL of water, 1 mL 80.6 mmol) of $NaHCO_3$ 5 % were added. The reaction was left under stirring at room temperature and after 10 minutes the pyridine **8** starts separating off the solution. After one day, the solid is filtrated, dried and characterized.

1-[(Hydroxyimino)(phenyl)methyl]pyridin-2(1H)-one ***8***, 40 mg (93 %), colourless crystals from water, m.p. 201–203 °C. IR: $\nu_{C=O}$ 1703, $\nu_{C=N}$ 1653 cm^{-1}. ^{1}H-NMR (DMSO) δ: 6.35 (t, 1H, *J* = 8 Hz, pyridone); 6.51 (d, 1H, *J* = 9 Hz, phenyl); 7.44 (s, 5H, phenyl); 7.46 (d, 1H, *J* = 9 Hz, pyridone); 7.57 (dd, 1H, *J* = 8, 1 Hz, pyridone); 12.04 (s, 1H, OH). ^{13}C-NMR (DMSO) δ: 105.7; 120.9; 125.3; 128.8; 129.9; 131.5; 137.8; 141.4; 146.1; 159.8. Anal. Calcd for $C_{12}H_{10}N_2O_2$ (214.22): C, 67.28; H, 4.71; N, 13.08. Found: C, 67.26; H, 4.70; N, 13.07.

General Procedure for the Reaction of the Salt 9 with Primary and Secondary Amines

A solution of the pyridinium salt **9** is prepared with 1.4 g (6 mmol) in 50 mL of methanol. To this solution 9 mmol of the amine hydrochlorides, dissolved in 20 mL of methanol, were added followed by 2 mL (14.2 mmol) of freshly distilled triethylamine dropwise. The reaction mixtures were left under stirring at room temperature for 5 days. After this period of time, methanol is removed at reduced pressure and the residues were taken up with toluene to ensure precipitation of insoluble chlorides. The organic phases were then washed with water and dried over anhydrous Na_2SO_4. Upon evaporation of the solvent, solid residues were submitted to chromatographic isolation and the solid compounds were recrystallized from proper solvents to afford the final products **11 a–j** that were fully characterized.

(1E,3E)-4-(3-Phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-amine ***11 a***, 0.92 g (72 %), yellow crystals from diisopropylether, m.p. > 100 °C (dec.). IR: $\nu_{C=N}$ 1660 cm^{-1}. ^{1}H-NMR (DMSO) δ: 5.98 (t, 1H, *J* = 12 Hz, CH=); 6.37 (d, 1H, *J* = 15 Hz, CH=); 7.30 (t, 5H, *J* = 12 Hz, CH=); 7.53 (d, 1H, *J* = 15 Hz, CH=); 7.57 (m, 3H, phenyl); 8.03 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 103.9; 106.2; 126.7; 128.7; 131.2; 142.2; 143.8; 167.3; 167.6; 176.3. Anal. Calcd for $C_{12}H_{11}N_3O$ (213.24): C, 67.59; H, 5.20; N, 19.71. Found: C, 67.58; H, 5.18; N, 19.70.

(1E,3E)-N-Methyl-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-amine ***11 b***, 1.28 g (94 %), yellow crystals from diisopropylether, m.p. 94–96 °C. IR: $\nu_{C=N}$ 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.84 (d, 3H, *J* = 7 Hz, N–CH_3); 4.30 (b, 1H, NH); 5.38 (t, 1H, *J* = 12 Hz, CH=); 6.08 (d, 1H, *J* = 15 Hz, CH=); 6.88 (dd, 1H, *J* = 12, 7 Hz, CH=); 7.41 (m, 3H, phenyl); 7.53 (dd, 1H, *J* = 15, 12 Hz, CH=); 8.10 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 96.1; 126.4; 126.9; 128.6; 130.5; 146.4; 152.5; 166.9; 176.9. Anal. Calcd for $C_{13}H_{13}N_3O$ (227.26): C, 68.70; H, 5.77; N, 18.49. Found: C, 68.72; H, 5.76; N, 18.48.

(1E,3E)-N,N-Dimethyl-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-amine ***11 c***, 1.42 g (98 %), yellow crystals from ethanol, m.p. 99–101 °C. IR: $\nu_{C=N}$ 1641 cm^{-1}. ^{1}H-NMR (DMSO) δ: 2.91 (s, 6H, N–Me_2); 5.29 (t, 1H, *J* = 12 Hz, CH=); 5.92 (d, 1H, *J* = 15 Hz, CH=); 7.18 (d, 1H, *J* = 15 Hz, CH=); 7.49 (t, 1H, *J* = 12 Hz, CH=); 7.55 (m, 3H, phenyl); 7.66 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 95.4; 96.0; 126.4; 126.9; 128.6; 130.5; 146.4; 152.5; 166.9; 176.9. Anal. Calcd for $C_{14}H_{15}N_3O$ (241.29): C, 69.69; H, 6.27; N, 17.41. Found: C, 69.71; H, 6.26; N, 17.39.

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(1E,3E)-N-Ethyl-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-amine ***11d***, 0.96 g (66%), yellow crystals from ethanol, m.p. >95 °C (dec.). IR: $\nu_{C=N}$ 1618 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.12 (m, 3H, CH_3); 3.47 (m, 2H, N–CH_2); 5.38 (t, 1H, *J* = 12 Hz, CH=); 5.86 (d, 1H, *J* = 15 Hz, CH=); 7.08 (t, 1H, *J* = 15 Hz, CH=); 7.17 (t, 1H, *J* = 12 Hz, CH=); 7.54 (m, 3H, phenyl); 7.98 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 21.7; 95.0; 126.4; 127.3; 128.8; 129.2; 129.5; 147.6; 167.2; 177.4. Anal. Calcd for $C_{14}H_{15}N_3O$ (241.29): C, 69.69; H, 6.27; N, 17.41. Found: C, 69.70; H, 6.25; N, 17.40.

(1E,3E)-4-(3-Phenyl-1,2,4-oxadiazol-5-yl)-N-propylbuta-1,3-dien-1-amine ***11e***, 1.38 g (90%), yellow crystals from diethylether, m.p. 82–84 °C. IR: $\nu_{C=N}$ 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.96 (t, 3H, *J* = 7 Hz, CH_3); 1.60 (m, 2H, CH_2); 3.07 (bs, 2H, *J* = 7 Hz, N–CH_2); 4.40 (bs, 1H, NH); 5.38 (t, 1H, *J* = 12 Hz, CH=); 6.03 (d, 1H, *J* = 15 Hz, CH=); 6.80 (dd, 1H, *J* = 15, 8 Hz, CH=); 7.44 (m, 3H, phenyl); 7.48 (d, 1H, *J* = 15 Hz, CH=); 8.10 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 24.7; 45.4; 97.3; 126.8; 127.3; 128.9; 130.9; 146.6; 148.6; 167.2; 177.3. Anal. Calcd for $C_{15}H_{17}N_3O$ (255.31): C, 70.56; H, 6.71; N, 16.46. Found: C, 70.57; H, 6.70; N, 16.44.

(1E,3E)-N-Isopropyl-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-amine ***11f***, 1.36 g (89%), yellow crystals from diiosopropylether, m.p. 88–89 °C. IR: $\nu_{C=N}$ 1618 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (d, 6H, *J* = 7 Hz, CH_3); 3.55 (m, 1H, N–CH); 4.31 (m, 1H, NH); 5.38 (t, 1H, *J* = 12 Hz, CH=); 6.03 (d, 1H, *J* = 15 Hz, CH=); 6.75 (dd, 1H, *J* = 15, 8 Hz, CH=); 7.50 (m, 3H, phenyl); 7.55 (d, 1H, *J* = 15 Hz, CH=); 8.08 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 23.6; 25.2; 96.3; 126.8; 127.3; 129.0; 130.9; 147.1; 151.8; 167.3; 177.2. Anal. Calcd for $C_{15}H_{17}N_3O$ (255.31): C, 70.56; H, 6.71; N, 16.46. Found: C, 70.55; H, 6.72; N, 16.47.

(1E,3E)-N-Benzyl-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-amine ***11g***, 1.58 g (87%), yellow crystals from ethanol, m.p. 115–117 °C. IR: $\nu_{C=N}$ 1646 cm^{-1}. ^{1}H-NMR (DMSO) δ: 4.29 (d, 1H, *J* = 6 Hz, N–CH_2); 5.43 (t, 1H, *J* = 12 Hz, CH=); 5.89 (d, 1H, *J* = 15 Hz, CH=); 7.26 (m, 7H, phenyl, CH=); 7.53 (m, 3H, phenyl); 7.97 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 96.3; 126.8; 127.1; 127.2; 127.3; 128.5; 128.6; 128.9; 129.0; 131.0; 147.2; 167.3; 177.2. Anal. Calcd for $C_{19}H_{17}N_3O$ (303.36): C, 75.23; H, 5.65; N, 13.85. Found: C, 75.25; H, 5.64; N, 13.85.

3-Phenyl-5-[(1E,3E)-4-(pyrrolidin-1-yl)buta-1,3-dien-1-yl]-1,2,4-oxadiazole ***11h***, 1.57 g (98%), yellow crystals from methanol, m.p. 115–116 °C. IR: $\nu_{C=N}$ 1630 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.86 (bs, 4H, pyrrolidine); 3.29 (bs, 4H, CH_2–N–CH_2 pyrrolidine); 5.22 (t, 1H, *J* = 12 Hz, CH=); 5.90 (d, 1H, *J* = 15 Hz, CH=); 7.35 (d, 1H, *J* = 12 Hz, CH=); 7.52 (m, 4H, phenyl, CH=); 7.99 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 24.7; 96.5; 97.3; 126.8; 127.3; 129.0; 130.9; 145.7; 148.6; 167.2; 177.3. Anal. Calcd for $C_{16}H_{17}N_3O$ (267.33): C, 71.89; H, 6.41; N, 15.72. Found: C, 71.90; H, 6.43; N, 15.73.

3-Phenyl-5-[(1E,3E)-4-(piperidin-1-yl)buta-1,3-dien-1-yl]-1,2,4-oxadiazole ***11i***, 1.67 g (99%), yellow crystals from methanol, m.p. 88–89 °C. IR: $\nu_{C=N}$ 1635 cm^{-1}. ^{1}H-NMR (DMSO) δ: 1.54 (m, 6H, piperidine); 3.23 (m, 4H, CH_2–N–CH_2 piperidine); 5.44 (t, 1H, *J* = 12 Hz, CH=); 5.91 (d, 1H, *J* = 15 Hz, CH=); 7.10 (d, 1H, *J* = 12 Hz, CH=); 7.53 (m, 4H, phenyl, CH=); 7.99 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 23.6; 25.2; 48.8; 95.8; 96.3; 126.8; 127.3; 128.9; 130.9; 147.1; 151.8; 167.3; 177.2. Anal. Calcd for $C_{17}H_{19}N_3O$ (281.35): C, 72.57; H, 6.81; N, 14.94. Found: C, 72.58; H, 6.82; N, 14.96.

4-[(1E,3E)-4-(3-Phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-yl]morpholine ***11j***, 1.68 g (99%), yellow crystals from methanol, m.p. 138–139 °C. IR: $\nu_{C=N}$ 1635 cm^{-1}. ^{1}H-NMR (DMSO) δ: 3.25 (m, 4H, morpholine); 3.63 (m, 4H, morpholine); 5.51 (t, 1H, *J* = 12 Hz, CH=); 6.00 (d, 1H, *J* = 15 Hz, CH=); 7.08 (d, 1H, *J* = 12 Hz, CH=); 7.53 (m, 4H, phenyl, CH=); 7.98 (m, 2H, phenyl). ^{13}C-NMR (DMSO) δ: 66.0; 97.7; 97.9; 127.2; 127.5; 129.4; 131.4; 146.9; 151.8; 167.7; 177.4. Anal. Calcd for $C_{16}H_{17}N_3O_2$ (283.33): C, 67.83; H, 6.05; N, 14.83. Found: C, 67.84; H, 6.03; N, 14.85.

General Procedure for the Reaction of the Salt 9 with 2-aminopyridine and 2-(N-methylamino)-pyridine

A solution of the pyridinium salt **9** is prepared with 1.0 g (4.3 mmol) in 80 mL of methanol. To this solution 2.5 equivalents of the 2-aminopyridine and 2-(*N*-methylamino)-pyridine, dissolved in 20 mL of methanol, were added dropwise. The reaction mixtures were left under stirring at room temperature for 5 days. After this period of time, methanol is removed at reduced pressure and the residues were taken up with DCM. The organic phases were washed with water and dried over anhydrous Na_2SO_4. Upon evaporation of the solvent, solid residues were submitted to chromatographic isolation and the solid compounds were recrystallized from proper solvents to afford the final products **12a**,**b** that were fully characterized.

N-[(1E,3E)-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-yl]pyridin-2-amine ***12a***, 1.19 g (95%), yellow crystals from ethanol, m.p. 184–185 °C. IR: $\nu_{C=N}$ 1647 cm^{-1}. ^{1}H-NMR (DMSO) δ: 6.03 (t, 1H, *J* = 12 Hz, CH=); 6.33 (d, 1H, *J* = 15 Hz, CH=); 6.87 (m, 2H, pyridine and CH=); 7.57 (m, 3H, phenyl); 7.65 (m, 2H, pyridine and CH=); 8.03 (m, 3H, phenyl and pyridine); 8.21 (d, 1H, *J* = 5 Hz, pyridine); 10.09 (d, 1H, *J* = 12 Hz, NH). ^{13}C-NMR (DMSO) δ: 102.8; 105.1; 110.0; 116.5; 126.8; 126.9; 129.1; 131.2; 138.2; 139.0; 145.1; 148.1; 152.7; 167.6; 176.4. Anal. Calcd for $C_{17}H_{14}N_4O$ (290.32): C, 70.33; H, 4.86; N, 19.30. Found: C, 70.34; H, 4.87; N, 19.32.

N-Methyl-N-[(1E,3E)-4-(3-phenyl-1,2,4-oxadiazol-5-yl)buta-1,3-dien-1-yl]pyridin-2-amine ***12b***, 1.28 g (98%), yellow crystals from ethanol, m.p. 157–158 °C. IR: $\nu_{C=N}$ 1620 cm^{-1}. ^{1}H-NMR (DMSO) δ: 3.38 (s, 3H, N–CH_3); 6.03 (t, 1H, *J* = 12 Hz, CH=); 6.37 (d, 1H, *J* = 15 Hz, CH=); 7.03 (dd, 1H, J = 7, 6 Hz, pyridine); 7.56 (d, 1H, *J* = 9 Hz, pyridine); 7.58 (m, 4H, phenyl and CH=); 7.77 (m, 2H, pyridine); 8.04 (m, 2H, phenyl); 8.30 (d, 1H, *J* = 12 Hz, CH=). ^{13}C-NMR (DMSO) δ: 32.0; 103.5; 104.5; 109.7; 117.4; 126.8; 126.9; 129.1; 131.2; 138.6; 142.1; 145.5; 147.8; 154.4; 167.6; 176.4. Anal. Calcd for $C_{18}H_{16}N_4O$ (304.35): C, 71.04; H, 5.30; N, 18.41. Found: C, 71.03; H, 5.29; N, 18.41.

Reaction followed at the NMR Between the Pyridinium salt 9 and Dimethylamine in CD_3OD

Pyridinium salt **9** (35 mg, 0.15 mmol) was dissolved in 1 mL CD_3OD and an excess dimethylmine was added. 1H NMR spectra were recorded after 1, 6, 12 and 24 h (see Figure 4) to monitor the reaction and evolution of intermediates and reaction products. Similarly it was done for the reactions conducted in $CDCl_3$.

HPLC Quantitative Analyses on the Reaction Between the Pyridinium Salt 9 and Morpholine

Pyridinium salt **9** (18 mg, 0.08 mmol) was dissolved in 25 mL CH_3CN and 9.5 mg (0.08 mmol) of morpholine were added along with 12 mg of internal standard. The reaction was conducted at 25 °C. HPLC analyses were done at fixed times and the plot of the reaction is reported in Figure 5.

HPLC Quantitative Analyses on the Reaction Between the Pyridinium Salt 9 and Morpholine with and without Norbornene at 60 °C in Chloroform as Solvent

Pyridinium salt **9** (18 mg, 0.08 mmol) was dissolved in 25 mL $CHCl_3$ and increasing amounts of morpholine ([Morpholine/[**9**] = 3, 6, 9, 18, 27, 36) were added along with 12 mg of internal standard in separated reactions. The reactions were conducted at 60 °C with and without excess norbornene. All the experiments were stopped at the 68% of conversion and HPLC analyses were performed. The plot of the reaction is reported in Figure 6.

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Synthesis of the 3-Phenyl-5-methyl-1,2,4-Oxadiazole[4,5-a] piridinium Chloride 21

To a methanol solution (40 mL) of 5.0 g (32 mmol) of benzhydroxymoyl chloride **1**, 8.0 mL (67 mmol) of 6-chloro-2-picoline were added and the mixture was cooled at 0 °C with an ice-bath. Triethylamine (30 mmol, 4.8 mL) dissolved in 20 mL methanol was added dropwise under stirring and the solution turns from colourless to pale yellow. The reaction is left under stirring overnight; after this period of time, methanol is removed at reduced pressure and the residue is taken up with benzene to dissolve the picoline excess. Upon filtration, the crude salt **21** is obtained along with the triethylamine hydrochloride. Purification of **21** is secured by dissolving the salt mixture with chloroform and leaving to crystallized the pyridinium salt **21** in colourless crystals. The product was submitted to complete characterization.

3-Phenyl-5-methyl-1,2,4-Oxadiazole[4,5-a]piridinium chloride ***21***, 4.74 g (60 %), colourless crystals from ethanol, m.p. 205 °C (dec.). IR: $\nu_{C=N}$ 1643 cm^{-1}. ^{1}H-NMR (DMSO) δ: 2.02 (s, 3H, CH_3); 7.85 (m, 2H, phenyl); 7.90 (m, 1H, phenyl); 8.03 (d, 2H, phenyl); 8.12 (t, 1H, J = 6 Hz, pyridine); 8.83 (d, 1H, J = 9 Hz, pyridine); 8.91 (t, 1H, J = 7 Hz, pyridine). ^{13}C-NMR (DMSO) δ: 18.5; 110.4; 122.7; 125.2; 128.8; 130.0; 130.7; 134.0; 147.6; 155.5; 161.2. Anal. Calcd for $C_{13}H_{11}N_2OCl$ (246.70): C, 63.29; H, 4.49; N, 11.36. Found: C, 63.28; H, 4.48; N, 11.37.

Synthesis of the 1-[(hydroxyimino)(phenyl) methyl]-6-methylpyridin-2(1H)-one 22

To a solution of 10 mg (0.43 mmol) of the salt **21** in 20 mL of water, 4 mL of $NaHCO_3$ 5 % were added. The reaction was left under stirring at room temperature and after few minutes the pyridine **22** starts separating off the solution. After one day, the solid is filtrated, dried and characterized.

1-[(Hydroxyimino)(phenyl)methyl]-6-methylpyridin-2(1H)-one ***22***, 0.10 g (98 %), colourless crystals from water, m.p. 217 °C (dec.). IR: $\nu_{C=O}$ 1705, $\nu_{C=N}$ 1642 cm^{-1}. ^{1}H-NMR (DMSO) δ: 2.02 (s, 3H, CH_3); 6.27 (d, 1H, J = 6 Hz, pyridone); 6.33 (d, 1H, J = 9 Hz, pyridone); 7.45 (s, 6H, pyridone and phenyl); 12.07 (s, 1H, OH). ^{13}C-NMR (DMSO) δ: 18.5; 105.3; 117.3; 124.9; 129.0; 129.9; 131.4; 141.1; 144.7; 145.3; 160.6. Anal. Calcd for $C_{13}H_{12}N_2O_2$ (228.25): C, 68.41; H, 5.30; N, 12.27. Found: C, 68.43; H, 5.31; N, 12.25.

Reaction Between the Picolinium salt 21 and Ammonia

To a solution of the salt **21** (400 mg, 1.6 mmol) in 50 mL of chloroform, ammonia (gas) was bubbled. After 3 days the solution was evaporated, taken up with water and extracted with DCM. The organic phase was dried and the residue, obtained upon evaporation of the solvent, was submitted to chromatographic separation. 2-Amino-6-methylpiridine **23** was isolated in 40 % and found identical to a commercially available reference compound (Sigma-Aldrich A75706) along with the benzamidoxime **24** (37 %), also identical to a reference compound available in our laboratory.

Reaction Between the Picolinium Salt 21 and Ammonia in the Presence of Norbornene

To a solution of the salt **21** (400 mg, 1.6 mmol) in 50 mL of chloroform and excess norbornene, ammonia (gas) was bubbled. After 3 days the solution was evaporated, taken up with water and extracted with DCM. The organic phase was dried and the residue, obtained upon evaporation of the solvent, was submitted to chromatographic separation. 2-Amino-6-methylpiridine **23** was isolated in 30 % and found identical to a commercially available reference compound (Sigma-Aldrich A75706) along with the benzamidoxime **24** (5 %), also identical to a reference compound available in our laboratory. Finally, the cycloadduct of BNO to norbornene **25** was isolated in 49 % yield, identical to an authentic sample prepared in our laboratory according to the known procedures.[34]

Acknowledgements

Financial support by the University of Pavia, MIUR is gratefully acknowledged. We also thank "VIPCAT – Value Added Innovative Protocols for Catalytic Transformations" project (CUP: E46D17000110009) for valuable financial support.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: nitrile oxides · 1,3-dipolar cycloadditions · Zincke salts · oxadiazoles · dienamino derivatives

[1] G. Romeo, U. Chiacchio, *Modern Heterocyclic Chemistry*, J. Alvarez-Builla, J. J. Vaquero, J. Barluenga, Ed.; Wiley-VCH, Eds.: **2011**, pp. 1047–1252.
[2] S. Chhama, S. Sanchit, *J. Drug Delivery Ther.* **2015**, *5*, 8–13.
[3] M. Arshad, T. A. Khan, M. A. Khan, *Int. J. Pharm. Sci. Res.* **2014**, *5*, 303–316.
[4] A. P. Piccionello, P. Musumeci, C. Cocuzza, C. G. Fortuna, A. Guarcello, P. Pierro, A. Pace, *Eur. J. Med. Chem.* **2012**, *50*, 441–448.
[5] C. Lueg, D. Schepmann, R. Gunther, E. K. Alexandros, E. L. Konstantinos, H.-L. Dimitra, C. F. Konstantina, *Bioorg. Med. Chem.* **2013**, *21*, 7481–7498.
[6] J. V. Dios Anjos, R. A. W. Neves Filho, S. C. do Nascimento, R. M. Srivastava, S. J. de Melo, D. Sinou, *Eur. J. Med. Chem.* **2009**, *44*, 3571–3576.
[7] M. Arshad, *Int. J. Pharm. Sci. Res.* **2014**, *5*, 1124–1137.
[8] B. Jayashankar, R. K. M. Lokanath, N. Baskaran, H. S. Sathish, *Eur. J. Med. Chem.* **2009**, *44*, 3898–3902.
[9] M. N. Purohit, C. Kunal, G. V. Pujar, Y. C. Mayur, S. M. Shantakumar, *Indian J. Heterocycl. Chem.* **2007**, *16*, 349–352.
[10] L.-L. Xu, J.-F. Zhu, X.-L. Xu, J. Zhu, L. Li, M.-Y. Xi, Z.-Y. Jiang, M.-Y. Zhang, F. Liu, M. Lu, Q.-C. Bao, Q. Li, C. Zhang, J.-L. Wei, X.-J. Zhang, L.-S. Zhang, Q.-D. You, H.-P. Sun, *J. Med. Chem.* **2015**, *58*, 5419–5436 and references therein.
[11] C. Grundman, P. Grünanger, *The Nitrile Oxides* Springer, Heidelberg, **1971**.
[12] A. Corsaro, G. Perrini, P. Caramella, F. Marinone Albini, T. Bandiera, *Tetrahedron Lett.* **1988**, *44*, 4917–4925.
[13] F. Marinone Albini, R. De Franco, T. Bandiera, P. Grünanger, P. Caramella, *Gazz. Chim. Ital.* **1990**, *120*, 1–7.
[14] P. Caramella, A. Gamba Invernizzi, E. Pastormerlo, P. Quadrelli, A. Corsaro, *Heterocycles* **1995**, *40*, 515–520.
[15] F. De Sarlo, *J. Chem. Soc. Perkin Trans I* **1974**, 1951–1953.
[16] F. De Sarlo, A. Guarna, *J. Chem. Soc. Perkin Trans II* **1976**, 626–628.
[17] For the synthesis of 1,2,4-oxadiazole-4-oxides see: a) P. Quadrelli, A. Gamba Invernizzi, M. Falzoni, P. Caramella, *Tetrahedron* **1997**, *53*, 1787–1796; b) P. Quadrelli, P. Caramella, *Curr. Org. Chem.* **2007**, *11*, 959–986.
[18] F. Eloy, R. Lenaers, *Chem. Rev.* **1962**, *62*, 155–183.
[19] P. von Ragué Schleyer, J. I. Wu, F. P. Cossio, I. Fernandez, *Chem. Soc. Rev.* **2014**, *43* 4909–4921.
[20] Th. Zincke, *Justus Liebigs Ann. Chem.* **1903**, *330*, 361–374.
[21] T. Focken, A. B. Charette, *Org. Lett.* **2006**, *8*, 2985–2988.
[22] A. M. Kearney, C. D. Vanderwal, *Angew. Chem. Int. Ed.* **2006**, *45*, 7803–7806; *Angew. Chem.* **2006**, *118*, 7967–7970.
[23] S. Kunugi, T. Okubo, N. Ise, *J. Am. Chem. Soc.* **1976**, *98*, 2282–2287.

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[24] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, *Gaussian 09* (Gaussian, Inc., Wallingford CT, 2009).
[25] A. Seegerer, J. Hioe, M. M. Hammer, F. Morana, P. J. W. Fuchs, R. M. Gschwind, *J. Am. Chem. Soc.* **2016**, *138*, 9864–9873.
[26] W.-C. Cheng, M. J. Kurth, *Org. Prep. Proced. Int.* **2002**, *34*, 585–608.
[27] C. D. Vanderwal, *J. Org. Chem.* **2011**, *76*, 9555–9567.
[28] M. Kathiresan, H.-J. Steinhoff, L. Walder, *Macromol. Chem. Phys.* **2017**, *218*, n/a.
[29] G. Das, T. Skorjanc, S. K. Sharma, F. Gandara, M. Lusi, D. S. Shankar Rao, S. Vimala, S. Krishna Prasad, J. Raya, D. Suk Han, R. Jagannathan, J.-C. Olsen, A. Trabolsi, *J. Am. Chem. Soc.* **2017**, *139*, 9558–9565.
[30] O. Domarco, I. Neira, T. Rama, A. Blanco-Gomez, M. D. Garcia, C. Peinador, J. M. Quintela, *Org. Biomol. Chem.* **2017**, *15*, 3594–3602.
[31] R. V. Shchepin, D. A. Barskly, D. M. Mikhaylov, E. Y. Chekmenev, *Bioconjugate Chem.* **2016**, *27*, 878–882.
[32] C. Grundmann, P. Grünanger, *The Nitrile Oxide* Springer-Verlag: Heidelberg, **1971**.
[33] For further information see the URL via the Internet at: http://www.niaid.nih.gov.
[34] A. Corsaro, G. Perrini, V. Pistarà, P. Quadrelli, A. Gamba Invernizzi, P. Caramella, *Tetrahedron* **1996**, *52*, 6421–6436.

Manuscript received: July 10, 2019
Revised manuscript received: July 31, 2019

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