Communication



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Posted Date: 10 September 2024

doi: 10.20944/preprints202409.0838.v1

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# Synthesis and Characterization of *N*-Nitrososydnonimine as NO. Donor

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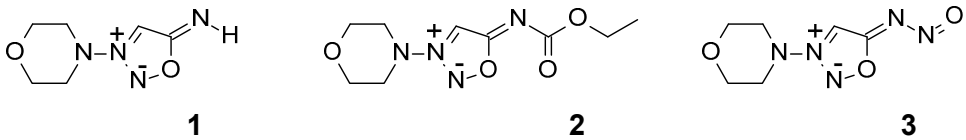
**Abstract:** *N*-Nitroso-3-morpholinosydnonimine **3** was prepared by nitrosation of SIN-1 and characterized by $^{1}$H-NMR, $^{13}$C-NMR and HRMS. Its structure, confirmed by single crystal X-ray diffraction analysis, was found in agreement with its mesoionic and aromatic character. Unlike SIN-1, which releases both nitric oxide and superoxide radical, decomposition of this nitrosylated sydonimine could yield nitric oxide as the only decomposition product, and thus without the formation of toxic peroxynitrite.

**Keywords:** sydnone; sydnonimine; imine; nitric oxide; SIN-1

## 1. Introduction

Sydnones are mesoionic dipolar heterocyclic chemical compounds with an 1,2,3-oxadiazole ring, which satisfy valence rules only if two atoms are assumed to carry formal, opposite charges delocalized across the ring [1]. They constitute an important class of molecules which possesses interesting chemical and biological properties, and, in recent years, sydnones are used for their capacity to undergo [3+2] cycloaddition reactions with terminal- and cyclo-alkynes [2]. Sydnonimines (or sydnone imines), such as 3-morpholino-sydnonimine (SIN-1, **1**, Figure 1), are a class of sydnone compounds which generate simultaneously both nitric oxide radical ($NO^{\cdot}$) and superoxide radical ($O_2^{\cdot-}$) in neutral oxygenated aqueous media [3]. As nitric oxide and superoxide radicals recombine in a near diffusion-controlled reaction to give the potent oxidant peroxynitrite anion ($ONOO^{-}$), known to decompose into highly reactive species such as carbonate, nitrogen dioxide or hydroxyl radicals reacting with a number of biological targets [4], sydnonimines are considered as peroxynitrite releasing molecules. In some cases, SIN-1 has been shown to be converted from a peroxynitrite donor in aerobic solutions, to a pure $NO^{\cdot}$ donor in vivo without concomitant superoxide production, probably by a mechanism involving one-electron oxidation of SIN-1 by heme proteins or other electron acceptors in biological systems, instead of molecular oxygen [5].



**1** **2** **3**

**Figure 1.** Molecular structures of SIN-1 (**1**), Mosildomine (**2**) and of *N*-nitrosomorpholinosydnonimine (**3**).

Sydnonimines are known to possess various pharmacological activities related to NO-release [6], and are an alternative to organic nitrates in the treatment of cardiovascular diseases, the use of which is often limited by the development of nitrate tolerance [7]. The release of nitric oxide from

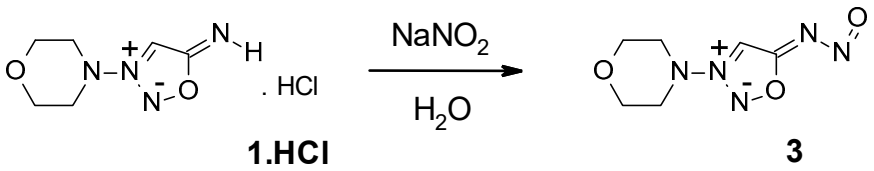
 doi:10.20944/preprints202409.0838.v1



sydnonimines proceeds either spontaneously, or after enzymatic conversion and subsequent decarboxylation to SIN-1, as in the case of Molsidomine, (*N*-ethoxycarbonyl-3-morpholino-sydnonimine 2, Figure 1) [8]. Here we present the synthesis, the characterization and the X-ray structure of an *N*-nitrosylated sydnonimine, *N*-nitroso-3-morpholinosydnonimine (compound **3**, Figure 1), whose decomposition could yield nitric oxide, and not superoxide, as decomposition product, without the formation of toxic peroxynitrite.

## 2. Results and Discussion

*N*-Morpholinosydnonimine hydrochloride (SIN-1.HCl) was synthesized as previously described from *N*-aminomorpholine and sodium formaldehyde bisulfite as starting material [9]. The imine intermediate was treated with potassium cyanide to give the corresponding nitrile compound, which was then nitrosated to yield a nitrosohydrazine. Cyclisation of the latter under acidic conditions gave SIN-1 hydrochloride with a yield of 50%. *N*-Nitroso-3-morpholinosydnonimine **3** was then obtained by a simple procedure by treatment of SIN-1 hydrochloride with sodium nitrite in water [10] (Scheme 1).




**Scheme 1.** Synthesis of *N*-nitrosomorpholinosydnonimine (**3**)§. § It should be noted that the preparation of compound **3** has been briefly reported (see reference 10), but no spectral or spectrometric characterization has been carried out.

Crystallization from methanol gave suitable crystals for X-ray analysis and all other spectral characterizations. Compound **3** crystallizes in the monoclinic space group $P2_1/n$ with one molecule in the asymmetric unit (Figure 2 and ESI for crystallographic data). The overall structure and the planar geometry of the oxadiazole ring atoms, as expected, is close to that for some already reported crystallographic structures of sydnone et sydnonimine [11], and in agreement with the aromaticity character of the mesoionic ring. The morpholine ring is in a typical chair conformation, and the plane defined by the four carbons of this ring forms an angle of 20.41° with that of oxadiazole. The exocyclic *N*-nitroso -N-N=O group does not align with the oxadiazole ring, but is slightly offset at an angle of 11.01°. As shown in Figure 1, the bond between oxazole-C5 and the exocyclic nitrogen atom of the *N*-nitroso group is written as a double bond, but the X-ray structure reveals a distance of 1.353 Å between these two atoms, *i.e.* a distance that corresponds more to that of a single bond between an aromatic carbon and a nitrogen ($C_{sp^2}$-*N* as found in aniline for example), than to a double bond like those found in imines, for example (1.279 Å). The overall 3D packing of compound **3** does not display intermolecular hydrogen bonds, but shows an ordered short contact network within the packing of molecules in the unit cell, in particular between oxygen atom of the nitroso group and morpholine protons (2.469, 2.474 and 2.480 Å) and the oxazole proton (2.328 Å). The $^{1}$H-NMR spectrum of **3** displayed two multiplets at 3.69 and 3.87 ppm attributed to the four morpholin protons, and a characteristic deshielded signal (singlet at 9.07 ppm) corresponding to the proton of the sydnone ring. Two signals at 53.24 and 64.66 ppm corresponding to the four morpholin carbons were observed in the $^{13}$C-NMR spectrum, as well as two others very weak and deshielded signals at 103.58 and 129.64 ppm, corresponding to the tertiary (-N-*C*-H) and quaternary (-O-*C*-N-) carbons of the mesoionic sydnone ring, respectively. These high chemical shift values are in agreement with the delocalization of the negative charge within the 1,2,3-oxadiazole ring system, and in accordance with chemical shifts of analogous sydnones.

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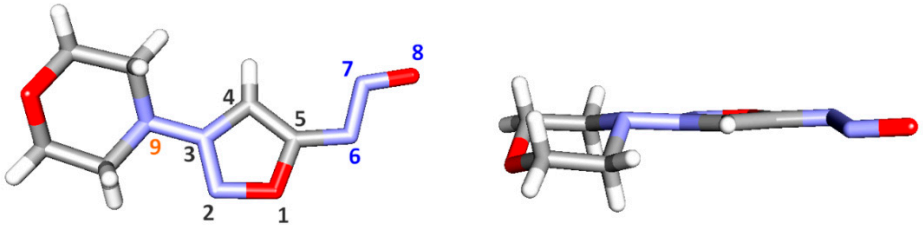


**Figure 2.** Two different views from X-ray structure of compound **3** and atom numbering (color code: C: gray, H: white, N: blue, O: red). See Table 1 (ESI) for crystal data (the cif file –CCDC 2381843– can be obtained free of charge from the Cambridge Crystallographic Data Center via https://www.ccdc.cam.ac.uk/).

The UV absorption spectrum of compound **3** in water showed two maximum absorptions at 320 and 251 nm. It remained stable in water, as shown by the uv spectrum recorded after several hours, but was shown to decompose in a first order manner in basic aqueous solution (NaOH) with half-time of 90 min and 16 min in 2.5 mM and 12.5 mM NaOH solution, respectively ($k_{OH^-}$ = 3.5 $M^{-1}.min^{-1}$) (Figure 3).

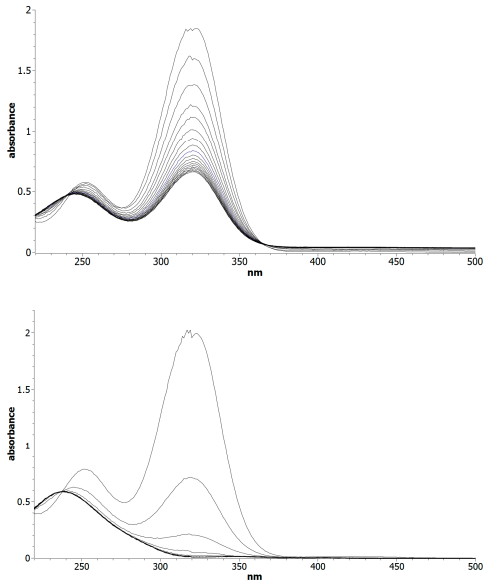


**Figure 3.** Repeated UV-visible spectral scans (30 min intervals) of decomposition of compound **3** (100 μM) in aqueous solution of NaOH 2.5 mM (left) and 12.5 mM (right).

The HRMS spectrum exhibited the [M+H$^+$]–ion at *m*/*z* 200.0796, corresponding to **3**-H$^+$ of molecular formula $C_6H_{10}N_5O_3$. However, the main pic in the mass spectrum was at *m*/*z* 140.0824 corresponding to formula $C_6H_{10}N_3O$, *i.e.* that of protonated SIN-1C [12], the known product of SIN-1-decomposition. Indeed, it has been shown that SIN-1 first spontaneously decomposes through ring opening into intermediate SIN-1A, which, in the presence of molecular oxygen, fragments to yield SIN-1C, nitric oxide, and superoxide as final products (Scheme 2A). It is therefore reasonable to assume that SIN1-C, product of decomposition of SIN-1 and also product of the decomposition of **3** under the conditions of mass spectrometry analysis, could also be the final product of decomposition of the latter in aqueous media. On theses bases, a postulated mechanism for the decomposition of sydnonimine **3** is proposed on Scheme 2B: a tautomeric form of **3** undergoes a ring opening to yield a *N*,*N*'-dinitroso intermediate (**3a**), which then releases through homolytic cleavage two NO· molecules and SIN-C. In that case, compound **3** would thus generate nitric oxide as sole radical product, and not superoxide as in the case of SIN-1 decomposition.

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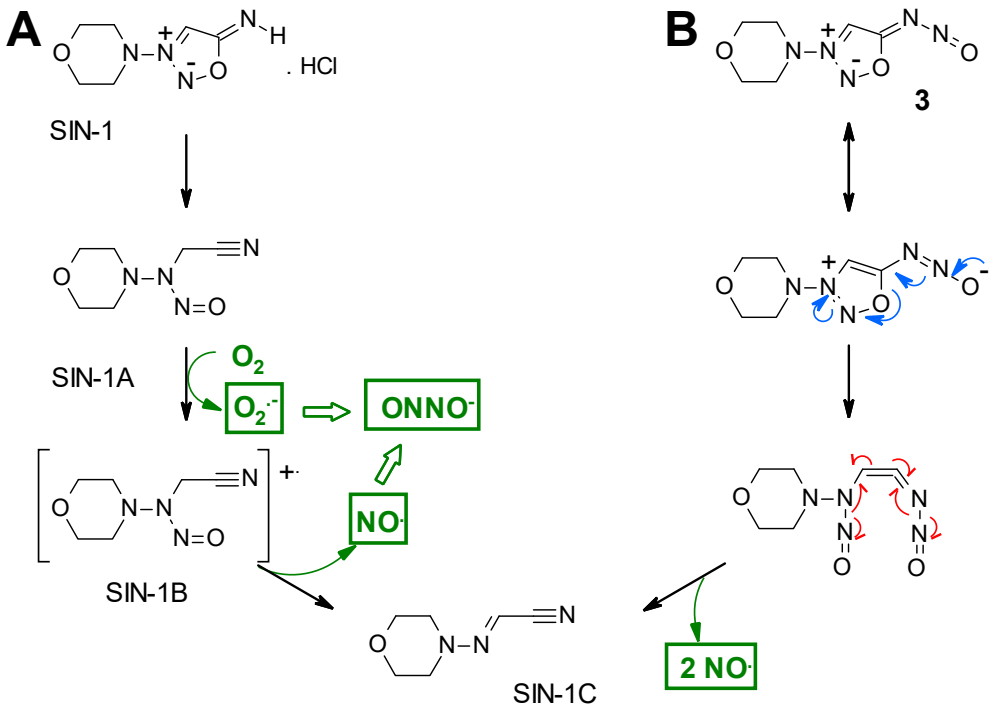


**Scheme 2. A.** Decomposition mechanism of SIN-1 to peroxynitrite [12]. **B.** Postulated mechanism of decomposition of *N*-nitroso-3-morpholinosydnonimine **3** to NO.

## 3. Materials and Methods

All reagents and solvents were purchased from commercial sources (Sigma Aldrich or Alfa Aesar) and were used without further purification. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on Bruker Advance 300 and 500 spectrometers, respectively, and the residual proton signals of deuterated DMSO were used as an internal reference (δ = 2.5 ppm and 39.52 ppm, respectively). Proton coupling patterns are abbreviated as "s" for singlet and "m" for multiplet. The high-resolution mass spectrum (HRMS) was recorded on a MAT 95XLspectrometer (ThermoFisher, Waltham, MA, USA). SIN-1 hydrochloride was synthesized from *N*-aminomorpholine and sodium formaldehyde bisulfite as previously described [9].

***N*-Nitroso-3-morpholinosydnonimine 3** – To an ice-cooled solution of SIN-1 hydrochloride (0.5 g, 2.4 mmol) in water (2.5 mL) was added a solution of sodium nitrite (0.2 g, 2.8 mmol) in water (7 mL), and the mixture was stirred for 6 hours at 0 °C and left at room temperature overnight. The solid formed was isolated by filtration on fritted-glass, washed with cold methanol to give 0.35 g of a yellow solid (yield 73 %). Recrystallization from methanol gave pure yellow crystals of compound **3** suitable for X-ray crystallography. HRMS Calculated for $C_6H_{10}N_5O_3$ (DCI-$CH_4$, M+H$^+$): 200.0784. Found: 200.0796. $^{1}$H-NMR (500 MHz, DMSO-$D_6$): δ ppm 3.69 (m, 4H), 3.88 (m, 4H), 9.07 (s, 1H). $^{13}$C-NMR (125.8 MHz, DMSO-$D_6$): δ ppm 53.24, 64.66, 103.58, 129.64.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: $^{1}$H-NMR spectrum of compound **3**; Figure S2: $^{13}$C-NMR spectrum of compound **3**; Figure S3: HRMS spectrum of compound **3**; Tables S1-S6: Crystal data, atomic coordinates, bond lengths and angles, anisotopic displacement parameters, hydrogen coordinates and torsion angles for compound **3**.

**Author Contributions:** NSM: X-Ray analysis; CL: Conceptualization, Investigation, Writing – review & editing; PH: Investigation, Conceptualization, Writing – original draft, Writing – review & editing.

**Funding:** This research was funded by the Centre Nationnal de la Recherche Scientifique (CNRS) and Toulouse 3 University.

**Conflicts of Interest:** The authors declare no conflicts of interest.

 doi:10.20944/preprints202409.0838.v1



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