Photoactivated Nitroxyl Donors

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Rapid Photoactivated Generation of Nitroxyl (HNO) under Neutral pH Conditions

Yang Zhou, Ruth B. Cink, Rohan S. Dassanayake, Alexander J. Seed, Nicola E. Brasch, and Paul Sampson**

Abstract: *Directly obtaining kinetic and mechanistic data for the reactions of nitroxyl (HNO) with biomolecules ($k \approx 10^3$–$10^7\ M^{-1}s^{-1}$) is not feasible for many systems because of slow HNO release from HNO donor molecules ($t_{1/2}$ is typically minutes to hours). To address this limitation, we have developed a photoactivatable HNO donor incorporating the (3-hydroxy-2-naphthalenyl)methyl phototrigger, which rapidly releases HNO on demand. A "proof of concept" study is reported, which demonstrates that, upon continuous xenon light excitation, rapid decomposition of the HNO donor occurs within seconds. The amount of HNO generated is strongly dependent on solvent and the rate of the reaction is dependent on the light intensity.*

Nitroxyl (HNO, nitrosyl hydride) is attracting increasing attention because it has chemical and biological properties distinct from the more widely studied nitric oxide (NO).[1] Furthermore HNO prodrugs show considerable promise as congestive heart failure therapeutics.[2] However, understanding the biological roles of HNO has been hindered by its propensity to dimerize rapidly in aqueous solution ($k = 8 \times 10^6\ M^{-1}s^{-1}$).[3] Thus, precursor molecules (HNO donors) are required to generate HNO in situ for chemical and biological studies.

Many HNO donors have been reported,[4] including Angeli's salt (AS), Piloty's acid (PA) and related derivatives,[5] *N*-substituted hydroxylamines,[6] primary amine-based diazeniumdiolates,[7] acyloxy nitroso compounds,[8] precursors of acyl nitroso species,[9] and metal nitrosyls.[10] Although HNO donors are widely used in biochemical studies,[4,9d] factors limiting their utility include requiring alkaline ($pH > 9$) conditions to trigger HNO release,[4a,11] and NO release at neutral pH conditions.[7c,8a,9b,c] Most importantly, in terms of mechanistic studies of HNO with biomolecules, HNO generation is invariably the rate-determining step,[12] since HNO reacts rapidly with biomolecules ($k \approx 10^3$–$10^7\ M^{-1}s^{-1}$)[13] but is typically released more slowly ($t_{1/2}$ ca. minutes to hours). Therefore, it is of great interest to develop HNO donors that rapidly release HNO upon demand for kinetic and mechanistic studies of HNO's most important reactions.

Photolysis has been widely used for rapid in situ generation of molecules of biological relevance.[14] To date, a limited number of photocontrollable HNO donors have been reported based on acyl nitroso intermediate formation by a retro-Diels–Alder reaction[9a–d] or the retrocycloaddition of a 1,2,4-oxadiazole-4-oxide.[9a,e,f] Limitations of these HNO donors include photodecomposition in ambient light,[15] and/or competition from NO formation,[9b–d] background thermal reactions, and secondary photocleavage processes.[16]

Herein, we present the first in a new family of photoactivatable *N*-alkoxysulfonamide HNO donors, **1**, incorporating the well-characterized *O*-(3-hydroxy-2-naphthalenyl)-methyl (HNM) photolabile protecting group. The HNM group was selected as the phototrigger because of its rapid photocleavage ($k_{release} \approx 10^5\ s^{-1}$ ($t_{1/2} \approx$ ms)) with good quantum and chemical yields.[17] The trifluoromethanesulfonamido moiety was chosen to facilitate HNO release by elimination of the excellent leaving group $CF_3SO_2^-$ ($pK_a(CF_3SO_2H) = -0.6$).[18] We hypothesized that rapid photo-uncaging of the HNM group from **1** would result in spontaneous release of HNO (Figure 1) with rapid hydration of the putative *o*-naphthoquinone methide intermediate.[17]

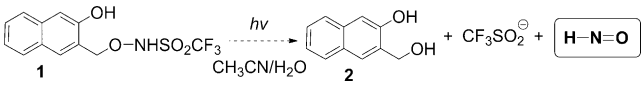

Figure 1. Proposed pathway for photoactivated HNO generation from target **1**.

Target **1** was successfully synthesized according to the route outlined in Scheme 1. After *O*-triisopropylsilyl (TIPS) protection of **3**, the resulting ester **4** was reduced using $LiAlH_4$. Bromodehydroxylation of the resulting alcohol **5** to give **6**, followed by S_N2 substitution using *N*-hydroxyphthalimide, afforded hydroxylamine adduct **7**. Deprotection to the free amine **8** followed by *N*-trifluoromethanesulfonation afforded the *O*-TIPS protected intermediate **9**. Finally, *O*-TIPS deprotection using KF gave target **1**. Importantly, compound **1** is stable to ambient light in both the solid state and in solution (Supporting Information, Figure S1), and is easily handled in air.

Upon photolysis of **1** in phosphate buffer/CH_3CN using a stopped-flow instrument with a xenon light source under strictly anaerobic conditions (Figure 2), **1** decomposed with

[*] Y. Zhou, Dr. R. S. Dassanayake, Prof. A. J. Seed, Prof. P. Sampson
Department of Chemistry and Biochemistry
Kent State University (KSU)
Kent, OH 44240 (USA)
E-mail: psampson@kent.edu
R. B. Cink, Prof. N. E. Brasch
School of Applied Sciences, Auckland University of Technology (AUT)
Private Bag 92006, Auckland 1142 (New Zealand)
E-mail: nbrasch@aut.ac.nz
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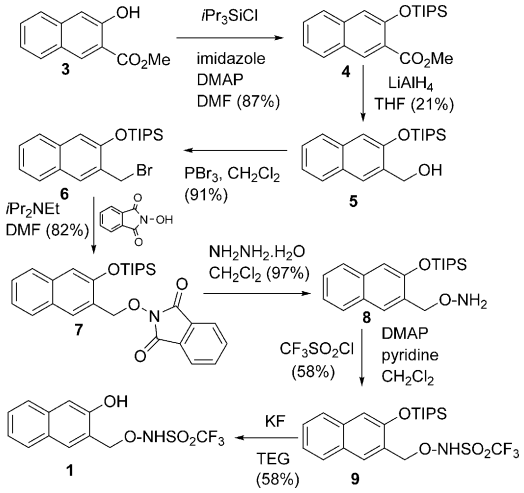

Scheme 1. Synthesis of HNO donor **1**.

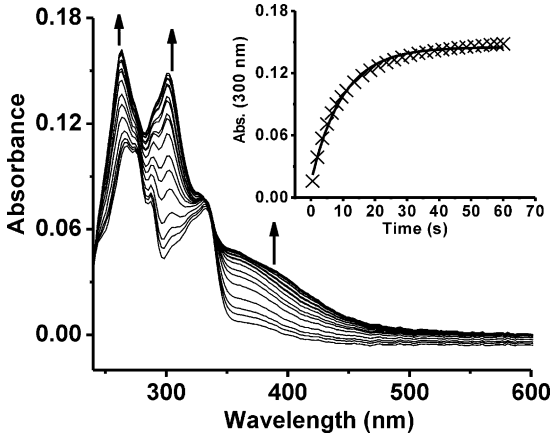

Figure 2. UV/Vis spectra for the photodecomposition of **1** (80.0 μM) using a xenon light (150 W, no monochromator) in an anaerobic mixture of phosphate buffer (5.0 mM, pH 7.00) and CH_3CN (40:60 v/v) at 25 °C. Inset: fit of absorbance data (300 nm) vs. time to a first-order rate equation, giving $k_{obs} = (9.7 \pm 0.1) \times 10^{-2}\ s^{-1}$. Note: Fitting the data to a first-order equation is an approximation only, since multiple reactions occur.

an observed first-order rate constant, $k_{obs} = (9.7 \pm 0.1) \times 10^{-2}\ s^{-1}$ ($t_{1/2} \approx 7$ s; inset to Figure 2). Reduction of the light intensity resulted in slower photodecomposition ($k_{obs} = (1.4 \pm 0.1) \times 10^{-2}\ s^{-1}$; $t_{1/2} \approx 50$ s; Supporting Information, Figure S2), showing that the decomposition of **1** is dependent on the intensity of the light source.

To unambiguously establish HNO generation upon irradiation of **1**, aquacobalamin ($H_2OCbl(III)^+$) was utilized as an HNO trapping agent.[12b] Control experiments showed that $H_2OCbl(III)^+$ reacts stoichiometrically with HNO to form nitroxylcobalamin (NO^--Cbl(III); phosphate buffer/CH_3CN, 40:60 v/v; Supporting Information, Figure S3) and both $H_2OCbl(III)^+$ and NO^--Cbl(III) are photostable under these experimental conditions (Supporting Information, Figure S4). Importantly, no reaction is observed between $H_2OCbl(III)^+$ and **1** in ambient light (Supporting Information, Figure S5); hence, HNO release from **1** can be initiated on

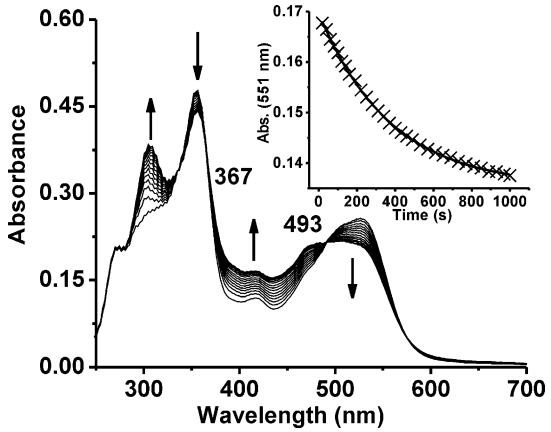

Figure 3. UV/Vis spectra for the reaction between $H_2OCbl(III)^+$ (30.0 μM) and **1** (80.0 μM) using xenon light (150 W, monochromator slit width = 3.0 mm) in an anaerobic mixture of phosphate buffer (5.0 mM, pH 7.00) and CH_3CN (40:60 v/v) at 25 °C. Inset: fit of absorbance data (551 nm) vs. time to a first-order rate equation, giving $k_{obs} = (2.7 \pm 0.1) \times 10^{-3}\ s^{-1}$. Note: The stopped flow instrument has a maximum data collection time of 1000 s.

demand. Upon photolysis of **1** in the presence of $H_2OCbl(III)^+$ (Figure 3), clean isosbestic points were observed at 367 and 493 nm, characteristic for the conversion of $H_2OCbl(III)^+$ to NO^--Cbl(III) (Supporting Information, Figure S6). Fitting the absorbance data at 551 nm to a first-order rate equation gave $k_{obs} = (2.7 \pm 0.1) \times 10^{-3}\ s^{-1}$ ($t_{1/2} \approx 4.3$ min; inset to Figure 3). As expected, the formation of NO^--Cbl(III) is considerably slower than decomposition of the HNO donor **1** under the same conditions ($1.4 \times 10^{-2}\ s^{-1}$), as a result of the inner filter effect from cobalamins.[19]

To probe our proposed photolytic pathway, the photolysis of **1** was monitored by ^{19}F NMR spectroscopy. $CF_3SO_2NH_2$ and $CF_3SO_2^-$ were generated in an approximately 39:61 ratio after 30 min of light exposure (Figure 4; Supporting Information, Figure S7). In addition to the anticipated diol **2**, 3-hydroxy-2-naphthaldehyde (**10**) was observed by ^1H NMR

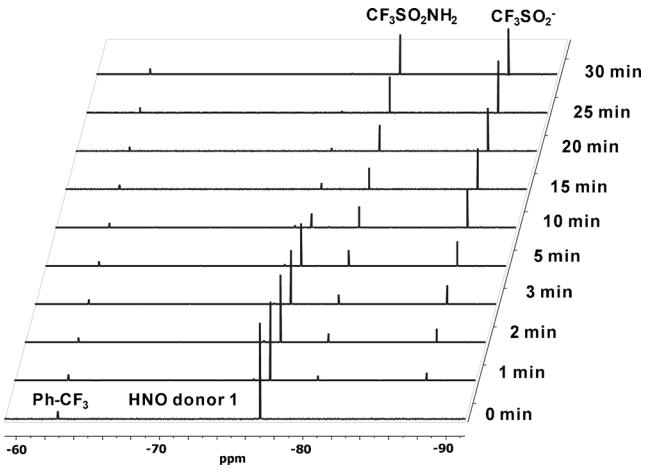

Figure 4. ^{19}F NMR spectra for the photodecomposition of **1** (3.89 mM, 350 nm, 4 W) in a mixture of phosphate buffer (0.10 M, pH 7.00) and CD_3CN (40:60 v/v).

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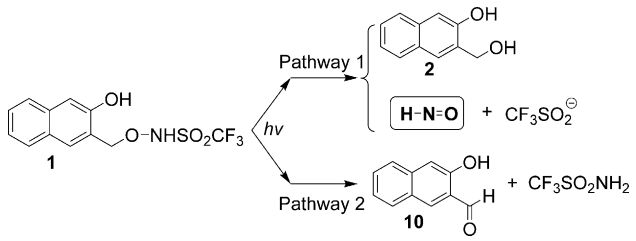
spectroscopy (Supporting Information, Figure S8). As shown in Scheme 2, the desired pathway leading to HNO generation (Pathway 1) was accompanied by a competing potentially solvent-assisted[20] O–N bond cleavage to form $CF_3SO_2NH_2$ and aldehyde **10**[21] (Pathway 2).



Scheme 2. Proposed pathways for the photolysis of HNO donor **1**.

Finally, to confirm that $CF_3SO_2^-$ formation directly infers HNO release, an equimolar solution (200.0 μM) of $H_2OCbl(III)^+$ and **1** was irradiated and the product solution analyzed (Supporting Information, Figure S9). The donor was only partially photodecomposed, to minimize additional unwanted chemistry arising from excitation of the products. The remaining unreacted $H_2OCbl(III)^+$ was subsequently converted to NO^--Cbl(III) by the addition of excess (2.5 mol equiv) Angeli's salt, an established HNO donor. The amount of NO^--Cbl(III) (equivalent to the amount of HNO; estimated as 10 ± 2%, based on an analysis of UV/Vis spectral changes) and $CF_3SO_2^-$ (16 ± 3%, based on the ^{19}F NMR spectrum of the product mixture) are in reasonable agreement (within experimental error; Supporting Information, Section 9) and are consistent with Scheme 2. HNO generation was also confirmed using TXPTS (Supporting Information, Section 11). Interestingly, $CF_3SO_2^-$ formation (and hence HNO generation) from **1** was solvent-dependent (Figure 5). A maximum of approximately 70% $CF_3SO_2^-$ was observed in a 5:95 v/v mixture of phosphate buffer and CD_3CN. Only a small amount of $CF_3SO_2^-$ (ca. 4%) was generated in neat CD_3CN, consistent with a mechanism involving excited-state deprotonation of the naphthol OH group by water.[22]

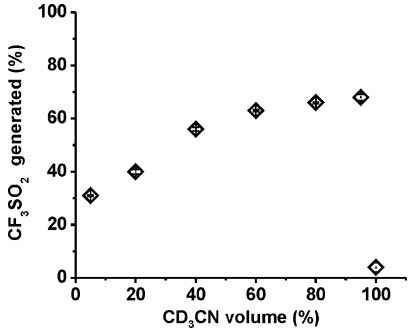


Figure 5. Solvent effect on the percentage of $CF_3SO_2^-$ generated (equivalent to the percent of HNO released, Scheme 2) upon irradiation of **1** (0.97 mM, 350 nm, 4 W) in a mixture of phosphate buffer (0.10 M, pH 7.00) and CD_3CN (v/v). Note: Data is the mean obtained from three independent measurements with a < 2% standard error.

In summary, the first example of a new class of photoactivated HNO donors incorporating the HNM phototrigger has been synthesized. Release of HNO does not occur in ambient light. It is anticipated that laser-induced HNO release from **1** will be extremely rapid.[17] Importantly, separate experiments on the rate of HNO release from $CF_3SO_2NHO(H)$ under the same experimental conditions show that this species is not photoactive and is unlikely to be an intermediate in the photolysis of **1**.[23] A concerted mechanism seems likely, although additional studies are required to further probe this question. Detailed kinetic and mechanistic studies using laser flash photolysis are currently underway. It is anticipated that this new class of HNO donors, which release HNO upon demand, will allow direct acquisition of kinetic and mechanistic data for biologically relevant reactions of HNO.

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Keywords: HNO donors · nitrosyl hydride · nitroxyl · photochemistry · photolysis

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Communications

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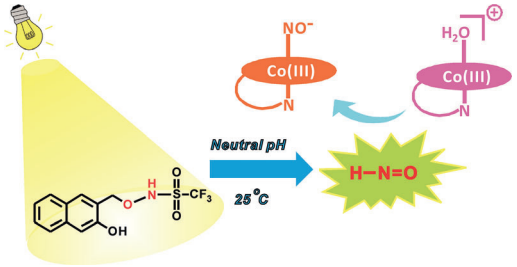
Rapid Photoactivated Generation of Nitroxyl (HNO) under Neutral pH Conditions



Lights, camera, action! The first example of a new class of photoactivatable nitroxyl (HNO) donors is presented. Under xenon light excitation, decomposition of the HNO donor occurs within seconds under neutral pH conditions. This class of HNO donors provides a platform for exploring the mechanisms of action of HNO in chemical and biochemical studies.

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