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Optimization of HNO Production from *N,O-bis*-Acylated Hydroxylamine Derivatives

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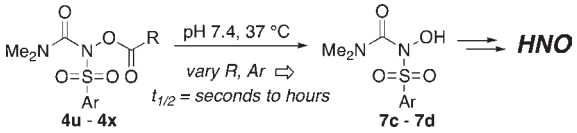
ABSTRACT



A wide range of *N,O-bis*-acylated hydroxylamine derivatives with chloro or arenesulfonyl leaving groups, and a related set of *N*-hydroxy-*N*-acylsulfonamides, have been synthesized and evaluated for nitroxyl (HNO) production. Mechanistic studies have revealed that the observed aqueous chemistry is more complicated than originally anticipated, and have been used to develop a new series of efficient HNO precursors (4u–4x, 7c–7d) with tunable half-lives.

Recent research has shown that nitroxyl (HNO), the one-electron reduced and protonated relative of nitric oxide (NO), has important and unique biological activity, especially as a potential alternative to current treatments of cardiac failure.[1−4] HNO is a reactive molecule (especially with thiols) that spontaneously dimerizes and subsequently dehydrates to form nitrous oxide (N_2O). Thus, in order to study HNO chemistry or biology, donor molecules for the generation of HNO *in situ* are required; however, the range of HNO donor molecules suitable for use under physiologically relevant conditions is currently quite limited.[5,6] These include Angeli's salt ($Na_2N_2O_3$),[7−9] Piloty's acid derivatives ($ArSO_2NHOH$),[10,11] primary amine-based diazeniumdiolates,[12] acyloxy nitroso compounds,[13] and precursors to acyl nitroso compounds.[14−16]

Acyl nitroso (**AN**) compounds are transient electrophiles that react with nucleophiles, including water, to produce HNO. Notable among the strategies that have been employed to generate acyl nitroso compounds is the work of Nagasawa and co-workers, who examined a series

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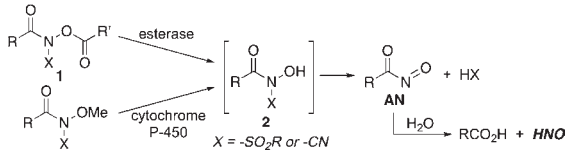
of esterase- and cytochrome P-450-activated precursors (Scheme 1).[17−24] These precursors, which were presumed to form *N*-hydroxy intermediate **2**, were used to demonstrate that HNO is a potent inhibitor of aldehyde dehydrogenase (AlDH) and has potential for the treatment of alcoholism. Indeed, Nagasawa also demonstrated that cyanamide (H_2NCN), an alcohol deterrent used clinically in Europe, Canada, and Japan, generates HNO following metabolic activation to an unstable *N*-hydroxycyanamide.[25−27]

Scheme 1. Enzymatically Activated Acyl Nitroso Precursors



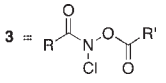
In addition, Fukuto, Nagasawa, and co-workers also reported the nonenzymatic production of HNO from several *N*,*O*-*bis*-acylated hydroxylamine derivatives **1**. Low HNO yields (< 15%, as determined by N_2O analysis) at neutral or basic pH were observed for *N*-cyano-*N*-benzoyloxybenzamide (**1**, X = CN, R,R′ = Ph) and also for *N*-4-chlorobenzenesulfonyl derivatives (**1**, X = 4-Cl-$PhSO_2$ with R,R′ = Me or with R = *t*-BuO and R′ = Me or Ph).[17,19,20] We wished to build upon these initial studies to optimize the nonenzymatic production of HNO at neutral pH from this class of precursors. Herein, we are pleased to report studies of a wide range of *bis*-acylated hydroxylamines **1** and also several *N*-hydroxy intermediates **2**, the latter of which have been synthesized and evaluated for HNO production for the first time. Mechanistic studies have revealed that the observed aqueous chemistry is more complicated than originally anticipated, and have been used to develop a new series of efficient HNO precursors with tunable half-lives.

We first examined compounds **1** with chloride as an alternative leaving group X. A series of *N*-chloro-*N*-acyloxyamides **3** were synthesized by chlorination of the corresponding *N*,*O*-*bis*-acylatated hydroxylamine with trichloroisocyanuric acid. These compounds were examined for HNO generation by gas chromatographic (GC) headspace analysis to quantify the amount of its dimerization product, N_2O, produced following decomposition in phosphate-buffered saline (PBS). (See Supporting Information (SI) for experimental details.)

Although low HNO yields are observed for all *N*-chloro compounds in PBS, yields are greatly enhanced when decompositions are carried out in deionized (DI) water (Table 1). The major organic byproduct observed in PBS is the corresponding *bis*-acylated hydroxylamine, formed by dechlorination of the precursor. We find that dechlorination is the dominant process in 3-(*N*-morpholino)-propanesulfonic acid (MOPS) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffers as well. *N*-Chloro compounds **3** have structures similar to those of the electrophilic chlorinating agents, *N*-chlorosuccinimide and trichloroisocyanuric acid, presumably making them susceptible to dechlorination.

Table 1. HNO Production from *N*-Chloro-*N*-acyloxyamides **3**

3 =



compd	R	R′	%HNO (PBS)[a]	%HNO (DI)[a]
3a	Ph	Ph	<1	22
3b	4-Cl-Ph	4-Cl-Ph	<1	24
3c	4-NO_2-Ph	4-NO_2-Ph	3	42
3d	Ph	4-NO_2-Ph	4	44
3e	Ph	2,6-F-Ph	[b]	36
3f	*t*-Bu	*t*-Bu	<1	28
3g	EtO	EtO	9	23
3h	Me	Me	11	60
3i	*t*-Bu	Me	4	36
3j	*t*-BuO	Me	29	74

[a] Donor compounds were incubated at 37 °C in PBS (pH 7.4) or DI water. HNO yields were determined from N_2O headspace analysis using a calibration standard following complete decomposition (SEM ± 5%; n = 3). [b] Not determined.

Given our observations that the use of *N*-chloro compounds **3** as HNO donors would be extremely limited, we focused on arenesulfonyl analogues **4** (Scheme 2), which were synthesized following modified literature procedures.[19] (See SI for experimental details concerning synthesis of all precursors.) Initially, we examined HNO generation in PBS from a series of *N*-arenesulfonyl-*N*-acetoxyacetamides **4a**−**4j**. An expected byproduct of HNO release from these compounds is sulfinic acid **5** (Scheme 2, Path 1a). HPLC analysis of the aqueous (PBS, pH 7.4) decomposition of derivative **4f** confirms sulfinic acid production (26%) that corresponds nicely to the HNO yield (26%) (Table 3). This analysis also indicates that the main non-HNO producing pathway is amide hydrolysis, producing an *O*-acylated Piloty's acid derivative **6** (Scheme 2, Path 2).

We were initially surprised to observe such facile hydrolysis of the amide functionality but realized that compounds **4** bear some similarity to *N*-alkyl-*N*-acylsulfonamides,

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Scheme 2. Reactivity of Potential HNO Precursors **4**

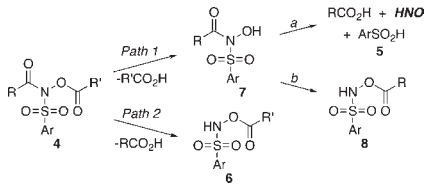

Table 2. HNO Production from *N*-Arenesulfonyl-*N*-acyloxamides **4**

compd[a]	R	R′	Ar	%HNO[b]
4a	Me	Me	Ph	2
4b	Me	Me	2,4,6-*i*-Pr-Ph	4
4c	Me	Me	4-Cl-Ph	3
4d	Me	Me	2-NO_2-Ph	10
4e	Me	Me	2-Cl-Ph	17
•4f	Me	Me	2-Br-Ph	26
4g	Me	Me	2-Br-4,6-F-Ph	29
4h	Me	Me	2,6-F-Ph	17
4i	Me	Me	2,6-Cl-Ph	42
4j	Me	Me	2,6-Br-Ph	53
4k	$CHCl_2$	Me	2,6-Cl-Ph	2
4l	Ph	Me	2,6-Cl-Ph	13
•4m	*i*-Pr	Me	2-Br-Ph	24
4n	*t*-Bu	Me	2-Br-Ph	<1
4o	EtO	Me	2,6-Cl-Ph	43
4p	BnO	Me	2,6-Cl-Ph	16
4q	*t*-BuO	Me	Ph	2
4r	*t*-BuO	Me	2-Br-Ph	5
4s	*t*-BuO	Me	2,6-Cl-Ph	3
4t	*t*-BuO	Me	2,6-Br-Ph	2
•4u	Me_2N	Me	2-Br-Ph	94
•4v	Me_2N	Me	Ph	99
•4w	Me_2N	CH_2Cl	Ph	99
•4x	Me_2N	*i*-Pr	Ph	97
AS			75	

[a] Compounds indicated with a bullet (•) are analyzed in more detail in Table 3. [b] Donor compounds were incubated at 37 °C in PBS (pH 7.4). HNO yields were determined from N_2O headspace analysis using a calibration standard following complete decomposition (SEM ± 5%; $n = 3$). The N_2O yield observed from Angeli's salt (**AS**) is provided for comparison. HNO was confirmed as the source of N_2O by complete quenching with added glutathione.

which are known to undergo facile hydrolysis.[28] In an attempt to inhibit this amide hydrolysis, we examined derivatives **4k**−**4x**, which contained either bulky substituents R (**4l**−**4n**), carbamate linkages (**4o**−**4t**), or carbamide linkages (**4u**−**4x**). Enhanced HNO production was observed only for urea derivatives **4u**−**4x** (Table 2), which will be discussed in more detail below.

HPLC decomposition analysis of derivative **4m** (R = *i*-Pr, R′ = Me) revealed *two O*-acylated Piloty's acid derivatives. The expected product of amide hydrolysis (**6**, R′ = Me) is detected in 58% yield along with a new product (**8**, R = *i*-Pr), detected in 19% yield (Table 3). This newly observed *O*-acylated Piloty's acid derivative is presumably derived via acyl migration from *N*-hydroxy intermediate **7** (Scheme 2, Path 1b) in another non-HNO producing pathway.

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Table 3. Decomposition Product Yields and Half-Lives of *N*-Arenesulfonyl-*N*-acyloxamides **4**

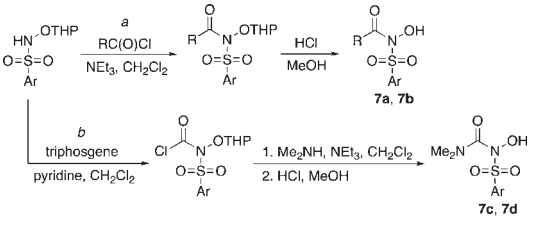
compd	%**5**[a]	%**6**[a]	%**8**[a]	$t_{1/2}$ (min)
4f	26	74	[b]	15
4m	23	58	19	31
4u	100[c]	0	0	52
4v	100[c]	0	0	65
4w	100[c]	0	0	<1
4x	100[c]	0	0	280

[a] Determined by HPLC and reported as relative yields (SEM ± 5%; $n = 3$). [b] In this case, byproducts **6** and **8** are identical. [c] Sulfinic acid **5** was the only HPLC detected product.

To confirm this proposed *N* to *O* acyl migration, we wished to examine the chemistry of *N*-hydroxy-*N*-acylsulfonamides **7** themselves. Although these species have been proposed as intermediates in the chemistry of *N*,*O*-*bis*-acylated hydroxylamine derivatives,[17−24] they have, to our knowledge, not been previously synthesized or evaluated for HNO production. We accomplished the synthesis of a series of *N*-hydroxy-*N*-acylsulfonamides **7** by either *N*-acylation and subsequent deprotection of an *N*-(tetrahydropyranyloxy)-arenesulfonamide to give **7a** (R = Me) or **7b** (R = *t*-Bu) (Scheme 3a) or, since the sulfonamide nitrogen could not be acylated directly using *N*,*N*-dimethylcarbamoyl chloride, a related sequence (Scheme 3b) to give **7c** or **7d**. These compounds can be handled and stored for short periods (days to weeks at −20 °C) but, as expected, readily decompose in aqueous solutions.

Decomposition analysis of **7a** (R = Me) revealed not only substantial HNO production (60%) along with a corresponding amount of sulfinic acid but also a significant amount (40%) of *O*-acylated Piloty's acid derivative **8** (R = Me), confirming potentially significant *N* to *O* acyl migration in these compounds (Table 4). Indeed, HPLC analysis of **7b** (R = *t*-Bu) indicates over 90% acyl migration demonstrating that even if amide hydrolysis were diminished in **4n** (R = *t*-Bu, R′ = Me), production of **7b** would result in very little HNO formation, as is observed (Table 2).

Scheme 3. Synthesis of *N*-Hydroxy-*N*-acylsulfonamides **7**



Given the high yields of HNO observed following decomposition of precursors **4u**–**4x**, we synthesized the related urea derivatives **7c**, **7d** and analyzed these compounds for HNO production. Acyl migration was not observed by HPLC in either case. **7c** cleanly decomposes to HNO and sulfinic acid **5** with a very short half-life ($t_{1/2}$ < 1 min) (Table 4). Precursor **7d**, which replaces the 2-Br-phenyl group in **7c** with an unsubstituted phenyl group, expectedly decomposes with a longer half-life ($t_{1/2}$ = 13 min). Again, essentially quantitative production of HNO and sulfinic acid is observed, but in this case a small amount of an additional intermediate, which decomposes over a slightly longer time frame, is detected by HPLC. This intermediate has been identified as acyl sulfone **9** (Ar = Ph) (Scheme 4) by coinjection with an authentic sample; we estimate that it is formed in < 5% yield.

Table 4. Decomposition Product Yields and Half-Lives of *N*-Hydroxy-*N*-acylsulfonamides **7**

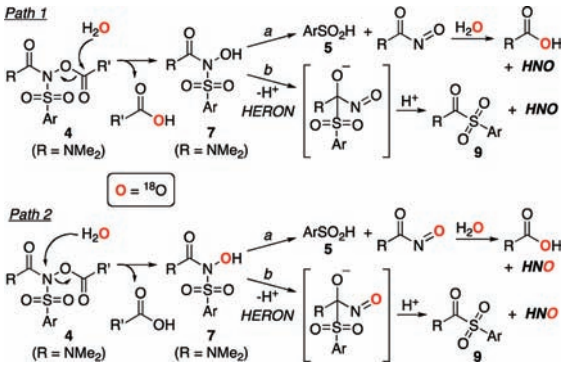
compd	R	Ar	%HNO[a]	%**5**[b]	%**8**[b]	$t_{1/2}$ (min)
7a	Me	2-Br-Ph	60	60	40	2
7b	*t*-Bu	2-Br-Ph	5	9	91	<1
7c	Me_2N	2-Br-Ph	96	100[c]	0	<1
7d	Me_2N	Ph	99	100[c]	0	13

[a] Donor compounds were incubated at 37 °C in PBS (pH 7.4). HNO yields were determined from N_2O headspace analysis using a calibration standard following complete decomposition (SEM ± 5%; n = 3). HNO was confirmed as the source of N_2O by complete quenching with added glutathione. [b] Determined by HPLC and reported as relative yields (SEM ± 5%; n = 3). [c] Sulfinic acid **5** was the only HPLC detected product.

Based on Glover's extensive studies of *bis*-heteroatom-substituted amides,[29] we propose that **9** forms along with HNO from **7d** via a HERON (heteroatom rearrangement on nitrogen) reaction as shown in Scheme 4, Path 1b (and 2b). Examination of **9** (Ar = Ph) itself demonstrates that in buffer (pH 7.4, 37 °C) it hydrolyzes cleanly to sulfinic acid **5** (Ar = Ph) with a $t_{1/2}$ of 22 min. A small amount of HERON product **9** is also observed by HPLC from decomposition of **4w**, which decomposes cleanly ($t_{1/2}$ < 1 min) to *N*-hydroxy compound **7d**, and ultimately HNO and sulfinic acid (Table 3).

Glover's work on *bis*-heteroatom-substituted amides also suggests that HNO production could be initiated by water attack at the amide nitrogen (Scheme 4, Path 2) in addition to the expected attack at the ester carbonyl (Scheme 4, Path 1). To probe this possibility, we examined the decomposition of precursor **4u** in ^{18}O-labeled water. Although somewhat complicated by the CO_2 formed from dissociation of the carbamic acid byproduct (Me_2NCO_2H), mass spectrometric analysis of the HNO-derived N_2O revealed very small amounts (<2%) of labeled N_2O (see SI), indicating an insignificant contribution from Scheme 4, Path 2.

Scheme 4. Potential HNO-Forming Reaction Pathways



Our mechanistic analysis of *N*,*O*-*bis*-acylated hydroxylamine derivatives **4a**–**4x** and related *N*-hydroxy compounds **7a**–**7d** has identified urea-based precursors **4u**–**4x** and **7c**–**7d** that avoid both amide hydrolysis (from **4**) and acyl migration (from **7**) and efficiently produce HNO. These experiments also indicate that Scheme 4, Path 1a is the major reaction pathway. The half-life of HNO generation at pH 7.4, 37 °C from these precursors can be tuned from seconds to hours by varying the ester R′ group and/or the sulfonyl leaving group (Tables 3, 4), providing flexibility in biological applications and the ability to probe the effects of either rapid or slow HNO production.

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Supporting Information Available. Experimental procedures, compound characterization data, and details concerning N_2O, HPLC, and UV–vis analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

(29) For a recent review, see: Glover, S. A. In *The Chemistry of Hydroxylamines, Oximes, and Hydroxamic Acids, Part 2*; Rappoport, Z., Liebman, J. F., Eds.; John Wiley & Sons: Chichester, West Sussex, 2009; pp 839–923.