# Fifty Years of Diazeniumdiolate Research. From Laboratory Curiosity to Broad-Spectrum Biomedical Advances

Larry K. Keefer
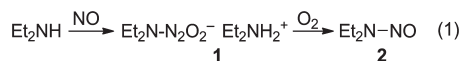
Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, Maryland 21702, United States

ABSTRACT: Here I show that a "pure" research project, seemingly totally lacking in practical application when it was first published, can years later spark a whole new scientific field with the potential to revolutionize clinical practice. A 1961 publication describing adducts of nitric oxide (NO) with certain nucleophiles attracted little notice at the time, but later work showing that the adducts could be hydrolyzed to regenerate the NO in bioactive form has provided the foundation for a host of biomedical applications. Crucial to the discovery of widely used tools for studying NO's chemical biology as well as for the design of a variety of promising therapeutic advances has been the increasingly detailed understanding of the physicochemical properties of these "diazeniumdiolates" (also known as NONOates).
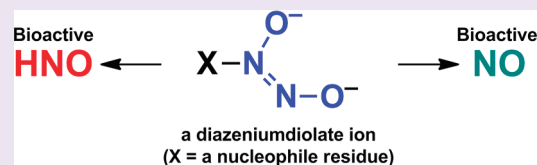
■ BASIC RESEARCH: SEEKING KNOWLEDGE FOR THE SAKE OF KNOWLEDGE

In 1961, a report emerged from the laboratory of inorganic chemist Russell Drago describing the reaction of nitric oxide (NO) with selected nucleophiles.[1] One of these was diethylamine, whose reaction with NO produced a white powder of formula **1** that, on standing exposed to air overnight, was converted to the potent carcinogen *N*-nitrosodiethylamine (**2**) (eq 1).[2] The authors speculated that the reaction proceeded by the mechanism shown in Figure 1, path A, which begins by reversing the synthesis reaction to regenerate NO from the solid material. On reading these reports, I hypothesized that this reaction might instead proceed by novel pathway B of Figure 1, which begins with oxygen directly abstracting the elements of NO^- from **1** to produce **2** plus diethylammonium nitrite.

$$Et_2NH \xrightarrow{NO} \underset{\mathbf{1}}{Et_2N\text{-}N_2O_2^-} \; Et_2NH_2^+ \xrightarrow{O_2} \underset{\mathbf{2}}{Et_2N\text{-}NO} \quad (1)$$

Confirmation that path B was operative would have been an interesting theoretical advance in the area of nitrosamine formation, a research theme I was pursuing at the time. Unfortunately for my hypothesis, however, this was not the case. NMR experiments supported path A, revealing that **1** simply regenerated the NO and diethylamine in solution, with the NO then being autoxidized to the well-known nitrosating agent N_2O_3, which recombined with the free amine to form **2**. This was not the newsworthy mechanism of nitrosamine formation I had hoped for, because everybody already knew that aerobic NO could nitrosate amines, so we published a short account of the study in a 1982 meeting proceedings[3] and pursued other, at the time seemingly more relevant, facets of nitrosamine chemistry.

The picture changed dramatically in 1987 and 1988, when publications from the biomedical sector claiming NO to be a key effector of blood vessel dilation,[4] neurotransmission in the brain,[5] immune response,[6,7] and anticoagulant activity[8] began to appear. Initially, I did not believe these reports, having learned a lot about the nefarious face of NO through its involvement as a metabolite and precursor of the carcinogenic *N*-nitroso compounds I had been studying. I realized, however, that if any part of that work was credible, something really important was breaking onto the biomedical scene.

■ PROBING THE CHEMISTRY OF 1 AND ANALOGOUS IONS AS A STARTING POINT FOR DEVELOPING NOVEL TOOLS FOR CHEMICAL BIOLOGY RESEARCH

If reports of NO's bioeffector roles were true, and if **1** and its analogues indeed hydrolyze to produce molecular NO in solution, then one might expect these ions to be pharmacologically active. As a platform for beginning tests of this hypothesis, my colleagues and I started by preparing a selection of salts of structure $X\text{-}N(O)\text{=}NO^-$ M^+ and systematically cataloguing their physicochemical properties. Many were found to precipitate in pure form from solutions of the X-H precursor on exposure to gaseous NO under basic conditions, although success sometimes proved to be quite dependent on empirically choosing the correct solvent. Exchanging the dialkyammonium ion of **1** and homologues for sodium tended to improve the salts' shelf life, with many examples proving indefinitely stable as solids at 4 °C. Structural studies confirmed the substantial double bond character of the N—N linkage of many $X\text{-}N(O)\text{=}NO^-$ ions, with the oxygens being *cis* to one another; this and other features of their structures led to their formal designation as "diazeniumdiolates",[9] although earlier nomenclature proposals referring to them as "NOC compounds" or "NONOates" continue in common use. These salts proved to be UV-active, with a strong chromophore at ~250 nm and ε_{max} of ~7 mM^{-1} cm^{-1} in
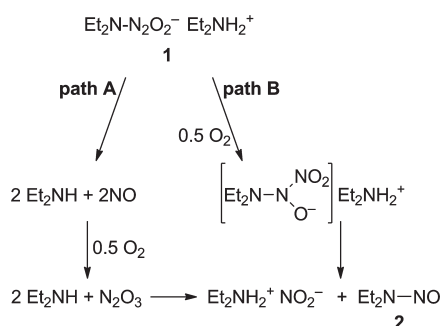
Figure 1. Two theoretically possible mechanisms by which diazenium-diolate salt **1** can be converted to nitrosamine **2**.
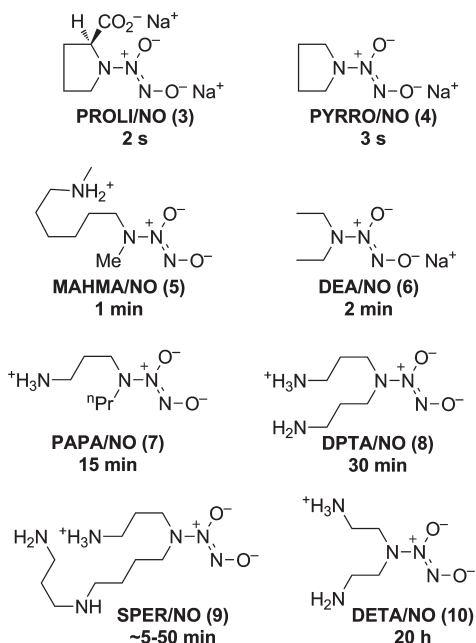

Figure 2. Structures of selected diazeniumdiolate salts and zwitterions, along with their half-lives in 0.1 M phosphate at pH 7.4 and 37 °C. Each of these generates close to the theoretical 2 mols of NO per mole of anionic diazeniumdiolate. The ~5- to 50-min half-life for **9** is attributable to a contribution from a substrate-squared term to the rate equation, i.e., to formation of a less reactive dimer as the concentration is increased.[14]

Figure 3. Observed maximal *in vitro* vasodilatory potencies $(1/EC_{50})$ of five ionic diazeniumdiolates correlated highly $(r = 0.995)$ with theoretical values computed from the compounds' rates and extents of NO release. (For details of the calculation, see ref 16).

the absence of electronic interaction with X, allowing for convenient characterization of their solution chemistry. They react with copper(II) to produce coordination complexes in which the metal binds the ligand's two oxygens without oxidizing it,[10] although reductive nitrosylation has been implicated in the reactions of diazeniumdiolate ions with other metal centers.[11,12]

Examples for which X is a secondary amine residue were found to generate NO in yields approaching the theoretical 2 mol/mol at physiological temperature and pH, with reproducible half-lives ranging from 2 s to 20 h, depending on the structure of the amine moiety (Figure 2).[13] The mechanism of the cleanly first-order (at constant pH) hydrolysis was elucidated, showing that the favored equilibrium protonation site is the terminal oxygen, while the kinetically significant tautomer has the proton at the amino nitrogen of the diazeniumdiolated secondary amines.[14] From the standpoint of stoichiometry, the acid-catalyzed hydrolysis reaction
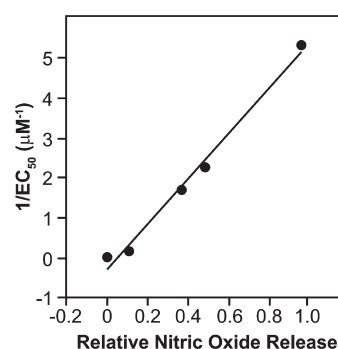
was confirmed to consume acid and slow itself down as the pH climbs in the absence of proper buffering capacity. Medium effects, such as micellar encapsulation and liposome formation, have been reported to affect hydrolysis rates, sometimes dramatically.[15] Examples of X-N(O)=NO⁻ ions for which X is carbon, oxygen, sulfur, or a primary amino group were also studied; they showed very similar structural and ultraviolet spectral characteristics,[9] though their reactivity patterns were much more diverse than those of the well-behaved secondary amine analogues, often generating little to no NO on hydrolysis, for example.

Two features of these fundamental chemistry studies set the diazeniumdiolate ions apart as advantageous tools for investigating the chemical biology of NO:

- In contrast to other NO donors, they require no redox activation, releasing NO at repeatable first-order rates on simple dissolution in buffered aqueous fluids, cell culture media, or organ baths.
- The broad array of reproducible NO generation rates allows researchers to probe the effects of exposure to short puffs of the bioeffector, to near-steady state fluxes, and to many half-lives of NO release in between.

■ PREDICTING BIOLOGICAL EFFECTS BASED ON A KNOWLEDGE OF THE DIAZENIUMDIOLATES' PHYSICOCHEMICAL PROPERTIES

Of special note was the finding that data on the rate and extent of NO release in simple buffers could be used for quantitatively predicting biological outcomes. Figure 3 illustrates an early example. Knowing that NO had been identified as the endothelium-derived relaxing factor,[4] we chose a set of five physicochemically diverse ionic diazeniumdiolates, determined their hydrolysis rate constants, measured the number of moles of NO that were produced on hydrolyzing one mole of each, studied their effects on blood vessel dilation in an organ bath, and published a paper in 1991 showing how the physicochemical parameters could be plugged into an equation that quantitatively correlated with vasodilatory activities in this series of compounds. [16]

As this increasingly refined understanding of the basic chemistry and its pharmacological implications was developing, colleagues in the biomedical realm became interested in studying their physiological effects *in vivo*. Cardiologist colleagues showed that the 2-min half-life for DEA/NO (**6**) in physiological buffer translated to a profound but brief drop in blood pressure on bolus
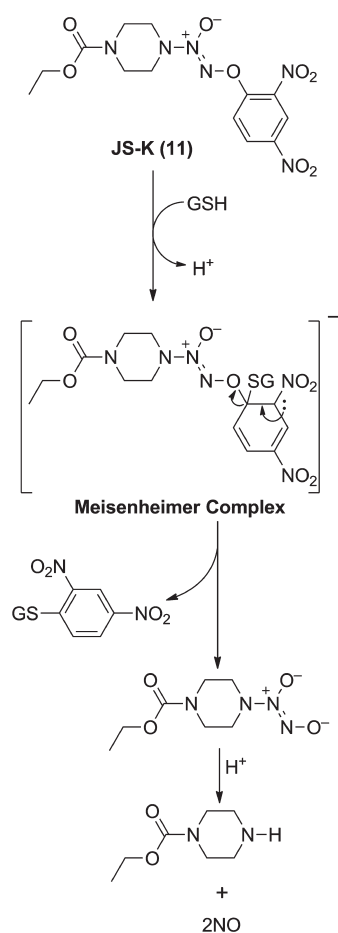
Figure 4. Activation of O^2-arylated diazeniumdiolate JS-K (**11**) as an NO donor by reaction with the thiol-containing peptide glutathione (GSH).[27]

Figure 5. Base-induced removal of glucose protecting groups for preparation of NO-releasing diazeniumdiolated poly(urethane) (from ref 31).

intravenous administration in rabbits, while 10-fold greater doses of the longer-lived analogue SPER/NO (**9**) had a blunted immediate effect but one that was correspondingly much more prolonged.[17] Similarly, the extent and time course of these agents' anticoagulant action *in vivo* closely matched predictions based on their solution chemistry.[18]

These studies showing the surprising extent to which the biological properties quantitatively matched expectation based on the fundamental chemistry in turn elicited interest among clinicians in possible therapeutic strategies. Neurosurgeons studying models of cerebral vasospasm following subarachnoid hemorrhage showed that intracarotid infusions of the 2-min compound DEA/NO (**6**) just upstream from the affected vessel induced the desired vasodilation.[19] Alternatively, polymer blends of the 20-h NO donor DETA/NO (**10**) were shown to prevent vasospasm in a rat femoral artery model.[20] In a vascular surgery application, ionically diazeniumdiolated materials applied to the outside of rat carotid arteries undergoing balloon angioplasty dramatically improved healing at the inner surface of the vessel, relative to untreated controls.[21]

■ PROTECTING GROUP STRATEGIES AND THE DESIGN OF PRODRUGS FOR INTERNAL USE

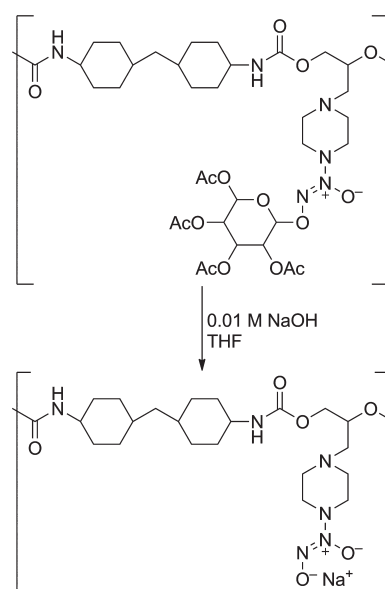Spontaneous generation of NO can be beneficial for the kinds of therapeutic applications mentioned above, but in most cases it is imperative to target the NO selectively to the given tissue or cell type where it is needed without exposing the myriad other NO-sensitive bodily compartments. One convenient means of doing this is to take advantage of the diazeniumdiolate ions' inherent nucleophilicity to generate neutral prodrug forms.[22] Initial studies with simple methylating and ethylating agents showed that alkylation occurred at the terminal oxygen (O^2) of the X-N(O)=NO$^-$ ion, in contrast to an earlier structural assignment that erroneously placed the alkyl group on the other oxygen; the N—N linkage consistently had even more double bond character than was seen in the corresponding ionic starting material, with the oxygens again being *cis* to one another.[22] The alkylation products proved remarkably stable toward oxidation. With a functional group consisting of three sequential nitrogens, two of which are oxygenated, one might have expected otherwise; nevertheless, no problems were seen in subjecting them to copper-mediated Click chemistry[23] or sodium periodate/ruthenium trichloride-induced oxidation of a methylene group to a carbonyl.[24] Diazeniumdiolate ions can be sulfonated at the O^2-position,[25] but the corresponding acylated analogues have so far defied attempts to isolate them.

S_NAr studies showed anionic diazeniumdiolate DEA/NO (**6**) to be intermediate between chloride and fluoride in its reactivity with 1-halo-2,4-dinitrobenzenes.[26] The resulting O^2-arylated diazeniumdiolates hydrolyzed smoothly in base.[26] These aryl derivatives also generate NO on reaction with the nucleophilic thiol group of glutathione (exemplified in Figure 4) and have been widely studied for their potential use as anticancer agents.[27] Initial hypotheses in this area focused on their potential to selectively remove highly NO-sensitive leukemia cells from the bloodstream without inflicting collateral toxicity in their normal hematopoietic counterparts,[28] but their potential anticancer activity has, for reasons that remain to be fully elucidated, proven far more general than that. In studies that will be reviewed in detail elsewhere, our lead O^2-arylated diazeniumdiolate JS-K (**11**) has moved into the late preclinical stage of evaluation as a broad-spectrum anticancer drug candidate, active in numerous
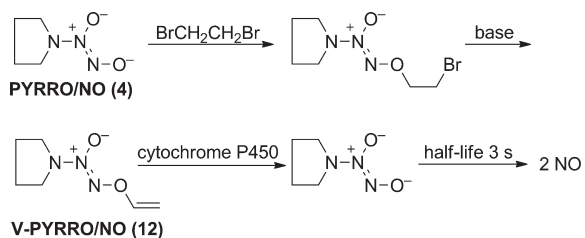
Figure 6. Synthesis and metabolism of V-PYRRO/NO (**12**), an O^2-vinylated diazeniumdiolate that is activated for NO release by cytochromes P450.
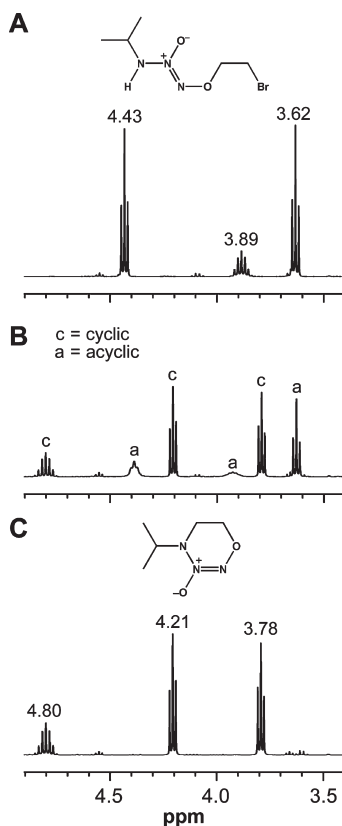

Figure 7. Proton spectra showing the conversion of the Z diazeniumdiolate structure in panel A at time zero to the cyclic E derivative of panel C after 2 h at 0 °C in basic D_2O/methanol-d_4 solution. Panel B shows the spectrum of the reaction mixture at the 15-min time point; note the line-broadening associated with the Z to E interconversion barrier (from ref 36).

in vivo rodent models of cancer including leukemia,[28] multiple myeloma,[29] and tumors of the prostate,[28] liver,[30] and lung.[27]

O^2-Glycosylation has also proven advantageous as a diazeniumdiolate protecting group strategy. O^2-Glucosylated DEA/NO was found to resist hydrolysis in neutral and acidic conditions but to hydrolyze remarkably rapidly in base.[31] This property proved useful during preparation of poly(urethane)s bearing ionic diazeniumdiolate groups. By O^2-glucosylating monodiazeniumdiolated piperazine and attaching its other nitrogen via a linker to the diol segment of the poly(urethane) backbone, the polymer could be processed through various heating and other steps that the ionic form could not survive,
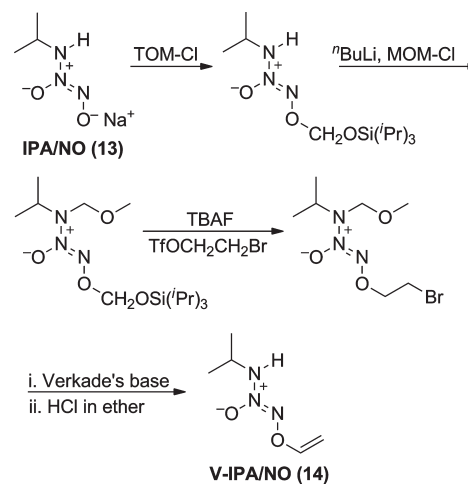

Figure 8. Use of TOM and MOM protecting groups in the synthesis of V-IPA/NO (**14**) (ref 37).

then treated with base to remove the saccharide residues and regenerate the capacity for NO release (Figure 5).[31] Additionally, preliminary work with small-molecule O^2-glycosylated diazeniumdiolates has revealed their possible utility in antimicrobial applications.[32]

Diazeniumdiolate ions can also be O^2-vinylated, as illustrated in Figure 6. The prototype in this series is V-PYRRO/NO (**12**, Figure 6), designed and shown to be oxidatively cleaved by cytochrome P450 enzymes concentrated in the liver to release NO selectively in that organ, with minimal effects elsewhere.[33] This strategy has shown considerable promise for treating fulminant liver failure,[33] acetaminophen toxicity,[34] and the ischemia/reperfusion injury that accompanies organ transplantation.[35]

Interestingly, the synthesis of O^2-vinylated derivatives as outlined in Figure 6 failed on attempted application to a primary amine diazeniumdiolate. In this case, the main product on treating the O^2-(2-bromoethyl)ated intermediate with base resulted from a cyclization reaction instead of dehydrohalogenation, as documented in the NMR spectra of Figure 7. This reaction provided the first clear evidence that *cis*-to-*trans* isomerization about the N(O)=NO bond was possible. Theoretical and experimental studies revealed that the barrier to such isomerization dropped from a prohibitive ~40 kcal/mol for the neutral molecule to an easily accessible value of ~20 kcal/mol on ionization of the N—H bond.[36] The pK_a for this process was estimated to be 12.4, lower than that needed to ionize the C—H bond en route to dehydrohalogenation.

To accomplish the conversion of primary amine diazeniumdiolate **13** to the desired vinyl derivative **14**, two new diazeniumdiolate protecting groups were developed and applied as shown in Figure 8.[37] Reaction of IPA/NO anion with (triisopropylsilyl)oxymethyl chloride (TOM-ylation) protected the O^2-position, stabilizing and solubilizing IPA/NO while the methoxymethyl (MOM) group was installed at the amino nitrogen to render it non-nucleophilic and prevent ring closure. Fluoride-induced removal of the TOM group in the presence of 2-bromoethyl triflate led to an O^2-bromoethylated diazeniumdiolate that on treatment with Verkade's base followed by acid-induced removal of the MOM group produced the desired O^2-vinylation product **14**.[37]

MOM-ylation at the O^2-position instead of N results in less hydrolytically sensitive diazeniumdiolates whose rates of spontaneous NO release are generally much slower than those of the corresponding ions. An example is diazeniumdiolated bovine serum albumin, which produces about 40 mols of NO per mole of protein with a half-life of about 3 weeks.[38] When placed in the pericardial sac surrounding the heart to suffuse the coronary arteries of pigs undergoing balloon angioplasty, this material not only reduced the cellular overgrowth that leads to narrowing of the arterial lumen but also beneficially remodeled the vessel by filling in the cracks in the wall that result from such balloon overstretch.[39]

Important synthetic opportunities were opened up when the sulfur analogue of the MOM group ($MeSCH_2^-$ instead of $MeOCH_2^-$) was introduced at the O^2-position. Treatment of the resulting thio-MOM derivative with sulfuryl chloride gives the corresponding O^2-chloromethyl compound, whose chloride can be displaced by carboxylate ions to produce a variety of esterase-sensitive prodrugs.[40] Among the important mutual prodrugs made possible using this strategy is a hybrid molecule whose hydrolysis yields aspirin and NO concurrently; this NONO-aspirin was similar to aspirin itself in terms of anti-inflammatory activity but largely devoid of aspirin's ulcerogenic side effects.[41]

Attaching esterase-sensitive protecting groups at the O^2-position has proven especially illuminating. Acetoxymethylating PYRRO/NO (**4**) generates a prodrug that is relatively stable in cell culture media but that enzymatically hydrolyzes rapidly once inside the cell to generate a concentrated intracellular flux of diazeniumdiolate ions whose antiproliferative effects on the cells are 2 to 3 orders of magnitude more potent than is seen when the unprotected ionic diazeniumdiolate is dissolved directly in the medium.[42] A particularly interesting effect of acetoxymethylation is described in the next section.

■ FROM NO TO HNO

Our initial 1991 study of vasodilatory activity on the part of five structurally diverse diazeniumdiolates (Figure 3) included the primary amine derivative IPA/NO (**13**), but this compound was both relatively unstable and much less potent as an NO generator and vasodilator than its secondary amine analogues.[16] For this reason, it remained little studied until news of the biological activities of nitroxyl (HNO), the one-electron reduction product of NO, began to break some years later.[43−45] Of special importance in the present connection was a 2005 paper by Miranda and colleagues addressing the following question: if IPA/NO (**13**) produced only 36% of the theoretical amount of NO on hydrolysis,[16] what happened to the other 64%? The answer: the missing nitrogenous product was HNO.[46]

To study the utility of diazeniumdiolated primary amines as HNO donors, the problem of their instability had to be overcome. The prototype in this series, IPA/NO (**13**), has a tendency to decompose on isolation and storage, sometimes producing a mixture of the corresponding diazoate plus other decomposition products that on visual inspection is indistinguishable from pure IPA/NO but at other times disintegrates explosively without warning or apparent provocation.

This problem can be largely controlled by exactingly avoiding excesses of acids and bases in the freshly synthesized material,[47] but conversion to stable and rigorously purifiable O^2-substituted derivatives has provided a more general solution. Alkylation of
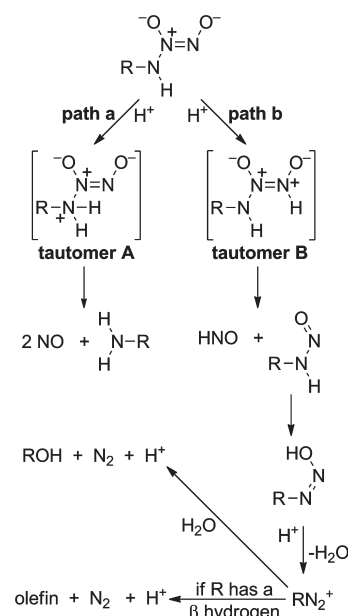
Figure 9. Partitioning of a diazeniumdiolated primary amine anion between its NO- and HNO-generating hydrolysis pathways (adapted from ref 51).
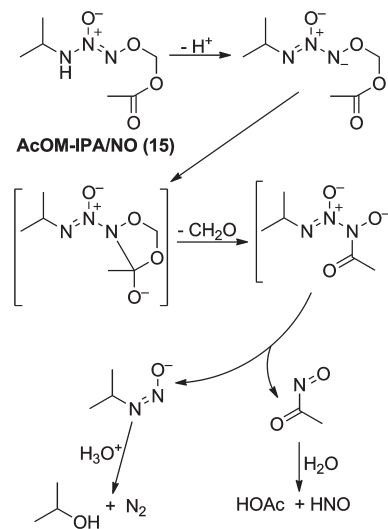
Figure 10. Spontaneous hydrolysis of the "pure" HNO donor AcOM-IPA/NO (from ref 50).

the ionic diazeniumdiolates occurs preferentially at the O^2-position,[48] providing such stable derivatives. The resulting RHN-N(O)=N-OR species proved to be an ambident nucleophile under basic conditions, undergoing alkylation at either end of the N−N−N system.[49] MOM-ylation of **13** led to an N,O bis-substituted product in the presence of excess derivatizing agent, with the O^2-monosubstituted intermediate also being isolable; **13** could also be O^2-glycosylated.[48]

Acetoxymethylation of IPA/NO unveiled some properties that were especially intriguing from the drug discovery point of view.[50] One was that the product hydrolyzed spontaneously in the absence of esterase with a half-life of 41 min at physiological temperature and pH, an order of magnitude longer than that of IPA/NO[50] and thus a considerable step toward the goal of
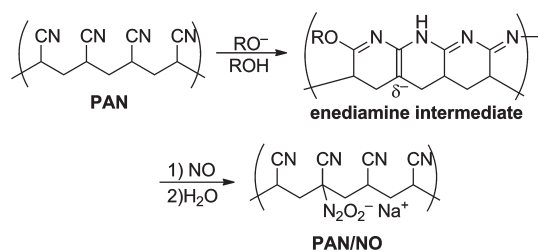
Figure 11. Diazeniumdiolation of poly(acrylonitrile) (PAN), a water-insoluble polymer that generates NO only at its surface while retaining all byproducts of its spontaneous hydrolysis within the solid polymer matrix (from ref 60).

providing an array of reliable half-lives for use as research tools in studying the chemical biology of HNO, much as has been the case with the NO donors discussed above.

The other major surprise was that hydrolysis of acetoxymethylated IPA/NO in the absence of esterase produced HNO without cogenerating NO.[50] We had expected the reaction to proceed by straightforward ester hydrolysis to produce **13**, which would then hydrolyze according to the mechanism outlined in Figure 9, which involves generation of similar fluxes of NO and HNO according to the indicated competing pathways.[51] Prodrug **15**'s behavior as a "pure" HNO donor has been ascribed to the mechanism shown in Figure 10.[50] Future work will seek to embellish on this chemistry as a platform for producing improved HNO donor research tools and drugs.

■ TACKLING THE NITROSAMINE TOXICITY ISSUE

Having begun researching diazeniumdiolates by studying them as precursors for carcinogenic nitrosamines (Figure 1),[3] we realized from the outset that this possible source of toxicity must be dealt with head-on during any effort to reap practical benefits from their chemistry. One way to do this is to employ diazeniumdiolates whose corresponding N-nitroso derivatives are considered noncarcinogenic; an example is PROLI/NO (**3**, Figure 2),[52] whose potential metabolite N-nitrosoproline is a normal constituent of human urine that has failed to induce tumors in any of the long-term toxicity studies published so far.[53–59] Another strategy is to attach the diazeniumdiolate functional group to primary amines or carbon-based nucleophiles, though care must still be taken to identify the byproducts of NO release and avoid their toxicity.

Yet another approach is to tie the NO-releasing moiety covalently to insoluble materials that limit NO exposure only to the cells and tissues with which they are in immediate physical contact while at the same time preventing release of any byproduct including nitrosated species into the surrounding medium. An example that combines the latter two approaches is diazeniumdolated poly(acrylonitrile) ("PAN/NO"), whose synthesis and structure are shown in Figure 11.[60] In addition to poly(urethane) (Figure 5) and poly(acrylonitrile), other insoluble materials whose surfaces can be beneficially converted to NO-releasing form for a variety of antiplatelet, antimicrobial, and wound healing applications by covalently diazeniumdiolating them include poly(ethyleneimine),[61] silicone rubber,[62] and silicon-based hydrogels.[63]

Studies of an O-alkylated diazeniumdiolate's photochemistry led to the surprising finding that its primary photoproducts resulted from cleavage of the formal N−N double bond to form

the corresponding nitrosamine plus an O-nitrene.[64] Although photochemical nitrosamine formation can be largely overcome by judicious selection of O^2-substituents that channel the reaction toward NO generation,[65] the findings reinforce the importance of excluding light as well as other potentially destructive stimuli during synthesis and storage so as to avoid unwanted contamination by impurities in general and carcinogenic nitrosamines in particular.

■ SOME REMAINING QUESTIONS

Many questions about diazeniumdiolate chemistry remain to be answered if we are to realize the full inherent potential of these compounds as tools for researching the chemical biology of NO and HNO as well as for rationally designing biomedical innovations. Below is a selection for your consideration and, hopefully, input:

- **How can it be explained that amines generally react smoothly with NO, whereas water and hydroxide apparently do not?** The product of a water/hydroxide diazeniumdiolation reaction would be Angeli's salt [NaO-N(O)=NONa], an HNO donor that is synthesized by nitrating hydroxylamine. Like most other ionic diazeniumdiolates, Angeli's salt is increasingly stabilized as the pH is raised, such that if it were to be formed in 1 M solutions of sodium hydroxide in contact with 3 atm of NO it would be spectrophotometrically detectable. But this does not happen. Why not?

- **What are the structural and stereoelectronic determinants of the partition between the NO and HNO generation pathways in the primary amine diazeniumdiolate series?** Are the results for IPA/NO (**13**), which generates substantial amounts of both NO and HNO on hydrolysis at physiological pH (Figure 9), typical for this series of ions? Perhaps future structure variation studies will reveal analogues with NO- or HNO-only buffer hydrolysis profiles among the diazeniumdiolated primary amine anions.

- **What are the structural and stereoelectronic determinants of hydrolysis half-life?** I find this to be an enduring mystery. Some generalizations are possible, with for example diazeniumdiolated pyrrolidines having relatively shorter lifetimes in solution, but how can the tremendous spread seen among the diazeniumdiolated di- and polyamines [e.g., 1 min for MAHMA/NO (**5**) and 1 day for DETA/NO (**10**)] be explained? My answer so far has been to rely completely on the empirical approach—synthesize a new compound and determine its hydrolysis rate experimentally—but I'm hopeful that someone will someday establish a means of rationally predicting such properties in advance.

■ CONCLUSION

I hope that what I have written has piqued your imagination. Much remains to be done if the full potential of the science presented here is to be realized, both in further probing the basic chemistry of the diazeniumdiolates, in using the knowledge thus gained to design additionally improved tools for chemical biology research, and in engineering important advances in medical practice. I am convinced that there are lives to be saved in the

technology described here and would warmly welcome your participation in the effort to exploit its full promise.

■ AUTHOR INFORMATION

Corresponding Author

E-mail: keeferl@mail.nih.gov.

■ ACKNOWLEDGMENT

■ KEY WORDS

Nitric oxide: a multifaceted diatomic bioeffector molecule; nitroxyl: the one-electron reduction product of NO, itself an important bioeffector molecule; NO: the formula for nitric oxide; HNO: the formula for nitroxyl; diazeniumdiolates: the formal name for compounds of structure $XN(O)=NOR$, where R represents an electrophile and X is a nucleophile residue; NONOate: a commonly used synonym for diazeniumdiolate; prodrug: (Wikipedia definition) "A ... pharmacological substance (drug) administered in an inactive (or significantly less active) form. Once administered, the prodrug is metabolized in vivo into an active metabolite, a process termed bioactivation."

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