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Carbon-Bound Diazeniumdiolates from the Reaction of Nitric Oxide with Amidines

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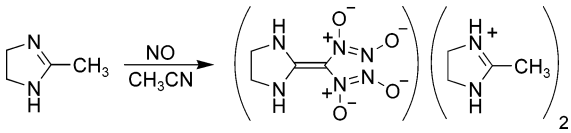
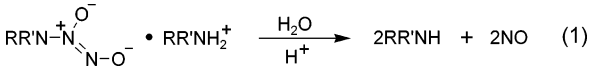


The enediamine tautomer of a variety of substituted amidine free bases reacts with nitric oxide (NO) to produce compounds containing a carbon-bound diazeniumdiolate [$R^1R^2R^3C-N(O)=NO^-$] functional group (previously called "nitrosohydroxylamines"). The new reaction has been shown to be quite general, although the nature of the products does vary. Amidines containing more than one replaceable hydrogen produce polydiazeniumdiolates as intermolecular salts, while those in which only one diazeniumdiolation can occur provide zwitterionic salts. These diazeniumdiolated amidines are shown to be useful NO donor compounds which undergo very slow spontaneous dissociation on dissolution in pH 7.4 phosphate buffer to produce mixtures of NO and nitrous oxide containing mostly NO. The most advantageous manifestation of the new discovery is the preparation of the monodiazeniumdiolated amidine zwitterions. Reaction of the medically relevant α-adrenergic agonists tetrahydrozoline and idazoxan produced monodiazeniumdiolated amidine zwitterions from which NO release was observed for up to 28 days and showed little sign of ending. The reaction should be applicable to a variety of pharmaceutical agents, including NO synthase inhibitors, antitumor agents, and antibacterials.

Introduction

The release of nitric oxide (NO) from diazeniumdiolate functional groups (designated by line formulas $-N(O)=NO^-$ or $-N_2O_2^-$) bound to a nitrogen atom via a simple dissociative mechanism (eq 1) has proven to be one of the most effective methods for the study of this highly reactive and biologically ubiquitous molecule.[1] Although the related carbon-bound analogues (often referred to as nitrosohydroxylamines) have been known for over a century, only recently have a few limited examples been demonstrated to be possible NO donors.[2] Most carbon-bound diazeniumdiolates have been prepared either by nitrosation of hydroxylamines or through the direct reaction of NO with carbanions, with one of the exceptions being our report that enamines may be used as neutral carbanion equivalents which react directly with NO.[3] Unlike the nitrogen-bound diazeniumdiolates, many of the carbon-bound variations of this functional group do not exhibit a propensity to release NO. Some structural variants of these materials decompose to release nitrous oxide (N_2O), while others are quite stable.[2]



The search for new NO-releasing examples, as well as the ongoing effort to identify those structural elements necessary for this mode of decomposition to predominate, has led us to search for new ways to introduce diazeniumdiolates into organic compounds by exploring the reactions of NO with various olefins. As we have discussed previously,[3] reactions of simple olefins and dienes with NO produce a complex set of outcomes that does not appear to have led to the production of diazenium-

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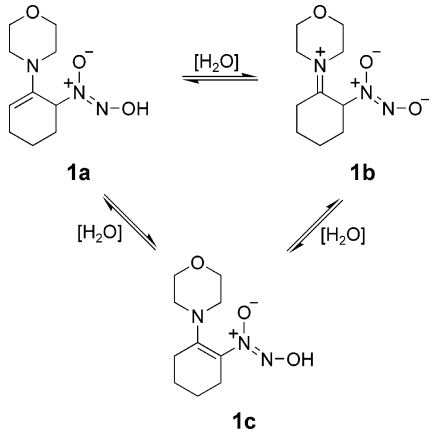
SCHEME 1

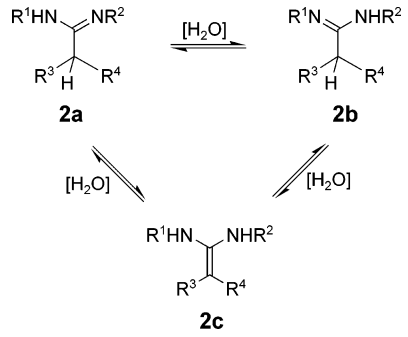


diolates. However, successful preparation of diazeniumdiolates from enamines demonstrated that electron-rich double bonds could react with specificity and suggested that other suitably substituted olefins might serve as useful precursors. In this report we describe the electrophilic reaction of NO with amidines via their enediamine tautomers to produce carbon-bound diazeniumdiolates and show that these compounds may serve as spontaneous NO donors.

Results and Discussion

Nitrogen-bound diazeniumdiolates are under development as NO donor compounds for biomedical applications in part because they can be prepared from a wide range of starting amines by relatively simple nonvigorous methods and can be reliably expected to generate NO without regard to the structure of the remaining parts of the molecule, which is thus available to achieve other medicinal effects.[4] While several individual carbon-bound diazeniumdiolates have been found to release NO spontaneously in aqueous solution[3,5−11] and one class of compounds (the cupferrons) has been found to release NO as a result of enzymatic oxidation,[12] at the outset of this study no general NO-releasing carbon-bound diazeniumdiolate structure had been identified. We were disappointed to discover only one NO-releasing enamine-derived diazeniumdiolate, cyclohexanone enamine **1**, but were encouraged by the observation that the standout compound was the one having an electronic structure containing a highly mobile double bond (see Scheme 1). The presence of the electron pair in the π cloud of **1** is not unlike the presence of the free pair of electrons in the p orbital of a *N*-diazeniumdiolated amine and suggests that this may be a key feature in providing the reversibility of the NO addition reaction that would be crucial to the development of a general class of NO donors. This feature also allows for the direct reaction of these compounds with NO without the need to employ a strong base. It is also worth noting that **1** represents a possible extension into the realm of the carbon diazeniumdiolates of an important theme first recognized over a decade ago with the description of the added stability of the zwitterionic polyamine diazeniumdiolates when compared to their intermolecular cousins.[13]

SCHEME 2



The amidine system (Scheme 2) presents a triad of tautomers strikingly similar to those of compound **1**. While enamine diazeniumdiolate tautomers **1a** and **1b** have been observed directly,[3] leaving the more fleeting existence of **1c** to be inferred from indirect observations, many observations and practical applications of amidine tautomers **2a** and **2b** exist but on rather few occasions has the transitory existence of enediamine tautomer **2c** been invoked.[14] Although substitution at the β-carbon by suitable electron-withdrawing groups can produce compounds (often called "ketene aminals") in which the enediamine tautomer is preferred,[15] most amidines may be considered enamine derivatives only with caution. While inclusion of an electron-withdrawing substituent was not necessary to achieve either the inverse electron demand Diels−Alder reaction[16] or the Michael reaction,[17] the alkylation reaction[18] of amidine derivatives appeared to require this structural feature. Nevertheless, the presence of the additional nitrogen on the olefin of tautomer **2c** could be expected to increase its reactivity with an electrophile such as NO, perhaps compensating for the low concentration of a species such as **2c** in any given solution to produce a reasonable reaction rate even in the absence of additional substitution.

Reaction of NO with Amidines. Nearly all amidines are isolated (and therefore supplied commercially) as their salts with strong mineral acids because they are

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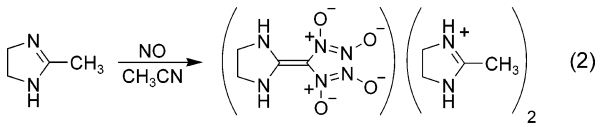
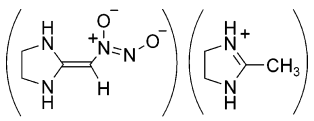
susceptible to base- (and thus self-) catalyzed hydrolysis. Since the diazeniumdiolates are sensitive to acid and form best under basic conditions,[2] we selected 2-methyl-2-imidazoline (lysidine, **3**) for initial study because it is readily available as the free base, which is a stable solid. In fact, the observation of deuterium incorporation at the methyl group of **3** is the earliest confirmation of the existence of the enediamine tautomer of amidines that we have found in the literature.[19] Unlike the formation of enamine diazeniumdiolate **1**, reaction of an ether solution of **3** with NO at a pressure of 4 atm did not provide a clean result, so in anticipation of the formation of an intramolecular salt, we employed identical reaction conditions to those used in the preparation of both the NO-releasing polyamine[13] and non-NO-releasing iminium[3] zwitterionic diazeniumdiolates. Reaction of **3** with NO in acetonitrile solution produced a good yield of bisdiazeniumdiolated imidazoline as the (in)termolecular imidazolinium salt **4** (eq 2). Bisdiazeniumdiolation was



(2)

3 **4**

immediately apparent from the molar extinction coefficient (ϵ) of the characteristic ultraviolet absorption which all diazeniumdiolates exhibit.[2] The observed value of ϵ (13.6 mM^{-1} cm^{-1} at $\lambda_{max} = 260$ nm) is fully consistent with that found for other known bisdiazeniumdiolates.[9] Since a bisdiazeniumdiolate could also form via reaction at the two amidine nitrogens, the NMR spectra of this material were examined closely to arrive at the structure of the dianion shown in **4**. If the NO had reacted at the amidine nitrogens, the hydrogens remaining on the methyl group would appear as a prominent singlet in the ^{1}H NMR unless they exchanged rapidly with the deuterium oxide solvent. However, had this exchange occurred, the presence of deuterium would be revealed by the observation of a multiplet due to C−D coupling in the ^{13}C NMR. Since no such coupling is observed, there can be no hydrogens remaining on the original methyl group. The sequential addition of two diazeniumdiolates to this amidine is consistent with all of the chemical properties of both the amidine and the resulting diazeniumdiolate functional groups. Initial formation of a monodiazeniumdiolate would lead to the formation of intermediate **5**, which would be expected to exist in the enediamine form shown since the diazeniumdiolate group is electron withdrawing.[9,10] Subsequent reaction with NO affords **4**,



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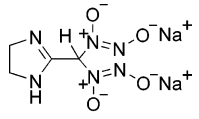
an olefin which is either not sufficiently reactive or too insoluble to proceed further. Ether was initially chosen as the solvent with the expectation that it would be an essential tool in avoiding the type of polydiazeniumdiolation that plagued the preparation of enamine NO-releasing compounds[3] because ionic materials tend to precipitate on formation, but the amidine system is apparently more self-limiting. Indeed, while structure **4** is drawn to represent this product because this is the result obtained by measuring the NMR spectrum of a simple (nonbuffered) solution in deuterium oxide, the disodium salt (prepared via a metathesis reaction with sodium methoxide) exhibits the spectrum of amidine tautomer **6** under these conditions. The ^{13}C NMR of **6**



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exhibits a 1:1:1 triplet at 88.5 ppm ($J_{CD} = 22$ Hz) due to the exchange of deuterium with the single hydrogen now present on what was originally the lysidine methyl group. The fact that the disodium salt exists entirely in the amidine tautomeric form at the pH generated upon its dissolution in deuterium oxide suggests that, perhaps for steric reasons, even the presence of two electron-withdrawing diazeniumdiolates does not overwhelmingly favor the enediamine tautomer. Additionally, although trisdiazeniumdiolation can occur,[8−10] it has in the past been accomplished only through use of bases stronger than the amidines to deprotonate the bisdiazeniumdiolated intermediates. Perhaps more noteworthy than any of the above is the observation that no diazeniumdiolation of the secondary amino groups occurred. The enamines used in the previous study[3] all contained only tertiary nitrogens, thus avoiding complications from the possible formation of nitrogen-bound diazeniumdiolates.

Acetamidine, the simplest member of the family, seemed the next logical target for study. As the free base it is an extremely unstable oil which is insoluble in the non-hydroxylic solvents normally required for successful diazeniumdiolation in the absence of added inorganic base.[20] A milky-white suspension of this oil in acetonitrile reacted vigorously with NO to produce a tan solid exhibiting an intense ultraviolet absorption maximum at 260 nm, characteristic of the diazeniumdiolate functional group, which was treated with caution as a result of the known high energy content of the polydiazeniumdiolates[9] that were thus indicated to be present. A solution of this reaction product in deuterium oxide exhibited a NMR spectrum containing five singlets, of which two could be definitively identified as representing acetamide and methane trisdiazeniumdiolate ion **8** (Scheme 3). The characteristic proton signal of **8** at 7.62 ppm was verified by spiking the sample with authentic material prepared previously.[10] We presume that the conversion of acetamidine to **8** follows the reaction pathway shown by analogy to that which leads to the production of trianion **8** from acetonitrile in the presence of sodium methoxide. Since the free acetamidine was obtained by treating a saturated aqueous solution of the hydrochloride with saturated sodium hydroxide solution and removing the

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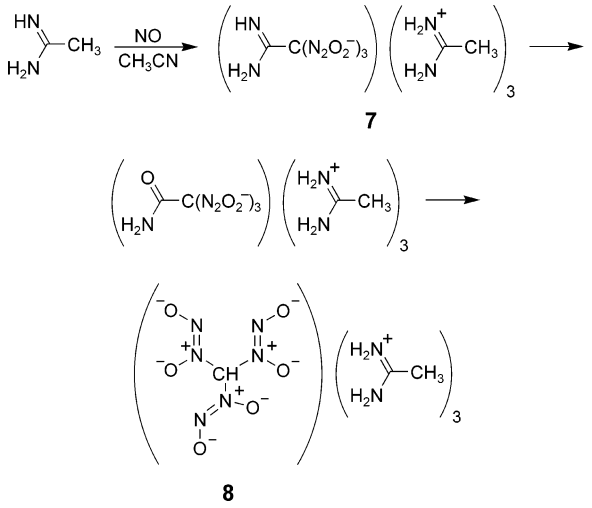
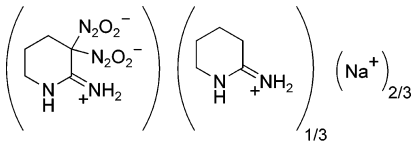
SCHEME 3



7 **8**

resulting oily layer, it undoubtedly contained sufficient water to at least partially hydrolyze intermediate **7** (which would itself not be detectable by ^{1}H NMR!). It would not contain any sodium methoxide, the strong base used in the preparation of **8** from acetonitrile,[10] leading us to discount the possibility that **8** forms from the acetonitrile used as the solvent in this reaction. It should be noted that no **8** forms from the acetonitrile used as the solvent in any of the other amidine diazeniumdiolations, indicating that neither the amidines nor sodium hydroxide are strong enough bases to cause the nitrile to react with NO. Formation of at least some **7** rather than the more limited bisdiazeniumdiolation that results in **4** can be attributed to the decreased steric demands of acetamidine as well as its increased base strength when compared to lysidine. In view of the facts that trisdiazeniumdiolates undergo explosive decomposition[9] and that convenient preparations of **8** (from acetone[9] and acetonitrile[10]) already exist, we chose not to conduct any further experimentation using acetamidine. Subsequent studies were limited to compounds in which trisdiazeniumdiolation at a single site was impossible.

Reaction of iminopiperidine with NO afforded the bisdiazeniumdiolate dianion accompanied by about 1.3 equiv of iminopiperidinium countercation and 0.7 equiv of sodium countercation that was presumably carried into the reaction mixture from the concentrated sodium hydroxide solution used to liberate the amidine from its hydrochloride salt. Repeated recrystallization of this material from methanol/ether afforded mixed salt **9** in which the bisdiazeniumdiolate exists as a zwitterion. This



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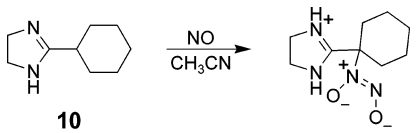
zwitterion apparently forms because iminopiperidine is more basic than lysidine and, unlike the latter, after bisdiazeniumdiolation it must still exist in the amidine form which remains sufficiently basic to compete successfully with a second molecule of iminopiperidine for the remaining proton. In contrast, the ability of the lysidine bisdiazeniumdiolate to exist in the enediamine form results in a decreased basicity relative to remaining unreacted lysidine and results in intermolecular salt formation. This explanation is supported by the formation of zwitterionic products in subsequent work described below.

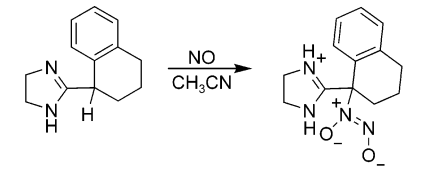
Preparation of 2-cyclohexyl-2-imidazoline (**10**) provided an opportunity to study an amidine containing only a single potentially replaceable hydrogen. It was initially surprising that the reaction of **10** with NO resulted in the formation of intramolecular (zwitterionic) salt **11**.



10 **11**

However, in retrospect, this result is fully consistent with the mechanism postulated for this reaction. If the addition of NO truly occurs via a fully formed enediamine tautomer, then a zwitterionic product like **11** will form from every amidine initially. Since this reaction proceeds without the addition of an auxiliary base, an intermolecular salt will ultimately form only if this zwitterionic product remains in solution long enough to allow proton transfer and if the addition of the diazeniumdiolate produces an amidine of lower basicity than the starting material.

Taken together, consideration of the above results would suggest that substrates capable of monodiazeniumdiolation would offer the simplest applications of this new reaction. Tetrahydrozoline diazeniumdiolate **12** and idazoxan diazeniumdiolate **13** were prepared to demonstrate the usefulness of the new method for the derivatization of compounds of medicinal value. Both of these



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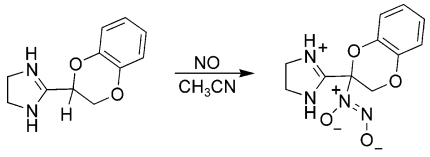

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substances were found to form as zwitterions. These two imidazoline-containing substrates are both α-adrenergic agonists but have widely disparate medicinal uses. Tetrahydrozoline is a common ingredient in nasal sprays that aim to relieve congestion,[21] while idazoxan has been used as a substitute for one of the components of the drug cocktail currently taken as the standard against which other proposed treatments for male impotence are mea-

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sured.[22] Both vasoconstriction (which the α-adrenergic agonist provides) and vasorelaxation (which NO produces) are required for male sexual function, suggesting a possible application for these compounds.

Since compounds **11**−**13** are monodiazeniumdiolates (as indicated by UV and elemental analysis), determination of the site at which the NO reacted could easily be performed using ^{1}H NMR. Attachment of the diazeniumdiolate at carbon produces a symmetric imidazolinium cation, resulting in the appearance of all four methylene protons as a sharp singlet in the NMR spectrum of these compounds. The only possible nitrogen-bound monodiazeniumdiolate in each case would not be symmetric, and the resulting NMR signal attributable to the ring protons would likely be an AA′BB′ multiplet. Indeed, the successful preparation of **12** in high yield and purity without the need for recrystallization serves as an important indicator of the preference of these systems to form *C*- rather than *N*-diazeniumdiolates. The total lack of even a hint of a multiplet in the NMR spectrum of this "crude" sample of **12** strongly attests to the absence of any mono-*N*-diazeniumdiolated product. Such evidence was less convincing in the case of **11** (which required recrystallization due to slight impurities in the starting amidine) and **13** (which produced a lower yield due to increased solubility of the product in the reaction solvent), although it is also true that no *N*-diazeniumdiolated product was observed in the portion of the product that was isolated from these preparations.

Reaction of NO with Enediamines. In response to the above results and previous success with enamines, it was natural to examine the reaction of NO with enediamines, compounds which bear no amino protons and are thus incapable of existing in an amidine tautomeric form. We prepared[23] 1,1-bis(dimethylamino)ethylene, its bismorpholino analogue, and its 2,2-dimethyl analogue. Reaction of each of these compounds with NO using the procedures developed above produced viscous dark orange and brown oils that proved to be complex mixtures. These results are identical to those obtained with simple olefins.[3] While it may eventually be possible to devise reaction conditions that minimize all the side reactions involved,[24] it seemed likely that, by analogy to the iminium diazeniumdiolates prepared from certain enamines,[3] the resulting diazeniumdiolates would not be NO donors, so these experiments have been deferred.

NO Donor Properties of the Diazeniumdiolated Amidines. Although most C-bound diazeniumdiolates do not release NO, many of those that contain adjacent substantial electron-donating functionality are NO donors.[2] Production of at least some NO occurred on dissolution of every diazeniumdiolated amidine prepared in this study in aqueous buffer at pH 7.4 and 37 °C, accompanied by the simultaneous release of nitrous oxide (N_2O). Release of NO was monitored by chemiluminescence, while N_2O was detected by gas chromatography. Quantification was difficult because the release was extremely slow, and extrapolation was effectively thwarted by the nonexistence of true half-lives. While N_2O can be produced via the dimerization of nitroxyl (HNO),[25] it is also a known degradation product of C-bound diazeniumdiolates in their nitrosohydroxylamine tautomeric form.[26] While the present study provides no direct evidence that can be used to distinguish between these two modes of N_2O generation, previous studies have postulated the release of nitroxyl by polydiazeniumdiolates accompanied by the formation of oxime byproducts.[9] These gases were not produced by strictly first-order processes because the amidine components of the salts are able to undergo hydrolysis simultaneously with the diazeniumdiolates. While the alkaline hydrolysis of most amidines is fast,[27] it appears from monitoring of NMR solutions that addition of the diazeniumdiolate group reduces this rate. The possible role of the resulting diazeniumdiolated amides as a source of NO cannot be evaluated since *C*-diazeniumdiolated amides have, to our knowledge, never been prepared. However, decomposition of these compounds is accompanied by a return of the NMR signals attributable to the starting amidines, suggesting that the major mechanism of NO release is simple hydrolysis of the diazeniumdiolated amidines themselves.

The compounds of potential medicinal value were studied in greater detail. Diazeniumdiolated tetrahydrozoline (**12**) solution in phosphate buffer at pH 7.4 and 37 °C generated NO at an initial rate of about 9.3×10^{-6} mol/min/mol which decreased steadily over a period of 8 days, producing a total NO release of ca. 0.03 mol/mol during that period of time. Diazeniumdiolated idazoxan (**13**) was also an extremely long-term NO-releasing agent, producing an initial flux of 4.0×10^{-5} mol of NO/min/mol which decreased steadily over a period of 28 days, producing a total NO release of ca. 0.2 mol/mol (10% of the total initially contained NO) during this short month. While N_2O could be detected in the stagnant headspace above pH 7.4 solutions of both these materials, it was not produced in large quantities and could not be quantified. It was only possible to measure the total amount of N_2O released from **4** (ca. 0.016 mol/mol over a period of 6 days) because it had the highest N_2O release of any of the amidine diazeniumdiolates prepared. Generation of these gases was not complete even after these relatively long periods, but the solutions were not monitored further since with the passage of time the results become somewhat less accurate due to diffusion, buffer pH changes, bacterial growth, and other influences. Attempts to speed up NO release using strong mineral acids probably altered the course of the hydrolysis since these solutions turned pale blue, an outcome indicative of the formation of *C*-nitroso compounds.

Implications for the Development of NO-Releasing Amidines of Importance in Biomedical Science. The release of substantial quantities of NO over lengthy periods of time without the production of much N_2O strongly recommends this new diazeniumdiolation reaction as a method for preparing NO-releasing versions of existing pharmaceutical or experimental agents that could benefit from the addition of this second bioactive

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agent. The α-adrenergic agonist derivatives described above are certainly one such class of medicinal compounds, but the greatest potential lies in the application of this method to permit experimentation with previously unavailable types of dual-acting drug candidates. We note, for example, that many simple amidines are inhibitors of nitric oxide synthase (NOS),[28] and there may be many potential applications for an agent that can both prevent uncontrolled endogenous production of NO (as happens during septic shock)[29] and yet maintain the low dose required for healthy physiology. Amidine-based protein kinase C[30] and thymidine phosphorylase[31] inhibitors have been tested for antitumor activity, and addition of NO-releasing activity could help these materials penetrate poorly vascularized tumors via vasodilation. Amidines of the type suitable for diazeniumdiolation are contained in cardiovascular, antidiabetic, antibacterial, antifungal, antiviral, and central nervous system drugs.[32] In addition, the amidine functional group is often introduced into peptides and proteins as the link produced when imidoesters are used either as cross-linking or conjugating reagents.[33]

The results reported guide the synthetic chemist toward the preparation of the zwitterionic monodiazeniumdiolates as the simplest and most economical (requiring no counterion) compounds discovered in this study. The medicinal chemist would be pleased to note that the same materials offer the largest NO-release potential since they are less prone to amidine hydrolysis and do not fission in such a way as to produce much N_2O.

Conclusion. The reaction of NO with a variety of substituted amidines proceeds via their enediamine tautomers to produce C-bound diazeniumdiolates which may serve as slow-release NO donors at physiological pH. The new reaction is among the few that have as yet been attributed to the existence of this relatively obscure "third tautomer" of the amidines and is one of a growing number of known electrophilic reactions of NO with neutral organic molecules. The most advantageous substrates appear to be those having a single substitutable hydrogen, leading to the production of zwitterionic monodiazeniumdiolates of excellent stability with minimal tendency toward the reduced NO-donating ability which results from amidine hydrolysis and/or cleavage to N_2O.

The identification of new NO-releasing C-bound diazeniumdiolates and methods for their preparation should stimulate further research aimed toward the identification of pharmaceutically relevant materials.

Experimental Section

Caution! Polydiazeniumdiolated species are energetic materials, examples of which have been reported to explode on heating[9] and can decompose spontaneously during reaction workup to produce large volumes of gas very rapidly.[3] The extent of formation of the diazeniumdiolate group in every freshly prepared sample should be ascertained immediately with a minimum of manipulation using the characteristic UV absorption at 240−260 nm, and those materials which appear to have formed excessively large quantities of polydiazeniumdiolates should never be placed in closed containers nor subjected to shock or heat until their stability has been verified.

General Information. The equipment used for conducting reactions with NO gas under anaerobic conditions has been described previously.[13] Nitric oxide was obtained in UHP grade from Matheson Tri-Gas (Montgomeryville, PA) and allowed to stand in a ballast tank at about 5 atm pressure over potassium hydroxide pellets for several hours before use. Amidines were purchased from Sigma-Aldrich Co. (Milwaukee, WI), except for idazoxan hydrochloride, which was purchased from Research Biochemicals International (Natick, MA), and 2-cyclohexyl-2-imidazoline, which was synthesized using a literature procedure.[34] Unless otherwise noted, the NMR spectra were recorded in nonbuffered D_2O solutions at 400 MHz for proton and 100 MHz for carbon. Ultraviolet data were obtained in 0.01 M NaOH solution. Chemiluminescence measurements of NO were performed with a Thermal Energy Analyzer model 502A (Thermedics, Inc., Woburn, MA) or a Sievers model 280i Nitric Oxide Analyzer (Sievers Instruments, Inc., Boulder, CO) using solutions in phosphate buffer at pH 7.4 at 37 °C as described previously.[13] Nitrous oxide emissions were checked using a Hewlett-Packard model 5890 gas chromatograph equipped with an electron capture detector. Melting points were obtained on a hot stage and are uncorrected.

General Procedure for the Preparation of Diazeniumdiolated Amidines. A solution of the amidine free base (prepared as described in the individual sections below, if necessary) in the indicated solvent was placed in a standard Parr thick-walled glass hydrogenation bottle. Argon gas was passed through the apparatus and bubbled through the solution for 5−10 min, the NO supply line was clamped into the bottle neck, and the headspace was further deoxygenated by 20−25 repeat cycles of argon pressurization to 5 atm followed by venting. Magnetic stirring was begun, and NO gas was admitted to a pressure of ca. 5 atm. After the indicated reaction period, excess NO was vented and argon bubbled through the resulting slurry (unless noted!) for 5 min. The products were then isolated as described in the individual sections. All are white to off-white powders that can be stored in the refrigerator for many months without degradation.

Reaction of 2-Methyl-2-imidazoline with NO To Produce 4. A solution of 2-methyl-2-imidazoline (lysidine, 5.00 g, 59.4 mmol) in 150 mL of acetonitrile (CH_3CN) was reacted with NO for 28 h at room temperature as described above. The product was isolated by filtration, washed with CH_3CN and then ether, and dried in vacuo to yield 3.59 g (49%) of **4**: mp 102−103 °C (dec); ^{1}H NMR δ 1.92 (6H, s), 3.51 (8H, s), 3.67 (4H, s); ^{13}C NMR δ 14.7 (2C), 47.6 (4C), 51.7 (2C), 163.5 (2C), 171.5 (2C); UV λ_{max} (ϵ) 260 nm (13.6 mM^{-1} cm^{-1}), 206 nm (22.5 mM^{-1} cm^{-1}).

Anal. Calcd for $C_{12}H_{24}N_{10}O_4$: C, 38.71; H, 6.50; N, 37.61. Found: C, 38.92; H, 6.55; N, 37.62.

To prepare the disodium salt 1.74 g of a 25% NaOMe in MeOH solution (Sigma-Aldrich, 8.06 mmol) was diluted with 0.5 mL of MeOH and to this was added 1.5 g of the above diimidazolium salt (**4**, 4.03 mmol). The solid slowly dissolved and then reprecipitated. The slurry was diluted with 2 mL of CH_3CN and filtered, and the resulting solid was dried in vacuo to produce 0.92 g (92% yield) of **6**: mp >180 °C (chars); ^{1}H NMR δ 2.76 (2H, t, J = 6.2 Hz), 3.38 (2H, t, J = 6.2 Hz); ^{13}C NMR δ 42.3, 44.8, 88.5 (triplet due to deuterium exchange, J_{CD} = 22 Hz), 166.6; UV λ_{max} (ϵ) 260 nm (13.9 mM^{-1} cm^{-1}).

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Anal. Calcd for $C_4H_6N_6O_4Na_2$: C, 19.36; H, 2.44; N, 33.87. Found: C, 19.44; H, 2.56; N, 33.66.

Reaction of Acetamidine with NO. A concentrated solution of sodium hydroxide was prepared by stirring a large excess of pellets in water for 1 h and then decanting the thick solution. This solution was cooled in an ice bath, and acetamidine hydrochloride was added until a large, viscous oily top layer of free base had formed. This acetamidine free base was removed with a glass pipet and used directly. A suspension of 5.00 g of acetamidine (86.1 mmol) in 150 mL of CH_3CN was reacted with NO for 20 h as described above. The product was isolated by filtration, washed with CH_3CN and then ether, and dried in vacuo to yield 5.95 g of tan powder which was a mixture of products and exhibited the following characteristics: ^{1}H NMR δ 2.05, 2.07, 2.22, 3.83, 7.62 (all singlets); UV λ_{max} 260 nm.

Reaction of 2-Iminopiperidine with NO To Produce 9. A nearly saturated solution of NaOH was prepared in an ice bath, and to this was added a cold, concentrated solution of iminopiperidine hydrochloride. The resulting white crystalline free base was removed by filtration (not washed with additional water!) and dried in vacuo. A solution of 5.00 g (50.9 mmol) of this iminopiperidine in 200 mL of CH_3CN was prepared and filtered to remove a small amount of insoluble residue. This solution was then treated with NO for 20 h as described above. The product was isolated by filtration, washed with CH_3CN and then ether, and dried in vacuo to yield 4.50 g (64%) of crude product which exhibited the following characteristics: ^{1}H NMR δ 1.8−1.9 (7.2H, m), 2.55−2.65 (2.6H, m), 2.85−2.95 (2H, m) 3.3−3.4 (2.6H, m), 3.5−3.6 (2H, m). Recrystallization of this material from methanol/ether afforded **9**: mp 160−170 °C (chars); ^{1}H NMR δ 1.8−1.9 (3.3H, m), 2.55−2.65 (0.7H, m), 2.85−2.95 (2H, m) 3.3−3.4 (0.7H, m), 3.5−3.6 (2H, m); ^{13}C NMR δ 19.0, 20.3, 23.0, 28.3, 29.0, 43.7, 44.1, 90.6, 162.7, 169.0; UV λ_{max} (ϵ) 259 nm (13.2 mM^{-1} cm^{-1}).

Anal. Calcd for $C_5H_9N_6O_4 \cdot 1/3(C_5H_{11}N_2) \cdot 2/3Na \cdot 2/3H_2O$: C, 28.85; H, 5.08; N, 33.75. Found: C, 28.70; H, 4.83; N, 34.19.

Reaction of 2-Cyclohexyl-2-imidazoline (10) with NO To Produce 11. A solution of 2-cyclohexyl-2-imidazoline (5.00 g, 32.8 mmol) in 300 mL of CH_3CN was treated with NO for 78 h as described above. The product was isolated by filtration, washed with CH_3CN and then ether, and recrystallized from ethanol to afford 6.66 g (97%) of **11**: mp 158−159 °C (dec); ^{1}H NMR δ 1.4−1.7 (6H, m), 1.9−2.1 (2H, m), 2.5−2.6 (2H, m), 4.00 (4H, s); ^{13}C NMR δ 23.9 (2C), 26.6, 34.3 (2C), 47.3 (2C), 73.0, 173.4; UV λ_{max} (ϵ) 245 nm (7.2 mM^{-1} cm^{-1}).

Anal. Calcd for $C_9H_{16}N_4O_2$: C, 50.93; H, 7.60; N, 26.40. Found: C, 51.18; H, 7.49; N, 26.39.

Reaction of Tetrahydrozoline with NO To Produce 12. A solution of tetrahydrozoline hydrochloride (10.0 g, 42.3 mmol) in 9.66 mL of 25% NaOMe in MeOH (42.3 mmol of NaOMe) was diluted with 200 mL of CH_3CN, and the precipitated sodium chloride was removed by filtration. The resulting solution was treated with NO for 24 h as described above to yield 9.0 g (82%) of **12**: mp 168−169 °C (dec); ^{1}H NMR δ 1.8−1.9 (2H, m), 2.3−2.45 (1H, m), 2.9−3.0 (3H, m), 4.00 (4H, s), 7.15−7.47 (4H, m); ^{13}C NMR δ 20.4, 30.6, 34.7, 47.7 (2C), 76.0, 129.7, 130.7, 131.8, 133.0, 133.1, 141.5, 173.7; UV λ_{max} (ϵ) 249 nm (broad, 9.3 mM^{-1} cm^{-1}), 215 nm (14.3 mM^{-1} cm^{-1}).

Anal. Calcd for $C_{13}H_{16}N_4O_2$: C, 59.99; H, 6.20; N, 21.52. Found: C, 60.05; H, 6.14; N, 21.48.

Reaction of Idazoxan with NO To Produce 13. A solution of idazoxan hydrochloride (1.00 g, 4.15 mmol) in 3 mL of MeOH was treated with 0.95 mL of 25% NaOMe in MeOH (4.15 mmol of NaOMe) and then diluted with 40 mL of CH_3CN. The precipitated sodium chloride was removed by filtration, and the resulting solution was treated with NO for 21 h as described above to yield 0.62 g (56%) of **13**: mp 152−154 °C (dec); ^{1}H NMR δ 4.04 (4H, s), 4.64 (1H, d, J = 12.2 Hz), 5.13 (1H, d, J = 12.2 Hz), 7.02−7.22 (4H, m); ^{13}C NMR δ 48.9 (2C), 67.8, 91.4, 120.3, 120.5, 126.1, 127.0, 141.8, 144.4, 166.8; UV λ_{max} (ϵ) 256 nm (broad, 8.4 mM^{-1} cm^{-1}), 216 nm (11.5 mM^{-1} cm^{-1}).

Anal. Calcd for $C_{11}H_{12}N_4O_4$: C, 49.81; H, 4.94; N, 21.12. Found: C, 50.22; H, 4.61; N, 20.98.

NO-Release Studies. NO release from all compounds was measured by a chemiluminescence method described previously.[13] Briefly, solutions of each in phosphate buffer at pH 7.4 were incubated continuously at 37 °C, and for short intervals their contents were swept with an inert gas into a chemiluminescence detector for quantification of NO. After a steady baseline was achieved, integration over several minutes provided the NO generation rate during that interval. Plots of these rates versus time at the midpoints of the corresponding intervals gave curves from which the yield of NO could be determined by integration. Extrapolation to infinite time was not possible due to the non-first-order nature of the NO generation decay curves. Nitrous oxide emissions were determined in much the same way except the electron capture detector of the gas chromatograph provided the signal, and only **4** produced enough gas to provide a useful plot, although N_2O was detected in the headspace of all the other samples.

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