New Nitric Oxide-Releasing Zwitterions Derived from Polyamines

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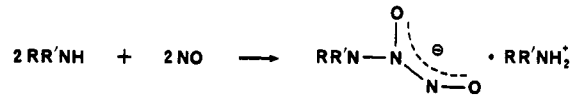
The reaction of nitric oxide (NO) with polyamines has been studied, resulting in the discovery of a new type of NO-releasing compound having the structure $RN[N(O)NO]^-(CH_2)_xNH_2^+R'$ (3). Numerous examples of these zwitterionic polyamine/NO adducts have been prepared and found to be very stable solids which release NO in solution. The new compounds contain as much as 45% NO by weight and are capable of releasing it all at rates which have been shown to vary in a predictable way with structure. The half-lives in buffered aqueous solution at pH 7.4 and 22 °C were shown to vary from extremely short (1.3 min for diamine 8, $MeN[N(O)NO]^-(CH_2)_4NH_2^+Me$) to very long (56 h for triamine 18, $H_2NCH_2CH_2N[N(O)NO]^-CH_2CH_2NH_3^+$). In general, the longest half-lives were achieved by triamine/NO adducts and derivatives of ethylenediamine ($x = 2$). For any given value of x, a small increase in the size of R resulted in a relatively large increase in half-life but changes in R' appeared to have little effect. Data are presented which should allow the selection of the proper compounds to achieve a wide range of desired NO generation rates. These NO-containing zwitterions should prove to be important resources in studies of the biology of NO and may also have important pharmaceutical and chemical applications.

The reaction of nitric oxide (NO) with amines to produce salts of structure 1 has been known for many years.[1] The



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anionic portions of these salts are of great interest since they spontaneously decompose in solution to regenerate NO,[1f,2] a molecule whose biological roles[3] are being discovered at a breathtaking pace. A limited number of these salts and those having sodium cations are available due to the work of Drago and co-workers,[1b,c] who isolated the more stable examples, most notably the diethylamine/NO adduct (DEA/NO, 1 with R = R' = Et) and the sodium salt of the isopropylamine/NO adduct (1, R = *i*-Pr, R' = H). These salts undergo slow decomposition even in the solid state unless stored at −78 °C. Nonetheless, they have proven to be valuable in biological studies[2,4] requiring a controlled, gradual release of NO.

Drago's group also studied[1e] the reaction of two diamines with NO and reported the production of *inter*molecular salt 2 (for the sake of brevity, the line formula $[N(O)NO]^-$ is used to represent the anionic functional group which is shown in its entirety in structure 1) as well as the analogous compound derived from piperazine. No special stability was reported for these materials, which were apparently not stable enough to give good combustion analyses.

$$CH_3N[N(O)NO]^-(CH_2)_2N[N(O)NO]^-CH_3$$

$$CH_3NH_2^+(CH_2)_2NH_2^+CH_3$$

2

We noted with interest that these two diamine/NO adducts were produced under conditions (i.e., in media in which any intermediate products would be expected to remain soluble) which would favor the reported *inter*molecular outcome and were intrigued by the possibility of producing *intra*molecular salts (zwitterions) of the general structure 3 by varying these conditions. Our expectation was that such materials would possess a much higher stability in the solid state and would thus prove more useful in biological studies.

$$RN[N(O)NO]^-(CH_2)_xNH_2^+R'$$

3

In this paper, we report the preparation of numerous examples of this new class of zwitterionic compounds and provide information about their tremendous potential as agents for the controlled generation of NO.

Results and Discussion

We began with the assumption that the reaction of NO with polyamines could be limited to one site since they are quite soluble in most organic solvents while the zwitterionic products were not expected to be and should thus precipitate before further reaction could occur. We also

(1) (a) Reilly, E. L. German Patent 1 085 166, July 14, 1960; *Chem. Abstr.* **1962**, *56*, 4594h. (b) Drago, R. S.; Paulik, F. E. *J. Am. Chem. Soc.* **1960**, *82*, 96–98. (c) Drago, R. S.; Karstetter, B. R. *J. Am. Chem. Soc.* **1961**, *83*, 1819–1822. (d) Drago, R. S.; Ragsdale, R. O.; Eyman, D. P. *J. Am. Chem. Soc.* **1961**, *83*, 4337–4339. (e) Longhi, R.; Ragsdale, R. O.; Drago, R. S. *Inorg. Chem.* **1962**, *1*, 768–770. (f) Ragsdale, R. O.; Karstetter, B. R.; Drago, R. S. *Inorg. Chem.* **1965**, *4*, 420–422.

(2) Maragos, C. M.; Morley, D.; Wink, D. A.; Dunams, T. M.; Saavedra, J. E.; Hoffman, A.; Bove, A. A.; Isaac, L.; Hrabie, J. A.; Keefer, L. K. *J. Med. Chem.* **1991**, *34*, 3242–3247.

(3) Moncada, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Rev.* **1991**, *43*, 109–142.

(4) Wink, D. A.; Kasprzak, K. S.; Maragos, C. M.; Elespuru, R. K.; Misra, M.; Dunams, T. M.; Cebula, T. A.; Koch, W. H.; Andrews, A. W.; Allen, J. S.; Keefer, L. K. *Science (Washington, D.C.)* **1991**, *254*, 1001–1003.

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assumed that the use of dilute solutions would favor formation of the *intra*molecular product (3).

The key experimental discovery was that while the use of dilute solutions in virtually any aprotic solvent would prevent the formation of *inter*molecular salts such as 2, more polar aprotic solvents were necessary to prevent formation of an alternative *inter*molecular product. The situation is best illustrated by the reaction of *N*-isopropyl-1,3-propanediamine with NO to form either salt 4 or zwitterion 5. Using the relative areas of the methyl

$$\underset{\mathbf{4}}{i\text{-PrN}[\text{N(O)NO}]^-(\text{CH}_2)_3\text{NH}_2{\cdot}i\text{-PrNH}_2{}^+(\text{CH}_2)_3\text{NH}_2}$$

$$\underset{\mathbf{5}}{i\text{-PrN}[\text{N(O)NO}]^-(\text{CH}_2)_3\text{NH}_3{}^+}$$

doublets in the NMR spectra, it was determined that mostly 4 (86%) forms in dilute ether solution, mostly 5 (96%) in dilute tetrahydrofuran (THF), and exclusively 5 in dilute acetonitrile. In line with our expectations, 4 proved to be a rather unstable solid while 5 could be manipulated and stored easily. Not all of the polyamine/NO reactions exhibit this dramatic variation with solvent and some[5] even form pure zwitterionic products in dilute ether solution. In practice, few of the reactions we studied exhibited this clear solvent effect because the amines themselves are small polar protic molecules which can serve to alter the solvent character. We soon adopted acetonitrile as the solvent of choice since reactions in this solvent appeared to be less subject to complications arising from this type of influence.

This new class of zwitterionic polyamine/NO adducts proved to have many advantageous properties when compared to the previously known materials of structures 1 and 2. Under an NO pressure of 70–80 psig, they form at room temperature in excellent yields with short (<1 d) reaction times. As solids they are all stable for weeks at room temperature in closed containers and yet will release NO rapidly in acidic solutions or more slowly in buffered near-neutral media. These facts encouraged us to undertake a systematic study of the various available polyamines in search of useful NO adducts.

Zwitterionic Diamine/NO Adducts. We begin with a study of a homologous series of *N,N'*-dimethyl diamines (3, R = R' = Me, products 6–9 in Table I). The half-life data show that increasing the length of the alkyl chain separating the nitrogens results in a more rapid release of NO. Ultimately when the chain is sufficiently long we would expect the compound to behave like the *inter*molecular salts. These early results clearly showed that these compounds had the potential to provide predictable, controlled release of NO at very fast or relatively slow rates.

To investigate the effect of alkyl substitution on solution stability, the ethylenediamine derivatives 10–12 were prepared. Compounds 11 and 12 have half-lives approximately *20 times* that of DEA/NO (1, R = R' = Et) which has a half-life of 16 min at 22 °C in pH 7.4 buffer. Despite the greatly enhanced stability of these materials, we were forced to switch to the propanediamine series to study larger alkyl group substitution because (perhaps for steric considerations) the ethylenediamine derivatives containing these larger groups (i.e., *N*-propylethylenediamine and *N*-isopropylethylenediamine) would not react with NO under the low pressure conditions employed.

(5) Keefer, L. K.; Hrabie, J. A. U.S. Patent 5 155 137, Oct. 13, 1992; *Chem. Abstr.* **1992**, *116*, 136242b.

Table I. Synthesized Zwitterions of the Form $RN[N(O)NO]^-(CH_2)_xNH_2{}^+R'$

product	x	R	R'	UV λ_{max} (nm)[a]	$\epsilon \times 10^{-3}$ (M^{-1} cm^{-1})[a]	$t_{1/2}$ (min)[b]
6	2	Me	Me	250	7.31	36.1
7	3	Me	Me	250	7.68	10.1
8	4	Me	Me	250	8.59	1.3
9	6	Me	Me	250	7.25	2.7
10	2	Me	H	252	7.80	40
11	2	Et	H	252	7.96	333
12	2	Et	Et	252	7.61	327
13	3	Me	H	250	7.77	13.7
14	3	Et	Et	250	8.55	71.8
15	3	Pr	H	250	8.05	76.6
5	3	*i*-Pr	H	250	7.44	93.0
16	3	*i*-Pr	*i*-Pr	250	7.89	88.5
17	3	cyclohexyl	H	250	9.13	115
18	2	$(CH_2)_2NH_2$	H	252	7.64	3,400
19	3	$(CH_2)_3NH_2$	H	252	7.86	284
20	3	$(CH_2)_4NH_2$	H	250	9.42	165

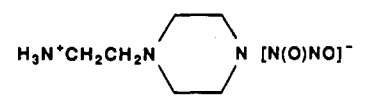
[a] Measured in 0.01 M NaOH. [b] Determined at 22 °C and pH 7.4 in 0.1 M phosphate buffer as described in the Experimental Section.

The solution half-lives of the NO adducts of the substituted propanediamines (5 and 13–17 in Table I) clearly show sharp increases with the size of R (the alkyl group on the $[N(O)NO]^-$-bearing nitrogen). The nature of this stabilization is not clear although a reasonable rationalization will be presented later. Particularly noteworthy is the almost complete absence of any influence of R' on the half-lives (compare 10 to 6, 11 to 12, 13 to 7, and 5 to 16).

Zwitterionic Triamine/NO Adducts. The magnitude of the half-life enhancement achieved by placing one proton-bearing nitrogen in the molecule prompted studies of the few triamines which have only one secondary nitrogen and were thus likely to give single pure NO adducts. Those zwitterions which could be isolated are listed in Table I as compounds 18–20. The unsymmetrical compounds (i.e., those having alkyl chains of unequal length on each side of the anion) proved to be very hygroscopic so only 20, the NO adduct of the important biomolecule spermidine, was isolated from that group. Compound 18, whose half-life is over 200 times that of DEA/NO, has the distinction of having the greatest solution stability of any amine/NO adduct yet prepared.

Products 18–20 are representative of the amazing preference that NO exhibits toward attachment at secondary amines. They each have two primary nitrogen sites yet no trace of primary amine/NO adducts could be found and the mass balance is excellent (isolated yields are 88–94%). Substitution by $[N(O)NO]^-$ at a primary nitrogen would have been particularly noticeable in the NMR spectra of compounds 18 and 19 since the resulting products would not have been symmetrical.

The triamine series also provided an opportunity to determine whether a tertiary amine could play a role in stabilizing these adducts. While we have thus far been unable to react NO with diamines containing a tertiary amine, it was possible to isolated triamine derivative 21.



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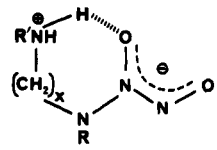
The half-life of **21** is 5.0 min (measured under the same conditions as those in Table I) which is similar to that of **9** and suggests no special stability due to the presence of the tertiary amine.

Larger Polyamines. Attempts to prepare zwitterionic NO adducts from larger polyamines were complicated by the presence of more than one reaction site in most such compounds. Most of these materials formed as oils or hygroscopic powders which we chose not to explore further. The singular exception was **22**, the NO adduct of spermine,

$$H_2N(CH_2)_3N[N(O)NO]^-(CH_2)_4NH_2^+(CH_2)_3NH_2$$
22

a well behaved compound of considerable biological interest which has already been used in several studies[2,4] of the biological effects of NO. Under the conditions of the present study it has a solution half-life of 230 min. Comparison with spermidine/NO adduct **20** suggests that the fourth nitrogen imparts little added stability.

Origin of the Exceptional and Unexpected Solution Stability of the Zwitterionic NO Adducts. Extensive studies of the structure of the RR'N[N(O)NO]$^-$ anion and the mechanism of the acid-catalyzed reversion to amine plus NO are currently underway. Pending the outcome of these studies, sufficient information exists to suggest a reasonable explanation for the results presented above. A recent study[6] of the chemistry of this anion has shown that alkylation occurs exclusively at the terminal oxygen. These alkylated compounds were found to be resistant to acid-catalyzed fragmentation, thus suggesting that removal of the negative charge from the [N(O)NO]$^-$ group prevents the protonation which is presumably the key step in this process. If this is the case, any substituents which lead to a reduction of the negative character of the group should increase the half-life of the anion. It is easy to see how the aminoalkyl groups of our zwitterions could do this via hydrogen bonding. The rapid decrease in stability with increasing chain length observed in compounds **6–8** can be attributed to the decreasing importance of hydrogen bonding in **23** as the ring size (i.e., x) increases. Similarly,



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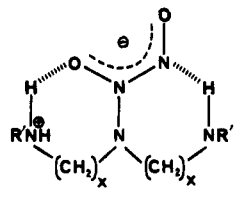

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structure **24** is representative of the further hydrogen bonding which is possible in the triamines.

The inability of **24** to accommodate a third hydrogen bond explains the observation that addition of a fourth nitrogen (as in **22**) does not result in a dramatic half-life enhancement. The slight additional stability observed may be attributed to the general accumulation of positive charge in the molecule. On purely electrostatic grounds, the presence of a greater number of potential cationic centers in the molecule should render protonation of the [N(O)NO]$^-$ group more difficult and thus raise the half-life.

The observed negligible influence of R' on the solution stability of these compounds is understandable by inspection of **23**. R' is isolated from the π-electron system and is apparently not capable of exerting a significant steric or electronic effect.

The increases in half-life which accompany increases in the size of R are somewhat more difficult to explain. The [N(O)NO]$^-$ group in structures **23** and **24** is shown in the cis configuration because X-ray and microwave studies[7] of the NO dimer suggest that this may be the favored structure although quantum-mechanical calculations[8] indicate little difference in energy between the cis and trans configurations. It is thus reasonable to attribute the influence of R to subtle changes in the electron distribution within the [N(O)NO]$^-$ system. Such changes could arise either as a result of the inductive electron donating ability of R or as a result of steric interaction between R and the nonbonding electron pair of the exocyclic nitrogen in **23**. We anticipate that a more detailed explanation will be available once our ongoing studies are completed.

Potential Utility in Biomedical Research. While we have not determined the extent of NO release for every one of the new zwitterions, data obtained in conjunction with the initial biological studies[2,9] have shown that **15** and **22** release the full 2 equiv of NO in solution. Using these same methods,[9] we have verified that compounds **6**, **10**, **12**, and **18** also release 2 equiv of NO. The data we have obtained show that this NO can be released rapidly (1–2 min) or slowly (several days), as desired, by appropriate choice of the NO compound. While our NO release studies have been performed at 22 °C, we can report that at 37 °C in phosphate buffered saline the half-lives for compounds **15** and **22** are 8 and 39 min, respectively.[2,9] This suggests that (with possible exceptions) the biologically relevant half-lives will be approximately $^1/_6$ to $^1/_9$ those reported in Table I.

The extended shelf life of these compounds was demonstrated by repeatedly obtaining combustion analysis data for samples of **15** that had been stored in a refrigerator for several months. No change in elemental composition was observed during this time.

Finally, these zwitterions bear a striking structural resemblance to the amino acids. This may render them more amenable to participation in biological processes.

Practical Benefits for the Chemist. NO is a difficult gas to handle due to its highly reactive and poisonous nature. It cannot be manipulated in the presence of oxygen, will attack most metals and plastics, and can only be purchased in relatively low pressure cylinders. In contrast, this new series of compounds can be handled in air without fear of exposure to toxic vapor. These compounds offer a remarkably compact storage medium for NO gas. It is truly amazing that compound **10** is 45% NO by weight and yet it is an easily handled solid. Indeed, calculation reveals that a 50-g bottle of **10** is equivalent to an entire lecture bottle of NO. We have shown that **10** can be used in a Kipp-type generator to produce a steady stream of NO in the same way that zinc is used in the production of hydrogen.

(6) Saavedra, J. E.; Dunams, T. M.; Flippen-Anderson, J. L.; Keefer, L. K. *J. Org. Chem.* **1992**, *57*, 6134–6138.

(7) Lee, T. J.; Rice, J. E.; Scuseria, G. E.; Schaefer, H. F. *Theoret. Chim. Acta* **1989**, *75*, 81–98, and references therein.

(8) Jones, W. H. *J. Phys. Chem.* **1992**, *96*, 594–603.

(9) Maragos, C. M.; Wang, J. M.; Hrabie, J. A.; Oppenheim, J. J.; Keefer, L. K. *Cancer Res.* **1993**, *53*, 564–568.

Conclusion. A wide variety of zwitterionic polyamine/NO adducts can be prepared easily and in high yield. While further studies of the structure and chemistry of these compounds are underway, the findings to date suggest that these compounds can play a key role in investigation of the biology of NO. Future studies will be devoted to the development of specific compounds to achieve a wide variety of pharmacological goals.

Experimental Section

The NMR spectra of all compounds were recorded in D_2O (^1H at 200 MHz; ^{13}C at 50 MHz) at the natural pD of their solutions. Due to the finite time required for data acquisition, the ^{13}C spectra of the shorter half-lived compounds often display small peaks attributable to the parent amines which are not listed here. Ultraviolet data were obtained in 0.01 M NaOH to avoid this degradation problem. Melting points were obtained on a hot stage and are uncorrected. No mass spectral data are provided since these compounds display the spectra of the parent amines due to rapid dissociation in high vacuum. Unless otherwise indicated, amines were purchased from either Carbolabs, Inc. (Bethany, CT) or Aldrich Chemical Co. (Milwaukee, WI). Fresh samples uncontaminated by absorbed CO_2 are important to both the yield of the reactions and the stability (shelf life) of the products. Reaction solvents were anhydrous grade (Aldrich) but all others were reagent grade. Nitric oxide was obtained from Matheson Gas Products and used as received. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA).

Caution! These compounds are designed to be physiologically active and should be handled with due care. While no signs of instability have been observed during routine handling under ambient conditions, exposure to CO_2, water, or acid vapor can lead to the release of large volumes of toxic NO gas. Closed containers of these materials should be stored in a freezer and the same precautions taken during their opening as with any substance that is capable of developing pressure on storage.

Apparatus for NO Reactions. Stainless steel (SS) is required for prolonged exposure to NO gas and amines degrade most types of stoppers and gaskets. Accordingly, we constructed a specialized reactor modeled after the standard Parr 3911 hydrogenation apparatus (Parr Instrument Co., Moline, IL). The reservoir was replaced by a type 304 SS gas sampling cylinder equipped with SS fittings (available from any "valve and fitting" plumbing supply company); the valves were the diaphragm-seal packless type (Aldrich) and the pressure gauges were SS (Air Products). The usual Parr clamp and bottle system was employed but was connected to the gas reservoir via a Teflon tube and mounted to allow stirring with a magnetic stirrer.

General Procedure for the Preparation of Intramolecular Di-, Tri-, and Tetramine NO Adducts (5–22). A solution of the appropriate amine in the desired solvent was placed in a standard Parr hydrogenation bottle. Nitrogen was passed through the apparatus and bubbled through the solution for 5–10 min, the bottle was clamped, and NO gas was admitted to a pressure of 5 atm. The solution was stirred for the indicated time with addition of NO as needed during the first 5–6 h to maintain the reservoir pressure. The reactions, which were neither heated nor cooled, appeared to warm only very slightly for the first hour and then returned to room temperature. Excess NO was then vented and N_2 was bubbled through the resulting white slurry for 5 min. The product was isolated by filtration, washed with the reaction solvent and then with ether, and dried in vacuo for several hours. All of the products are amorphous, voluminous white powders which (except as indicated) are air-stable. Since we have as yet found no method for recrystallization of these compounds, all of the analytical data given were obtained using the products as isolated directly from the reaction mixtures.

1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-isopropyl-1-triazene (5)[10] was prepared by treating *N*-isopropyl-1,3-propanediamine (10.0 g, 86.0 mmol) in 200 mL of THF with NO for 74 h: yield 0.66 g (4.5%); mp 114–115 °C dec; ^1H NMR δ 1.07 (6 H, d, J = 6.3 Hz), 1.68 (2 H, m), 3.02–3.14 (4 H, m), 3.22 (1 H, septet, J = 6.3 Hz); ^{13}C NMR δ 22.2 (2 C), 27.2, 40.6, 50.1, 57.1.

Anal. Calcd for $C_6H_{16}N_4O_2$: C, 40.90; H, 9.15; N, 31.79. Found: C, 40.98; H, 9.15; N, 31.79.

When this preparation was repeated using a solution of 10 g of the amine in 150 mL of ether and a 4-d reaction time, there was obtained 0.88 g of white solid which NMR showed to be 86% *inter*molecular salt **4**. This material was not stable enough to obtain combustion analytical data. **4**: mp 98–101 °C dec; ^1H NMR δ 1.07 (6 H, d, J = 6.3 Hz), 1.26 (6 H, d, J = 6.7 Hz), 1.68 (2 H, m), 1.86 (2 H, m), 2.87 (2 H, t, J = 7.3 Hz), 3.02–3.14 (6 H, m), 3.16–3.35 (2 H, m); ^{13}C NMR δ 21.7 (2 C), 22.2 (2 C), 27.2, 30.1, 40.5, 40.6, 45.4, 50.1, 53.0, 57.1.

1-Hydroxy-2-oxo-3-(*N*-methyl-2-aminoethyl)-3-methyl-1-triazene (6) was prepared by reacting *N,N'*-dimethylethylenediamine (5.00 g, 56.7 mmol) in 200 mL of THF with NO for 47 h: yield 3.87 g (46%); mp 116–117 °C dec; ^1H NMR δ 2.73 (3 H, s), 2.82 (3 H, s), 3.05 (2 H, m, M_2 portion of an AA'M_2 system), 3.27 (2 H, m, AA' portion of an AA'M_2 system); ^{13}C NMR δ 35.8, 44.9, 48.5, 53.1.

Anal. Calcd for $C_4H_{12}N_4O_2$: C, 32.43; H, 8.17; N, 37.82. Found: C, 32.53; H, 8.23; N, 37.75.

1-Hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (7) was prepared by treating *N,N'*-dimethyl-1,3-propanediamine (9.78 g, 95.7 mmol) in 200 mL of THF with NO for 27 h: yield 5.37 g (35%); mp 111–112 °C dec; ^1H NMR δ 1.70 (2 H, m), 2.68 (3 H, s), 2.76 (3 H, s), 3.00–3.13 (4 H, m); ^{13}C NMR δ 25.8, 35.8, 45.3, 50.1, 54.4.

Anal. Calcd for $C_5H_{14}N_4O_2$: C, 37.02; H, 8.69; N, 34.55. Found: C, 37.12; H, 8.74; N, 34.45.

1-Hydroxy-2-oxo-3-(*N*-methyl-4-aminobutyl)-3-methyl-1-triazene (8) was synthesized by reacting *N,N'*-dimethylputrescine (Pfaltz and Bauer, 5.00 g, 43.0 mmol) in 150 mL of CH_3CN with NO for 23 h: yield 6.47 g (85%); mp 112–114 °C dec; ^1H NMR δ 1.40 (2 H, m), 1.74 (2 H, m), 2.67 (3 H, s), 2.74 (3 H, s), 2.97 (4 H, m); ^{13}C NMR δ 26.0, 26.2, 35.6, 45.2, 51.7, 56.7.

Anal. Calcd for $C_6H_{16}N_4O_2$: C, 40.90; H, 9.15; N, 31.79. Found: C, 41.18; H, 9.20; N, 31.63.

1-Hydroxy-2-oxo-3-(*N*-methyl-6-aminohexyl)-3-methyl-1-triazene (9) was synthesized by treating *N,N'*-dimethyl-1,6-hexanediamine (5.00 g, 34.7 mmol) in 150 mL of CH_3CN with NO for 22 h: yield 6.08 g (86%); mp 108–110 °C dec; ^1H NMR δ 1.25–1.45 (6 H, m), 1.55–1.72 (2 H, m), 2.67 (3 H, s), 2.72 (3 H, s), 2.85–3.01 (4 H, m); ^{13}C NMR δ 28.2 (2 C), 28.3, 28.4, 35.6, 45.1, 52.0, 57.3.

Anal. Calcd for $C_8H_{20}N_4O_2$: C, 47.04; H, 9.86; N, 27.43. Found: C, 47.26; H, 9.94; N, 27.29.

1-Hydroxy-2-oxo-3-(2-aminoethyl)-3-methyl-1-triazene (10) was prepared by treating *N*-methylethylenediamine (10.0 g, 135 mmol) in 150 mL of CH_3CN with NO for 27 h: yield 14.8 g (82%); mp 115–116 °C dec; ^1H NMR δ 2.82 (3 H, s), 3.00 (2 H, m, M_2 portion of an AA'M_2 system), 3.24 (2 H, m, AA' portion of an AA'M_2 system); ^{13}C NMR δ 39.2, 45.0, 54.1.

Anal. Calcd for $C_3H_{10}N_4O_2$: C, 26.86; H, 7.51; N, 41.77. Found: C, 27.10; H, 7.58; N, 41.80.

1-Hydroxy-2-oxo-3-(2-aminoethyl)-3-ethyl-1-triazene (11) was synthesized by treating *N*-ethylethylenediamine (5.00 g, 56.7 mmol) in 150 mL of CH_3CN with NO for 26 h: yield 5.51 g (66%); mp 105–106 °C dec; ^1H NMR δ 0.99 (3 H, t, J = 7.1 Hz), 2.97–3.08 (4 H, m), 3.25 (2 H, m, AA' portion of an AA'M_2 system); ^{13}C NMR δ 14.0, 39.2, 51.5, 53.0.

Anal. Calcd for $C_4H_{12}N_4O_2$: C, 32.43; H, 8.17; N, 37.82. Found: C, 32.52; H, 8.19; N, 37.88.

1-Hydroxy-2-oxo-3-(*N*-ethyl-2-aminoethyl)-3-ethyl-1-triazene (12) was prepared by reacting *N,N'*-diethylethylenediamine (5.00 g, 43.0 mmol) in 150 mL of CH_3CN with NO for 21 h: yield 6.80 g (90%); mp 127–128 °C dec; ^1H NMR δ 0.99 (3 H, t, J = 7.1 Hz), 1.29 (3 H, t, J = 7.3 Hz), 2.97–3.08 (4 H, m), 3.11 (2 H, q, J = 7.3 Hz), 3.27 (2 H, m, AA' portion of an AA'M_2 system); ^{13}C NMR δ 13.4, 13.9, 45.8, 46.5, 51.5, 52.1.

Anal. Calcd for $C_6H_{16}N_4O_2$: C, 40.90; H, 9.15; N, 31.79. Found: C, 41.00; H, 9.23; N, 31.73.

1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene(13) was prepared by treating *N*-methyl-1,3-propanediamine (10.0 g, 113 mmol) in 150 mL of CH_3CN with NO for 22

(10) *Nomenclature Note*: The compounds have been named in the neutral form which would result if the site of proton attachment was assumed to be the same as the preferred site of alkylation.

15.9 g (94%); mp 117–118 °C dec; ^{1}H NMR δ 1.61 (2 H, m), 2.76 (3 H, s), 3.02 (2 H, t, J = 4.8 Hz), 3.07 (2 H, t, J = 6.7 Hz); ^{13}C NMR δ 27.1, 40.5, 45.3, 54.5.

Anal. Calcd for $C_4H_{12}N_4O_2$: C, 32.43; H, 8.17; N, 37.82. Found: C, 32.57; H, 8.23; N, 37.59.

1-Hydroxy-2-oxo-3-(*N*-ethyl-3-aminopropyl)-3-ethyl-1-triazene (14) was synthesized by reacting *N,N'*-diethyl-1,3-propanediamine (5.00 g, 38.4 mmol) in 150 mL of CH_3CN with NO for 24 h: yield 6.71 g (92%); mp 114–116 °C dec; ^{1}H NMR δ 0.97 (3 H, t, J = 7.2 Hz), 1.27 (3 H, t, J = 7.3 Hz), 1.71 (2 H, m), 2.96 (2 H, q, J = 7.2 Hz), 3.00–3.14 (6 H, m); ^{13}C NMR δ 13.4, 13.8, 25.8, 45.7, 48.0, 51.7, 53.5.

Anal. Calcd for $C_7H_{18}N_4O_2$: C, 44.19; H, 9.54; N, 29.45. Found: C, 44.23; H, 9.47; N, 29.39.

1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-propyl-1-triazene (15) was prepared by treating *N*-propyl-1,3-propanediamine (10.0 g, 86.1 mmol) in 300 mL of CH_3CN with NO for 23 h: yield 12.4 g (82%); mp 98–99 °C dec; ^{1}H NMR δ 0.90 (3 H, t, J = 7.3 Hz), 1.34 (2 H, sextet, $J_1 = J_2$ = 7.3 Hz), 1.69 (2 H, quintet, $J_3 = J_4$ = 6.8 Hz), 2.88 (2 H, t, J = 7.3 Hz), 3.03 (2 H, t, J = 6.8 Hz), 3.06 (2 H, t, J = 6.8 Hz); ^{13}C NMR δ 13.6, 22.3, 26.9, 40.6, 53.8, 58.9.

Anal. Calcd for $C_6H_{16}N_4O_2$: C, 40.90; H, 9.15; N, 31.79. Found: C, 40.93; H, 9.21; N, 31.85.

1-Hydroxy-2-oxo-3-(*N*-isopropyl-3-aminopropyl)-3-isopropyl-1-triazene (16) was prepared by reacting *N,N'*-diisopropyl-1,3-propanediamine (5.00 g, 31.6 mmol) in 150 mL of CH_3CN with NO for 42 h: yield 3.52 g (51%); mp 114–116 °C dec; ^{1}H NMR δ 1.07 (6 H, d, J = 6.2 Hz), 1.30 (6 H, d, J = 6.4 Hz), 1.67 (2 H, m), 3.06–3.14 (4 H, m), 3.22 (1 H, septet, J = 6.2 Hz), 3.38 (1 H, septet, J = 6.4 Hz); ^{13}C NMR δ 21.1 (2 C), 22.2 (2 C), 26.1, 45.8, 50.1, 53.4, 57.1.

Anal. Calcd for $C_9H_{22}N_4O_2$: C, 49.52; H, 10.16; N, 25.66. Found: C, 49.59; H, 10.21; N, 25.59.

1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-cyclohexyl-1-triazene (17) was prepared by reacting *N*-cyclohexyl-1,3-propanediamine (5.00 g, 32.0 mmol) in 150 mL of CH_3CN with NO for 42 h: yield 4.06 g (59%); mp 116–117 °C dec; ^{1}H NMR δ 1.05–1.38 (5 H, m), 1.55–1.80 (7 H, m), 2.88 (1 H, m), 2.98–3.15 (4 H, m); ^{13}C NMR δ 27.0 (2 C), 27.1, 28.1, 32.5 (2 C), 40.7, 49.6, 64.4.

Anal. Calcd for $C_9H_{20}N_4O_2$: C, 49.98; H, 9.32; N, 25.91. Found: C, 50.54; H, 9.43; N, 25.41.

1-Hydroxy-2-oxo-3,3-bis(2-aminoethyl)-1-triazene (18) was prepared by treating diethylenetriamine (5.00 g, 48.5 mmol) in 150 mL of CH_3CN with NO for 23 h: yield 7.14 g (90%); mp 109–110 °C dec; ^{1}H NMR δ 2.82 (4 H, m, M_2 portion of an $AA'M_2$ system), 3.18 (4 H, m, AA' portion of an $AA'M_2$ system); ^{13}C NMR δ 39.7 (2 C), 55.8 (2 C).

Anal. Calcd for $C_4H_{13}N_5O_2$: C, 29.44; H, 8.03; N, 42.92. Found: C, 29.50; H, 8.05; N, 42.96.

1-Hydroxy-2-oxo-3,3-bis(3-aminopropyl)-1-triazene (19) was prepared by reacting 3,3'-iminobis(propylamine) (5.00 g, 38.1 mmol) in 150 mL of CH_3CN with NO for 23 h: yield 6.87 g (94%); mp 99–100 °C dec; ^{1}H NMR δ 1.60 (4 H, tt, J = 7.0, 7.3 Hz), 2.88 (4 H, t, J = 7.3 Hz), 3.00 (4 H, t, J = 7.0 Hz); ^{13}C NMR δ 29.1 (2 C), 40.8 (2 C), 54.2 (2 C).

Anal. Calcd for $C_6H_{17}N_5O_2$: C, 37.69; H, 8.96; N, 36.62. Found: C, 37.59; H, 8.97; N, 36.52.

1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-(4-aminobutyl)-1-triazene (20) was prepared by treating spermidine (5.00 g, 34.4 mmol) in 150 mL of CH_3CN with NO for 23 h and isolating the hygroscopic product by the usual method but in a glove bag under N_2: yield 6.19 g (88%); mp 92–94 °C dec; ^{1}H NMR δ 1.32–1.47 (2 H, m), 1.51–1.71 (4 H, m), 2.79–2.88 (4 H, m), 2.91–3.02 (4 H, m); ^{13}C NMR δ 26.0, 29.0, 29.7, 40.9, 42.5, 54.3, 56.3.

Anal. Calcd for $C_7H_{19}N_5O_2$: C, 40.96; H, 9.33; N, 34.12. Found: C, 40.91; H, 9.40; N, 34.05.

Piperazine derivative 21 was prepared by treating 1-(2-aminoethyl)piperazine (4.00 g, 31.0 mmol) in 150 mL of CH_3CN with NO for 18 h: yield 2.23 g (38%); mp 101–102 °C dec; ^{1}H NMR δ 2.6–2.8 (6 H, m), 3.0–3.3 (6 H, m); ^{13}C NMR δ 39.0, 53.9 (2 C), 54.1 (2 C), 56.7.

Anal. Calcd for $C_6H_{15}N_5O_2$: C, 38.09; H, 7.99; N, 37.01. Found: C, 37.83; H, 8.05; N, 36.73.

Spermine/NO Adduct (22). The previously published procedure[2] was considerably improved by a change in solvent. Thus, following the general procedure described above, 5.0 g of spermine (Sigma, 24.7 mmol) in 150 mL of CH_3CN was treated with NO for 20 h: yield 6.39 g (98%); mp 105–107 °C dec; ^{1}H NMR δ 1.38 (2 H, m), 1.58 (4 H, m), 1.75 (2 H, m), 2.70–2.85 (8 H, m), 2.96 (4 H, m); ^{13}C NMR δ 26.4, 27.7, 30.0, 31.6, 41.0, 41.1, 48.6, 50.6, 54.4, 56.4

Kinetic Studies. Stock solutions of each compound in 0.01 M NaOH were added via syringe to pH 7.4 phosphate buffer (0.1 M) contained in a standard UV cell to produce final solutions having compound concentrations in the range of 90–120 μM. Since the compounds do not decompose at high pH, this technique, coupled with the use of a microprocessor-controlled diode array spectrophotometer, allowed data collection to begin within 10 s of the start of the decomposition. The rate of NO release was determined by following the disappearance of the characteristic UV absorption (λ_{max} = 250–252 nm) which all these compounds exhibit. The temperature in the spectrophotometer cavity (22 ± 2 °C) was not thermostated. In each case, clean first-order kinetics was observed and the rate constant was obtained from the 15–30 absorbance readings via a standard linear regression analysis. The correlation coefficients for the plots of $\ln(A - A_\infty)$ vs t were invariably 0.998 or better. All reactions were run at least twice so that the time interval between absorbance readings could be adjusted to allow monitoring over several half-lives for all compounds except 18 which was too stable to permit this. Since the decomposition of 18 could not be usefully followed for more than 1 half-life under these conditions, the value obtained for its half-life is of low accuracy. In duplicate runs the k values were reproducible within ±5%. The half-life data given in Table I were calculated using the formula $t_{1/2} = 0.6931\ (1/k)$.

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