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Bis-diazeniumdiolates of Dialkyldiamines: Enhanced Nitric Oxide Loading of Parent Diamines

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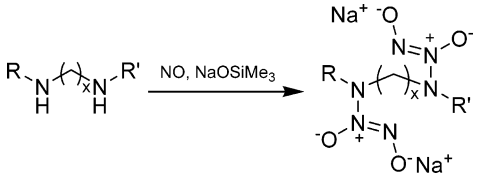
ABSTRACT



The synthesis and characterization of a series of symmetric bis-dialkyldiamine-based diazeniumdiolates, $RN[N(O)NO^-Na^+](CH_2)_xN[N(O)NO^-Na^+]R'$, are reported. Preparation of corresponding intramolecular diazeniumdiolates of the form $RN[N(O)NO]^-(CH_2)_xNH_2^+R'$ with alkyl groups > $(CH_2)_4$-CH_3 have been shown previously to lack stability. In contrast, sodium-stabilized bis-diazeniumdiolates of such lipophilic species can be readily formed when these same diamines are reacted with NO in basic media. The resulting compounds release 4 mol of NO per mole of original diamine. This approach enables the synthesis of more lipophilic NO donors than previously possible.

Nitric oxide (NO) is known to regulate vascular tone,[1] kill cancer cells,[2] prevent the activation of platelets,[3] and serve as a neurotransmitter.[4] As more is learned about the biological roles of NO, a substantial body of research has focused on synthesizing compounds that will store NO and subsequently release this free-radical species under physiological conditions.[5]

Among a host of organic NO donor species reported to date,[5,6] diazeniumdiolates have emerged as attractive candidates for direct use as pharmacological agents[2,5−7] as well as dopants within polymeric materials to create more biocompatible polymers.[8,9] Diazeniumdiolates (nucleophilic NO adducts) have been developed and can be classified within three broad categories: intramolecularly stabilized species (**2**), anionic (**3**), and protected (**4**) (Figure 1), all of which have been tested in vivo and shown to be biologically active. For example, anionic diazeniumdiolates, such as the diazeniumdiolate of proline (PROLI/NO), have been shown

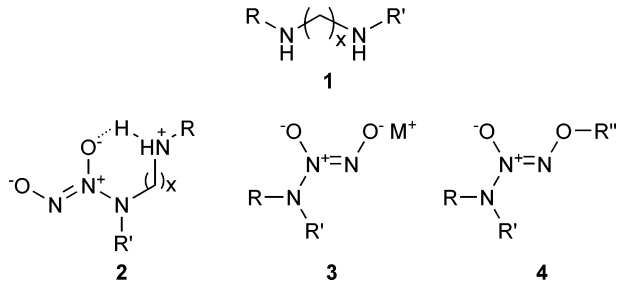


Figure 1. Structures of diamines and diazeniumdiolates where R, R′, and R″ are side groups, *x* is an integer, and M^+ is a postively charged species.

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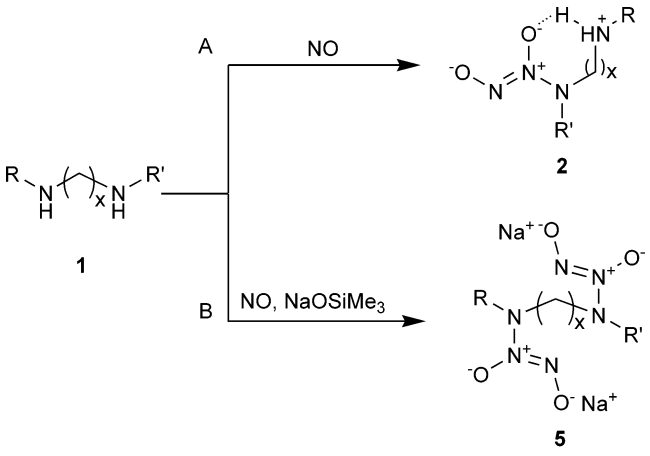
to prevent cerebral spasms,[10] and the protected adduct of PYRRO/NO, V-PYRRO/NO, has been shown to be a liver-selective NO donor.[11] In addition, numerous zwitterionic diazeniumdiolates such as (*Z*)-1-[*N*-methyl-*N*-[6-(*N*-methyl-ammoniohexyl)amino]]diazen-1-ium-1,2-diolate (MAHMA/NO) (see Figure 1, compound **2**; where R,R′ = CH_3, $x = 6$) and the diazeniumdiolate of diethylene triamine (DETA/NO) have been used to inhibit platelet aggregation, lower blood pressure,[12] and prevent overproliferation of cells in the blood vessel wall after arterial injury.[11,13]

Because of the recent success in using diamines as NO storage and delivery agents, we sought to improve the NO-loading efficiency of symmetrical parent diamines, especially more lipophilic dialkyldiamines that could potentially be doped into polymers to create NO releasing polymeric materials that are thromboresistant owing to NO's potent inhibition of platelet function. In this work, we demonstrate that NO can be reacted with symmetrical dialkyldiamine compounds, including MAHMA as well as much more lipophilic analogues (of the form $RNH(CH_2)_6NHR$), to generate doubly loaded diazeniumdiolate adducts that can release 4 mol of NO per mole of initial diamine species. In addition, we show that this methodology is not limited to diamines with a hexamethylene spacer by achieving similarly enhanced NO loading with substrates of the form $RNH(CH_2)_3NHR$. Finally, we compare the initial rates of NO release of these bis-adducts with their zwitterionic counterparts.

Hrabie et al. originally demonstrated that diamines of the form $RNH(CH_2)_xNHR'$ can react with NO under medium-pressure conditions to form diazeniumdiolates, with stabilization believed to exist as a result of hydrogen bonding between the terminal oxygen of the diazeniumdiolate moiety and the hydrogen on the ammonium nitrogen (Scheme 1A).[14] However, as the lipophilicity of these parent diamine compounds increases, the ability to form stable diazeniumdiolates of this type dramatically decreases.[9] Indeed, the most lipophilic intramolecular diamine diazeniumdiolates (**2f**,**g**) cannot be isolated in the presence of air. Exposure to oxygen results in immediate decomposition to the corresponding dialkylhexamethylenediamine ammonium nitrite salt. The nitrite salt decomposition product has also been observed by Drago and co-workers for the anionic diazeniumdiolate of diethylamine.[15] However, longer air exposure times at room temperature were required (i.e., days) before this decomposition was observed compared to **2f** and **2g**, which were found to decompose within seconds.[9,15]

Scheme 1



	x	R=R'
a	6	CH_3
b	6	CH_2CH_3
c	6	$(CH_2)_2CH_3$
d	6	$(CH_2)_3CH_3$
e	6	$(CH_2)_4CH_3$
f	6	$(CH_2)_5CH_3$
g	6	$(CH_2)_{11}CH_3$
h	3	CH_3
i	3	$CH(CH_3)_2$

The reason for this lack of diazeniumdiolate stability with increased side chain length is not yet known. However, destabilization presumably occurs because the hydrogen bond formed between the hydrogen on the ammonium nitrogen and the oxygen on the diazeniumdiolate is significantly weakened. This may be attributed to the flexibility of the longer alkyl chains disrupting the H-bonding interaction. Normally, this hydrogen bond stabilizes the zwitterionic complexes.[14] In view of this, we sought to eliminate this dependency on the hydrogen bond stabilization and use exogenous cations (sodium) to stabilize the corresponding diazeniumdiolates of the dialkyldiamines. Beyond obtaining stable diazeniumdiolates of the most lipophilic diamine structures, the use of exogenous cations has the potential to greatly enhance the NO storage capability of these species, by enabling the formation of bis-diazeniumdiolate type structures (see **5a**−**i**).

Incorporating sodium trimethylsilanolate salt during the NO addition reaction with the dialkyldiamines allows air-stable bis-diazeniumdiolates to be prepared (Scheme 1B). For all diamines examined, the sodium-stabilized bis-diazeniumdiolates (**5a**−**i**) were the only products observed. The products can store twice as much NO in one molecule and, more importantly, increasingly more lipophilic NO donors can be synthesized that remain stable in the presence

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of air. For example, an NO donor with a parent structure of **1g** (with a log $P > 12$; P = octanol/water partition coefficient) can be synthesized and stored under ambient conditions without significant loss of NO. Symmetrical diamines with three methylenes between the amine nitrogens can also be reacted with NO to yield the corresponding stable bis-diazeniumdiolates (**5h** and **5i**).

NMR Spectra. Spectroscopic data strongly suggests that stable bis-diazeniumdiolates were formed. Compared to the starting diamine, the NO-reacted species show symmetrical downfield shifts in the ^{1}H NMR of the methylene protons adjacent to the NO-reacted nitrogens, with integrals corresponding to eight protons around each nitrogen, indicative of the proposed structures. In contrast, if a monodiazeniumdiolate were formed, two distinct proton resonances would be observed, one from each of the methylene protons adjacent to the diazeniumdiolated nitrogen and the other from the nondiazeniumdiolated nitrogen (see the Supporting Information).

UV−vis Spectra. In addition, the UV absorption λ_{max} of the complexes formed from reaction of the diamines with NO in the presence of sodium trimethylsilanoate were found to exist at 247 nm and had molar absorptivities (ϵ) in the range from 12000 to 17000 M^{-1} cm^{-1}, twice that of the corresponding zwitterionic complexes, where formation of only one dizeniumdiolate is achieved.[14,16]

Microanaylsis. Further authenticity of the bis-diazeniumdiolates was confirmed by derivatizing representative compounds (**5a**, **5b**, **5d**, **5h**) via O^2-methylation.[11,17] Full characterization, including combustion analyses and exact mass measurements, were performed for the resulting products (**6a**, **6b**, **6d**, **6h**) supporting the identification of the target bis-diazeniumdiolate species (see the Supporting Information).

Decomposition. In general, discrete diazeniumdiolates have been shown to decompose and release NO by a proton-driven mechanism.[16] To monitor the decomposition of the bis-diazeniumdiolates under investigation with time at pH 7.4, UV−vis spectroscopy was used. Figure 2 shows representative spectra of the decomposition of **5d** as a function of time under physiological conditions (i.e., PBS buffer). Note the decrease in the absorbance at 247 nm, corresponding to the diazeniumdiolate absorption band. In addition, the spectrum has an isobestic point, suggesting a one-step decomposition pathway as previously reported for diazeniumdiolates.[16]

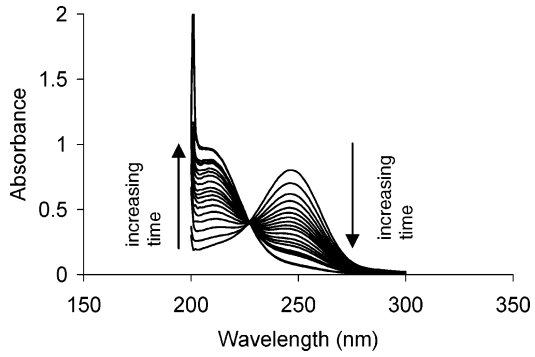

Figure 2. UV−vis spectra of **5d** as a function of time in PBS buffer (pH 7.4) at 22 °C.

NO Release. All sodium stabilized bis-diazeniumdiolates are hydrolyzed at pH 7.4 to release essentially 4 mol of NO per mole of diamine (see Table 1). These values were determined via chemiluminescence, after adding a given amount of the bis-diazeniumdiolate to PBS buffer. The NO released was detected and integrated over time, until no further release of NO was observed.

Kinetics. The decomposition of *N*-based diazeniumdiolates have been shown to be proton driven.[16] The half-lives of the anionic bis-diazeniumdiolates prepared in this work, at pH 7.4 and 37 °C, are summarized in Table 1. The term "apparent" half-life is used to describe those compounds that have limited solubility in PBS buffer owing to their high lipophilicity. Due to the insolubility of some of the diazeniumdiolates under investigation, chemiluminescence, rather than UV absorbance, was used to monitor the NO loss with time. This also makes it possible to differentiate between the dissociation of the first diazeniumdiolate from the second (i.e., half-life 1 and half-life 2).

Table 1. Summary of Characteristics of the Bis-diazeniumdiolates

	R	x	$t_{1/2}$[a] (s)	ratio[b] of diamine/NO
5a	CH_3	6	59.8 ± 2.0	1:3.7
5b	CH_2CH_3	6	102.9 ± 5.4	1:3.8
5c	$(CH_2)_2CH_3$	6	107.8 ± 5.4	1:3.9
5d	$(CH_2)_3CH_3$	6	93.3 ± 2.3	1:4.0
5e	$(CH_2)_4CH_3$	6	102.6 ± 1.3	1:3.8
5f	$(CH_2)_5CH_3$	6	88.9 ± 4.8	1:4.0
5g	$(CH_2)_{11}CH_3$	6	50.4 ± 5.7	1:4.0
5h	CH_3	3	93.8 ± 9.1	1:3.7
5i	$CH(CH_3)_2$	3	155.0 ± 0.5	1:3.9

[a] "Apparent" $t_{1/2}$ and $t_{1/2}$ for diazeniumdiolates under investigation in PBS buffer at 37 °C and pH 7.4. [b] Measurements are within ≤0.3 standard deviation.

The "apparent" half-lives for the bis-diazeniumdiolates were found to be shorter than that of their corresponding zwitterionic forms (see Tables 1 and ref 14). One plausible explanation for the longer half-lives of the zwitterionic species (**2a**−**e**) is that the more hydrophobic side groups of the ring structure of such a structure impedes protons from reacting with the amine to heterolytically cleave the N−N bond. For the sodium-stabilized bis-diazeniumdiolates, the parent diamine backbone is more linear and does not provide shielding from the aqueous environment.

Beyond the differences in the half-lives of the bis-diazeniumdiolates with their corresponding zwitterionic

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counterparts, the kinetics of the bis-diazeniumdiolate decomposition is more complex than that of the corresponding zwitterions (see Table 1). For example, all zwitterionic compounds (**2a**−**e**) follow first-order kinetics as do bis-diazeniumdiolates **5a**−**d** and **5h**−**i**; however, the most lipophilic bis-diazeniumdiolate compounds **5e**−**g** do not. The NO release curves for the more lipophilic bis-diazeniumdiolates can be described as biphasic where the rates of NO release are greater initially then decrease as function of time (see the Supporting Information). Attempts to fit either zero-order, first-order, or second-order kinetics to the release profiles was not possible at the $R^2 \geq 0.99$ level. The detailed kinetics of the decomposition of the bis-diazeniumdiolates is currently under investigation; however, it seems likely that the dissociation of one diazeniumdiolate group, yielding a single secondary amine can alter the decomposition of the second diazeniumdiolate group on the same molecule owing to local pH changes around the structure (i.e., differing pK_a of the amines within the compound).

Finally, we examined the effect of the methylene spacer length between the diamine nitrogens on the half-lives of the bis-diazeniumdiolates. It has been previously reported for the zwitterionic analogues that a decrease in the methylene spacer length leads to an increase in the half-lives of the corresponding diazeniumdiolates under physiological conditions. In the same manner, a decrease in the methylene spacer between the diamine nitrogens also leads to an increase in the half-lives of the bis-diazeniumdiolate species (compare compounds **5a** and **5h**, Table 1). The rationale for this enhance half-life as a function of methylene spacer length for zwitterionic diazeniumdiolates is apparently due to the stability of an 11-membered ring compared to an 8-membered ring.[14] The reason for the enhanced stability for the bis-diazeniumdiolates as a function of decreased methylene spacer is not straightforward; however, it seems likely that either the electron distribution of the compounds with the shorter methylene spacer chain is more favorable or the basicity of the nitrogen bearing the diazeniumdiolate is less than that of the bis-compounds with a hexamethylene spacer, leading to a longer half-life.

The potential advantages of the bis-diazeniumdiolates described herein are numerous. Higher NO loading per parent diamine increases the NO loading efficiency of diamine compounds, thus providing twice the NO delivery capability compared to their zwitterionic counterparts. More importantly, however, the use of an exogenous cation and base provides the ability to synthesize far more lipophilic stable diazeniumdiolates (i.e., didodecyldiamines) that could not be synthesized by previous methods. Such NO donors are attractive candidates to add to hydrophobic polymeric materials used to prepare a wide variety of biomedical devices (catheters, extracorporeal tubings, implantable sensors, etc.). Indeed, use of these new bis-diazeniumdiolates could aid in the manufacturing process (owing to better air stability) as well as the in vivo thromboresistivity observed with such polymers because of the enhanced levels of NO that could be released from the surfaces of materials doped with such compounds. Efforts to demonstrate the utility of these new compounds in creating more thromboresistant polymeric coatings are currently in progress within this laboratory.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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