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# $O^2$-Functionalized Methylamine Diazeniumdiolates: Evidence for *E* ⇄ *Z* Equilibration in an Acyclic System

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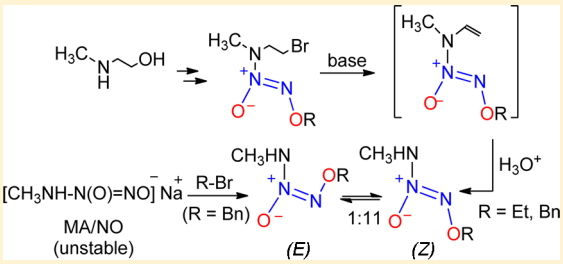
Supporting Information

**ABSTRACT:** Diazeniumdiolates that have the structure RHN−N(O)=NOR′ are of interest as prodrug (caged) forms of the bioeffectors nitric oxide (NO) and nitroxyl (HNO). Previous work has focused on examples possessing α-branched R groups, with isopropylamine (IPA)/NO (R = isopropyl) being the smallest examined to date. To probe the effect of minimizing the alkyl-group size on the chemistry of IPA/NO, we prepared the corresponding methylamine derivative as a sodium salt that was highly unstable but could be trapped in very low overall yield as the stable $O^2$-benzyl derivative. To prepare enough for efficient characterization, we devised an alternate synthesis involving a novel N-dealkylation route. $CH_3HN$−N(O)=NOBn, synthesized in high yield and crystallized as the *Z* isomer as determined by X-ray crystallography, was observed to exist as a 11:1 mixture of two isomeric forms in dynamic equilibrium in solution. Similar results were seen for the $O^2$-ethyl derivative, whose two equilibrium constituents were partially separated by HPLC to reveal essentially identical UV and mass spectra, indicating them to be *Z* and *E* isomers of $CH_3HN$−N(O)=NOEt. The results could lead the way to a fuller understanding of the chemistry of the acyclic (*E*)-diazeniumdiolates.




## INTRODUCTION

We are pursuing an increasingly refined understanding of the fundamental physicochemical properties of the N-bound diazeniumdiolates,[1] which have the structure $R^1R^2N$−N(O)=$NOR^3$, as a basis for the rational design of therapeutically useful prodrugs of nitric oxide[2] (NO) and nitroxyl[3] (HNO) as well as novel tools for probing the chemical biology of these important bioeffectors.[4] One aspect that to date has been difficult to elucidate fully concerns the factors that control the cis/trans stereochemistry of the oxygens in the −N(O)=$NOR^3$ group. Arulsamy and co-workers very recently addressed this issue as it relates to compounds in which the diazeniumdiolate nitrogen is attached to carbon,[5,6] but to date we know of only one report in which an *E* configuration has been confirmed in a nitrogen-bound diazeniumdiolate; this special case ($R^1$ = isopropyl, $R^2$ = H, $R^3$ = 2-bromoethyl)[7] in which base-induced removal of the N−H proton led to an anion whose *E* isomer was trapped by an intramolecular alkylation process to form cyclic product **2**[8] (see Scheme 1). In an effort to obtain a macroscopically observable pair of acyclic *Z* and *E* isomers in sufficient equilibrium concentrations that the *E* form's properties can be directly characterized and compared with those of the Z isomer, we hypothesized that minimizing steric interactions among the R groups might achieve that objective. Here we provide evidence that replacing the isopropyl group of anion **1** in Scheme 1 with a methyl group ($R^3$ = benzyl, ethyl) provides just such an equilibrium mixture of isomers.

## RESULTS AND DISCUSSION

**Direct Synthesis of MA/NO.** Preparation of primary-amine diazeniumdiolates has been an enduring challenge on account of their low thermodynamic stabilities.[9] Initial attempts toward the synthesis of methylamine analog **3** (see Scheme 2) involved the reaction of NO with a solution of methylamine in ether/acetonitrile precooled to −80 °C. Filtration of the resulting methylammonium salt (obtained in very low yield) and subsequent treatment with sodium methoxide afforded a white solid in ∼3% overall yield (Scheme 2). All of the manipulations were performed at −80 °C, and the solids were found to be thermally unstable, often decomposing explosively at ambient temperature. Nevertheless, the powder survived long enough under cold conditions to allow the anion in **3** to be trapped by reaction with benzyl bromide at −80 °C to

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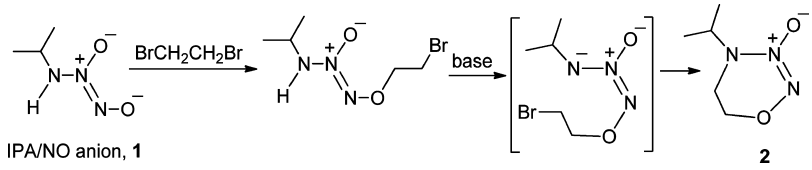
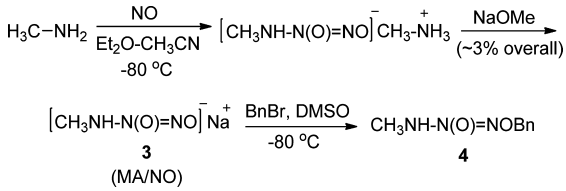
**Scheme 1. Synthesis of a Crystallographically Confirmed (*E*)-Diazeniumdiolate, 2**



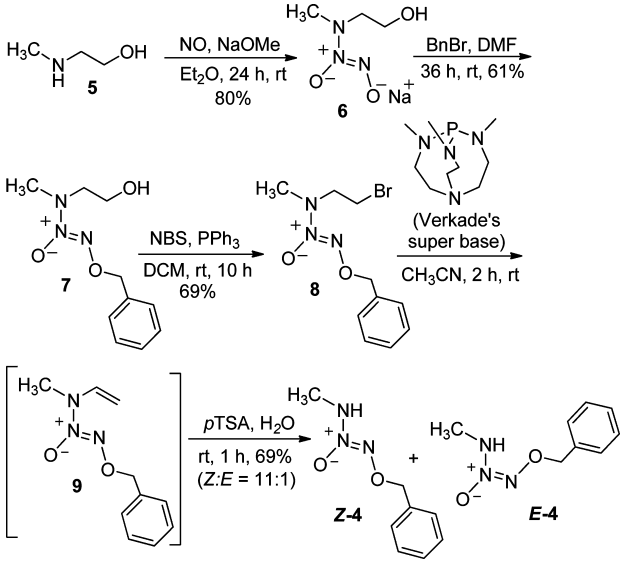

**Scheme 2. Reaction of Methylamine with NO**




produce the $O^2$-benzyl derivative **4** in trace quantities. Evidently, however, a more convenient alternative synthetic route for the preparation of caged MA/NO prodrugs was desired for systematic studies of the chemistry of MA/NO.

**Dehydrohalogenation-Mediated Preparation of $O^2$-Protected MA/NO.** Accordingly, we devised the dealkylation route outlined in Scheme 3 for the preparation of derivatized

**Scheme 3. N-Dealkylation Route to $O^2$-Benzyl-Protected MA/NO**




MA/NO analogues starting with the sodium salt of N-diazeniumdiolated 2-(methylamino)ethanol (**6**),[10] which was used as a synthon. Preparation of the $O^2$-benzyl-protected diazeniumdiolate **7** and subsequent bromination with NBS/$Ph_3P$ afforded the corresponding *N*-bromoethyl analogue **8**. Recently, we reported the use of 2,8,9-trimethyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane (Verkade's super base) for dehydrohalogenation of diazeniumdiolate-containing compounds under mild conditions.[11] Reaction of **8** with this base in acetonitrile was facile, but the desired *N*-vinyl derivative **9** was found to be unstable and could not be isolated. Acidic hydrolysis of the in situ-generated **9** with *p*-toluenesulfonic acid followed by purification of the crude reaction mixture afforded a 69% isolated yield of $O^2$-benzyl-protected MA/NO **4**, whose physicochemical properties were identical to those of the material obtained in the low-temperature benzylation of the MA/NO anion in **3**.

**Isomer Equilibration in 4.** Careful examination of the $^1$H NMR spectral pattern of **4** in $CDCl_3$ revealed the presence of a pair of doublets at 2.98 and 3.05 ppm in a 11:1 ratio along with two overlapped singlets at 5.18 ppm, indicating the existence of this $O^2$-benzyl MA/NO analogue as a mixture of two isomeric forms in solution. Similarly, the proton-coupled $^{13}$C NMR spectrum of **4** also exhibited two sets of six peaks, further supporting the existence of two isomeric forms of this analogue. Recrystallization of the product mixture from diethyl ether at 4 °C followed by X-ray crystallographic analysis revealed it to exist in the *Z* conformation (***Z*-4**) (Figure 1A). We believe the minor isomer to be the *E* conformation (***E*-4**), although it could not be isolated by recrystallization. Low-temperature NMR analysis of a freshly prepared solution of recrystallized ***Z*-4** in $CDCl_3$ at −20 °C revealed the *Z*:*E* isomeric ratio of **4** to be ∼66:1, indicating that the crystalline material contained no more than 1.5% ***E*-4** (Figure 1B). At room temperature, the sample was found to equilibrate to a 11:1 ***Z*-4**:***E*-4** mixture, similar to the purified product mixture, indicating that the two isomers exist in a dynamic equilibrium in solution at room temperature (see the Supporting Information).

**LC/MS Identification and Partial Chromatographic Separation of *Z* and *E* Isomers.** With the same synthetic protocol, $O^2$-ethyl-protected MA/NO (**13**) was also prepared in high yield starting from **10** via the formation of the corresponding *N*-bromoethyl (**11**) and *N*-vinyl (**12**) intermediates (Scheme 4). NMR analysis of this product was consistent with that of the $O^2$-benzyl analogue and suggested the formation of **13** as an 11:1 mixture of two isomers, presumably also having the *Z* and *E* conformations. Injection of a purified mixture of these two isomers on an HPLC column allowed a reasonable separation of the two peaks, which were fused at the baseline (Figure 2A). Isolation of either form followed by reinjection resulted in a chromatogram containing both peaks with an integration ratio matching that of the original mixture (Figure 2B,C). These results were confirmed using HRMS detection. The measured *m*/*z* values for the $[M + H]^+$ ions of the two peaks were both 120.07692, which is in excellent agreement (<1.5 ppm) with the calculated value for the molecular formula $C_3H_9N_3O_2$, *m*/*z* 120.07675 (Figure 3). The *m*/*z* value for the sodium adduct was extracted and plotted as a function of time. This analysis showed that the taller peak readily forms a sodium adduct, while such an adduct was not detected for the smaller peak (see the Supporting Information). These data are consistent with the view that **13** exists as a mixture of *Z* and *E* isomers, with the *Z* isomer's cis oxygens being uniquely favorably oriented for bidentate coordination to the alkali metal ion (see the Supporting Information). This isomerization is presumably mediated through a proton shift from the amine nitrogen (N3) to the diazeniumdiolate nitrogen (N2) and/or direct ionization of the N3−H bond, thus rendering largely single-bond character to the N1−N2 bond; this allows free rotation about this bond, as indicated by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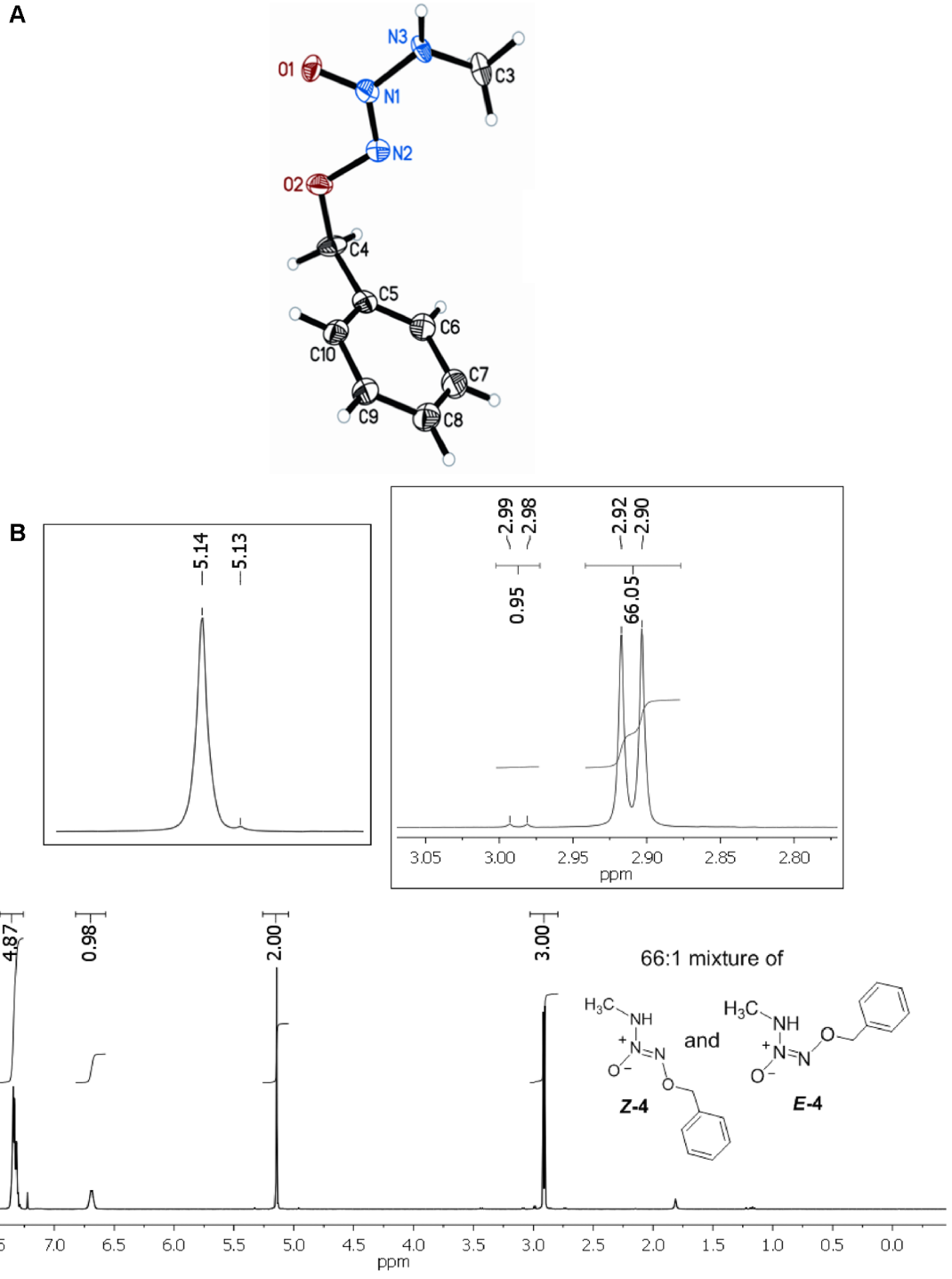


**Figure 1.** (A) Molecular structure and numbering scheme for the crystals of ***Z*-4** (displacement ellipsoids are shown at the 50% level). (B) 1D $^{1}$H NMR spectra of recrystallized ***Z*-4** in $CDCl_3$ at −20 °C, indicating that ***Z*-4** and ***E*-4** exist in a 66:1 ratio in solution.

theoretical calculations reported previously.[8,12] (See Figure 1A for the numbering scheme).

**UV Spectroscopic Determination of p$K_a$.** The potential acidity of the NH bond in a purified mixture of ***Z*-13** and ***E*-13** was examined by UV spectroscopy using buffers of different pH, following the similar procedure reported previously for the analysis of $O^2$-methyl IPA/NO.[12] The extinction coefficients were determined by dissolving a known amount of **13** in a given quantity of ethanol, diluting with a large excess of 0.01 M phosphate buffer for runs at pH 7.4−12, and then recording the spectrum within 1−2 s of dilution at room temperature. A single peak was found for **13** at $\lambda_{max}$ = 241 nm at all pH from 1 to 11. As the pH was increased above 11, the peak intensity decreased, and a new peak due to the ionization of the NH proton emerged at $\lambda_{max}$ = 278 nm. Reacidification of the alkaline solution restored the 241 nm peak at its original intensity.

The p$K_a$ of the process was measured by adjusting the pH in small increments between 11.0 and 12.0 until the 241 and 278 nm peaks were each at half-maximal intensity (Table 1). As the extinction coefficients for the un-ionized and anionic forms of **13** were found to be 11.0 and 10.0 $mM^{-1}$ $cm^{-1}$, respectively,


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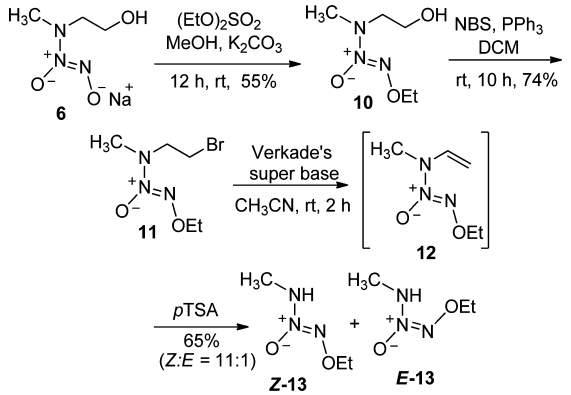
**Scheme 4. Preparation of a Mixture of $O^2$-Ethylated MA/NO Isomers**



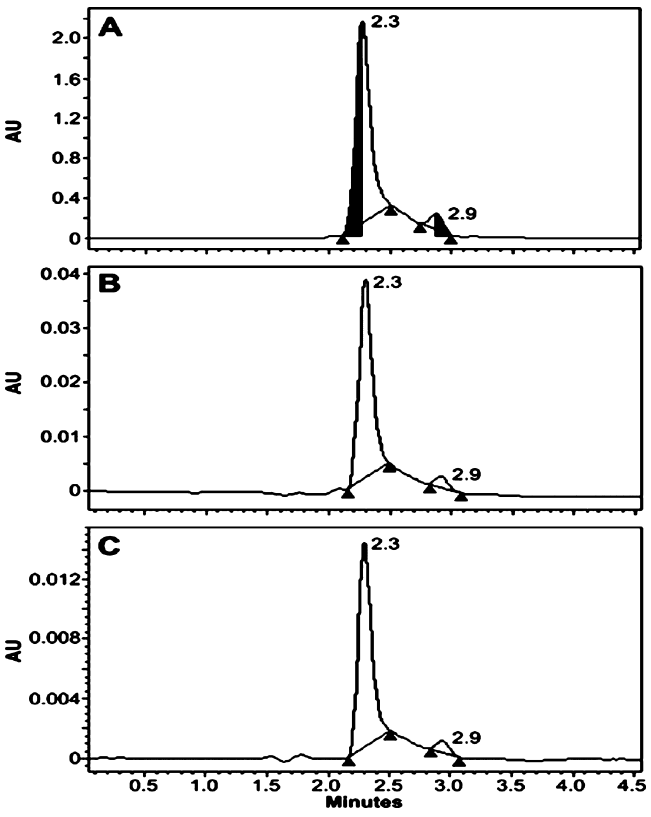




**Figure 2.** HPLC traces of a mixture of the two isomers of **13** observed at 240 nm. (A) Chromatogram of the mixture of isomers purified by silica gel chromatography. Both peaks have a $\lambda_{max}$ at 240 nm. (B) Chromatogram obtained when the shaded portion of the first peak was collected at 0 °C and immediately injected into the HPLC column, showing re-equilibration. (C) Chromatogram obtained similarly to (B), showing re-equilibration upon collection of the shaded portion of the second peak.

the pH was adjusted so that the apparent extinction coefficients were ~5.5 and ~5.0 $mM^{-1}$ $cm^{-1}$, respectively. Half-maximal absorption intensities for both forms were observed at a pH of ~11.70, and this pH value was taken as the $pK_a$ for **13**.

**Kinetic Studies.** Ionization of the N−H bond in a mixture of these two isomers at room temperature in $D_2O$ was also examined by $^1H$ NMR spectroscopy. Samples were run in $D_2O$ adjusted to a pD value[13] of either ~8.5, ~10.5, or ~13.0 by adding NaOD to solutions in $D_2O$. Prominent exchange line broadening of the *N*-methyl and *O*-methylene signals was observed for both isomers at pD 10.5 (Figure 4C). The observed spectrum at pD 13.0 indicated ionization of the acidic primary-amine proton to form the corresponding charge-delocalized monoanionic species having a line-broadened singlet and quartet for the *N*-methyl and *O*-methylene groups, respectively (Figure 4D). Kinetic data for the exchange between the anion and its conjugate acid were determined from the signal broadening of the peak centered at 2.95 ppm using previously described saturation-transfer and dynamic NMR spectroscopic methods.[8,14] The rate constant for exchange, ~$1.2 \times 10^3$ $s^{-1}$, is similar to that of a structurally analogous primary-amine diazeniumdiolate that was shown to have an acidic NH bond.[12] The interconversion barrier was found to be 17.4 kcal/mol, in close agreement with the results of previously reported theoretical calculations for a similar molecule.[8] Reacidification of this solution to pD 3.5 using DCl revealed a re-equilibration with a *Z*/*E* ratio and spectral patterns identical to those of the parent mixture at pD 7.5. Analysis of this sample after 14 h at room temperature indicated no significant change in the isomer ratio.

## ■ CONCLUSIONS

Our results reveal important similarities as well as differences between the well-characterized *i*PrHN−N(O)=NOR derivatives and their methylamine analogues. While the sodium salt of the former (R = $Na^+$) can be stored indefinitely without incident,[15] salts of its analogue MA/NO have to date defied isolation in pure form, even when the MA/NO reaction was carried out at cryogenic temperatures. Both the IPA/NO and MA/NO anions can be O-alkylated to produce stable derivatives, but because the instability of MA/NO has precluded isolation of the salts in reasonable yield, we had to devise an indirect dealkylative synthesis in order to produce enough MeHN−N(O)=NOR (R = benzyl, ethyl) for efficient characterization. The O-alkylated IPA/NO and MA/NO derivatives were very similar to each other in their UV properties, the $pK_a$'s for ionization of their N−H bonds, and the rate constants for protonation/deprotonation at pHs near the $pK_a$ values. Additionally, there was evidence that the O-alkylation products of both IPA/NO and MA/NO can isomerize from the favored *Z* conformation (cis oxygens) to the *E* form, but with one critical difference: the *E* isomer of IPA/NO had to be trapped in an intramolecular cyclization reaction to confirm its existence, while that of MeHN−N(O)=NOR proved to be directly observable as a constituent of the equilibrium mixture. The isomers of the MA/NO derivatives were partially separable. It may be that conditions can be found that will permit a future MA/NO derivative to be isolated as a pure *E* form, facilitating further insights into the chemistry (and possibly the biology) of diazeniumdiolates having this thus far little-studied configuration.

## ■ EXPERIMENTAL SECTION

**Instrumentation.** All reactions were performed under an inert atmosphere. All chemicals were purchased from commercial sources and used without further purification. UV spectra were recorded on a diode array spectrophotometer. NMR spectra were collected with a 400 MHz spectrometer using appropriate deuterated solvents; chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. The analytical HPLC/MS analyses were performed using systems with a photodiode array UV−vis detector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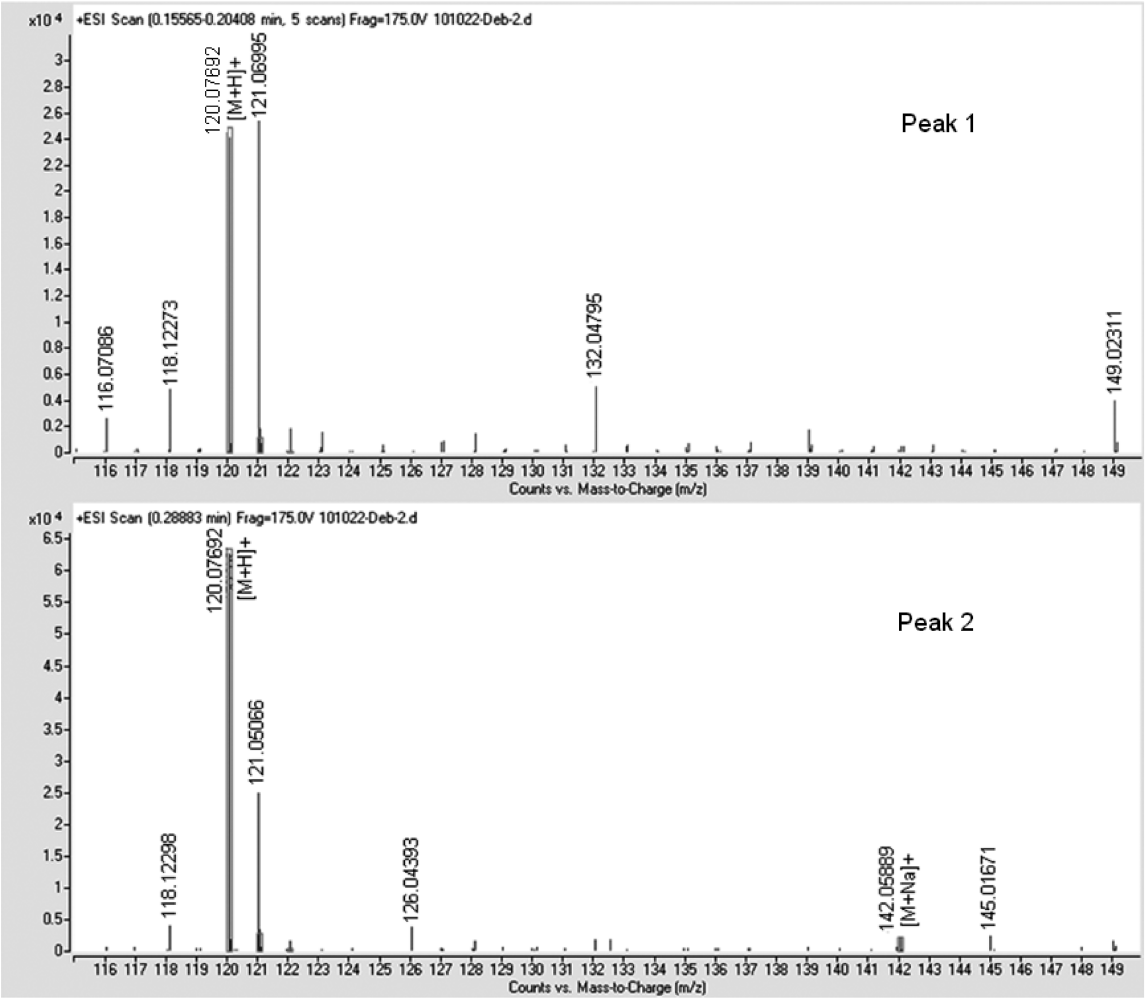


**Figure 3.** HRMS spectra for the two peaks of compound **13** in the extracted-ion chromatogram presented in the Supporting Information. Peak 1 has $[M + H]^+$ at $m/z$ 120.07682. Peak 2 has $[M + H]^+$ at $m/z$ 120.07692 and $[M + Na]^+$ at $m/z$ 142.05889. The ion at $m/z$ 121.051 is due to purine, which was added as an internal reference to obtain high-resolution spectra of our analytes. All other ions are attributable to background interference.

and an accurate-mass quadrupole time-of-flight (Q-TOF) mass spectrometer.

**Reaction of Methylamine with NO.** The equipment used for conducting reactions with NO gas under anaerobic conditions has been described previously.[16,17] Nitric oxide was obtained in ultrahigh-purity grade and allowed to stand in a ballast tank at a pressure of ~5 atm over potassium hydroxide pellets for a minimum of several hours before use. A 2 M solution of methylamine in THF (20 mL) was diluted with 20 mL of diethyl ether and 10 mL of acetonitrile. The resulting solution was placed in a standard thick-walled glass hydrogenation bottle, cooled to −80 °C, flushed with nitrogen, charged with 50 psi NO, and stirred for 2 h. After completion of the reaction, ~500 mg (~10% yield) of the methylammonium salt of MA/NO was collected by filtration using a precooled Büchner funnel. While this product was still moist and cold, it was promptly suspended in ether, cooled to −80 °C, and treated with 750 μL of 4.6 M sodium methoxide in methanol; care was taken to use less than 1 equiv of sodium methoxide as estimated from the mass of the crude methylammonium salt. The resulting product was filtered while cold to afford ~140 mg (~3% yield) of **3** as a white powder, which was quickly stored under nitrogen at −20 °C. UV (0.01 M NaOH) $\lambda_{max}$ ($\varepsilon$): 249 nm (8.3 $mM^{-1}$ $cm^{-1}$). (***Caution!*** Compound **3** and its methylammonium analogue are unstable and decompose, often explosively, at ambient temperature.)

***O*²-Benzyl [*N*-(2-Hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (7).** Compound **6**[10] (3.00 g, 19.1 mmol) was dissolved in 15 mL of anhydrous DMF and cooled to 0 °C with constant stirring. A solution of benzyl bromide (3.89 g, 22.8 mmol) in 5 mL of anhydrous DMF was slowly added. The reaction mixture was then allowed to warm to room temperature, stirred for an additional 24 h, quenched with $H_2O$, and extracted with ether (3 × 20 mL). The combined organic layers were washed with water and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the crude product was purified on a silica gel column (15:85 hexanes/ethyl acetate) to afford 2.62 g (61% yield) of **7** as a hygroscopic, pale-yellow oil. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 7.40−7.32 (m, 5H), 5.22 (s, 2H), 3.66−3.62 (m, 2H), 3.34 (t, $J$ = 5.2 Hz, 2H), 2.97 (s, 3H), 2.36 (br s, 1H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 135.5, 128.6, 128.5, 128.5, 75.7, 59.4, 57.1, 42.0. UV (MeOH) $\lambda_{max}$ ($\varepsilon$): 244 nm (10.0 $mM^{-1}$ $cm^{-1}$). Anal. Calcd for $C_{10}H_{15}N_3O_3 \cdot 0.3H_2O$: C, 52.07; H, 6.82; N, 18.22. Found: C, 52.09; H, 6.66; N, 18.08.

***O*²-Benzyl [*N*-(2-Bromoethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (8).** Compound **7** (1.37 g, 6.08 mmol) was dissolved in 15 mL of dichloromethane and cooled to 0 °C with constant stirring. Triphenylphosphine (1.91 g, 7.30 mmol) was dissolved in 5 mL of dichloromethane and added to the reaction mixture, which was then stirred for 15 min. NBS (1.29 g, 7.30 mmol) was added in portions to the reaction mixture over a period of 10 min, after which the mixture was allowed to warm to room temperature and stirred overnight. Solvent was removed on a rotary evaporator, and the crude product was purified on a silica gel column (80:20 hexanes/ethyl acetate) to afford 1.20 g (69% yield) of **8** as a dark-yellow oil. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 7.40−7.34 (m, 5H), 5.23 (s, 2H), 3.60 (t, $J$ = 6.8 Hz, 2H), 3.35 (t, $J$ = 6.8 Hz, 2H), 3.02 (s, 3H). $^{13}$C NMR ($CDCl_3$, 100


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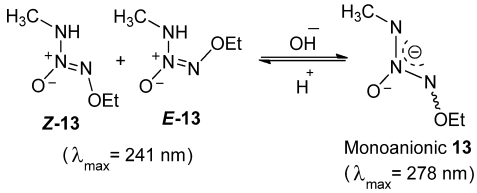
**Table 1. UV Absorbances of the Un-ionized and Monoanionic Forms of 13 at 241 and 278 nm as Functions of pH**




| | | absorbance (AU) | |
|---|---|---|---|
| entry | pH | 241 nm | 278 nm |
| 1 | 7.40 | 0.94[a] | 0 |
| 2 | 11.00 | 0.88 | 0.35 |
| 3 | 11.20 | 0.72 | 0.36 |
| 4 | 11.40 | 0.52 | 0.39 |
| 5 | 11.60 | 0.48 | 0.41 |
| 6 | **11.70** ($pK_a$) | **0.47** | **0.42** |
| 7 | 11.75 | 0.42 | 0.44 |
| 8 | 11.80 | 0.29 | 0.51 |
| 9 | 11.90 | 0.23 | 0.68 |
| 10 | 12.00 | 0.17 | 0.80 |
| 11 | 14.00 | 0 | 0.85[b] |

[a] $\varepsilon$ = 11.0 $mM^{-1}$ $cm^{-1}$. [b] $\varepsilon$ = 10.0 $mM^{-1}$ $cm^{-1}$.

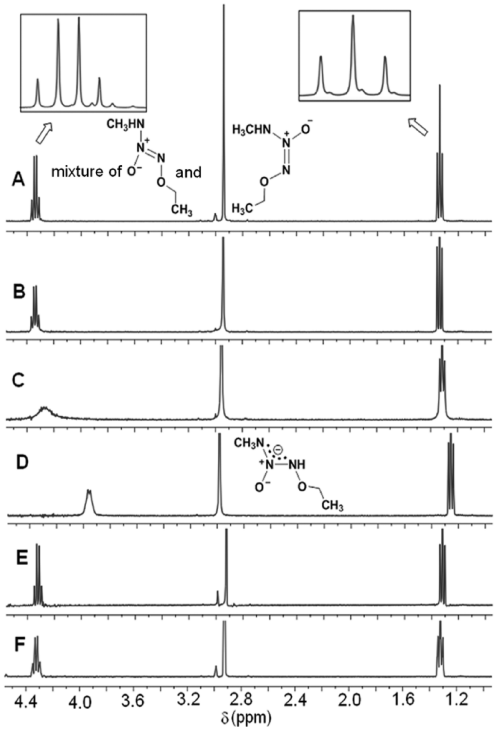



**Figure 4.** $^1$H NMR spectra of the mixture of ***Z*-13** and ***E*-13** at 400 MHz revealing the slow exchange process accompanying ionization of the N−H bond at room temperature. Spectra A−D were run in $D_2O$ adjusted to measured pD values of ∼7.5, ∼8.5, ∼10.5, and ∼13.0, respectively, by addition of NaOD. Spectrum E was run after the pD of the sample used in **D** was adjusted back to 3.5 from 13.0 by addition of DCl. Spectrum F shows the status of the pD 3.5 solution in $D_2O$ run after 14 h.

MHz): $\delta$ 135.6, 128.7, 128.7, 128.6, 75.8, 55.9, 41.8, 27.3. UV (EtOH) $\lambda_{max}$ ($\varepsilon$): 246 nm (9.17 $mM^{-1}$ $cm^{-1}$). Anal. Calcd for $C_{10}H_{14}N_3O_2Br$: C, 41.68; H, 4.90; N, 14.58. Found: C, 41.44; H, 4.98; N, 14.29.

**Isomeric Mixture of *O*$^2$-Benzyl (*N*-Methylamino)diazen-1-ium-1,2-diolate (*Z*-4 and *E*-4).** Compound **8** (0.50 g, 1.7 mmol) was dissolved in 4 mL of anhydrous acetonitrile and slowly added to a stirring solution of 2,8,9-trimethyl-2,5,8,9-tetraaza-1-phosphabicyclo-[3.3.3]undecane (0.41 g, 1.91 mmol) in 3 mL of anhydrous acetonitrile precooled to 0 °C. Subsequently, the reaction mixture was warmed to room temperature and stirred for another 2 h while the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was recooled to 0 °C, and a solution of *p*-toluenesulfonic acid (0.33 g, 1.74 mmol) in 1 mL of $H_2O$ was added. The resulting mixture was stirred for 1 h, concentrated under reduced pressure, and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with saturated brine solution and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the crude product was purified on a silica gel column (40:60 hexanes/ethyl acetate) to afford 0.22 g (69% yield) of an 11:1 mixture of the *Z* and *E* isomers of **4** as a white solid. Mp 48−50 °C. $^1$H NMR of ***Z*-4** ($CDCl_3$, 400 MHz): $\delta$ 7.40−7.10 (m, 5H), 6.37−6.33 (m, 1H), 5.18 (s, 2H), 2.98 (d, *J* = 5.6 Hz, 3H). $^{13}$C NMR of ***Z*-4** ($CDCl_3$, 100 MHz): $\delta$ 135.7, 128.6, 128.6, 128.5, 75.4, 34.3. UV (MeOH) $\lambda_{max}$ ($\varepsilon$): 245 nm (12.1 $mM^{-1}$ $cm^{-1}$). Anal. Calcd for $C_8H_{11}N_3O_2$: C, 53.03; H, 6.12; N, 23.19. Found: C, 53.06; H, 6.02; N, 23.14.

**O$^2$-Ethyl [*N*-(2-Hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (10).** Compound **6**[10] (3.00 g, 19.1 mmol) was dissolved with constant stirring in a suspension of 1.00 g of finely powdered potassium carbonate in 20 mL of precooled anhydrous methanol. To this mixture, a solution of diethyl sulfate (4.11 g, 28.7 mmol) in 10 mL of anhydrous methanol was slowly added. The reaction mixture was allowed to warm to room temperature, stirred for an additional 10 h, and filtered. The methanol was removed on a rotary evaporator, and the residue was extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with saturated brine solution and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the crude product was purified on a silica gel column (20:80 hexanes/ethyl acetate) to afford 1.71 g (55% yield) of **10** as a hygroscopic, pale-yellow oil. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 4.17 (q, *J* = 7.2 Hz, 2H), 3.72−3.57 (m, 2H), 3.26 (t, *J* = 5.2 Hz, 2H), 2.89 (s, 3H), 2.71 (br s, 1H), 1.25 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 69.7, 59.3, 57.0, 42.0, 14.2. UV (MeOH) $\lambda_{max}$ ($\varepsilon$): 242 nm (11.7 $mM^{-1}$ $cm^{-1}$). Anal. Calcd for $C_5H_{13}N_3O_3{\cdot}0.2H_2O$: C, 36.01; H, 8.10; N, 25.20. Found: C, 35.77; H, 7.82; N, 24.95.

**O$^2$-Ethyl [*N*-(2-Bromoethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (11).** Compound **10** (3.00 g, 18.4 mmol) was dissolved in 15 mL of dichloromethane and cooled to 0 °C with constant stirring. Triphenylphosphine (9.64 g, 36.8 mmol) was dissolved in 5 mL of dichloromethane and added to the reaction mixture, which was stirred for 15 min. NBS (6.51 g, 36.80 mmol) was added in portions to the reaction mixture over a period of 10 min, after which the mixture was allowed to warm to room temperature and stirred overnight. Solvent was removed on a rotary evaporator, and the crude product was purified on a silica gel column (80:20 hexanes/ethyl acetate) to afford 2.75 g (74% yield) of **11** as a yellow oil. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 4.20 (q, *J* = 7.2 Hz, 2H), 3.53 (t, *J* = 7.2 Hz, 2H), 3.35 (t, *J* = 6.8 Hz, 2H), 2.95 (s, 3H), 1.28 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 69.8, 55.8, 42.0, 27.3, 14.3. UV (EtOH) $\lambda_{max}$ ($\varepsilon$): 244 nm (10.6 $mM^{-1}$ $cm^{-1}$). Anal. Calcd for $C_5H_{12}N_3O_2Br$: C, 26.56; H, 5.35; N, 18.59. Found: C, 26.81; H, 5.43; N, 18.25.

**Isomeric Mixture of O$^2$-Ethyl (*N*-Methylamino)diazen-1-ium-1,2-diolate (*Z*-13 and *E*-13).** Compound **11** (0.50 g, 2.21 mmol) was dissolved in 4 mL of anhydrous acetonitrile and slowly added to a stirring solution of 2,8,9-trimethyl-2,5,8,9-tetraaza-1-phosphabicyclo-[3.3.3]undecane (0.57 g, 2.65 mmol) in 3 mL of anhydrous acetonitrile precooled to 0 °C. Subsequently, the reaction mixture was warmed to room temperature and stirred for another 2 h while the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was recooled to 0 °C, and a solution of *p*-toluenesulfonic acid (0.42 g, 2.21 mmol) in 1 mL of $H_2O$ was added. The resulting mixture was stirred for 1 h, concentrated under reduced pressure, and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with saturated brine solution and dried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over anhydrous sodium sulfate. After concentration under reduced pressure, the crude product was purified on a silica gel column (40:60 hexanes/ethyl acetate) to afford 0.17 g (65% yield) of an 11:1 mixture of the *Z* and *E* isomers of **13** as a pale-yellow oil. $^1$H NMR of **Z-13** ($CDCl_3$, 400 MHz): $\delta$ 4.25 (q, $J$ = 8.4 Hz, 2H), 3.02 (d, $J$ = 5.6 Hz, 3H), 1.37 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR of **Z-13** ($CDCl_3$, 100 MHz): $\delta$ 69.4, 34.3, 14.3. UV (MeOH) $\lambda_{max}$ ($\varepsilon$): 245 nm (11.1 $mM^{-1}$ $cm^{-1}$). HRMS (ESI) $m/z$: calcd for $C_3H_9N_3O_2$ $[M + H]^+$, 120.07675; found, 120.07692; $\Delta$ = 1.4 ppm. Anal. Calcd for $C_3H_9N_3O_2 \cdot 0.076EtOAc$: C, 31.54; H, 7.70; N, 33.40. Found: C, 31.14; H, 7.65; N, 33.00.

**HPLC Analysis.** HPLC separations of the mixture of the two isomeric forms of **13** (Figure 2) were performed using an LC instrument connected to a photodiode array UV−vis detector. A reversed-phase C18 column (150 mm × 4.6 mm) with a particle size of 5 $\mu$m and a guard column of the same material, both at 25 °C, were used for the stationary phase. The mobile phase used for the separation was composed of an isocratic solution of 30% acetonitrile in water containing 0.1% formic acid. The eluate was monitored at a wavelength of 250 nm. The fractions were collected manually at room temperature and immediately cooled to 0 °C and preserved at that temperature until further analysis.

## ■ ASSOCIATED CONTENT

### Supporting Information

Full $^1$H and $^{13}$C NMR spectra for new compounds, crystallographic data for compound **Z-4** (CIF), details of the dynamic NMR experiment, p$K_a$ studies, and chromatographic isomer separation. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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