ABSTRACT: Recent theoretical studies have suggested that the parent diazeniumdioliate ion, H₂N⁻N(O)⁺NO⁻ ("diazeniumdiolated ammonia"), might be stable enough to be isolated and that it could potentially serve as a uniquely advantageous prodrug form of bioactive nitroxyl (HNO). Here, we report on an attempt to isolate its O²-benzylated derivative by aminolysis of the C⁻N bond in PhC(NH₂)⁻N(O)⁺NOBn. The reaction proved remarkably sluggish in comparison to aminolysis of unsubstituted benzamidine, and the desired product could not be isolated, apparently because of base sensitivity of the NH₂ group. Consistent with this interpretation, O-benzylhydroxylamine and N₂O were recovered from the reaction mixture in high yields, along with N,N'‑dibutylbenzamidine. Theoretical calculations rationalize the observed slow aminolysis by demonstrating that the diazeniumdiolate group greatly suppresses the electrophilicity of the adjacent C⁻N carbon center, rendering attack at that position endothermic. The data provide significant insights into the challenges inherent to the pursuit of diazeniumdiolated ammonia.

INTRODUCTION
Reaction of nitric oxide (NO) with a variety of aliphatic amines generates diazeniumdiolate ions [R¹R²N⁻N(O)⁺NO⁻] ("diazeniumdiolated ammonia").¹ ² Secondary amine derivatives (R¹ ≠ H ≠ R²) have become important tools in studying the chemistry and biology of NO by virtue of their tunable rates and extents of hydrolysis to regenerate the starting materials, as shown in eq 1.² More recently, primary amine diazeniumdiolates have been shown to hydrolyze according to two mechanisms running in parallel, one mimicking the NO-forming pathway of eq 1 and the other initiated by N-protonation at a different site to generate similar amounts of a second nitrogenous bioeffect of current intense interest, nitroxyl (HNO).³ The divergent primary amine pathways are shown in eq 2.

If replacing one N-alkyl group of the "pure" NO donor shown in eq 1 with a proton converts it to a mixed NO/HNO prodrug, one might wonder whether a "pure" HNO donor might result if both alkyl groups were removed, producing the parent diazeniumdiolate structure, "diazeniumdiolated ammonia" (eq 3). Indeed, recent calculations have indicated that the species would produce NO only very slowly, suggesting that HNO release would take over as the default hydrolysis pathway, potentially producing HNO with nontoxic dinitrogen and water as the only byproducts (eq 3).⁴

Here we describe our initial attempts to prepare a derivative of diazeniumdiolated ammonia for studies of its fundamental physicochemical properties. The data provide important information about its reactivity.
RESULTS AND DISCUSSION

Synthetic Approach. Unlike secondary and primary amines, ammonia has shown no tendency to react directly with NO. Placing a solution of ammonia in methanol or tetrahydrofuran in contact with 3 atm of NO led to no observable gas uptake, precipitation, or development of an ultraviolet peak at the characteristic diazeniumdiolate wavelength of 250 nm.

Even if such a direct NO/NH₃ reaction were to occur, the product might be too reactive to isolate. While secondary amine diazeniumdiolate salts are generally stable in the absence of protonation sources, their primary amine counterparts are highly pH-sensitive and prone to rapid decomposition. Primary amine diazeniumdiolate ions can be stabilized by O-derivatization, however, so we decided to pursue O-benzylated derivative H₂N−N(O)−NOBn (1) as our initial synthetic target.

As starting material, we chose known compound 2, already available from a previous study. Early attempts at hydrolyzing 2 under either basic or acidic conditions were unsuccessful, so we resorted to aminolysis by n-butylamine as a possible means of breaking the C=N bond to free the desired ammonia derivative. This reaction was chosen on the basis of a previous report on the aminolysis of benzamidine with n-butylamine to produce N-butylbenzamidine and N,N′-dibutylbenzamidine, which were fully characterized. Our expectation was that 2 would follow a similar reaction course to produce 1 in addition to the alkylated benzamidines.

Aminolysis of 2. Accordingly, 2 was dissolved in n-butylamine to make a 3.7 mM solution and heated at 70 °C. The time course of the reaction was monitored by LC−MS. After 1 h, significant amounts of one expected product, N-butylbenzamidine 3 (along with its further aminolysis product N,N′-dibutylbenzamidine 4), were seen, but there was no trace of the desired product 1. After 30 h, the starting material was almost gone, the same two butylated amidines were the major products, and a tiny amount of mono-N-butylated 2 (structure 5 in Scheme 1) was detected, again without any evidence of the presence of 1.

To probe the origins of the significant signal associated with the void volume in these chromatograms, a different chromatographic system was used to identify the remaining products and establish a material balance. Again, no trace of 1 was seen. Instead, a near-quantitative yield of O-benzhydrylhydroxylamine (6) was found by LC−MS, along with a high recovery of nitrous oxide as determined by gas chromatography (Figure S1, Supporting Information). The overall transformation is shown in Scheme 1, while its time course is shown in Figure 1.

Products were identified as follows: 3, m/z obsd = 177.1382, m/z calcd = 177.1386; 4, m/z obsd = 233.2018, m/z calcd = 233.2012; 5, m/z obsd = 327.1815, m/z calcd = 327.1816. Product 6, m/z = 124.0764, separated with distinct chromatographic conditions, was identified and quantified by LC−MS comparison to an authentic standard, m/z = 124.0761. The structures of product 3 and product 4 were confirmed via...
purification and 1H NMR (Figures S2 and S3, Supporting Information, respectively).

**Mechanistic Considerations.** While various mechanisms can be conceived, we rationalize the above results as follows. The first step is postulated to be attack by the amine at the C=NX carbon of 2 to generate tetrahedral intermediate 7, shown in Scheme 2. Of its three C–N bonds, cleavage of the NH₂ group of 7 to form 5 was very slow, with only a small yield being observed after many hours. Cleavage to expel the butylamine substituent would simply regenerate starting material 2. The fastest nondegenerate reaction was loss of the diazeniumdiolated nitrogen to release 3 and 1, with the latter undergoing a series of deprotonation/tautomerization steps as represented in Scheme 1, ultimately leading to nitrous oxide and hydroxylamine 6 in good yields.

**Kinetic Comparisons.** To probe the effect of N-diazeniumdiolating an amidine group on its susceptibility to nucleophilic attack at the C=NX carbon, we reacted benzamidine itself with butylamine under the conditions used in the aminolysis of 2. The results are shown in Figure 2. Benzamidine quickly gave way to 3, which was then converted to 4. The time courses of reacting benzamidine vs 2 with butylamine are compared in Figure 3. It is apparent that the presence of the diazeniumdiolate substituent has a pronounced dampening effect on the electrophilicity of the imine carbon, since a significant quantity of 2 remains even after a 30-h reaction.

**Computational Chemistry Study Comparing Aminolysis Rates of 2 and Benzamidine.** In an effort to explain the unexpectedly sluggish aminolysis rate of 2, we performed a computational chemistry study comparing rate-determining aminolysis reaction energetics of 2 and benzamidine. The theoretical methodology we have used has been shown to provide very reliable predictions of chemical properties for these types of systems when compared against experimental observations.4,8 For facility we have used methylamine (instead of n-butylamine) and the O₂-methyl (instead of benzyl) derivatives of 2 (2a) and 1 (1a), as we expect that the results will be commensurate. Lowest energy structures were located for all species occurring in reactions 4 and 5, with the only structural constraint being a Z conformation for the N(O)NO fragment in 2a and 1a.

\[
2a + \text{MeNH}_2 \rightarrow 1a + \text{PhC(=NMMe)NH}_2 \tag{4}
\]

\[
\text{PhC(=NH)NH}_2 + \text{MeNH}_2 \\
\rightarrow \text{PhC(=NMMe)NH}_2 + \text{NH}_3 \tag{5}
\]

The resultant computed changes in free energies, ΔG₃₁₀(H₂O solvent), for the aminolysis reactions of 2a and benzamidine are 3.78 and −5.45 kcal/mol, respectively. Therefore, the slow aminolysis rate of 2 (assuming a similar behavior to 2a) can be simply attributed to the endothermicity of the first reaction step while the exothermicity of benzamidine aminolysis gives rise to a comparably faster rate of reaction. Experimental confirmation of these predicted free energy changes should be possible in principle; however, the essentially immediate decomposition of 1 via release of N₂O would make such a determination for 2 difficult. In fact, it may be that this quick degradation helps drive...
the aminolysis of 2 forward, albeit very slowly; i.e., the reaction is akin to a slow leak.

Although the observed slow aminolysis rate of 2 relative to that of benzamidine can be rationalized via reaction energetics, it is also instructive to investigate the electrophilicity of the imine carbon center in the two systems. Figure 4 shows the optimized structures of 2a and benzamidine together with selected bond lengths and Mulliken atom charges.

When comparing 2a against benzamidine we find that the imine C=N bond length in the former (1.323 Å) is noticeably longer than that of the latter (1.304 Å) while the formally designated single C=N bond length of 2a (1.352 Å) is shorter than its benzamidine counterpart (1.382 Å). Furthermore, the C−NH₂ group is more nearly planar in 2a as indicated by the dihedral angle between the two planes defined by atoms H−N−C and H′−N−C (θ = 159°) which is noticeably larger than that for benzamidine (θ = 139°). The results suggest that in 2/2a there is some resonance of the type shown in Scheme 3 that results in delocalization of the π bond. The 60:40 ratio of the two resonance forms was crudely estimated by comparing the C−N bond length differences between benzamidine and 2a.

Indeed, the computed Mulliken atomic charges support this notion whereby the imine nitrogen in 2a has a more negative charge (−0.73) than in benzamidine (−0.53). Additionally, the imine carbon is less positive in 2a (+0.18) than in benzamidine (+0.29).

The comparative bond lengths and charges indicate that nucleophilic attack on the imine double bond is more difficult for 2/2a than benzamidine since (i) the π electrons are delocalized over three centers in 2/2a and (ii) the electrophilicity of the 2/2a imine carbon is subdued, relative to benzamidine, due to its lower positive charge. Therefore, we must conclude that the diazeniumdiolate group acts to greatly suppress the reactivity of the imine C≡N group.

**CONCLUSION**

Our results show that the presence of a diazeniumdiolated amine group attached to an imine nitrogen of an amide dramatically slows the rate-determining step of its aminolysis, namely nucleophilic attack at the imine carbon, while also playing a dominant role in the subsequent product-determining step. Thus, presumed tetrahedral intermediate 7 formed on attack of butylamine on the C≡N carbon of 2 eliminated the elements of 1 far more rapidly than it expelled ammonia. Theoretical calculations confirmed that the presence of a diazeniumdiolate group increases the electron density at the imine carbon of 2a, rendering the initial nucleophilic attack endothermic.

The results also point to base sensitivity on the part of the desired O²-protected diazeniumdiolate 1, whose NH₂ group’s acidity allows the two protons to be ionized or to tautomerize readily to neighboring basic sites en route to complete removal. Loss of N₂O from the resulting intermediate leaves O-benzylhydroxyamine in near-quantitative yield.

While the desired diazeniumdiolated ammonia derivative 1 was neither isolated from the above-described experiments nor directly observed as an intermediate, our data strongly suggest that it was generated in essentially theoretical yield, only to be rapidly destroyed through a series of prototropic and base-induced reactions to form N₂O and O-benzylhydroxyamine 6, both of which were also recovered in high yields. As for attempts to isolate diazeniumdiolated ammonia derivatives for studies of their physicochemical properties and possible pharmacological utility, future work will seek to avoid the basic conditions that proved problematic in our aminolysis reactions, focusing instead on neutral and acidic conditions in an effort to minimize the influence of the prototropic equilibria that lead to fragmentation of the H₂N−N(O)−NOR.

**EXPERIMENTAL SECTION**

**General Methods.** Compound 2 was prepared as previously described. All other compounds used here including authentic compound 6 were obtained from commercial sources.

**Aminolysis Conditions.** In their respective reactions either 7 mg of compound 2 or 3 mg of benzamidine was dissolved in 7 mL of neat butylamine, for a final concentration of 3.7 mM, in a tightly sealed reaction vessel and heated to 70 °C. At each reported time point the reaction was allowed to cool to room temperature before aliquots were removed for dilution and analysis. Each aliquot was diluted 100-fold in acetonitrile and analyzed by LC−MS/MS. All compounds were separated and identified with an HPLC coupled with a Quadrupole Time-of-Flight (Q-TOF) mass spectrometer. Separations of all compounds, with the exception of compound 6, were performed on a
reversed-phase C18 column, 3 μm, 2.1 × 150 mm, at a flow rate of 0.2 mL/min under H2O/acetonitrile/0.1% formic acid gradient conditions. Separation of compound 6 was performed on a polar end-capped C18 column, 3-μm, 2.1 × 150 mm, at a flow rate of 0.2 mL/min under H2O/acetonitrile gradient conditions. Positive ions were generated by a dual electrospray source with a 150 V fragmenter voltage.

**Time Course of N2O Evolution.** Reactions were run according to the conditions presented above with the following exceptions. Increased quantities of compound 2 (14 mg) and butylamine (14 mL) were used. Each reaction was run under a constant stream of N2, which flowed out of a condensing column and into a gas chromatography system with an electron capture detector. A high surface area carbon molecular sieve column (2 m × 2.0 mm i.d., 80/100 mesh) was used with helium as carrier gas. The GC operation conditions were as follows: injector and detector temperatures were at 250 °C, oven temperature was programmed from 90 to 200 °C at 20 °C/min and held at 200 °C for 1.1 min. Helium flow was 30 mL/min, and nitrogen was used as makeup gas at 2 mL/min.

**Computational Chemistry.** Computations were performed using the second-order Moller–Plesset perturbation theory (MP2)\(^\text{15}\) method together with the correlation-consistent aug-cc-pVDZ (ADZ) basis set.\(^\text{16,17}\) Water solvent effects were modeled by use of the polarized continuum model (PCM),\(^\text{14}\) whereby the density of tesserae on the cavity surface was set to 240. Geometries were optimized using analytical gradients,\(^\text{12}\) and Hessians were computed seminumerically with analytic gradients so that Gibbs free energies at 310 K could be determined. Molecular structures were illustrated using MacMolPlt.\(^\text{17}\) All computations were performed using the GAMESS package.\(^\text{18}\)

### ASSOCIATED CONTENT

1. **Supporting Information**
   
   Time course of N2O generation in the aminolysis of 2; selected NMR spectra; Cartesian coordinates and energies for all the computed structures. 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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