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Chemistry of the Diazeniumdiolates. O- versus N-Alkylation of the RNH[N(O)NO]$^-$ Ion

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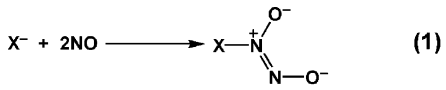
Abstract: Monomethylation of the potentially ambident RNH[N(O)NO]$^-$ ion (R = isopropyl or cyclohexyl) has been shown to occur at the terminal oxygen to yield the novel diazeniumdiolate structural unit, RNHN(O)═NOMe. The NH bond of the product proved acidic, with a pK_a of 12.3 in aqueous solution. The ultraviolet spectrum showed a large bathochromic shift on ionization (λ_{max} 244 → 284 nm, ϵ_{max} 6.9 → 9.8 mM^{-1} cm^{-1}). Deprotonation led to a pH-dependent line broadening in the ^{1}H NMR spectrum of *i*PrNHN(O)═NOMe, suggesting a complex fluxionality possibly involving isomerizations around the N−N bonds. Consistent with this interpretation, evidence for extensive delocalization and associated changes in bond order on ionizing RNHN(O)═NOR′ were found in density functional theory calculations using Gaussian 03 with B3LYP/6-311++G** basis sets. With MeNHN(O)═NOMe as a model, all N−N and N−O bonds lengthened by 0.04−0.07 Å as a result of ionization except for the MeN−N linkage, which shortened by 7%. These anions can be N-alkylated to generate R^{1}R^{2}NN(O)═NOR^3 derivatives that would otherwise be difficult to access synthetically. Additionally, some RNHN(O)═NOR′ species may display unique and beneficial pharmacological properties. As one example, an agent with R = isopropyl and R′ = β-D-glucosyl was prepared and shown to generate nitric oxide in the presence of glucosidase at pH 5.

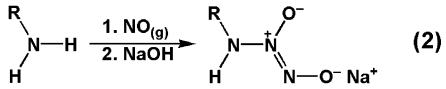
Introduction

The multifaceted bioregulatory agent nitric oxide (NO) binds to a variety of nucleophiles[1] according to eq 1 to produce stable solids that regenerate NO when dissolved in physiological fluids and are hence of interest in the drug discovery realm.[2] For example, certain primary amines are known to undergo this reaction (eq 2), and the isopropylamine derivative, **1a**, has been shown to possess both cytostatic[3] and vasorelaxant[4] activity.

We have been interested in alkylating ions of structure **1** as a "prodrug" approach[2] to targeting NO release in vivo. We report now on the characterization of this alkylation reaction: the regiochemistry of attack and structure of the products; the ionization constant and structure of the anions resulting from dissociation of the N−H bond in products of formula RNHN(O)═NOR′; reaction of such anions with a second electrophile to produce additional novel compound types; and the potential utility of this chemistry in drug design.



(1)



(2)

1a: R = isopropyl
1b: R = cyclohexyl

Experimental Section

Compounds **1a** and **1b** were prepared by reacting nitric oxide with isopropylamine and cyclohexylamine, respectively. They were isolated as the sodium salts, as previously described.[5] Unless otherwise indicated, proton NMR spectra were obtained in deuteriochloroform. Low- and

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high-resolution mass spectral measurements were carried out on a VG-Micromass model 7070 spectrometer. Gas chromatographic analyses were carried out on a Shimadzu model 4BM gas chromatograph equipped with a Hewlett-Packard 18652A A/D converter coupled to the recorder of a flame ionization detector; columns were packed with 10% Carbowax 20M (+2% KOH) on 80/100 Gaschrom Q, 3% OV-210, or 3% OV17 on 80/100 Supelcoport (glass). Ultraviolet spectra were run as aqueous solutions on an HP8451A diode array spectrophotometer. Chemiluminescence measurements were made with a Thermal Energy Analyzer model 610 (Thermedics, Inc.). Elemental analyses were performed by Galbraith Laboratories, Inc., and Atlantic Microlab, Inc.

***O*^{2}-Methyl 1-(Isopropylamino)diazen-1-ium-1,2-diolate (2a).** A solution of 9.2 g (0.065 mol) of **1a** (sodium salt) in 65 mL of anhydrous methanol was cooled to 0 °C. To the cold solution were added 5 mL (0.07 mol) of freshly distilled dimethyl sulfate, and the mixture was stirred in the cold for 1 h. The ice bath was removed, and the resulting solution was stirred at 25 °C for 4 h. The solvent was removed on a rotary evaporator. The residue was taken up in 5% aqueous sodium hydroxide solution and stirred for 30 min to decompose any excess dimethyl sulfate. The basic solution was washed with ether, then neutralized with 10% hydrochloric acid, and extracted with dichloromethane. The organic layer was dried over sodium sulfate and filtered through a pad of magnesium sulfate. The solvent was removed in vacuo. The residual oil crystallized on standing at 0 °C. It was recrystallized from ether to give 3.64 g (42%) of **2a**: mp 29−30 °C; UV λ_{max} 244 nm (ϵ 6.5 mM^{-1} cm^{-1}); NMR δ 1.18 (6H, d, J = 5 Hz), 3.93 (1H, m), 3.98 (3H, s), 5.90 (1H, b); MS *m*/*z* (relative intensity) 133 (M$^+$, 6), 132 (11), 118 (32), 102 (18), 88 (42), 87 (7), 86 (10), 85 (8), 73 (6), 61 (9), 60 (5), 57 (12), 56 (17), 49 (21), 47 (20), 45 (32), 44 (7), 43 (100); exact mass calcd for $C_4H_{11}N_3O_2$ (M$^+$) 133.0851, found (M$^+$) 133.0859. Anal. Calcd for $C_4H_{11}N_3O_2$: C, 36.08; H, 8.33; N, 31.56. Found: C, 35.89; H, 8.26; N, 30.97.

***O*^{2}-Methyl 1-(Cyclohexylamino)diazen-1-ium-1,2-diolate (2b).** A slurry of 4.23 g (0.023 mol) of **1b** (sodium salt) in 50 mL of methanol was cooled to 0 °C. To this were added dropwise 2.85 mL (0.03 mol) of dimethyl sulfate. The resulting solution was stirred at 25 °C overnight. The solvent was removed on a rotary evaporator. The residue was taken up in 50 mL of 5% aqueous sodium hydroxide solution and stirred for 30 min to destroy any excess dimethyl sulfate. The solution was washed with ether, neutralized with 10% HCl, extracted with dichloromethane, filtered through magnesium sulfate, and evaporated. The crude product was chromatographed on silica gel and eluted with 5:1 dichloromethane/ethyl acetate to give 713 mg (18%) of crystalline **2b**, which was recrystallized from aqueous ethanol: mp 84−5 °C; NMR δ 1.09−2.01 (10H, m), 3.64 (1H, m), 3.96 (3H, s), 5.98 (1H, b); UV λ_{max} 243 nm (ϵ 7.9 mM^{-1} cm^{-1}); MS *m*/*z* (relative intensity) 173 (M$^+$, 9), 158 (19), 151 (3), 150 (3), 149 (14), 128 (27), 125 (5), 123 (4), 112 (5), 111 (8), 109 (5), 108 (3), 107 (3), 99 (10), 97 (14), 94 (8), 90 (8), 84 (14), 83 (100), 82 (35), 81 (16), 71 (24), 67 (19), 62 (57); exact mass calcd for $C_7H_{15}N_3O_2$ (M$^+$) 173.1165, found (M$^+$) 173.1174. Anal. Calcd for $C_7H_{15}N_3O_2$: C, 48.55; H, 8.67; N, 24.28. Found: C, 48.47; H, 8.74; N, 24.32. An analytical sample could also be obtained by recrystallization from petroleum ether.

***O*^{2}-Methyl 1-(*N*-Isopropyl-*N*-methylamino)diazen-1-ium-1,2-diolate (3).** To a solution of 200 mg (1.5 mmol) of **2a** in 1.5 mL of anhydrous tetrahydrofuran and 0.5 mL of *N*,*N*-dimethylformamide were added 200 mg of finely powdered sodium hydroxide. The resulting mixture was stirred at room temperature for 15 min, then treated with 0.187 mL (3 mmol) of methyl iodide, and stirred for 12 h at 25 °C under nitrogen. To the reaction mixture was added 10 mL of water. The product was extracted with ether. The solution was dried over sodium sulfate, filtered, and concentrated under vacuum. The residue was purified on silica gel using 5:1 dichloromethane/ethyl acetate as the eluant. Fractions were monitored by gas chromatographic analysis on a 3% OV-210 packed glass column with a helium flow rate of 60 mL/min and a column temperature of 110 °C. The fractions containing the desired product were combined and concentrated. The residual oil was vacuum distilled to give a 60% yield of **3**. Methylation was also carried out in tetrahydrofuran using *n*-butyllithium or sodium methoxide as the base: bp 52 °C at 1 mmHg; UV λ_{max} 236 nm (ϵ 6.4 mM^{-1} cm^{-1}); NMR δ 1.13 (6H, d, J = 6.1 Hz), 2.84 (3H, s), 3.86 (1H, septet, J = 6.1 Hz), 4.03 (3H, s); MS *m*/*z* (relative intensity) 147 (M$^+$, 3), 132 (32), 102 (47), 97 (11), 95 (8), 91 (7), 88 (3), 87 (14), 85 (24), 71 (70), 70 (9), 69 (19), 68 (4), 67 (8), 60 (12), 57 (51), 56 (36), 55 (32), 49 (13), 45 (27), 43 (100), 42 (34); exact mass calcd for $C_5H_{13}N_3O_2$ (M$^+$) 147.1007, found (M$^+$) 147.0982. Anal. Calcd for $C_5H_{13}N_3O_2$: C, 40.82; H, 8.84; N, 28.57. Found: C, 40.94; H, 8.88; N, 28.50.

One-Pot Preparation of 3 from 1a. A slurry of 1.28 g (0.009 mol) of **1a** in 20 mL of anhydrous tetrahydrofuran was treated with 0.93 mL (0.015 mol) of methyl iodide. The resulting mixture was heated at reflux under nitrogen for 8 h, cooled to room temperature, and dissolved in 50 mL of water. The solution was extracted with dichloromethane, dried over sodium sulfate, filtered through a layer of magnesium sulfate, and evaporated in vacuo to give 202 mg of a yellow oil. The crude product was purified as described above to give 143 mg (11%) of **3**, the physical and spectral properties of which were identical to those obtained from the monomethylation of **2a**.

***O*^{2}-Methyl 1-(*N*-Allyl-*N*-isopropylamino)diazen-1-ium-1,2-diolate (4).** To a solution of 399 mg (3 mmol) of **2a** in 20 mL of anhydrous tetrahydrofuran were added 1 g of powdered sodium hydroxide and 0.433 mL (5 mmol) of allyl bromide. The mixture was heated at reflux under nitrogen for 2 h. The mixture was evaporated to dryness, and the residue was extracted with dichloromethane. The solution was washed with aqueous sodium bisulfite, dried over sodium sulfate, and filtered through a layer of magnesium sulfate. Evaporation of the solvent gave 454 mg of a brown oil. The oil was chromatographed through silica gel and eluted with 5:1 dichloromethane/ethyl acetate to give 346 mg of an orange oil. This oil was further purified by fractional vacuum distillation to give 180 mg of pure **4**: bp 74 °C at 1.9 mmHg; UV λ_{max} 236 nm (7.6 mM^{-1} cm^{-1}); NMR δ 1.16 (6H, d, J = 6.4 Hz), 3.47 (1H, septet, J = 6.2 Hz), 3.65 (2H, m), 4.02 (3H, s), 5.20 (2H, m), 5.32 (1H, m); MS *m*/*z* (relative intensity) 173 (M$^+$, 9), 158 (18), 132 (4), 129 (4), 128 (40), 113 (4), 102 (6), 101 (4), 87 (9), 86 (13), 85 (4), 84 (4), 83 (4), 82 (13), 69 (40), 68 (8), 60 (40), 58 (4), 57 (7), 56 (27), 55 (9), 54 (4), 45 (4), 44 (9), 43 (100), 42 (18); exact mass calcd for $C_7H_{15}N_3O_2$ (M$^+$) 173.1164, found (M$^+$) 173.1166. Anal. Calcd for $C_7H_{15}N_3O_2$: C, 48.55; H, 8.67; N, 24.28. Found: C, 48.44; H, 8.74; N, 24.20.

***O*^{2}-Methyl 1-(*N*-Ethyl-*N*-isopropylamino)diazen-1-ium-1,2-diolate (5).** (a) A solution of 340 mg (2.6 mmol) of **2a** in 10 mL of anhydrous tetrahydrofuran was stirred with 200 mg of finely powdered sodium hydroxide. The slurry was treated with 0.28 mL (3.5 mmol) of iodoethane then heated at reflux for 1 h. The progress of the reaction was monitored by gas chromatography. An additional 0.28 mL of iodoethane was added to the mixture, which was heated at reflux overnight. The reaction mixture was evaporated, and the residue was taken up in dichloromethane. The solution was washed with water, dried over sodium sulfate, filtered, and evaporated to give 129 mg of an orange oil. The crude product was eluted from silica gel with dichloromethane to give 116 mg (28%) of **5**: bp 70 °C at 2 mmHg; NMR δ 1.08 (3H, t, J = 6.8 Hz), 1.14 (6H, d, J = 6.2 Hz), 3.09 (2H, q, J = 6.8 Hz), 3.41 (1H, m), 4.06 (3H, s); UV λ_{max} 235 nm (ϵ 8.5 mM^{-1} cm^{-1}); MS *m*/*z* (relative intensity) 161 (M$^+$, 5), 160 (2), 147 (3), 146 (54), 117 (5), 116 (100), 101 (14), 99 (47), 88 (5), 74 (26), 71 (22), 70 (27), 58 (8), 56 (37), 45 (14), 44 (20), 43 (99), 42 (32); exact mass calcd for $C_6H_{15}N_3O_2$ (M$^+$) 161.1164, found (M$^+$) 161.1170. Anal. Calcd for $C_6H_{15}N_3O_2$: C, 44.72; H, 9.32; N, 26.09. Found: C, 44.48; H, 9.12; N, 25.97.

(b) The above reaction was carried out in tetrahydrofuran with 1 equiv of 25% methanolic sodium methoxide as follows. To a solution of 660 mg (4.69 mmol) of **2a** in 10 mL of tetrahydrofuran was added

1.02 mL (4.69 mmol) of methanolic sodium methoxide. Ethyl iodide (780 μL; 10 mmol) was added, and the resulting solution was heated at reflux for 2 h. The solution was evaporated to dryness, extracted with dichloromethane, and washed with water. The solution was dried over sodium sulfate, filtered, and evaporated. The residue was purified as described above to give 233 mg (29%) of **5**.

***O*^{2}-Methyl 1-(*N*-Cyclohexyl-*N*-methylamino)diazen-1-ium-1,2-diolate (6).** To a solution of 106 mg (0.613 mmol) of **2b** in 2 mL of tetrahydrofuran was added 0.22 mL (1 mmol) of 25% sodium methoxide in methanol. The resulting solution was heated at reflux for 15 min and then cooled to 25 °C. To the reaction mixture were added 95 μL (1 mmol) of dimethyl sulfate, and the mixture was heated at reflux for 2 h. After evaporation to dryness, the residue was extracted with dichloromethane. The extracts were dried over sodium sulfate, filtered, and concentrated on a rotary evaporator. The crude product was purified by column chromatography (neutral alumina, dichloromethane) to give 76 mg (66%) of **6**: NMR δ 1.27 (4H, m), 1.65 (2H, m), 1.72 (4H, m), 2.85 (3H, s), 3.37 (1H, b), 4.03 (3H, s); UV λ_{max} 235 nm (ϵ 7.2 mM^{-1} cm^{-1}); MS *m*/*z* (relative intensity) 187 (M$^+$, 7), 173 (4), 172 (40), 142 (77), 84 (21), 83 (99), 82 (33), 81 (18), 75 (10), 71 (7), 70 (14), 61 (100), 57 (22), 55 (91), 49 (33); exact mass calcd for $C_8H_{17}N_3O_2$ (M$^+$) 187.1320, found 187.1332. Anal. Calcd for $C_8H_{17}N_3O_2$: C, 51.33; H, 9.09; N, 22.45. Found: C, 51.40; H, 9.12; N, 22.35.

***O*^{2}-Methoxymethyl 1-(*N*-Isopropylamino)diazen-1-ium-1,2-diolate (7).** A slurry of 8.5 g (0.06 mol) of **1a** in 60 mL of tetrahydrofuran was cooled to 0 °C. To the cold mixture were added 10 mL of methanol. This was followed by the dropwise addition of 5.3 mL (0.07 mol) of chloromethyl methyl ether in 10 mL of tetrahydrofuran. The reaction mixture was stirred at 25 °C for 72 h, then evaporated to dryness under vacuum to give a solid residue which was extracted with dichloromethane and filtered through a layer of magnesium sulfate. The solvent was evaporated, and the crude product was chromatographed on basic alumina with dichloromethane to give 4.16 g (43%) of product: bp 31 °C at 1.5 mmHg; NMR δ 1.19 (6H, d, J = 6.5 Hz), 3.49 (3H, s), 4.01 (1H, m), 5.18 (2H, s); UV λ_{max} (pH 7) 234 nm (ϵ 6.7 mM^{-1} cm^{-1}), (pH 14) 279 nm (ϵ 7.2 mM^{-1} cm^{-1}); MS (chemical ionization, positive ion spectrum, NH_3) *m*/*z* (relative intensity), 181 ([M + NH_4^+], 100), 164 ([M + H$^+$], 24), 145 (13), 129 (18), 116 (20), 72 (54), 58(12), 45(9); exact mass calcd for $C_5H_{14}N_3O_3$ ([M + H]$^+$) 164.1034, found ([M + H]$^+$) 164.1016. Anal. Calcd for $C_5H_{13}N_3O_3$: C, 36.81; H, 7.98; N, 25.77. Found: C, 37.01; H, 7.84; N, 25.52.

***O*^{2}-Methoxymethyl 1-(*N*-Isopropyl-*N*-methylamino)diazen-1-ium-1,2-diolate (8).** To a solution of 163 mg (1 mmol) of **7** in 3 mL of anhydrous tetrahydrofuran was added 1.5 equiv of sodium methoxide (25% in methanol) followed by addition of 0.14 mL (1.5 mmol) of dimethyl sulfate. The resulting solution was kept at 37 °C overnight. The solvent was removed on a rotary evaporator. The residue was taken up in dichloromethane and washed with 5% aqueous sodium hydroxide solution. The crude product was purified by column chromatography using silica gel and 5:1 dichloromethane/ethyl acetate as the eluant. Pure **8** was obtained in 44% yield (78 mg): NMR δ 1.14 (6H, d, J = 6.5 Hz), 1.88 (3H, s), 3.49 (3H, s), 4.01 (1H, m), 5.23 (2H, s); UV λ_{max} 227 nm (ϵ 7.4 mM^{-1} cm^{-1}); MS (chemical ionization, positive ion spectrum, NH_3) *m*/*z* (relative intensity), 178 ([M + H$^+$], 55), 176 (2), 148 (6), 147 (83), 133 (4), 120 (11), 117 (17), 116 (4), 104 (4), 103 (94), 102 (9), 89 (6), 86 (17), 72 (43), 58 (11), 56 (10), 45 (100); exact mass calcd for $C_6H_{16}N_3O_3$ ([M+H]$^+$) 178.1191, found ([M + H]$^+$) 178.1197. Anal. Calcd for $C_6H_{15}N_3O_3$: C, 40.67; H, 8.47; N, 23.73. Found: C, 40.76; H, 8.55; N, 23.60.

Sodium 1-(*N*-Isopropyl-*N*-methylamino)diazen-1-ium-1,2-diolate. A solution of 12 mL (8.42 g; 0.115 mol) of *N*-methyl-*N*-isopropylamine in 10 mL of ether was placed in a 50-mL Ace Thread Parr bottle. The solution was degassed, cooled to −80 °C and charged with 40 psi of nitric oxide. A white precipitate formed within 4 h. (Note: When the reaction mixture was allowed to warm to room temperature, the white precipitate went back into solution.) The product was collected by filtration, washed with ether, and dried under nitrogen to give 915 mg of isopropylmethylammonium 1-(*N*-isopropyl-*N*-methylamino)diazen-1-ium-1,2-diolate. When a small amount of this material was placed in a vial at room temperature, white fumes quickly began to evolve. Some material was kept at dry ice temperature during transit to the analytical laboratory, and despite its instability, reasonable values were obtained. Calculated for $C_8H_{22}N_4O_2 \cdot {}^3/_4H_2O$: C, 43.72; H, 10.78; N, 25.49. Found: C, 43.29; H, 10.21; N, 25.46. The remainder of the cold isopropylmethylammonium salt was quickly treated with 10 M NaOH for cation exchange to give a white paste. This was treated with 100 mL of ether, and the light crystalline material was collected by filtration: NMR δ 1.06 (6H, d, J = 6.35 Hz), 2.73 (s, 3H), 3.22 (1H, septet, J = 6.35 Hz); UV λ_{max} 250 nm (ϵ 6.5 mM^{-1} cm^{-1}).

***O*^{2}-Methoxymethyl 1-(*N*-Isopropyl-*N*-methoxymethylamino)diazen-1-ium-1,2-diolate (9).** A solution of 157 mg (0.96 mmol) of **7** in 1 mL of tetrahydrofuran and 0.1 mL of *N*,*N*-dimethylformamide was cooled to 0 °C and mixed with 0.25 mL (1.2 mmol) of 25% methanolic sodium methoxide under a stream of nitrogen. After the solution was stirred in the cold for 10 min, 88 μL (1.2 mmol) of chloromethyl methyl ether were introduced, and the mixture was stirred at room temperature overnight. After dilution with 10 mL of ether, the mixture was filtered. The filtrate was washed with water, dried over anhydrous sodium sulfate, filtered through a layer of magnesium sulfate, and evaporated to give 109 mg of colorless oil. The crude product was purified on a silica gel column. Elution with 5:1 dichloromethane/ethyl acetate was monitored by gas chromatography on an OV-17 column. Pure **9** was obtained in 34% yield (67 mg): NMR δ 1.22 (6H, d, J = 6.4 Hz), 3.41 (3H, s), 3.50 (3H, s), 3.83 (1H, septet, J = 6.4 Hz), 4.67 (2H, s), 5.26 (2H, s); UV λ_{max} 228 nm (ϵ 7.3 mM^{-1} cm^{-1}); MS *m*/*z* (relative intensity) 207 (M$^+$, 48), 177 (26), 149 (9), 97 (16), 91 (15), 83 (26), 69 (53), 55 (100). Anal. Calcd for $C_7H_{17}N_3O_4$: C, 40.58; H, 8.21; N, 20.29. Found: C, 40.95; H, 8.07; N, 20.18.

***O*^{2}-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) 1-(Isopropylamino)diazen-1-ium-1,2-diolate.** A solution of 12 mL of water and 12 mL of 5% aqueous sodium bicarbonate was cooled to 0 °C and used to dissolve 824 mg (5.84 mmol) of **1a** sodium salt. This was followed by the addition of 2.26 g (5.5 mmol) of acetobromoglucose in 20 mL of acetone. After stirring at room temperature for 2 h, the reaction mixture was concentrated on a rotary evaporator and the residue was extracted with dichloromethane. The organic layer was dried over sodium sulfate, filtered through magnesium sulfate, and evaporated under vacuum to give 2.28 g of a glass. The crude material was dissolved in 10 mL of 5:1 dichloromethane/ethyl acetate, loaded on a 2.7-cm × 29-cm glass column packed with silica gel, and flash chromatographed using 5:1 dichloromethane/ethyl acetate as the eluant to give 391 mg of pure product: UV (10 mM NaOH) λ_{max} (ϵ) 276 nm (11.8 mM^{-1} cm^{-1}); ^{1}H NMR δ 1.19 (6H, d, J = 6.4 Hz), 2.02 (3H, s), 2.04 (6H, s), 2.08 (3H, s), 3.78−3.85 (1H, m), 3.94−4.05 (1H, m), 4.12−4.31 (2H, m), 5.14−5.32 (3H, m), 6.10 (1H, d, J = 9.2 Hz). Anal. Calcd for $C_{17}H_{27}N_3O_{11}$: C, 45.43; H, 6.06; N, 9.35. Found: C, 45.74; H, 6.20; N, 8.95.

***O*^{2}-(β-D-Glucopyranosyl) 1-(Isopropylamino)diazen-1-ium-1,2-diolate (10).** To a solution of the acylated adduct (62 mg; 0.138 mol) in 5 mL of methanol were added 5 μL of 25% methanolic sodium methoxide with stirring at room temperature. The progress of the reaction was followed on thin-layer chromatography (silica gel) using 5:1 dichloromethane:ethyl acetate as the solvent indicating that the reaction was practically complete in 30 min. DOWEX-50W−H$^+$ (500 mg) was added to the solution, which was stirred for 30 min and filtered through a membrane. The solvent was partially removed under a stream of nitrogen then placed under vacuum to give 37 mg of a white glass: ^{1}H NMR δ 1.18 (6H, d, J = 6.5 Hz), 3.54−3.97 (7H, m), 5.20−5.24 (1H, dd, J = 2.1 and 4.0 Hz); UV (1 M HCl) λ_{max} (ϵ) 236 nm (9.2 mM^{-1} cm^{-1}); UV (1 M NaOH) λ_{max} (ϵ) 278 nm (10.2 mM^{-1} cm^{-1});

no change in the absorptivity of either solution was observed after the cuvette was allowed to stand at room temperature for 3.5 h.

Enzymatic Hydrolysis of 10. A solution of 12.4 units (12.4 units/mg) of β-glucosidase from almonds (Sigma) in 3 mL of pH 5 buffer was placed in a cuvette and warmed to 37 °C, whereupon 20 μL of 3.2 mM **10** in methanol were added to the enzyme preparation. Cleavage and subsequent decomposition of the diazeniumdiolate were followed via the loss of the 238-nm chromophore as well as by a previously described chemiluminescence procedure.[6]

X-ray Diffraction Analysis of 2b. A crystal with approximate dimensions $0.5 \times 0.1 \times 0.1$ mm^3 was mounted on a glass fiber. Data were collected at −170 °C on a Bruker P4 diffractometer equipped with a SMART 1000 CCD area detector and incident beam graphite monochromator using a Mo sealed tube source ($\lambda = 0.710\,73$ Å). Integration and final cell refinement were performed with SAINT.[7] Data were corrected for absorption using SADABS.[8] The structure was solved and refined using the SHELXTL suite of programs.[9] Crystal data and refinement details are summarized in Table S6.

Variable Field NMR Experiments. Data used to calculate rates for the pH-dependent fluxionality were recorded on 300- and 400-MHz Varian XL Fourier transform NMR spectrometers. Samples were run in D_2O adjusted to pD values of either 8.6, 12.1, or 13.0 by adding NaOD to solutions in D_2O, and at pD 14 by dissolving **2a** in 1 M NaOD. Spectra were recorded at 25 °C in a 5-mm NMR tube. The sweep width and pulse width were 4803.1 Hz and 5.85 μs and 6410.3 Hz and 3.95 μs for the 300- and 400-MHz spectrometers, respectively. A delay time of 1 s and decoupler power of 25 were used for all spectra. The number of acquisitions for these spectra was 16. ^{1}H NMR spectra were acquired on a single sample at pD = 13.0 at both 300 and 400 MHz. Using standard saturation transfer, the broadened isopropyl methinyl signal centered at 3.7 ppm was measured while the isopropyl methyl resonance centered at 0.96 ppm was irradiated for 2 s and the bandwidth at half-maximum intensity of the isopropyl methine resonance at 3.7 ppm was determined.

Calculation of Structural Parameters. The calculational results described in this paper were obtained using Gaussian03[10] run on a PC platform. The ground states for each optimization corresponded to stationary points with all positive vibrational frequencies. We,[11] and others,[12] have found that Becke's three-parameter functionals and triple-ζ basis sets lead to accurate predictions for the vibrational energies and give ground-state structural results similar to those from G2 and QCISD calculations. Calculated energies in Hartrees were −395.081 170 79 for MeNHN(O)NOMe and −394.510 470 52 for [MeNN(O)NOMe]$^-$.

Results and Discussion

N- versus O-Alkylation of the RNH[N(O)NO]$^-$ Ion (1). Ions of structure **1** have a potentially labile NH proton that we suspected might be replaced by an attacking electrophile, $(R')^+$, to give an RR′N[N(O)NO]$^-$ ion instead of (or in addition to) the cis RNHN(O)=NOR′ species expected by analogy to the results with the secondary amine adduct.[13] To determine the preferred site(s) of electrophilic attack on the RNH[N(O)NO]$^-$ ion, **1a** was treated with less than a full equivalent of dimethyl sulfate in methanol-d_4 solution. Only one alkylation product could be detected by proton NMR spectrometry. Its *O*-methyl signal at δ 3.69 appeared at substantially lower field than that of the independently prepared 1-(*N*-isopropyl-*N*-methylamino)-diazen-1-ium-1,2-diolate ion, whose *N*-methyl singlet was seen at δ 2.72. To determine whether **1** could be N-deprotonated to a dianion whose nitrogen center is expected to be more nucleophilic than oxygen, we repeated the experiment except that 1 mol of sodium methoxide was added before mixing in 0.23 equiv of the methylating agent. Again, no NMR signal for the l-(*N*-isopropyl-*N*-methylamino)diazen-1-ium-1,2-diolate ion could be detected. We conclude that monomethylation of **1a** as described above gave the O-alkylated derivative as the only detectable product. When a preparative scale reaction of **1a** with dimethyl sulfate in methanol was conducted, the product was obtained in 42% isolated yield after recrystallization. Its electronic spectrum was similar to that of the $R_2NN(O)$=NOR′ derivatives characterized earlier,[13] consistent with the view that alkylation had occurred at the terminal oxygen of anion **1a** to generate **2a**.

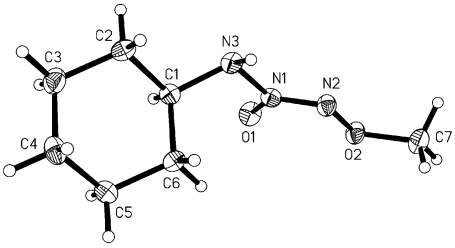
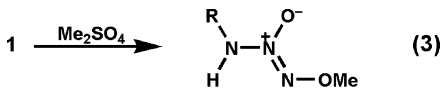


Figure 1. ORTEP representation of **2b** with non-hydrogen atoms shown as thermal ellipsoids at the 50% probability level and hydrogen atoms (unlabeled) shown as small circles of an arbitrary radius.



(3)

2a: R = isopropyl
2b: R = cyclohexyl

Crystal Structure Investigation. The structure of the compound produced on similar methylation of **1b** (eq 3) was confirmed as **2b** by X-ray crystallography. The N1−N2 distance of 1.275(2) Å (see Figure 1 for numbering system) is that of a typical N−N double bond. The two oxygens are cis to each other in a planar N_2O_2 group. The nitrogenous substituent occupies an equatorial position on the cyclohexyl ring, which is in the expected chair conformation. All in all, the structural parameters for primary amine derivative **2b** (Figure 1) showed great similarity to those of its secondary amine analogues.[13]

Acid−Base Properties of the RNHN(O)=NOR′ Functional Group. During the course of the preparative reactions described above, it was noted that alkylation of **1a** samples containing

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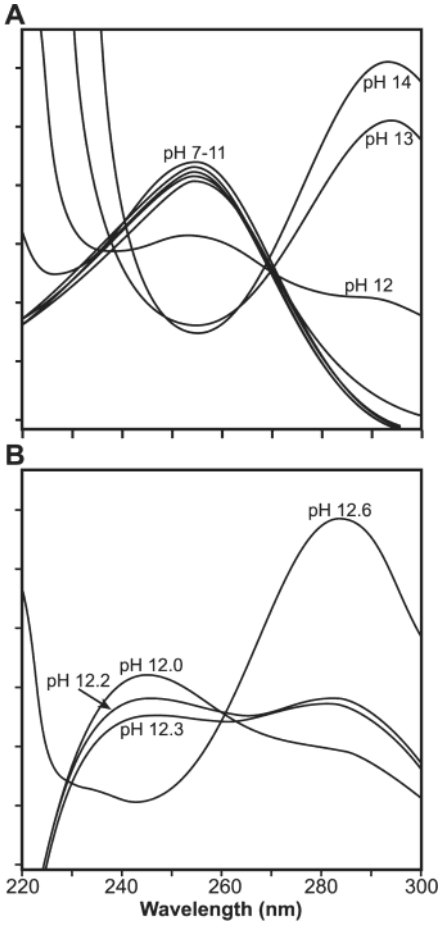

Figure 2. Ultraviolet spectra of **2a** at a concentration of 0.13 mM in various buffers: 0.01 M phosphate for pH 7−11; 0.12 M borate for pH 12−12.6; and 0.1 or 1.0 M sodium hydroxide for pH 13 and 14, respectively. Panel B shows the changes in the region of the pK_a.

increasing amounts of sodium hydroxide (added as a stabilizer) gave increasing relative yields of a second product identified by gas chromatography and NMR as a dialkylated derivative. As one example, when the reaction of **1a** with excess dimethyl sulfate was monitored by gas chromatography and NMR, dialkylation product **3** was found in roughly a 1:4 abundance relative to **2a**. This suggested that, once formed, the RNHN(O)═NOR′ functional unit was capable of reacting with the sodium hydroxide to ionize the NH bond and that the resulting anionic nitrogen was sufficiently increased in nucleophilicity to undergo a second alkylation.

To examine more directly the potential acidity of the NH bond, we dissolved **2a** in buffers of differing pH and recorded the resulting ultraviolet spectra. As shown in Figure 2, a single peak was found at 244 nm at all pHs from 1 to 11. This position is reminiscent of the λ_{max} values for the secondary amine analogues of **2a**. As the pH was increased above 11, this peak decreased in intensity and a new one emerged at 284 nm. The latter peak was the only one seen at pH 13 and above. Since this bathochromic shift was not produced on basifying the dimethylation product **3** mentioned in the previous paragraph, we conclude that the process leading to the appearance of the 284-nm peak is ionization of the NH proton. Reacidification of the alkaline solution restored the 244-nm peak at its original intensity, showing that the anion has enough stability in aqueous solution that it can be reversibly reprotonated.

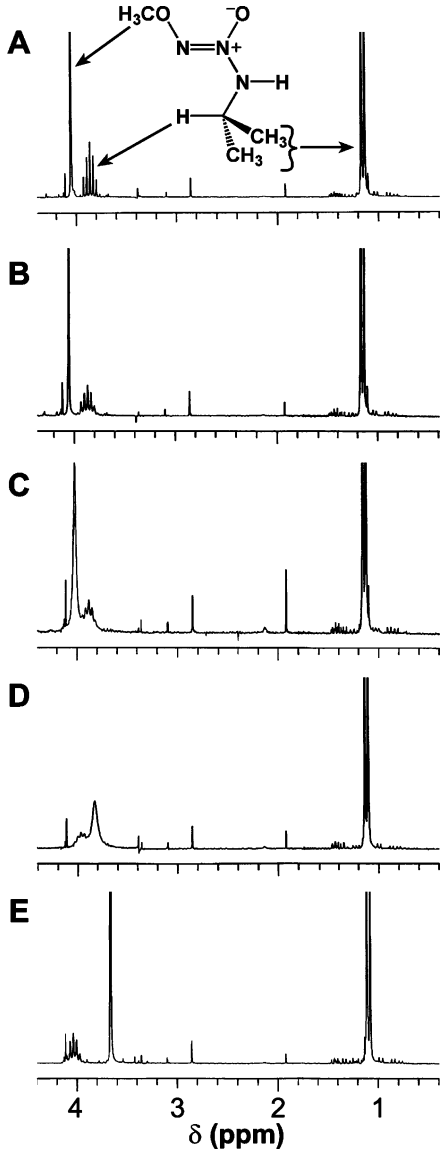

Figure 3. ^{1}H NMR spectra of **2a** at 300 MHz revealing the slow exchange process accompanying ionization of its N−H bond. Spectra A, B, C, and D were run in 0.1 M phosphate in D_2O adjusted to measured pH values of 8, 10, 11, and 12, respectively, by adding NaOD. Spectrum E was run on a solution prepared by dissolving 0.15 mmol of **2a** in 1.0 mL of 1.0 M NaOD. Note the marked line broadening of the methinyl and *O*-methyl signals in the region of pD 11−12, as well as the transposition of the methinyl and *O*-methyl signals in proceeding from low to high pH.

The pK_a for this process was measured by adjusting the pH in smaller increments between 12.0 and 12.6 until the 244- and 284-nm peaks were each at half-maximal intensity (Figure 2). Thus, since the extinction coefficients for the un-ionized and anionic forms of **2a** are 6.9 and 9.8 mM^{-1} cm^{-1}, respectively, we adjusted the pH such that the apparent extinctions were 3.5 and 4.9 mM^{-1} cm^{-1}, respectively. This pH value, 12.3, was taken as the pK_a for **2a**. Similar results were observed for **2b**.

Structure of the (RN[N(O)NO]R′)$^-$ Anion. Major changes also occurred in the proton NMR spectrum upon ionization of **2a**. As shown in Figure 3, the *O*-methyl singlet moved upfield by 0.39 ppm when the solvent was changed from phosphate at a pD of about 8 to 1 M NaOD in D_2O. By contrast, the isopropyl methinyl proton moved downfield from 3.84 to 4.03 ppm on ionization. Remarkably, both peaks broadened considerably in

Table 1. Experimental NMR Parameters Allowing Estimation of the Exchange Rate Constant k in **2a** Solutions at pH ≥ pK_a

field	$\Delta\nu$ (s^{-1})	W^* (s^{-1})	W_o (s^{-1})	k (s^{-1})
300 MHz	52.5	6.98	3.25	1000
400 MHz	70	10.69	2.13	900

the region around pD 11 to 12, pointing to a dynamic exchange involving the anion and/or its conjugate acid, **2a**, at pH ≈ pK_a.

To estimate the rate of this fluxional process, we made the tentative assumption that the exchange was occurring between two equally populated structures at or near the pK and used saturation transfer methods along with dynamic NMR spectroscopy[14] to extract rate information from the broadened signal centered at 3.7 ppm. In a single sample at pD = 13.0, the isopropyl methyl resonance centered at 0.96 ppm was irradiated and the bandwidth at half-maximum intensity of the isopropyl methinyl resonance at 3.7 ppm was determined at two different field strengths. The rate constant k for the exchange process was approximated using the equation, $k = \pi(\Delta\nu)^2/2(W^* - W_O)$, where W^* is the width at half-maximum intensity of the exchange-broadened signal, $\Delta\nu$ is the shift difference between the limiting resonance frequencies, and W_O is the width at half-height in the absence of exchange. For these experiments, the limiting resonances were determined at pH = 8.6 and 14. The observed and derived data are shown in Table 1, with the two determinations giving similar values for the exchange rate constant of $\sim 1 \times 10^3$ s^{-1}. This probably represents a lower limit for k, since it is not certain that the shift difference seen at the highest pH (14) that we could use was at its maximum, though the peaks at that pH showed little sign of exchange broadening.

It is possible that this new fluxionality is a consequence of changes in rotational energy barriers associated with the electronic reorganization that accompanies deprotonation. In particular, one might expect the N1−N2 bond to lengthen substantially and the N1−N3 bond to shorten even more, giving rise to interconverting stereoisomers that can be individually observed if the temperature is low enough and/or the field strength is high enough. Some possibilities are shown in eq 4.

To more fully understand the electronic structure and the nature of the deprotonation product of **2a**, we have used density functional theory, B3LYP/6-311++G**, to investigate Z-MeNHN(O)=NOMe and the Z,Z isomer (eq 4) of its corresponding anion, $[MeNN(O)NOMe]^-$. A comparison between the structure of **2b** and that of its methyl analogue, MeNHN(O)NOMe, is shown in Figure 4. For MeNHN(O)NOMe, the ground state has a C_1 symmetry that is very close to a planar C_s configuration. There is excellent agreement between the observed and calculated structures shown in Figure 4. Not only are the bond lengths in accord with one another, but the observed and calculated conformations, as reflected by the dihedral angles, are of similar magnitudes and sign. Similar structural trends have been observed before for the adducts produced by the diazeniumdiolation of secondary amines,[11] supporting the use of this level of theory and basis sets for these species.

The $[MeNN(O)NOMe]^-$ anion has a nearly planar ONNO framework, corresponding to a local minimum at this level of

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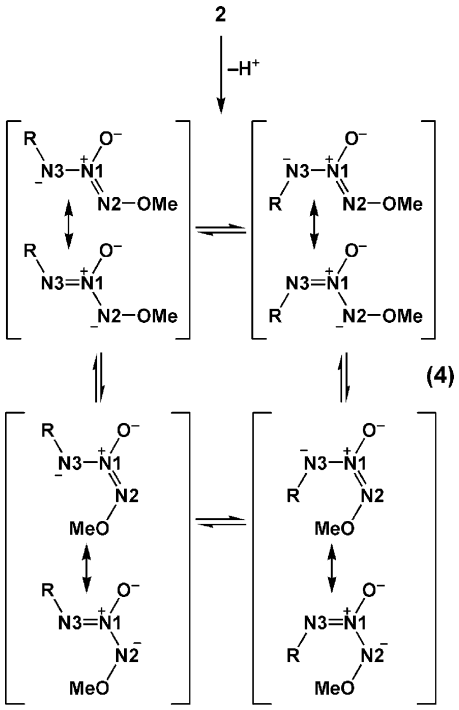


theory. In Figure 5, $[MeNN(O)NOMe]^-$ is juxtaposed with its protonated form and as anticipated from the simple resonance considerations of eq 4, deprotonation leads to considerable structural changes. Thus, the N1−N2 bond lengthens while the N1−N3 bond shortens to the extent that the N1−N3 linkage is ~0.008 Å shorter than the N1−N2 bond for the anion, whereas in the neutral species N1−N3 is 0.135 Å longer than N1−N2. In addition, the N1−O1 bond lengthens substantially upon deprotonating N3, and the optimized ground state for the anion corresponds to a nearly planar geometry instead of the nonplanar framework found in MeNHN(O)NOMe. The calculations support the view that changes in bond order could account for the changes in the electronic and NMR spectra seen on deprotonation. Further theoretical studies on the potential energy

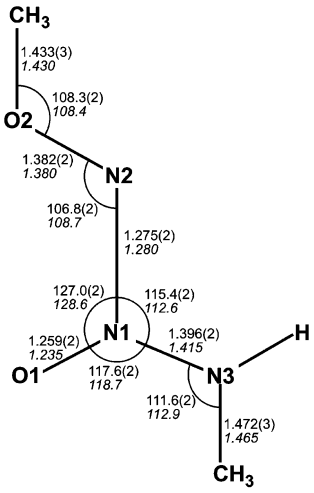


Figure 4. Experimental values for the crystallographically determined structure of CyNHN(O)NOMe, in normal type, contrasted with the calculated values (B3LYP/6-311++G**) for MeNHN(O)NOMe, in italics.

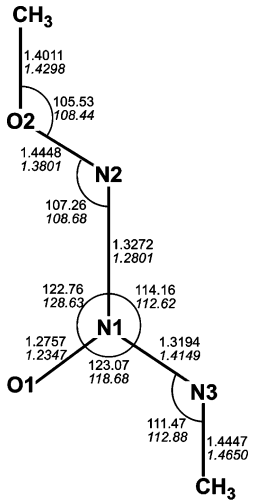


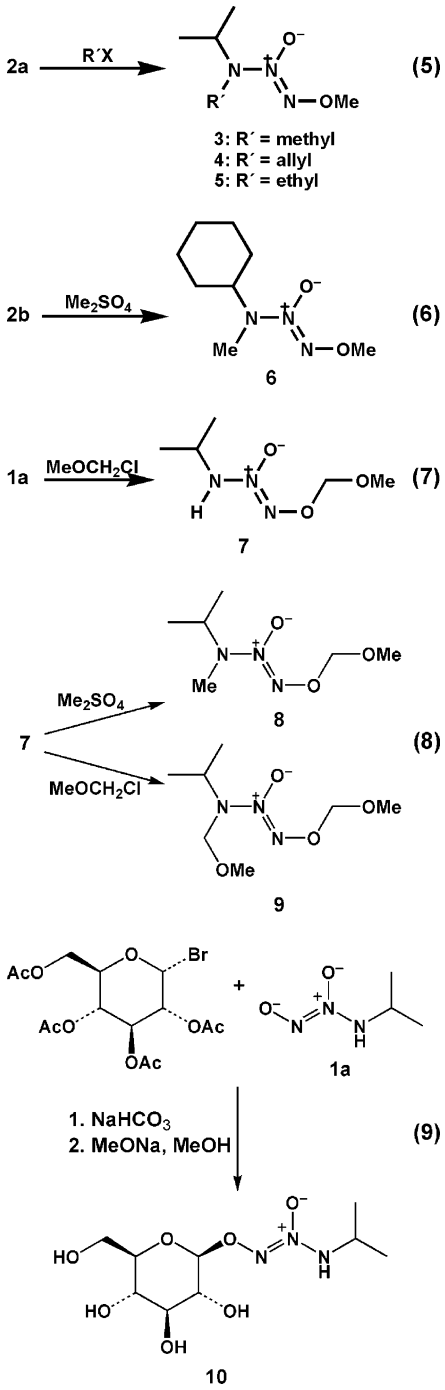
Figure 5. Calculated values (B3LYP/6-311++G**) for MeNHN(O)-NOMe, in italics, contrasted with those of [MeNN(O)NOMe]$^-$, in normal type.

surfaces of MeNHN(O)NOMe and its anion are underway and will be reported elsewhere soon.

Synthetic Utility and Drug Discovery Implications. Our findings concerning the relative nucleophilicities of the different heteroatoms in ions of structure **1** should permit facile design of novel diazeniumdiolates for biological screening. As examples, the reactions of eqs 5−8 have led to new compounds **3**−**9**. Of interest in this connection are target molecules that cannot be prepared under previously described diazeniumdiolation conditions. Compound **9**, for instance, is a derivative of the unstable amine *i*PrNHCH$_2$OMe. Many other structures that might serve as prodrugs of such amines as well as of nitric oxide on suitable metabolism can be conceived.

Of particular importance from the drug design standpoint is the expected toxicological profile of the primary amine derivatives relative to their secondary amine analogues. The N═N bonds of R^{1}R^{2}NN(O)═N−O$^-$ ions and their O^2-alkylation products can undergo net cleavage to R^{1}R^{2}NNO species[15] photolytically[16] or on hydrolysis in aerobic media.[17] If neither R^1 nor R^2 is H, the result is a stable *N*-nitroso compound, many (but not all) of which can be metabolized to carcinogenic form.[18] This is not the case when one of the R groups is H, however. This is because the intermediate primary nitrosamines are unstable, hydrolyzing rapidly via diazoates and diazonium ions under physiological conditions before distribution to remote parts of the body can occur.

For this reason, we are exploring molecules of structure RNHN(O)═NOR′ as possible prodrugs of nitric oxide with advantageous toxicological characteristics. To illustrate the promise of such chemistry in the drug design arena, β-D-glucoside **10** was prepared as in eq 9 and examined by ultraviolet spectrophotometry in 1 M hydrochloric acid (λ_{max} 236 nm) and 10 mM sodium hydroxide (λ_{max} 278 nm) as well as at neutral pH. No change was seen in the absorbance during a 3.5-h observation period in any of the spectra. In the presence of β-glucosidase at pH 5 and 37 °C, however, the 236-nm peak rapidly decreased and a total of 1 mol of NO was generated over the ensuing 2 h per mol of starting **10**. Similar results were reported with a secondary amine-bound glucoside analogue by Wu et al.[19] Further work on the prodrug potential of RNHN(O)═NOR′ derivatives is in progress.



Acknowledgment. We thank Mr. John Klose for the NMR determination of the pK_a of **2a** and Mr. John Roman for collecting the mass spectral data. D.S.B. gratefully acknowledges support from the National Science and Engineering Research

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Supporting Information Available: Crystallographic files for **2b**; optimized geometries for MeNHN(O)NOMe and its conjugate anion at the B3LYP/6-311++G** level. This material is available free of charge via the Internet at http://pubs.acs.org.

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