Acid-Catalyzed Rearrangements of *N*-Nitrosodehydromorpholine

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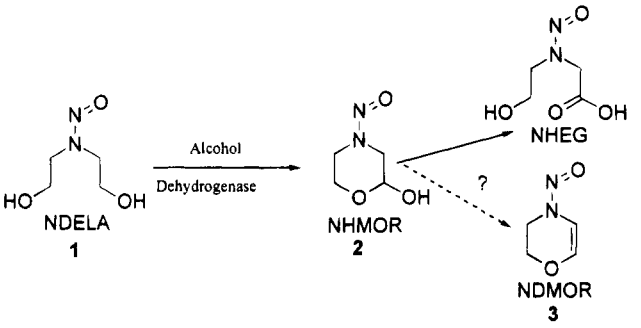
N-Nitrosodehydromorpholine, a potential metabolic intermediate in the carcinogenic activation of *N*-nitrosodiethanolamine, and *N*-nitrosomorpholine, was prepared from the hemiacetal *N*-nitroso-2-hydroxymorpholine (**2**) by tosylation and *in situ* base-catalyzed elimination. The attempted acid-catalyzed hydration of **3** gives neither **2** nor the unstable α-hydroxynitrosamine, *N*-nitroso-3-hydroxymorpholine (**4**). The product of this transformation is *N*-(2-hydroxyethyl)-2-oximinoethanamide (**5**) as confirmed by an X-ray crystallographic determination. Treatment of **3** with gaseous HCl in CH_2Cl_2 gives 1-aza-4-oxa-3-oximinocyclohexene (**6**), which rearranges to **5** upon treatment with aqueous acid. The initial rearrangement was proven to be intermolecular by means of a crossover experiment employing ^{15}NO and C-5-D_2 isotopomers of **3**.

Introduction

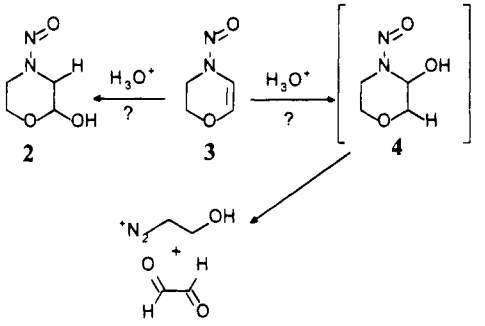
N-Nitrosodiethanolamine (NDELA) is a potent, widespread animal carcinogen derived from the inadvertent nitrosation of a number of substrates carrying the diethanolamine moiety.[1,2] The only known biochemical transformations of NDELA involve its successive oxidative metabolism. One hydroxyl group is oxidized first to an aldehyde, which cyclizes to the more stable hemiacetal *N*-nitroso-2-hydroxymorpholine (NHMOR) and subsequently to *N*-nitroso-*N*-(2-hydroxyethyl)glycine (NHEG) (Scheme 1).[3]

Although NHMOR is mutagenic[4,5] and possesses the interesting chemical property of transferring its *N*-nitroso group to other amines,[6–8] neither it nor NHEG is carcinogenic.[9] In probing possible biologically significant transformations of NHMOR, we have investigated its possible reversible conversion to *N*-nitroso-2,3-didehydromorpholine (NDMOR), a process well known for the related 2-hydroxydihydropyran. Several vinyl nitrosamines, *N*-nitrosoenamines, are known to be extremely potent carcinogens,[10] and even the minor biochemical conversion of NDELA into such a species could represent an effective activation pathway. For example, either hydration of the double bond of NDMOR to generate the unstable α-hydroxynitrosamine, *N*-nitroso-3-hydroxymorpholine **4**, shown in Scheme 2, or its epoxidation[11] would result in highly reactive intermediates capable of alkylating DNA. Because **3** is both a *N*-nitrosoenamine and an enol ether, the regiochemistry of its hydration cannot be easily predicted. In this paper, we report the synthesis of NDMOR and the interesting finding that it undergoes two successive rearrangements in dilute aqueous acid and neither hydrates to NHMOR nor generates the α-hydroxy nitrosamine **4** at detectable levels.

Scheme 1



Scheme 2



The chemistry of *N*-nitrosoenamines has been explored to only a limited extent, and no investigation of their reactions with aqueous acids has been reported. *N*-Nitrosoenamines have been synthesized through β-elimination reactions of β-(chloroethyl)-,[12–14] β-[(tosyloxy)ethyl]-,[14–18] and β-(phenylselenoxy)nitrosamines.[18] Because of the relatively high acidity of the nitrosamine α-hydrogen, *N*-nitrosoenamines can also be produced by base-

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(15) Seebach, D.; Enders, D. *Chem. Ber.* **1975**, *108*, 1293–1320.
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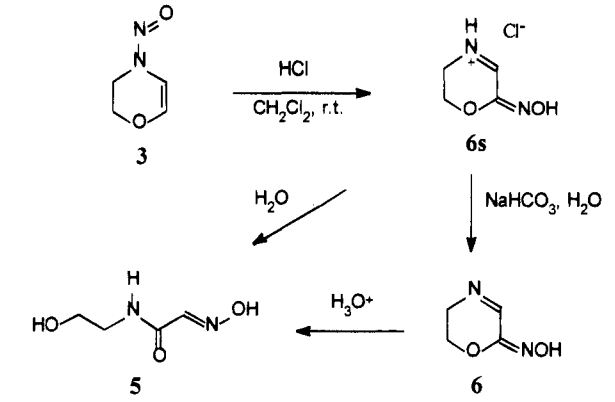
catalyzed dehydration of β-hydroxynitrosamines.[19,20] *N*-Nitrosoenamines undergo both electrophilic and nucleophilic addition at the double bond because of the ability of the nitrosamine function to stabilize both positive and negative charge at the α-carbon.[18] Kupper and Michejda have demonstrated that electrophilic reagents add to *N*-nitrosoenamines in such a way as to add the nucleophilic partner to the carbon adjacent to the nitrogen atom, whereas nucleophilic reagents attack the β-carbon atom.[18] In their pioneering work on the synthetic utility of α-nitrosamino carbanions, Seebach and Enders produced *N*-nitrosoenamines from either *in situ* tosylate elimination following hydroxyalkylation (condensation with an aldehyde or a ketone) or through the rearrangement of carbanions derived from allylic nitrosamines.[15-17] This group and others have shown that acid treatment of *N*-nitrosoenamines results in their rearrangement to oximino imines.[15-17] Lyle and co-workers[22] have proposed an intermolecular pathway for this transformation, but definitive proof is absent. We show here, by means of a crossover experiment using isotopically labeled substrates, that this rearrangement is definitely intermolecular.

Results and Discussion

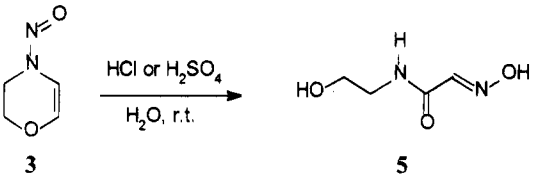
NDMOR, 1-aza-4-oxa-1-nitroso-2-cyclohexene (**3**), was synthesized from NHMOR using a modified version of Seebach and Enders' procedure,[15] since attempts directed at the dehydration of NHMOR under various conditions were not successful. NHMOR reacted rapidly with *p*-toluenesulfonyl chloride in the presence of triethylamine at 0 °C in methylene chloride, yielding presumably the corresponding tosylate. The tosylate proved to be unstable and underwent elimination in the presence of triethylamine at room temperature, to give **3** as a mixture of *N*-nitroso *Z*/*E* isomers in 71–79% yield. Each isomer exhibited a pair of methylenes (δ 3.9–4.6) and vinyl protons (δ 6.14–7.22, doublets) in its ^{1}H NMR spectrum. The IR spectrum of the mixture showed no OH, and the ^{13}C NMR spectrum was consistent with the assigned structure.

Our initial expectation was that NDMOR would easily hydrate to give NHMOR (Scheme 2) since this chemistry is a well-known property of enol ethers. This hydration would proceed through an oxygen-stabilized carbocation (oxonium ion) generated by protonation at C-3. The ability of the nitrosamine function to stabilize an adjacent positive charge, however, raises the possibility of protonation at C-2. In this case, the reverse hydration would lead to a reactive α-hydroxy nitrosamine **4** (Scheme 2).

Scheme 3



NHMOR does not react with neutral water at room temperature. It slowly decomposes in aqueous sodium hydroxide solution. On the other hand, when **3** is treated with at least 1 equiv of hydrochloric acid in water at room temperature, a very polar compound is formed in high yield (91%). The same substance forms from the reaction of **3** with sulfuric acid in an aqueous solution in 83% yield. Spectroscopic analyses of this substance reveal the presence of OH and C=O groups. Elemental analysis gives the formula $C_4H_8N_2O_3$. The proton NMR gives a pattern consistent with the presence of a hydroxyethyl group. The ^{13}C NMR spectrum gave a peak at δ 162, which, taken together with other spectroscopic data, is consistent with the presence of an amide. On the basis of this information we tentatively assigned the structure **5** *N*-(2-hydroxyethyl)-2-oximinoethanamide to this compound (equation 1). Proof of this structure was provided



(1)

by an X-ray crystallographic analysis.[23] The crystal structure of **5** indicated that the carbon nitrogen double bond of the oximino group possesses an *E*-configuration, and no intramolecular hydrogen bonding is evident.

The oximino amide **5** is formally a combination of **3** and a water molecule. In order to better understand the mechanism leading to the formation of **5**, the reaction of NHMOR with HCl in nonaqueous medium was investigated (Scheme 3). Treatment of **3** with 1 equiv of dry hydrogen chloride in methylene chloride at room temperature generated a yellow precipitate. The precipitate exhibited the characteristics of a salt, and its spectroscopic analysis was consistent with the generation of an oximinoimine having structure **6s**. Treatment with aqueous sodium bicarbonate gave the corresponding free base, 1-aza-4-oxa-3-oximinocyclohexene (**6**). Structures **6s** and **6** were assigned in analogy with the known chemistry of *N*-nitrosoenamines and their spectroscopic properties. An interesting feature of this transformation is that the use of less than 1 equiv or a catalytic amount of acid resulted in incomplete nitroso migration. For example, treatment of **3** with 0.38 equiv of dry hydrogen chloride in methylene chloride, followed by neutralization with an aqueous solution of sodium bicarbonate, resulted in the formation of 32% of **6**, with a recovery of 57% of **3**. In fact, the nitroso migration converted the nonbasic

(19) Loeppky, R. N.; McKinley, W. A.; Hazlitt, L. G.; Outram, J. R. *J. Org. Chem.* **1982**, *47*, 4833–4841.
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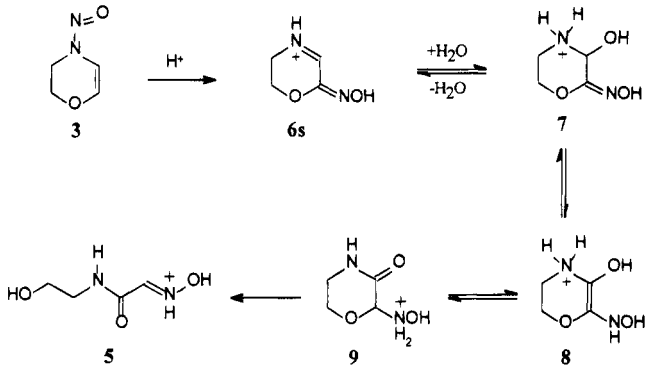
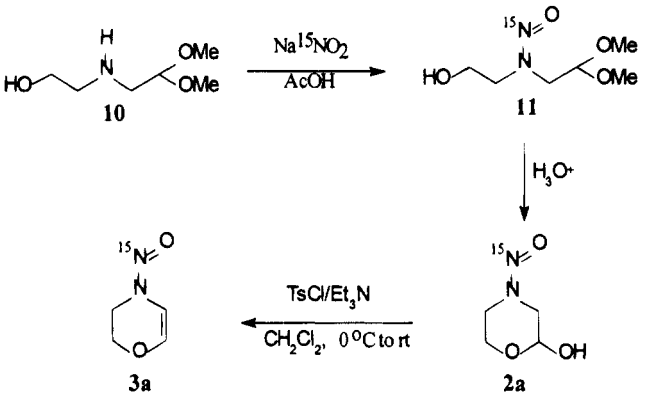
(23) Crystal data for **5**: (ORTEP drawing provided in supplementary material) $C_4H_8N_2O_3$, FW = 132.12, crystal dimensions 0.20 × 0.40 × 0.50 mm; orthorhombic, space group $P2_12_12_1$, a = 4.64950(10) Å, b = 8.7370(3) Å, c = 15.2322(5) Å, V = 618.77(3) Å^3, Z = 4, D_{calc} = 1.418 g/cm^3, λ = 1.540 56 Å, μ = 1.00 mm^{-1}, $F(000)$ = 280. The intensity data were collected on a Nonius diffractometer, using the $\theta - 2\theta$ scan mode ($2\theta_{max}$ = 150°). A total of 769 reflections were measured, and all were unique. The last least-squares cycle was calculated with 17 atoms, 107 parameters, and 748 reflections with $I > 2.0\sigma(I)$. R = 0.040 (R_w = 0.059). The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

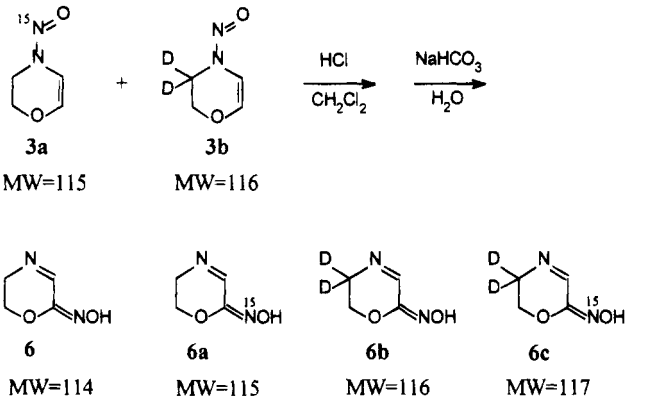
Scheme 4



Scheme 5



Scheme 6



nitrosamine **3** to the basic imino oxime, consuming 1 equiv of acid. The use of a weaker acid, such as acetic acid, proved ineffective in catalyzing the rearrangement.

Interestingly, direct reaction of the isomerized product **6s** with water, or treatment of its free base **6** with hydrochloric acid in an aqueous solution (pH 2–3), all led to the formation of *N*-(2-hydroxyethyl)-2-oximinoethanamide (**5**). Therefore, the initial step for the reaction of **3** with an acid under aqueous conditions, probably also involved the migration of the nitroso group.

Scheme 4 depicts a proposed mechanism for the formation of **5** from **3** under aqueous acidic conditions. After migration of the nitroso group, the imino group of **6s** reacts further with a molecule of water, yielding an α-hydroxy oxime **7**. The double bond isomerization of **7** to **8** and the ketonization is followed by the breakage of the ring to give the final product. This mechanistic scheme includes several equilibria involving the isomerized product **6s** and intermediates **7**–**9**. The equilibrium between **7** and **9** which lies decidedly on the side of **9**, is typical of isomerization reactions of α-hydroxy imines. The ring opening of **9**, which could be reversible, appears to drive the overall equilibrium in favor of the formation of the final product. Interestingly, treatment of **3** with 0.39 equiv of hydrochloric acid in water also gives **5**, but in only 34% yield. Most of the starting material (**3**) is recovered (60%). The percentage yield of **5** does not increase with a longer reaction time. Obviously, the newly formed basic product **5** consumes 1 equiv of acid, preventing **3** from further isomerization.

An important question connected to the rearrangement reaction of **3** to **6s** (Scheme 3) is how the migration of the *N*-nitroso group takes place. The isomerization of a structural analog, 1-nitroso-1,2,3,4-tetrahydropyridine, to 3-oximino-3,4,5,6-tetrahydropyridine under acidic conditions has been proposed to be an intermolecular process.[22] On the other hand, the Fischer–Hepp rearrangement, which involves treatment of a *N*-nitroso secondary aromatic amine with HCl to give a *p*-nitroso secondary aromatic amine, is believed to be intramolecular.[24] In order to distinguish an intramolecular process from an intermolecular pathway, we designed a crossover experiment utilizing two different isotopically labeled species of **3**. One of these compounds was labeled with a ^{15}N isotope on the nitroso group, and the other compound was labeled on the ring with two deuterium atoms. An intramolecular isomerization would retain the labeling within the starting molecules, whereas an intermolecular rearrangement would lead to the scrambling of the labels.

The ^{15}N-labeled molecule, 1-aza-2-oxa-1-[^{15}N]nitroso-2-cyclohexene (**3a**), was prepared by the procedure shown in Scheme 5. (2,2-Dimethoxyethyl)(2-hydroxyethyl)-amine (**10**), obtained from the alkylation of ethanolamine with chloroacetaldehyde dimethyl acetal,[7] was nitrosated with sodium [^{15}N]nitrite in acetic acid at room temperature to give 2,2-(dimethoxyethyl)(2-hydroxyethyl)[^{15}N]-nitrosamine (**11**). The deprotection of the aldehydic group of **11** by acidic hydrolysis afforded *N*-[^{15}N]nitroso-2-hydroxymorpholine (**2a**) which was converted to **3a** using a procedure similar to the procedure described earlier for the preparation of **3**.

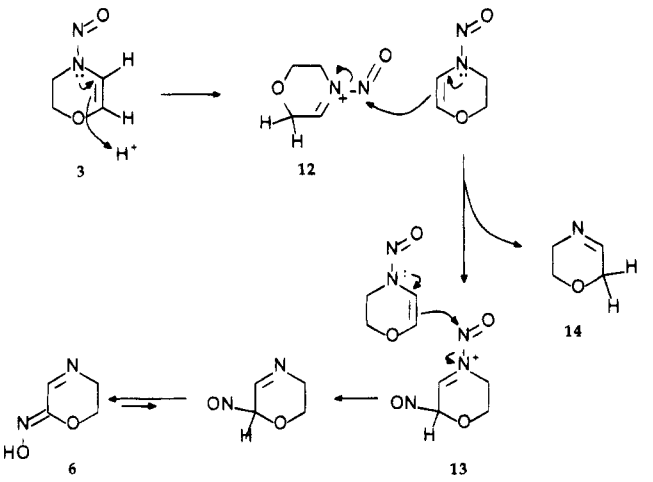
The dideuterated species, 6,6-dideuterio-1-aza-4-oxa-1-nitroso-2-cyclohexene (**3b**), was obtained from *N*-nitroso-2-hydroxy-5,5-dideuteriomorpholine. The latter compound was synthesized by a method that we recently developed.[25]

Scheme 6 presents the details for the reaction of the two isotopically labeled molecules with hydrogen chloride. One equivalent of each 1-aza-2-oxa-1-[^{15}N]nitroso-2-cyclohexene (**3a**) and 6,6-dideutero-1-aza-4-oxa-1-nitroso-2-cyclohexene (**3b**), were mixed in methylene chloride and treated with hydrogen chloride. The precipitate was collected and neutralized with an aqueous solution of sodium bicarbonate. After isolation and purification, the product was analyzed by spectroscopic methods, especially GC/MS and high resolution MS. The reaction produces four isotopomers of 1-aza-4-oxa-3-oximinocyclohexene, **6**, **6a**, **6b**, and **6c**. Two of these isotopomers, **6** and **6c**, are generated from cross migrations of the corresponding nitroso groups to the other rings, *i.e.*, intermolecular processes, while the other two isoto-

(24) Williams, D. H. L. *Nitrosation*; Cambridge University Press: Cambridge, 1988; pp 113–128.

(25) Loeppky, R. N.; Xiong, H. *J. Labeled Cmpds. Radiopharms* **1994**, *24*, 1099–1110.

Scheme 7



pomers, **6a** and **6b**, could be the result of either intermolecular or intramolecular migrations. The approximately 1:1:1:1 ratios[26] of the four isotopomers indicates that the rearrangement of NDMOR is a completely intermolecular process.

These results provide evidence for the mechanism for the *N*-nitroso migration of NDMOR shown in Scheme 7 and support the hypothesis of Lyle, Krueger, and Gunn[22] on the isomerization of 1-nitroso-1,2,3,4-tetrahydropyridine. The initial step in the nitroso migration involves the protonation of the double bond of **3** at C-2, yielding a nitrosamine-stabilized carbocation **12**. This carbocation, a *N*-nitrosoimminium ion, nitrosates **3** at C-2 to give another cation **13**. The cation intermediate **13** then serves as a NO^+ carrier and transfers the nitroso group to **3**, regenerating **13** and leaving 1-aza-4-oxa-3-oximinocyclohexene **6** as the rearranged product. The yield data show that the nitroso transfer from **13** to **3** is very efficient and that very little **14** is formed. It was not detected in the product mixture.

While it is easy to rationalize the intermolecular nature of the transnitrosative rearrangement, it is striking that this transformation occurs without significant side transformations. In water we anticipated that the C=C would hydrate to give either NHMOR or the α-hydroxy nitrosamine **4**. Since the initial protonation occurs at the oxygen-bearing carbon to generate **12** it is somewhat surprising that the nitroso transfer from this species to the C=C of a substrate is faster than nucleophilic attack of water at either the α-carbon or the *N*-nitroso nitrogen atom. The former process would yield an α-hydroxy nitrosamine which is presumed to decompose with ease as shown in Scheme 2. Yet, under the conditions of the transformation we find no decomposition products expected of such an intermediate. Attack of water at the nitrogen atom of **12** or **14** would generate the imine and nitrous acid. The latter could be an intermediate in the nitroso transfer, but the efficiency of the rearrangement argues against it because of the well-known ability of nitrous acid to undergo decomposition in acid. Further experiments are underway to determine whether **3** can be hydrated to an α-hydroxy nitrosamine.

Conclusion

NDMOR can be readily prepared from *N*-nitroso-2-hydroxymorpholine by tosylation and *in situ* elimination. The reaction of this α,β-unsaturated nitrosamine with an acid in aqueous solution results in migration of *N*-nitroso group and the breakage of the ring, yielding *N*-(2-hydroxyethyl)-2-oximinoethanamide (**5**). When the reaction is carried out under nonaqueous conditions, 1-aza-4-oxa-3-oximinocyclohexene (**6**) is produced. Acid-catalyzed hydration of **6** leads to **5**. Reactions of two isotopically labeled species with hydrogen chloride in methylene chloride indicated that the nitroso migration is an intermolecular process. NDMOR contains both an enol ether and an *N*-nitrosoenamine functionality, and the chemistry revealed here shows that either the latter functional group can better stabilize a positive charge than the former, a somewhat surprising observation when one considers the electron-withdrawing nature of the nitroso group, or the *N*-nitroso transfer occurs so rapidly after the protonation of the double bond at C-1 that protonation at C-2 to generate the oxygen-stabilized carbocation cannot compete.

(26) The amount of individual isotopomer was based on the GC/MS analyses.

Experimental Section

Caution. Many nitrosamines are potent chemical carcinogens, and all should be handled with extreme caution. We routinely use a solution of anhydrous HBr in glacial acetic acid to wash all glassware and decontaminate other washable items exposed to nitrosamines. All transformations involving these substances are carried out in a hood, and laboratory personnel wear two pairs of surgical-type gloves on each hand with a layer of talc between them to inhibit nitrosamine transfer through the rubber.

General. Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. Gas chromatography was performed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a Supelco SPB-1 fused silica capillary column (30 M × 0.25 mm, 0.25 μm film) and interfaced with a Hewlett-Packard 3396 Series II integrator. GC/MS analyses were done on a Hewlett-Packard 5890A gas chromatograph coupled with a Hewlett-Packard 5970 series mass selective detector. High pressure liquid chromatography was performed with a Waters chromatograph consisting of Waters Maxima 820 system controller, Waters Model 490 programmable multiwavelength detector, two Waters Model 510 pumps, and a Waters Model 710B WISP autosampler. Unless stated otherwise, the HPLC was done on a DuPont Zorbax ODS column (4.6 mm × 25 cm). Product purification was typically performed by column chromatography using glass columns packed with Merck flash silica gel 60 (230–400 mesh).

^1H NMR and proton-decoupled ^{13}C NMR spectra were taken on a Bruker AMX 500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C). IR spectra were recorded on a Nicolet 20 DXB FTIR spectrometer. High resolution mass spectra were performed by the Midwest Regional Center for Mass Spectrometry at the University of Nebraska–Lincoln.

All commercial chemicals were used as received without further purification. Methylene chloride was dried by refluxing over calcium hydride and freshly distilled before use.

1-Aza-4-oxa-1-nitroso-2-cyclohexene (3). A round-bottomed flask equipped with an addition funnel was charged with *N*-nitroso-2-hydroxymorpholine (1.993 g, 15.08 mmol). Methylene chloride (10 mL) and triethylamine (8 mL) were added sequentially. *p*-Toluenesulfonyl chloride (3.496 g, 18.33 mmol) dissolved in 10 mL of methylene chloride was added dropwise at 0 °C. The mixture was stirred at 0 °C for 30 min and then warmed to room temperature and stirred at room temperature for 10 h. Precipitates were separated from the reaction mixture by filtration and washed with methylene chloride (10 mL × 2). The filtrate was concentrated to dryness. The residue was chromatographed, using hexane/ethyl acetate (5:1, v/v) as eluent, to give 1-aza-4-oxa-1-nitroso-2-cyclohexene

(3) (1.365 g, 79% yield): ^{1}H NMR ($CDCl_3$)[27,28] *E*-isomer (49%) δ 7.22 (d, J = 4.9 Hz, 1 H), 6.14 (d, J = 4.9 Hz, 1 H), 4.16 (t, J = 5.1 Hz, 2 H), 3.90 (t, J = 5.1 Hz, 2 H); *Z*-isomer (51%) δ 7.10 (d, J = 5.0 Hz, 1 H), 6.29 (d, J = 5.0 Hz, 1 H), 4.51 (t, J = 4.8 Hz, 2 H), 4.37 (t, J = 4.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$)[29] *E*-isomer δ 131.2, 108.7, 63.6, 38.5; *Z*-isomer δ 136.1, 100.9, 65.3, 46.5; IR (neat, mixture of *E* and *Z* isomers) 3125, 2996, 2940, 2887, 1648, 1631, 1433, 1400, 1354, 1296, 1221, 1166, 1067, 995, 860, 741 cm^{-1}; HRMS calcd for $C_4H_6N_2O_2$ 114.0429, found 114.0427.

***N*-(2-Hydroxyethyl)-2-oximinoethanamide (5).** 1-Aza-4-oxa-1-nitroso-2-cyclohexene (**3**) (0.343 g, 3.00 mmol) was dissolved in 10 mL of water. Hydrochloric acid (37%, 0.304 g, 3.08 mmol), diluted with 5 mL of water, was added dropwise at room temperature. The mixture was stirred at room temperature for 4 h, and then sodium bicarbonate (0.285 g, 3.39 mmol) was added. Water was completely removed under vacuum. The residue was washed with methanol (10 mL), and the insoluble material was filtered off and washed with methanol (10 mL × 2). Solvent was evaporated from the combined methanol solution. The residue was chromatographed, using methylene chloride/methanol (5:1, v/v) as eluent, to afford *N*-(2-Hydroxyethyl)-2-oximinoethanamide (**5**) (0.352 g, 89% yield): mp 100–100.5 °C (crystallized from acetone/methylene chloride); ^{1}H NMR (DMSO-d_6) δ 11.88 (s, 1 H), 8.00 (t, J = 5.2 Hz, 1 H), 7.44 (s, 1 H), 4.71 (s, 1 H), 3.42 (t, J = 5.6 Hz, 2 H), 3.20 (dt, J = 5.2, 5.6 Hz, 2 H); ^{13}C NMR (DMSO-d_6) δ 162.0, 143.8, 59.6, 41.4; IR (KBr) 3381, 3210–2750 (br), 1659, 1619, 1541, 1484, 1421, 1276, 1218, 1102, 1028, 1002, 923, 795, 747, 636 cm^{-1}; EIMS m/z (rel inten) 115 ([M – OH]$^+$, 9), 101 (100), 89 (24), 85 (25), 72 (35); HRMS calcd for ($C_4H_8N_2O_3$ – OH) 115.0508, found 115.0510.

Anal. Calcd for $C_4H_8N_2O_3$: C, 36.36; H, 6.10; N, 21.20. Found: C, 36.34; H, 5.93; N, 21.12.

1-Aza-4-oxa-3-oximinocyclohexene (6). 1-Aza-4-oxa-1-nitroso-2-cyclohexene (**3**) (0.526 g, 4.61 mmol) was mixed with methylene chloride (20 mL). A diethyl ether solution of dry hydrogen chloride (1.0 M, 5.0 mL, 5.0 mmol) was added dropwise at room temperature. A yellow precipitate formed immediately. The mixture was stirred at room temperature for 30 min. The precipitate was collected by filtration and washed with dry methylene chloride (10 mL × 3). (Attempts to recrystallize the solid using various solvent systems were not successful.) The solid was dried under vacuum at room temperature to afford 1-aza-4-oxa-3-oximinocyclohexene hydrochloride (**6s**) (0.631 g, 91% yield): mp 159–160 dec; ^{1}H NMR (DMSO-d_6) δ 12.69 (s, 1 H), 11.80–10.20 (br, 1 H), 8.61 (s, 1 H), 4.37 (t, J = 4.9 Hz, 2 H), 3.88 (t, J = 4.9 Hz, 2 H); ^{13}C NMR (DMSO-d_6) δ 157.9, 146.7, 63.0, 43.0. A part of the yellow solid (0.401 g, 2.66 mmol) was dissolved in an aqueous solution (15 mL) of sodium bicarbonate (0.268 g, 3.19 mmol) at room temperature. This mixture was stirred for 10 min, and the water removed under vacuum. The residue was chromatographed, using methylene chloride/methanol (15:1, v/v) as eluent, to give 1-aza-4-oxa-3-oximinocyclohexene (**6**) (0.282 g, 93% yield): mp 170–171 dec (recrystallized from ethyl acetate/hexane); ^{1}H NMR (DMSO-d_6) δ 10.76 (s, 1 H), 7.72 (t, J = 2.2 Hz, 1 H), 4.10 (t, J = 5.2 Hz, 2 H), 3.69 (td, J = 5.2, 2.2 Hz, 2 H); ^{13}C NMR (DMSO-d_6) δ 152.5, 145.5, 63.4, 47.6; IR (KBr) 3200–2400 (br), 1651, 1628, 1505, 1470, 1439, 1397, 1338, 1298, 1257, 1223, 1102, 1063, 1029, 993, 952, 894, 830, 759, 616 cm^{-1}; EIMS m/z (rel inten) 114 (M$^{\cdot+}$, 86), 84 (9), 67 (100); HRMS calcd for $C_4H_6N_2O_2$ 114.0429, found 114.0429.

Anal. Calcd for $C_4H_6N_2O_2$: C, 42.11; H, 5.30; N, 24.55. Found: C, 42.07; H, 5.15; N, 24.44.

(2,2-Dimethoxyethyl)(2-hydroxyethyl)[^{15}N]nitrosamine[30] (11). (2,2-Dimethoxyethyl)(2-hydroxyethyl)amine (**10**)[7] (1.041 g, 6.98 mmol) was dissolved in glacial acetic acid (10 mL) at 0 °C. Solid $Na^{15}NO_2$ (0.487 g, 6.96 mmol, 99 atom % ^{15}N) was added. The mixture was stirred at room temperature for 20 h. Acetic acid was then removed under vacuum. The residue was dissolved in an aqueous solution of saturated sodium bicarbonate (30 mL). This solution was then extracted with ethyl acetate (50 mL × 3). The combined organic phases were dried over anhydrous magnesium sulfate. After filtration and removal of the solvent, the residue was chromatographed, using hexane/ethyl acetate (1:1, v/v) as eluent, to give (2,2-dimethoxyethyl)(2-hydroxyethyl)[^{15}N]nitrosamine (**11**) (1.024 g, 82% yield): ^{1}H NMR ($CDCl_3$) *E*-isomer (40%) δ 4.73 (t, J = 5.4 Hz, 1 H), 4.32 (dd, J = 5.4, 2.7 Hz, 2 H), 3.82 (t, J = 5.2 Hz, 2 H), 3.70 (q, J = 5.2 Hz, 2 H), 3.44 (s, 6 H), 2.82 (t, J = 5.2 Hz, 1 H, hydrogen-bonded OH); *Z*-isomer (60%) δ 4.59 (t, J = 5.5 Hz, 1 H), 4.30 (td, J = 5.3, 2.4 Hz, 2 H), 3.97 (q, J = 5.3 Hz, 2 H), 3.73 (d, J = 5.5 Hz, 2 H), 3.42 (s, 6 H), 3.23 (t, J = 5.3 Hz, 1 H, hydrogen-bonded OH); ^{13}C NMR ($CDCl_3$) *E*-isomer δ 102.6, 59.4, 54.6 (d, J = 6.4 Hz), 54.3, 48.9; *Z*-isomer δ 100.5, 60.8, 56.5 (d, J = 5.9 Hz), 55.4, 48.3; IR (neat) 3419, 2992, 2945, 2838, 1444, 1424, 1349, 1299, 1192, 1152, 1124, 1074, 1020, 980 cm^{-1}; EIMS m/z (rel inten) 148 ([M – CH_3O]$^+$, 7), 116 (1), 86 (9), 75 (100), 56 (4); HRMS calcd for [$C_6H_{14}{}^{15}NNO_4$ – CH_3O] 148.0740, found 148.0740.

***N*-[^{15}N]Nitroso-2-hydroxymorpholine[30] (2a).** (2,2-Dimethoxyethyl)(2-hydroxyethyl)[^{15}N]nitrosamine (**11**) (0.963 g, 5.37 mmol) was dissolved in an aqueous solution of sulfuric acid (10%, 6 mL) at 0 °C. The mixture was stirred for 1 h at 0 °C and 2 h at room temperature. This mixture was then cooled to 0 °C and neutralized with an aqueous solution of saturated sodium bicarbonate. This aqueous solution was extracted with ethyl acetate (15 mL × 4). The combined organic phases were dried over anhydrous magnesium sulfate. After filtration and removal of solvent, the residue was chromatographed to afford *N*-[^{15}N]nitroso-2-hydroxymorpholine (**2a**) (0.660 g, 92% yield): ^{1}H NMR ($CDCl_3$) *E*-isomer (55%) δ 5.23 (dd, J = 5.0, 2.3 Hz, 1 H), 4.68 [s, 1 H (OH)], 4.43 (dt, J = 13.3, 2.3 Hz, 1 H), 4.14 (ddd, J = 13.3, 5.0, 1.7 Hz, 1 H), 4.03 (ddd, J = 11.6, 6.7, 4.3 Hz, 1 H), 3.87–3.78 (m, 2 H), 3.59 (ddd, J = 11.6, 5.9, 4.3 Hz, 1 H); *Z*-isomer (45%) δ 5.01 (dd, J = 4.7, 3.0 Hz, 1 H), 4.52 [s, 1 H (OH)], 4.32–4.24 (m, 3 H), 3.94 (dd, J = 13.7, 3.0 Hz, 1 H), 3.87–3.78 (m, 2 H); ^{13}C NMR ($CDCl_3$) *E*-isomer δ 91.3, 59.5, 53.7 (d, J = 6.0 Hz), 39.7 (d, J = 1.6 Hz); *Z*-isomer δ 90.6, 61.0, 48.9 (d, J = 6.1 Hz), 44.5 (d, J = 1.6 Hz); IR (neat) 3382, 2981, 2937, 2887, 1446, 1415, 1354, 1283, 1248, 1198, 1157, 1115, 1094, 1070, 1048, 1011, 967, 929, 891, 844, 799, 761 cm^{-1}; EIMS m/z (rel inten) 133 (M$^{\cdot+}$, 4.4), 116 (2), 102 (57), 73 (51), 56 (100); HRMS calcd for $C_4H_8{}^{15}NNO_3$ 133.0505, found 133.0503.

1-Aza-2-oxa-1-[^{15}N]nitroso-2-cyclohexene (3a). The title compound was prepared from **2a** by the procedure for the synthesis of **3**: ^{1}H NMR ($CDCl_3$) *E*-isomer (49%) δ 7.22 (d, J = 4.9 Hz, 1 H), 6.14 (d, J = 4.9 Hz, 1 H), 4.16 (t, J = 5.2 Hz, 2 H), 3.90 (t, J = 5.2 Hz, 2 H); *Z*-isomer (51%) δ 7.10 (d, J = 5.0 Hz, 1 H), 6.29 (d, J = 5.0 Hz, 1 H), 4.51 (t, J = 4.7 Hz, 2 H), 4.37 (t, J = 4.7 Hz, 2 H); ^{13}C NMR ($CDCl_3$) *E*-isomer δ 131.3 (d, J = 3.0 Hz), 108.8 (d, (J = 6.8 Hz), 63.7, 38.5; *Z*-isomer δ 136.2, 100.9, 65.3, 46.5 (d, J = 5.9 Hz); IR (neat) 3125, 2998, 2939, 2887, 1659, 1630, 1449, 1416, 1396, 1347, 1291, 1272, 1220, 1161, 1067, 982, 859, 740 cm^{-1}; HRMS calcd for $C_4H_6{}^{15}NNO_2$ 115.0400, found 115.0399.

6,6-Dideuterio-1-aza-4-oxa-1-nitroso-2-cyclohexene (3b). The title compound (about 95 atom % deuterium as estimated by ^{1}H NMR and MS) was prepared from *N*-nitroso-2-hydroxy-5,5-dideuteriomorpholine[24] by the procedure for the synthesis of **3**: ^{1}H NMR ($CDCl_3$) *E*-isomer (51%) δ 7.22 (d, J = 4.9 Hz, 1 H), 6.14 (d, J = 4.9 Hz, 1 H), 4.15 (s, 2 H); *Z*-isomer (49%) δ 7.10 (d, J = 5.0 Hz, 1 H), 6.29 (d, J = 5.0 Hz, 1 H), 4.36 (s, 2 H); ^{13}C NMR ($CDCl_3$) *E*-isomer δ 131.3, 108.8, 63.6, 38.5 (m, $-CD_2-$); *Z*-isomer δ 136.2, 100.9, 65.2, 46.5 (m, $-CD_2-$); IR (neat) 3125, 2932, 2886, 2351 (weak), 2327 (weak), 2149

(27) The equilibrium ratio of *E* and *Z* isomers of **3** in D_2O was 42:58, clearly showing two sets of proton signals. The exact assignments of proton chemical shifts of **3** shown as follows were made by comparison with the ^{1}H NMR spectra of two deuterium-labeled species of **3**, 6,6-dideuterio-1-aza-4-oxa-1-nitroso-2-cyclohexene and 5-deuterio-1-aza-4-oxa-1-nitroso-2-cyclohexene.

(28) The assignments of *E* and *Z* isomers were based on their differences in chemical shifts. The protons resonate at higher fields when *cis* to the oxygen of the *N*-nitroso group.

(29) The *E* and *Z* assignments of carbon chemical shifts were based on ^{1}H–^{13}C chemical shift correlation.

(30) For the corresponding nonisotopically labeled compound, see ref 7.

(weak), 2125 (weak), 1652, 1629, 1429, 1382, 1349, 1292, 1265, 1219, 1058, 959, 901 cm^{-1}; HRMS calcd for $C_4H_4D_2N_2O_2$ 116.0555, found 116.0552.

Reaction of 3a and 3b with Hydrogen Chloride. 1-Aza-2-oxa-1-[15N]nitroso-2-cyclohexene (**3a**) (80 mg, 0.70 mmol)) and 6,6-dideuterio-1-aza-4-oxa-1-nitroso-2-cyclohexene (**3b**) (81 mg, 0.70 mmol) were mixed in methylene chloride (5 mL). A diethyl ether solution of hydrogen chloride (1.0 M, 1.4 mL, 1.4 mmol) was added dropwise at room temperature. The mixture was stirred at room temperature for 30 min. Solvent was removed. The solid residue was dissolved in an aqueous solution (5 mL) of sodium bicarbonate (0.140 g, 1.67 mmol) at room temperature. This mixture was stirred at room temperature for 10 min. Water was then removed under vacuum. The residue was chromatographed, using methylene chloride/methanol (15:1, v/v) as eluent, to give a mixture of isotopomers, **6**, **6a**, **6b**, and **6c** (144 mg, 89% yield): HRMS calcd for $C_4H_6N_2O_2$, $C_4H_6{}^{15}NNO_2$, $C_4H_4D_2N_2O_2$, and $C_4H_4D_2{}^{15}NNO_2$ 114.0429, 115.0400, 116.0555, and 117.0525, found 114.0431, 115.0405, 116.0551, and 117.0529.

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Supporting Information Available: ORTEP drawing of **5** and ^{13}C NMR spectra of **3** and **6s** (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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