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The Synthesis of Deuterium-Labeled *N*-Nitrosodiethanolamine and *N*-Nitroso-2-hydroxymorpholine

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Summary

Two deuterated derivatives of *N*-nitrosodiethanolamine and two deuterated derivatives of *N*-Nitroso-2-hydroxymorpholine were prepared. 1,1,1',1'-$^2H_4$-*N*-Nitrosodiethanolamine **1a** was prepared in 99% isotopic purity by simple base catalyzed H-D exchange. 2,2,2',2'-$^2H_4$-*N*-Nitrosodiethanolamine **1b** was synthesized in 98% isotopic purity by the $LiAlD_4$ reduction of dimethyl iminodiethanoate, followed by acid catalyzed nitrosation. 5,5-$^2H_2$-*N*-Nitroso-2-hydroxymorpholine **2a** was prepared in 99% isotopic purity through a series of steps involving 1) the selective base catalyzed H-D exchange of the CH_2 adjacent to the ester function of 2,2-diethoxyethylcarboethoxymethylnitrosamine, 2) its reduction with diisobutylaluminum hydride at -78° C followed by 3) acid catalyzed hydrolysis of the acetal and cyclization to the hemiacetal. 2-^2H-*N*-Nitroso-2-hydroxymorpholine **2b** (98% isotopic purity) was prepared through the $LiAlD_4$ reduction of 2-hydroxyethylcorboethoxymethylnitrosamine at -78° C.

Key words: Deuterium, nitrosamine, synthesis, *N*-nitrosodiethanolamine, *N*-nitroso-2-hydroxymorpholine

Introduction

N-Nitrosodiethanolamine (NDELA, **1**), a potent liver carcinogen in several species of animals (1), is one of the most widespread *N*-nitroso compounds in the human environment (2). As a nitrosamine, NDELA possesses several unusual features. It is excreted in the urine unchanged to the extent of 85-90% except at the lowest dose levels (1b) and carbon fragments from it are bound to DNA at very low levels (1d).

The *in vitro* metabolism of NDELA by rat liver preparations has been shown to produce the corresponding aldehyde, *N*-2-hydroxyethyl-*N*-nitrosoaminoethanal (3a,b). This hydroxy aldehyde preferentially exists as the cyclic hemiacetal, *N*-nitroso-2-hydroxymorpholine (NHMOR,

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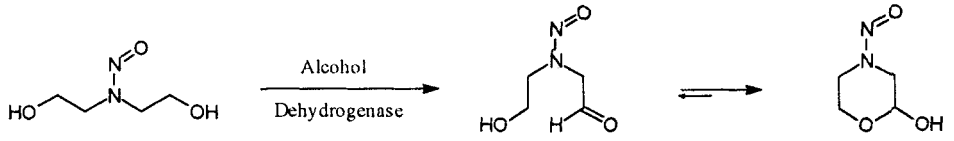
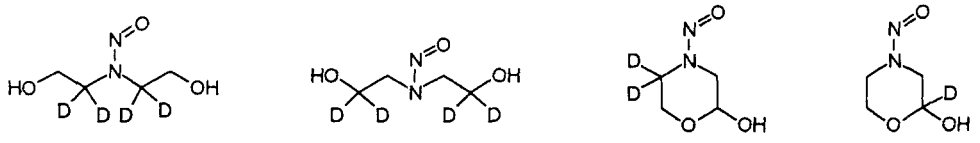
2) (Figure 1). Unlike dialkyl nitrosamines, NHMOR is chemically unstable and possesses the unusual property of transferring its *N*-nitroso functional group to other amines (4). It has been demonstrated that NHMOR is a direct acting mutagen (3c). *In vivo* NHMOR is further metabolized to the biologically inert *N*-(2-hydroxyethyl)-*N*-nitrosoglycine (3a,b). Although NHMOR is a direct acting mutagen and reacts with DNA to deaminate bases and form "glyoxal adducts" with guanine (4b) it is not carcinogenic when administered orally to rats and is only very weakly carcinogenic in AJ mice (5). These results and others raise questions regarding the hypothesis that NHMOR is a proximate carcinogen of NDELA.



1 (NDELA) 2 (NHMOR)

Figure 1

In order to elucidate the mechanism of carcinogenic activation of NDELA and the role of NHMOR, we required these molecules labeled with deuterium atoms at the specific positions shown in Figure 2, 1a-b, 2a-b. We report here the synthesis of these specific deuterium-labeled isotopomers of NDELA and NHMOR. The synthesis of several of these compounds involves the preparation of multi-functionalized nitrosamines as the synthetic precursors and the use of selective functional group transformations not previously reported for molecules containing the N-NO group.



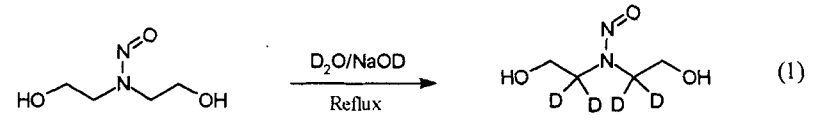
1a 1b 2a 2b

Figure 2

Results and Discussion

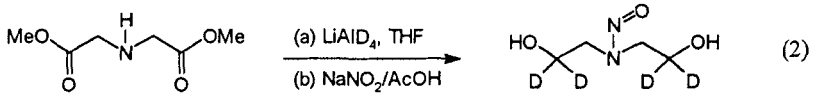
It has been postulated that a N-NO function can stabilize either a positive or a negative charge at the α-position. This feature allows for the preparation of α-deuterated nitrosamines by base catalyzed hydrogen/deuterium isotope exchange (6). We found that the exchange method can be used for preparing compound 1a directly from *N*-nitrosodiethanolamine without protecting the

hydroxyl groups (equation 1). For example, a D_2O solution of *N*-nitrosodiethanolamine (0.5-0.65 M) was refluxed for five hours in the presence of NaOD (0.5-0.65 M) (7). Removal of the solvent and flash column chromatography gave **1a** with an isotopic purity of 96 atom% deuterium (8). Higher isotopic purity (>99 atom% deuterium) of **1a** was obtained simply by evaporating the solvent from the reaction mixture and adding fresh D_2O, followed by refluxing for another five hours. No hydrogen isotope exchange was observed for the α-protons of *N*-nitrosodiethanolamine when the reaction was carried out at room temperature for a week.



(1)

1a



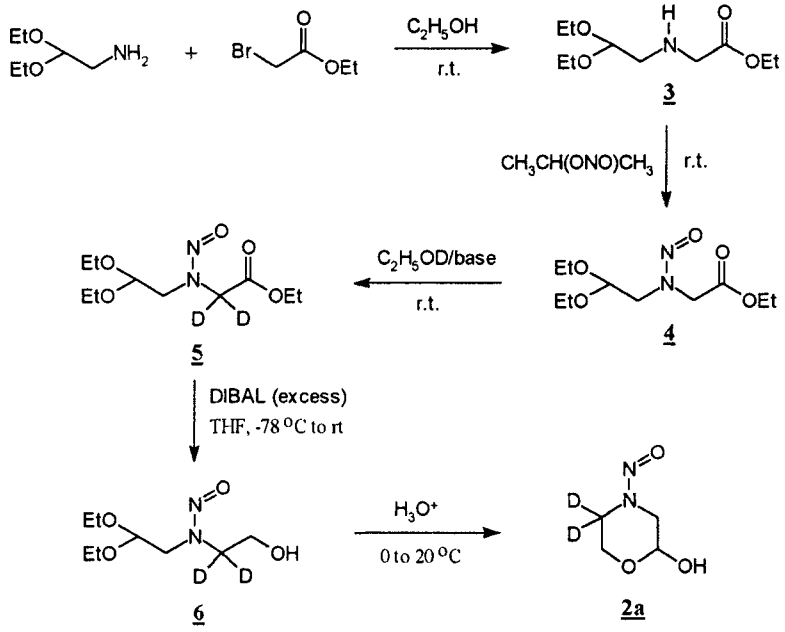
(2)

1b

The method of choice for preparing **1b** is based on the reduction of an appropriate substrate with a deuteride-transfer reagent (equation 2). Thus, dimethyl iminodiethanoate, obtained from the esterification of iminodiethanoic acid (9), was treated with $LiAlD_4$ in THF. The resulting 2,2,2',2'-$^2H_4$-diethanolamine was nitrosated *in situ* with $NaNO_2$ under acidic conditions. Workup afforded **1b** in 68% overall isolated yield, with an isotopic purity higher than 98 atom% deuterium.

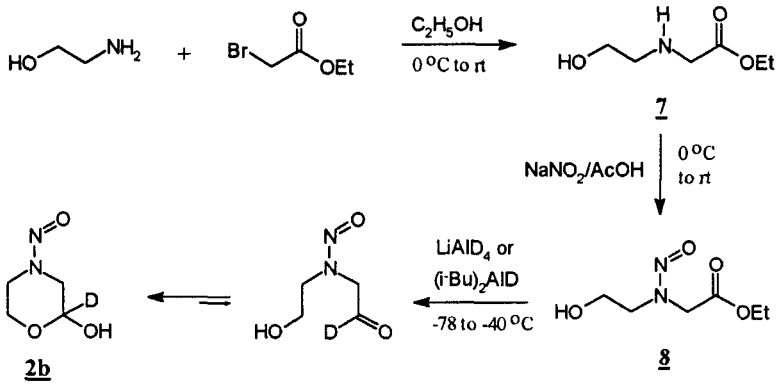
The synthetic pathway for preparing **2a** is summarized in Scheme I. The reaction of an excess of 2,2-diethoxyethylamine with ethyl bromoacetate in ethanol yielded ethyl (2,2-diethoxyethyl)aminoethanoate **3**, in 80% isolated yield (10). Compound **3** was then smoothly nitrosated at room temperature by using isopropyl nitrite (11) as the nitrosation reagent, generating the key multi-functionalized nitrosamine, 2,2-diethoxyethylcarboethoxymethylnitrosamine **4**, in essentially quantitative yield. As expected, the methylene group of compound **4** [$(EtO)_2CHCH_2N(NO)CH_2COOEt$] activated by both an ester and a nitrosamino functionalities readily underwent hydrogen isotope exchange in the presence of a base. For example, a C_2H_5OD solution of **4** (1.0 M) in the presence of catalytic amount of C_2H_5ONa (2-5 mol%) was stirred at room temperature for 10 hours to give cleanly the desired deuterium-labeled intermediate, ethyl

2,2-diethoxyethyl-*N*-nitroso-1,1-$^2H_2$-aminoethanoate **5**, in quantitative yield. After one exchange run, the isotopic purity of **5** was about 93 atom% D. Higher isotopic purity (>98 atom% D) of **5** was obtained by evaporating the reaction solvent and replacing with fresh C_2H_5OD to allow a second exchange. A weaker base, such as Et_3N, was also found to catalyze the hydrogen isotope exchange, but the reaction required longer times (3 days) to complete.



Scheme I

The hydrogen isotope exchange did not take place without the presence of a base. The deuterated intermediate **5** was used directly for the next reaction. The reduction of the ester functional group of **5** to the corresponding primary hydroxy group represents a key step of the synthetic sequence. Lithium aluminum hydride ($LiAlH_4$) proved not to be a reagent of choice because it also destroyed the *N*-nitroso group under the reaction conditions for achieving the desired transformation. It was found that the reduction of **5** with an excess of diisobutylaluminum hydride in THF afforded the corresponding primary alcohol, *N*-2,2-diethoxyethyl-*N*-nitroso-1,1-$^2H_2$-aminoethanol **6**. After deprotection of the aldehydic group of **6** by acidic hydrolysis *in situ*, 5,5-$^2H_2$-*N*-nitroso-2-hydroxymormpholine **2a** was obtained in 72% overall isolated yield, with a final isotopic purity of 95 atom% deuterium.



Scheme II

Scheme II illustrates the synthetic approach to the deuterium-labeled compound **2b**. An excess of ethanolamine was treated with ethyl bromoacetate in ethanol, yielding ethyl *N*-2-hydroxyethylaminoethanoate **7** (12). This compound was then nitrosated *in situ* with sodium nitrite under acidic conditions to afford the key nitrosamine, ethyl (2-hydroxyethyl)nitrosoaminoethanoate **8**, in 55% overall yield. The next synthetic operation involved the reduction of the ester group of **8** with a deuteride-transfer reagent to produce the corresponding deuterium-labeled aldehyde. It was found that treatment of **8** with diisobutylaluminum deuteride (2.5 eq.) accomplished the transformation, affording *N*-2-hydroxyethyl-*N*-nitroso-1-^{2}H-aminoethanal, which was isolated as its preferred hemiacetal form, ^{2}H-*N*-Nitroso-2-hydroxymorpholine **2b**, in 67% yield. The careful control of the reaction temperature proved to be important. The reduction was found to be slow and incomplete at -78° C. On the other hand, over-reduction occurred to give the corresponding alcohol if the reaction temperature was higher than -40° C. Interestingly, lithium aluminum deuteride was also found to effect the reduction. Thus, compound **8** was reacted with lithium aluminum deuteride (1.1 eq.) in THF at -78° C. Workup at low temperature gave **2b** in 64% isolated yield (13). A similar temperature effect was observed for the $LiAlD_4$ reduction. Lithium aluminum deuteride did not react with *N*-nitroso functional group under the low temperature conditions.

In summary, several efficient procedures have been developed for preparing individual deuterium-labeled molecules of *N*-nitrosodiethanolamine and *N*-nitroso-2-hydroxymorpholine. Some of the labeled compounds can be synthesized by the reductions of the appropriate intermediates with deuteride-transfer reagents, while the others can be prepared by the hydrogen isotope exchanges of the suitable precursors. The hydrogen isotope exchange method represents a useful

synthetic approach to deuterium-labeled materials at low monetary costs. Because the nitroso functional group can be readily cleaved from nitrosamines (14), the present approaches also have potential applications to the synthesis of deuterium-labeled secondary amines and their derivatives. The majority of nitrosamines are potent carcinogens (15), and the several new nitrosamines utilized as synthetic intermediates in this work should also be treated accordingly and handled with care.

Experimental

General. 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 x 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 multi-wavelength detector, two Waters Model 510 pumps, and Waters Model 710B WISP autosampler. Unless stated otherwise, the HPLC was done on a DuPont Zorbax ODS column (4.6 mm x 25 cm). Product purification was typically performed by distillation or by column chromatography using glass columns packed with Merck flash silica gel 60 (230-400 mesh).

^{1}H NMR and proton-decoupled ^{13}C NMR spectra were taken on a Bruker AMX 500 spectrometer (500 MHz for ^{1}H 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 without further purification. All deuterium containing reagents were purchased from Aldrich Chemical Company. THF and diethyl ether were dried by refluxing over lithium aluminum hydride and freshly distilled before use.

1,1,1',1'-$^{2}H_4$-*N*-Nitrosodiethanolamine 1a: A two-necked round-bottom flask, equipped with a condenser and a magnetic stirring bar, was charged with 100 g of deuterium oxide (D_2O). The flask was cooled to 0° C in an ice bath. Sodium (1.199 g, 52.13 mmol) sliced into small pieces was slowly added with caution. After sodium metal was completely dissolved, *N*-nitrosodiethanolamine (8.391 g, 62.55 mmol) was added. The reaction mixture was brought to reflux for five hours, after which time, the isotopic purity of the desired product was about 96 atom% deuterium. Solvent was removed under vacuum, and 50 g of fresh D_2O was added. The

mixture was brought to reflux for another five hours. After the mixture had been cooled to room temperature, the solvent was removed under vacuum. The residue was redissolved in 40 mL of methanol. The insoluble base was filtered off, and washed with methanol (30 mL x 3). The methanol solution was concentrated to 15 mL, and the concentrated solution was purified by flash column chromatography, eluting with methylene chloride/methanol (95:5 v/v) to give 1,1,1',1'-$^2H_4$-*N*-Nitrosodiethanolamine **1a** (7.103 g, 82% yield) with an isotopic purity of 99+ atom% deuterium: ^1H NMR (D_2O) δ 3.95 (s, 2 H), 3.72 (s, 2 H); ^{13}C NMR (acetone-d_6) δ 59.6, 57.8, 54.9 (quint., J=21.2 Hz), 46.5 (quint., J=21.4 Hz); IR (neat) 3364 (br), 2943, 2887, 2249 (weak), 2157 (weak), 2125 (weak), 1423, 1296, 1116, 1057, 919 cm^{-1}; EIMS *m/z* (relative intensity) 107 (3, $[M-CH_3O]^+$), 93 (21), 75 (44), 60 (35), 46 (100); HRFAB calcd for $C_4H_6D_4N_2O_3$+H 139.1021, found 139.1021.

2,2,2',2'-$^2H_4$-*N*-Nitrosodiethanolamine 1b: A well stirred suspension of lithium aluminum deuteride (10 g, 238 mmol) in THF (200 mL) was cooled to -78° C. Dimethyl iminodiethanoate (15.4 g, 95.6 mmol), mixed with 50 mL of THF, was added dropwise at -78° C under nitrogen atmosphere. The mixture was stirred at -78° C for 10 min, then gradually warmed to room temperature and stirred for another 2 h. The mixture was cooled to 0° C, and D_2O (20 mL) was added very slowly to quench the reaction. Another 50 mL of distilled water was slowly added at 0° C. This mixture was warmed to room temperature, and the precipitates were filtered off and washed with methanol (100 mL x 3). The solvent was evaporated from the filtrate and the residue was dissolved in 50 mL of glacial acetic acid. Sodium nitrite (20 g, 290 mmol) dissolved in 50 mL of water was added at 0° C. The mixture was stirred at 0° C for 30 min, then warmed to room temperature and stirred for another 2 h. Most of the solvent and acetic acid were removed under vacuum. The residue was then neutralized with a saturated aqueous solution of sodium bicarbonate. Water was removed under vacuum, and the residue was extracted with acetonitrile (100 mL). The insoluble inorganic salts were filtered off and washed with acetonitrile (50 mL x 3). The combined acetonitrile solution was concentrated to 20 mL. The residue was chromatographed, using methylene chloride/methanol (95:5, v/v) as an eluent to afford 2,2,2',2'-$^2H_4$-*N*-nitrosodiethanolamine **1b** (9.02 g, 68% overall yield) with an isotopic purity of at least 98 atom% deuterium: ^1H NMR (D_2O) δ 4.30 (s, 2H), 3.86 (s, 2 H); ^{13}C NMR (acetone-d_6) δ 58.9 (quint., J=21.7 Hz), 57.1 (quint., J=21.8 Hz), 55.3, 46.9; IR (neat) 3354 (br), 2987, 2944, 2780, 2221, 2121, 2101, 1451, 1426, 1370, 1311, 1212, 1133, 1089, 1018, 971, 839 cm^{-1}; EIMS *m/z* (relative intensity) 105 (7, $[M-CHD_2O]^+$), 94 (56), 74 (96), 58 (75), 47 (100);

HRFAB calcd for $C_4H_6D_4N_2O_3$+H 139.1021, found 139.1021.

Ethyl *N*-2,2-diethoxyethylaminoethanoate 3 (10): A round-bottom flask was charged with 2,2-diethoxyethylamine (40 g, 0.30 mol) and 100 mL of ethanol. Ethyl bromoacetate (16.7 g, 0.10 mol), mixed with 50 mL of ethanol, was added dropwise at room temperature. The mixture was stirred for 4 h. The solvent was evaporated and the residue was poured into a 200 mL mixture of diethyl ether and 40% aqueous solution of sodium hydroxide (1:1, v/v) with stirring at 0° C. The organic layer was separated from the aqueous layer, and subsequently extracted with diethyl ether (100 mL x 3). The combined ether solution was dried over anhydrous magnesium sulfate. After filtration and removal of the solvent, the residue was distilled under reduced pressure to give ethyl *N*-2,2-diethoxyethylaminoethanoate 3 (bp 88-89° C at 0.6 mm Hg, 17.58 g, 80% yield): ^{1}H NMR ($CDCl_3$) δ 4.59 (t, J=5.47 Hz, 1 H), 4.19 (q, J=7.14 Hz, 2 H), 3.74-3.67 (m, 2 H), 3.58-3.52 (m, 2 H), 3.44 (s, 2 H), 2.76 (d, J=5.47 Hz, 2 H), 1.72 [s, 1 H (NH)], 1.28 (t, J=7.14 Hz, 3 H), 1.22 (t, J=7.03 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 172.2, 102.1, 62.1, 60.6, 51.5, 50.8, 15.3, 14.1; IR (neat) 3338, 2977, 2931, 2904, 2877, 1741, 1465, 1446, 1372, 1345, 1197, 1129, 1062, 1028 cm^{-1}.

Ethyl *N*-2,2-diethoxyethyl-*N*-nitrosoaminoethanoate 4: A round-bottom flask was charged with ethyl *N*-2,2-diethoxyethylaminoethanoate (12.41 g 56.61 mmol). Isopropyl nitrite (25.31 g, 284.1 mmol) was added at room temperature. The mixture was stirred at room temperature until no starting material was left, as monitored by GC (total 16 h). The excess isopropyl nitrite and generated isopropyl alcohol were removed using a rotary evaporator. The residue was further evacuated by an oil pump to give ethyl *N*-2,2-diethoxyethyl-*N*-nitrosoaminoethanoate 4 (14.00 g, 100% yield): ^{1}H NMR ($CDCl_3$) *Z*-isomer (74%): δ 4.75 (t, J=5.20 Hz, 1 H), 4.36 (d, J=5.20 Hz, 2 H), 4.35 (s, 2 H), 4.16 (q, J=7.14 Hz, 2 H), 3.79-3.73 (m, 2 H), 3.59-3.53 (m, 2 H), 1.26 (t, J=7.14 Hz, 3 H), 1.21 (t, J=7.04 Hz, 6 H); *E*-isomer (26%): δ 5.04 (s, 2 H), 4.46 (t, J=5.27 Hz, 1 H), 4.25 (q, J=7.14 Hz, 2 H), 3.79 (d, J=5.27 Hz, 2 H), 3.71-3.65 (m, 2 H), 3.51-3.45 (m, 2 H), 1.31 (t, J=7.14 Hz, 3 H), 1.19 (t, J=7.02 Hz, 6 H) (see reference 16); ^{13}C NMR ($CDCl_3$) *Z*-isomer: δ 165.7, 101.6, 63.3, 61.4, 55.0, 46.9, 15.1, 13.9; *E*-isomer: δ 168.1, 98.9, 63.5, 61.7, 53.9, 47.0, 15.1, 14.0; IR (neat, mixture of *E* and *Z* isomers): 2979, 2935, 2900, 1749, 1483, 1373, 1341, 1204, 1129, 1112, 1065, 1032, 972 cm^{-1}; EIMS *m/z* (relative intensity) 203 (3, $[M\text{-}EtO]^+$), 186 (1.5), 173 (25), 144 (9), 116 (7), 103 (100), 75 (87); HRFAB calcd for $C_{10}H_{20}N_2O_5$+Li 255.1532, found 255.1543.

Ethyl *N*-2,2-diethoxyethyl-*N*-nitroso-1,1-$^2H_2$-aminoethanoate 5: A dry, round-bottom flask was charged with C_2H_5OD (50 mL) and sodium (0.063 g, 2.74 mmol) was added. After

the sodium metal was completely consumed, ethyl *N*-2,2-diethoxyethyl-*N*-nitrosoaminoethanoate **4** (14.0 g, 56.4 mmol) was added. The mixture was stirred at room temperature for 10 h, at which time the isotopic purity of the desired product was about 93 atom% deuterium. The solvent was removed under vacuum, and 50 mL of fresh C_2H_5OD was added. The mixture was stirred at room temperature for another 10 h. and the solvent was completely removed under vacuum. The residue was dissolved in anhydrous diethyl ether, and the insoluble catalytic base was filtered off under anhydrous conditions. Evaporation of solvent gave ethyl *N*-2,2-diethoxyethyl-*N*-nitroso-1,1-$^2H_2$-aminoethanoate **5** (14.1 g, 99% yield) with an isotopic purity of 98 atom% deuterium: ^1H NMR ($CDCl_3$) *Z*-isomer (74%): δ 4.75 (t, J=5.20 Hz, 1 H), 4.36 (d, J=5.20 Hz, 2 H), 4.16 (q, J=7.14 Hz, 2 H), 3.79-3.73 (m, 2 H), 3.59-3.53 (m, 2 H), 1.26 (t, J=7.14 Hz, 3 H), 1.21 (t, J=7.04 Hz, 6 H); *E*-isomer (26%): δ 4.46 (t, J=5.27 Hz, 1 H), 4.25 (q, J=7.14 Hz, 2 H), 3.79 (d, J=5.27 Hz, 2 H), 3.71-3.65 (m, 2 H), 3.51-3.45 (m, 2 H), 1.31 (t, J=7.14 Hz, 3 H), 1.19 (t, J=7.02 Hz, 6 H) (see reference 16); ^{13}C NMR ($CDCl_3$) *Z*-isomer: δ 165.7, 101.5, 63.2, 61.4, 54.9, 46.4 (m, weak, -CD_2-), 15.1, 13.9; *E*-isomer: δ 168.1, 98.9, 63.5, 61.7, 53.9 (m, weak, -CD_2-), 46.9, 15.1, 14.0; IR (neat, mixture of *E* and *Z* isomers) 2979, 2934, 2899, 2250 (weak), 2142 (weak), 1747, 1457, 1373, 1330, 1257, 1196, 1157, 1116, 1068, 897, 879 cm^{-1}; HRFAB calcd for $C_{10}H_{18}D_2N_2O_5$+Li 257.1658, found 257.1668.

5,5-$^2H_2$-*N*-Nitroso-2-hydroxymorpholine 2a (17): A round-bottom flask was charged with ethyl *N*-2,2-diethoxyethyl-*N*-nitroso-1,1-$^2H_2$-aminoethanoate **5** (14.1 g, 56.4 mmol) and THF (150 mL). Diisobutylaluminum hydride (1.0 M solution in THF, 180 mL, 180 mmol) was added dropwise at -78° C. The mixture was stirred at -78° C for 1 h, then gradually warmed to room temperature, and stirred at room temperature for 3 h. D_2O (20 mL) was added very slowly at 0° C to quench the reaction. After being stirred at 0° C for 30 min, the mixture was directly hydrolyzed by slowly adding hydrochloric acid (150 mL, 6 N) at 0° C, followed by stirring the mixture at 20° C for 20 h. The acid was neutralized first with sodium carbonate and then with a saturated aqueous solution of sodium bicarbonate. The insoluble inorganic salts were filtered off, the filtrate concentrated to 100 mL at 20° C under vacuum, and extracted with ethyl acetate (100 mL x 5). The combined organic extracts were dried over anhydrous magnesium sulfate. After filtration and evaporation of solvent, the residue was chromatographed, using hexane/ethyl acetate (40:60, v/v) as eluent to give 5,5-$^2H_2$-*N*-Nitroso-2-hydroxymorpholine **2a** (5.446 g, 72% yield) with an isotopic purity of about 95 atom% deuterium: ^1H NMR ($CDCl_3$) *Z*-isomer (55%): δ 5.23 (dd, J=5.0, 2.9 Hz, 1 H), 4.51 [s, 1 H (OH)], 4.43 (dd, J=13.2, 2.9 Hz, 1 H), 4.14 (dd,

J=13.2, 5.0 Hz, 1 H), 4.03 (d, J=12.0 Hz, 1 H), 3.59 (d, J=12.0 Hz, 1 H); *E*-isomer (45%): δ 5.01 (dd, J=4.7, 3.0 Hz, 1 H), 4.35 [s, 1 H (OH)], 4.27 (d, J=11.8 Hz, 1 H), 3.94 (dd, J=13.7, 3.0 Hz, 1 H), 3.80 (dd, J=13.7, 4.7 Hz, 1 H), 3.79 (d, J=11.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) *Z*-isomer: δ 91.3, 59.3, 53.7, 39.4 (m, -CD_2-); *E*-isomer: δ 90.5, 60.8, 48.4 (m, -CD_2-), 44.6; IR (neat, mixture of *Z* and *E* isomers) 3376 (br), 2938, 2886, 2250 (weak), 2140 (weak), 2122 (weak), 1425, 1337, 1278, 1243, 1179, 1114, 1096, 1059, 1035, 1012, 908, 890 cm^{-1}; EIMS *m/z* (relative intensity) 134 (2.2, $M\cdot^+$), 104 (22), 74 (100), 58 (70); HRMS calcd for $C_4H_6D_2N_2O_3$ 134.0660, found 134.0661.

Ethyl *N*-2-hydroxyethyl-*N*-nitrosoaminoethanoate 8: A round-bottom flask was charged with ethanolamine (18.32 g, 0.30 mol) and ethanol (150 mL). Ethyl bromoacetate (16.70 g, 0.10 mol), diluted with 50 mL of ethanol, was added dropwise at 0° C. The mixture was stirred at 0° C for 1 hour, then gradually warmed to room temperature, and stirred at room temperature for 2 h. Glacial acetic acid (85 mL) was added at 0° C. Sodium nitrite ($NaNO_2$, 51.75 g, 0.75 mol), dissolved in 100 mL of water, was added dropwise at 0° C. The mixture was stirred at 0° C for 2 h, then warmed to room temperature, and stirred at room temperature for 10 h. The solvent and most of the acetic acid was removed under vacuum at 20° C. The residue was poured into 200 mL of ethyl acetate and neutralized with a saturated aqueous solution of sodium bicarbonate. The aqueous layer was extracted with ethyl acetate (150 mL x 4). The combined organic extracts were dried over anhydrous magnesium sulfate. After filtration and removal of the solvent, the residue was separated by flash column chromatography, eluting with hexane/ethyl acetate (40:60, v/v) to afford ethyl *N*-2-hydroxyethyl-*N*-nitrosoaminoethanoate 8 (9.724 g, 55% overall yield): ^1H NMR ($CDCl_3$) *E*-isomer (16%): δ 5.05 (s, 2 H), 4.26 (q, J=7.1 Hz, 2 H), 3.80 (t, J=5.1 Hz, 2 H), 3.68 (q, J=5.1 Hz, 2 H), 3.28 [t, J=5.1 Hz, 1 H (hydrogen-bonded OH)], 1.31 ((t, J=7.1 Hz, 3 H); *Z*-isomer (84%): δ 4.39 (t, J=5.0 Hz, 2 H), 4.29 (s, 2 H), 4.20 (q, J=7.2 Hz, 2 H), 3.94 (q, J=5.0 Hz, 2 H), 3.56 [t, J=5.0 Hz, 1 H (hydrogen-bonded OH)], 1.29 (t, J=7.2 Hz, 3 H) (see reference 16); ^{13}C NMR ($CDCl_3$) *E*-isomer: δ 168.6, 61.8, 58.7, 54.1, 47.5, 13.8; *Z*-isomer δ 166.8, 61.9, 59.7, 55.5, 46.9, 13.7; IR (neat, mixture of *E* and *Z* isomers) 3432 (br), 2985, 2945, 2885, 1742, 1452, 1375, 1336, 1219, 1152, 1048, 978 cm^{-1}; EIMS *m/z* (relative intensity) 145 (27, [M-CH_3O]), 133 (36), 116 (32), 103 (25), 86 (32), 72 (100), 56 (32); HRFAB calcd for $C_6H_{12}N_2O_4$+H 177.0875, found 177.0880.

2-^{2}H-*N*-Nitroso-2-hydroxymorpholine 2b (17): Lithium aluminum deuteride (0.748 g, 17.81 mmol) (18) was dissolved in 60 mL of THF. Ethyl *N*-2-hydroxyethyl-*N*-nitrosoaminoethanoate 8 (2.752 g, 15.62 mmol) was added via a syringe at -78° C. The mixture was stirred at -

78° C for 1 h, then gradually warmed to -40° C, and stirred at this temperature for 3 hours. D_2O (2.5 mL) was added very slowly at -40° C to quench the reaction. The mixture was gradually warmed to 0° C. Another 20 mL of water was added at 0° C. The precipitates were filtered off and washed with water (20 mL x 2) and ethyl acetate (50 mL). The aqueous layer was extracted with ethyl acetate (50 mL x 6). The combined organic layers were dried over anhydrous magnesium sulfate. After filtration and evaporation of solvent, the residue was chromatographed, eluting with hexane/ethyl acetate (40:60, v/v) to give ^{2}H-*N*-nitroso-2-hydroxymorpholine **2b** (1.342 g, 65% yield) with an isotopic purity of at least 98 atom% deuterium: ^{1}H NMR ($CDCl_3$) *E*-isomer (55%): δ 5.09 [s, 1 H (OH)], 4.41 (d, J=13.2 Hz, 1 H), 4.13 (d, J=13.2 Hz, 1 H), 4.03 (ddd, J=11.3, 7.2, 4.0 Hz, 1 H), 3.87 (ddd, J=9.9, 5.9, 4.0 Hz, 1 H), 3.83-3.77 (m, 1 H), 3.59 (ddd, J=10.1, 5.9, 4.2 Hz, 1 H); *Z*-isomer (45%): δ 4.94 [s, 1 H (OH)], 4.32-4.23 (m, 3 H), 3.92 (d, J=13.7 Hz, 1 H), 3.83-3.77 (m, 1 H), 3.80 (d, J=13.7 Hz, 1 H); ^{13}C NMR *E*-isomer: δ 90.8 (t, J=25.6 Hz), 59.3, 53.6, 39.8; ^{13}C NMR *Z*-isomer: δ 90.1 (t, J=25.6 Hz), 60.8, 48.9, 44.5; IR (neat, mixture of *E* and *Z* isomers) 3381 (br), 2992, 2939, 2884, 2180(weak), 2106 (weak), 1427, 1367, 1286, 1182, 1092, 1062, 971, 912 cm^{-1}; EIMS *m/z* (relative intensity) 133 (1.5, $M\cdot^+$), 103 (54), 74 (53), 56 (100); HRMS calcd for $C_4H_7D_1N_2O_3$ 133.0598, found 133.0596.

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References and Notes

1. (a) Druckrey, H.; Preussmann, R.; Ivankovic, S.; Schmahl, D. *Z. Krebsforsch*, 69, 103 (1967). (b) Lijinsky, W.; Reuber, M. D.; Manning, W. B. *Nature (London)*, 288, 589 (1980). (c) Preussmann, R.; Habs, M.; Habs, H.; Schmahl, D. *Cancer Res.* 42, 5167 (1982). (d) Lijinsky, W.; Kovatch, R. M. *Carcinogenesis*, 6, 1679 (1985).

2. (a) Fan, T. Y.; Morrison, J.; Rounbehler, D. P.; Ross, R.; Fine, D. H.; Miles, W.; Sen, N. P. *Science*, 196, 70 (1977). (b) National Research Council, *The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds*, Part 1, Chap. 7 (1981). National Academy Press, Washington, DC.

3. (a) Airoldi, L.; Bonfanti, M.; Fanelli, A.; Bove, B.; Benfenati, E.; Gariboldi, P. *Chem. Biol. Interactions*, 51, 103 (1984). (b) Hecht, S. S. *Carcinogenesis*, 5, 1745 (1984). (c) Eisenbrand, G.; Denkel, E.; Pool, B. *J. Cancer Res. Clin. Oncol.* 108, 76 (1984).

4. (a) Loeppky, R. N.; Tomasik, W.; Kerrick, B. E. *Carcinogenesis*, 8, 941 (1987). (b) Loeppky, R. N., Lee, M. P. and Mueller S., IARC Scientific Publication 125, 429 (1994).

5. Hecht, S. S.; Lijinsky, W.; Kovatch, R. M.; Chung, F.-L.; Saavedra, J. E. *Carcinogenesis*, 10, 1475 (1989).

6. (a) Keefer, L. K.; Fodor, C. H. *J. Am. Chem. Soc.* 92, 5747 (1970). (b) Kupper, R.; Michejda, C. J. *J. Org. Chem.* 45, 2919 (1980).

7. The isotopic purity of the product is proportional to the refluxing time for the initial five hours, as monitored by ^{1}H NMR. After five hours of refluxing, the isotopic purity shows no significant increase with increased reaction time.

8. The isotopic purities of deuterium reported in this paper were all estimated by ^{1}H NMR, GC/MS, and/or high resolution MS.

9. (a) Ellis-Davies, G. C. R.; Kaplan, J. H. *J. Org. Chem.* 53, 1966 (1988). (b) *CA*, 98, 178752h (1983).

10. (a) Lowe, G.; Sproat, B. S. *J. Biol. Chem.* 255, 3944 (1980). (b) Valls, N.; Segarra, V. M.; Bosch, J. *Heterocycles*, 24, 943 (1986).

11. Levin, N.; Hartung, W. H. *Org. Syntheses*, Horning, E. C., Ed., *Coll. Vol. III,* 191 (1955).

12. Kashima, C.; Harada, K. *J. Chem. Soc. Perkin Trans. I*, 1521 (1988).

13. The isotopic purity of the product depends upon the isotopic purity of the reducing reagents used. Commercial lithium aluminum deuteride has isotopic purity as high as 98 atom% deuterium, and is recommended for the reaction.

14. (a) Eizember, R. F.; Vogler, K. R.; Souter, R. W.; Cannon, W. N.; Wege, P. M., II. *J. Org. Chem.* 44, 784 (1979). (b) Seebach, D.; Wykypiel, W. *Synthesis* 423 (1979).

15. Magee, P. N.; Montesano, R.; Preussmann, R. "*Chemical Carcinogens*", Searle, C. E., Ed.; American Chemical Society: Washington, D. C., p 491-625 (1976).

16. The assignments of *E* and *Z* isomers were based on their differences in chemical shifts. The protons *cis* to the N-N=O group appear at higher fields. (a) Looney, C. E.; Phillips, W. D.; Reilly, E. L. *J. Am. Chem. Soc.* 79, 6136 (1957). (b) Karabatsos, G. J.;Taller, R. A. *J Am. Chem. Soc.* 86, 4373 (1964). (c) D'Agostino, J. T.; Jaffe, H. H. *J. Org. Chem.* 36, 992, (1971).

17. For the corresponding non-isotopically labeled compound, see references 3b and 4.

18. When diisobutylaluminum deuteride was used as the reducing reagent, an excess amount (2.5 folds) was used.