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The Synthesis of Highly Reactive, Multi-Functional α,β-Epoxy- and α-Acetoxy-Nitrosamines

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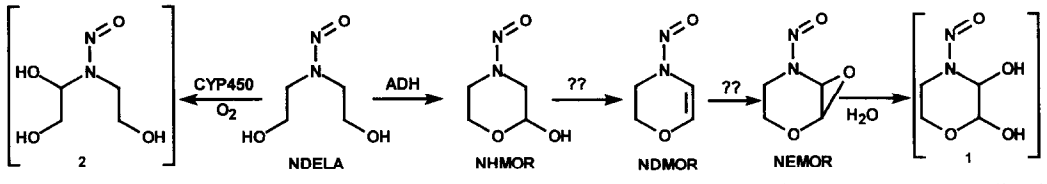
Abstract: The synthesis of the reactive acetates, *trans*-3-acetoxy-2-hydroxy-*N*-nitrosomorpholine **3** and *N*-(1-acetoxy-2-hydroxyethyl)-*N*-nitrosoethanolamine **12,** of two α-hydroxynitrosamines has been accomplished through the ring opening of the corresponding epoxides, NEMOR and **10** which were prepared by dimethyldioxirane oxidation of the vinyl nitrosamines.

The mode of metabolic activation of the potent animal carcinogen *N*-nitrosodiethanolamine (NDELA), which is a trace contaminant of many commercial formulations based on ethanolamines, has been the subject of considerable conjecture and numerous investigations.[1] Most nitrosamines undergo activation through enzymatic hydroxylation at the carbon adjacent to the amine nitrogen[2], but evidence for this α-hydroxylation pathway for NDELA has been lacking.[1c] Recently we have obtained evidence that the carcinogenic activation of NDELA and related ethanolnitrosamines may involve enzymatic oxidation at both the *N*-bound (α) carbon and the OH containing carbon (β), or competitively only α-hydroxylation. In order to test these hypotheses and adequately probe the chemistry and DNA adducts produced from these putative reactive metabolites, we have undertaken the synthesis of reactive α,β-epoxy- and α-acetoxynitrosamines[3] related to NDELA and one of its two known metabolites, *N*-nitroso-2-hydroxymorpholine, NHMOR. Both α,β-epoxy- and α-acetoxynitrosamines are expected to be hydrolyzed to unstable α-hydroxynitrosamines, progenitors of reactive diazonium ions and aldehydes, which can also react with DNA.[3,4] Possible activation schemes for NDELA[1] are shown in Scheme 1. We present here the synthesis of the acetates of the α-hydroxynitrosamines **1** and **2,** related to NHMOR and NDELA respectively, which are required for testing of these hypotheses. To our knowledge, this represents the first synthesis of reactive α-acetoxynitrosamines carrying other nucleophilic functionality.

Scheme 1



The synthesis of 3-acetoxy-2-hydroxy-*N*-nitrosomorpholine proceeded from NDELA as outlined in Scheme 2. Our preparation of NHMOR has been improved significantly over our previous method which involved time consuming and lower yield coupling of 2-aminoethanol and 1,1-dimethoxy-2-chloroethane.[1d] NHMOR was made in 90% yield, after purification, by employing the Dess-Martin reagent[5] to oxidize

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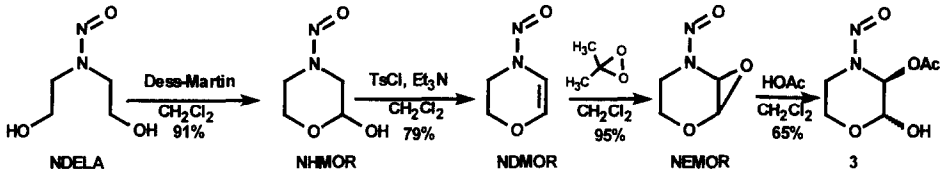
NDELA. The reaction can be extended to the preparation of other α-nitrosaminoaldehydes in good yields.[6] Dehydration of NHMOR was performed through tosylation and *in situ* base-catalyzed elimination.[7]

Scheme 2



The epoxidation of NDMOR was first examined with commonly used epoxidation reagents *m*-chloroperbenzoic acid (*m*CPBA) and *t*-butyl hydroperoxide. In both cases the reaction did not stop at the epoxide stage and the major products separated were *m*-chlorobenzoate ester **4** and *t*-butyl ether **5**, respectively.[6] It became obvious that the epoxide of NDMOR is very reactive and can be easily destroyed by even a weak nucleophile. We attempted the preparation of **3** by the direct reaction of NDMOR and commercially available peracetic acid without success. Only a complex mixture of products with no trace of the desired compound was obtained under a variety conditions.

Dimethyldioxirane (DMD) has been shown to be very effective in the production of reactive epoxides.[8] This reagent was used by Guengerich and coworkers[4] to generate methylnitrosaminooxirane **6**, a highly reactive compound, in a low state of purity in about 20% yield. We have modified and optimized their procedure, and by utilizing absolutely anhydrous conditions produced **6**, NEMOR and several other α,β-epoxynitrosamines in nearly quantitative yields.[9] Removal of the acetone, after the oxidation, and replacement with dry, pure CH_2Cl_2 gave essentially pure solutions of **6** and other simple α,β-epoxy nitrosamines which could be stored below 0 °C without decomposition. NEMOR is relatively stable in dry CH_2Cl_2 at 0 °C for several days. Reaction of NEMOR with 1.1 equivalents of glacial acetic acid easily affords 3-acetoxy-2-hydroxy-*N*-nitrosomorpholine **3**, which was purified by silica gel chromatography (CH_2Cl_2 : Ethyl Acetate 9:1, 65% yield). NMR assignments for NEMOR, **3** and **4** are given in the Fig. 1. Structural assignment for **3** was greatly facilitated by the fact that the related m-chlorobenzoate **4** was obtained in crystalline form and its structure elucidated by means of X-ray diffraction. The ring of **4** exists in a nearly perfect chair-form with the HO and OAc groups occupying *trans*-diaxial orientation. H-2 and H-3 are not coupled but H-2 is coupled to the OH enabling its assignment. COSY

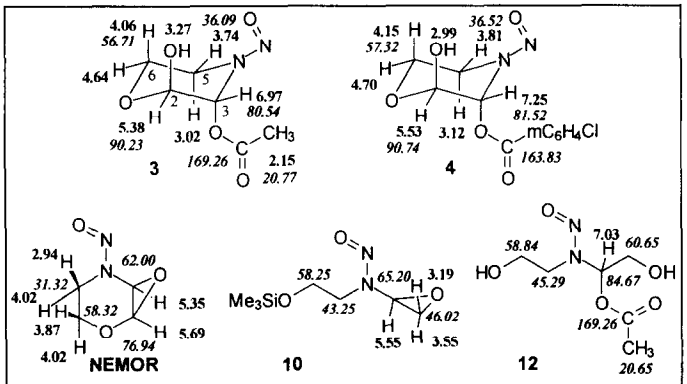
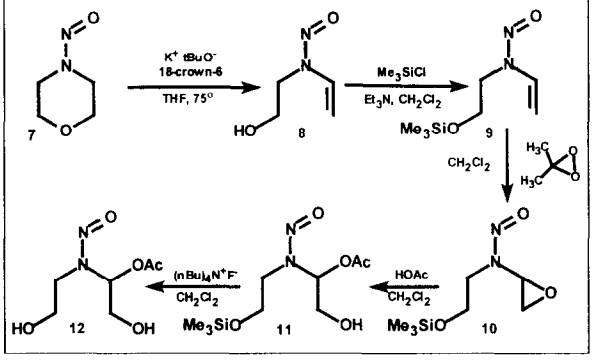


Figure 1. ^1H (bold) and ^{13}C (italics) NMR chemical shifts for α,β-epoxy and α-acetoxynitrosamines.

and coupling constant magnitudes permit assignments of axial and equatorial geminal H,[10] however, the assignment which geminal pair is on which carbon is uncertain, but based on similar compounds.[7]

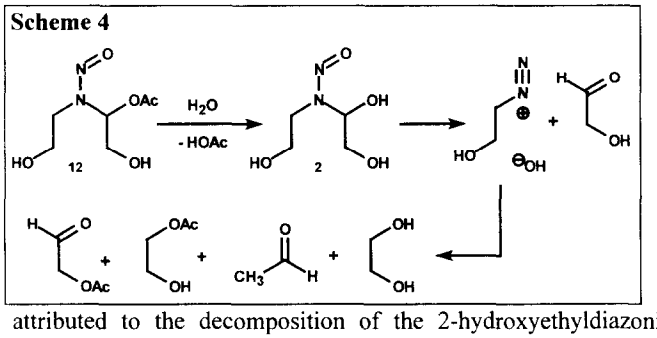
N-1-Acetoxy-2-hydroxyethyl-*N*-nitrosoethanolamine **12**, was synthesized as shown in Scheme 3. 2-Hydroxyethylvinylnitrosamine **8** is most efficiently prepared (85%) by the K^+ $tBuO^-$-18-crown-6 catalyzed ring opening of *N*-nitrosomorpholine in refluxing THF.[11] The conversion of **8**, to its trimethylsilyl derivative **9** is required to facilitate the epoxidation of the double bond. The epoxidation of **9** was carried out using DMD by a procedure analogous to that utilized for the preparation of NEMOR. The epoxide **10** was obtained in 95% yield (according to the NMR spectrum). ^1H and ^{13}C spectral data for **10** are given in Fig. 1 and are completely consistent with the assigned structure as is its MS which shows a molecular ion at 204. While the signals corresponding to the epoxide fragment can clearly be identified in the ^1H spectrum,[10] the shifts of the coupled CH_2's are too close to facilitate assignment.

Scheme 3



Reaction of **10** with 1 equiv. of glacial acetic acid in CH_2Cl_2 (30 min, rt) produced the masked acetate **11** in 65% yield. Except for the characterization of **11**, **10** could be converted to **12** through the two steps shown in Scheme 3 in a single flask (2 equiv. HOAc), but prior to this finding the deprotection of **11** required optimization. Removal of the silyl group from **11** is accomplished smoothly by stirring **11** with 1 equiv. of $(n\text{-}Bu)_4N^+ F^-$ (TBNF) in CH_2Cl_2 for 10-12 hours (rt), followed by a short silica gel column separation (CH_2Cl_2 : ethyl acetate 3:1). An excess amount of TBNF resulted in the complete decomposition of the product. The α-acetoxynitrosamine **12** is much more reactive than **3** and does not survive either preparative TLC or long column purification. In spite of its unusually high reactivity, good NMR spectral data could be obtained for α-acetoxy-N-nitrosodiethanolamine **12** and these are given in Fig. 1. As was the case for **10**, the CH_2 protons are not well resolved, even at 500 MHz. In spite of this difficulty, the ^{13}C data and the resolved proton shifts are in complete accord with the assigned structure.

Scheme 4



As is mentioned, **12** hydrolyzes ($t_{1/2}$ 8.5 min, 25 °C, pH 7) much more rapidly than does **3**. While the reasons for this unanticipated reactivity are being addressed separately, we have identified the hydrolysis products of **12** as ethylene glycol, 2-hydroxyethyl acetate, acetaldehyde, glycolaldehyde, its acetate, and acetic acid. The majority of these products can be attributed to the decomposition of the 2-hydroxyethyldiazonium ion produced from the short-lived α-hydroxynitrosamine which arises from the hydrolysis of the acetate as shown in Scheme 4. Approximately 50% of the diazonium ion rearranges to acetaldehyde by a hydride migration concerted with the loss of N_2. From a toxicological perspective this rearrangement is detoxifying because it limits the yield of effective electrophilic species which can bind to DNA.

Of the two epoxides, NEMOR is the more reactive. Hydrolysis of NEMOR and **3** give similar products, glyoxal, ethylene glycol, and acetaldehyde, as anticipated from the formation of the 2-hydroxyethyldiazonium ion from **1**. Preliminary experiments have shown that both the epoxides and the α-acetoxynitrosamines can be used effectively in nucleotide and DNA alkylation studies. The successful syntheses of both the α,β-epoxy- and the α-acetoxynitrosamines, show that modern synthetic methodology can be used to prepare these reactive model compounds, bearing other nucleophilic functionality, which are important in elucidating the carcinogenic activation mechanisms of important carcinogens. The hydrolysis products indicate that these compounds are good models for the putative in vivo metabolites of NDELA and related nitrosamines.

Warning: Nitrosamines are potent chemical carcinogens, and α,β-epoxy- and α-acetoxynitrosamines are potentially extremely hazardous "contact carcinogens" due to their direct alkylating capacity and should be handled with extreme caution. Safety protocols are available from the corresponding author.

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9. Epoxidation: A flame-dried 50 mL flask, equipped with a rubber septum and gas inlet and outlet needles, was charged with 50 mg of NDMOR in 5mL of CH_2Cl_2. DMD,[8] distilled with acetone, dried over $MgSO_4$, filtered under N_2, and dried over activated 4 Å molecular sieves for 3 h, (approximately 0.03 M, total 70 mL) was added intermittently to the solution through a syringe with stirring at room temperature in the dark. After completion of the oxidation, the solvents were removed by a stream of dry N_2 (3-4 h).
10. All NMR spectra measured in $CDCl_3$. Coupling constants (Hz): NEMOR, $J_{2\text{-}3}$ 2.3, others m; **3**, (*E* NNO isomer) $J_{2\text{-}3}$ 0, $J_{5e\text{-}5a}$ 12.04, $J_{5e\text{-}6a}$ 3.89, $J_{5a\text{-}6a}$ 11.97, $J_{5a\text{-}6e}$ 3.58, $J_{6e\text{-}6a}$ 11.77; **10**, (epoxide; others m) J_{ab} 3.6, J_{ax} 3.39, J_{bx} 1.86.
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