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Nitroxyl (HNO) release from new functionalized *N*-hydroxyurea-derived acyl nitroso-9,10-dimethylanthracene cycloadducts

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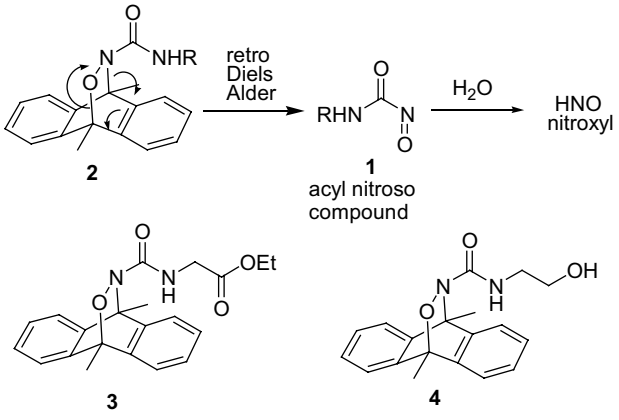
Abstract—A thermal retro-Diels–Alder decomposition of *N*-hydroxyurea-derived acyl nitroso compounds and 9,10-dimethylanthracene cycloadducts followed by acyl nitroso compound hydrolysis produces nitrous oxide, evidence for the formation of nitroxyl, the one-electron reduced form of nitric oxide that has drawn considerable attention for its potential roles in biological systems. EPR and NMR spectroscopy provide further evidence for nitroxyl formation and kinetic information, respectively. Such compounds may prove to be useful alternative nitroxyl donors.

An increasing amount of evidence highlights the biological importance of nitroxyl (HNO), the one-electron reduced form of nitric oxide (NO).[1] While nitroxyl-releasing compounds mimic the action of NO donors under some conditions, recent studies reveal distinct biological activities for HNO and NO.[2] For example, HNO and NO show differences in their ability to promote oxidative DNA damage.[3] Another group of studies highlight the discrete effects mediated by nitroxyl and nitric oxide in both normal and failing heart models.[4,5] Specifically, nitroxyl stimulates calcitonin gene-related peptide release while nitric oxide increases cyclic guanylate monophosphate (cGMP) and these results suggest the potential of nitroxyl donors for the treatment of heart failure.[4–6] These studies have prompted both the theoretical and experimental examination of the thermodynamic properties and chemical reactivity of HNO.[7–14]

Angeli's salt (sodium trioxodinitrate, $Na_2N_2O_3$) is currently the most widely used HNO donor. However, with the described emerging picture, new nitroxyl donors will be of increasing importance as both biochemical and pharmacological tools and potential therapeutic agents. Hydrolysis of acyl nitroso species (**1**, Scheme 1) produces HNO and represents a reliable chemical approach for HNO formation.[15] Earlier work shows that cycloadducts (**2**, Scheme 1) of the acyl nitroso species derived from *N*-hydroxyureas and 9,10-dimethylanthracene (9,10-DMA) undergo a retro-Diels–Alder reaction to form acyl nitroso species that hydrolyze to HNO, carbon dioxide, and the corresponding amine.[16] While the cycloadducts of *N*-hydroxyureas derived from simple alkyl and aryl amines release HNO under mild conditions at useful rates, these compounds demonstrate poor water solubility.[16] We wish to report the synthesis and



Scheme 1.

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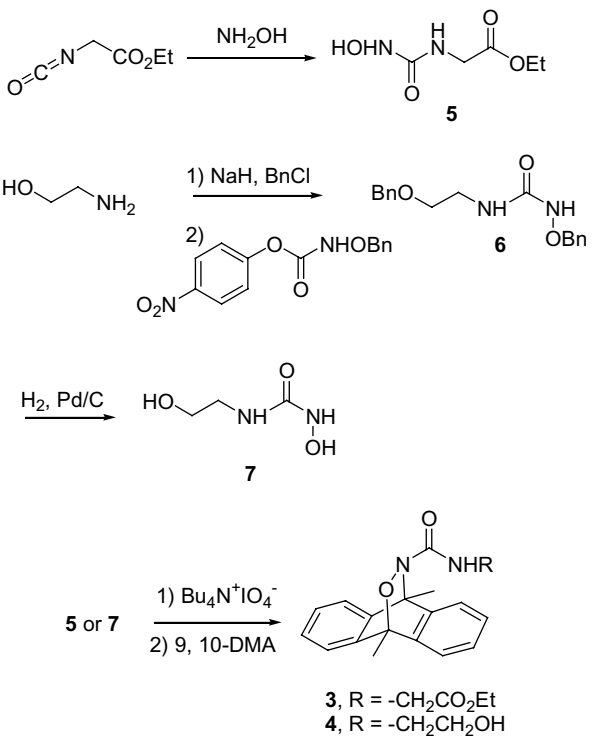


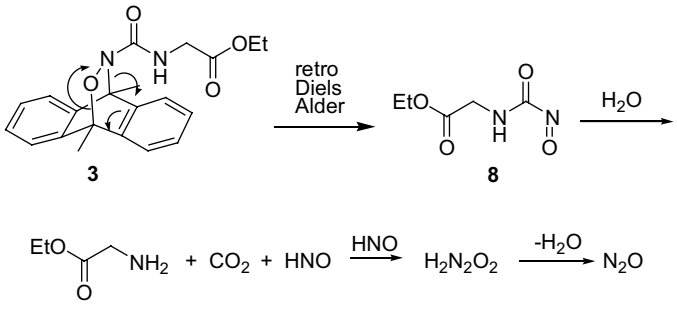
Scheme 2.

evaluation of 9,10-DMA cycloadducts of the acyl nitroso species derived from the *N*-hydroxyureas of ethyl glycine (**3**) and ethanolamine (**4**) that demonstrate improved water solubility and HNO release properties.

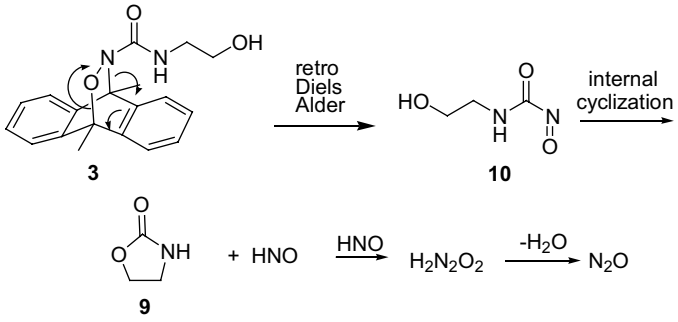
The synthesis of cycloadducts **3** and **4** requires the properly substituted *N*-hydroxyurea derivatives. Addition of hydroxylamine to ethyl isocyanatoacetate yields the glycine derived *N*-hydroxyurea in 91% yield (**5**, mp 96–98 °C, Scheme 2). Protection of the hydroxyl group of ethanolamine as the benzyl ether[17] followed by condensation with 4-nitrophenyl-*N*-hydroxy-*O*-benzylcarbamate gives the fully protected ethanolamine derived *N*-hydroxyurea (**6**) and hydrogenation gives the ethanolamine derived *N*-hydroxyurea (**7**, 66% yield for three steps, Scheme 2). Tetra-*n*-butyl ammonium periodate oxidation of the *N*-hydroxyureas (**5** and **7**) in the presence of 9,10-DMA yields the cycloadducts **3** and **4** (14% and 44% yield after chromatography, respectively, Scheme 2). These compounds were characterized by both proton and carbon NMR spectroscopy, mass spectrometry, and elemental analysis.[18] While **3** does not show appreciable water solubility, the ester group provides a point for further derivation for improving the solubility (conversion to the salt or the attachment of hydrophilic groups). Cycloadduct **4** readily dissolves in water to yield a 1 mM solution.

Thermal decomposition of cycloadduct **3** in a 1:1 mixture of water–acetonitrile at 40 °C under argon yields a mixture of nitrous oxide (42%) and carbon dioxide after 3 h as determined by gas chromatography of the reaction headspace (Scheme 3).[16] Nitrous oxide and carbon dioxide likely form through the initial retro-Diels–Alder dissociation of **3** to 9,10-DMA and the acyl nitroso species (**8**, Scheme 3). Hydrolysis of **8** produces HNO and a carbamic acid that further decomposes to carbon dioxide and presumably ethyl glycine. The dimerization and dehydration of HNO produces nitrous oxide and provides strong evidence for the intermediacy of HNO in this process (Scheme 3).[19] Monitoring the disappearance of the unique methyl singlets of **3** over time at 37 °C by nuclear magnetic resonance (NMR) spectroscopy in the presence of 1,3-cyclohexadiene, to trap the reactive acyl nitroso species as previously demonstrated,[16] shows that **3** decomposes in a first order manner ($k = 1.33 \times 10^{-2}\,\mathrm{min}^{-1}$, $t_{1/2} = 53$ min). Compound **3** decomposes at a rate similar to other cycloadducts of 9,10-DMA and the acyl nitroso species derived from alkyl substituted *N*-hydroxyureas.[16]

Thermal decomposition of cycloadduct **4** in distilled water at 40 °C under argon similarly produces nitrous oxide (86%) but no carbon dioxide after 3 h (Scheme 4). Examination of the reaction by NMR spectroscopy also shows the formation of 2-oxazolidone (**9**, Scheme 4). The intramolecular cyclization of the acyl nitroso species (**10**, Scheme 4), formed by the retro-Diels–Alder decomposition of **4**, explains the formation of both nitrous oxide (through nitroxyl as above) and 2-oxazolidone and the absence of carbon dioxide (Scheme 4). Monitoring the disappearance of the methyl singlets of **4** over time at 37 °C in water by NMR spectroscopy reveals that **4** also decomposes in a first order manner ($k = 2.84 \times 10^{-2}\,\mathrm{min}^{-1}$, $t_{1/2} = 24$ min). As the cycloadducts exist in equilibrium with the acyl nitroso compounds and 9,10-DMA, the immediate trapping of the acyl nitroso compound (**10**) by an internal nucleophilic



Scheme 3.



Scheme 4.

hydroxyl group may accelerate the observed rate of decomposition of **4**. Solvent effects could also play a role in the different observed decomposition rates of compounds **3** and **4**. Compound **4** decomposes at a rate similar to other cycloadducts of 9,10-DMA and the acyl nitroso species derived from aryl substituted *N*-hydroxyureas.[16]

Electron paramagnetic resonance (EPR) spectroscopic experiments provide further evidence for HNO release from cycloadducts **4**. Anaerobic incubation of **4** (1 mM) with methemoglobin (1 mM) in phosphate buffer produces iron nitrosyl hemoglobin over 2 h (Fig. 1) as indicated by the increasing characteristic absorbance of ferrous nitrosyl complexes near $g = 2$. These results provide a second independent line of evidence for HNO production from **4** as HNO reductively nitrosylates a number of ferric heme proteins, including methemoglobin, to yield the ferrous–NO complexes.[20] The resonance near $g = 6$ indicates the presence of un-reacted methemoglobin suggesting incomplete HNO trapping by methemoglobin under these conditions. While these studies clearly indicate HNO formation from the decomposition of **3** and **4**, the carcinogenic and mutagenic properties of 9,10-DMA, the reaction by-product, likely limit the use of these compounds to in vitro studies of HNO chemistry and biology.[21] However, this work clearly indicates that a retro-Diels–Alder reaction of properly constructed cycloadducts represent a reliable method of HNO generation.

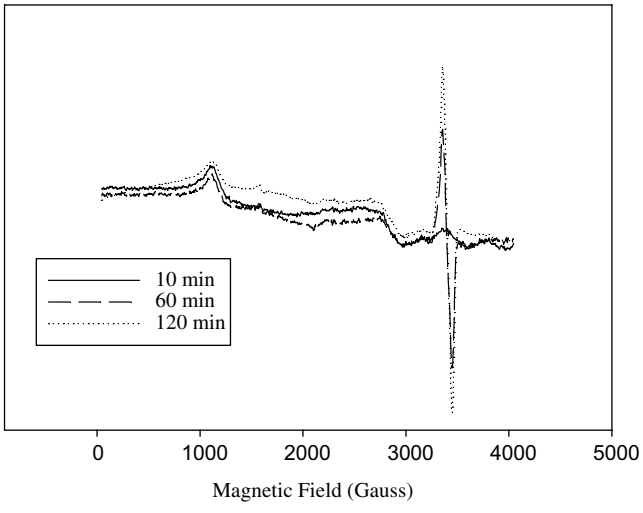


Figure 1. EPR spectra at 130 K of the anaerobic incubation of **4** with methemoglobin over time. Solid line (10 min), dashed line (60 min), and dotted line (120 min).

In summary, cycloadducts **3** and **4** generate HNO through a retro-Diels–Alder reaction followed by hydrolysis of the resulting acyl nitroso species. Functionalization of the hydroxyurea portion of these compounds improves their water solubility and provides a point of further derivatization while not affecting the rate of nitroxyl release at 37 °C as compared to similar compounds. Such compounds provide alternative HNO donors to Angeli's salt that may be useful in distinguishing the biological effects of HNO from those of NO in some systems.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.08.062.

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18. For **3**: mp 92–94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (m, 4H), 7.29 (m, 4H), 4.89 (s, 1H), 4.07 (q, J = 7.1 Hz, 2H), 3.33 (s, 2H), 2.59 (s, 3H), 2.29 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 170.0, 163.3, 141.6, 140.5, 127.1, 127.0, 121.3, 120.4, 79.4, 64.4, 60.7, 41.4, 16.3, 14.1, 13.1; ESI-MS: m/z = 389 $(M^+ + Na)^+$; Anal. Calcd for $C_{22}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.74; H, 6.07; N, 7.65. For **4**: mp 171–174 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.42 (m, 4H), 7.25 (m, 4H), 3.00 (t, J = 5.9 Hz, 2H), 2.82 (t, J = 5.9 Hz, 2H), 2.41 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) 163.2, 141.1, 139.9, 128.7, 128.6, 123.0, 122.0, 78.9, 64.2, 60.0, 41.2, 16.0, 13.8; ESI-MS: m/z = 347 $(M^+ + Na)^+$; Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.42; H, 6.19; N, 8.64.
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