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Photochemically-Triggered Decarboxylation/Deamination of *o*-Nitrodimethoxyphenylglycine

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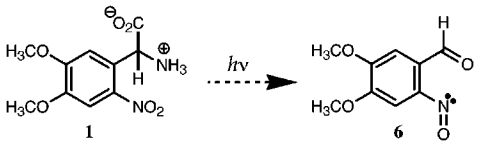
ABSTRACT



***o*-Nitrodimethoxyphenylglycine (1) decomposes on photolysis at 366 nm to release ammonia and carbon dioxide, via a five-step mechanism (Scheme 1). We have obtained spectroscopic and kinetic data from experiment and calculation which support our mechanism. We have also confirmed that a carbonic anhydrase inhibitor bearing 1 as a photolabile cage releases a tight-binding inhibitor on photolysis at 366 nm.**

Photolabile molecular cages have been proven to be widely applicable in biochemical systems and in organic synthesis. Photolabile synthetic amino acids have been used to activate a protein on photolysis[1] and to regulate protein function, thus aiding in the identification of sites of covalent modification at cageable side chains[2] and critical sites in cellular signal transduction.[3] Incorporation of synthetic amino acids such that the photolabile bond is in the backbone of the protein has allowed site-specific proteolysis, toward the goal of regulation of ion channel proteins.[4] Photolabile protecting groups have also been used in solid-phase oligonucleotide, organic, and peptide synthesis.[5−7]

The requirement of photolysis for activation allows the introduction of ligands of known concentration and directed localization.[8] Photolabile cages may therefore be of particular interest in the design of site-specific drugs, which are activated by light upon delivery. A hydrophobic inhibitor of carbonic anhydrase II[9] (CA) has been modified with *o*-nitrodimethoxyphenylglycine (*o*-NDMPG, **1**), a polar caging group.[10] Nitrophenyl derivatives such as **1** have found utility in many biochemical systems.[1−8] The benzylic carboxylate group of our cage increases its water solubility at neutral pH. Delivering the inhibitor as a prodrug is particularly useful because, in addition to allowing control of activation, the polar caging group increases the water solubility of the drug[10] without compromising activity of the inhibitor.[9] Methoxy substituents have been shown to increase the rate of photolysis of nitrophenyl derivatives,[5,6] thus their inclusion in our cage. While many uses of such photolabile cages have been identified, the intermediates and products of photodeprotection have not been fully characterized.

Our proposed mechanism for photodeprotection (Scheme 1), based on the work of England et al.,[4] Holmes,[6] and McCray and Trentham,[8] begins with the generation of *aci*-nitro intermediate **2** (Scheme 1) via n → π^* excitation of **1** and intramolecular hydrogen atom transfer from the α carbon of the amino acid. Intermediate **2** cyclizes, affording **3**. The subsequent collapse of **3**, in the presence of a proton source,

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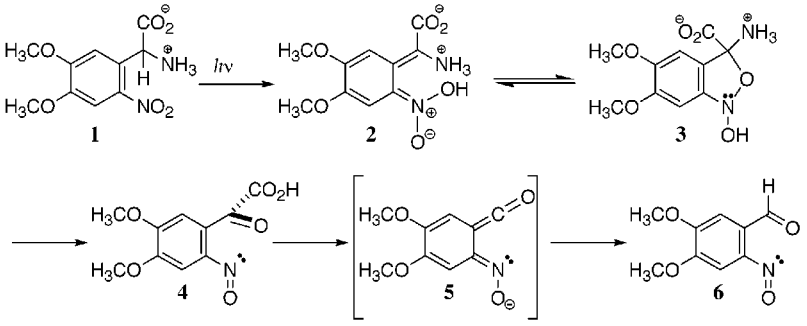
Scheme 1. Proposed Mechanism of Photodecomposition of *o*-Nitrodimethoxyphenylglycine



results in the elimination of ammonia, cleaving the "photolabile" bond and forming α-ketoacid **4.** Decarboxylation of **4** gives transient ketene **5**, with the *o*-nitroso substituent serving as an electron sink. Although **5** should be a high-energy structure, minimization of the conformation of **4** using molecular mechanics (MOPAC) in CAChe[11] showed that the carbon−carbon bond to the carboxylic acid is properly aligned for decarboxylation (Scheme 1). In the last step, the *o*-nitroso substituent is restored and a proton is abstracted from the solvent to form *o*-nitrosobenzaldehyde derivative **6**.[12]

MOPAC/ZINDO calculations[11] afforded calculated electronic spectra for **1**−**6**. On the basis of the differences evident in these spectra, we expected to be able to follow the progress of our mechanism at specific wavelengths. By photolyzing **1** in both protic and aprotic solvents and by varying pH, we were able to gather further evidence in support of the structures along the decomposition pathway.

Photolysis of **1** in $CHCl_3$[13] implicated intermediate **2** as the predominant photoproduct in this aprotic medium (Scheme 1). Successive irradiation at 366 nm over 5 min yielded the spectra shown in Figure 1. The disappearance of **1** and appearance of **2** is evident from the increase in absorption at 400 nm. We believe that *aci*-nitro intermediate **2** is in equilibrium with cyclic intermediate **3**.

The ammonium group of **3** is a good leaving group, but the neutral *N*-hydroxyl does not supply adequate driving force for the decomposition to **4**. Furthermore, **3** may not exist as a zwitterion in $CHCl_3$. Neutral **3** lacks the necessary leaving group, disfavoring further decomposition, so for these two reasons we do not expect to obtain **6** in $CHCl_3$. We determined that the rate of appearance of **2**, based on the change in absorbance at 400 nm with time, was 0.0073 ± 0.0010 s^{-1}.[14]

Photolysis of **1** at 366 nm in acidic CH_3OH gave 4,5-dimethoxy-2-nitrosobenzaldehyde (**6**, Scheme 1).[12] The spectra obtained on photolysis as a function of time indicate the disappearance of absorbances corresponding to **1** at 300 and 345 nm. The *N*-hydroxyl group of **3** is partially deprotonated, and the ammonia leaving group is protonated, facilitating formation of **4**. The increase in absorbance at 322 nm reflects formation of **6** at a rate of 0.010 ± 0.001 s^{-1}.

Photolysis of **1** at 366 nm in 20 mM KH_2PO_4 in the presence of dilute HCl also resulted in direct product formation. At both pH 1 and pH 2, absorbances for **1** at 300 and 345 nm decrease in intensity upon irradiation, and a peak characteristic of product appears near 320 nm (Figure 2). The rate of product formation at pH 2, as measured by the change in absorbance at 319.5 nm, was 0.00099 ± 0.00016 s^{-1}. The rate of product formation at pH 1, followed at 322 nm, was 0.0033 ± 0.0007 s^{-1}.

The relative rates of photodecomposition of **1** in protic solvents support our mechanistic scheme. The faster rate at

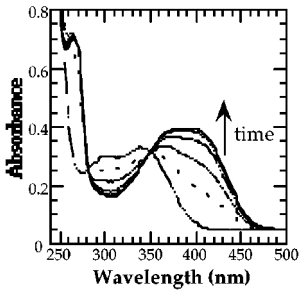


Figure 1. Absorbance spectra of **1** in $CHCl_3$, obtained after 0, 60, 120, 180, 240, and 300 s of photolysis.

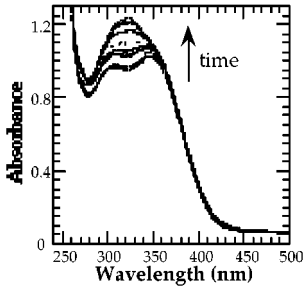
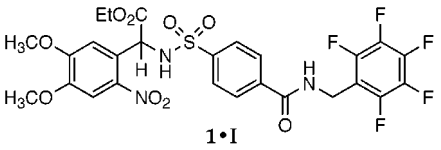


Figure 2. Absorbance spectra of **1** in 20mM KH_2PO_4 (pH 1), obtained after 0, 30, 60, 90, 150, 210, 270, 360, 420, 540, and 660 s of photolysis.

low pH is consistent with our hypothesis that the amino group of **3** must be protonated to facilitate formation of **4**. In water, the rate was slower than in CH_3OH, which is consistent with the fact that ionic intermediates **2** and **3** are stabilized by solvation.

Caged inhibitor **1·I**[10] was photolyzed in the manner described above, to release free **I**. We confirmed that **I** binds to CA via a competitive fluorescence-based assay.[15] To a



1•I

solution of CA (40 nM) saturated with dansylamide (DNSA, 20 μM) in 20 mM KH_2PO_4 (pH 8) was added **1·I** (200 nM). This solution was examined by fluorescence spectrophotometry, via measurement of emission from CA·DNSA on excitation of CA at 280 nm. As the solution was photolyzed, **I** was released from the caged form resulting in a time-dependent decrease in fluorescence (measured using a SPEX Fluorolog-3 spectrofluorimeter; Figure 3), as free **I** displaced DNSA from the active site of CA. These data were analyzed to afford a rate of deprotection of 0.0028 ± 0.0007 s^{-1}.[16]

In conclusion, we have obtained spectroscopic and kinetic evidence that support the mechanism shown in Scheme 1 for the photolysis of *o*-NDMPG. The rate and products of photolysis are solvent and pH-dependent. We have also confirmed that a CA inhibitor bearing **1** as a photolabile cage (**1·I**) releases the active form of the inhibitor (**I**) on photolysis. We are now in the process of developing an alternate delivery mechanism for our caged CA inhibitor, involving attachment of **1·I** to a gold surface via a photolabile tether.

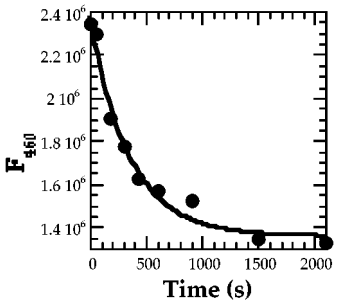


Figure 3. Deprotection of **1·I** monitored by the decrease in fluorescence observed on displacement of dansylamide from the active site of carbonic anhydrase by **I**.

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Supporting Information Available: Experimental procedure for the synthesis of **1** and spectra and kinetic data not shown in Figures 1−3. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Version 4.0.2, Oxford Molecular Group, 1998. Uncertainties in the calculated electronic transitions are 10−20%.

(12) ^{1}H NMR (Bruker DRX-400, C*D*Cl_3) δ 10.5 (s, 1H), 7.63 (s, 1H), 7.44 (s, 1H), 4.05 (s, 3H), 4.04 (s, 3H).

(13) Typical conditions involved photolysis of 67 μM *o*-NDMPG in solvent with a handheld UV lamp at 366 nm, directly in a cuvette. The reaction was followed with a JASCO V-550 UV/vis spectrophotometer, at room temperature.

(14) The rate of disappearance of *o*-NDMPG in $CHCl_3$ was also determined at 302 nm to be 0.0066 ± 0.0013 s^{-1}.

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(16) We have also followed deprotection of **1·I** by UV/vis spectrophotometry at 395 nm and have obtained a rate of 0.0046 ± 0.0003 s^{-1} via that approach.