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Caged *trans*-4-Hydroxy-2-nonenal

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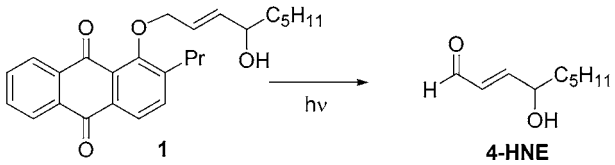
ABSTRACT



A caged 4-hydroxy-2-nonenal (4-HNE) has been prepared and its photochemistry investigated. Upon photolysis, 1 releases 4-HNE in up to 100% yield. From these photolyses, 4-HNE could be isolated in up to 91% yield. 4-HNE is produced under either aerobic or anaerobic conditions. The caging strategy does not require prior preparation of 4-HNE and, therefore, represents a three-step synthetic route to the bioactive enal in 48% overall yield.

The use of photolabile protecting groups to "cage" bioactive molecules that can then be released photochemically is of great interest in the chemical and biological communities.[1] An advantage of this technology is the temporal and spatial control it allows in the distribution of bioactivity. Caged molecules have been used extensively in the investigation of biochemical pathways and mechanisms, such as calcium uptake or the behavior of neurotransmitters.[2]

Similar chemistry has potential uses in photodynamic therapy (PDT). Traditional PDT relies on the generation of singlet oxygen via a photosensitization involving porphyrins.[3] Due to dependence on oxygen, this technique has limited use in hypoxic tissues or in cases where the therapy itself generates a hypoxic condition.[4] A photoactive agent that acts independently of oxygen would be an important advance in the development of PDT strategies.

A number of α,β-unsaturated aldehydes, such as *trans*-4-hydroxy-2-nonenal (4-HNE), display biological activity with significance to human health.[5] The biochemistry of such molecules is under intensive investigation.[6] Given the interest in biologically active aldehydes and the need to develop new oxygen independent PDT strategies, development of a photoactivatable 4-HNE was pursued; preliminary results are reported below.

Blankespoor has investigated the photochemistry of 1-alkoxyanthraquinones.[7] Hydrogen abstraction, followed by single electron transfer (SET), produces a zwitterion, **3**, that is rapidly trapped by solvent (Scheme 1). Upon oxidation, a

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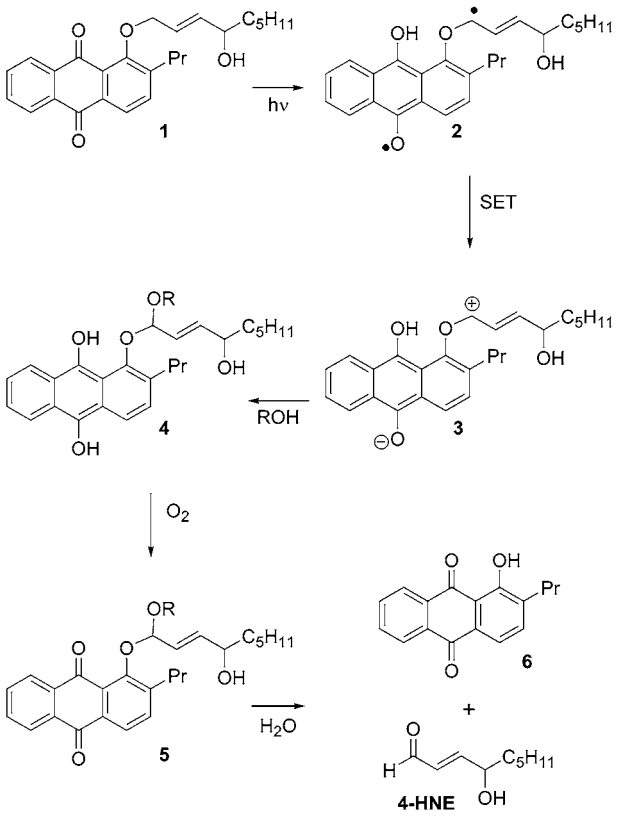
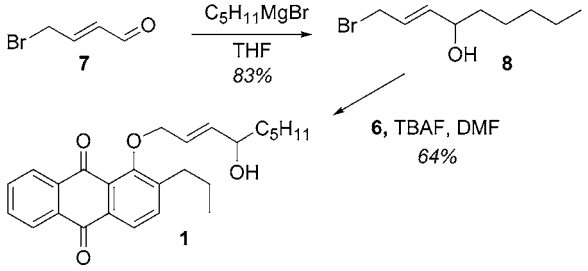
Scheme 1. Proposed Mechanism for Release of Caged 4-HNE



Scheme 2. Synthesis of Caged 4-HNE



1-hydroxyanthraquinone, **6**, and the aldehyde corresponding to the alkyl group are produced in high yields. The use of this photochemistry to produce α,β-unsaturated aldehydes has not been reported. A possible strategy toward a photoreleasable 4-HNE was to modify this photochemistry as shown in Scheme 1. Thus, **1** would serve as a potential caged 4-HNE. Initial experiments showed that α,β-unsaturated aldehydes, including 4-HNE, can be photoreleased using this photochemistry. However, the incorporation of an allyloxy group in the 1-position significantly alters the reaction pathway(s) under some conditions.

The synthesis of **1** was carried out by alkylation of **6**[7b] with 1-bromo-2-nonen-4-ol (**8**). This bromide was prepared by addition of pentylmagnesium bromide to 4-bromocrotonaldehyde (**7**).[8] Each of these reactions proceeded smoothly in high yield (Scheme 2).

Initially, the investigation of the photochemistry of **1** under aerobic conditions was carried out before the experimentally more complex anaerobic experiments. When irradiated at $\lambda > 350$ nm[9] in methanol in the presence of oxygen, **1** converted to **6** and 4-HNE. The aerobic photochemistry of **1** was examined under a variety of conditions (Table 1). For example, when a 10 mM solution of **1** in 4:1 CH_3OH/H_2O was irradiated, the starting anthraquinone was consumed in 2 h (entry 11). However, acetal product **9**[10] was formed under these conditions. Treatment of the crude reaction mixture with aqueous acetic acid afforded 4-HNE in near-quantitative yield (entries 9 and 11). The yield of the photoreactions was measured by integrating ^{1}H NMR signals relative to an internal standard.

Generally, yields of 4-HNE increased with increasing concentrations of water and with the addition of acetic acid. Under aerobic conditions with acetic acid present, high yields of 4-HNE were produced directly from the photolysis (entries 14−21).

The formation of the diacetal **10a** was surprising and indicated that the inclusion of an α,β-unit of unsaturation to the 1-alkoxy group opened up alternative photochemical reaction pathways in addition to those reported by Blankespoor.[7] Based on these observations and previous reports, it seemed likely that **10a** would become the predominant product under anaerobic conditions, where oxidation to the 1-hydroxyanthraquinone is slow.[7a] Indeed, **10a** was isolated in moderate yield (36%) when **1** was irradiated in degassed methanol, but 4-HNE and **6** were both isolated in 39% yield. **10a** was prone to slow hydrolysis on a silica column, in humid air, or in dilute aqueous acid and proved impossible to purify completely. These observations are important because they indicate that the presence of oxygen was not necessary for release of 4-HNE.

The photochemistry at 419 nm was expected to mirror that using the medium-pressure Hg arc lamp ($\lambda > 350$ nm).

However, irradiation of **1** at 419 nm afforded 4-HNE and **9** in a combined yield of only 47% (Table 1, entry 10). A preparative anaerobic reaction afforded **10a** in 42% yield; 4-HNE and **6** were isolated in 30% and 32% yield, respectively.

This observation suggested that **10a** might be photochemically converted to 4-HNE when irradiated at shorter wavelengths; this was confirmed by the finding that irradiation of **10a** in methanol using 366 nm light rapidly produces 4-HNE and **6** (in the dark, **10a** was much slower to decompose to 4-HNE and **6**). This is possibly the result of a photosolvolysis similar to that reported by Wan in the 9-fluorenol series (Scheme 3).[11]

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(9) Irradiations were carried out using one of two methods: for $\lambda > 350$ nm, reactions were carried out in Pyrex vessels using a medium-pressure Hg lamp and a uranium oxide doped glass filter. For $\lambda = 419$ nm, a Rayonet reactor equipped with 16 419 nm lamps was used.

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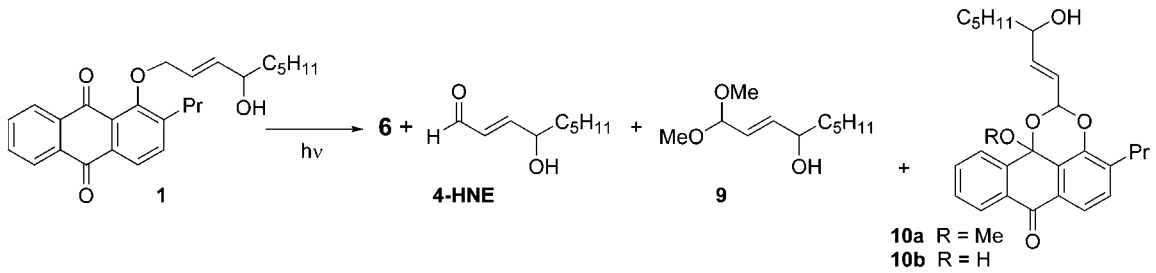
Table 1. Photorelease of 4-Hydroxynonenal from **1**



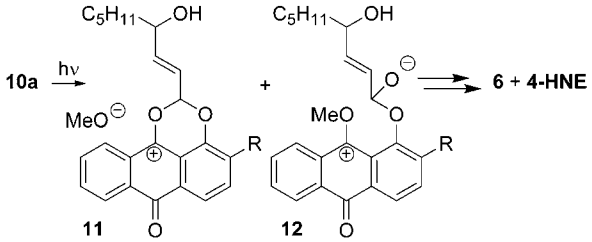
entry	solvent	method[a]	**1** (mM)	4-HNE[b]	**9**[b]	**10a**[b]	4-HNE after hydrolysis[b,c]
1	CD_3OD	A	10	15	42	tr	50
2	CD_3OD	B	4	30	0	*f*	95
3	CD_3OD	B	10	29	0	*f*	82
4	CD_3OD	B	25	26	0	*f*	47
5	CD_3OD/D_2O (15:1)	A	4	50	40	0	77
6	CD_3OD/D_2O (15:1)	A	10	35	26	0	64
7	CD_3OD/D_2O (15:1)	A	25	26	18	14	49
8	CD_3OD/D_2O (9:1)	A	1				(91)[e]
9	CD_3OD/D_2O (8:1)	A	10	67	*d*	0	91
10	CD_3OD/D_2O (8:1)	B	10	35	12	*f*	100
11	CD_3OD/D_2O (4:1)	A	10	75	15	0	100
12	CD_3CN/D_2O (4:1)	B	10	29	0	*f*	100
13	CD_3CN/D_2O (2:1)	B	10	23	0	*f*	100
14	$CD_3OD/D_2O/CD_3CO_2D$ (8:1:0.5)	A	10	59	*d*	20	71
15	$CD_3CN/D_2O/CD_3CO_2D$ (8:1:0.5)	B	10	61	0	*f*	99
16	$CD_3OD/D_2O/CD_3CO_2D$ (8:1:0.5)	A	4	58	*d*	tr	100
17	$CD_3OD/D_2O/CD_3CO_2D$ (8:1:0.5)	A	25	36	26	24	59
18	$CD_3OD/D_2O/CD_3CO_2D$ (3:2:1)	A	10	100	0	0	
19	$CD_3CN/D_2O/CD_3CO_2D$ (1:1:1)	B	10	100	0	0	
20	CD_3CO_2D/D_2O (8:1)	A	10	86	0	0	
21	CD_3CO_2D/D_2O (8:1)	B	10	100	0	0	

[a] Method A: O_2 saturated, irradiation with a 450 W medium-pressure Hg lamp and UO_2 glass filter, 2 h. Method B: O_2 saturated, irradiation with 16 419 nm lamps in a Rayonet reactor, 2.5 h. [b] Yields determined by ^1H NMR and integration relative to an internal standard (see the Supporting Information).[c] In some cases, the crude photolysis mixture was stirred with aqueous acetic acid prior to analysis. [d] Solvent peak interferes with quantitative analysis but **9** was formed. [e] Isolated yield. [f] A mixture of uncharacterized acetal products. See text. tr = trace.

Figure 1 shows the UV spectra of **1** and **10a**. **1** had a λ_{max} at 351 nm with the absorption band tailing into the visible region. **10a** had a λ_{max} at 288 nm and a shoulder at about 335 nm. Because **10a** did not absorb as well at 419 nm as at 366 nm, the compound accumulated when the photolysis was carried out at the longer wavelength. Prolonged irradiation of **10a** at 419 nm (>24 h) resulted in conversion to 4-HNE.

With the idea that hemiacetal **10b** would be less stable than **10a** and would hydrolyze readily to give 4-HNE and **6**, we assessed the reaction in an anaerobic acetonitrile/water solvent system. However, 4-HNE was initially formed in low to average yield (λ > 419 nm). Proton spectra of the reaction mixture had numerous new NMR signals in the vinyl region

Scheme 3. Proposed Photosolvolysis of **10a**



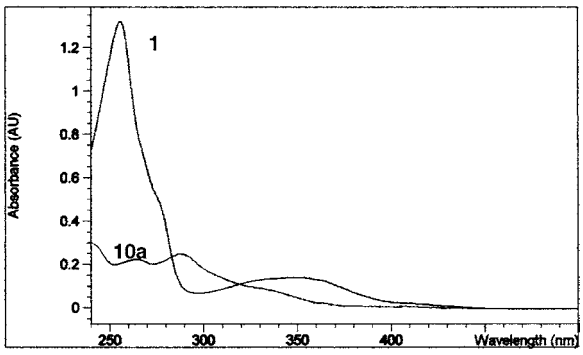


Figure 1. UV−vis spectra of **1** (ϵ_{366} = 3953) and **10a** (ϵ_{366} = 585). Each compound was dissolved in MeCN (30 μM).

similar to those seen for **10a** (entries 12, 13, and 15). We believe these NMR signals are due to **10b**, but this compound could not be isolated.[12] Treatment of a crude reaction mixture containing this product (along with 4-HNE and **6**) with aqueous acetic acid, or irradiation of the crude mixture ($\lambda > 350$ nm) produced 4-HNE in quantitative yield. Thus, the putative hemiacetal **10b** behaved exactly as did **10a** when irradiated or treated with acid.

Preparative-scale reactions were then run in an attempt to isolate the possible hemiacetal product **10b** and confirm its structure. However, this product decomposed readily and could not be isolated.

The photolysis of **1** also proved to be a preparatively useful method for obtaining pure 4-HNE in good yield. When **1** was irradiated in 9:1 methanol/water, 4-HNE was isolated in 91% yield. This synthesis required three steps from known materials to produce 4-HNE in 48% overall yield. Other advantages of this reaction are the stability of the immediate precursor—**1** is indefinitely stable in air at ambient temperature—and ease of purification after the final step.

(12) A reviewer noted that it is possible that the diradical **2** could be trapped by oxygen to give a peroxyacetal that would hydrolyze to the same products as would the structure we attribute to **10b**. However, the yield of **10b** is highest under anaerobic conditions and is only a minor component of the reaction mixture in the presence of oxygen. Thus, while we cannot rule out the possibility of trapping by oxygen, the evidence available does not support the idea.

A caged *trans*-4-hydroxy-2-nonenal (4-HNE) capable of releasing the bioactive aldehyde under either aerobic or anaerobic conditions using visible light has been prepared. Photolysis of **1** in nucleophilic solvents gave 4-HNE in excellent yield. In addition to the first example of a photoreleasable 4-HNE, this chemistry provided a short synthesis of the lipid oxidation product. Also, several new photochemical pathways have been identified in the photolysis of 1-allyloxy-9,10-anthraquinones. We continue to investigate the photochemistry of 1-alkoxy-9,10-anthraquinones bearing α,β-unsaturation on the alkyl group, the development of water-soluble derivatives of **1**, and the utility of these caged bioactive aldehydes.

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Supporting Information Available: Synthetic procedures, purification protocols, melting points, spectroscopic data, and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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