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A Three-Component Photoreversible Tag for Thiols

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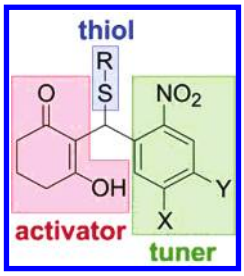
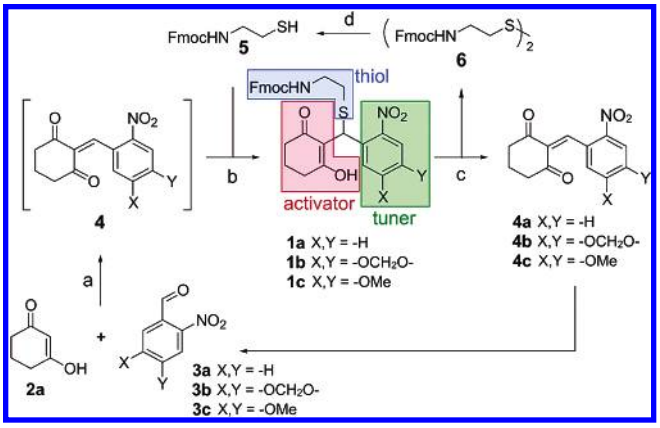


A one-pot coupling of a 1,3-diketone, an aldehyde, and an alkanethiol has been developed to produce a protected sulfide. Through use of an *o*-nitrophenylbenzaldehyde, this method provides a one-step route to a photochemically reversible thiol-protecting group. The kinetics of photolysis were established using ^{1}H NMR analysis, which allows for the rate to be based on the entire reaction scheme.

The modification of functional groups with photocleavable moieties is a versatile tool for organic synthesis,[1] the development of small molecule libraries,[2] and the decoding of proteomic and genomic queries.[3] Modifications by appropriate photocleavable moieties allows for orthogonal protection in complex synthetic pathways and *in situ* deprotection where exposure to a photon flux is allowed. While the development of photocleavable protecting groups for alcohols and amides has been established for solution, bead, and surface arrays,[3b,4] only a few methods exist for thiols. Established methods focus on coupling of a thiol to an alkyl halide or an α,*β*-unsaturated diketone.[5,6] While effective, these reactions are often complicated by the synthetic complexity of forming appropriate thiol-reactive labels.

SCHEME 1. Synthesis and Processing of *o*-Nitrobenzyl Derivatives (1) as a Photoremovable Thiol Modification[a]



[a] Key: (a) CH_2Cl_2, dried SiO_2; (b) addition of **5**, CH_2Cl_2; (c) $CDCl_3$, $\lambda = 365$ nm; (d) Zn, TFA, CH_2Cl_2/MeOH or 1,4-dithiothreitol.

To achieve a photocleavable Michael acceptor, we propose the in situ generation of a 2-ene-1,3-dione via a Knoevenagel condensation.[7] Surprisingly, this approach has not yet been advanced as a general tool for thiol modifications. Using this methodology, we developed a one-pot, three-component method to generate a photocleavable-protected thiol (Scheme 1). Thioether **1** represents the general components of this system: a "tuner", an "activator", and a target thiol. The tuner is an *o*-nitrobenzaldehyde, the cleavage wavelength of which has been demonstrated to be attenuated by ether functionalities at positions 4 and 5.[8] The activator is a reactive nucleophilic 1,3-diketone that forms an enedione upon Knoevenagel condensation with the tuner aldehyde. To investigate the utility of this *o*-nitrophenyl protecting group, a simple alkylthiol, ethanethiol, was protected by combining 5,5-dimethylcyclohexane-1,3-dione (**2b**) and 6-nitroveratraldehyde (**3c**) to yield the protected alkylthiol **7** in 63% yield. As shown in Scheme 1, diketone activator **2** and aldehyde tuner **3** couple via Knoevenagel condensation to form intermediate **4** *in situ*. Enone **4** then serves as a Michael acceptor for thiol **5**. The protected thiol is deprotected by light irradiation, yielding disulfide **6** and the photolysis byproduct **4**. Enone **4** is further hydrolyzed to *o*-nitrobenzaldehyde **3** according to ^{1}H-NMR.

Traditionally, the extent of photolytic cleavage is quantified by UV−vis spectroscopy,[9] time-resolved FTIR

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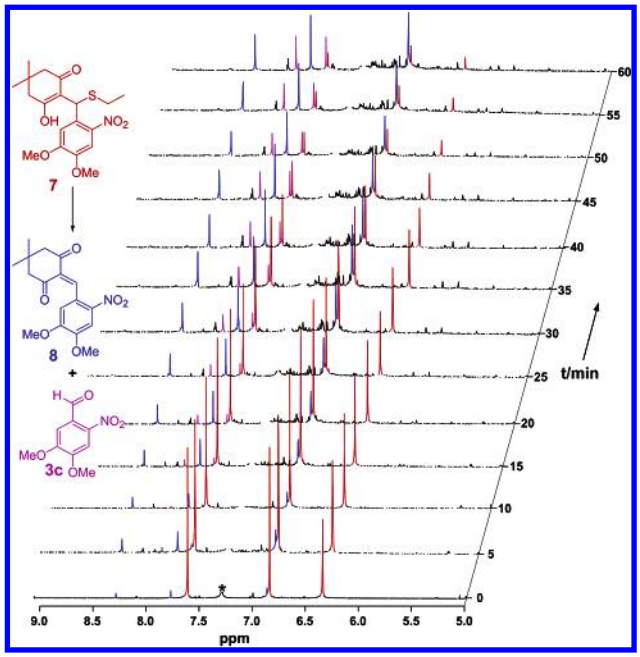

FIGURE 1. ^{1}H NMR analysis of photolytic cleavage of **7** (red) with periodic irradiation at λ = 365 nm in $CDCl_3$ to **8** (blue) and **9** (magenta). The $CDCl_3$ peak has been removed (*) to simplify the spectra.

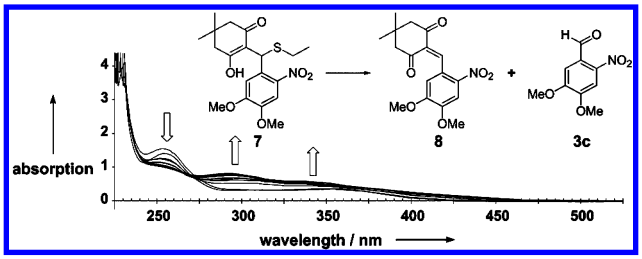

FIGURE 2. UV−vis spectral monitor of the photolysis of **7** at λ = 365 nm in $CDCl_3$.

spectroscopy,[9a,10] or HPLC.[11] This thiol protecting group, however, provides a convenient handle to monitor photolytic cleavage by ^{1}H NMR. Figure 1 displays an ^{1}H NMR time course of the photolytic deprotection of ethanethiol at 365 nm over 60 min. The change in hybridization of the β-carbon of protected ethanethiol **7** to photolytic product **8**, from $sp^3 \rightarrow sp^2$, provided a means to monitor the photolytic reaction. Upon photolytic cleavage, the proton signal at δ 6.33 ppm (**7**) disappears and a new signal at δ 8.28 ppm appears. In addition to the formation of disulfide and enone **8**, a third product 6-nitroveratraldehyde **3c** arises from the hydrolysis of enone **8**. Both photolytic byproducts had ^{1}H NMR and ^{13}C NMR identical to authentic samples. Photolytic deprotection could also be followed by UV−vis. Figure 2 depicts the photolysis of protected thiol **7** between 220 and 600 nm over 60 min. Photolytic cleavage provides the formation of photolytic byproduct **8** whose absorption increases at 295 nm and 340 nm. The absence of a clear isosbestic point at 275 nm indicates the formation of multiple products (**8** and **3c**), which is in agreement with the ^{1}HNMR.

While the rates of *o*-nitrophenyl photocleavage reactions are often linked to decomposition of *aci*-nitro intermediates,[12] photolytic deprotection has been shown to be orders of magnitude slower than the breakdown of the *aci*-nitro group.[9a] By comparison of the disappearance of starting material and the formation of the product with ^{1}H NMR, we were able to monitor photolytic kinetics of the complete reaction. This obviated the need to determine whether the rate-determining step consists of the decomposition of *aci*-nitro group or the decay of secondary intermediates.

To demonstrate the utility of this methodology, an 11-membered set of protected thiols was prepared from Fmoc-cystamine, four diketone activators (**2a**−**d**), and three nitrobenzadehydes (**3a**−**c**) (Figure 3). The yields ranged from 41% to 96%. Initially, the derivatives containing 1,3-indandione (**11a** and **11b**) were isolated and purified. However, upon standing the 1,3-indandione Knoevenagel products proved to be poor Michael acceptors. As a result, the product readily undergoes β-elimination of the thiol, reverting to the enone intermediate.

Each of the purified compounds of the library was subjected to photolysis at 365 nm. The percent of alkanethiol cleaved ranged from 55% to 86%.[14] The rate of each deprotection was monitored by ^{1}H NMR. The rates of photocleavage are on the same order as current literature.[9a] As expected, the 6-veratraldehyde derivatives cleaved twice as fast as the 6-nitro and 6-nitropiperonal derivatives. This increased rate of thiol cleavage and amount of thiol cleaved, as compared to the nitro and piperonal derivatives, may be attributed to the 3,4-dimethoxy substituents on the nitro benzaldehyde in **1c**, **9c**, and **10c**.[8]

We also sought to demonstrate the utility of this protecting group by assessing its stability under conditions used in organic synthesis.[1c] The protected thiol **7** was found to be stable under basic conditions (NaH, Pyr, TEA, piperidine, and NaOMe); Lewis acid (TsOH); and strong acid (TFA). The protecting group was stable to heat (<100 °C). The photolytic cleavage may also be performed in the presence of other protecting groups such as *N*-(9-fluorenylmethoxycarbonyl) (Fmoc).

We have demonstrated rapid entry into a three-component protection strategy that provides flexibility for additional functionality or specific photochemical reactivity. The three component thiol protecting group is also stable to many conditions commonly used in organic synthesis. This methodology should find useful applications in organic synthesis, the development of small molecule libraries,and the decoding proteomic and genomic queries.

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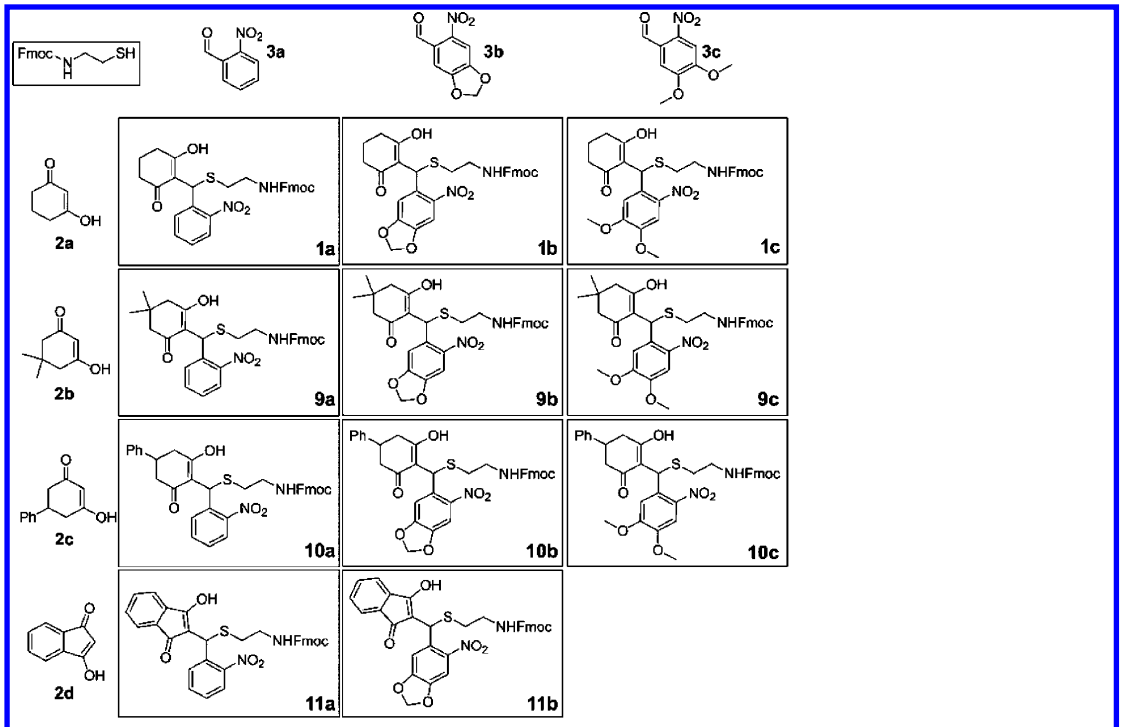
(14) The percent thiol cleaved was calculated based monitoring the disappearance of the benzylic proton at δ 6.33 ppm and standardizing it to doublet of the Fmoc protecting group.



Entry	Protected Thiol	Yield of Protected Thiol[a]	Thiol Cleaved[b]	1st Order Kinetics of Thiol Cleavage (s^{-1})	Φ^c
1	1a	84%	62%	$3.04\times10^{-4} \pm 6.99\times10^{-5}$	2.42×10^{-13}
2	1b	66%	59%	$2.97\times10^{-4} \pm 1.4\times10^{-5}$	2.36×10^{-13}
3	1c	57%	55%	$4.26\times10^{-4} \pm 2.22\times10^{-5}$	3.39×10^{-13}
4	9a	94%	61%	$5.25\times10^{-4} \pm 1.1\times10^{-5}$	4.18×10^{-13}
5	9b	41%	66%	$3.19\times10^{-4} \pm 1.79\times10^{-5}$	2.54×10^{-13}
6	9c	68%	82%	$5.50\times10^{-4} \pm 4.07\times10^{-5}$	4.37×10^{-13}
7	10a	96%	64%	$3.04\times10^{-4} \pm 1.7\times10^{-5}$	2.42×10^{-13}
8	10b	41%	56%	$2.87\times10^{-4} \pm 1.61\times10^{-5}$	2.28×10^{-13}
9	10c	63%	87%	$5.67\times10^{-4} \pm 6.12\times10^{-5}$	4.51×10^{-13}
10	11a	65%			
11	11b	41%			

FIGURE 3. Set of protected alkanethiols. Key: (a) yield of purified protected thiol from Knoevenagel condensation and Michael addition; (b) percent thiol cleaved was determined by ^{1}HNMR analysis after 50 min of irradiation; (c) quantum yield (Φ) determined by measuring the number of reactions/photon. This can be compared to 8.71×10^{-14} for 1-(2-nitrophenyl)ethylcholine ($\Phi_r = 0.27$).[13]

Experimental Section

General Procedure for Knoevenagel Condensation: Michael Addition. 2-[(4,5-Dimethoxy-2-nitrophenyl)ethylsulfanylmethyl]-3-hydroxy-5,5-dimethylcyclohex-2-enone (7) (Representative Example). 1,3-Cyclohexadione **2b** (500 mg, 3.6 mmol) was added to 6-nitrobenzadehyde **3c** (1.29 g, 6.1 mmol) in CH_2Cl_2 (20 mL) containing dried silica gel (50 mg). The reaction vessel was purged with Ar, and ethanethiol (0.29 mL, 4.0 mmol) was added in CH_2Cl_2 (5 mL). After 4 h, the reaction was concentrated to 10% volume and purified via flash chromatography (hexanes, EtOAc) to yield a yellow foam **7**: 63% yield; R_f (1:2 hexanes/ethyl acetate) 0.44; ^{1}H NMR ($CDCl_3$) δ (ppm) 0.95 (3H, s), 1.00 (3H, s), 1.24 (3H, t, 7.2 Hz), 2.17 (H, d, J = 16.3 Hz), 2.25 (H, d, 16.9), 2.40 (H, d, 17.6 Hz), 2.52 (H, d, 17.6 Hz), 2.59−2.72 (2H, m), 3.80 (3H, s), 3.81 (3H, s), 6.18 (H, s), 6.90 (H, s), 7.44 (H, s), 10.70 (H, s); ^{13}C NMR ($CDCl_3$) δ (ppm) 14.0, 27.7, 27.6, 29.1, 31.9, 39.7, 44.1, 50.6, 56.4, 56.6, 108.9, 110.8, 110.9, 129.6, 140.8, 148.1, 153.0, 174.5, 197.1;[1] IR (thin film) (max/cm^{-1}) 2961, 1614, 1578, 1521, 1274, 1058; MS [M − H]$^-$ 393.98; HRMS (FAB) calcd for $C_{19}H_{26}O_6NS$ 396.1475 [M + H]$^+$, found 396.1479 *m/z*.

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Supporting Information Available: Experimental procedures, characterization data, and ^{1}H NMR and ^{13}C NMR for compounds **1a**−**c**, **6**−**8**, **9a**−**c**, and **10a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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