OL Organic Letters

pubs.acs.org/OrgLett Letter

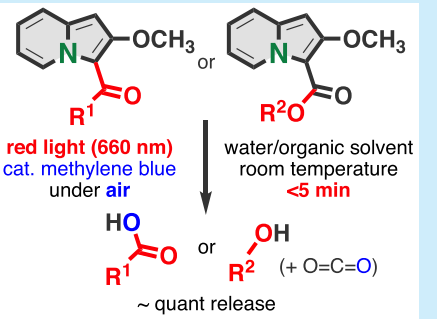
Indolizines Enabling Rapid Uncaging of Alcohols and Carboxylic Acids by Red Light-Induced Photooxidation

Kenji Watanabe,* Nodoka Terao, Isao Kii, Reiko Nakagawa, Takashi Niwa, and Takamitsu Hosoya*

Cite This: https://dx.doi.org/10.1021/acs.orglett.0c01799 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The irradiation of red light-emitting-diode light (λ = 660 nm) to 3-acyl-2-methoxyindolizines in the presence of a catalytic amount of methylene blue triggered the photooxidation of the indolizine ring, resulting in a nearly quantitative release of alcohols or carboxylic acids within a few minutes. The method was applicable for photouncaging various functional molecules such as a carboxylic anticancer drug and a phenolic fluorescent dye from the corresponding indolizine conjugates, including an insulin−indolizine−dye conjugate.



Caged compounds, which are deactivated (bio)functional molecules protected by photodegradable groups,[1] are widely used in molecular biology and drug discovery research.[2,3] Photoirradiation of caged compounds triggers the release of active molecules, thus enabling the restoration of the original (bio)functions in the desired spatiotemporal field. Thus, caged compounds have been effectively used as molecular tools for mechanistic investigation of biological phenomena. Typically, ultraviolet light has been used to photodegrade the protecting groups of caged compounds. However, the low tissue permeability of ultraviolet light has impeded the application of this technique to *in vivo* experiments. To overcome this problem, the use of light with a longer wavelength (λ = 650−900 nm) with higher tissue permeability has been recommended.[4,5] However, the energy of such light is insufficient to achieve deprotection via the cleavage of covalent bonds. Indeed, there are only a few examples of molecules that undergo cleavage of covalent bonds under the irradiation of long-wavelength light.[6−12]

The use of singlet oxygen ($^1O_2$) is another potential strategy for addressing this issue, as $^1O_2$ can be easily generated by the irradiation of long-wavelength light to a suitable photosensitizer (PS).[13] The reactivity of $^1O_2$ is also sufficiently high to cleave covalent bonds, as exhibited in the oxidative cleavage of carbon−carbon (C−C) double bonds (Figure 1A).[14−17] This strategy has previously been applied to uncage bioactive molecules. For instance, You et al. developed PS-conjugated aminoacrylates to release an anticancer drug via oxidation with $^1O_2$ generated by the irradiation of long-wavelength light (λ = 690 nm; Figure 1B).[18,19] Schnermann et al. also developed cyanine-based photocaged compounds in which the alkene moiety was photooxidized to release hydrolyzable precursors of aromatic alcohols or amines (Figure 1C).[20−22] These systems were successfully applied to the *in vivo* photouncaging of functional molecules in model mice, demonstrating the utility of this approach. Nevertheless, these methods require long irradiation times (>30 min) to achieve the cleavage of C−C double bonds with high efficiency. To develop more advanced systems, such as phototherapy in deep tissues where only weak light is reachable, improvement in the photoreactivity of caged compounds is required. In principle, increasing the electron density of the alkene moiety accelerates the photooxidative reaction. However, introducing electron-donating groups often makes alkene moieties significantly unstable, particularly under acidic conditions, thus undermining the practical utility of the method. Herein, we report that designer indolizine derivatives serve as efficient caged compounds for the alcohols and carboxylic acids released by short irradiation of red light in the presence of suitable PS.

First, we focused on the C−C double bond in electron-rich heterocycles as a covalent bond reactive with $^1O_2$. In particular, we paid attention to indolizines,[23−26] as it was reported by Xu et al. that $^1O_2$ underwent electrophilic addition to the C3 carbon in indolizine by photoirradiation with a xenon lamp (500 W) in the presence of PSs, affording pyridinyl acrylates (Figure 1D).[27,28] When an acyl group was attached at the C3 position, it could be expected that it would be released as a carboxylic acid after intramolecular rearrangement, though the

Received: May 28, 2020

https://dx.doi.org/10.1021/acs.orglett.0c01799
Org. Lett. XXXX, XXX, XXX−XXX

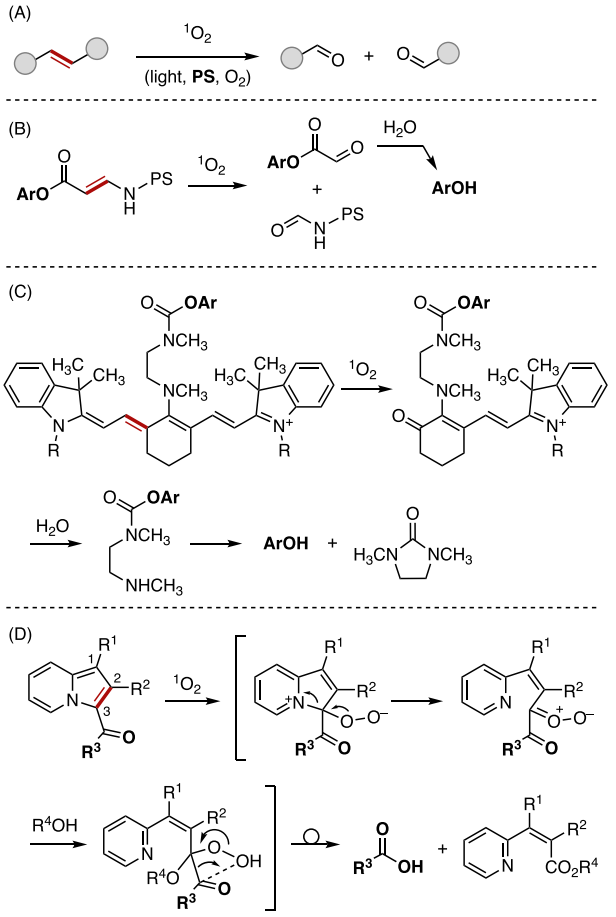


Figure 1. Photouncaging methods by singlet oxygen ($^1O_2$). (A) Cleavage of a C−C double bond with $^1O_2$. (B) Photooxidation of a PS-conjugated aminoacrylate. (C) Photooxidation of a cyanine-based cage compound. (D) Xu's proposed mechanism for photooxidation of 3-acylindolizines.

behavior of this group has not been previously confirmed. On the basis of this proposed mechanism, we designed 3-acyl-2-alkoxyindolizines as a photocaged compound of carboxylic acids. The introduction of a 2-alkoxy group in addition to a nitrogen atom attached directly to the C2−C3 double bond of indolizine was assumed to increase its electron density, whereas the π-conjugation could contribute to maintaining the chemical stability.

On the basis of this concept, we first attempted the photooxidation of 2-methoxy-3-(phenyloxycarbonyl)indolizine (**1**) under irradiation with 660 nm light-emitting-diode (LED) light in the presence of various PSs in deuterated methanol under air atmosphere. An extensive screening study revealed that methylene blue (1 mol %) served as an effective PS, quantitatively liberating phenol within 30 s (Figure 2A; see Table S1 and Figure S1 for full screening results). The phenol could be formed via photooxidation with $^1O_2$ to afford monophenyl carbonate followed by spontaneous decarboxylation. Indeed, the reaction did not proceed under argon atmosphere, whereas a comparable result was obtained under 1% oxygen atmosphere. This result demonstrated the high reactivity of **1** and the potential applicability of the present system to low-oxygen regions, such as tumor tissues. To further confirm the involvement of $^1O_2$ in this event, indolizine **1** was treated under argon with 1.2 equiv of dioxynaphthalene

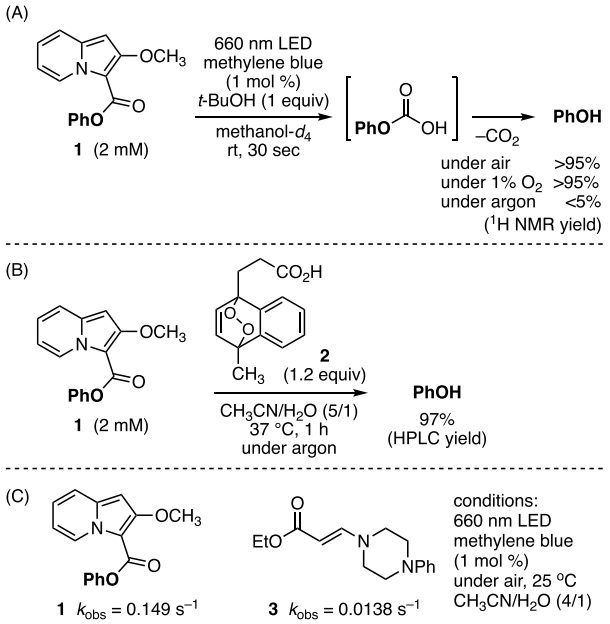


Figure 2. Photooxidation experiments. (A) Photooxidation of indolizine **1**. (B) Oxidation of **1** with $^1O_2$ generated from endoperoxide **2**. (C) Comparison of the apparent rate constants (k_{obs}) for the photooxidation of **1** and **3**.

2, a reagent known to generate $^1O_2$,[29] and phenol was obtained almost quantitatively (Figure 2B). The photoinduced $^1O_2$-dependent rapid uncaging of **1** was also supported by treatment with an excess amount of hydrogen peroxide (100 equiv) at room temperature, resulting in sluggish conversion to afford phenol only in 13% yield after 5 h (Figure S2).

The apparent rate constant (k_{obs}) for the release of phenol from **1** was determined to be 0.149 s^{-1} by quantifying the amount of phenol produced as a function of the photoirradiation time and analyzing the data using a pseudo-first-order rate eq (Figure 2C; Figure S3). For comparison, the k_{obs} of the photooxidation of aminoacrylate **3** was determined to be 0.0138 s^{-1} under the same conditions (Figure S4), demonstrating the faster photooxidation of 2-methoxyindolizine **1** compared with that of **3**.

Next, to examine the substrate scope of this method, we prepared various 3-acylindolizines (Figure 3). Friedel−Crafts

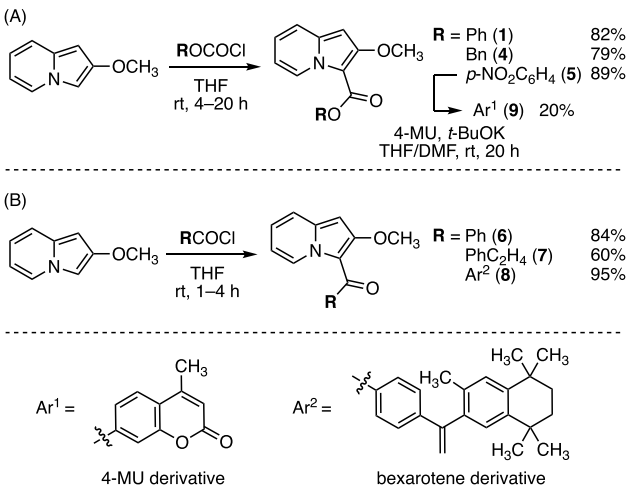


Figure 3. Synthesis of various 3-acyl-2-methoxyindolizines.

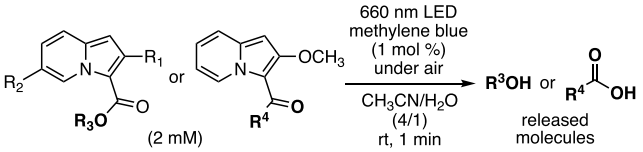
https://dx.doi.org/10.1021/acs.orglett.0c01799
Org. Lett. XXXX, XXX, XXX−XXX

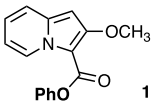
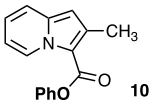
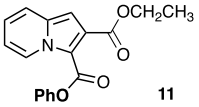
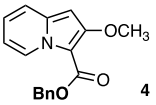
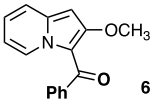
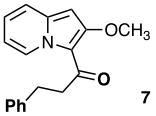
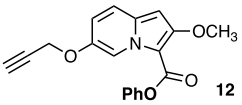
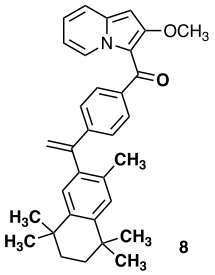
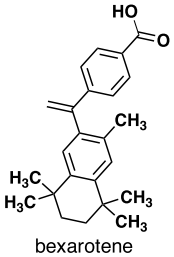
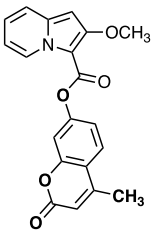
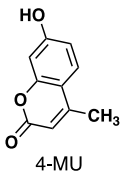
acylation of indolizines is known to occur more easily in electron-rich C3 carbon compared with C1 carbon.[30] Indeed, the acylation of 2-methoxyindolizine, which was prepared by extensively modifying the reported method (Table S2),[31] with various chloroformates (Figure 3A) and acyl chlorides (Figure 3B) proceeded smoothly to afford C3-acylated 2-methoxyindolidines **1** and **4**–**8**. These included a derivative of bexarotene, an anticancer drug, in high yields (Figure 3B). These substrates were bench-stable, and the benzoyl derivative **6** showed particularly high tolerance to strong acid and base (Figure S2). In addition, an ester exchange reaction of *p*-nitrophenyl carbonate **5** with alcohols was also feasible under basic conditions (Figure 3A). For example, 4-methylumbelliferone (4-MU) ester derivative **9** was prepared using this method. These methods allowed for the effortless protection of carboxylic acids and alcohols by the 2-methoxyindolizines.

Having obtained various 3-acylindolizines, we next examined their uncaging reactions by irradiation of 660 nm LED light to the substrates in the presence of methylene blue (1 mol %) for 1 min at room temperature in aqueous acetonitrile under air atmosphere (Table 1). The importance of the electron-donating substituent at the C2 position was demonstrated in the reactions of 3-(phenoxycarbonyl)indolizines **1**, **10**, and **11** bearing 2-methoxy, 2-methyl, and 2-ethoxycarbonyl groups, respectively (entries 1–3). The release of phenol from C2–methoxy substrate **1** was the fastest, whereas the photouncaging reaction of indolizine **11** with the C2 electron-withdrawing group was considerably slower than that of the others. In addition to phenol, aliphatic alcohol was efficiently uncaged, as demonstrated in the photooxidation of benzyl ester **4** (entry 4). It is noteworthy that aromatic and aliphatic carboxylic acids could be uncaged with excellent efficiency under the same conditions, thus significantly expanding the scope of the method (entries 5 and 6). The photooxidation of **12** with a clickable terminal alkyne moiety proceeded without difficulty to afford phenol (entry 7). The release of bexarotene from caged compound **8** also proceeded smoothly without affecting the olefinic moiety, which is potentially susceptible to oxygenation (entry 8). Moreover, restoration of the strong fluorescence of 4-MU was achieved by the photoirradiation of fluorescent caged compound **9**. Using this caged dye, a time course analysis in serum as a biological crowding media was conducted. Photoirradiation of **9** in dimethyl sulfoxide containing 20 v/v% serum with methylene blue (10 mol %) was completed within 5 min to afford 4-MU almost quantitatively (Table 1, entry 9; Figure S5). We also confirmed the stability of **9** in this medium by observing that only 17% of 4-MU was released after 10 days in darkness (Figure S2). These results indicate the applicability of the photoinduced uncaging reaction of indolizine-caged compounds in a complex biological mixture.

Finally, the applicability of this uncaging system to a larger biomolecule was examined. We designed insulin derivative **13** conjugated with three indolizine–4-MU as a model molecule. This molecule was prepared by copper-catalyzed cycloaddition reaction of the known triazidoinsulin derivative **14**[32] and 4-MU-caged alkyne **15** (Scheme 1). The photoirradiation of conjugate **13** in dimethyl sulfoxide/H_2O (4/1) containing methylene blue (10 mol %) for 1 min resulted in the quantitative release of 4-MU, as determined by fluorescence measurement (Scheme 1). The complete consumption of **13** and generation of the counterpart peptide **16** was also confirmed by deconvoluted electrospray ionization-mass spectrometry analysis (Scheme 1; Figure S7). This result demonstrates the applicability of the indolizines to the photoinduced release of functional molecules from biomacromolecules, such as peptides.[33]

Table 1. Scope of Indolizines for Photooxidative Uncaging

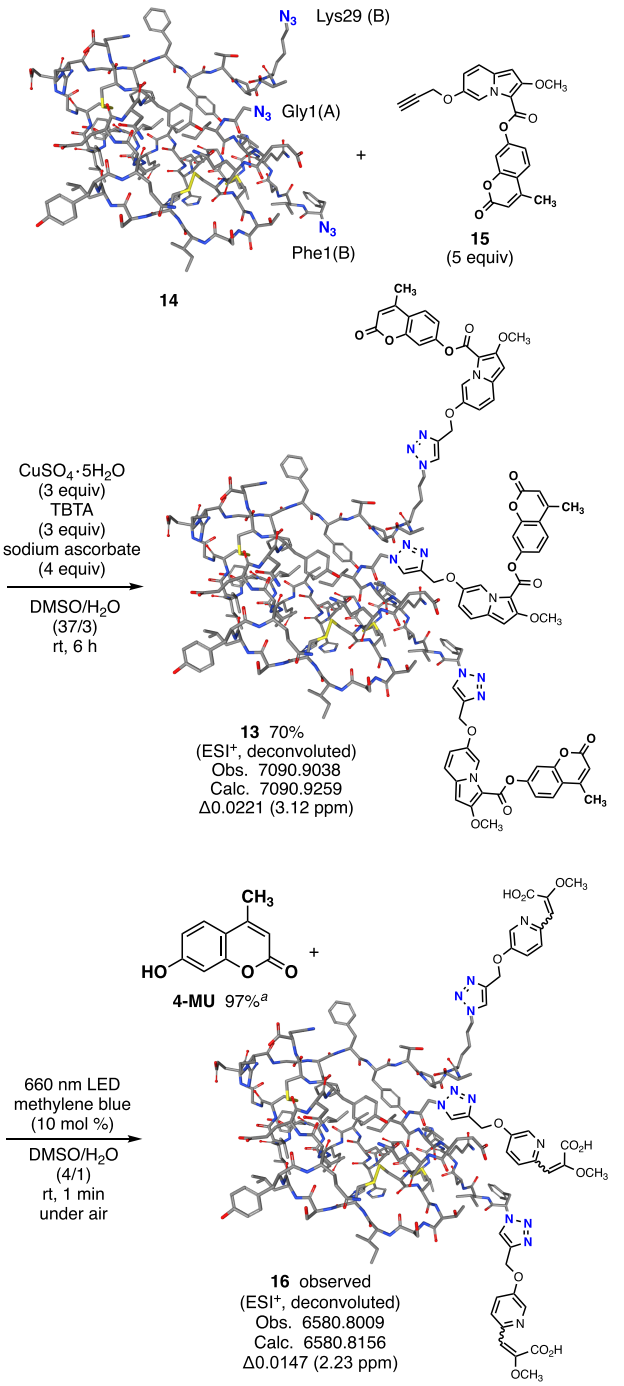


entry	substrate	released molecule	yield (%)[a]
1		PhOH	98
2		PhOH	71
3		PhOH	7
4		BnOH	92
5		$PhCO_2H$	99
6		$Ph(CH_2)_2CO_2H$	99
7		PhOH	98
8	  bexarotene		97
9	  4-MU		97 (98)[b]

[a]Yields of released molecules determined by HPLC analysis. [b]Methylene blue (10 mol %), DMSO/serum (4/1), 660 nm LED, 5 min. Yield determined by fluorescence measurements shown in parentheses.

https://dx.doi.org/10.1021/acs.orglett.0c01799
Org. Lett. XXXX, XXX, XXX–XXX

Scheme 1. Preparation and Uncaging of 4-MU by Photooxidation of Insulin−Indolizine−4-MU Conjugate 13



[a]Yield determined by fluorescence measurement.

In summary, we developed an efficient method for the uncaging of functional alcohols and carboxylic acids via the photooxidation of 3-acyl-2-methoxyindolizines. The reactions were promoted by irradiation with red LED light along with the assistance of PS. In our experiments, the high oxidative susceptibility of the designed indolizines allowed for the rapid and nearly quantitative release of various alcohols and carboxylic acids, including functional molecules. Because the uncaging is triggered by highly biopermeable red light and because a wide variety of functional alcohols and carboxylic acids can be easily derived to the corresponding caged 3-acylindolizines, this system has great advantage over the conventional methods. A number of further studies are currently underway, including the expansion of the method to the uncaging of molecules with other functional groups (such as amino groups), synthesis of an indolizine platform bearing an intramolecular PS moiety, and application of the method to molecular cell biology research and development of antibody−drug conjugates bearing photocleavable indolizine linkers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c01799.

Experimental procedures for synthesis of substrates, photoreactions, kinetic studies, and characterization for new compounds including ^{1}H and ^{13}C NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Kenji Watanabe − *Laboratory for Chemical Biology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe 650-0047, Japan*; Email: kenji.watanabe.vs@riken.jp

Takamitsu Hosoya − *Laboratory for Chemical Biology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe 650-0047, Japan; Laboratory of Chemical Bioscience, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU), Chiyoda-ku, Tokyo 101-0062, Japan*; orcid.org/0000-0002-7270-351X; Email: thosoya.cb@tmd.ac.jp

Authors

Nodoka Terao − *Laboratory for Chemical Biology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe 650-0047, Japan*

Isao Kii − *Laboratory for Chemical Biology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe 650-0047, Japan; RIKEN, Cluster for Science, Technology and Innovation Hub, Kobe 650-0047, Japan; Laboratory for Drug Target Research, Integrated Bioscience Division, Institute of Agriculture, Shinshu University, Nagano 399-4598, Japan*

Reiko Nakagawa − *Laboratory for Phyloinformatics, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe 650-0047, Japan*

Takashi Niwa − *Laboratory for Chemical Biology, RIKEN Center for Biosystems Dynamics Research (BDR), Kobe 650-0047, Japan*; orcid.org/0000-0002-6209-5362

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c01799

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Hidefumi Mukai and Ms. Chizuru Kiyota (RIKEN BDR) for their kind support for the LC/MS measurement. This research was supported by Japan Agency for Medical Research and Development (AMED) under Grant Nos. JP19am0401021 (Science and Technology Platform Program for Advanced Biological Medicine) and

https://dx.doi.org/10.1021/acs.orglett.0c01799
Org. Lett. XXXX, XXX, XXX−XXX

JP19am0101098 (Platform Project for Supporting Drug Discovery and Life Science Research, BINDS); JSPS KAKENHI Grant No. JP19K05707 (C; K.W.); the Cooperative Research Project of Research Center for Biomedical Engineering; and the Pioneering Project "Chemical Probe" from RIKEN.

REFERENCES

(1) Klán, P.; Solomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Photoremovable protecting groups in chemistry and biology: reaction mechanisms and efficacy. *Chem. Rev.* **2013**, *113*, 119−191.
(2) Nani, R. R.; Gorka, A. P.; Nagaya, T.; Kobayashi, H.; Schnermann, M. J. Near-IR light-mediated cleavage of antibody-drug conjugates using cyanine photocages. *Angew. Chem., Int. Ed.* **2015**, *54*, 13635−13638.
(3) Nani, R. R.; Gorka, A. P.; Nagaya, T.; Yamamoto, T.; Ivanic, J.; Kobayashi, H.; Schnermann, M. J. In vivo activation of duocarmycin−antibody conjugates by near-infrared light. *ACS Cent. Sci.* **2017**, *3*, 329−337.
(4) Chin, A. L.; Zhong, Y. L.; Tong, R. Emerging strategies in near-infrared light triggered drug delivery using organic nanomaterials. *Biomater. Sci.* **2017**, *5*, 1491−1499.
(5) Silva, J. M.; Silva, E.; Reis, R. L. Light-triggered release of photocaged therapeutics−Where are we now? *J. Controlled Release* **2019**, *298*, 154−176.
(6) Peterson, J. A.; Wijesooriya, C.; Gehrmann, E. J.; Mahoney, K. M.; Goswami, P. P.; Albright, T. R.; Syed, A.; Dutton, A. S.; Smith, E. A.; Winter, A. H. Family of BODIPY photocages cleaved by single photons of visible/near-infrared light. *J. Am. Chem. Soc.* **2018**, *140*, 7343−7346.
(7) Šťacko, P.; Muchová, L.; Vítek, L.; Klán, P. Visible to NIR light photoactivation of hydrogen sulfide for biological targeting. *Org. Lett.* **2018**, *20*, 4907−4911.
(8) Lv, W.; Li, Y.; Li, F.; Lan, X.; Zhang, Y.; Du, L.; Zhao, Q.; Phillips, D. L.; Wang, W. Upconversion-like photolysis of BODIPY-based prodrugs via a one-photon process. *J. Am. Chem. Soc.* **2019**, *141*, 17482−17486.
(9) Anderson, E. D.; Gorka, A. P.; Schnermann, M. J. Near-infrared uncaging or photosensitizing dictated by oxygen tension. *Nat. Commun.* **2016**, *7*, 13378.
(10) Kobayashi, H.; Choyke, P. L. Near-infrared photoimmunotherapy of cancer. *Acc. Chem. Res.* **2019**, *52*, 2332−2339.
(11) Al-Afyouni, M. H.; Rohrabaugh, T. N., Jr.; Al-Afyouni, K. F.; Turro, C. New Ru(II) photocages operative with near-IR light: new platform for drug delivery in the PDT window. *Chem. Sci.* **2018**, *9*, 6711−6720.
(12) Wang, X.; Kalow, J. A. Rapid aqueous photouncaging by red light. *Org. Lett.* **2018**, *20*, 1716−1719.
(13) Ye, H.; Zhou, Y.; Liu, X.; Chen, Y.; Duan, S.; Zhu, R.; Liu, Y.; Yin, L. Recent advances on reactive oxygen species-responsive delivery and diagnosis system. *Biomacromolecules* **2019**, *20*, 2441−2463.
(14) Anderson, V. C.; Thompson, D. H. Triggered release of hydrophilic agents from plasmalogen liposomes using visible light or acid. *Biochim. Biophys. Acta, Biomembr.* **1992**, *1109*, 33−42.
(15) Ruebner, A.; Yang, Z.; Leung, D.; Breslow, R. A cyclodextrin dimer with a photocleavable linker as a possible carrier for the photosensitizer in photodynamic tumor therapy. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 14692−14693.
(16) Baugh, S. D. P.; Yang, Z.; Leung, D. K.; Wilson, D. M.; Breslow, R. Cyclodextrin dimers as cleavable carriers of photodynamic sensitizers. *J. Am. Chem. Soc.* **2001**, *123*, 12488−12494.
(17) Jiang, M. Y.; Dolphin, D. Site-specific prodrug release using visible light. *J. Am. Chem. Soc.* **2008**, *130*, 4236−4237.
(18) Bio, M.; Nkepang, G.; You, Y. Click and photo-unclick chemistry of aminoacrylate for visible light-triggered drug release. *Chem. Commun.* **2012**, *48*, 6517−6519.
(19) Bio, M.; Rajaputra, P.; Nkepang, G.; You, Y. Far-red light activatable, multifunctional prodrug for fluorescence optical imaging and combinational treatment. *J. Med. Chem.* **2014**, *57*, 3401−3409.
(20) Gorka, A. P.; Nani, R. R.; Zhu, J. J.; Mackem, S.; Schnermann, M. J. A near-IR uncaging strategy based on cyanine photochemistry. *J. Am. Chem. Soc.* **2014**, *136*, 14153−14159.
(21) Gorka, A. P.; Nani, R. R.; Schnermann, M. J. Harnessing Cyanine Reactivity for Optical Imaging and Drug Delivery. *Acc. Chem. Res.* **2018**, *51*, 3226−3235.
(22) Yamamoto, T.; Caldwell, D. R.; Gandioso, A.; Schnermann, M. J. A cyanine photooxidation/β-elimination sequence enables near-infrared uncaging of aryl amine payloads. *Photochem. Photobiol.* **2019**, *95*, 951−958.
(23) Borrows, E. T.; Holland, D. O. The chemistry of the pyrrocolines and the octahydropyrrocolines. *Chem. Rev.* **1948**, *42*, 611−643.
(24) Prostakov, N. S.; Baktibaev, O. B. Indolizines. *Usp. Khim.* **1975**, *44*, 1649−1687; *Russ. Chem. Rev.* **1975**, *44*, 748−766.
(25) Singh, G. S.; Mmatli, E. E. Recent progress in synthesis and bioactivity studies of indolizines. *Eur. J. Med. Chem.* **2011**, *46*, 5237−5257.
(26) Teng, L.; Liu, X.; Guo, P.; Yu, Y.; Cao, H. Visible-light-induced regioselective dicarbonylation of indolizines with oxoaldehydes via direct C−H functionalization. *Org. Lett.* **2020**, *22*, 3841−3845.
(27) Tian, J.-Z.; Zhang, Z.-G.; Yang, X.-L.; Fun, H.-K.; Xu, J.-H. Photooxygenation of indolizines via selective excitation of their charge transfer complexes with molecular oxygen. *J. Org. Chem.* **2001**, *66*, 8230−8235.
(28) Li, Y.; Hu, H.-Y.; Ye, J.-P.; Fun, H.-K.; Hu, H.-W.; Xu, J.-H. Reaction modes and mechanism in indolizine photooxygenation reactions. *J. Org. Chem.* **2004**, *69*, 2332−2339.
(29) Mukai, K. Antioxidant activity of foods: development of singlet oxygen absorption capacity (SOAC) assay method. *J. Nutr. Sci. Vitaminol.* **2019**, *65*, 285−302.
(30) Bobrovskii, S. I.; Lushnikov, D. E.; Bundel, Y. G. Structures and ambiphilic reactivities of indolizines. 5. Acylation of 2-methylindolizine. *Chem. Heterocycl. Compd.* **1989**, *25*, 1360−1364.
(31) Kakehi, A.; Ito, S.; Watanabe, K.; Kitagawa, M.; Takeuchi, S.; Hashimoto, T. Preparation of new nitrogen-bridged heterocycles. Synthesis and some reactions of 2,3-dihydroindolizin-2-one derivatives. *J. Org. Chem.* **1980**, *45*, 5100−5104.
(32) Boga, S. B.; Krska, S. W.; Lin, S.; Pissarnitski, D.; Yan, L.; Kekec, A.; Tang, W.; Pierson, N. A.; Strulson, C. A.; Streckfuss, E.; Zhu, X.; Zhang, X.; Kelly, T.; Parish, C. A. Site-selective synthesis of insulin azides and bioconjugates. *Bioconjugate Chem.* **2019**, *30*, 1127−1132.
(33) The stability of **13** in serum containing DMSO (2 v/v%) at 37 °C was examined in the presence or absence of glutathione (100 equiv, 2 mM) under air without irradiation. The time-dependent release of 4-MU was monitored, showing that less than 4% of 4-MU was released after 87 h in the dark regardless of the presence or absence of glutathione (Figure S8). This result indicates sufficient stability of 3-acyl-2-methoxyindolizines to glutathione and components in serum.

https://dx.doi.org/10.1021/acs.orglett.0c01799
Org. Lett. XXXX, XXX, XXX−XXX