Organic LETTERS

Letter

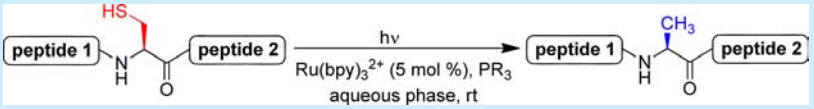
pubs.acs.org/OrgLett

Visible-Light-Induced Specific Desulfurization of Cysteinyl Peptide and Glycopeptide in Aqueous Solution

Xiao-Fei Gao, Jing-Jing Du, Zheng Liu, and Jun Guo*

Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, CCNU-uOttawa Joint Research Centre, College of Chemistry, Central China Normal University, 152 Luoyu Road, Wuhan, Hubei 430079, P. R. China

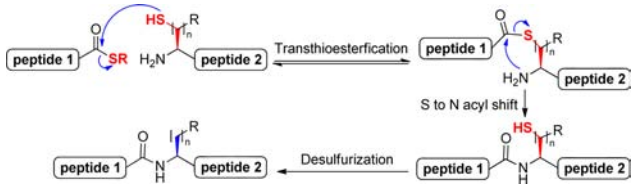
Supporting Information



ABSTRACT: Visible-light-induced specific desulfurization of cysteinyl peptides has been explored. The photocatalytic desulfurization catalyzed by $Ru(bpy)_3^{2+}$ can proceed efficiently at room temperature in aqueous solution or in binary mixtures of aqueous/organic solvent and be compatible with the presence of residues of amino acids, carbohydrates, and various sulfur-containing functional groups. This approach was successfully applied to synthesize linear and cyclic peptides through the ligation−desulfurization protocol.

Over the past several decades, methods of chemical synthesis of peptides and proteins have been greatly developed. The most remarkable breakthrough was made by Merrifield in 1963 for applying solid phase peptide synthesis (SPPS).[1] Stepwise SPPS is limited to peptides bearing less than ca. 50 amino acids, because longer peptide synthesis is often plagued by side products and coupling efficiency. Thus, a segment condensation strategy has been employed to facilitate longer peptides and proteins. The most widely used method for condensation of peptide segments is a native chemical ligation (NCL) protocol, developed by Kent and co-workers in the 1990s.[2] The general mechanistic process of NCL—transthioesterification and S to N acyl transfer—is outlined in Scheme 1. Even though the NCL protocol has led to many proteins being chemically synthesized,[3] the low abundance of cysteine in peptides and proteins has limited the ligation site of NCL. To overcome the limitation of cysteine dependence, synthesis and application of cysteine surrogates[4] or selenium analogs[5] have emerged, which expanded the peptide ligation at more amino acid sites through a ligation−desulfurization/deselenization[6] protocol.

Scheme 1. Native Chemical Ligation and Desulfurization of Unprotected Peptide Segments



Hoffmann and co-workers have reported desulfurization reactions between mercaptans and trialkylphosphites under reflux or ultraviolet irradiation conditions.[7] It was then suggested that the reaction is a radical process.[8] However, these reported conditions[7−9] failed to effectively desulfurize peptides in an aqueous phase.[10] Raney nickel or palladium under H_2 has been utilized to desulfurize peptides,[4a,11] but these desulfurization conditions are often haunted by their tolerance of special functional groups or low recovery of product.[4d,12] In 2007, Wan and Danishefsky developed an important protocol of heat-induced radical desulfurization for peptide synthesis,[10] which involved tris(2-carboxyethyl)phosphine (TECP, Figure 1) and radical initiator. In this protocol, an elevated temperature (37−65 °C) is required to activate the initiator and trigger the reaction,[4d,10] and a large excess of initiator (VA-044 or V-50) and TCEP is usually introduced to complete the desulfurization process of peptides.[6,10,13]

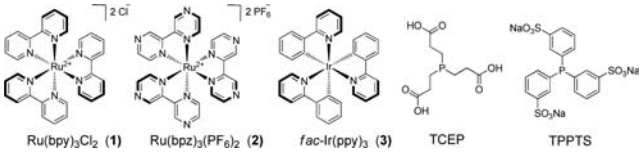


Figure 1. Structures of photocatalysts and phosphines.

Visible-light-photoredox catalysis has recently become an important strategy for organic synthesis. A milestone was achieved in this field due to the seminal research by MacMillan,[14] Yoon,[15] Stephenson,[16] and other[17] groups. This photoredox catalysis, which is based on the ability of transition metal complexes or organic dyes to engage in the single-electron transfer (SET) process with substrates upon photoexcitation, has been widely used in organic synthesis.[14−17] The thiyl radical, which plays important roles in various fields,[18] can be generated by visible-light-photoredox catalysis from thiol-containing substrates to perform additive or oxidative reactions.[19]

Received: January 28, 2016
Published: February 19, 2016

DOI: 10.1021/acs.orglett.6b00292
Org. Lett. 2016, 18, 1166−1169

Based on these results, we tried to explore a specific desulfurization method using visible-light-photoredox catalysis for peptide chemistry. Therefore, we examined the reaction of cysteine with water-soluble phosphine and 5 mol % $Ru(bpy)_3Cl_2$ (**1**) in D_2O, under visible-light irradiation, and monitored the reaction process by ^1H NMR. Initially, the most common water-soluble phosphine (TCEP) was utilized, and only a trace amount of desired product (<3%) was detected. Surprisingly, when another water-soluble phosphine -3,3′,3″-phosphinidynetris-(benzenesulfonic acid) trisodium salt (TPPTS, Figure 1) was used, the cysteine was almost quantitatively converted into alanine (Figure 2 and Figure S1).

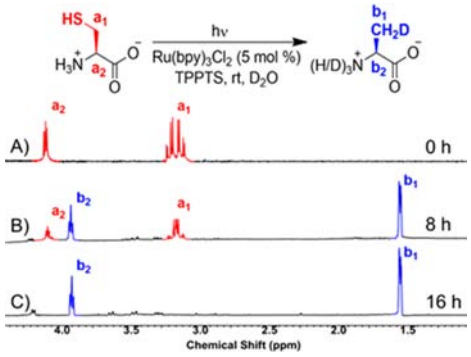
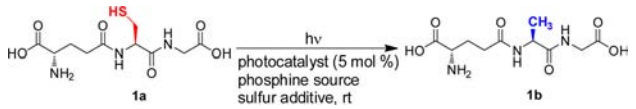


Figure 2. Model study of the visible-light-induced desulfurization of cysteine by ^1H NMR. Conditions: cysteine (20 mM) with TPPTS (50 mM), $Ru(bpy)_3Cl_2$ (5 mol %), in D_2O at rt after (A) 0 h, (B) 8 h, and (C) 16 h under the irradiation of a 36 W household bulb.

Encouraged by this result, we continued to optimize the reaction conditions using glutathione (GSH) as a model substrate (Table 1). In the absence of phosphine TPPTS, photocatalyst $Ru(bpy)_3Cl_2$, or visible light, no product was observed (entries 1−3). These findings suggest that all these conditions are essential in this system. The yield (85%) of product was good when 50 mM TPPTS was employed (entry 4). The yield was slightly improved as the amount of TPPTS increased (entry 5). Trace product (<3%) was detected when TPPTS was replaced by TCEP in this photoreaction (entry 6). As for different solutions applied, not only water and phosphate buffer (entries 4 and 7) but also mixtures of PB/organic solvent (entries 8−9) are suitable for this photoredox reaction, and all provide the desired product in good yields. This photoredox catalysis is also compatible with *tert*-butyl mercaptan (TBM) and guanidine hydrochloride (Gn·HCl) (entries 10−11), and a longer reaction time is required for a high concentration of Gn·HCl. For different photocatalysts (see Supporting Information, Table S1, entries 1−3), $Ru(bpy)_3Cl_2$ (**1**) was found to be the most efficient catalyst; $Ru(bpz)_3(PF_6)_2$ (**2**) also resulted in the desired product, but in a relatively lower yield; *fac*-$Ir(PPy)_3$ (**3**) only led to the desired product in a very low yield, probably due to its poor solubility in aqueous solution. The scavenger reagents were also evaluated (see Supporting Information, Table S1, entries 4−6), and TBM was found to be a good scavenger reagent. The reaction completed within 5 h when the concentration of substrate is 1 mM (see Supporting Information, Table S1, entries 7−9). Therefore, for this photoredox desulfurization reaction the optimized conditions consist of 1 mM peptide substrate, 50 mM of TPPTS, 5 mol % of **1**, and additives at room temperature.

Table 1. Screening and Control Experiments[a]



entry	phosphine source (mM)	solvent	sulfur additive	yield (%)[b]
1	–	H_2O	–	N.D.
2[c]	50	H_2O	–	N.D.
3[d]	50	H_2O	–	N.D.
4	50	H_2O	–	85
5	100	H_2O	–	88
6[e]	100	H_2O	–	<3
7	50	PB	–	81
8	50	PB/CH_3CN	–	87
9	50	PB/MeOH	–	84
10	50	PB	TBM	83
11[f]	50	PB	TBM	79

[a]Reaction conditions: **1a** (20.0 mM), TPPTS, photocatalyst **1** (5 mol %), phosphine, sulfur additive (80.0 mM), 16 h. [b]Yield was determined by ^1H NMR. [c]In the absence of light. [d]In the absence of **1**. [e]The TPPTS was replaced by TCEP. [f]Gn·HCl (200 mM). PB = phosphate buffer (pH = 7.4, 200 mM), TBM = *tert*-butyl mercaptan, N.D. = not detected.

With the optimized conditions in hand, we next investigated the longer peptide substrates, beginning with peptide **2a**. In order to reduce the reaction time, 1 mM of peptide substrate was used. Under household bulb irradiation for 5 h, the cysteinyl peptide **2a** completely disappeared, and the expected alanyl peptide **2b** was obtained with an isolated yield of 89%. The process was monitored by HPLC and ESI-MS as shown in Figure 3. Notably,

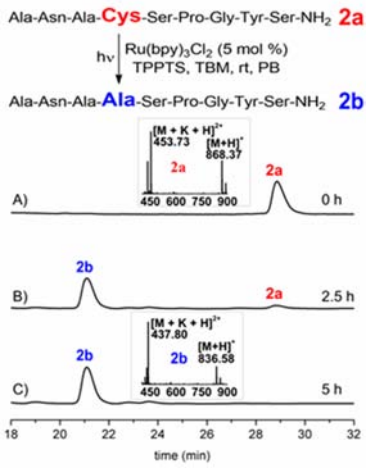
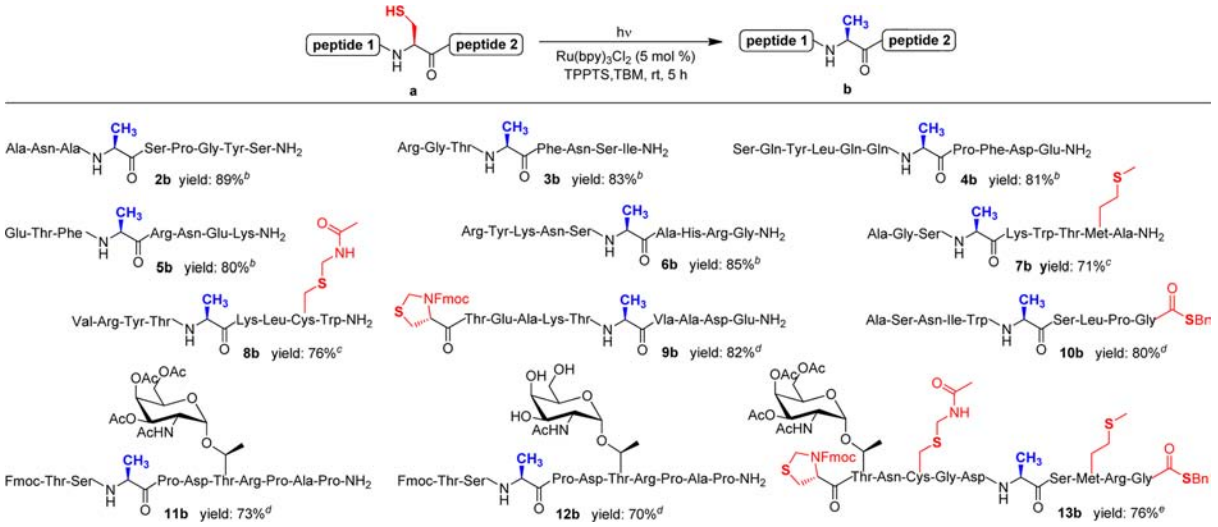


Figure 3. Model study of the visible-light-induced desulfurization of **2a** by HPLC and ESI-MS. Conditions: **2a** (1 mM), TPPTS (50 mM), $Ru(bpy)_3Cl_2$ (5 mol %), and TBM (80 mM), in phosphate buffer (200 mM, pH = 7.4) at rt after (A) 0 h, (B) 2.5 h, and (C) 5 h under the irradiation of a 36 W household bulb.

for this photoredox desulfurization procedure, 50 mM of TPPTS was used as a phosphine reagent, while for the conventional desulfurization procedure with radical initiator,[4d,10] a higher concentration of TCEP (250−500 mM) was always used. If TPPTS was replaced by TCEP in this photoredox desulfurization reaction, only a high concentration of TCEP (500 mM) can give the product **2b** effectively. The different reactivity between TPPTS and TCEP in the photoredox reactions is probably caused by their different electronic effect. Compared with the

DOI: 10.1021/acs.orglett.6b00292
Org. Lett. 2016, 18, 1166−1169

Scheme 2. Visible-Light-Induced Transformation of Cysteinyl Peptide to Alanyl Peptide[a]



[a]Desulfurization conditions: Cysteinyl peptide (1 mM), TPPTS (50 mM), $Ru(bpy)_3Cl_2$ (5 mol %), TBM (80 mM), PB or PB mixed with organic solvent, 36 W household bulb irradiation, 5 h; yield of isolated product. [b]In PB. [c]In PB, Gn·HCl (200 mM). [d]In CH_3CN/PB (1:1, v/v). [e]In MeOH/PB (1:1, v/v). PB = phosphate buffer, 200 mM, pH = 7.4.

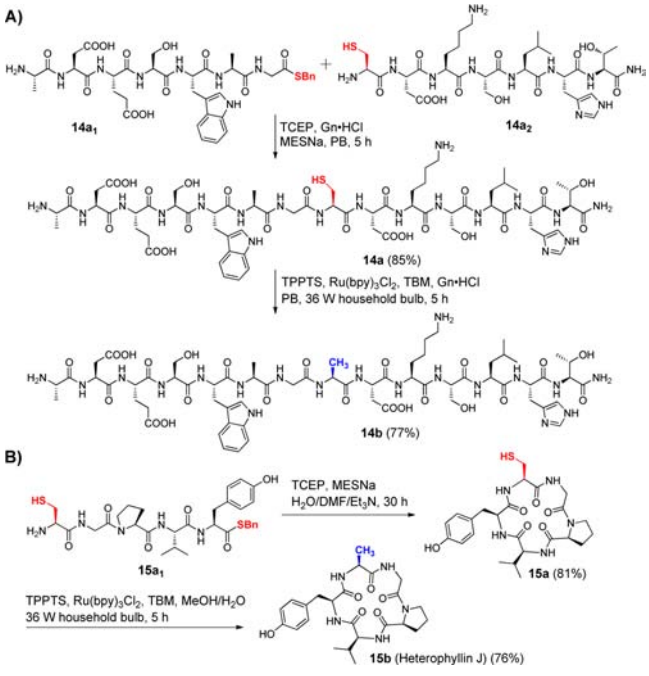
heat-induced radical desulfurization, the present visible-light-induced radical desulfurization has merits such as proceeding at room temperature, with low concentrations of initiator and phosphine (for more details, see Table S2).

Next, the visible-light-induced desulfurization procedure was applied to a range of peptides with different functional residues, and all the corresponding desulfurization peptides were obtained in good yields (Scheme 2). Remarkably, this protocol removed cysteine's thiol group in the presence of other sulfur-containing functional groups, such as thioether (Met in **7b**, Cys(Acm) in **8b**, and Thz in **9b**) and thioester (in **10b**). The protected and unprotected carbohydrate was intact during the phtotoredox catalysis (in **11b** and **12b**). Furthermore, we prepared and evaluated peptide **13a** bearing a range of suspicious sensitive functional groups (Met, Acm, Thz, thioester, and carbohydrate); the desired desulfurization peptide **13b** was obtained with a good yield of 76%. Moreover, this desulfurization protocol can proceed in both aqueous buffer and a mixture of aqueous buffer and organic solvent (CH_3CN or MeOH).

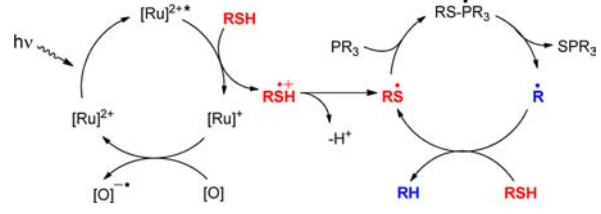
We then combined this photodesulfurization protocol with native chemical ligation (NCL) for peptide synthesis (Scheme 3). The linear peptide **14a** and the cyclic peptide **15a** were successfully prepared by native chemical ligation and were desulfurized to the desired peptide **14b** and Heterophyllin J (**15b**), a natural compound isolated from plant *Dianthus chinensis*,[20] in good yield via photoredox desulfurization reaction.

Based on a previous study,[7−9,19c] a plausible mechanism for this visible-light induced desulfurization process is outlined in Scheme 4. Initially, photoactive catalyst $[Ru]^{2+}$ accepts a photon from visible light to populate the excited state $[Ru]^{2+*}$ via metal-to-ligand charge transfer (MLCT), and this activated $[Ru]^{2+*}$ intermediate will be efficiently reduced by the thiol group to generate thiol radical cation and $[Ru]^{+}$. The $[Ru]^{+}$ can be oxidized back to the $[Ru]^{2+}$ by the oxidation species,[21,22] which rejuvenated the photoactive catalyst, and the thiol radical cation deprotonates to generate a thiyl radical. Concurrent with the photoredox pathway, in another catalytic cycle the addition of the thiyl radical to the phosphine generates a phosphoranyl radical.[23] Subsequently, β scission of the phosphoranyl radical provides an alkyl radical, which abstracts a hydrogen atom from a mercaptan to generate the desulfurization product and thiyl radical.

Scheme 3. Synthesis of Linear and Cyclic Peptides through Ligation/Desulfurization Protocol



Scheme 4. Proposed Mechanism of $[Ru]^{2+}$ Catalyzed Desulfurization



DOI: 10.1021/acs.orglett.6b00292
Org. Lett. 2016, 18, 1166−1169

In conclusion, we have developed a visible-light-initiated desulfurization method using a photocatalyst. This mild and specific desulfurization reaction is compatible with various related functional groups, able to incorporate into ligation of polypeptides and glycopeptides synthesis, and will be further applied in complex peptide and protein chemistry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00292.

Detailed experimental procedures, HPLC traces, ESI-MS and characterization data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jguo@mail.ccnu.edu.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the National Natural Science Foundation of China (Nos. 21272084, 21402058) and Central China Normal University for support of this research.

■ REFERENCES

(1) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.

(2) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. *Science* **1994**, *266*, 776−779.

(3) For selected reviews on NCL, see: (a) Dirksen, A.; Dawson, P. E. *Curr. Opin. Chem. Biol.* **2008**, *12*, 760−766. (b) Hackenberger, C. P. R.; Schwarzer, D. *Angew. Chem., Int. Ed.* **2008**, *47*, 10030−10074. (c) Gamblin, D. P.; Scanlan, E. M.; Davis, B. G. *Chem. Rev.* **2009**, *109*, 131−163. (d) Kent, S. B. H. *Chem. Soc. Rev.* **2009**, *38*, 338−351. (e) Payne, R. J.; Wong, C.-H. *Chem. Commun.* **2010**, *46*, 21−43. (f) Monbaliu, J.-C. M.; Katritzky, A. R. *Chem. Commun.* **2012**, *48*, 11601−11622. (g) Unverzagt, C.; Kajihara, Y. *Chem. Soc. Rev.* **2013**, *42*, 4408−4420. (h) Zheng, J.-S.; Tang, S.; Huang, Y.-C.; Liu, L. *Acc. Chem. Res.* **2013**, *46*, 2475−2484.

(4) For examples using a ligation/desulfurization protocol, see: (a) Yan, L. Z.; Dawson, P. E. *J. Am. Chem. Soc.* **2001**, *123*, 526−533. (b) Crich, D.; Banerjee, A. *J. Am. Chem. Soc.* **2007**, *129*, 10064−10065. (c) Chen, J.; Wan, Q.; Yuan, Y.; Zhu, J.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 8521−8524. (d) Haase, C.; Rohde, H.; Seitz, O. *Angew. Chem., Int. Ed.* **2008**, *47*, 6807−6810. (e) Kumar, K. S. A.; Haj-Yahya, M.; Olschewski, D.; Lashuel, H. A.; Brik, A. *Angew. Chem., Int. Ed.* **2009**, *48*, 8090−8094. (f) Yang, R.; Pasunooti, K. K.; Li, F.; Liu, X.-W.; Liu, C.-F. *J. Am. Chem. Soc.* **2009**, *131*, 13592−13593. (g) El Oualid, F.; Merkx, R.; Ekkebus, R.; Hameed, D. S.; Smit, J. J.; de Jong, A.; Hilkmann, H.; Sixma, T. K.; Ovaa, H. *Angew. Chem., Int. Ed.* **2010**, *49*, 10149−10153. (h) Hojo, H.; Ozawa, C.; Katayama, H.; Ueki, A.; Nakahara, Y.; Nakahara, Y. *Angew. Chem., Int. Ed.* **2010**, *49*, 5318−5321. (i) Ding, H.; Shigenaga, A.; Sato, K.; Morishita, K.; Otaka, A. *Org. Lett.* **2011**, *13*, 5588−5591. (j) Merkx, R.; de Bruin, G.; Kruithof, A.; van den Bergh, T.; Snip, E.; Lutz, M.; El Oualid, F.; Ovaa, H. *Chem. Sci.* **2013**, *4*, 4494−4498. (k) Thompson, R. E.; Chan, B.; Radom, L.; Jolliffe, K. A.; Payne, R. J. *Angew. Chem., Int. Ed.* **2013**, *52*, 9723−9727. (l) Guan, X.; Drake, M. R.; Tan, Z. *Org. Lett.* **2013**, *15*, 6128−6131.

(5) For examples using a ligation/deselenization protocol, see: (a) Metanis, N.; Keinan, E.; Dawson, P. E. *Angew. Chem., Int. Ed.* **2010**, *49*, 7049−7053. (b) Malins, L. R.; Giltrap, A. M.; Dowman, L. J.; Payne, R. J. *Org. Lett.* **2015**, *17*, 2070−2073. (c) Mitchell, N. J.; Malins, L. R.; Liu, X.; Thompson, R. E.; Chan, B.; Radom, L.; Payne, R. J. *J. Am. Chem. Soc.* **2015**, *137*, 14011−14014. (d) Reddy, P. S.; Dery, S.; Metanis, N. *Angew. Chem., Int. Ed.* **2016**, *55*, 992−995.

(6) For selected reviews on a ligation−desulfurization/deselenization protocol, see: (a) Rohde, H.; Seitz, O. *Biopolymers* **2010**, *94*, 551−559. (b) He, Q.-Q.; Fang, G.-M.; Liu, L. *Chin. Chem. Lett.* **2013**, *24*, 265−269. (c) Wong, C. T. T.; Tung, C. L.; Li, X. C. *Mol. BioSyst.* **2013**, *9*, 826−833. (d) Malins, L. R.; Mitchell, N. J.; Payne, R. J. *J. Pept. Sci.* **2014**, *20*, 64−77. (e) Malins, L. R.; Payne, R. J. *Aust. J. Chem.* **2015**, *68*, 521−537.

(7) Hoffmann, F. W.; Ess, R. J.; Simmons, T. C.; Hanzel, R. S. *J. Am. Chem. Soc.* **1956**, *78*, 6414.

(8) Walling, C.; Basedow, O. H.; Savas, E. S. *J. Am. Chem. Soc.* **1960**, *82*, 2181−2184.

(9) González, A.; Valencia, G. *Tetrahedron: Asymmetry* **1998**, *9*, 2761−2764.

(10) Wan, Q.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 9248−9252.

(11) Brik, A.; Ficht, S.; Yang, Y.-Y.; Wong, C.-H. *J. Am. Chem. Soc.* **2006**, *128*, 15026−15033.

(12) Node, M.; Nishide, K.; Shigeta, Y.; Obata, K.; Shiraki, H.; Kunishige, H. *Tetrahedron* **1997**, *53*, 12883−12894.

(13) El Oualid, F.; Merkx, R.; Ekkebus, R.; Hameed, D. S.; Smit, J. J.; de Jong, A.; Hilkmann, H.; Sixma, T. K.; Ovaa, H. *Angew. Chem., Int. Ed.* **2010**, *49*, 10149−10153.

(14) Nicewicz, D. A.; MacMillan, D. W. C. *Science* **2008**, *322*, 77−80.

(15) Ischay, M. A.; Anzovino, M. E.; Du, J.; Yoon, T. P. *J. Am. Chem. Soc.* **2008**, *130*, 12886−12887.

(16) Narayanam, J. M. R.; Tucker, J. W.; Stephenson, C. R. J. *J. Am. Chem. Soc.* **2009**, *131*, 8756−8757.

(17) For selected reviews on visible light photocatalysis, see: (a) Yoon, T. P.; Ischay, M. A.; Du, J. *Nat. Chem.* **2010**, *2*, 527−532. (b) Narayanam, J. M. R.; Stephenson, C. R. J. *Chem. Soc. Rev.* **2011**, *40*, 102−113. (c) Teply, F. *Collect. Czech. Chem. Commun.* **2011**, *76*, 859−917. (d) Shi, L.; Xia, W. *Chem. Soc. Rev.* **2012**, *41*, 7687−7697. (e) Xuan, J.; Xiao, W.-J. *Angew. Chem., Int. Ed.* **2012**, *51*, 6828−6838. (f) Maity, S.; Zheng, N. *Synlett* **2012**, *23*, 1851−1856. (g) Prier, C. K.; Rankic, D. A.; MacMillan, D. W. C. *Chem. Rev.* **2013**, *113*, 5322−5363. (h) Ravelli, D.; Fagnoni, M.; Albini, A. *Chem. Soc. Rev.* **2013**, *42*, 97−113. (i) Xi, Y.; Yi, H.; Lei, A. *Org. Biomol. Chem.* **2013**, *11*, 2387−2403. (j) Meggers, E. *Chem. Commun.* **2015**, *51*, 3290−3301.

(18) (a) Dénès, F.; Pichowicz, M.; Povie, G.; Renaud, P. *Chem. Rev.* **2014**, *114*, 2587−2693. (b) Nauser, T.; Koppenol, W. H.; Schoeneich, C. *Free Radical Biol. Med.* **2015**, *80*, 158−163.

(19) For examples of a thiyl radical generated by visible-light photocatalysis, see: (a) Cheng, Y.; Yang, J.; Qu, Y.; Li, P. *Org. Lett.* **2012**, *14*, 98−101. (b) Yu, C.; Lee, K.; You, Y.; Cho, E. J. *Adv. Synth. Catal.* **2013**, *355*, 1471−1476. (c) Tyson, E. L.; Ament, M. S.; Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 2046−2050. (d) Keshari, T.; Yadav, V. K.; Srivastava, V. P.; Yadav, L. D. S. *Green Chem.* **2014**, *16*, 3986−3992. (e) Xuan, J.; Feng, Z.-J.; Chen, J.-R.; Lu, L.-Q.; Xiao, W.-J. *Chem. - Eur. J.* **2014**, *20*, 3045−3049. (f) Keylor, M. H.; Park, J. E.; Wallentin, C.-J.; Stephenson, C. R. J. *Tetrahedron* **2014**, *70*, 4264−4269. (g) Bhat, V. T.; Duspara, P. A.; Seo, S.; Abu Bakar, N. S. B.; Greaney, M. F. *Chem. Commun.* **2015**, *51*, 4383−4385. (h) Zhang, G.; Liu, C.; Yi, H.; Meng, Q.; Bian, C.; Chen, H.; Jian, J.-X.; Wu, L.-Z.; Lei, A. *J. Am. Chem. Soc.* **2015**, *137*, 9273−9280.

(20) Yang, Y.-B.; Tan, N.-H.; Zhang, F.; Lu, Y.-Q.; He, M.; Zhou, J. *Helv. Chim. Acta* **2003**, *86*, 3376−3379.

(21) Zou, Y.-Q.; Chen, J.-R.; Liu, X.-P.; Lu, L.-Q.; Davis, R. L.; Jorgensen, K. A.; Xiao, W.-J. *Angew. Chem., Int. Ed.* **2012**, *51*, 784−788.

(22) Visible-light-promoted desulfurization reaction using GSH as a substrate was also conducted without a degassing procedure. The desired desulfurization product was obtained in good yield (78%), which is slightly lower than that of the degassing procedure (85%; see Table 1, entry 4). Based on this result, we speculated that the oxidant species in the catalysis cycle probably is the remaining oxygen in the solution.

(23) Giles, J. R. M.; Roberts, B. P. *J. Chem. Soc., Perkin Trans. 2* **1981**, 1211−1220.

DOI: 10.1021/acs.orglett.6b00292
Org. Lett. 2016, 18, 1166−1169