Bioorthogonal Chemistry

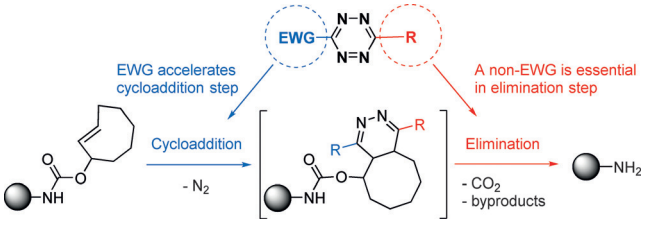
Deutsche Ausgabe: DOI: 10.1002/ange.201608009
Internationale Ausgabe: DOI: 10.1002/anie.201608009

Optimized Tetrazine Derivatives for Rapid Bioorthogonal Decaging in Living Cells

*Xinyuan Fan, Yun Ge, Feng Lin, Yi Yang, Gong Zhang, William Shu Ching Ngai, Zhi Lin, Siqi Zheng, Jie Wang, Jingyi Zhao, Jie Li, and Peng R. Chen**

Abstract: *The inverse-electron-demand Diels–Alder (iDA) reaction has recently been repurposed as a bioorthogonal decaging reaction by accelerating the elimination process after an initial cycloaddition between trans-cyclooctene (TCO) and tetrazine (TZ). Herein, we systematically surveyed 3,6-substituted TZ derivatives by using a fluorogenic TCO–coumarin reporter followed by LC-MS analysis, which revealed that the initial iDA cycloaddition step was greatly accelerated by electron-withdrawing groups (EWGs) while the subsequent elimination step was strongly suppressed by EWGs. In addition, smaller substituents facilitated the decaging process. These findings promoted us to design and test unsymmetric TZs bearing an EWG group and a small non-EWG group at the 3- and 6-position, respectively. These TZs showed remarkably enhanced decaging rates, enabling rapid iDA-mediated protein activation in living cells.*

The inverse-electron-demand Diels–Alder ligation reaction (iDA) between *trans*-cyclooctene (TCO) and tetrazine (TZ) has been widely used for diverse labeling purposes owing to its high reaction rate and good biocompatibility.[1] Recently, the iDA process has been redirected as an attractive bioorthogonal decaging reaction for the activation of prodrugs,[2] proteins,[3a] and kinases[3b] as well as the detection of mRNA[4] in living systems. The advantages of such small-molecule-enabled decaging strategies over previous photodecaging methods include the low cytotoxicity, high tunability, and excellent compatibility with deep tissues and intact animals.[5] Optimization of the recently emerged TCO–TZ decaging pair with an increase in reaction rates is highly desirable for the temporal manipulation and/or investigation of fast biological processes. Herein, we systematically surveyed a range of TZ derivatives bearing different substituents at the 3- and 6-positions for triggering the elimination process after the initial cycloaddition with TCO.[6] The intriguing results further guided us to design and test a range of unsymmetric TZs, which led to significantly enhanced decaging rates that allow the instantaneous activation of proteins in living cells (Scheme 1).



Scheme 1. Optimizing TZ derivatives for rapid iDA decaging on proteins in living cells.

We began our studies by designing and synthesizing a range of 1,2,4,5-TZs bearing symmetric substituents at the 3- and 6-positions using a previously reported method[7] with a small modification: Hydrazine hydrate was used instead of anhydrous hydrazine to avoid problems caused by the tedious handling and the limited availability of anhydrous hydrazine.[7b] The size of the substituents was varied for examining steric effects (**1a**–**1f**; Figure 1 A) whereas the electron-withdrawing or -donating abilities of the substituents were varied to examine the electronic effects on the iDA decaging reaction (**1g**–**1k**). To evaluate and compare their decaging activities, we first developed a fluorogenic TCO-conjugated coumarin reporter for facile detection of the decaging efficiency. 7-Amino-2-methylcoumarin was chosen because its fluorescence can be effectively quenched by protection of its amine group.[8] Reporter **2** was thus designed and synthesized (see the Supporting Information, Figure S1). As TZ-mediated decaging can liberate free 7-amino-2-methylcoumarin **3**, which leads to the restoration of its fluorescence, the decaging activity of TZ derivatives can be monitored by measuring the restored fluorescence intensity (Figure 1 B). Compounds **1a**–**1f** were first subjected to this fluorogenic assay to evaluate steric effects. As indicated by the restored fluorescence intensities in Figure 1 C, smaller alkyl groups led to higher decaging rates. The smallest alkyl TZ derivative, **1a**, exhibited the highest decaging activity whereas the presence of a bulky *tert*-butyl group almost completely inhibited the iDA decaging reaction.

Electron-rich and electron-deficient TZ derivatives (**1g** and **1h**) were examined next. Interestingly, neither **1g** nor **1h** was able to restore any fluorescence, indicating their poor efficiency in triggering the iDA decaging reaction. The lower

[*] Dr. X. Fan, Y. Ge, F. Lin, Y. Yang, G. Zhang, W. S. C. Ngai, Z. Lin, S. Zheng, J. Wang, Dr. J. Zhao, Dr. J. Li, Prof. Dr. P. R. Chen
Beijing National Laboratory for Molecular Sciences
Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education
Synthetic and Functional Biomolecules Center
College of Chemistry and Molecular Engineering
Peking University, Beijing 100871 (China)
E-mail: pengchen@pku.edu.cn
Dr. X. Fan, G. Zhang, J. Wang, Dr. J. Zhao, Prof. Dr. P. R. Chen
Peking-Tsinghua Center for Life Sciences
Beijing 100871 (China)

Supporting information for this article can be found under: http://dx.doi.org/10.1002/anie.201608009.

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

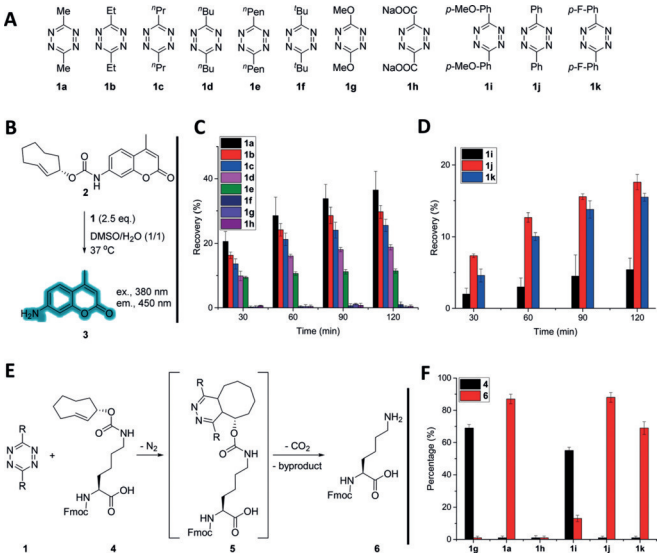


Figure 1. Systematic study of TZ derivatives in the iDA decaging reaction. A) Chemical structures of the differently substituted TZs. B) 7-Amino-2-methylcoumarin-based fluorogenic assay to evaluate the decaging activity of TZs. C) Fluoroscence of **2** restored by TZs bearing alkyl substituents. D) Fluoroscence of **2** restored by TZs bearing aryl groups. E) The iDA decaging reaction analyzed by LC-MS (**5** is a mixture of isomers resulting from tautomerization). Procedure: A mixture of **1** (0.5 μM) and **4** (0.2 μM, with internal standard) in 0.1 mL DMSO/H_2O (1:1) was allowed to react for 1 min at 37 °C; then the crude reaction mixture was directly injected into the LC-MS for analysis. F) Amounts of unreacted **4** and decaged product **6** after the iDA decaging reaction between **4** and TZs.

decaging activity of electron-rich TZs is probably due to the increased LUMO energy, which may hinder the initial cycloaddition step.[6e,9] The lower activity of electron-deficient TZs was, however, difficult to interpret as it is known that iDA cycloadditions can be accelerated by lowering the LUMO energy of the TZ.[6e,9] As aryl-substituted TZs are commonly used in iDA ligation reactions, we next tested the decaging efficiencies of aryl-substituted TZs (Figure 1 D).[1] The fluorescence was recovered to a small extent, indicating that these TZ derivatives possess only moderate decaging activity. Notably, both EDG- (**1i**) and EWG-substituted (**1k**) aryl-TZs displayed lower decaging activities than phenyl-TZ (**1j**), further indicating that both EDGs and EWGs hinder the decaging process.

The iDA decaging reaction includes two consecutive processes, that is, the initial iDA cycloaddition step and a subsequent elimination step. For a more comprehensive understanding of how the 3,6-substituents affect the reactivity of the TZ during these chemical processes, we determined the relative amounts of starting material and decaged product by LC-MS analysis. The reactions between different TZs and 2-ax-TCOK (**4**) were monitored (Figure 1 E). It is noteworthy that the decaging rate with TCOK (**4**) is generally much greater than that with coumarin **2**. A large amount of decaging product **6** was observed in the presence of **1a** or **1j**, indicating that both the cycloaddition and elimination steps proceeded efficiently (Figure 1 F). Unreacted **4** was observed as the major component when **1g** or **1i** was used, confirming that the poor decaging activity of EDG-substituted TZs is due to slow cycloaddition. For the reactions with EWG-substituted TZs, however, **4** was completely consumed while only a small amount of **6** could be detected. In particular, neither **4** nor **6** were observed in the LC spectrum when **1h** was tested. Instead, strong MS signals corresponding to cycloaddition intermediate **5** were detected (Figure S8). These results suggest that while EWGs can accelerate the cycloaddition step by lowering the LUMO energy, the elimination step is greatly suppressed. Together, the overall decaging activity of EWG-substituted TZs remains low. The isolation of **4** and **6** from various crude reaction mixtures corroborated the LC-MS results (Figure S10).

These intriguing findings prompted us to synthesize and test TZs bearing different substituents at the 3- and 6-position. For example, an EWG can be anchored at one side to accelerate the initial cycloaddition step while a small alkyl group is introduced at the other side to facilitate the subsequent elimination step. Such unsymmetric TZs may ultimately enhance the overall decaging rate (Figure 2 A).

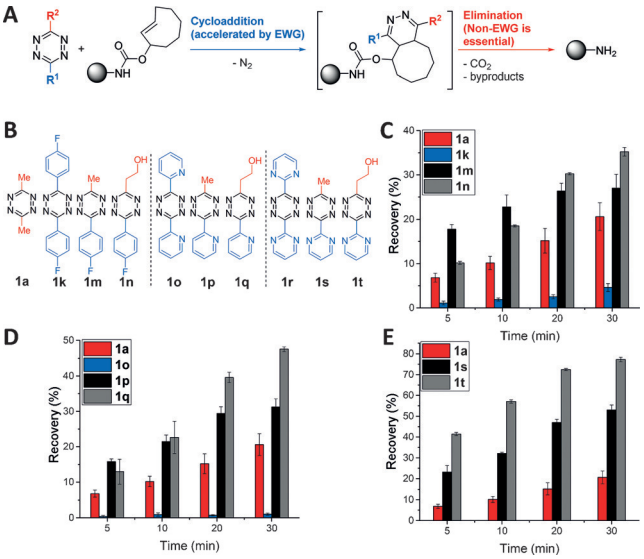


Figure 2. Unsymmetric TZs for rapid iDA-mediated decaging. A) The EWG in the TZ facilitates the initial cycloaddition step, and non-EWGs (preferably small alkyl groups) on the other side of the TZ render the subsequent elimination step efficient. B) Chemical structures of the designed unsymmetric TZs, with their symmetric analogues for comparison. C–E) Decaging activities evaluated with the fluorogenic assay. **1r** was not examined owing to its extremely poor solubility.

Several unsymmetric TZs (**1m**, **1n**, **1p**, **1q**, **1s**, and **1t**; Figure 2 B) were next synthesized, and their symmetric analogues (**1a**, **1k**, **1o**, and **1r**) were also prepared for comparison. The decaging activities of these TZ derivatives were evaluated using the coumarin assay. As expected, all of these TZs exhibited superior decaging activities compared with their symmetric counterparts (Figure 2 C–E). In particular, with **1t**, more than 80 % of the coumarin fluorescence was recovered within 30 min, whereas only 20 % of the fluorescence was restored for the most efficient decaging agent that is currently in use (**1a**). In general, hydroxyethyl-

 © 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

derived TZs (**1n**, **1q**, and **1t**) showed higher activities than methyl-substituted ones (**1m**, **1p**, and **1s**) in terms of the decaging rate.

The decaging activities of these new TZs were next examined on proteins. Purified GFP with TCOK at residue Y40 (GFP-Y40TCOK) was treated with **1s** and **1t** for 5 min followed by MS analysis. The disappearance of the GFP-Y40TCOK peak and the appearance of the GFP-Y40K peak indicated the high decaging efficiency of **1s** and **1t** for proteins (>90%). For comparison, only 58% of GFP-Y40TCOK were decaged by **1a** under the same conditions (Figure S2). As the decaging efficiency inside cells is affected by many factors, such as solubility, membrane permeability, stability, and accessibility to the reaction site as well as the intracellular activity of the decaging agent, we next tested the new TZs for protein decaging in living cells. A cytotoxicity study was first performed, which showed that all of the TZs (200 mM) exerted negligible toxicity on cells for up to 72 h (Figure S3).

Next, our previously developed firefly luciferase (fLuc) based decaging assay was used as the model protein system. The catalytic lysine residue (K529) on fLuc was replaced by the caged lysine 2-ax-TCOK by genetic code expansion,[10] which completely eliminated its catalytic function. Treatment with the decaging agent liberates free lysine, which restores the fLuc activity. The decaging efficiency can therefore be evaluated by measuring the recovered luminescence intensity (Figure 3A).[11] The most efficient decaging reagents obtained from the coumarin assay, **1s** and **1t**, were examined (Figure 3B). Remarkably, the enzyme activity was restored to 80% within 2 min by **1s**, indicating its extremely fast decaging efficacy in living cells. By comparison, **1a** was only able to restore 10% of the enzyme activity in 2 min. Moderate decaging efficiencies were observed for **1t**, which is probably due to its relatively poor membrane permeability owing to the presence of a hydroxy group.[12] We also tested **1p**, which has been used for elegant prodrug activation by Robillard and coworkers.[2] High activation efficiencies were achieved with **1p**, but they were still lower than that of **1s** (Figure 3B). Encouraged by the outstanding activity of **1s**, we next performed time- and concentration-dependent studies to further optimize the decaging conditions (Figure 3C and D). Approximately 90% of the fLuc activity could be recovered by treatment with a 50 μM solution of **1s** within 4 min (Figure 3D). As this decaging method is a gain-of-function approach from a clean background, even 10% enzyme activation efficiency may be sufficient for downstream studies.[13] We then examined the lowest concentration required for intracellular protein decaging. A decaging efficiency of 7% was achieved within 4 min in the presence of only 1 μM **1s**, whereas 18% decaging can be achieved with 2.5 μM **1s** (Figures 3D and S4).

To further confirm that **1s** enables decaging reactions on intracellular proteins, GFP-Y40TCOK expressed in HEK293T cells was subjected to **1s**-mediated decaging and LC-MS analysis. The conversion of GFP-Y40TCOK (27944 Da) into decaged protein GFP-Y40K (27791 Da) was very high (ca. 90%) in the presence of 50 μM **1s** after 4 min. A small peak assigned to the iDA cycloaddition

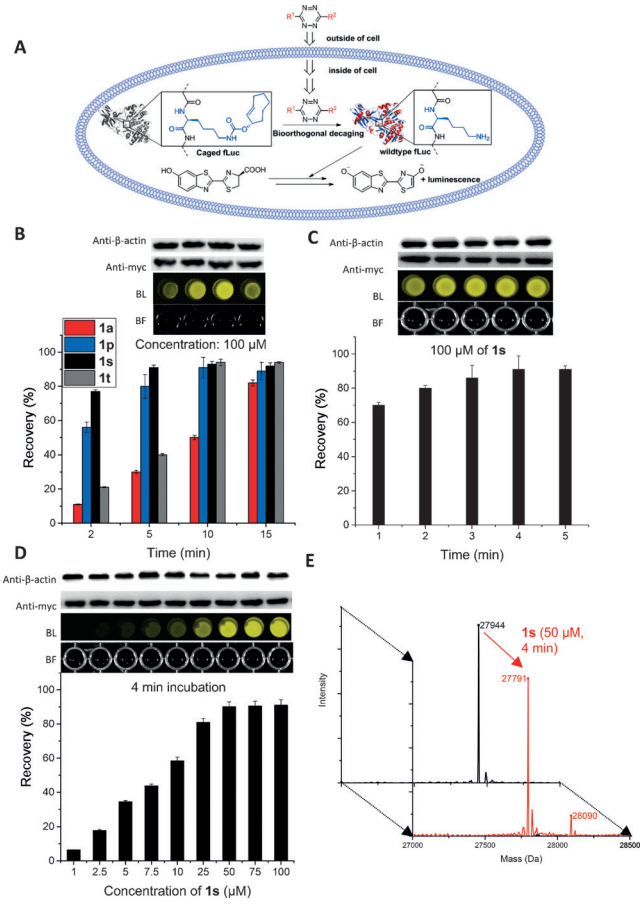


Figure 3. Protein activation with the optimized TZs in living cells. A) fLuc-K529TCOK activation in living cells. B) Comparison of the fLuc activation with different TZs. C) Time-course experiment of intracellular fLuc activation with **1s**: About 90% of the fLuc activity could be restored with 100 μM **1s** in as little as 4 min. D) Concentration dependence of intracellular fLuc activation with **1s**: About 90% of the fLuc activity could be restored within 4 min with 50 μM **1s**. E) MS analysis of the decaging reaction on GFP-Y40TCOK in living cells with **1s** (50 μM, 4 min). BF = bright field, BL = bioluminescence.

product (28090 Da) was also detected (Figures 3E and S2). For comparison, only 21% of decaged GFP was observed when **1a** was used under the same conditions (Figure S5), with unreacted GFP-Y40TCOK being the major component. Photodecaging reactions have been widely used for protein activation owing to their fast decaging rates.[5] To compare our new TZ with the photodecaging approach, cells expressing GFP-Y40ONBK[14] were subjected to UV irradiation (365 nm, ca. 8 mW cm^{-2}) for 4 min before purification and analysis by LC-MS. Only 38% of the GFP had undergone photodecaging, which is higher than the conversion achieved with **1a** (50 μM, 4 min), but lower than that for **1s**-mediated chemical decaging (50 μM, 4 min; Figure S6).

In conclusion, we have developed a coumarin-based fluorogenic assay to compare the iDA decaging reactions triggered by a range of TZs bearing different substituents with various steric and electronic properties. We found that small and relatively electron-neutral groups offer better decaging reagents. Subsequent LC-MS analysis suggested that the

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

initial cycloaddition step is hindered by EDGs but accelerated by EWGs, whereas the following elimination step was strongly suppressed by EWGs. Based on these observations, we designed and synthesized a panel of unsymmetric TZs bearing an EWG and a small alkyl group at the 3- and 6-position, respectively. Remarkably, all of these TZs showed significantly enhanced decaging activities compared with their symmetric counterparts. In particular, **1t** showed excellent decaging efficiency in vitro whereas **1s** showed the highest decaging activity inside living cells (> 90% recovery with 50 μM **1s** within 4 min). Further studies demonstrated that a concentration as low as 1 μM is sufficient for intracellular protein activation with **1s**. The decaging efficiency was further confirmed by studies with GFP, and found to be even better than that of the photodecaging approach using UV irradiation. Together, our optimized TZ derivatives allow for rapid chemical decaging and the recovery of protein activity within a complex intracellular environment.

Experimental Section

A tetrazine (0.025 μmol) was dissolved in a mixture of DMSO and H_2O. Compound **2** (0.01 μmol) was added to this mixture. The reaction mixture was shaken at 37°C. The fluorescence intensity (F1) was measured at given time points ($\lambda_{ex} = 380$ nm, $\lambda_{em} = 450$ nm). As a control experiment, the fluorescence (F2) for the maximum amount of coumarin that could be released from probe **2** was also measured under the same conditions. The decaging efficiency (%) was calculated according to (F1/F2) × 100%.

Acknowledgements

This work was supported by the National Key Research and Development Program (2016YFA0501500) and the National Natural Science Foundation of China (21225206, 21432002, and 21521003). X.F. thanks the Center for Life Sciences (CLS) and the China Postdoctoral Science Foundation (2016M591004) for support.

Keywords: amino acids · decaging · Diels–Alder reactions · protein activation · protein engineering

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 14046–14050
Angew. Chem. **2016**, *128*, 14252–14256

[1] a) M. L. Blackman, M. Royzen, J. M. Fox, *J. Am. Chem. Soc.* **2008**, *130*, 13518–13519; b) N. K. Devaraj, R. Weissleder, S. A. Hilderbrand, *Bioconjugate Chem.* **2008**, *19*, 2297–2299; c) N. K. Devaraj, S. Hilderbrand, R. Upadhyay, R. Mazitschek, R. Weissleder, *Angew. Chem. Int. Ed.* **2010**, *49*, 2869–2872; *Angew. Chem.* **2010**, *122*, 2931–2934; d) J. C. Jewett, C. R. Bertozzi, *Chem. Soc. Rev.* **2010**, *39*, 1272–1279; e) R. Rossin, P. R. Verkerk, S. M. van den Bosch, R. C. Vulders, I. Verel, J. Lub, M. S. Robillard, *Angew. Chem. Int. Ed.* **2010**, *49*, 3375–3378; *Angew. Chem.* **2010**, *122*, 3447–3450; f) B. M. Zeglis, P. Mohindra, G. I. Weissmann, V. Divilov, S. A. Hilderbrand, R. Weissleder, J. S. Lewis, *Bioconjugate Chem.* **2011**, *22*, 2048–2059; g) D. S. Liu, A. Tangpeerachaikul, R. Selvaraj, M. T. Taylor, J. M. Fox, A. Y. Ting, *J. Am. Chem. Soc.* **2012**, *134*, 792–795; h) J. L. Seitchik, J. C. Peeler, M. T. Taylor, M. L. Blackman, T. W. Rhoads, R. B. Cooley, C. Refakis, J. M. Fox, R. A. Mehl, *J. Am. Chem. Soc.* **2012**, *134*, 2898–2901; i) M. Grammel, H. C. Hang, *Nat. Chem. Biol.* **2013**, *9*, 475–484; j) A.-C. Knall, C. Slugovc, *Chem. Soc. Rev.* **2013**, *42*, 5131–5142; k) R. Selvaraj, J. M. Fox, *Curr. Opin. Chem. Biol.* **2013**, *17*, 753–760; l) I. Nikić, T. Plass, O. Schraidt, J. Szymański, J. A. Briggs, C. Schultz, E. A. Lemke, *Angew. Chem. Int. Ed.* **2014**, *53*, 2245–2249; *Angew. Chem.* **2014**, *126*, 2278–2282; m) J. W. Wollack, B. J. Monson, J. K. Dozier, J. J. Dalluge, K. Poss, S. A. Hilderbrand, M. D. Distefano, *Chem. Biol. Drug Des.* **2014**, *84*, 140–147; n) D. A. MacKenzie, A. R. Sherratt, M. Chigrinova, L. L. Cheung, J. P. Pezacki, *Curr. Opin. Chem. Biol.* **2014**, *21*, 81–88; o) D. M. Patterson, L. A. Nazarova, J. A. Prescher, *ACS Chem. Biol.* **2014**, *9*, 592–605; p) A. Sachdeva, K. Wang, T. Elliott, J. W. Chin, *J. Am. Chem. Soc.* **2014**, *136*, 7785–7788; q) R. J. Blizzard, D. R. Backus, W. Brown, C. G. Bazewicz, Y. Li, R. A. Mehl, *J. Am. Chem. Soc.* **2015**, *137*, 10044–10047; r) H. E. Murrey, J. C. Judkins, C. W. A. Ende, T. E. Ballard, Y. Z. Fang, K. Riccardi, L. Di, E. R. Guilmette, J. W. Schwartz, J. M. Fox, D. S. Johnson, *J. Am. Chem. Soc.* **2015**, *137*, 11461–11475.

[2] a) R. M. Versteegen, R. Rossin, W. ten Hoeve, H. M. Janssen, M. S. Robillard, *Angew. Chem. Int. Ed.* **2013**, *52*, 14112–14116; *Angew. Chem.* **2013**, *125*, 14362–14366; b) R. Rossin, S. M. van Duijnhoven, W. Ten Hoeve, H. M. Janssen, L. H. Kleijn, F. J. Hoeben, R. M. Versteegen, M. S. Robillard, *Bioconjugate Chem.* **2016**, *27*, 1697–1706; c) J. M. Mejia Oneto, I. Khan, L. Seebald, M. Royzen, *ACS Cent. Sci.* **2016**, *2*, 476–482.

[3] a) J. Li, S. Jia, P. R. Chen, *Nat. Chem. Biol.* **2014**, *10*, 1003–1005; b) G. Zhang, J. Li, R. Xie, X. Fan, Y. Liu, S. Zheng, Y. Ge, P. R. Chen, *ACS Cent. Sci.* **2016**, *2*, 325–331.

[4] H. Wu, S. C. Alexander, S. Jin, N. K. Devaraj, *J. Am. Chem. Soc.* **2016**, *138*, 11429–11432.

[5] a) N. Wu, A. Deiters, T. A. Cropp, D. King, P. G. Schultz, *J. Am. Chem. Soc.* **2004**, *126*, 14306–14307; b) A. Deiters, D. Groff, Y. Ryu, J. Xie, P. G. Schultz, *Angew. Chem. Int. Ed.* **2006**, *45*, 2728–2731; *Angew. Chem.* **2006**, *118*, 2794–2797; c) J. Hemphill, C. J. Chou, J. W. Chin, A. Deiters, *J. Am. Chem. Soc.* **2013**, *135*, 13433–13439; d) J. Y. Kang, D. Kawaguchi, I. Coin, Z. Xiang, D. D. O'Leary, P. A. Slesinger, L. Wang, *Neuron* **2013**, *80*, 358–370; e) A. S. Baker, A. Deiters, *ACS Chem. Biol.* **2014**, *9*, 1398–1407; f) J. Luo, R. Uprety, Y. Naro, C. J. Chou, D. P. Nguyen, J. W. Chin, A. Deiters, *J. Am. Chem. Soc.* **2014**, *136*, 15551–15558; g) W. Ren, A. Ji, H. W. Ai, *J. Am. Chem. Soc.* **2015**, *137*, 2155–2158.

[6] a) J. Sauer, D. K. Heldmann, J. Hetzenegger, J. Krauthan, H. Sichert, J. Schuster, *Eur. J. Org. Chem.* **1998**, 2885–2896; b) M. L. Blackman, M. Royzen, J. M. Fox, *J. Am. Chem. Soc.* **2008**, *130*, 3760–3761; c) M. T. Taylor, M. L. Blackman, O. Dmitrenko, J. M. Fox, *J. Am. Chem. Soc.* **2011**, *133*, 9646–9649; d) A. Darko, S. Wallace, O. Dmitrenko, M. M. Machovina, R. A. Mehl, J. W. Chin, J. M. Fox, *Chem. Sci.* **2014**, *5*, 3770–3776; e) M. R. Karver, R. Weissleder, S. A. Hilderbrand, *Bioconjugate Chem.* **2011**, *22*, 2263–2270.

[7] a) J. Yang, M. R. Karver, W. Li, S. Sahu, N. K. Devaraj, *Angew. Chem. Int. Ed.* **2012**, *51*, 5222–5225; *Angew. Chem.* **2012**, *124*, 5312–5315; b) H. Wu, J. Yang, J. Šečkutė, N. K. Devaraj, *Angew. Chem. Int. Ed.* **2014**, *53*, 5805–5809; *Angew. Chem.* **2014**, *126*, 5915–5919.

[8] S. S. Matikonda, D. L. Orsi, V. Staudacher, I. A. Jenkins, F. Fiedler, J. Chen, A. B. Gamble, *Chem. Sci.* **2015**, *6*, 1212–1218.

[9] a) J. Schoch, M. Staudt, A. Samanta, M. Wiessler, A. Jäschke, *Bioconjugate Chem.* **2012**, *23*, 1382–1386; b) Y. Liang, J. L. Mackey, S. A. Lopez, F. Liu, K. N. Houk, *J. Am. Chem. Soc.* **2012**, *134*, 17904–17907; c) R. A. Foster, M. C. Willis, *Chem. Soc. Rev.* **2013**, *42*, 63–76; d) J. A. Wagner, D. Mercadante, I. Nikić, E. A. Lemke, F. Gräter, *Chem. Eur. J.* **2015**, *21*, 12431–12435; e) Y. F. Yang, Y. Liang, F. Liu, K. N. Houk, *J. Am. Chem. Soc.* **2016**, *138*, 1660–1667.

 © 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[10] a) Y.-X. Chen, G. Triola, H. Waldmann, *Acc. Chem. Res.* **2011**, *44*, 762 – 773; b) C. H. Kim, J. Y. Axup, P. G. Schultz, *Curr. Opin. Chem. Biol.* **2013**, *17*, 412 – 419; c) K. Lang, J. W. Chin, *Chem. Rev.* **2014**, *114*, 4764 – 4806; d) G. Zhang, S. Zheng, H. Liu, P. R. Chen, *Chem. Soc. Rev.* **2015**, *44*, 3405 – 3417.
[11] J. Zhao, S. Lin, Y. Huang, J. Zhao, P. R. Chen, *J. Am. Chem. Soc.* **2013**, *135*, 7410 – 7413.
[12] Y. Kim, Y. Choi, R. Weissleder, C. H. Tung, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5054 – 5057.
[13] J. A. Zorn, J. A. Wells, *Nat. Chem. Biol.* **2010**, *6*, 179 – 188.
[14] ONBK = N^6-(((2-nitrobenzyl)oxy)carbonyl)-L-lysine, a commonly used photocaged lysine.

Received: August 16, 2016
Published online: October 13, 2016

 © 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim