

## Rapid Cross-Metathesis for Reversible Protein Modifications via Chemical Access to Se-Allyl-selenocysteine in Proteins

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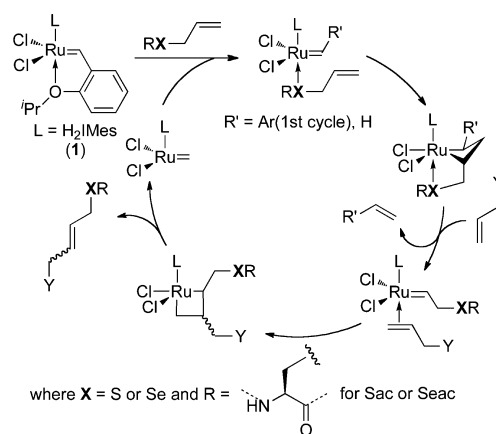
**S** Supporting Information

**ABSTRACT:** Cross-metathesis (CM) has recently emerged as a viable strategy for protein modification. Here, efficient protein CM has been demonstrated through biomimetic chemical access to Se-allyl-selenocysteine (Seac), a metathesis-reactive amino acid substrate, via dehydroalanine. On-protein reaction kinetics reveal a rapid reaction with rate constants of Seac-mediated-CM comparable or superior to off-protein rates of many current bioconjugations. This use of Se-relayed Seac CM on proteins has now enabled reactions with substrates (allyl GlcNAc, *N*-allyl acetamide) that were previously not possible for the corresponding sulfur analogue. This CM strategy was applied to histone proteins to install a mimic of acetylated lysine (KAc, an epigenetic marker). The resulting synthetic H3 was successfully recognized by antibody that binds natural H3-K9Ac. Moreover, Cope-type selenoxide elimination allowed this putative marker (and function) to be chemically expunged, regenerating an H3 that can be rewritten to complete a chemically enabled “write (CM)–erase (ox)–rewrite (CM)” cycle.

Developing strategies for site-specific protein modifications is an ongoing challenge in chemical biology.<sup>1–3</sup> For over a decade, allylic alcohols have been identified as reactive in olefin metathesis.<sup>4,5</sup> However, only in recent years has a generalized allylic heteroatom effect begun to emerge in cross-metathesis (CM).<sup>5</sup> A mechanism involving pre-coordination to the metal center of catalyst **1** has been proposed to explain the observed reactivity of privileged substrates, such as amino acid S-allyl-cysteine (Sac, Scheme 1).<sup>6</sup> This “heteroatom-relay” has enabled CM to be applied as a bioconjugation technique in selective protein modifications,<sup>7–11</sup> and so greatly increased the benchmark for complexity in the substrate scope. Such putative beneficial effects of allylic chalcogens suggest a likely increase in the reactivity of higher chalcogens as Lewis bases that may be more preferred, softer ligands for ruthenium complexes. Indeed, simple allyl selenides are reactive substrates in aqueous CM.<sup>11</sup> We show here that installation into peptides and proteins of the simplest amino acid residue containing an allyl selenide moiety, the unnatural amino acid Se-allyl-selenocysteine (Seac), enables rapid and efficient CM.

Possible approaches to incorporating Seac into proteins were considered that relied on installation of the Se heteroatom either co- (either using Seac directly or from a precursor such as selenocysteine (Sec)) or post-translationally. Direct co-transla-

**Scheme 1.** Allyl Chalcogen-Assisted Cross-Metathesis

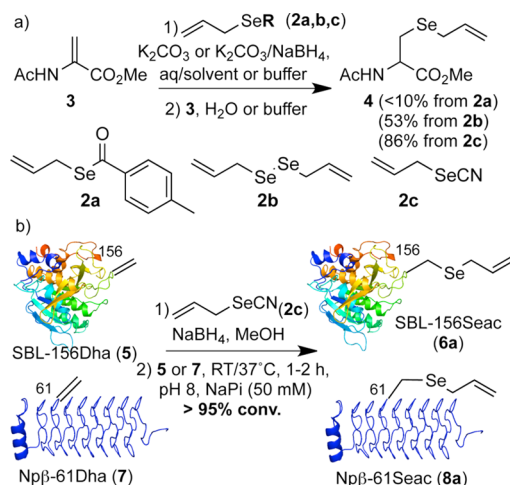


tional incorporation of Seac has not yet been demonstrated, likely due to rapid oxidative cellular metabolism, also observed for selenomethionine and S-methyl-selenocysteine.<sup>12–14</sup> Despite the precedence for incorporation of Sec into recombinant proteins in *Escherichia coli*, this approach requires more complex genetic manipulation of both DNA and RNA<sup>15,16</sup> that limits widespread utility. Moreover, our attempts to convert Sec to Seac via a desulfurative 2,3-sigmatropic rearrangement failed in model systems (see Supporting Information (SI) p S9). Notably, a recent study has revealed a biosynthetic mechanism for selenocysteine generation via conjugate C–Se bond formation from a dehydroalanyl-tRNA<sup>Sec</sup> intermediate.<sup>17</sup> Inspired by nature, we therefore considered an alternative biomimetic route to incorporate Seac into proteins via dehydroalanine (Dha), which has recently emerged as a versatile chemical handle for protein modifications.<sup>18–22</sup>

First, the generation of an appropriate conjugate, allyl selenolate nucleophile, was explored on model Dha 3 (Scheme 2a). *In situ* generation of allyl selenolate via cleavage of methyl benzoselenoate **2a** failed (Table S1). Next, we considered reductive access. Diallyl diselenide (**2b**) is unstable and exists in mixture with diallyl selenide.<sup>23</sup> Moreover, allyl selenolate has previously only been reductively generated under aprotic conditions.<sup>23</sup> Nevertheless, treatment of **2b** with a stoichiometric amount of NaBH<sub>4</sub> in degassed MeOH generated allyl selenolate; subsequent addition of this solution to Dha 3 in water yielded

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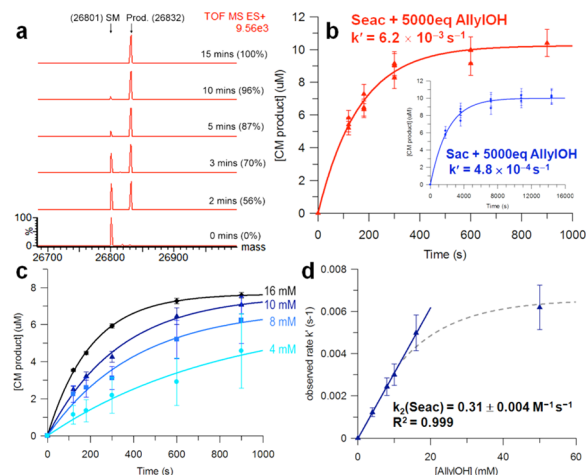
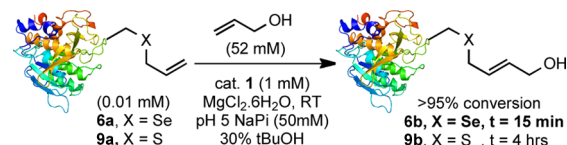
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**Scheme 2. Biomimetic Synthesis of Se-Allyl-selenocysteine via Conjugate Addition of Allyl Selenolate to Dehydroalanine (Table S1)**


53% of the desired Seac product **4** (Table S1). Allyl selenocyanate (**2c**), a potentially more stable,<sup>24</sup> alternative allyl selenolate source, was investigated next. Pleasingly, due to cleaner pre-reduction, the desired addition product **4** was obtained with an improved yield of 86% after 1 h at room temperature (Scheme 2a). Notably, although allyl selenolate is sensitive to acidic conditions (allylselenol quickly decomposes to **2b** in the absence of radical inhibitor<sup>23</sup>), under buffered basic conditions the lifetime of allyl selenolate is apparently long enough to successfully react with Dha in aqueous media.

With promising results observed on amino acid models, we next explored installation of Seac via this biomimetic strategy in two differing protein structural motifs/folds: three-layer  $\alpha/\beta$ -Rossmann-fold protein subtilisin from *Bacillus lentus* (SBL) and all- $\beta$ -helix protein 275–276 from *Nostoc punctiforme* (termed Npβ),<sup>25</sup> respectively. Accordingly, Dha substrate proteins SBL-156Dha (**5**) and Npβ-61Dha (**7**) were synthesized from corresponding cysteine mutants following reported bis-alkylative elimination procedures.<sup>21</sup> Use of selenolate derived from **2a** or **2b** failed on proteins and led to only a mixture of products. However, allyl selenolate solution generated from **2c** allowed successful biomimetic conjugate addition (Scheme 2b); LC-MS analysis revealed >95% conversion of SBL-156Dha (**5**) to the desired product SBL-156Seac (**6a**) after 1 h at room temperature. Even addition to the more sterically demanding<sup>26</sup> 61 site in Npβ-61Dha (**7**) single Dha mutant under slightly elevated temperature (37 °C) afforded expected Seac-containing protein with >95% conversion after 2 h (**8a**, see SI). Importantly, resulting SBL-derivative proteins retained fold and functional (peptidase) activity (see SI p S23). Generation of Dha and addition likely leads to the formation of a 1:1 dr of adducts.<sup>26</sup>

With model Seac-tagged protein **6a** in hand, CM with allyl alcohol was tested using catalyst **1** under previously optimized reaction conditions<sup>11</sup> (which can be used at pH 4–8). This reaction was strikingly rapid and was complete after only 15 min at room temperature (Scheme 3). In comparison, the sulfur-equivalent protein substrate SBL-156Sac (**9a**) required 4 h to reach completion under the same reaction conditions. Rapid protein modification reactions are rare but powerful.<sup>27,28</sup> We conducted a quantitative comparison of the enhanced rate observed here for CM of SBL-156Seac with popular bioconjugation techniques such as Staudinger ligation,<sup>29,30</sup>

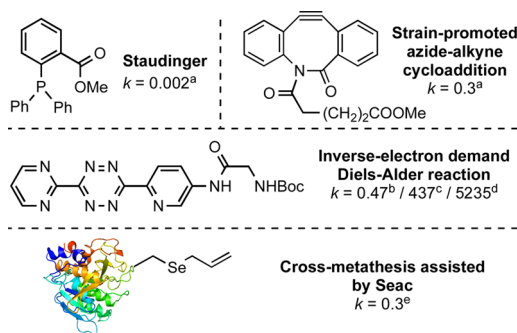
**Scheme 3. Enhanced CM of Seac-SBL **6a**, cf. Sac-SBL **9a****


**Figure 1.** Kinetic studies of CM between SBL-156Seac (**6a**) and allyl alcohol. (a) LC-MS of reaction showing conversions over time. (b) Changes in [product] as a function of time. Inset shows kinetics of CM with SBL-156Sac (**9a**); note difference in time scale. Conditions: [protein] = 0.01 mM, [cat.] = 1 mM, [allyl alcohol] = 52 mM, [MgCl<sub>2</sub>·6H<sub>2</sub>O] = 26 mM in 3:7 tBuOH/NaPi (50 mM, pH 5) at room temperature. (c) CM of **6a** with various concentrations of allyl alcohol. (d) Determination of the second-order CM rate constant for **6a**.

azide–alkyne cycloaddition,<sup>31,32</sup> and inverse electron-demand Diels–Alder cycloaddition,<sup>33–36</sup> which have typically been studied in small-molecule models (“off-protein”). Under pseudo-first-order conditions with respect to the protein, starting material and product were monitored by LC-MS at several time points during reactions (Figure 1a).<sup>37</sup> Data analyzed using nonlinear, single-exponential regression gave pseudo-first-order rate constants (Figure 1b).

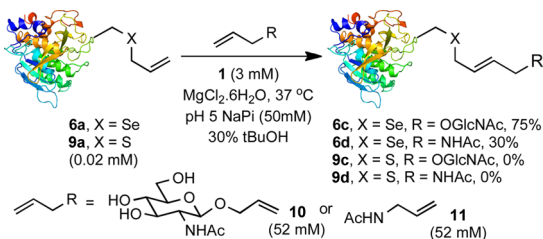
Determination of pseudo-first-order rate constants at various allyl alcohol concentrations and analysis of the results using linear regression revealed second-order rate constants for Seac-mediated CM. The on-protein second-order rate constant for the CM of allyl alcohol with Seac-protein was determined to be  $0.31 \pm 0.004 \text{ M}^{-1} \text{ s}^{-1}$  (Figure 1c,d), whereas the value for on-protein Sac-mediated CM in an identical protein context (site 156) was some 10-fold lower ( $0.031 \pm 0.0015 \text{ M}^{-1} \text{ s}^{-1}$ ) under the same conditions (Figures S21 and S22). Interestingly, Figure 1d indicated an initially linear increase in observed rate with respect to allyl alcohol concentration and gradually plateaued at concentrations >20 mM; this suggests that the catalyst plays a critical role under these conditions, presumably becoming “saturated” at high concentrations of allyl alcohol, leading to a shift in rate-limiting step (see also SI section 2.10 for off-protein catalyst dependency). Preliminary computations (using methyl allyl chalcogenides, see SI section 3) suggest a reaction profile in which the Ru-carbene intermediate after “Se-relay” is unusually stable and enhanced in stability over “S-relay”; this intermediate is also accessed through a lowered transition state.

When comparing the second-order rate constants with those from widely used bioconjugation techniques in chemical biology,



**Figure 2.** Selected rate constants ( $M^{-1} s^{-1}$ ) of conjugations. All rate constants are second-order in units of  $M^{-1} s^{-1}$  with <sup>a</sup>benzyl azide, <sup>b</sup>norbornene, <sup>c</sup>bicyclo[6.1.0]non-4-yn-9-ylmethanol, <sup>d</sup>*trans*-cyclooctene, and <sup>e</sup>allyl alcohol.

#### Scheme 4. Cross-Metathesis of SBL 6a and 9a with Challenging Substrates 10 and 11

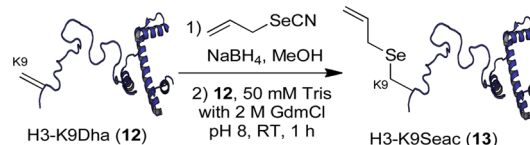


the rate of on-protein Seac-assisted CM was found to be  $\sim 150$ -fold faster than that of off-protein Staudinger ligation ( $k = 0.002 M^{-1} s^{-1}$ )<sup>38</sup> and comparable to faster variants of off-protein strain-promoted azide–alkyne cycloaddition ( $k = 0.002$ – $0.9 M^{-1} s^{-1}$ )<sup>39–41</sup> and certain tetrazine-based cycloadditions ( $k > 0.15 M^{-1} s^{-1}$ ).<sup>27</sup> Notably, however, recent select examples of catalyzed oxime ligations<sup>42</sup> and tetrazine-based reactions are still more rapid (Figure 2).<sup>27,28,43,44</sup> It seems likely that local environment strongly affects on-protein reactions compared with off-protein; indeed, here, crowded (e.g., Np $\beta$ -61Seac) sites showed negligible reactivity (see SI). However, detailed on-protein measurements are rare,<sup>42,44</sup> so other relevant direct comparisons are not possible. Although here directly comparable reaction conditions could not be achieved, we extrapolate (see SI section 2.10) off-protein rates for Seac-mediated CM that are at least comparable and may be enhanced, consistent with CM's steric sensitivity.<sup>5</sup>

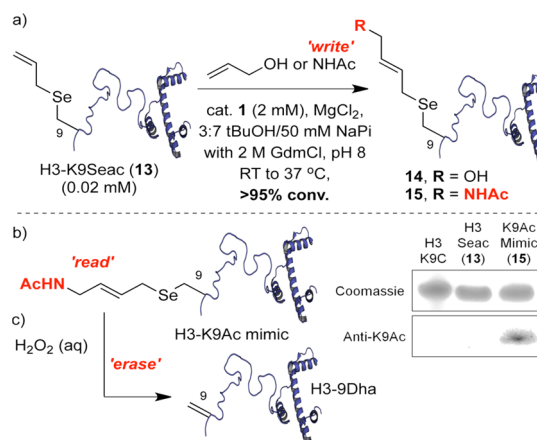
Next, having established usefully enhanced on-protein rates, we examined substrate breadth. Model Seac-tagged protein SBL-156Seac **6a** was tested with challenging metathesis substrate GlcNAc **10**; this olefin is essentially unreactive in sulfur-relayed CM with SBL-156Sac (**9a**). Pleasingly, reaction reached 75% conversion after 1.5 h at 37 °C (Scheme 4). Another biologically relevant substrate, *N*-allyl acetamide (**11**), was used in CM with protein **6a**. Acetamide **11** has been suggested to poison catalyst **1**, and also fails with SBL-156Sac (**9a**).<sup>11,45,46</sup> Despite poisoning,<sup>47</sup> after 20 min at 37 °C, LC-MS revealed conversion to desired **6d** (Scheme 4), albeit in 30% yield.

After demonstrating CM on SBL-Seac, we tested Se-relayed CM on more functionally relevant protein systems. Histone proteins are key chromatin components; post-translational modifications (e.g. K-Ac) are critical regulators of structure and function.<sup>48,49</sup> First, Seac was installed into key epigenetic site 9 of histone H3. After generation of H3-9Dha,<sup>22</sup> subsequent allyl

#### Scheme 5. Synthesis of H3-9Seac (13)



#### Scheme 6. CM Reactions on H3-9Seac and the Chemical “Write–Read–Erase” Cycle via a K9Ac Mimic “Mark” and Anti-K9Ac Western Blots of H3 Proteins (Also See SI)



selenolate addition to Dha **12** proceeded successfully to yield Seac protein **13** after 1 h at room temperature (Scheme 5).

With H3-9Seac (**13**) in hand, we carried out model CM with allyl alcohol. Coloration implying metal coordination to **13** was observed; such coordination<sup>6,50</sup> can necessitate metal scavenging prior to MS analysis. Use of 3-mercaptopropionic acid<sup>51,50</sup> allowed ready MS monitoring of H3 CM reactions. Pleasingly, this revealed CM of H3-K9Seac and allyl alcohol with nearly full conversion to CM-modified H3 **14** (Scheme 6a). This ability to modify site 9 suggested a strategy for chemical recapitulation (under benign conditions) of a “write–read–erase” cycle that is observed (enzyme-mediated) in histone epigenetics.<sup>48,49</sup> As a “write” step we used the more challenging *N*-allyl acetamide olefin in CM; complete conversion was observed to **15** (Scheme 6a). As a “read” step, CM-modified H3 (**15**) was analyzed by an antibody raised to natural epigenetic marker *N*-acetyl lysine at position 9 of H3. We were pleased to find that this antibody successfully cross-reacted and recognized the CM-installed modification at position 9 as a K9Ac PTM mimic (Scheme 6b). Next, as an “erase” step the CM-installed K9Ac mimic “mark” was removed with full conversion using Cope-type elimination through the generation of labile selenoxide using mild peroxide oxidation to regenerate Dha. Met-containing H3 may be prone to side reaction;<sup>52</sup> this was tested and confirmed through the use of a Met-free H3 variant in which the mark was cleanly expunged without side reaction (see SI sections 2.11–2.14). Finally, a full chemical “write–read–erase–rewrite–read” cycle was performed (SI section 2.12) to demonstrate iterative histone “switching”.

In conclusion, we have demonstrated efficient chemical incorporation of Seac into proteins. This has facilitated rapid cross-metathesis for protein modifications; determination of on-protein rates shows that these outstrip or are comparable to many of the so-called “click” reactions in chemical biology. Using such allyl selenide (Seac) proteins, we were able to access a broader substrate scope in Se-relayed CM than was possible with

allyl sulfides. Direct access to Sec in proteins creates opportunities for potential PTM mimicry via CM as demonstrated here: a K9Ac mimic was successfully installed, recognized, and removed (write—read—erase) in histone H3. Although, to the best of our knowledge, such a chemical “write—read—erase” approach has not been explored before, one could envisage a system based on creation, e.g., of disulfide-linked modification mimics<sup>53</sup> and subsequent reductive cleavage. This synthetic manipulation of biology also highlights opportunities for new metathesis catalysts; uses of CM on protein/cell systems may be enabled by tuned solubilities, compatibilities, and permeabilities. We note too that this work provides access to a protected (allylated) Sec in proteins (that could be revealed using, e.g., Pd(0))<sup>54</sup> and also further motivates developments in genetic incorporation of allyl chalcogenide amino acids into proteins to access more generally the wider biological applications of cross-metathesis.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Full procedures and protein ESI-MS. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- (1) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974.
- (2) Chalker, J. M.; Bernardes, G. J. L.; Davis, B. G. *Acc. Chem. Res.* **2011**, *44*, 730.
- (3) Takaoka, Y.; Ojida, A.; Hamachi, I. *Angew. Chem., Int. Ed.* **2013**, *52*, 4088.
- (4) Hoye, T. R.; Zhao, H. *Org. Lett.* **1999**, *1*, 1123.
- (5) Lin, Y. A.; Davis, B. G. *Beilstein J. Org. Chem.* **2010**, *6*, 1219.
- (6) Lin, Y. A.; Chalker, J. M.; Floyd, N.; Bernardes, G. J. L.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 9642.
- (7) Brik, A. *Adv. Synth. Catal.* **2008**, *350*, 1661.
- (8) Kirshenbaum, K.; Arora, P. S. *Nat. Chem. Biol.* **2008**, *4*, 527.
- (9) Lin, Y. A.; Chalker, J. M.; Davis, B. G. *ChemBioChem* **2009**, *10*, 959.
- (10) Ai, H.-W.; Shen, W.; Brustad, E.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2010**, *49*, 935.
- (11) Lin, Y. A.; Chalker, J. M.; Davis, B. G. *J. Am. Chem. Soc.* **2010**, *132*, 16805.
- (12) Foster, S. J.; Kraus, R. J.; Ganther, H. E. *Arch. Biochem. Biophys.* **1986**, *251*, 77.
- (13) Ganther, H. E. *Carcinogenesis* **1999**, *20*, 1657.
- (14) Stolz, J. F.; Basu, P.; Santini, J. M.; Oremland, R. S. *Annu. Rev. Microbiol.* **2006**, *60*, 107.
- (15) Arnér, E. S. J.; Sarioglu, H.; Lottspeich, F.; Holmgren, A.; Böck, A. *J. Mol. Biol.* **1999**, *292*, 1003.
- (16) Aldag, C.; Bröcker, M. J.; Hohn, M. J.; Prat, L. H., G.; Plummer, A.; Söll, D. *Angew. Chem., Int. Ed.* **2013**, *52*, 1441.
- (17) Palioura, S.; Sherrer, R. L.; Steitz, T. A.; Söll, D.; Simonovic, M. *Science* **2009**, *325*, 321.
- (18) Levengood, M.; van der Donk, W. A. *Nat. Protoc.* **2007**, *1*, 3001.
- (19) Wang, J.; Schiller, S. M.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2007**, *46*, 6849.
- (20) Bernardes, G. J. L.; Chalker, J. M.; Errey, J. C.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 5052.
- (21) Chalker, J. M.; Gunnoo, S. B.; Boutoureira, O.; Gerstberger, S. C.; Fernández-González, M.; Bernardes, G. J. L.; Griffin, L.; Hailu, H.; Schofield, C. J.; Davis, B. G. *Chem. Sci.* **2011**, *2*, 1666.
- (22) Chalker, J. M.; Lercher, L.; Rose, N. R.; Schofield, C. J.; Davis, B. G. *Angew. Chem., Int. Ed.* **2012**, *51*, 1835.
- (23) Riague, E. H.; Guillemin, J.-C. *Organometallics* **2001**, *21*, 68.
- (24) Crich, D.; Krishnamurthy, V.; Brebion, F.; Karatholuvhu, M.; Subramanian, V.; Hutton, T. K. *J. Am. Chem. Soc.* **2007**, *129*, 10282.
- (25) Vetting, M. W.; Hegde, S. S.; Hazleton, K. Z.; Blanchard, J. S. *Protein Sci.* **2007**, *16*, 755.
- (26) Fernández-González, M.; Boutoureira, O.; Bernardes, G. J. L.; Chalker, J.; Young, M.; Errey, J.; Davis, B. G. *Chem. Sci.* **2010**, *1*, 709.
- (27) Lang, K.; Davis, L.; Torres-Kolbus, J.; Chou, C.; Deiters, A.; Chin, J. W. *Nat. Chem.* **2012**, *4*, 298.
- (28) Lang, K.; Davis, L.; Wallace, S.; Mahesh, M.; Cox, D. J.; Blackman, M. L.; Fox, J. M.; Chin, J. W. *J. Am. Chem. Soc.* **2012**, *134*, 10317.
- (29) Köhn, M.; Breinbauer, R. *Angew. Chem., Int. Ed.* **2004**, *43*, 3106.
- (30) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.
- (31) Hong, V.; Steinmetz, N. F.; Manchester, M.; Finn, M. G. *Bioconjugate Chem.* **2010**, *21*, 1912.
- (32) van Hest, J. C. M.; van Delft, F. L. *ChemBioChem* **2011**, *12*, 1309.
- (33) Blackman, M. L.; Royzen, M.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 13518.
- (34) Devaraj, N. K.; Weissleder, R.; Hilderbrand, S. A. *Bioconjugate Chem.* **2008**, *19*, 2297.
- (35) Schoch, J.; Wießler, M.; Jäschke, A. *J. Am. Chem. Soc.* **2010**, *132*, 8846.
- (36) Devaraj, N. K.; Weissleder, R. *Acc. Chem. Res.* **2011**, *44*, 816.
- (37) Aliquots taken, quenched (Sephadex G-25) prior to LC-MS. No precipitation observed; % conversion used for concentrations. For validation of ESI-MS in protein reaction monitoring see ref 11.
- (38) Lin, F. L.; Hoyt, H. M.; van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686.
- (39) Debets, M. F.; van Berkel, S. S.; Dommerholt, J.; Dirks, A. J.; Rutjes, F. P. J. T.; van Delft, F. L. *Acc. Chem. Res.* **2011**, *44*, 805.
- (40) Debets, M. F.; van Berkel, S. S.; Schoffelen, S.; Rutjes, F. P. J. T.; van Hest, J. C. M.; van Delft, F. L. *Chem. Commun.* **2010**, *46*, 97.
- (41) Jewett, J. C.; Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 3688.
- (42) Rashidian, M.; Mahmoodi, M. M.; Shah, R.; Dozier, J. K.; Wagner, C. R.; Distefano, M. D. *Bioconjugate Chem.* **2013**, *24*, 333.
- (43) Plass, T.; Milles, S.; Koehler, C.; Szymański, J.; Mueller, R.; Wießler, M.; Schultz, C.; Lemke, E. A. *Angew. Chem., Int. Ed.* **2012**, *51*, 4166.
- (44) Seitchik, J. L.; Peeler, J. C.; Taylor, M. T.; Blackman, M. L.; Rhoads, T. W.; Cooley, R. B.; Refakis, C.; Fox, J. M.; Mehl, R. A. *J. Am. Chem. Soc.* **2012**, *134*, 2898.
- (45) Fürstner, A.; Langemann, K. *J. Am. Chem. Soc.* **1997**, *119*, 9130.
- (46) Fürstner, A.; Seidel, G.; Kindler, N. *Tetrahedron* **1999**, *55*, 8215.
- (47) The reaction mixture colored consistent with catalyst degradation and no further reaction was observed (see SI).
- (48) Kouzarides, T. *Cell* **2007**, *128*, 693.
- (49) Bannister, A. J.; Kouzarides, T. *Cell Res.* **2011**, *21*, 381.
- (50) Spicer, C. D.; Davis, B. G. *Chem. Commun.* **2011**, *47*, 1698.
- (51) Macdonald, J. E.; Veinot, J. G. C. *Langmuir* **2008**, *24*, 7169.
- (52) Guo, J.; Wang, J.; Lee, J. S.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2008**, *47*, 6399.
- (53) Chatterjee, C.; McGinty, R. K.; Fierz, B.; Muir, T. M. *Nat. Chem. Biol.* **2010**, *6*, 267.
- (54) Shimizu, I. In *Handbook of Organopalladium Chemistry for Organic Synthesis*; Negishi, E.-I., Ed.; John Wiley & Sons, Inc.: New York, 2003; pp 1981–1994.