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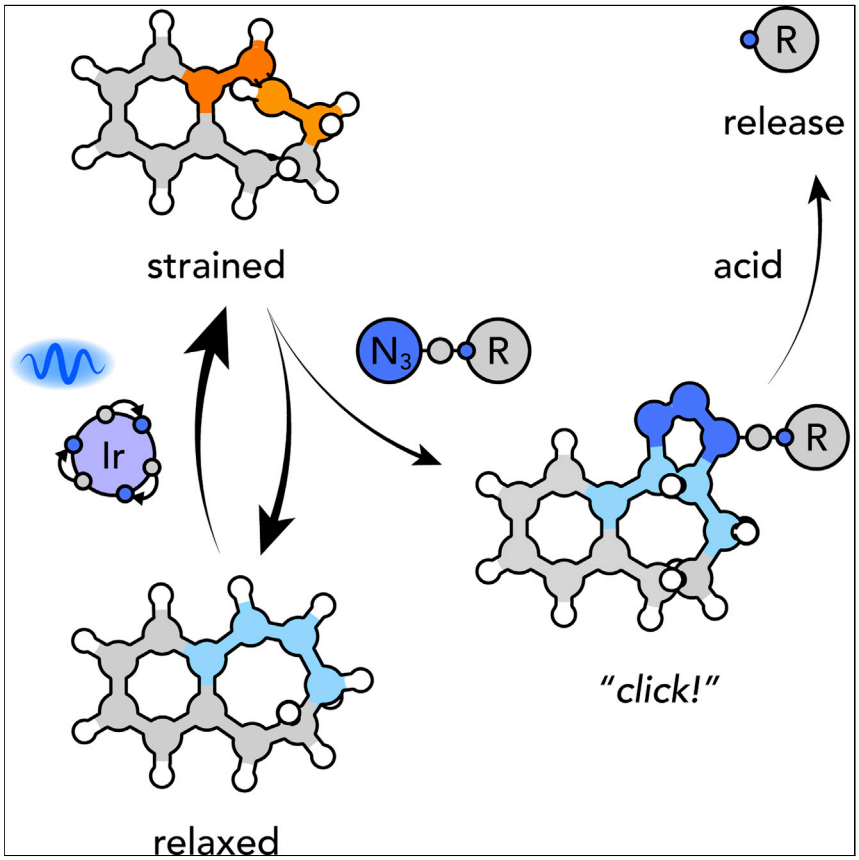
# Light Harvesting for Rapid and Selective Reactions: Click Chemistry with Strain-Loadable Alkenes




Strain-loadable alkenes are cycloalkenes that, when irradiated in the presence of a visible-light-absorbing photocatalyst, undergo double-bond isomerization. Because of engineered geometrical constraints, this isomerization results in significant molecular strain. Weaver and colleagues exploit this strain to dramatically accelerate the cycloaddition with azides, which are otherwise unreactive, in mixed molecular environments.

Kamaljeet Singh, Christopher J. Fennell, Evangelos A. Coutsias, Reza Latifi, Steve Hartson, Jimmie D. Weaver

jimmie.weaver@okstate.edu

HIGHLIGHTS

Engineered alkenes for the capture and conversion of photochemical to strain energy

Strain-free reagents for strain-induced conjugation allow easy synthetic handling

Visible-light-mediated strain loading provides ultimate functional-group tolerance

Light-induced conjugation provides spatial-temporal control opportunity

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# Light Harvesting for Rapid and Selective Reactions: Click Chemistry with Strain-Loadable Alkenes

Kamaljeet Singh,[1] Christopher J. Fennell,[1] Evangelos A. Coutsias,[2] Reza Latifi,[1] Steve Hartson,[3] and Jimmie D. Weaver[1,4,*]

## SUMMARY

**Intramolecular strain is a powerful driving force for rapid and selective chemical reactions, and it is the cornerstone of strain-induced bioconjugation. However, the use of molecules with built-in strain is often complicated as a result of instability or selectivity issues. Here, we show that such strain, and subsequent cycloadditions, can be mediated by visible light via the harvesting of photochemical energy. Through theoretical investigations and molecular engineering of strain-loadable cycloalkenes, we demonstrate the rapid chemoselective cycloaddition of alkyl azides with unstrained cycloalkenes via the transiently (reversibly) formed *trans*-cycloalkene. We assess this system via the rapid bioconjugation of azide-functionalized insulin. An attractive feature of this process is the cleavable nature of the linker, which makes a catch-and-release strategy possible. In broader terms, we show that conversion of photochemical energy to intramolecular ring strain is a powerful strategy that can facilitate complex chemical transformations, even in biomolecular systems.**

## INTRODUCTION

The study of biomolecules in their native environments is facilitated by selective chemistry, empowering research into a wide array of areas.[1–3] To be useful, such reactions need to be extremely rapid and have minimal reactivity with native functional groups, a balance that is highly desirable yet highly difficult to strike. Strain-induced couplings have proven useful in bioconjugation (Figure 1A) but often require significant effort to ensure that the reactivity is used productively. The use of external stimuli, such as light, can offer an additional level of control not available in other methods.[4–7] The ability to use light to toggle chemistry on and off offers the convenience of spatial and temporal control. Several examples of light-induced bioconjugation systems have been developed over the last decade (Figure 1B), and most commonly, these make use of short-wavelength light. This light is typically used to convert an otherwise unreactive molecule to a reactive molecule that undergoes bond formation with a non-natural functional group. In this manner, upon UV irradiation, tetrazoles can extrude $N_2$ to form a highly reactive nitrile imine dipole,[8–11] which undergoes cycloaddition with alkenes. This strategy has been used by Lin to label proteins within *Escherichia coli* cells.[12] In addition, Lin has also shown that reactive nitrile ylides generated from the photolysis (302 nm) of 2H-azirines[13] can be used for the PEGylation of a lysozyme.[14] Similarly, cyclopropenones have been used as a photolabile surrogate for strained cycloalkynes,[15] which, upon unmasking, undergo rapid reaction with azides. Although these works have begun to demonstrate the utility of "photochemical click" reactions, the use of

### The Bigger Picture

Probing, isolating, and/or manipulating biologically relevant macromolecules is central to the study of their function in living systems. However, the synthetic tools available for performing the chemistry necessary for such studies are often difficult to use or limited in utility. In the approach presented here, light is converted to molecular strain energy, which can in turn be used for performing rapid and highly selective chemistry on macromolecular systems. Because it involves chemically stable and chemoselective reactions, this research not only opens up new possibilities for biomolecular functionalization and manipulation but also promises to make such experiments accessible to a broader class of researchers. The central concept of strain-loadable alkenes is general and provides a firm foundation for light-activated chemistry in complex environments.

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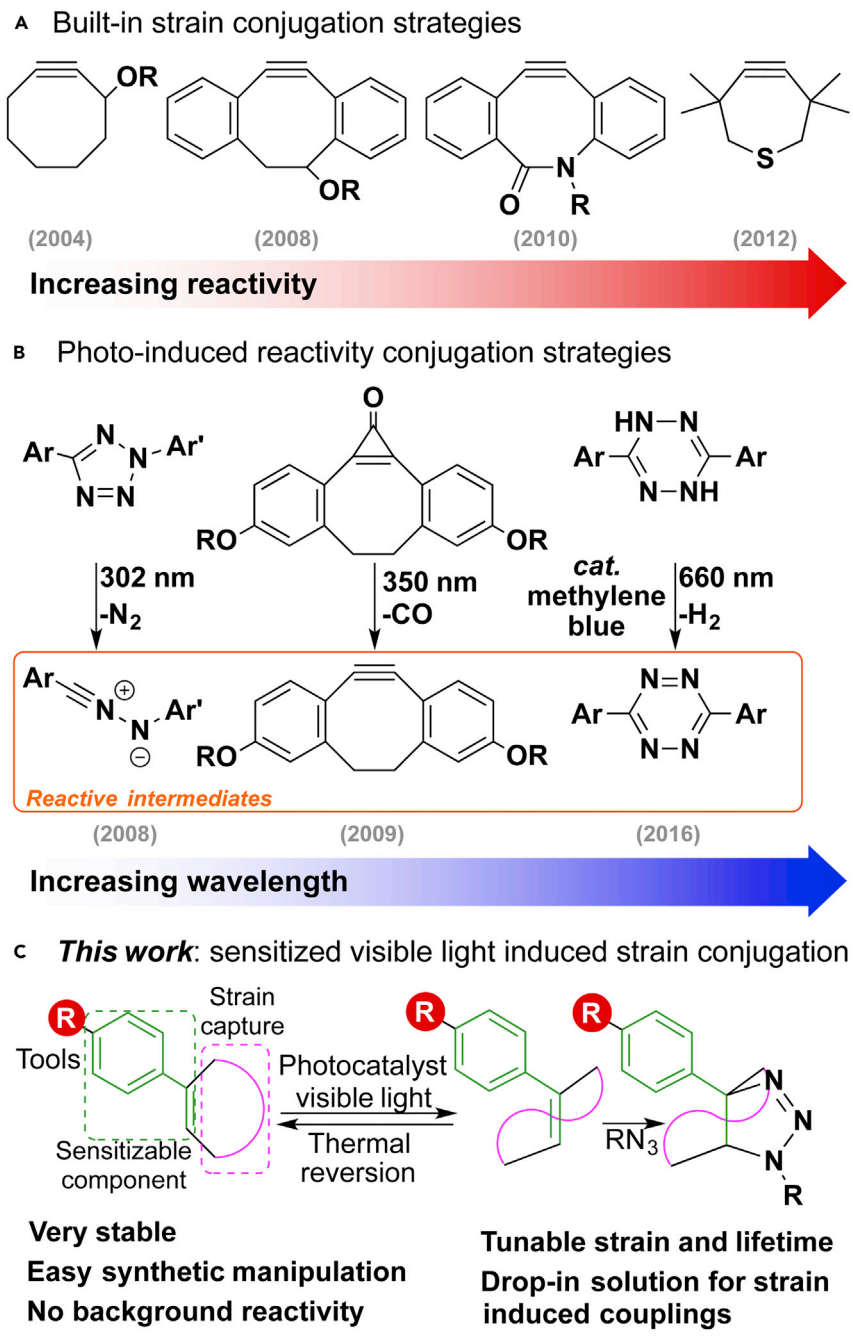


**Figure 1. Conjugation Strategies**

high-energy UV light is a significant drawback because of its often detrimental effects on living organisms. Two-photon processes, which utilize lower energy photons, could help alleviate the need for high-energy photons, but they can suffer from low quantum yields, and these strategies are still under development.[9,16] Tetrazines have been a popular motif in conjugation chemistry because of their highly reactive nature, which allows them to undergo rapid [4 + 2] cycloaddition reactions.[17,18] The cycloaddition of tetrazines with *trans*-cyclooctenes provides the greatest rate constants to date (from $2 \times 10^3$ to $1 \times 10^6$ $M^{-1}$ $s^{-1}$),[19,20] partly as a

[1]107 Physical Science, Department of Chemistry, Oklahoma State University, Stillwater, OK 74078, USA

[2]Department of Applied Mathematicise and Statistics, Stony Brook University, New York, NY 11794, USA

[3]Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078, USA

[4]Lead Contact

*Correspondence: jimmie.weaver@okstate.edu

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result of the strained nature of the *trans*-cyclooctene, the most strained isolable alkene known. However, the built-in reactivity of both partners can decrease the practicality of this system via non-trivial syntheses and handling concerns.[17] Very recently, Fox and co-workers[21] have shown that visible light and catalytic methylene blue can be used to oxidize dihydrotetrazine *in situ* to give the highly reactive tetrazine, which undergoes rapid conjugation, circumventing some of the aforementioned issues. However, in all of these examples, the light stimulation serves to irreversibly unmask a reactive molecule, and little additional control is gained in comparison with non-stimulated strain-induced reactions. In order for the field of light-mediated bioconjugation to move forward, we need new strategies that provide enhanced control of reactivity.

In 2014, we showed that we could use catalytic amounts of *fac*-tris-[2-phenylpyridinato-$C^2$,*N*]iridium(III) [Ir(ppy)$_3$] and blue light-emitting diodes (LEDs) to facilitate the isomerization of substituted styrenes toward the less conjugated *Z* isomers.[22] We were curious to find out if we could find an alkene that, upon isomerization, would capture some of the photochemical energy in the form of ring strain that could be utilized for bimolecular couplings (Figure 1C). If successful, we would be able to apply visible light as a trigger that would generate a reactive alkene potentially capable of undergoing bioconjugation. It was expected that this might circumvent some of the circuitous syntheses of popular strained molecules currently being used for bioorthogonal coupling. Furthermore, unlike other photo-initiated processes that utilize photochemical energy to irreversibly unmask the reactive group, our system would simply capture photochemical energy, which would give rise to a steady, catalytic concentration of a more reactive form of the same molecule, a currently unexplored strategy.

## RESULTS AND DISCUSSION

At the outset, we considered a variety of coupling partners,[23] and azides stood out as a good candidate. They are relatively inert toward biological functional groups, result in minimal perturbation to the system, and are known to undergo reaction with alkenes to form triazolines.[24,25] It is also known that electron-deficient[26,27] and strained[28,29] alkenes react faster with azides. Bach[30] calculated the energy barrier for the cycloaddition of methyl azide to cyclooctyne and *E*-cyclooctene, and found that addition of methyl azide to cyclooctyne has a lower barrier than *E*-cyclooctene by 3.1 kcal/mol. In addition, Houk and co-workers performed a theoretical study on the cycloaddition of strained alkenes and azides and concluded that only *trans*-cyclooctene would be sufficiently rapid for use at room temperature, perhaps somewhat diminishing interest in the alkene azide cycloaddition.[28] However, in this study, only isolable *trans*-cycloalkenes were considered, leaving the possibility for potential discovery of a smaller alkene that would react more rapidly. Early photochemical studies have shown that the *trans*-cycloalkenes could be formed via excitation to the excited state with UV light, which suggest that formation could be possible with visible light and a photocatalyst.[31–35]

We initiated our study by using (*E*)-1-phenylcyclooct-1-ene (*E*-**PC8**; Scheme 1A). We were pleased to see the isomerized alkene (*Z*)-1-phenylcyclooct-1-ene (*Z*-**PC8**) was formed under these conditions. Although the photostationary state equilibrium was disappointing from a synthetic view (19%–29%), it was encouraging from an energetic standpoint. Caldwell and co-workers have estimated the energy difference for the *E* and *Z* isomers of phenyl cyclooctene at 13.3 kcal/mol,[36] suggesting that we were able to skew the product distribution away from the thermodynamic

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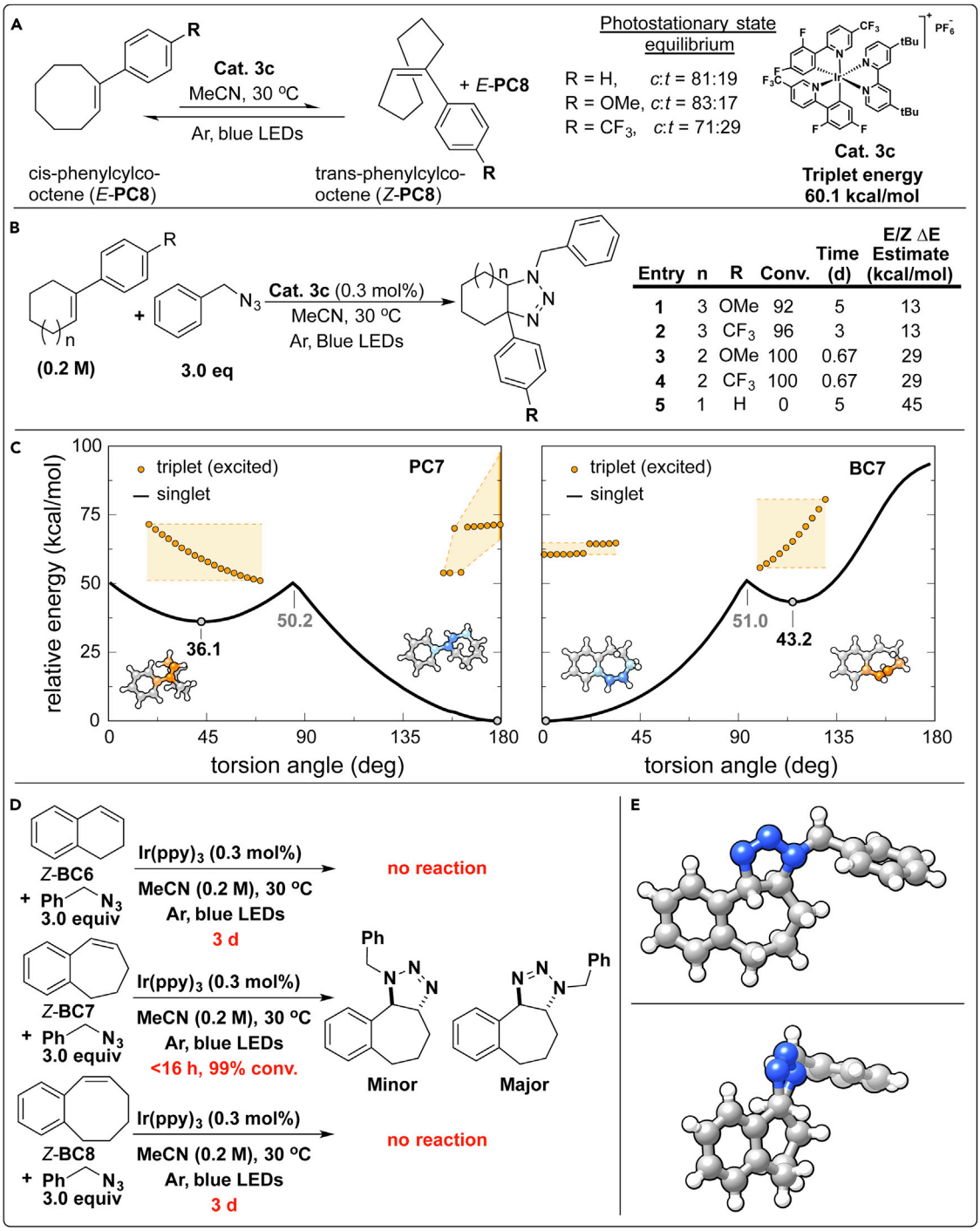


**Scheme 1. The Development of the Molecular Mousetrap**

(A) First effort to capture photochemical energy as ring strain.

(B) Attempts to chemically capture strained alkenes. Significant dimerization was observed for entries 3 and 4; see Supplemental Information for details.

(C) Calculated torsion angle energy landscapes about the ring alkene bonds in (left) PC7 and (right) BC7.

(D) Reaction results for BC6, BC7, and BC8 triazoline formation.

(E) Two views of the crystal structure of the major product, showing the *trans*-ring fusion.

equilibrium by nearly ten orders of magnitude using visible light. Because our intention was to generate the reactive alkenes *in situ* rather than isolate the unstable molecules, we were not dissuaded.

We next evaluated the ability to form triazolines as a function of alkene ring size (Scheme 1B). It was anticipated that the *trans*-cycloalkene would be continually consumed by the azide, and the photocatalytic system would reestablish the equilibrium. In contrast to their reactivity upon UV-light irradiation,[37] alkyl azides are generally stable to visible light, which is important to avoid competing types of photochemistry. We were pleased to see that 7- and 8-membered *trans*-alkenes (entries 1–4) afforded the desired triazoline product.[38] Although the rates of the reaction displayed some electronic dependence in the **PC8** system, in general, it was disappointingly slow (3–5 days). In contrast, despite no observation of the *trans* isomer ($^1$H nuclear magnetic resonance [NMR]) in the **PC7** series, the reaction required less than 0.67 days to reach completion. This suggested a strong correlation between ring strain and reaction rate and provided evidence that a transient alkene could result in faster coupling than the **PC8** system. Unfortunately, significant dimerization[39] was also observed in the case of the **PC7** system. Meanwhile, cyclohexene, **PC6**, failed to give any conversion even after 5 days of irradiation (entry 5).

In order to better understand the observed reactivity and guide future efforts, we performed a computational evaluation of the energy landscapes showing conversion between the *Z* and *E* forms of **PC6**, **PC7**, and **PC8**. We generated both singlet and triplet excitation landscapes with MP2/cc-pVTZ calculations[23] in order to assess both the strain energy loaded upon isomerization and gauge how features of the excitation process could influence the reactive behavior of these systems. As expected, *Z*-**PC7** (Scheme 1C; Figure S10) has over twice the strain energy of *Z*-**PC8** (Figure S12), supporting our observed reactivity. Although **PC6** has a similar excitation energy, the *trans* isomer, when accessible, is only transiently stable with a reversion barrier to the *cis* isomer near room temperature thermal energy. The predicted short-lived nature helps explain the inability of **PC6** to undergo bimolecular triazoline formation, indicating that reactivity is most likely limited to intramolecular processes.

Given that a transient amount of the *trans*-cycloheptene gave a substantial rate increase in comparison with observable amounts of the **PC8** system, we sought to modify these systems to increase the concentration of the reactive conformer(s). We envisioned that rigidification of the cycle could potentially result in increased strain energy and increased barriers to interconversion. These changes, along with potentially more efficient energy transfer due to the fusion of the aryl ring that reinforces conjugation with the double bond, might lead to increased concentration of more reactive strained species. Thus, we fused the essential aryl group to the ring system, giving benzocyclohexene, -heptene, and -octene (*Z*-**BC6**, *Z*-**BC7**, and *Z*-**BC8**; Scheme 1D). We performed similar energy landscape calculations on these compounds (Scheme 1C; Figures S7, S9, and S11), and they partly support these expectations. In the case of **BC7**, there is indeed a roughly 7 kcal/mol increase in strain energy in relation to **PC7** upon isomerization; however, the interconversion barrier is essentially unchanged. The forced conjugation in **BC7** lowers the triplet excitation energy from the unstrained singlet conformer in relation to the **PC7** excitation energy, which exists over a 30 kcal/mol band as a result of thermally accessible ground state conformers. This lower excitation energy of **BC7** will most likely result in an increased excited-state population when under visible-light irradiation. Calculations on **BC6** and **BC8** (Figures S7 and S11) predict poor triazoline formation reactivity for

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both. In **BC6**, a strained form is geometrically disallowed. In **BC8**, because of atomic crowding in the octene ring, conjugation is severely broken and raises the excitation energy well above the triplet energy of the photocatalyst used. We synthesized and tested the photocatalytic reactivity of these three aryl fused-ring systems (Scheme 1D), and the results are fully consistent with the calculations. Of these, **BC7** certainly shows the greatest promise given its predicted higher strain energy and greater potential concentration of reactive species, both supporting an expected increase in the rate of the subsequent bimolecular cycloadditions. Our attempts to trap the transient *E*-**BC7** with benzyl azide were successful. We were pleased to find that the reaction went to completion in less than 1 day, with only two regioisomers as the sole products. Both are single diastereomers with *trans*-ring junctions.[23] An X-ray structure of the major product (Scheme 1E) clearly shows the *trans*-ring fusion of the triazoline, which is most readily explained by a concerted or nearly concerted [3 + 2] cycloaddition of the azide and *E*-**BC7**. Control reactions indicate the necessity of the photocatalyst and indicate that there is no background reaction with *Z*-**BC7**.[23]

Despite our initial excitement over these preliminary findings, there were a number of issues that might preclude its utility for bioconjugation purposes that needed to be addressed. These issues included slow kinetics, problematic dimerization of alkene, use of excess azide, strict use of inert environment, use of pure MeCN, the unknown toxicity of iridium and its potential to cause undesired redox chemistry, and the instability of the triazoline motif. Fortuitously, the dimerization of the **BC7** system proved to be much slower than that of the **PC7** system, so slow that it was no longer problematic. We then focused on increasing the rate of the reaction and utilizing the azide as the limiting reagent. Although the reaction works with either alkene or azide as the limiting component, we assumed that the azide was the most valuable component of the reaction mixture. This seemed reasonable given the overwhelming number of examples of incorporation of azides into biomolecules, and functioning under azide limiting conditions would allow this chemistry to function as a drop-in solution and require little to no alterations of existing biological systems. Optimization included solvent, photocatalyst structure and loading, alkene loading, photon dependency, substrate concentration, and temperature effects.[23] We found that use of dimethylformamide (DMF), commercially available *fac*-tris-Ir(4′-$CF_3$-ppy)$_3$ (**Cat. 3**) at 1.2 mol %, 60°C, and 3 equiv of the alkene partner all led to increases in the rate of the reaction. Under optimal reaction conditions, both the photocatalyst and the alkene fell out of the rate expression,[23] such that no further rate increase was observed by increasing the concentration of these reagents. Under these conditions, a first-order rate constant was determined to be $k = 2.4 \pm 0.9 \times 10^{-3}$ $s^{-1}$, with a half-life of only $t_{1/2} = 4.8$ min.[23] Furthermore, we expect that this is a lower estimate for the rate constant because it was performed with an easily monitored substrate, which is known to react more slowly than others. In direct competition experiments between the photoconjugation of several azides with **BC7** and Cu-catalyzed azide alkyne cycloaddition (CuAAC), the photoconjugation reaction was found to be up to 100-fold faster.[23] CuAAC has been used for bioconjugation, which suggests that the photoconjugation of **BC7** takes place with sufficient rates for bioconjugation applications.

In order to better understand the nature of the reaction, we began to investigate the scope of the reaction by using these optimized conditions (Scheme 2). In bioconjugation, the surface of biomolecules can severely limit access to the azide substrate, so we were pleased to see that the reaction works well across a range of sterically hindered azides, even with sterically demanding 1-azidoadamantane (**4i**). Often, the modest isolated yields are a reflection of the separation of the minor

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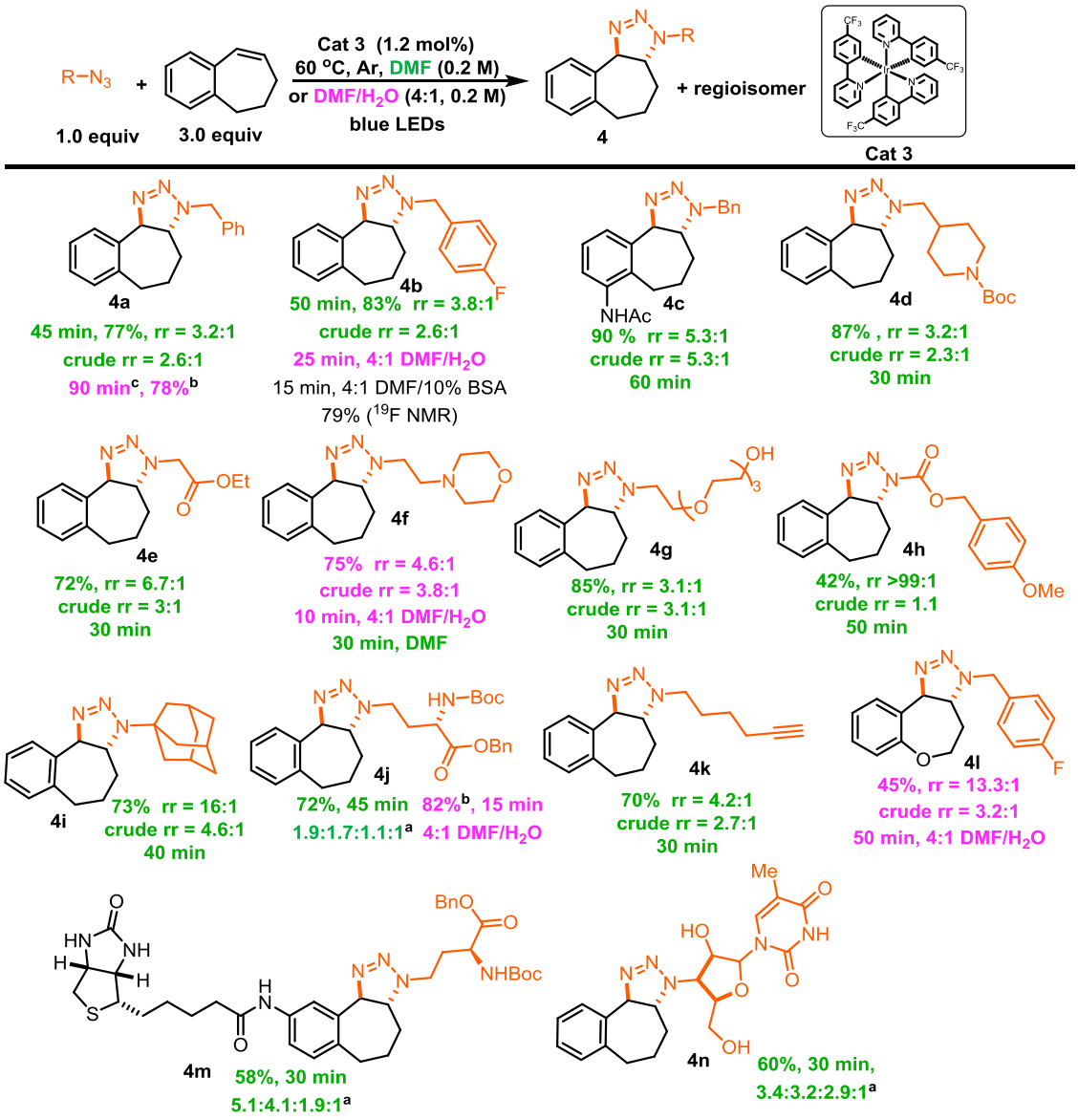


**Scheme 2. Visible-Light-Mediated [3 + 2] Cycloaddition of Benzofused Cycloheptenes and Azides**

regioisomer during purification, not a lack of reactivity. Despite the frequent use of photocatalysts in promoting redox reactions,[40–42] the conditions proved to be tolerant of a number of biologically relevant functional groups, such as amino acid derivatives (**4j** and **4m**), amines (**4d**, **4f**, and **4l**), amides (**4c** and **4m**), alcohols (**4g** and **4n**), esters (**4e**), electron-rich arenes (**4h**), and purine bases or sugar molecules (**4n**). In addition, it tolerates a number of unnatural functional groups such as aryl-bromides (**4l**) and -fluorides (**4b**), and alkynes (**4k**). This functional-group tolerance is most likely a result of the mild conditions and the fast quenching of the excited-state photocatalyst by the alkene. In other words, excess alkene serves to protect sensitive functional groups by dissipating the photochemical energy in the form of bond motion, much like the protective action of cinnamic acid-based sunscreens.[43] Smooth intermolecular coupling to give **4k** demonstrates the orthogonal nature of

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these reaction conditions toward traditional alkyne and azide cycloadditions that would be expected to give the fused triazole product. The doubly orthogonal nature of the reaction makes it even more attractive, because alkynes could be used later for further functionalization via traditional click chemistry.[44]

Although the geometry of the ring is a prerequisite to reactivity, initial exploration suggests that the aryl ring (**4c** and **4m**) and the seven-membered ring (**4l**) can both tolerate substitution. In the future, further evaluation will focus on the development of probes and fine-tuning of the reactivity and improvement in the physicochemical properties of the molecule. Acetamides (**4c** and **4m**) were found to be well tolerated in the reaction and allowed the facile coupling of a biotin-labeled alkene with a protected amino acid derivative (**4m**). This example highlights a particular strength of this approach to strain-induced coupling: derivatization of the alkene is rapid and trivial, and it provides biochemical tools that are indefinitely stable until subjected to the reaction conditions. In the case of conjugation chemistry, it may be advantageous if the stereochemical environment of the substrate does not affect the rate of conjugation, in case it resulted in failed couplings. Fortunately, stereocenters on the azide do not hamper the cycloaddition (**4j**, **4m**, and **4n**).

Finally, knowing that we want to use this chemistry on biological systems in which proteins might interfere in undesired ways, we subjected **BC7** and 4-fluorobenzyl azide to normal reaction conditions but, instead of pure DMF, a 4:1 DMF:10% aqueous BSA solution was used. Gratifyingly, under these conditions 79% ($^{19}$F NMR yield) of the desired product **4b** was formed. Given the possibility of interference or unselective reaction on the protein surface, a high yield of the desired product indicates that the BSA interfered with neither yield nor chemoselectivity.[45] Serendipitously, it also led us to discover that the reaction was further accelerated by the presence of water, given that the reaction takes nearly 50 min to complete in dry DMF but <15 min (upper limit) in the presence of the BSA solution. Under these conditions, the reaction mixture was homogeneous, but at higher water loadings the photocatalyst precipitates, which precludes using this specific set of reaction components in a purely aqueous system. However, we believe the aqueous solubility can be increased via a number of strategies, and this is currently being pursued in our laboratory. We were pleased to find that this acceleration was a general trend (Scheme 2, conditions B), with most substrates experiencing a roughly 3-fold increase in rate, although we have made no attempts to directly quantify this acceleration. The source of this acceleration could be a decrease in the transition state volume ($\Delta V^{\ddagger}$), a case in which the use of water has been shown to lead to an acceleration.[46,47] Alternatively, it is possible that the *trans*-alkene adopts a zwitterionic form to relieve strain and is stabilized by a more polar solvent, leading to a greater concentration of the reactive form.

Tetrazines are known to have better reactivity than azides and undergo cycloaddition with strained alkenes. Thus, we briefly explored this possibility. We exposed *E*-**PC8** to 3,6-diphenyl-1,2,4,5-tetrazine in the presence of **Cat. 3c** and MeCN at 45°C, and no cycloaddition product was observed.[23] Importantly, no strained *Z*-**PC8** was detected (as is the case in the absence of the tetrazine) and both the reactants were recovered unchanged after 18 hr of the irradiation. One explanation for this is that the fuchsia-colored 3,6-diphenyl-1,2,4,5-tetrazine could itself absorb in the visible-light region and might be responsible for the observed lack of reactivity.

In contrast to triazoles, triazolines are not aromatic and are quite susceptible to further chemistry. Traditionally, the observed rate of triazoline decomposition was

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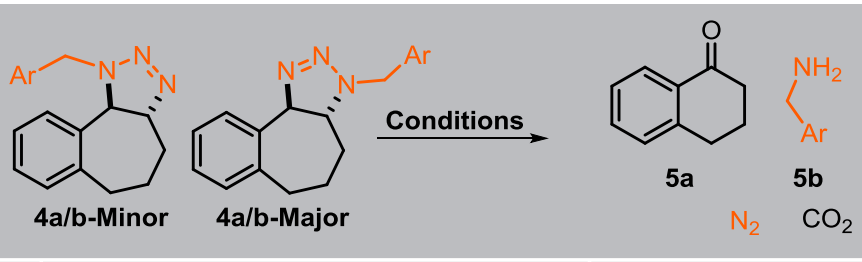
**Table 1. Stability Studies of Triazoline Products**

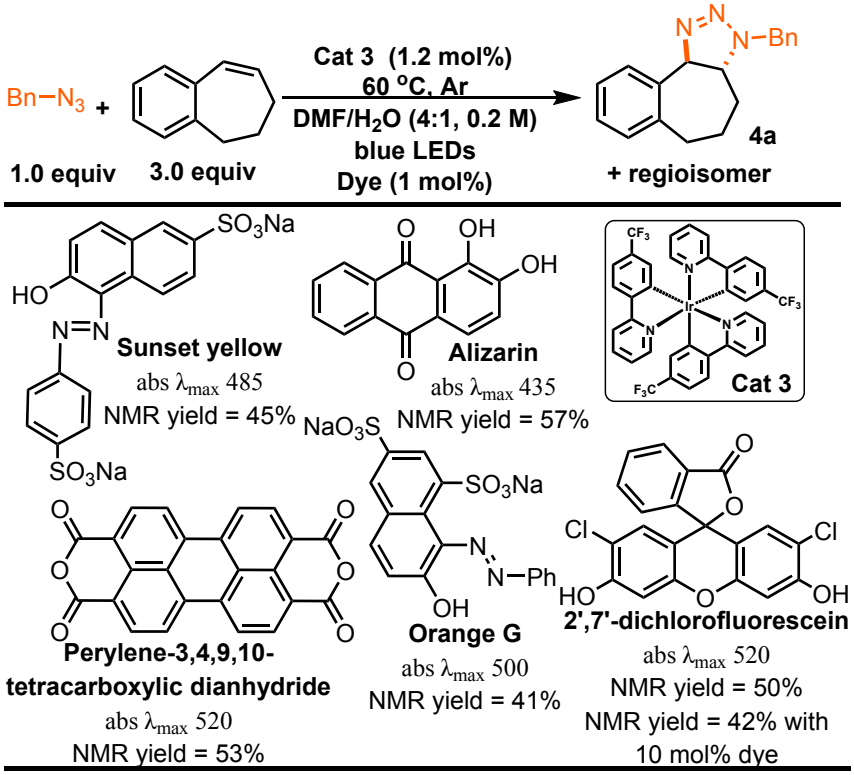



| Entry | 4a and 4b Conditions | Result |
|---|---|---|
| 1 | **4a** Cat-Ir(ppy)$_3$, 60°C, Ar, DMF, blue LEDs | no decomposition, 24 hr |
| 2 | **4a** isolated compounds, 4°C, 2 years | **4a**-major, no decomposition; **4a**-minor, decomposition |
| 3 | **4a** DMF, exposed to air | **4a**-minor, decomposition |
| 4 | **4a** silica chromatography | **4a**-minor, some decomposition |
| 5 | **4a** basic alumina chromatography | no decomposition |
| 6 | **4b** TFA, 5 min | rapid decomposition, **5b** 38% |

comparable with that of its formation, possibly deterring investigation of its use in conjugation chemistry.[1] However, the use of **BC7** under photocatalytic conditions effectively amplifies the rate of triazoline formation, such that appreciable decomposition does not occur on the reaction timescale, allowing isolation of the triazoline products. Because triazolines can be formed and isolated, the inherently greater lability of triazolines might be a unique asset if decomposition could be induced intentionally.[48] In other words, rapid conjugation could then be followed by controlled release of the amine product. In fact, Gamble and co-workers have demonstrated that hydrolysis-susceptible triazolines formed via the 1,3-dipolar cycloaddition of azide and *trans*-cyclooctene is a viable prodrug activation strategy.[49]

To investigate the stability, we subjected **4a** to a number of conditions (Table 1). Extended reaction time (24 hr) led to no detectable decomposition (entry 1). However, upon isolation and storage at 4°C, the major isomer showed no decomposition for up to 2 years (and counting). The minor regioisomer is notably less stable and decomposes both upon storage (entry 2) and in solution (~7 days) even in the absence of the photocatalyst (entry 3). Decomposition is further accelerated when chromatography on silica is attempted (entry 4). Basic alumina was found to be an appropriate chromatography medium; it did not lead to decomposition of the minor isomer (entry 5). However, upon exposure of the products to acidic environments, we observed rapid decomposition and gas evolution, presumably $N_2$ and $CO_2$. The isolation of α-tetralone from decomposed mixtures was surprising but has precedent[50] and presumably arises from ring contraction, followed by hydrolysis, oxidation, and an oxidative decarboxylation. Upon brief exposure of the crude reaction mixture containing **4a** to trifluoroacetic acid (TFA) (entry 6), a 38% yield of **5b** was obtained, suggesting that this might be a viable release strategy. Further studies aimed at increasing this yield via optimization of the alkene structure and conditions are underway. As an aside, it could also provide an alternative route to the Staudinger reaction, which produces hard-to-separate triphenylphosphine oxide during the reduction of azides.

Given the frequent use of dyes as fluorophores for labeling cells in many bioconjugation reactions, the effect of fluorophores on the reaction outcome was evaluated. It was anticipated that the outcome of the reactions could potentially




**Scheme 3. Reaction Outcome in the Presence of Various Fluorophores**

be effected partially, or even completely, in the presence of dyes as a result of competitive absorption of photons or possibly undesired quenching of the excited photocatalyst by the dye. Hence, experiments were performed in which various dyes, absorbing at different wavelengths, were added to the reaction mixture. The results are tabulated below (Scheme 3). The desired triazoline product **4a** was formed, albeit with lower efficiencies than the standard conditions in the absence of any extra dye. Increasing the amount of dye (2,7-dichlorofluorescein) present in the reaction to more than 8-fold that of the photocatalyst, resulted in a 42% NMR yield of the desired triazoline product, **4a**, within 6 hr of irradiation. With some effort, it should be possible to design a system that selectively excites a photocatalyst even in the presence of another dye,[23] which is supported by the results of Scheme 3.

In order to showcase the applicability of this novel photoconjugation approach, we applied the reaction conditions on a small tripeptide. A commercially available tripeptide was modified with an azide and then conjugated with the **BC7** under standard conditions. Within 4 hr of the irradiation, complete conversion to the desired conjugated tripeptide mass was observed by liquid chromatography-mass spectrometry.[23]

Although we had observed that the reaction could take place selectively in the presence of BSA, we also investigated the ability to perform the chemistry directly on a biomolecule. This would demonstrate that the photoconjugation takes place at sufficient rate to be useful even with large molecules, which have smaller diffusion constants and are present at much lower concentrations, typical of proteins. The N termini of insulin (bovine), a peptide hormone that plays a key role in the

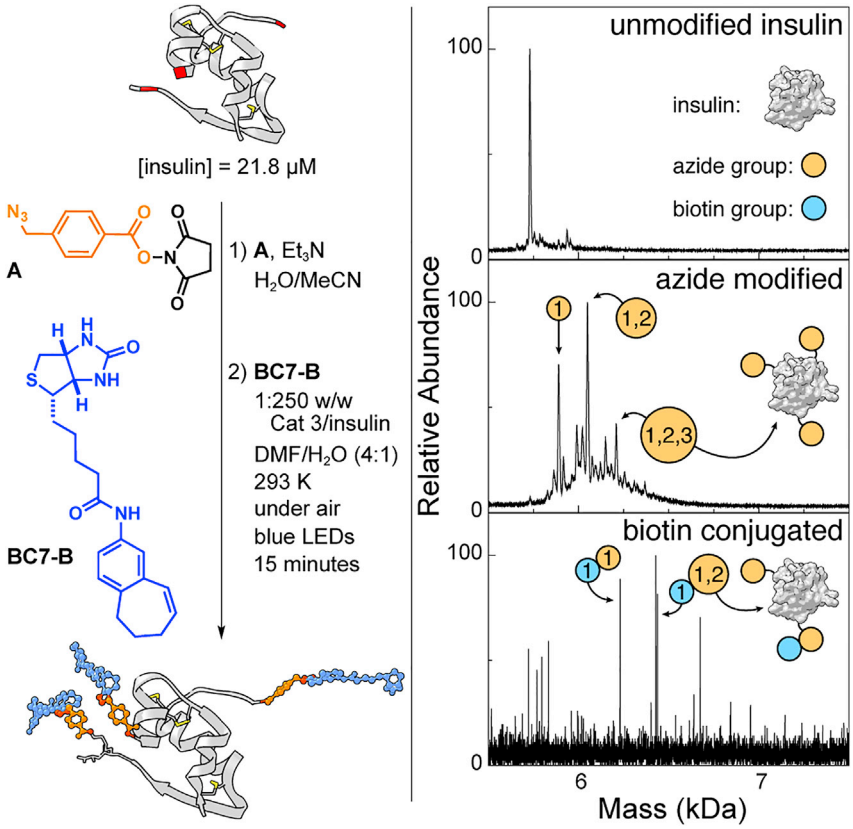



**Figure 2. Demonstration of Biotin Conjugation to Insulin via Strain-Loadable Alkene Coupling**

There are three modifiable sites on the insulin structure highlighted in red: the N termini on the A and B strands and the lysine residue on the B strand. The matrix-assisted laser desorption/ionization spectra on the right show modification and identification of two variants of the monoconjugated protein.

metabolism of glucose and is made up of two peptide chains connected by disulfide bridges, were unselectively modified with an azide-modified benzoylating reagent (**A**; Figure 2). The mixture of un-, mono-, di-, and tri-modified insulin (21.8 μM) was next subjected to the photoconjugation reaction (**Cat. 3**, 0.1 μg), with biotin-modified benzocycloheptene (**BC7-B**, 1.2 mM), which took place at room temperature and with no degassing. In less than 15 min, all of the modified insulin was consumed. New masses consistent with the mono- and di-conjugated product were detected.[51] The conjugation of insulin was further supported by the SDS-PAGE experiment and subsequent silver staining. New bands of slightly higher molecular weight than the unmodified insulin and azide-modified insulin were detected.[23] When the alkene was left out of the irradiated reaction mixture (12 hr, not shown), the modified insulin was broken into its constituent chains, highlighting the protective nature of the alkene and suggesting that even redox-sensitive biomolecules can tolerate the reaction conditions, provided the alkene is present to quench the photocatalyst. It is quite possible that the tertiary structure of insulin was lost as a result of the solvent. The use of DMF is due to the low solubility of this particular photocatalyst in water, and it is expected that modifications to the structure of the photocatalyst can lead to increased aqueous solubility, which could eliminate this problem. As a side note, it could also lead to diminished redox potentials, which could make it even more compatible with redox-sensitive substrates. Furthermore, photoconjugation is not necessarily limited to Ir-based photocatalysts. Recently,

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Gilmour and co-workers have shown that riboflavin was also a competent photocatalyst for photoisomerization of alkenes, indicating that it might be possible to use the water-soluble vitamin with the appropriate modifications to accomplish the photoconjugation.[52]

In an attempt to move toward more biocompatible conditions (i.e., mostly aqueous solvent), we performed a reaction with a more water-soluble catalyst [4-F Ir(ppy)$_2$bpy]$^+$PF$_6^-$ (1.0 mg of catalyst is completely miscible in 1.0 mL of 3:2 DMF/water) than **Cat. 3** (less than 10 ppm in 3:2 DMF/water). However, the catalyst has lower triplet energy (51.4 versus 56.4 kcal/mol), which was expected to decrease the overall efficiency of the reaction.[53] Indeed, when a more aqueous-rich 3:2 DMF/water solvent system was used, the reaction produced the desired triazoline product **4a**, but required 9 hr of irradiation to reach completion. This result suggests that designing a photocatalyst that is more water soluble and emits at a higher energy will allow this reaction to take place in water.

## Conclusions

In conclusion, we have taken the idea of energy harvesting from concept to practice by providing conditions that allow kinetically and thermodynamically stable benzo-fused cycloheptene, *Z*-**BC7**, to undergo fast and chemoselective cycloaddition with substituted azides upon exposure to visible light. The reaction takes place under mild conditions that can be used to work with biological molecules. The operative mechanism involves energy transfer from a photocatalyst to *Z*-**BC7**—which results in isomerization to an extremely strained and kinetically unstable alkene, *E*-**BC7**, meaning that no background reaction occurs in the absence of photocatalyst or light—and provides a strategy for temporal control that is simply not available to strain-promoted reactions. The designed relief mechanism of **BC7** is unique among photo-initiated processes. In contrast to others, it provides a steady-state concentration of highly reactive alkene throughout the duration of the reaction. The reaction tolerates a broad range of azides that give triazoline products. A unique aspect of this conjugation strategy is that the products can be conveniently and purposefully decomposed by the addition of weak acid sources, releasing the amine that corresponds to the azide. Finally, we have shown that the conjugation reaction can be performed on modified proteins such as insulin (which are reasonably soluble and stable in organic solvents) in short reaction times and at biologically relevant concentrations and temperatures. From the initial synthetic concept to an applied tool ready for further development, this work presents a powerful and flexible strategy for complex chemical transformations via the conversion of photochemical energy to intramolecular ring strain, facilitating transformations even on challenging bimolecular systems.

## EXPERIMENTAL PROCEDURES

An NMR tube was charged with a solution of azide (1.0 equiv), alkene **S2** or **7** (3.0 equiv), photocatalyst **3** (1.2 mol %) in DMF (0.2 M) or DMF/water (4:1, 0.2 M), and a sealed capillary containing deuterated benzene. The tube was capped with a rubber septum, and the reaction mixture was degassed with argon bubbling for 10–15 min. The tubes were then sealed with Parafilm and placed in the light bath. The progress of the reaction was monitored by $^1$H NMR. To minimize unintentional reaction, the tube was quickly covered with aluminum foil after removal from the light bath and kept under dark until being loaded into the NMR instrument. After completion of the reaction, the mixture was diluted with EtOAc and washed with water. The organic layer was dried over $MgSO_4$ and concentrated to obtain the

crude product, which was purified by normal phase chromatography. Normal phase chromatography was performed with a Teledyne Isco automated chromatography system with basic alumina as the stationary phase and either EtOAc/hexanes or MeOH/$CH_2Cl_2$ as the mobile phase unless otherwise noted.

## DATA AND SOFTWARE AVAILABILITY

The structure of triazoline **4a** in this article has been deposited in the Cambridge Crystallographic Data Center under accession number CCDC: 1525656.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, 18 schemes, 13 figures, 1 table, and $^{1}H$ and $^{13}C$ NMR data and can be found with this article online at https://doi.org/10.1016/j.chempr.2017.11.007.

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## AUTHOR CONTRIBUTIONS

Conceptualization, J.D.W. and K.S.; Methodology, J.D.W. and K.S.; Investigation, K.S.; Formal Analysis, C.J.F. and E.C., Writing – Original Draft, J.D.W.; Writing – Review & Editing, J.D.W., K.S., and C.J.F.; Funding Acquisition, J.D.W.; Resources, J.D.W., S.H., and R.L.; Supervision, J.D.W.

## DECLARATION OF INTERESTS

A provisional patent concerning this technology has been filed.

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