HHS Public Access

Author manuscript

Org Lett. Author manuscript; available in PMC 2021 December 18.

Published in final edited form as:

Org Lett. 2020 December 18; 22(24): 9415–9420. doi:10.1021/acs.orglett.0c02714.

Probing the Mechanism of Photoaffinity Labeling by Dialkyldiazirines through Bioorthogonal Capture of Diazoalkanes

Jessica G. K. O'Brien,
Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716, United States

Andrew Jemas,
Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716, United States

Papa Nii Asare-Okai,
Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716, United States

Christopher W. am Ende,
Pfizer Worldwide Research and Development, Groton, Connecticut 06340, United States

Joseph M. Fox
Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716, United States

Abstract

Dialkyldiazirines have emerged as reagents of choice for biological photoaffinity labeling studies. The mechanism of crosslinking has dramatic consequences for biological applications where instantaneous labeling is desirable, as carbene insertions display different chemoselectivity and are much faster than competing mechanisms involving diazo or ylide intermediates. Here, deuterium labeling and diazo compound trapping experiments are employed to demonstrate that both carbene and diazo mechanisms operate in the reactions of a dialkyldiazirine motif that is commonly utilized for biological applications. For the fraction of intermolecular labeling that does involve a carbene mechanism, direct insertion is not necessarily involved, as products derived from a carbonyl ylide are also observed. We demonstrate that a strained cycloalkyne can intercept diazo compound intermediates and serve as a bioorthogonal probe for studying the contribution of the diazonium mechanism of photoaffinity labeling on a model protein under aqueous conditions.

Corresponding Authors jmfox@udel.edu, Christopher.amende@pfizer.com.

The authors declare the following competing financial interest(s): C.W.A. is an employee of Pfizer Inc.

ASSOCIATED CONTENT

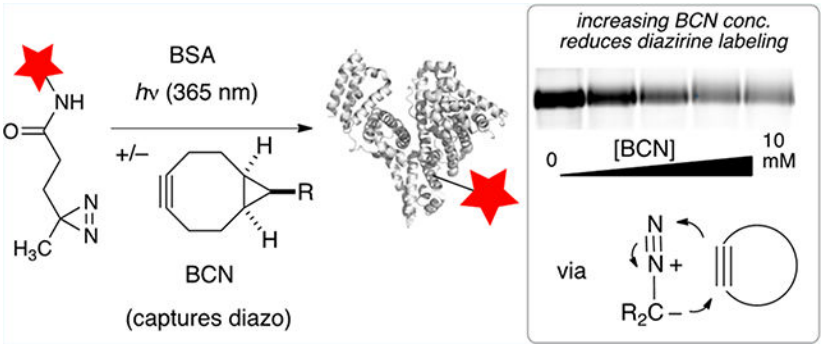
Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c02714.

Experimental procedures, characterization details, and copies of ^{1}H and ^{13}C NMR spectra for new compounds (PDF)

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c02714

Graphical Abstract



Photoaffinity labeling has become a ubiquitous tool in chemical biology and drug discovery.[1] Aromatic diazirines, arylazides, and benzophenone derivatives have long served in photoaffinity labeling applications,[1–3] and recent probes based on nitrile imines[4] and photoredox catalysis[5] offer new possibilities. A consideration for the design of any photoaffinity label (PAL) is the minimization of size and hydrophobicity in order to ameliorate any detrimental impact of the PAL on the function, solubility, permeability, or subcellular localization of the biological molecules being studied.[6] Accordingly, dialkyldiazirines have emerged as favored probes for photoaffinity labeling in cellular experiments[7–9] with applications that include probing small molecule–protein interactions,[6,7] protein–protein interactions,[10] nucleic acid–protein interactions,[11] metabolic oligosaccharide engineering,[12] lipid–protein interactions,[13,14] and the study of biological membranes.[15]

While their physiochemical properties are advantageous for chemical biology, there are important photochemical differences between dialkyldiazirines and more traditional aromatic diazirines. Analogs of α-trifluoromethyl-α-phenyldiazirine are well established biological probes that upon irradiation give high intermolecular labeling yields via formal carbene addition even with aliphatic CH bonds,[16] and because α-trifluoromethyl-α-phenylcarbene lacks α-CH bonds, it cannot participate in intramolecular α-C–H insertion processes. α-Trifluoromethyl-α-phenyldiazirine also leads to ~35% of α-trifluoromethyl-α-phenyldiazomethane.[16] While this side product undergoes photochemistry with shorter wavelength UV (~300 nm),[17] it does not absorb at the wavelengths typically used for diazirine activation (>350 nm) and displays high stability in the dark under neutral or acidic conditions.[16] Photochemical reactions of dialkyldiazirines are relatively challenging to study because of the susceptibility of both alkylcarbenes and their photoexcited nitrogenous precursors to undergo intramolecular rearrangements with α-hydrogens.[18] However, several dialkylcarbenes have been characterized and both singlet and triplet ground states have been observed.[19]

When diazirines are applied in photoaffinity labeling, it is frequently assumed that carbene insertion reactions are responsible for the bimolecular capture of biological targets, but further consideration is required with dialkyldiazirine precursors (**1**, Figure 1A). Like aromatic diazirines, aliphatic diazirines partially rearrange to diazo compounds upon

irradiation.[18] However, aliphatic diazo compounds (**2**) are readily protonated at neutral pH to give diazonium compounds (**4**) which are reactive alkylating agents.[20,21] While both carbene (**3**) and diazonium mechanisms can lead to intermolecular labeling, the chemoselectivity of alkyldiazonium labeling is likely to differ from that of a carbene mechanism. Labeling by alkyldiazonium **4** is known to favor esterification reactions,[22–25] whereas the reactions of free carbenes are less selective and include aliphatic C–H insertion as a possibility.[26] Moreover, crosslinking reactions are extremely rapid for the carbene pathway,[26] whereas the alkylation reactions of alkyldiazonium ions are much slower; for example, the reaction of a methanediazonium ion with water has a relatively long half-life of ~0.4 s in water/THF solution.[27,28] Thus, in biological target identification experiments, alkyldiazonium ions are likely to display a much larger labeling radius than more reactive carbene intermediates. Additionally, when alkyldiazonium ions do modify proteins they are likely to modify carboxylic acid residues,[22–25] producing esters which may be subsequently hydrolyzed by esterases[22–24] in live cell experiments and potentially in the proteomic workflow. Understanding the relative contributions of carbene and diazonium intermediates is therefore critical to the interpretation of biological data from photoaffinity experiments.

In seminal studies, Kirmse, Platz and coworkers studied the photolyses of spirobicyclic diazirines including norbornane-derived **7**, which upon irradiation in MeOH gives a mixture of products that could derive from either carbene **8** or diazo compound **9** (Figure 2A).[18] By studying the changes in product distribution in the presence of dipolarophile traps for diazocompounds and by analyzing levels of deuterium incorporation for photolyses in MeOD, it was possible to deduce that diazirine **7** produces both carbene **8** and diazo compound **9** in significant amounts. These studies with bicyclic diazirines not only demonstrated the importance of diazonium ions to intermolecular ether formation but also revealed a structural dependence on the contribution of carbenes to bimolecular chemistry.

While "linear" dialkyldiazirines have emerged as reagents of choice for photoaffinity labeling (e.g., **10**[12] and **11**,[7] Figure 2B), studies on the mechanism of intermolecular labeling are lacking for the substrate types that are most often used for biological experiments. Another mechanistic consideration for such systems is that carbonyl groups are generally used to attach the probe to a molecule of interest, which opens a pathway for carbenes to react via carbonyl ylides (**5**) and subsequently formed oxocarbenium ions (**6**) (Figure 1). Recent LC-MS/MS studies on diazirine photo crosslinking of proteins have uncovered a preference for labeling carboxylic acid residues, suggesting an important role of diazo intermediates.[25,29,30] Here, we present direct evidence that both carbene and diazonium mechanisms operate in the reactions of a dialkyldiazirine motif commonly utilized for photoaffinity labeling. We demonstrate that a strained cycloalkyne can intercept diazocompound intermediates[20] and serve as a bioorthogonal probe for studying the contribution of the diazonium mechanism of photoaffinity labeling on a model protein under aqueous conditions.

Our study began by observing the products formed when methanol solutions of diazirine **12** (60 mM) were irradiated at 350 nm in the air to produce three alkenes **13–15**, lactone **16**, and the methanol adduct **17**[31] (Figure 3A). Based on corrected GC yields there was a 64% yield of the intramolecular products **13–16** and a 32% yield of the intermolecular product

17. Alkene products **13–15** may arise via carbene **18** or via diazo **21**/diazonium **22** (Figure 3B,C). For carbene **18**, the alkenes can form either by 1,2 hydride (1,2 H–) shift or via the intermediacy of ylide **19** and oxocarbenium **20**. Among these mechanisms, only the carbene concerted 1,2 H-shift does not involve protonation. Thus, for irradiations carried out in methanol-*d*, the D/H ratio reports on the contribution of the carbene 1,2 H-shift mechanism for the formation of alkenes **13–15**,[18] as alkenes **13**-*d*, **14**-*d*, and **15**-*d* arise from protonation/elimination of diazo **21** or ylide **19**. As shown in Figure 3C, the level of deuterium incorporation for **13**, **14**, and **15** was 48%, 31%, and 50%, respectively. Hence, the carbene concerted 1,2 H-shift mechanism is responsible for at least 50–69% of the alkene products **13–15**.

Additionally, the diazirinyl amide **12a** was prepared and irradiated in MeOH (350 nm, 2 h) and by NMR analysis was observed to give alkene (**13a–15a**) and ether (**17a**) products in 61% yield and ratios that were similar to what was observed with diazirinyl ester **12**. Lactone **16** was not observed, nor was the cyclic butyrolactam, 5-methyl-1-propylpyrrolidin-2-one.[32]

Dipolarophiles such as fumaronitrile and diethyl fumarate participate in cycloaddition reactions with diazo compounds formed photochemically from diazirines.[33] These dipolarophiles have been used as mechanistic probes in the reactions of spirocyclic diazirines,[18,34] but are also potent electrophiles toward biological nucleophiles. We therefore sought to identify a bioorthogonal dipolarophile that could intercept intermediate diazo compounds under biologically relevant conditions. Cycloalkynes including the cyclooctyne BCN participate in a number of bioorthogonal reactions[35] including 3 + 2 cycloadditions with diazo compounds.[20] As shown in Figure 4A, the photolysis of diazirine **12** in MeOH in the presence of BCN (1.1 equiv) produces cycloadduct **23** as a mixture of diastereomers in 22% isolated yield, thus demonstrating the ability of BCN to intercept diazo intermediate **21**. As depicted in Figure 4B,D, the inclusion of dipolarophile in photolyses of **12** (60 mM) in MeOD also has influence on D-incorporation for alkene products **13–15**. Interception of diazo **21** by BCN is expected to lead to a decrease in the yield and the D/H ratio for alkenes **13–15**. In the absence of BCN, alkene products were formed in combined 52% yield and with 31–50% D incorporation. However, the photolysis of 60 mM **12** in the presence of 70 mM BCN gave alkenes **13–15** with a yield decrease to 33% and with only 6–9% D incorporation.[36] This decrease in alkene yield and D-incorporation is consistent with the capture by BCN of diazo **21**, which is partly responsible for the formation of alkenes **13**-*d*, **14**-*d*, and **15**-*d*. Similar decreases in yield and D-incorporation were noted for the photolysis in the presence of fumaronitrile.[18]

As depicted in Fig 4C,D, the inclusion of dipolarophiles in photolyses of **12** also decreases the yield of bimolecular product **17**. The yield of ether **17** without added dipolarophile is 29%, whereas irradiation of **12** (60 mM) with either BCN (70 mM) or fumaronitrile (200 mM) gives **17** in 14% yield. Thus, intercepting diazo intermediate **21** leads to a major reduction in bimolecular product formation. These results illustrate the importance of diazo compound protonation/alkylation for intermolecular product formation.

Persistently formed in the photolyses is lactone **16**, which is proposed to arise via protonation of ylide **19** and subsequent hydrolysis of oxocarbenium **20** by adventitious water. Intermediates **19** and **20** may arise from either the carbene pathway (via cyclization of **18**) or the diazo pathway (via cyclization of either **21** to **19**, or **22** to **20** (Figure 3B)). Consistent with all of these mechanisms, **16** shows >99% D-incorporation when photolyses are carried out in MeOD. The formation of ylide **19** from carbene **18** provides a possible explanation for the incomplete ability of dipolarophiles to suppress D-incorporation in alkenes **13–15**, as would be expected if the carbene 1,2 H-shift was the sole pathway (Figure 2).[37] The formation of **19** from **18** also explains the incomplete suppression of bimolecular adduct **17** (Figure 4B,C). Thus, while carbene **18** may lead to the formation of an intermolecular product **17**, this is most likely preceded by an intramolecular reaction to produce ylide **19** based on earlier conclusions that intramolecular H-shifts are generally too rapid for intermolecular reactions to compete.[18,38–40] Also consistent with the formation of ylide **19** from carbene **18** is the incomplete ability of dipolarophiles to suppress the formation of lactone **16**, suggesting that **16** arises from a combination of the carbene and diazo pathways.

We next sought to use BCN to probe the mechanism of protein photoaffinity labeling. Rhodamines are known to non-selectively bind to bovine serum albumin (BSA), and Jewett had previously demonstrated fluorescent labeling of BSA with a rhodamine–diazirine conjugate.[41] Accordingly, we prepared a fluorescent diazirine, **TAMRA-DAz**, and the water-soluble **BCN-PEG$_{12}$** (Figure 5A). BSA was reduced by DTT and alkylated with iodacetamide before usage to minimize cysteine labeling by the BCN reagent.[42,43] As shown in Figure 5B, irradiating a solution of BSA (22 μM) with **TAMRA-DAz** (24 μM) in PBS/tris buffer for 15 min at 365 nm led to fluorescent labeling as judged by SDS-PAGE (lane 3), whereas controls without light gave no labeling (lanes 2, 9, 10). The inclusion of **BCN-PEG$_{12}$** (2–10 mM) in photolysis experiments with BSA led to decreased labeling by **TAMRA-DAz**, with up to a 69% decrease at the highest **BCN-PEG$_{12}$** concentration (lanes 4–7). However, no suppression of fluorescence was observed upon irradiation in the presence of **24**, an analog of **BCN-PEG$_{12}$** that lacks the reactive alkyne (lane 8). As further evidence that the diazonium mechanism is partly responsible for protein labeling, an additional control was carried out in the presence of sodium acetate, which would be expected to decrease labeling by competing for the diazonium intermediate.[22–25] As shown in Figure S1, inclusion of NaOAc (1 M) during the irradiation of **TAMRA-DAz** with BSA led to a 45% decrease in labeling.

These protein labeling results are consistent with the results in methanol that show that intermolecular labeling can be suppressed by capturing diazoalkane intermediates, and support the hypothesis that the diazonium alkylation pathway is a major pathway in photoaffinity labeling. The inability to completely quench labeling by addition of **BCN-PEG$_{12}$** does suggest a role for the a carbene intermediates in diazirine photoaffinity labeling. However, the mechanism by which the carbenes studied here label proteins is unclear and may not involve commonly assumed carbene insertion reactions, but could instead involve the intermediacy of ylide and oxocarbenium intermediates. These mechanistic details have consequences for chemical biology applications, as alkylations of diazonium or

oxocarbenium ions are much slower than carbene insertion reactions which may in turn negatively impact the radius of labeling in protein identification experiments. Additionally, reactions of diazo compounds with proteins are known to selectively react with carboxylic acid residues to give esters,[22–25] which may not be stable in live cell applications due to native esterases and/or the proteomic workflow employed.[22–24]

The long-recognized problem of detangling the chemistry of carbenes from the reactions of their precursors remains incredibly relevant today.[18,39,44–48] The field of chemical biology will benefit from future studies to develop photoaffinity tags with desirable physiochemical properties that can label biological targets via a "real" carbene insertion mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by NIH GM132460 and Pfizer Inc. J.G.K.O. and A.J. were supported as CBI fellows through T32-GM133395, and J.G.K.O. thanks the University of Delaware for fellowship support. We thank R. Knowles and his group for sending us analytical samples of **13a** and 5-methyl-1-propylpyrrolidin-2-one. Instrumentation was supported by NIH Awards P20GM104316, P30GM110758, S10OD025185, S10RR026962, and S10OD016267 and NSF Awards CHE-0840401, CHE-1229234, and CHE-1048367.

REFERENCES

(1). Halloran MW; Lumb J-L Recent Applications of Diazirines in Chemical Proteomics. Chem. - Eur. J 2019, 25, 4885–4898. [PubMed: 30444029]

(2). Mishra PK; Yoo C-M; Hong E; Rhee HW Photo-crosslinking: An Emerging Chemical Tool for Investigating Molecular Networks in Live Cells. ChemBioChem 2020, 21, 924. [PubMed: 31794116]

(3). Dubinsky L; Krom BP; Meijler MM Diazirine based photoaffinity labeling. Bioorg. Med. Chem. 2012, 20, 554–570. [PubMed: 21778062]

(4). Herner A; Marjanovic J; Lewandowski TM; Marin V; Patterson M; Miesbauer L; Ready D; Williams J; Vasudevan A; Lin Q 2-Aryl-5-carboxytetrazole as a New Photoaffinity Label for Drug Target Identification. J. Am. Chem. Soc. 2016, 138, 14609–14615. [PubMed: 27740749]

(5). Loh YY; Nagao K; Hoover AJ; Hesk D; Rivera NR; Colletti SL; Davies IW; MacMillan DWC Photoredoxcatalyzed deuteration and tritiation of pharmaceutical compounds. Science 2017, 358, 1182–1187. [PubMed: 29123019]

(6). MacKinnon AK; Taunton J Target Identification by Diazirine Photo-Cross-linking and Click Chemistry. Curr. Protoc. Chem. Biol. 2009, 1, 55–73. [PubMed: 23667793]

(7). Li Z; Hao P; Li L; Tan CYJ; Cheng X; Chen GYJ; Sze SK; Shen H-M; Yao SQ Design and Synthesis of Minimalist Terminal Alkyne-Containing Diazirine Photo-Crosslinkers and Their Incorporation into Kinase Inhibitors for Cell- and Tissue-Based Proteome Profiling. Angew. Chem., Int. Ed. 2013, 52, 8551–8556.

(8). Das J Aliphatic Diazirines as Photoaffinity Probes for Proteins: Recent Developments. Chem. Rev. 2011, 111, 4405–4417. [PubMed: 21466226]

(9). Chang C-F; Mfuh A; Gao J; Wu H-Y; Woo CM Synthesis of an electronically-tuned minimally interfering alkynyl photo-affinity label to measure small molecule-protein interactions. Tetrahedron 2018, 74, 3273–3277.

(10). Murale DP; Hong SC; Haque MM; Lee J-S, Photo-Affinity Labeling (PAL) in Chemical Proteomics: a Handy Tool to Investigate Protein-Protein Interactions (PPIs). Proteome Sci. 2016, 15 DOI: 10.1186/s12953-017-0123-3.

(11). Song C-X; He C Bioorthogonal Labeling of 5-Hydroxymethylcytosine in Genomic DNA and Diazirine-Based DNA Photo-Cross-Linking Probes. Acc. Chem. Res. 2011, 44, 709–711. [PubMed: 21539303]

(12). Bond MR; Zhang H; Kim J; Yu S-H; Yang F; Patrie SM; Kohler JJ Metabolism of Diazirine-Modified N-Acetylmannosamine Analogues to Photo-Cross-Linking Sialosides. Bioconjugate Chem. 2011, 22 (22), 1811–1823.

(13). Feltes M; Moores S; Schaffer JE Synthesis and characterization of diazirine alkyne probes for the study of intracellular cholesterol trafficking. J. Lipid Res. 2019, 60, 707–716. [PubMed: 30617147]

(14). Xia Y; Peng L Photoactivatable Lipid Probes for Studying Biomembranes by Photoaffinity Labeling. Chem. Rev. 2013, 113, 7880–7929. [PubMed: 23947482]

(15). Peng T; Hang HC Bifunctional Fatty Acid Chemical Reporter for Analyzing S-Palmitoylated Membrane Protein–Protein Interactions in Mammalian Cells. J. Am. Chem. Soc. 2015, 137, 556–559. [PubMed: 25575299]

(16). Brunner J; Senn H; Richards FM 3-Trifluoromethyl-3-phenyldiazirine. J. Biol. Chem. 1980, 255 (8), 3313–3318. [PubMed: 7364745]

(17). Chee G-L; Yalowich JC; Bodner A; Wu X; Hasinoff BB A diazirine-based photoaffinity etoposide probe for labeling topoisomerase II. Bioorg. Med. Chem. 2010, 18, 830–838. [PubMed: 20006518]

(18). Kirmse W; Meinert T; Modarelli DA; Platz MS Carbenes and the O-H Bond: Bicycloalkylidenes. J. Am. Chem. Soc. 1993, 115, 8918–8927.

(19). Platz MS A Perspective on Physical Organic Chemistry. J. Org. Chem. 2014, 79, 2341–2353. [PubMed: 24571434]

(20). Mix KA; Aronoff MR; Raines RT Diazo Compounds: Versatile Tools for Chemical Biology. ACS Chem. Biol. 2016, 11, 3233–3244. [PubMed: 27739661]

(21). McGarrity JF In The Chemistry of Diazonium and Diazo Groups; Patai S, Ed.; Wiley: Chichester, 1978.

(22). McGrath NA; Andersen KA; Davis AKF; Lomax JE Diazo compounds for the bioreversible esterification of proteins. Chem. Sci. 2015, 6, 752–755. [PubMed: 25544883]

(23). Mix KA; Lomax JE; Raines RT Cytosolic Delivery of Proteins by Bioreversible Esterification. J. Am. Chem. Soc. 2017, 139, 14396–14398. [PubMed: 28976737]

(24). Ressler VT; Mix KA; Raines RT Esterification Delivers a Functional Enzyme into a Human Cell. ACS Chem. Biol. 2019, 14, 599–602. [PubMed: 30830748]

(25). Iacobucci C; Götze M; Piotrowski C; Arlt C; Rehkamp A; Ihling C; Hage C; Sinz A Carboxyl-Photo-Reactive MS-Cleavable Cross-Linkers: Unveiling a Hidden Aspect of Diazirine-Based Reagents. Anal. Chem. 2018, 90, 2805–2809. [PubMed: 29376325]

(26). Kirmse W Carbene Chemistry, 2nd ed.; Academic Press: New York, 1971.

(27). McGarrity JF; Smyth T Hydrolysis of Diazomethane—Kinetics and Mechanism. J. Am. Chem. Soc. 1980, 102, 7303–7308.

(28). Pearce M The Rates of Diazomethane Formation from Methylnitrosoamides. The Stability of Diazomethane Solutions towards Aqueous Alkalis. Helv. Chim. Acta 1980, 63, 887–891.

(29). Jumper CC; Bomgarden R; Rogers J; Etienne C; Schriemer DC High-Resolution Mapping of Carbene-Based Protein Footprints. Anal. Chem. 2012, 84, 4411–4418. [PubMed: 22480364]

(30). Ziemianowicz DS; Bomgarden R; Etienne C; Schriemer DC Amino Acid Insertion Frequencies Arising from Photoproducts Generated Using Aliphatic Diazirines. J. Am. Soc. Mass Spectrom. 2017, 28, 2011–2021. [PubMed: 28799075]

(31). King S Orthoester-Dependent Alcoholysis of Lactones. Facile Preparation of 4-Alkoxybutanoates and 5-Alkoxypentanoates. J. Org. Chem. 1994, 59, 2253–2256.

(32). Nguyen ST; Zhu Q; Knowles RR PCET-Enabled Olefin Hydroamidation Reactions with N-Alkyl Amides. ACS Catal. 2019, 9 (5), 4502–4507. [PubMed: 32292642]

(33). Fairfax DJ; Austin DJ; Xu SL; Padwa A Alternatives to a-Diazo Ketones for Tandem Cyclization-Cycloaddition and Carbenoid-Alkyne Metathesis Strategies. Novel Cyclic Enol-

Ether Formation via Carbonyl Ylide Rearrangement Reactions. J. Chem. Soc., Perkin Trans. 1 1992, 2837–2844.

(34). Knoll W; Mieusset J-L; Arion VB; Brecker L; Brinker UH 2H-Azirines from a Concerted Addition of Alkylcarbenes to Nitrile Groups. Org. Lett. 2010, 12, 2366–2369. [PubMed: 20397663]

(35). Dommerholt J; Schmidt S; Temming R; Hendriks LJA; Rutjes FPJT; van Hest JCM; Lefeber DJ; Friedl P; van Delft FL Angew. Chem., Int. Ed. 2010, 49, 9422–9425.

(36). For each alkene, the decrease in yield corresponded within 3% of the expected yield based on the D/H ratio. See Table S9 in the Supporting Information.

(37). Complete suppression of D-incorporation was observed for intramolecular products in studies by Kirmse and Platz with bicyclic diazirine 9, which lacks a carbonyl and therefore cannot form a carbonyl ylide intermediate. See ref 18.

(38). Sugiyama MH; Celebi S; Platz MS A Significant Barrier to 1,2 Hydrogen Migration in Singlet 1-Phenylethylidene. A Laser Flash Photolysis Study. J. Am. Chem. Soc. 1992, 114, 966–973.

(39). Modarelli DA; Morgan S; Platz MS Carbene Formation, Hydrogen Migration, and Fluorescence in the Excited States of Dialkyldiazirines. J. Am. Chem. Soc. 1992, 114, 7034–7041.

(40). Modarelli DA; Platz MS Interception of Dimethylcarbene with Pyridine: A Laser Flash Photolysis Study. J. Am. Chem. Soc. 1991, 113, 8985–8986.

(41). Ahad AM; Jensen SM; Jewett JC A Traceless Staudinger Reagent To Deliver Diazirines. Org. Lett. 2013, 15, 5060–5063. [PubMed: 24059816]

(42). van Geel R; Prujin GJM; van Delft FL; Boelens WC Preventing Thiol-Yne Addition Improves the Specificity of Strain-Promoted Azide–Alkyne Cycloaddition. Bioconjugate Chem. 2012, 23, 392–398.

(43). Tian H; Sakmar TP; Huber T A simple method for enhancing the bioorthogonality of cyclooctyne reagent. Chem. Commun. 2016, 52, 5451–5454.

(44). Fox JM; Scacheri JEG; Jones KGL; Jones M Jr.; Shevlin PB; Armstrong B; Sztyrbicka R The Phenylcarbene Rearrangement as a Source of Real Carbenes. Tetrahedron Lett. 1992, 33, 5021–5024.

(45). Chen N; Jones M Jr.; White WR; Platz MS Equilibrium between Homocub-1(9)-ene and Homocub-9-ylidene. J. Am. Chem. Soc. 1991, 113, 4981–4992.

(46). Mansoor AM; Stevens IDR Hot radical effects in carbene reactions. Tetrahedron Lett. 1966, 7, 1733–1737.

(47). Chang KT; Shechter H Roles of Multiplicity and Electronic Excitation on Intramolecular Reactions of Alkylcarbenes in Condensed Phase. J. Am. Chem. Soc. 1979, 101, 5082–5084.

(48). Chambers GR; Jones M Jr. Intermolecular reactions of 4,4dimethylcyclohex-2-enylidene. J. Am. Chem. Soc. 1980, 102, 4516–4518.

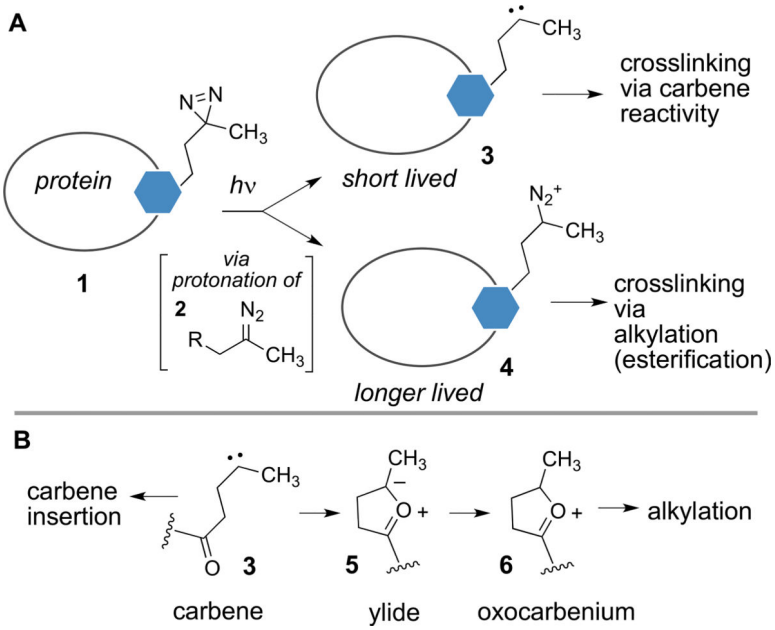


Figure 1.

Potential mechanisms for photoaffinity labeling with dialkyldiazirine probes. (A) Both carbene and diazonium intermediates can potentially lead to crosslinking. (B) For diazirines with pendant carbonyls, carbene mechanisms do not necessarily involve direct insertion, but may instead involve formation of ylide and oxocarbenium intermediates.

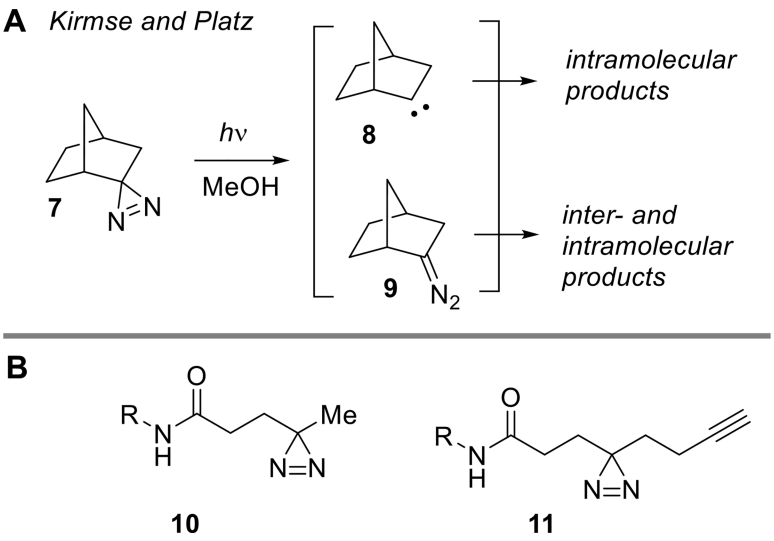


Figure 2.

(A) Studies of spirobicyclic diazirines by Kirmse and Platz. (B) Structures of "linear" alkyl diazirines more commonly used in biological photoaffinity labeling.

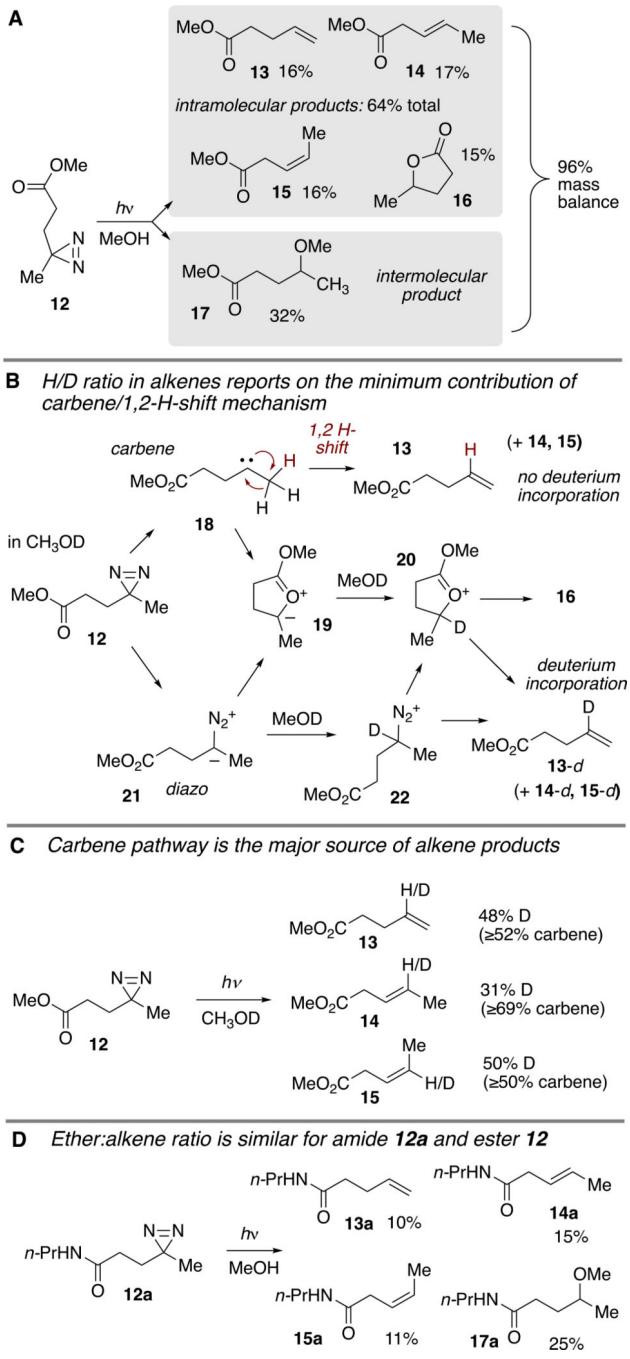
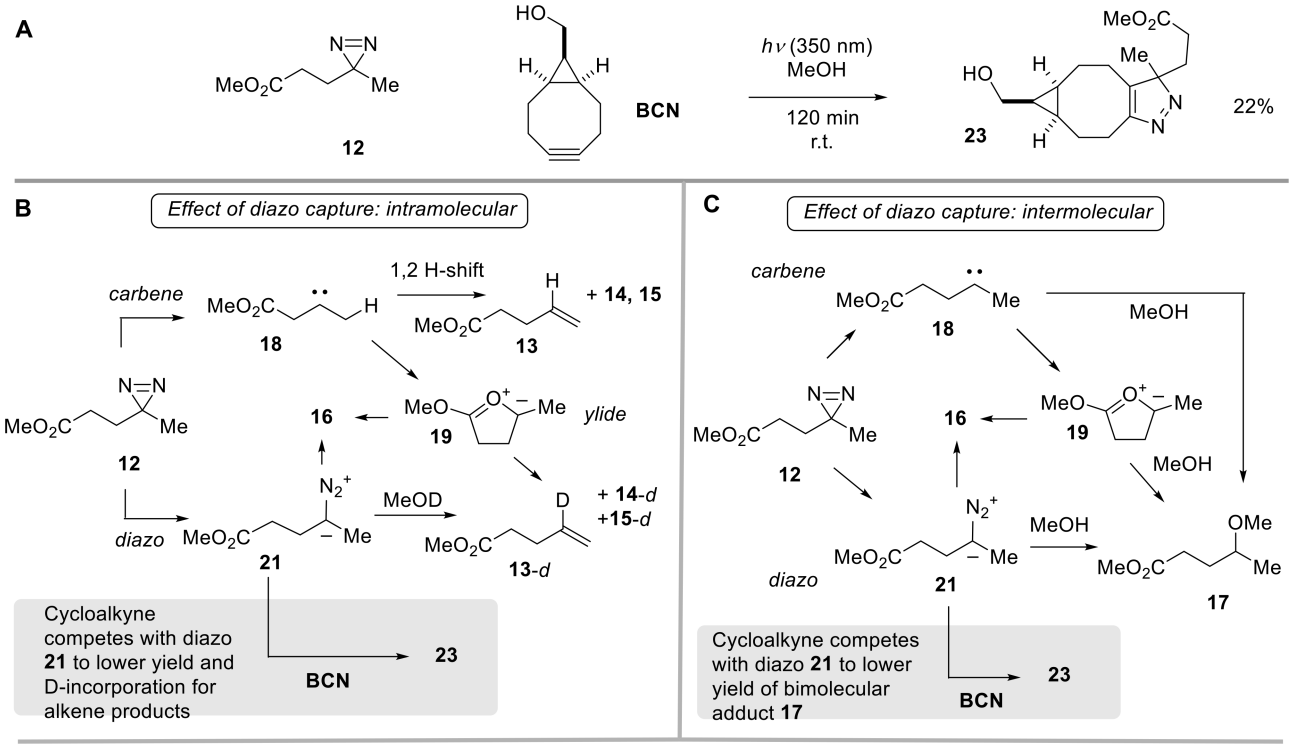
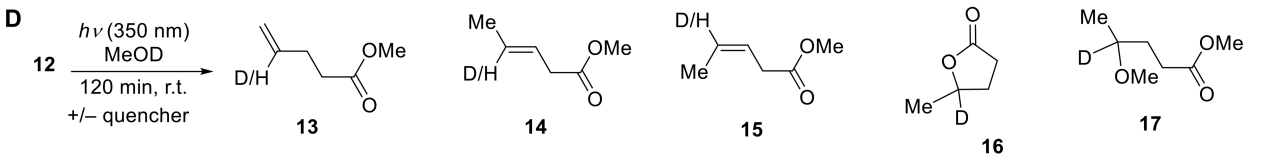


Figure 3.

(A) Products from photolysis of diazirine **12**. (B) Mechanistic pathways of intramolecular product formation for photolyses in MeOD leading to deuterated and non-deuterated alkenes **13–15**. (C) D/H ratio serves as a reporter of the carbene 1,2 H-migration pathway. (D) The ratio of alkene:ether products from amide **12a** is similar to that from ester **12**.





MeOD alone	15% yield 48% D	20% yield 31% D	17% yield 50% D	18% yield	29% yield
200 mM fumaronitrile	11% yield 10% D	16% yield 7% D	11% yield 3% D	14% yield	14% yield
70 mM BCN	10% yield 9% D	13% yield 7% D	10% yield 6% D	10% yield	14% yield

Figure 4.

(A) Preparative reaction of diazirine **12** with the cycloalkyne BCN to produce cycloadduct **23** is proposed to occur by [3 + 2] cycloaddition with intermediate diazo compound **21**, which (B) influence formation of intramolecular products by decreasing the yield and level of D-incorporation for alkenes **13–15**, and (C) on intermolecular chemistry by decreasing the yield of product **17**. (D) Effect of product formation by dipolarophiles BCN and fumaronitrile.

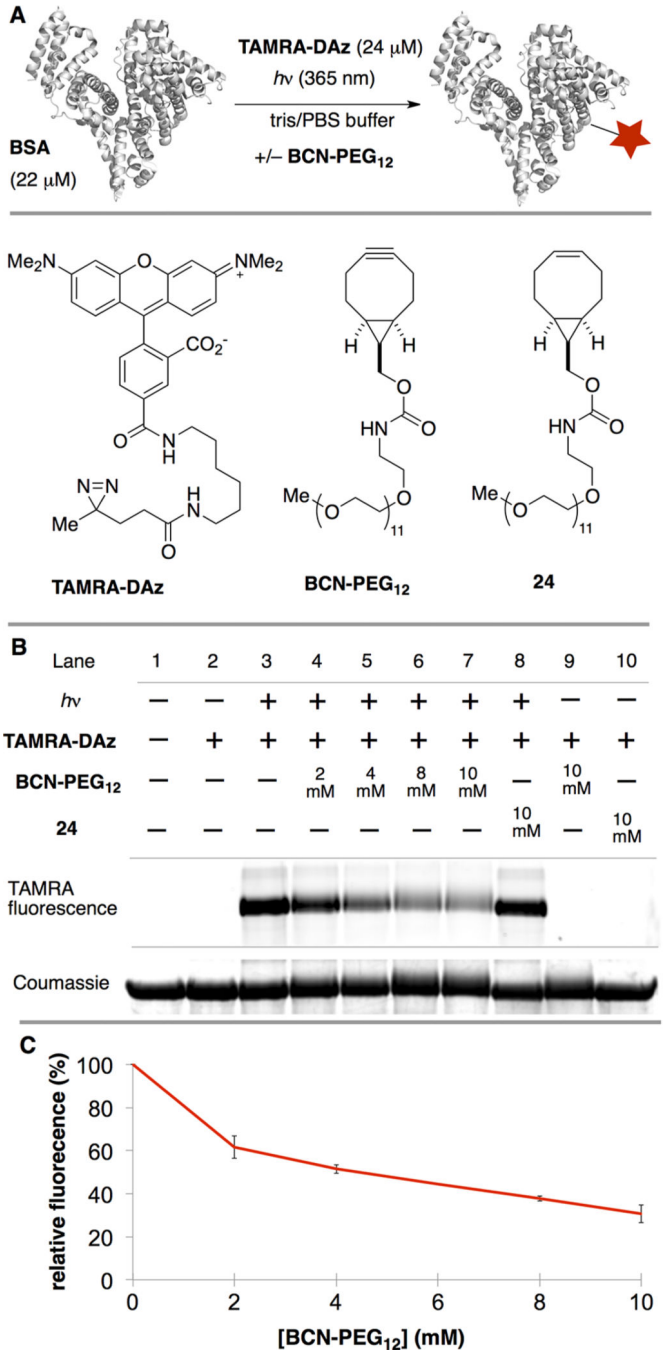


Figure 5.

(A) Experiment involving **BSA**, **TAMRA-DAz**, **BCN-PEG$_{12}$**, and **24**. (B) Lane assignments in SDS-PAGE. (C) Relative fluorescence with 0 to 10 mM **BCN-PEG$_{12}$**.