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A Bioorthogonal Ligation of Cyclopropenones Mediated by Triarylphosphines

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Supporting Information

ABSTRACT: Bioorthogonal chemistries have been widely used to probe biopolymers in living systems. To date, though, only a handful of broadly useful transformations have been identified because of the stringent requirements placed on the reactants. Here we report a novel bioorthogonal ligation between cyclopropenones and functionalized phosphines. These components are stable in physiological buffers and react rapidly with one another to form covalent adducts. The cyclopropenone ligation is also distinct from other bioorthogonal chemistries in that it makes use of readily accessible, commercially available reagents and proceeds via a nucleophilic reaction pathway. On the basis of these features, the cyclopropenone ligation is poised to join the ranks of chemistries with utility in living systems.

A complete understanding of organismal biology requires tools and technologies to examine biomolecules in their native habitats. Among the most powerful methods for probing biomolecules in cells and tissues is the bioorthogonal chemical reporter strategy. This approach relies on the installation of unique functional groups (reporters) into target biomolecules. The reporters can be detected in a second step using selective (bioorthogonal) reactions. The reporter and detection reagent must both be nontoxic and unreactive toward endogenous biological functionality, yet react rapidly with one another in physiological environments. Popular reporters include organic azides and terminal alkynes, although several other candidates have been reported in recent years.[1]

While the bioorthogonal toolbox is expanding at a rapid pace, many bioorthogonal reagents remain too large or unstable for tagging biomolecules in live cells. Moreover, the majority of common bioorthogonal reagents are incompatible with one another. This has stymied our ability to "see" multiple biomolecules in real time and in living systems. To address these limitations, new bioorthogonal reactions and combinations of such reactions are being developed. Our group has been exploring bioorthogonal transformations based on cyclopropenes.[2,3] These small, strained rings are broadly compatible with a variety of cellular functionality. Indeed, cyclopropenes have been installed in numerous biomolecules and detected via cycloaddition reactions with complementary probes.[3−9]

To further expand the bioorthogonal toolkit, we recently turned our attention to a related class of microcycles: cyclopropenones. These scaffolds are attractive for biomolecule tagging because of their small size and biocompatibility. Cyclopropenones are also present in natural products[10−12] and synthetic drugs,[12−17] suggesting that they possess some degree of metabolic stability. Cyclopropenones are not normally found in mammals but are tolerated by such organisms, making them well-suited for bioorthogonal applications in higher eukaryotes. Indeed, diarylcyclopropenones have been employed as photolabile masking groups in live cells.[18,19]

While cyclopropenones are stable in physiologically relevant environments, they react with soft nucleophiles (e.g., triarylphosphines) to produce activated electrophiles; these intermediates can be easily trapped to form stable adducts (Figure 1).[20] Like cyclopropenones, phosphines are extremely rare in mammalian systems and are also remarkably bioinert.[21,22] In fact, triarylphosphines are components of the Staudinger ligation—one of the first bioorthogonal reactions on record.[23,24] This reaction similarly exploits phosphine reactivity to generate activated intermediates that are subject to rapid covalent trapping. We aimed to capitalize on these features by designing a polar, bioorthogonal transformation with electrophilic cyclopropenones and nucleophilic phosphines.

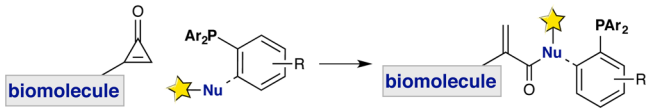

Figure 1. Phosphine-mediated cyclopropenone ligation. The triarylphosphine activates the cyclopropenone for attack by a pendant or exogenously added nucleophile.

Key to the success of the proposed reaction would be the generation and trapping of activated ketene ylide **1** (Figure 2). This intermediate would form upon initial Michael-type addition of triarylphosphine **2** to cyclopropenone **3** and subsequent ring opening. Ketene ylides can be long-lived and even isolated under ambient, moisture-free conditions.[20] However, these moieties are susceptible to rapid reaction with both oxygen and amine nucleophiles.[20,25−28] We hypothesized that intermediate **1** could be formed under aqueous conditions and competitively trapped with strong nucleophiles (e.g., amines) even in the presence of water. The selectivity could be further enhanced by tethering the desired

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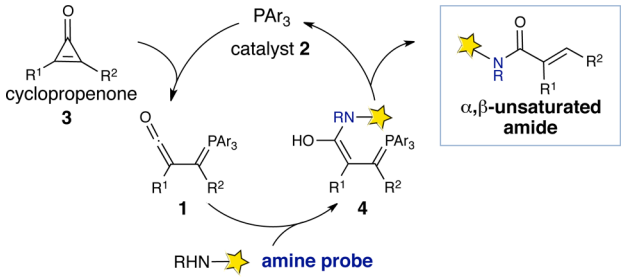
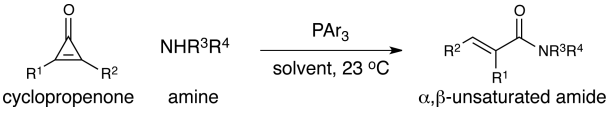
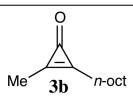
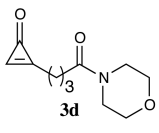


Figure 2. Proposed catalytic cycle for the tricomponent ligation.

Table 1. Scope of the Cyclopropenone Ligation



Entry	Cyclopropenone	Amine[a]	PAr_3 (mol%)	Solvent	Time	Yield (%)
1	 **3a**	$NH_2{}^iPr$	PPh_3 (100 mol%)	PhH	90 min	99
2		$NH_2{}^iPr$	PPh_3 (10 mol%)	PhH	16 h	93
3	 **3b**	$NH_2{}^iPr$	PPh_3 (100 mol%)	PhH	10 d	83[b]
4	 **3c**	$NH_2{}^iPr$	PPh_3 (100 mol%)	PhH	45 min	83
5		$NH_2{}^iPr$	PPh_3 (10 mol%)	PhH	10 h	79
6	 **3d**	$NH_2{}^iPr$	$P(3\text{-}SO_3NaPh)_3$ **2a** (10 mol%)	PBS[c]	30 min	75[d]
7			$P(3\text{-}SO_3NaPh)_3$ **2a** (10 mol%)	PBS[c]	30 min	81[d]

[a]1.5 equiv of amine was used. [b]The product was isolated as a 1.3:1 mixture of regioisomers. [c]Containing 20% MeCN. [d]10 equiv of amine was used.

nucleophile to the phosphine core or using an excess of an exogenously delivered probe. The latter case also offered a unique platform for bioorthogonal organocatalysis: following the trapping of ketene ylide **1** to yield hemiaminal **4**, an elimination–proton transfer sequence would provide the ligated product with concomitant regeneration of the phosphine.[29]

To evaluate the proposed transformation, we initially turned to commercially available cyclopropenone, phosphine, and amine reagents. Diphenylcyclopropenone (**3a**) was dissolved in benzene and incubated with triphenylphosphine and isopropylamine (Table 1, entry 1). The desired α,β-unsaturated amide was isolated in excellent yield with no observed decomposition or Baylis–Hillman intermediates. The reaction also proceeded efficiently when the ketene ylide was preformed and stored for 24 h prior to the addition of isopropylamine (Figures S11–S13).[26] Consistent with the proposed mechanism, lower catalyst loading slowed the reaction but did not significantly impact the overall yield (entry 2).

Encouraged by these results, we began to optimize the reagents for bioorthogonal application. We first synthesized two model alkyl-substituted cyclopropenones (**3b** and **3c**) that are smaller in size than **3a** and thus more attractive for biomolecule labeling. These cyclopropenones were synthesized via alkylation of the corresponding acetals.[16,30] Both **3b** and **3c** reacted with amines in the presence of triphenylphosphine (Table 1, entries 3–5), with the less substituted scaffold (**3c**) providing the fastest conversions. Very good yields of the desired amides (79–83%) were observed in all cases. Importantly, **3b** and **3c** were stable in physiological buffers and toward cellular concentrations of cysteine (Figures S2–S5).[31,32] The more water-soluble cyclopropenone **3d** was found to be similarly robust (Figures S6 and S7).

The ligation reaction was noticeably faster in aqueous solution. When cyclopropenone **3d** and water-soluble triarylphosphine **2a** were combined in the presence of primary or secondary amines, the reaction was complete in 30 min, even when catalytic amounts of phosphine were used (Table 1, entries 6 and 7). Good yields of the ligation adducts were observed in all cases, with amine trapping preferred over ketene hydrolysis even under aqueous conditions.[33] In the absence of phosphine, direct addition of the amine to the cyclopropenone was not observed (Figures S8 and S9).[34] Furthermore, the α,β-unsaturated ligation products were stable in physiological buffers and showed no additional modification by nucleophiles such as cysteine (Figure S10). Collectively, these results suggested that cyclopropenones could be efficiently ligated in biological settings using amine probes and triarylphosphine reagents.

To evaluate the ligation in a more relevant context, we appended cyclopropenone scaffolds to a model protein (lysozyme) using standard *N*-hydroxysuccinimide (NHS)

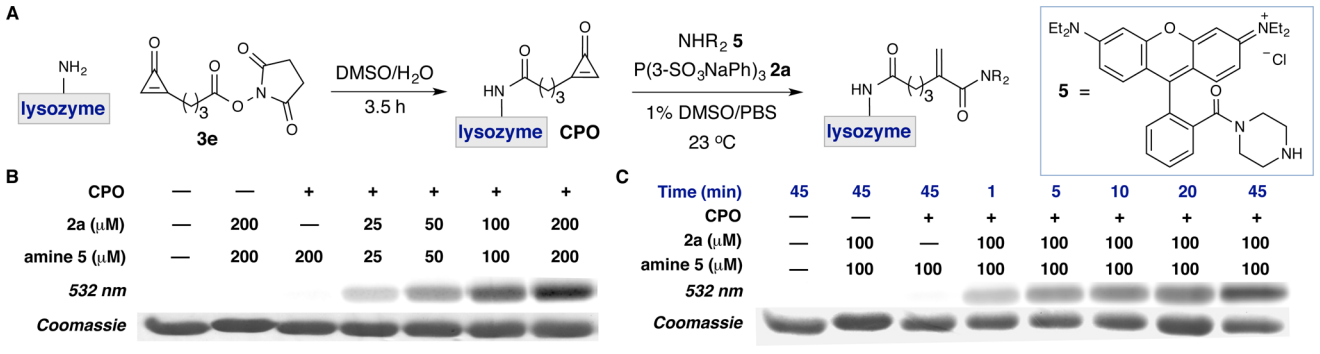


Figure 3. Cyclopropenones can be ligated on model proteins. (A) Cyclopropenone NHS ester **3e** was appended to the model protein lysozyme. The modified protein (**CPO**) was subsequently reacted with amine–rhodamine conjugate **5** in the presence of water-soluble triarylphosphine catalyst **2a**. (B) Gel analysis of **CPO** incubated with equimolar concentrations of **2a** (25–200 μM) and **5** (25–200 μM) or no reagent (−) for 20 min. (C) Gel analysis of **CPO** incubated with **2a** (100 μM) and **5** (100 μM) or no reagent (−) for 1–45 min.

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ester coupling conditions (Figures 3A and S14).[35] The modified protein sample (**CPO**) was then reacted with triarylphosphine **2a** in the presence of a widely available rhodamine probe (**5**). The resulting adducts were analyzed via gel electrophoresis and in-gel fluorescence imaging (Figures 3 and S15) along with mass spectrometry (Figure S17). As depicted in Figure 3B−C, the ligation was both dose- and time-dependent, and no reaction was observed in the absence of either the cyclopropenone scaffold or triarylphosphine. When 100 μM concentrations of phosphine and amine were used, the reaction was complete within 45 min. Sparing amounts of phosphine were also sufficient to ligate **CPO**, with excess amine providing more efficient reactions in all cases (Figure S15). Lower concentrations of amine resulted in diminished signal intensity, likely as a result of ketene hydrolysis (Figures 3B, S15, and S17). Thus, the cyclopropenone ligation is best performed with excess exogenous amine.

While suitable for some applications, the tricomponent cyclopropenone ligation (Figure 3) would be difficult to transition to biomolecule tagging in live cells, as three reactants must converge at a single spot. To access a more tractable *bimolecular* reaction for use in living systems, we envisioned combining the nucleophile and phosphine into a single reagent (P,X-probe **6**; Figure 4A). Such a probe would rapidly trap ketene **1** in an intramolecular fashion, mitigating against undesired reactions with water or endogenous biological nucleophiles. We synthesized model P,N-probe **6a** bearing a pendant aniline nucleophile. When **6a** was incubated with cyclopropenone **3c**, amide **7** was isolated as the major product (Figure 4B).

Given the efficiency of ketene trapping observed with **6a**, we anticipated that the bimolecular cyclopropenone ligation would be suitable for use in more complex environments. Indeed, when the model protein **CPO** was treated with **6a**, ligated adducts were readily detected, with no hydrolysis or undesired ketene addition products observed (Figure 4C). The ligation also proceeded readily in biological media and common buffers (Figures 4C and S18). As a further check on the selectivity, we assayed the ligation in a more complex environment. **CPO** was incubated in lysate from HEK293 cells and then treated with rhodamine-conjugated P,N-probe **6b**. As shown in Figures 4D and S16, ligated adducts were readily detected, with no diminishment in signal due to competitive nucleophile addition. These results indicated that ketene trapping by the tethered amine is rapid and further suggested that weaker nucleophiles (e.g., phenols) could potentially afford efficient bimolecular ligation. Indeed, when P,O-probe **6c** was combined with cyclopropenone **3c**, ester formation was observed (20 min, 69% yield). The P,O-probe was also capable of ligating the model protein **CPO** with no competitive hydrolysis (Figure S19). These data suggest that the cyclopropenone ligation can be used to access a range of biomolecule linkages.

In conclusion, a novel bioorthogonal ligation is reported. This transformation exploits the electrophilic nature of cyclopropenone reagents and their susceptibility to attack by nucleophilic triarylphosphines. The reaction produces activated ketene intermediates that can be efficiently trapped with exogenous amine nucleophiles to form stable amide adducts. This tricomponent ligation is advantageous in terms of reagent accessibility (similar to the copper-catalyzed "click" reaction). A plethora of functionalized amine fluorophores and affinity tags are commercially available, making this a versatile and immediately useful reaction. Moreover, the phosphine and

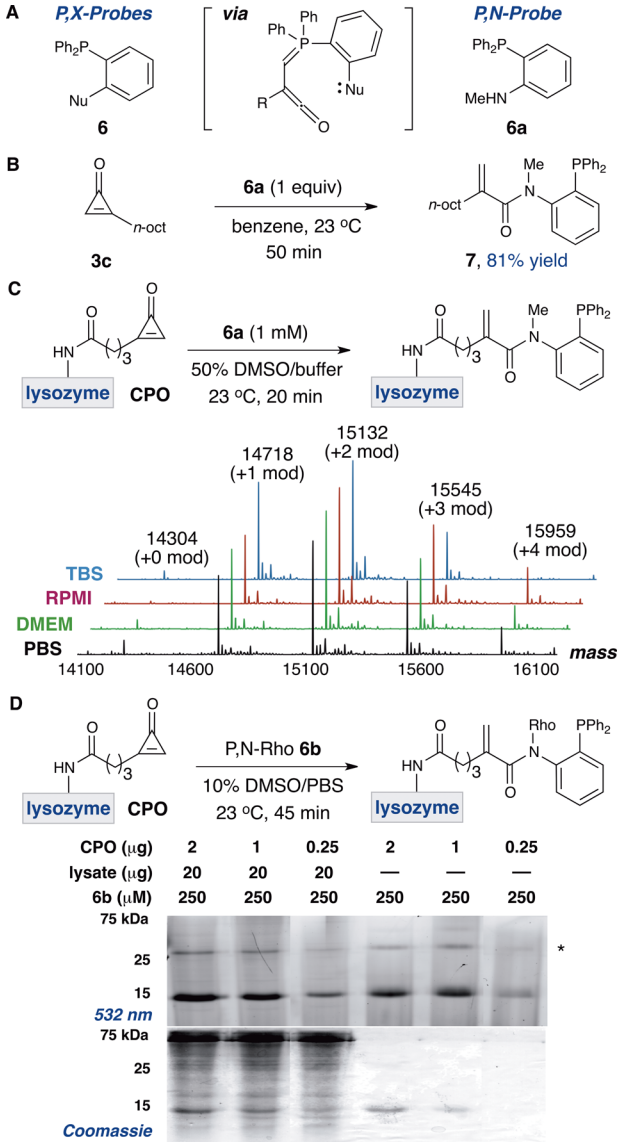

Figure 4. (A) Bimolecular P,X-probes react via intramolecular ketene trapping. (B) P,N-probes react efficiently with cyclopropenones. (C) Cyclopropenone-modified lysozyme **CPO** reacts selectively with P,N-probe **6a**. ESI-MS shows 1−4 ligations and no intermolecular ketene addition products or cyclopropenone after 20 min. (D) Gel analysis of **CPO** (2−0.25 μg) incubated with P,N-rhodamine probe **6b** (250 μM) and HEK293 lysate (20 μg) or no lysate (−) for 45 min. *denotes **CPO** dimer.

trapping nucleophile can be merged into a single scaffold. Such probes offer added utility in complex settings.

The cyclopropenone−phosphine ligation is also distinct from other bioorthogonal ligations. First, this ligation is one of the few bioorthogonal reactions described to date that proceed via a nucleophilic reaction manifold. Most bioorthogonal reactions, and nearly all of the ones reported in the last 15 years, comprise cycloadditions. The most notable exception is the Staudinger ligation, one of the first bioorthogonal reactions on record and still one of the most selective.[21,23,24] Second, the mechanism of the cyclopropenone ligation suggests that the reaction will be orthogonal to most bioorthogonal cycloadditions and thus useful for multicomponent labeling. As mentioned earlier, many popular bioorthogonal motifs are incompatible with one another and cannot be used concurrently to probe multiple

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biomolecules or cellular processes in vivo. Third, the α,β-unsaturated products afforded by the ligation can potentially be further exploited for biomolecule tagging. Last, the cyclopropenone scaffold is small and highly tunable. Collectively, these features will enable new biological applications and inspire new reaction development.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b06969.

Experimental details, full spectroscopic data for all new compounds, and additional images (PDF)

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Notes

The authors declare no competing financial interest.

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