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Note

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Cyclopropeniminium ions exhibit unique reactivity profiles with bioorthogonal phosphines

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ABSTRACT: We report a new ligation of cyclopropeniminium ions with bioorthogonal phosphines. Cyclopropeniminium scaffolds are sufficiently stable in biological media and, unlike related isomers, react with functionalized phosphines via formal 1,2-addition to a π-system. The ligation can be performed in aqueous solution and is compatible with existing bioorthogonal transformations. Such mutually compatible reactions are useful for multicomponent labeling.

Bioorthogonal chemistries are powerful tools for investigating biomolecules in living systems.[1,2,3] These transformations involve reagents that react selectively with one another while remaining inert to biological species. Such chemistries have enabled numerous applications *in vitro* and *in vivo*,[4] including biomolecule imaging,[5,6,7] antibody-drug conjugation,[8,9] and on-demand drug release.[10,11,12] While the number and examples of bioorthogonal reagents continue to grow, limitations remain.[13,14,15,16] Many scaffolds are too large for routine use with biological targets. Several of the most popular reagents also cross-react, hindering multicomponent labeling applications.

The need for additional biocompatible reagents has inspired several pursuits over the years.[17,18] We recently reported cyclopropenone (CpO) scaffolds with broad biological utility.[19,20] CpO motifs are relatively small and stable, and they react selectively with functionalized (and bioorthogonal) phosphines. The ligation proceeds via a unique ketene-ylide intermediate that can be trapped with a variety of nucleophiles (Figure 1).[21] Modifications to the cyclopropenone core can also alter reactivity. For example, we showed that sulfur heteroanalogs (CpS) react more rapidly with substituted phosphines.[22]

We hypothesized that additional CpO analogs would broaden the scope of bioorthogonal reactivity.[23,24] We were drawn to cyclopropeniminium (CpN^+) motifs based on their previous use in aqueous environments (Figure 2).[25,26,27] Mono-*N*-substituted scaffolds (e.g., I–III) are not stable across the entire span of physiological pH values.[28] *N,N*-Disubstituted scaffolds, by contrast, are robust in a range of environments.[29,30] CpN^+ analogs have even been used as transfection reagents, suggesting compatibility with live cells.[31] Furthermore, Hamada and colleagues showed that cyclopropenimines can react with triarylphosphines[32]—reagents that have been well vetted in living systems.[33,34,35]

To evaluate CpN^+ motifs as candidate bioorthogonal reagents, we first synthesized a panel of symmetric probes (**2a**–**c**, Scheme 1). The desired motifs were obtained from the corresponding cyclopropenones via carbonyl activation and amine displacement.[25,36] To access **2a–b**, commercially available alkynes were reacted with difluorocarbene and then hydrolyzed using the general procedure of Olah, *et al.*[20,37] The resulting cyclopropenones were then alkylated with Meerwein's reagent and subsequently treated with diethylamine. The aryl-substituted probe **2c** was similarly accessed using commercially available diphenylcyclopropenone (**1c**). The overall yields for **2a**–**c** were modest (Scheme S1), likely due to inefficient carbonyl activation. In each case, though, the remaining cyclopropenone could be readily recovered and re-subjected to the reaction sequence.

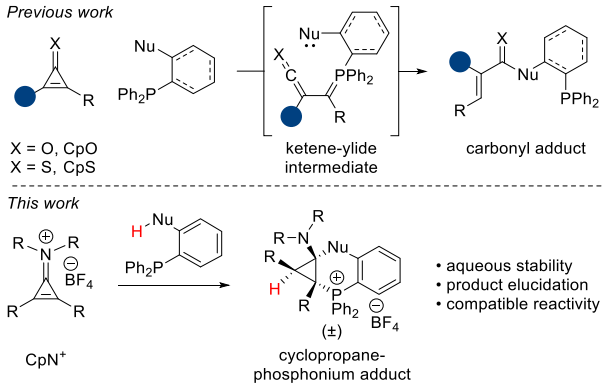


Figure 1. Cyclopropenone analogs can be ligated with bioorthogonal phosphines. Cyclopropenone (CpO) and cyclopropenethione (CpS) scaffolds react with substituted phosphines to provide carbonyl adducts. In this work, we examine the reactivity of analogous cyclopropeniminium (CpN^+) ions.

With the CpN^+ compounds in hand, we tested their stabilities in aqueous solution (Table S1). Compounds **2a**–**c** were dissolved in buffer (pH 7.4) and monitored via nuclear magnetic resonance (NMR) spectroscopy. Compounds **2a**–**b** were stable for >2 d (Figures S1–S2), suggesting that they are suita-

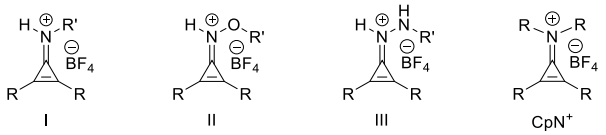
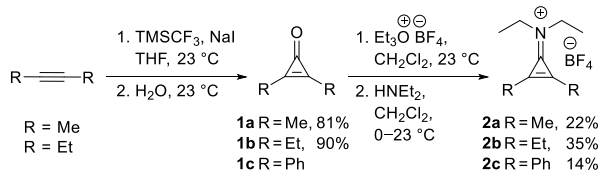


Figure 2. Cyclopropeniminium analogs are candidate bioorthogonal probes.

Scheme 1. Synthesis of CpN$^+$ probes



ble for biological use. Compound **2c**, by contrast, degraded over 24 h (Figure S3). Aryl-substituted CpO and CpS derivatives were also previously observed to be less stable than their bis-alkylated counterparts.[20,22] Alkyl-substituted CpN$^+$ probes are susceptible to degradation at higher pH values, but they are sufficiently long-lived for most applications ($t_{1/2}$ ~13 d at pH 8.4, Table S2).

CpN$^+$ species were further examined in the presence of biologically relevant nucleophiles. Compounds **2a**–**c** were incubated with amines or L-glutathione (5 mM) in aqueous buffer and analyzed via NMR spectroscopy. Analogs **2a**–**b** were stable to exogenous amines, while **2c** degraded under similar conditions (Figure S4–S6). The probes also exhibited varying degrees of reactivity with cellular thiols (Figures S7–S9). The half-life of **2c** in the presence of L-glutathione was ~7 h, while the half-life of **2b** (the most robust probe) was ~41 h. Despite being less stable than their CpO or CpS counterparts, CpN$^+$ probes are still suitable for use in non-reducing environments.[20,22] The reaction between CpN$^+$ motifs and L-glutathione also liberates diethylammonium ions and bioorthogonal CpO scaffolds (Figure S7–S9), a process that could potentially be exploited for caged probe release.

CpN$^+$ analogs were hypothesized to react with functionalized phosphines via initial conjugate addition, ultimately undergoing ring opening similar to CpO and CpS derivatives (Figures 1 and S10).[21,38,39] However, when **2b** was incubated with phosphine **3a**, the expected iminium product was not observed. Instead, a species with hemiaminal and phosphonium character was formed (**4a**, Figure 3A). Unfortunately, this product was found to revert to starting materials over time (Figure S11). A stable product was formed in the reaction of **2b** and phosphine **3b**, and spectral analyses confirmed the presence of a cyclopropane-phosphonium adduct (**4b,** Figure 3B). The ^{13}C-^{31}P coupling constants in the ^{13}C NMR spectrum were smaller than typical J_{C-P} values (Figure S12). Cyclopropane carbon resonances are typically less affected by phosphorous nuclei.[40] Additionally, ^{1}H-^{1}H COSY analysis revealed $^4J_{H-H}$ coupling between the endocyclic methine and exocyclic methylene protons (Figure S13). Such long-range interactions have been observed in related structures.[41] NMR analyses also suggested a single diastereomer was formed. Unfortunately, ^{1}H-^{1}H NOESY analysis could not distinguish among the possibilities (Figure S14). To unambiguously assign the stereochemistry, crystals of **4b** were grown and analyzed via X-ray diffraction (Figure 3C). The structure confirmed the presence of a cyclopropane-phosphonium bicycle (Figure S15, Table S2). Interestingly, the phosphorus and sulfur atoms were positioned on the same face of the ring as the methine proton.

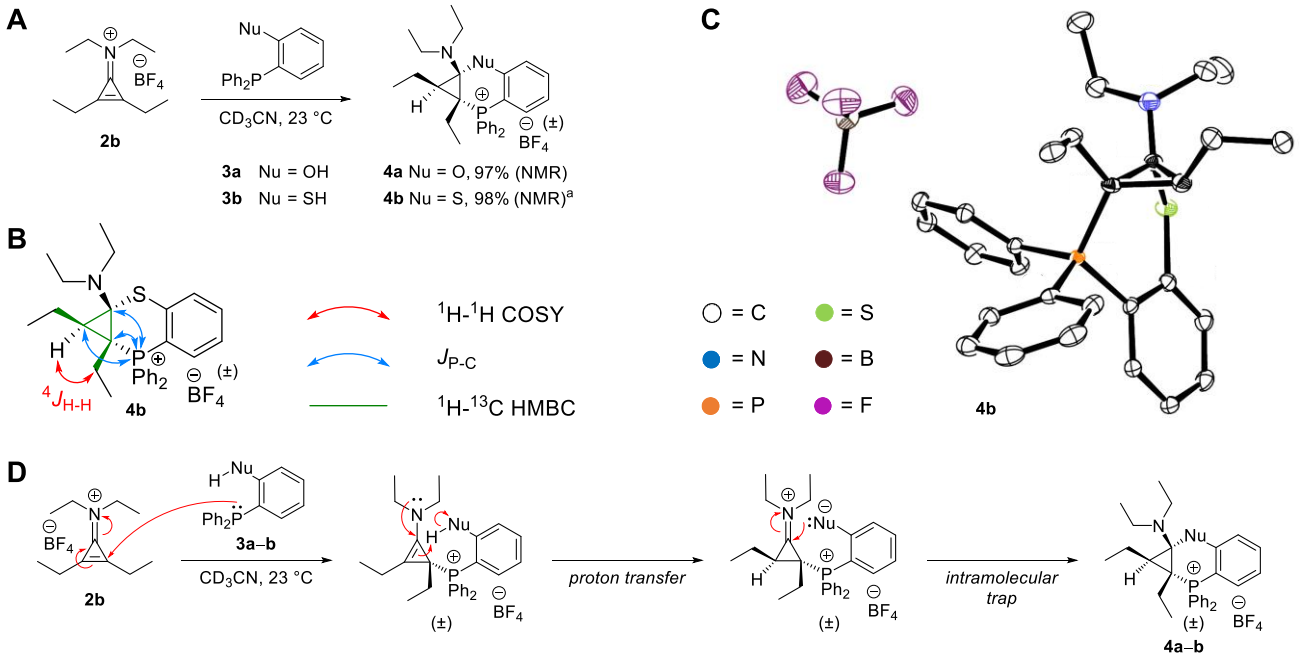
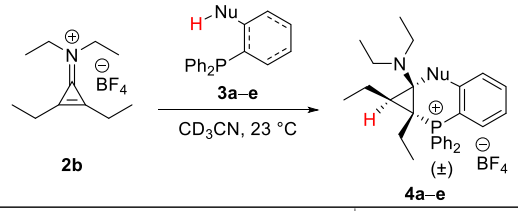
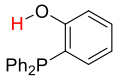
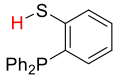
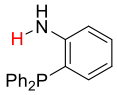
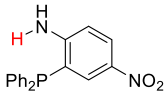
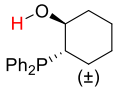


Figure 3. The CpN$^+$-phosphine ligation. (A) CpN$^+$ **2b** reacts with phosphines **3a**–**b** to yield cyclopropane-phosphonium adducts **4a**–**b**. (B) Structure of **4b** as suggested by correlative NMR analyses. Key connections are indicated. (C) ORTEP diagram of **4b** showing thermal ellipsoids at the 50% probability level (hydrogens have been omitted for clarity). (D) Proposed mechanism for the formation of **4a**–**b**. Phosphines **3a**–**b** react with **2b** via a conjugate addition. Subsequent intramolecular proton transfer and nucleophilic attack provide the observed adducts. [a]Isolated yield 46%.

Table 1. CpN$^+$ species reactivity with functionalized phosphines



	Phosphine	pK_a	k_2 (M^{-1} s^{-1})
3a		~ 10	7.1 ± 0.8 x 10^{-3}
3b		~ 6	2.3 ± 0.3 x 10^{-3}
3c		~ 31	N.R.
3d		~ 21	N.R.
3e		~ 16	N.R.

The CpN$^+$-phosphine ligation is also compatible with aqueous solvent. When **2b** and phosphine **3b** were mixed in CH_3CN containing 50% water, conversion to the expected adduct **4b** was observed (Figures S16 and S17). Thiohemiaminal **4b** was also stable over time in aqueous solution (Figure S18). To further probe the longevity of **4b** in biological environs, we incubated the compound with L-glutathione (Figure S19). No degradation was observed over 24 h. Related thiohemiaminal motifs comprise natural products and drug scaffolds, providing further evidence for their biocompatibility.[42,43]

The CpN$^+$-phosphine ligation appears to be a highly unusual formal [4+2] bis-nucleophilic cycloaddition. The observed stereoselectivity can be explained via the mechanism shown in Figure 3D. We propose that phosphines **3a**–**b** first react with **2b** to form enamine intermediates. Intramolecular proton transfer to a single face of the enamine dictates the observed stereochemistry. The deprotonated nucleophile can then attack the iminium species, generating a product with the phosphonium, nucleophile, and proton positioned on a single face of the cyclopropane ring.

The proposed mechanism is further supported by reactivity data. When **2b** was incubated with a panel of functionalized phosphines (**3a**–**e**), reactions were only observed with the phenol and thiophenol conjugates (**3a**–**b**, Table 1 and Figure S20). No reaction was observed with the corresponding aniline probes **3c**–**d** (Figures S21 and S22), even when the less sterically encumbered CpN$^+$ **2a** was used (Figure S23). The trends in reactivity correlate with the pK_a values of the pendant nucleophiles, with efficient ligation observed with the most acidic residues (pK_a ~ 10, 6 for **3a**–**b**, respectively). Intramolecular proton transfer is likely, as no ligation was observed when triphenylphosphine and exogenous thiophenol were combined with **2b** (1:1, data not shown). Slight reactivity was observed only when >100 equivalents of thiophenol were present (Figure S24). Further evidence for the proposed mechanism comes from a lack of reactivity between **2b** and phosphine **3e**. The more nucleophilic cyclohexyl phosphine[44] has been shown to robustly ligate CpO and CpS derivatives[20] In the case of CpN$^+$ species, though, no reactivity was observed (Figure S25), likely due to inefficient proton transfer (Figure S26).

The unique reactivity profile of CpN$^+$ motifs suggested that they could be used in tandem with other bioorthogonal reagents, even the structurally related cyclopropenones. CpO derivatives react quickly with phosphine **3e** (k_2 = 0.34 ± 0.06 M^{-1} s^{-1}), but the same phosphine does not ligate **2b**.[20] Conversely, phosphine **3b** reacts readily with **2b** (k_2 = 2.3 ± 0.3 x 10^{-3} M^{-1} s^{-1}) but only sluggishly with similar cyclopropenones ($k_2 \leq 10^{-5}$ M^{-1} s^{-1}).[20] To examine whether the CpO- and CpN$^+$-phosphine ligations could be used concurrently, cyclopropenone **1b**, CpN$^+$ **2b**, and phosphines **3b** and **3e** were mixed together, and the reactions were monitored by ^{1}H- and ^{31}P-NMR spectroscopy (Figures S27-S28). The expected ligation products formed, but a minor cross product was also observed (Figure S29). This product would be unlikely to form under biologically relevant conditions, when lower reactant concentrations are typically employed (Figure S29). Nonetheless, to showcase the exquisite compatibility of the CpO and CpN$^+$ probes, we performed the ligations sequentially. Compounds **1b**, **2b**, and **3e** were first incubated, and only the expected carbonyl adduct was observed (Figures 4, S30–S32). Phos-

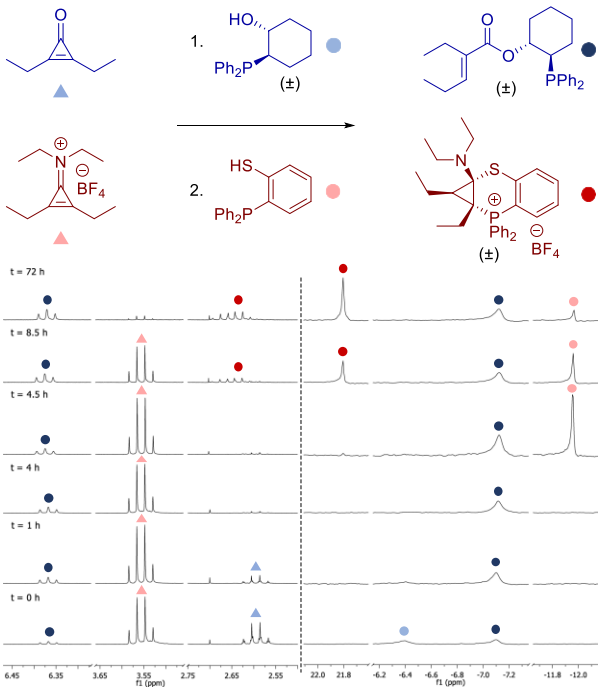


Figure 4. Compatible CpO- and CpN$^+$-phosphine ligations. CpO **1b** (blue triangle), CpN$^+$ **2b** (pink triangle), and phosphine **3e** (light blue circle) were mixed in 20% C_6D_6/CD_3CN. A single adduct formed (dark blue circle). After 4 h, phosphine **3b** (light pink circle) was added, providing the second adduct (red circle). Full spectra are provided in Figures S30 and S31.

phine **3b** was then added, and the cyclopropane adduct formed with no cross products observed. Considering that the CpO and CpN^+ scaffolds differ by just a single heteroatom, it is noteworthy that they can be used for multi-component labeling.

In conclusion, we investigated *N*,*N*-dialkylcyclopropeniminium (CpN^+) ions as new bioorthogonal reagents. A panel of CpN^+ scaffolds was designed and synthesized. We tested the motifs in the presence of cellular nucleophiles, such as thiols and amines. We determined that dialkylated CpN^+ analogs react readily with functionalized phosphines to form cyclopropane-phosphonium adducts. The unique reactivity profiles of CpN^+ motifs enabled the reagents to be used in tandem with CpO probes. Collectively, cyclopropeniminium ions will be useful additions to the bioorthogonal toolkit and are poised for a variety of applications. Future work will address methods to install the probes on a range of target biomolecules for deployment in biological settings.

EXPERIMENTAL SECTION

General synthetic procedures. Compounds **3a**,[45] **3b**,[46] **3c**,[47] and **3d**–**e**[20] were prepared according to literature procedures. All reagents and solvents were used as received, unless otherwise specified. Anhydrous organic solvents were prepared by degassing with argon and passing through two 4 x 36 in. columns of anhydrous neutral A2 (8 x 12 mesh; LaRoche Chemicals: activated at 350 °C for 12 h under a flow of argon). Column chromatography was carried out using Silicycle 60 Å (230–400 mesh) silica gel. Thin layer chromatography (TLC) was carried out with Merck Millipore 250 mm silica gel F-254 plates. Plates were visualized using UV light or $KMnO_4$ stain. Organic solutions were concentrated under reduced pressure using a Büchi rotary evaporator.

^1H, ^{13}C, and ^{31}P NMR spectra were obtained using Bruker instruments: DRX400, DRX500 equipped with a cryoprobe, or AVANCE600 equipped with a cryoprobe. ^1H NMR spectra were acquired at 400 MHz, 500 MHz, or 600 MHz, ^{13}C spectra were acquired at 125 MHz or 151 MHz, and ^{31}P spectra were acquired at 162 MHz or 243 MHz. Spectra were internally referenced to residual solvent signals (7.27 ppm for ^1H and 77.16 ppm for ^{13}C for $CDCl_3$, 3.31 ppm for ^1H and 49.0 ppm for ^{13}C for CD_3OD, 1.94 ppm for ^1H and 1.32 ppm for ^{13}C for CD_3CN, and 4.79 ppm for ^1H for D_2O). ^{31}P NMR spectra were referenced by indirect absolute chemical shift to residual protic solvent signals. All spectra were acquired at 298 K. Chemical shifts are reported in ppm, and coupling constants (*J*) are reported in Hz. In some cases, internal standards were used (trimethylsilylacetylene for ^1H NMR and triphenylphosphine oxide for ^{31}P NMR). Mass spectra were acquired at the University of California, Irvine Mass Spectrometry Facility. Crystallographic analysis was performed at the University of California, Irvine X-Ray Crystallography Facility.

Preparation of 2,3-dimethylcycloprop-2-en-1-one (1a). Compound **1a** was prepared following the procedure of Shih, *et al*., with some modifications.[19] To an oven-dried sealed tube containing a stir bar was added NaI (1.65 g, 11.0 mmol). The NaI was gently flame-dried under vacuum and then allowed to cool to room temperature. A solution of 2-butyne (0.39 mL, 5.0 mmol) in anhydrous THF (15 mL) was added under an atmosphere of N_2. Trifluoromethyltrimethylsilane (1.47 mL, 10.0 mmol) was added, and the tube was sealed. The solution was stirred rapidly at room temperature for 2 d, then diluted with H_2O (60 mL) and extracted into CH_2Cl_2 (3 x 60 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was dry-loaded onto silica (3 g) and purified by flash column chromatography (eluting with 30% acetone/CH_2Cl_2) to afford compound **1a** as a yellow oil (0.33 g, 4.0 mmol, 80%). Spectra matched those previously reported.[48]

Preparation of 2,3-diethylcycloprop-2-en-1-one (1b). Compound **1b** was prepared following the procedure of Shih, *et al*., with some modifications.[19] To an oven-dried sealed tube containing a stir bar was added NaI (0.804 g, 5.36 mmol). The NaI was gently flame-dried under vacuum and then allowed to cool to room temperature. A solution of 3-hexyne (0.39 mL, 5.0 mmol) in anhydrous THF (7.3 mL) was added under an atmosphere of N_2. Trifluoromethyltrimethylsilane (0.72 mL, 4.9 mmol) was added, and the tube was sealed. The solution was stirred rapidly at room temperature for 2 d, then diluted with H_2O (30 mL) and extracted into CH_2Cl_2 (3 x 30 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was dry-loaded onto silica (2 g) and purified by flash column chromatography (eluting with 5% acetone/ethyl acetate) to afford compound **1b** as a yellow oil (0.24 g, 4.0 mmol, 91%). Spectra matched those previously reported.[49]

Preparation of *N*-(2,3-dimethylcycloprop-2-en-1-ylidene)-*N*-ethylethanaminium tetrafluoroborate (2a). To an oven-dried round bottom flask containing a stir bar was added cyclopropenone **1a** (25 mg, 0.31 mmol) and anhydrous CH_2Cl_2 (2 mL) under an atmosphere of N_2. Triethyloxonium tetrafluoroborate (1 M in CH_2Cl_2, 0.31 mL, 0.31 mmol) was added dropwise, and the reaction was stirred at room temperature for 20 min. A solution of diethylamine (0.032 mL, 0.31 mmol) was prepared in anhydrous CH_2Cl_2 (1 mL). Both solutions were chilled to 0 °C. The diethylamine solution was then added dropwise to the solution of alkylated **1a**, and the reaction was monitored by TLC (25% acetone/CH_2Cl_2). After 2 h, the reaction was concentrated *in vacuo*, and the residue was purified by flash column chromatography (eluting with 5% toluene/25% acetone/CH_2Cl_2) to afford compound **2a** as an orange oil (43 mg, 0.19 mmol, 22%). ^1H NMR (400 MHz, CD_3OD) δ 3.64 (q, *J* = 7.3 Hz, 4H), 2.52 (s, 6H), 1.36 (t, *J* = 7.3 Hz, 6H). $^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) δ 150.0, 143.5, 47.8, 12.2, 8.5. HRMS (ESI-TOF) m/z calculated for $C_9H_{16}N$ $[M]^+$ 138.1283, found 138.1281.

Preparation of *N*-(2,3-diethylcycloprop-2-en-1-ylidene)-*N*-ethylethanaminium tetrafluoroborate (2b). To an oven-dried round bottom flask containing a stir bar was added cyclopropenone **1b** (50 mg, 0.45 mmol) and anhydrous CH_2Cl_2 (3 mL) under an atmosphere of N_2. Triethyloxonium tetrafluoroborate (1 M in CH_2Cl_2, 0.45 mL, 0.45 mmol) was added dropwise, and the reaction was stirred at room temperature for 20 min. A solution of diethylamine (0.066 mL, 0.64 mmol) was prepared in anhydrous CH_2Cl_2 (1 mL). Both solutions were chilled to 0 °C. The diethylamine solution was then added dropwise to the solution of alkylated **1b**, and the reaction was monitored by TLC (10% acetone/CH_2Cl_2). After 2 h, the reaction was concentrated *in vacuo*, and the residue was purified by flash column chromatography (eluting with 5% toluene/10% acetone/CH_2Cl_2) to afford compound **2b** as a yellow oil (12 mg, 0.046 mmol, 11%). ^1H NMR (400 MHz, CD_3CN) δ 3.58 (q, *J* = 7.3 Hz, 4H), 2.84 (qt, *J* = 7.5, 1.1 Hz, 4H) 1.33 (t, *J* = 7.5 Hz, 6H), 1.29 (t, *J* = 7.3 Hz, 6H). $^{13}C\{^1H\}$ NMR

(151 MHz, CD_3CN) δ 150.8, 148.3, 49.6, 18.5, 13.6, 11.0. HRMS (ESI-TOF) m/z calculated for $C_{11}H_{20}N$ $[M]^+$ 166.1596, found 166.1598.

Preparation of *N*-(2,3-diphenylcycloprop-2-en-1-ylidene)-*N*-ethylethanaminium tetrafluoroborate (2c). To an oven-dried round bottom flask containing a stir bar was added cyclopropenone **1c** (53 mg, 0.26 mmol) and anhydrous CH_2Cl_2 (3 mL) under an atmosphere of N_2. Triethyloxonium tetrafluoroborate (1 M in CH_2Cl_2, 0.26 mL, 0.26 mmol) was added dropwise, and the reaction was stirred at room temperature for 20 min. A solution of diethylamine (0.037 mL, 0.34 mmol) was prepared in anhydrous CH_2Cl_2 (1 mL). Both solutions were chilled to 0 °C. The diethylamine solution was then added dropwise to the solution of alkylated **1c** at 0 °C, and the reaction was monitored by TLC (5% acetone/CH_2Cl_2). After 2 h, the reaction was concentrated *in vacuo*, and the residue was purified by flash column chromatography (eluting with 5% toluene/15% acetone/CH_2Cl_2) to afford compound **2c** as a yellow solid (13 mg, 0.035 mmol, 14%). ^{1}H NMR (400 MHz, CD_3CN) δ 8.15–8.11 (m, 4H), 7.88–7.83 (m, 2H), 7.80–7.74 (m, 4H), 3.96 (q, *J* = 7.3 Hz, 4H), 1.47 (t, *J* = 7.3 Hz, 6H). ^{13}C{^{1}H} NMR (151 MHz, CD_3CN) δ 144.8, 136.2, 135.8, 133.7, 131.1, 121.5, 50.7, 14.6. HRMS (ESI-TOF) m/z calculated for $C_{19}H_{20}N$ $[M]^+$ 262.1596, found 262.1593.

Preparation of 1a-(diethylamino)-1,7a-diethyl-7,7-diphenyl-1,1a,7,7a-tetrahydrobenzo[b]cyclopropa[e][1,4]-oxaphosphinin-7-ium tetrafluoroborate (4a). To an oven-dried scintillation vial was added cyclopropeniminium **2b** (18.3 mg, 0.072 mmol) and anhydrous MeCN (2.0 mL). Once dissolved, phosphine **3a** (40 mg, 0.144 mmol) was added and the reaction vessel was flushed with Ar. The reaction was then stirred under an inert atmosphere, and reaction was monitored by TLC (10 % acetone/CH_2Cl_2). After 72 h, the solution was concentrated *in vacuo* and purification was attempted by flash column chromatography (eluting with 5% toluene/10% acetone/CH_2Cl_2). While isolable, compound **4a** was found to revert to starting materials **2b** and **3a** at room temperature, preventing full characterization of compound **4a** (see Figure S11). ^{31}P{^{1}H} NMR (162 MHz, CD_3CN) δ 16.3. HRMS (ESI-TOF) m/z calculated for $C_{29}H_{35}NPO$ $[M]^+$ 444.2456, found 444.2500.

Preparation of 1a-(diethylamino)-1,7a-diethyl-7,7-diphenyl-1,1a,7,7a-tetrahydrobenzo[b]cyclopropa[e][1,4]-thiaphosphinin-7-ium tetrafluoroborate (4b). To an oven-dried scintillation vial was added cyclopropeniminium **2b** (26 mg, 0.11 mmol). and anhydrous MeCN (5.3 mL). Once dissolved, phosphine **3b** (34 mg, 0.12 mmol) was added and the reaction vessel was flushed with Ar. The reaction was then stirred under an inert atmosphere, and the reaction was monitored by TLC (10 % acetone/CH_2Cl_2). After 12 h, the solution was then concentrated *in vacuo* and purified by flash column chromatography (eluting with 5% toluene/10% acetone/CH_2Cl_2) to afford compound **4b** as a white solid (27 mg, 0.049 mmol, 46%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.99–7.96 (m, 2H), 7.89–7.79 (m, 8H), 7.72–7.67 (m, 2H), 7.58–7.54 (m, 1H), 7.33 (dd, *J* = 12.4, 7.9 Hz, 1H), 2.97 (dq, *J* = 15.1, 7.5 Hz, 1H), 2.89 (dq, *J* = 13.8, 7.0 Hz, 1H), 2.69 (dq, *J* = 13.8, 7.0 Hz, 1H), 2.47–2.28 (m, 4H), 1.99 (app quint, *J* = 7.1 Hz, 1H), 1.24 (t, *J* = 7.4 Hz, 3H), 1.21 (t, *J* = 7.2 Hz, 3H), 1.04 (t, *J* = 7.3 Hz, 3H), 0.68 (t, *J* = 7.5 Hz, 3H). ^{31}P{^{1}H} NMR (243 MHz, CD_3OD) δ 20.5. ^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ 144.6 (d, *J* = 6.5 Hz), 137.3 (d, *J* = 10.5 Hz), 137.3 (d, *J* = 2.3 Hz), 136.7–136.6 (m), 136.1 (d, *J* = 9.8 Hz), 135.4 (d, *J* = 9.8 Hz), 133.0 (d, *J* = 7.5 Hz), 132.0 (d, *J* = 12.9 Hz), 132.0 (d, *J* = 12.5 Hz), 129.3 (d, *J* = 12.3 Hz), 119.3 (d, *J* = 96.4 Hz), 118.3 (d, *J* = 89.0 Hz), 117.6 (d, *J* = 85.5 Hz), 69.8 (d, *J* = 3.9 Hz), 49.7, 49.6, 41.4 (d, *J* = 4.0 Hz), 35.2 (d, *J* = 72.5 Hz), 20.4 (d, *J* = 5.0 Hz), 18.4 (d, *J* = 1.1 Hz), 15.2, 14.7, 14.4, 12.0. HRMS (ESI-TOF) m/z calculated for $C_{29}H_{35}NPS$ $[M]^+$ 460.2228, found 460.2208.

Reaction compatibility of CpO- and CpN$^+$-phosphine ligations. For the simultaneous ligation experiment, cyclopropenone **1b** (20 mM), cyclopropeniminium ion **2b** (20 mM), cyclohexyl phosphine **3e** (20 mM), thiophenol phosphine **3b** (20 mM), and trimethylsilylacetylene (4 mM, internal standard for ^{1}H NMR) were combined in CD_3CN (containing 14% C_6D_6) in an oven-dried NMR tube. The reaction was monitored by ^{1}H- and ^{31}P{^{1}H}-NMR spectroscopy. For the sequential ligation experiment, cyclopropenone **1b** (20 mM), cyclopropeniminium ion **2b** (20 mM), phosphine **3e** (24 mM), triphenylphosphine oxide (2 mM, internal standard), and trimethylsilylacetylene (2 mM, internal standard) were combined in CD_3CN (containing 20% C_6D_6) in an oven-dried NMR tube. The reaction was monitored by ^{1}H- and ^{31}P-NMR spectroscopy. When **1b** was consumed, phosphine **3b** (18 mM) was added to the solution. The reaction was monitored by ^{1}H- and ^{31}P{^{1}H}-NMR spectroscopy.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS publication website at DOI: http://pubs.acs.org.

Additional images and NMR spectra (PDF).

X-ray crystallographic data of compound **4b** (CIF).

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Notes

The authors declare no competing financial interests.

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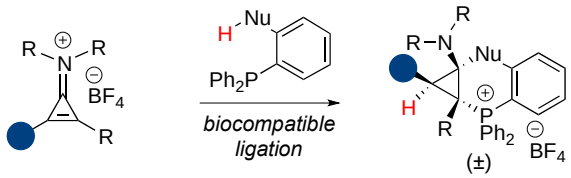
TOC Graphic



cyclopropane-phosphonium adduct