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Biomolecule-Compatible Dehydrogenative Chan-Lam Coupling of Free Sulfilimines

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Abstract

Inspired by the discovery of an S=N bond in the collagen IV network and its essential role in stabilizing basement membranes, sulfilimines have drawn much attention in the fields of chemistry and biology. However, its further uptake is hindered by the lack of mild, efficient and environmentally benign protocols by which sulfilimines can be constructed under biomolecule-compatible conditions. Here, we report a terminal oxidant-free copper-catalyzed dehydrogenative Chan-Lam coupling of free diaryl sulfilimines with arylboronic acids with excellent chemoselectivity and broad substrate compatibility. The mild reaction conditions and biomolecule-compatible nature allow the employment of this protocol in the late-stage functionalization of complex peptides, and more importantly, as an effective bioconjugation method as showcased in a model protein. A combined experimental and computational mechanistic investigation reveals an inner sphere electron transfer process circumvents the sacrificial oxidant employed in traditional Chan-Lam coupling reactions. An energetically viable concerted pathway was located wherein a copper hydride facilitates hydrogen atom abstraction from the isopropanol solvent to produce dihydrogen via a four-membered transition state.

Graphical Abstract

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Supporting Information

Detailed experimental procedures, characterization data, NMR spectra of new compounds, detailed computational study, and calculated structures are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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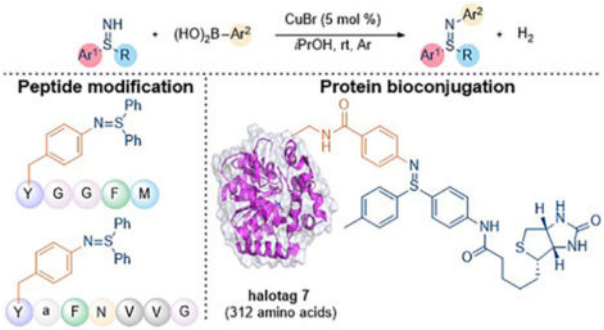

INTRODUCTION

Sulfilimines, the aza-analogues of sulfoxides, are a class of sulfur (IV)-derived scaffolds, which possess a unique sulfurstereocentre if two carbon-based substituents on sulfur are not identical. They have been exploited in organic chemistry as building blocks, directing groups, chiral axillaries, among others.[1] Moreover, chiral sulfilimines have also been developed as ligands for transition-metal catalysts.[2] In recent years, sulfilimines have been added to the toolbox used by medicinal chemists owing to their structural similarity to sulfoxides yet possessing an additional site for derivatization.[2–3]

In 2009, Hudson and coworkers discovered a surprising sulfilimine bond covalently crosslinking hydroxylysine-211 and methionine-93 to adjoin triple-helical protomers of collagen IV, which is a highly conserved major component of basement membranes across the animal kingdom.[4] Moreover, this unique bond plays a key role in the structural integrity of basement membranes.[5] The discovery of the first sulfilimine bond in naturally occurring biomacromolecules has aroused tremendous interest in this unusual structural motif in biological contexts. For example, Chang, Toste and coworkers have introduced an elegant biocompatible tagging strategy to oxidize methionines to the corresponding sulfilimines by treatment of oxaziridines, and successfully achieved antibody-drug conjugates as well as identification of reactive methionine residues in whole proteomes.[6] In addition, the Tang group has developed a molecular probe which can image HOBr based on formation of an intramolecular S=N bond in live cells and zebrafish.[7]

Considering the unique and intriguing properties of collagen IV derived from sulfilimine crosslinks, derivatization of peptides and proteins with this S(IV)-derived motif could endow them with novel features. In this regard, *N*-Ar diaryl sulfilimines, in sharp contrast to their *S*-alkyl counterparts, represent a promising scaffold of great value, since they are more stable toward acid, base, water, or elevated temperature.[1a] However, their uptake in medicinal chemistry as well as chemical biology was hampered by the lack of general and biocompatible synthetic methods. Conventionally, *N*-Ar diaryl sulfilimines could be prepared via oxidative imination of the corresponding sulfides,[1h,8] but the requisite strong oxidants jeopardize its application on biomolecules. Friedel-Crafts-typed arylation of *NH*-sulfilimines with aryl fluorides, chlorides or nitrates successfully leads to the formation of arylated products,[9] but substrate scope is limited to the electron-deficient arenes, thereby preventing its application on peptides or proteins. In 2019, the Hashmi group has disclosed

an elegant palladium-catalyzed arylation strategy of *NH*-sulfilimines with aryl bromides or iodides.[1h] Unfortunately, the strongly basic condition (KO*t*Bu) as well as the high reaction temperature (110 °C) renders this protocol unsuitable for functionalization of peptides or proteins.

Therefore, a general, mild and efficient synthetic route for *N*-Ar diaryl sulfilimines that could be applied to peptides and proteins is an unmet challenge.

With inexpensive and bio-friendly catalysts, an absence of acid, base, or elaborate ligands, mild and environmentally benign reaction conditions, as well as the excellent functional group compability, we hypothesized that copper-catalyzed Chan-Lam coupling[10] of free sulfilimines could be a powerful tool to install diaryl sulfilimines on complex peptides or even proteins. Indeed, the Bolm group has developed Chan-Lam couplings of sulfur(VI)-based sulfoximines[11] and sulfondiimines[12] using dry air as external oxidant in alcoholic solvents at ambient temperature. Ball and coworkers have introduced a seminal copper-catalyzed Chan-Lam coupling of the pyroglutamate amide N-H bond at a naturally encoded pyroglutamate-histidine dipeptide sequences.[13] However, due to the cross-nucleophile coupling nature, conventional Chan-Lam couplings typically require the use of oxidants to facilitate the oxidation of Cu(I) to Cu(II),[14] which could be problematic especially in biological contexts, wherein proteins and cells are sensitive to oxidative stress.[15] In this regard, a terminal oxidant-free Chan-Lam coupling process is an appealing alternative (Figure 1a). A source of inspiration of our design came from the classic electron-transfer triggered sulfoxide isomerization in metal complexes, in which an electron could be transferred from a metal center to sulfur(IV) atom to form sulfur-based radical anion (Figure 1b).[16] Though this process, which also serves as a key step in the transformation of cytochrome c from an electron-transfer carrier to a peroxidase associated with the methionine 80 sulfoxide ligation, has been known for decades,[17] it has not been previously explored in small molecule catalysis. We envisioned that electron transfer process from copper catalyst to sulfur (IV) atom of sulfilimines, which are structurally similar to sulfoxides, could be leveraged to circumvent the sacrificial oxidant required in traditional Chan-Lam coupling reactions. As part of our investigations into Chan-Lam coupling,[18] herein we report an unprecedented copper-catalyzed dehydrogenative Chan-Lam coupling of free diaryl sulfilimines with arylboronic acids, which does not require a terminal oxidant. It permits facile access to a myriad of *NAr*-diaryl sulfilimines and allows the synthesis of sulfilimine-modified peptides and protein under biomolecule-compatible conditions.

RESULTS AND DISCUSSION

Condition Optimization.

In pursuit of a terminal oxidant-free coupling process, the model reaction between *NH*-diphenyl sulfilimine **1a** and *p*-tolylboronic acid **2a** was carried out under an atmosphere of argon to preclude the influence of air. While the model reaction proceeded in ethanol (0.3 M) with $Cu(OAc)_2$ (10 mol %), only 11% assay yield of desired product **3aa** was obtained (entry 1, Table 1). Even so, an analysis of the redox balance of this process is consistent with two turnovers which was supported by results with other copper sources (entries 1–5). After a survey of copper catalyst sources, CuBr was determined to be superior with 71% yield

of **3aa** obtained (compare entry 6 vs entries 1–5). Forging ahead with CuBr as the optimal copper source, three other commonly used alcoholic solvents (MeOH, *i*PrOH and *t*BuOH) were investigated (entries 7–9). When *i*PrOH was employed as the solvent, **3aa** was obtained in 86% yield (entry 8). Other alcohols, unfortunately, produced **3aa** in much lower yields (entries 7, 9). Concentration was also discovered to be a key factor. Interestingly, when the reaction concentration was decreased from 0.3 M to 0.1 M, the assay yield of **3aa** could be improved from 86% (entry 8) to 99% (entry 11). Subsequently, the amount of **2a** could be successfully decreased to 1.5 equivalents, and the assay yield of **3aa** remained the same (entry 12). However, utilization of 1.2 equivalents of **2a** resulted in slightly diminished yield (85%, entry 13). Gratifyingly, the copper loading could be successfully lowered to 5 mol %, and **3aa** could be generated in 99% assay yield and 99% isolated yield (entry 14). Further reduction of the copper source to 2.5 mol %, however, led to lower yield (75% yield **3aa**, entry 15). A control experiment was also conducted in the absence of any copper source, and no desired product formed, which confirmed the essential role of the copper species in the transformation (entry 16). Therefore, the optimal condition for copper-catalyzed Chan-Lam coupling of free sulfilimines was determined to be: free sulfilimine **1a** as limiting reagent, boronic acid **2a** (1.5 equiv) as coupling partner, CuBr (5 mol %) as catalyst, in *i*PrOH (0.1 M) under an argon atmosphere at room temperature for 24 h. Interestingly, despite no need for external oxidant, the model reaction led to formation of **3aa** in slightly diminished yield (81%) under an air atmosphere (entry 17), which paves the road for convenient operation of our method on bioconjugation of proteins.

Substrate Scope.

With the optimal conditions established, generality of arylboronic acid substrates was first investigated (Table 2). The parent phenylboronic acid **2b** coupled with **1a** to yield the corresponding sulfilimines **3ab** near quantitatively. Arylboronic acids possessing electron-withdrawing groups, such as 4-F (**2c**), 4-Cl (**2d**), 4-Br (**2e**), or 4-CF_3 (**2f**), proved to be compatible coupling partners under the optimal conditions, providing **3ac–af** in 72–95% yields. A *meta*-substituted arylboronic acid **2g** was also well-suited. The neutral and oxidant-free conditions enabled a range of functionalized arylboronic acids to be tolerated, including those contained aldehyde (**2h**), ketone (**2i**), ester (**2j**), and even the polymerizable vinyl group (**2k**) (54–97%). To our delight, heteroarylboronic acids, such as 3-pyridyl (**2l**), 3-quinolinyl (**2m**) and 6-quinolinyl (**2n**), proceeded smoothly to furnish the corresponding products **3al–an** in usable yields (33–65%) by increasing the stoichiometry of **2a** and extending the reaction time. Moreover, a tyrosine-derived boronic acid **2o** was also compatible with the transformation, providing **3ao** in 87% yield. Remarkably, the coupling protocol displayed excellent chemoselectivity favoring arylation of the *NH*-sulfilimine **1a** over arylation of the amide N–H bond.

We then turned our attention to the substrate scope of diaryl sulfilimines using 4-tolylboronic acid (**2a**) as the coupling partner. Electron-neutral **1a** and **1b** furnished **3aa** and **3ba** in 99% and 88% yield, respectively. *NH*-Sulfilimines bearing electron-withdrawing substituents, such as 4-F (**1c**), 4-Cl (**1d**), 4-CF_3 (**1e**) and 2-Br (**1f**) performed very well, furnishing the corresponding *N*-aryl sulfilimines (**3ca–fa**) in yields ranging from 75% to 84% under slightly modified conditions. The coupling chemistry proceeded smoothly with

3,5-dimethyl substituent appended to the sulfilimine, giving **3ga** in 84% yield, albeit with an extended reaction time. Other functional groups, such as 4-NHAc (**1h**) and 4-COOMe (**1i**) were also viable, providing **3ha** and 3**ia** in 80% and 64% yield respectively. This chemistry was also well accommodated by heteroaryl *NH*-sulfilimines to generate **3ja**–**na** in 64–77% yields. Notably, the substrate scope of this chemistry could even be expanded to *S*-aryl-*S*-alkyl sulfilimines, and **3op** and **3pp** were successfully obtained in 73% and 64% yield respectively.

Owing to the instability of the *N*-arylated sulfilimines bearing an *ortho*-substituted aryl group on nitrogen, such as 1-naphthyl or 2-tolyl, we have successfully developed the sequential coupling/oxidation cascade to directly afford the corresponding *N*-aryl sulfoximines **4aq** and **4ar** (Figure 2), which provides a step-economic approach to prepare NAr-sulfoximines.

Inspired by the reactivity as well as the excellent chemoselectivity observed with tyrosine-derived substrate **3ao**, the suitability of the newly devised copper-catalyzed Chan-Lam coupling for use in sulfilimine-modified peptides was investigated (Table 3). The introduction of second amino acid to the tyrosine-based boronic acid **2o** was nitially investigated. As depicted in Table 3, Tyr containing amino acids at *N*-terminal positions bearing diverse functionalities were not detrimental, affording dipeptides **6aa**–**ae** in good to excellent yields. These results highlight the excellent chemoselectivity of our protocol, favoring C-N bond coupling of sulfilimine *N*-H bonds over C-O, C-S, or C-N bond formation of hydroxyls O-H bonds, thiol S-H bonds, amide N-H bonds, or indole N-H bonds. It is noteworthy that methionine (Met, **5b**) and cysteine (Cys, **5c**), which were not amendable in the previous sulfide imination strategy,[6] could be successfully employed in our coupling protocol. It is also apparent that the position of the phenylboronic acid containing residue at either the *N*-terminus or *C*-terminus (**6af–ah**) does not significantly affect the efficiency. We next investigated the compatibility and superiority of this Chan-Lam coupling to modify more complex peptides. The Tyr($B(OH)_2$) derivative of oglufanide, a tripeptide immuno-modulator in the treatment of hepatitis C (**5i**),[19] coupled with **1a** to furnish **6ai** in 85% yield. The aspartame derivate **5j**, as well as two anti-cancer tripeptides, tyroserleutide and tyroservatide (**5k**–**l**),[20] were also well-suited to the coupling, providing **6aj**–**l** in good yields. Of note, an angiotensin converting enzyme (ACE) inhibitor tripeptide used to decrease angiotensin I[21] performed well under the optimal conditions to produce **6am** in 68% yield. To evaluate the robustness of our coupling protocol, even more complex Tyr(($B(OH)_2$)-containing oligopeptides beyond tripeptides were explored. At the outset, a series of bioactive tripeptides [glutathione, tripeptide-10,[22] melanostatin,[23] and two ACE inhibitors (Gly-Pro-Met-NH_2 and Gly-Pro-Leu-NH_2)[24]] were decorated with a Tyr(($B(OH)_2$) residue at the *N*-terminus to afford **5n–r**. These substrates proved successful partners in the coupling reactions with **1a** to generate the corresponding products **6an–r**. The terminal oxidant-free Chan-Lam coupling was an effective tool to install diphenyl sulfilimine moieties on two endogenous opioid receptor agonists endomorphin-1 (**6as**) and met-enkephalin (**6at**) in 61% and 58% yield, respectively. Remarkably, the chemistry was well accommodated even with deltorphin I (**5u**), an exogenous opioid receptor agonist,[25]

providing the heptapeptide derivative **6au** in 63% yield under slightly modified reaction condition, highlighting the breadth and expediency of this procedure.

Chan-Lam Coupling-Based Bioconjugation in Model Protein Halotag 7.

Encouraged by the broad tolerance to different peptides as depicted in Table 3, we then sought to develop the copper-catalyzed Chan-Lam coupling of free sulfilimines as a selective protein conjugation method based on C-N bond formation of free sulfilimines. A major chemical challenge for such an unprecedented bioconjugation method under pH-neutral physiological conditions is the relatively weak nucleophilicity of free sulfilimines, which demands exceptional level of chemoselectivity relative to more nucleophilic amino acids, such as cysteine, lysine, tyrosine, or serine. **Halotag 7**, the 33 kDa monomeric protein, is a genetically engineered derivative of a dehalogenase, which can efficiently and specifically form a covalent bond with a synthetic ligand.[26] Therefore, it was selected as a model protein substrate for this study. As illustrated in Figure 3a, we initialized the investigation by installing a PEG linker tethered with a phenylboronic acid motif on D106 of **halotag 7**, to provide the viable protein substrate, namely **halotag 7'**, for the subsequent coupling protocol (see details in Figure S4 and S5). The boronic acid group serves as a point for further functionalization of the protein via the copper-catalyzed Chan-Lam coupling with free sulfilimine **1q**. In practice, exposure of 330 μM **halotag 7'** to the standard catalytical condition resulted in the formation of cross-coupling product **7** with 43% conversion based on intact protein mass analysis.

As discovered by Hudson group, the sulfilimine covalent crosslink in collagen IV appears as an adaptation of the extracellular matrix in response to mechanical stress in metazoan evolution, and thus serves as a key reinforcement that stabilizes networks as well as basement membranes.[5] Thus, the stability of sulfilimines in the context of proteins under various physiologically relevant conditions stands as an interesting problem. Nevertheless, our biomolecule-compatible terminal oxidant-free Chan-Lam coupling offers an enabling platform to tackle this issue. Purified protein **7** was incubated under a variety of biologically-relevant conditions at room temperature. As disclosed in Figure 3b and 3c, the sulfilimine bond in protein **7** (50 μM) was relatively stable in PBS buffer (pH 7.4) or under oxidative conditions (5 mM sodium periodate and 5 mM hydrogen peroxide), as evidenced by no statistically significant variation in biotinylation levels after 12 h as quantified by Western blots. Nevertheless, the S=N bond is sensitive to acidic (pH 5.0, 0.2 mM NaH_2PO_4), basic (pH 10.0, 50 mM NMM), reductive (5.0 mM DTT), and Dulbecco's Modified Eagle Medium (DMEM, with 10% v/v fetal bovine serum) conditions, with a considerable decrease of biotinylated protein **7** (25 to 60% retained). Among all of the factors examined, high temperature caused the most degradation of the sulfilimine motif, leading to significant cleavage of sulfilimine bond in very short period of times (10 or 30 min), which is consistent with the previous observations from Wells.[27] In addition, significant degradation of sulfilimine-based covalent linker in protein **7** occurred upon treatment with sodium selenite (5 mM), attesting to selective reduction of S=N bond in protein under physiological conditions based on Tang's pioneering report.[7b]

Mechanistic Studies.

To gain mechanistic insight into the unprecedented terminal oxidant-free Chan-Lam cross-coupling reaction, several control experiments were performed. The oxidant in the conventional Chan-Lam coupling is believed to facilitate the oxidation of Cu(I) to Cu(II) or Cu(III) in the catalytic cycle.[10] Due to the terminal oxidant-free nature of the Chan-Lam coupling of free sulfilimines,[28] along with our report on oxidant-free photoredox Chan-Lam coupling of free sulfoximines,[18a] the impact of light on the newly devised coupling reaction was initially explored. When the model reaction between **1a** and **2a** was performed in the dark under otherwise identical conditions, **3aa** was still obtained in 94% yield (Figure 4a), suggesting that light is not integral to this process. To discover the oxidation states of the copper species, spectroscopic studies were conducted under an atmosphere of argon. Electron paramagnetic resonance spectroscopy (EPR) (Figure 5a) of the reaction mixture revealed a Cu(II) signal. When CuBr was only dissolved in *i*PrOH, or mixed with *p*-tolylboronic acid **2a**, no noticeable signal was observed via EPR, in agreement with the existence of a Cu(I) species. Surprisingly, a Cu(II) peak appeared when CuBr was mixed with free sulfilimine **1a** together in *i*PrOH. This unexpected result supports that **1a** could facilitate the oxidation of Cu(I) to Cu(II), replacing the role of oxidant in classical Chan-Lam coupling. To further verify this finding, a series of UV-Vis experiments was performed (Figure 5b). There was no obvious UV absorption of CuBr solution above 400 nm, whereas a $CuBr_2$ solution exhibited a characteristic band on 590 nm, which is consistent with the d−d transition of Cu(II) species. In addition, the UV band shifted to 760 nm after **1a** was added into $CuBr_2$ solution, presumably due to coordination of **1a** to the Cu(II). As expected, a characteristic UV absorption of Cu(II) species appeared at 690 nm when CuBr was mixed with **1a**, supporting the pivotal role of **1a** in the transformation of Cu(I) to Cu(II). Furthermore, addition of radical scavengers [2.0 equivalents of tetramethylpiperidine *N*-oxide (TEMPO) or 1,4-dinitrobenzene (*p*-DNB)] to the model reactions only had a modest effect affording **3aa** in 89% and 74% yield, respectively (Figure 4b). This result implies that free radicals are not major intermediates in the transformation, although *caged radical pairs* are still plausible. We hypothesized that the byproduct of the transformation might be dihydrogen gas, which was confirmed by the examination of the reaction headspace via gas chromatography (GC) (see details in Table S2 and Figure S7–S10). To shed light on the source of H_2, mass spectroscopy was used to monitor the gaseous byproduct formed while deuterated methanol was employed as the solvent. A signal corresponding to HD was observed, supporting that one hydrogen atom of H_2 arises from the alcoholic solvent. When competitive external ligands, such as 4,4-di-*tert*-butyl bipyridine (dtbpy) or 2,9-dimethyl-1,10-phenanthroline (neocuproine), were added to the reaction system containing CuBr, the reaction rates were inhibited dramatically (Figure 4c). Together with UV-vis studies (see above), this result supports coordination of sulfilimine **1a** to copper catalyst playing a pivotal role in the catalytic cycles. All told, the data is consistent with an inner sphere electron transfer process enabling the formal oxidation of Cu(I) to Cu(II).

To understand the origin of the hydrogen evolution in this reaction, a DFT study was initiated. Initial calculations were conducted using Gaussian 16[29] with B3LYP/6-31G(d), Cu:SDD[30] to explore different pathways. Key steps were further evaluated

with SMD-2-propanol-BP86-d3/6-311G(d,p), Cu:SDD//BP86-d3/6-311G(d,p), Cu:SDD,[31] a method previously used to calculate pathways with copper hydrides.[32]

With the presence of copper (II) confirmed through EPR and UV-Vis, a Born-Haber cycle (Figure S13) was used to calculate the redox potential of Cu^{+} and **1a** to Cu^{2+} and the anion radical of **1a**.[33] The favorable potential of 0.5 V vs SHE indicates that *NH*-sulfilimines can oxidize Cu(I) to Cu(II) providing a possible explanation for the presence of Cu(II) in the reaction solution.

Three possible mechanisms for the formation of hydrogen gas were considered. The first formed hydrogen through a five-membered transition state; the second formed hydrogen through an H• arising from isopropanol (*i*PrOH); the third formed hydrogen through a four-membered transition state arising from initial formation of a copper hydride. The five-membered pathway formed hydrogen gas directly through a union between the hydrogen atoms of *i*PrOH and **1a** when coordinated to copper (Figure S14). This pathway proceeds from a Cu(I) to a Cu(III) species. The transition state barrier for this pathway is 91.1 kcal/mol, which is far too high to be feasible. From Cu(II), the corresponding five-membered transition state could not be located, and versions with a frozen core indicated any such transition state would be very high in energy.

The remaining pathways were investigated commencing from the triplet Cu(II) species **I** as illustrated in Figure 5c and 5d. A radical pathway (Figure 5c) can form H• by dissociation from the sulfilimine (**II**). The H• then abstracts the hydrogen from *i*PrOH via transition state **III** with a barrier of 21.9 kcal/mol. While transition state **III** is accessible at room temperature, the formation of a radical is inconsistent with the experimental results showing that TEMPO does not inhibit the reaction. Another pathway (Figure 5d) forms hydrogen gas through a copper hydride species. The copper hydride (**V**) arises from insertion into the N-H of the sulfilimine via transition state **IV**. From **V**, a four-membered transition **VI** allows the copper hydride to abstract H^{+} from coordinated *i*PrOH to form hydrogen gas. The formation of copper hydride and the further formation of hydrogen gas is plausible at room temperature, since the largest barrier in the pathway is 21.1 kcal/mol (**IV**).

On the base of the combined experimental results and computational studies, a plausible mechanism is outlined in Figure 5e. Initially, CuBr binds to *NH*-sulfilimines **1** to give Cu(I) species **A**, which undergoes an inner-sphere electron transfer process to yield Cu(II) intermediate **B** featuring a radical anion *NH*-sulfilimine ligand. Meanwhile, the solvent *i*PrOH coordinates to the copper centre to generate **B**. Subsequently, an insertion of Cu(II) into the N-H bond of the sulfilimine leads to the formation of three-membered transition state **C**. Cu(II)-facilitated homolysis of O–H bond of *i*PrOH occurs to give a formal Cu(III) hydride species **D**, which can release the hydrogen gas, as revealed by headspace GC analysis, via a feasible four-membered transition state. The resultant Cu(III) species **F** can undergo transmetallation with boronic acid **2** to produce arylated Cu(III) species **G**, followed by reductive elimination and ligand exchange with **1** to generate the product **3**, and close the overall catalytic cycle.

CONCLUSION

In summary, a terminal oxidant-free copper-catalyzed dehydrogenative Chan-Lam cross coupling of *NH*-sulfilimines with arylboronic acids has been achieved. This method obviates the need for stoichiometric oxidants of classic Chan-Lam couplings, enables the facile syntheses of a variety of *N*-arylated diaryl sulfilimines, and is also compatible with complex peptide scaffolds. Furthermore, leveraging the exceptional chemoselectivity, we have showcased its capability as an efficient bioconjugation tool on a model protein under biomolecule-compatible conditions. A combined experimental and computational investigation reveals that the free sulfilimines could facilitate the oxidation of Cu(I) to Cu(II) via an inner sphere electron transfer process, which allows the C-N bond formation in the absence of external oxidants. The protocol described herein represents an appealing alternative to classic oxidative C-N coupling strategies, enabling greater substrate generality and eliminating byproducts from oxidants. Our simple copper catalytic system provides a promising solution toward addressing the challenge associated with construction of sulfilimine covalent crosslink in the context of proteins, which is currently under investigation in our laboratory.

EXPERIMENTAL SECTION

Typical Procedure for the Terminal Oxidant-Free Chan-Lam Coupling Reaction.

To an oven-dried microwave vial equipped with a stir bar was added free sulfilimine **1** (0.3 mmol, 1.0 equiv), boronic acid **2** (0.45 mmol, 1.5 equiv), CuBr (2.2 mg, 0.015 mmol, 5 mol %) under an argon atmosphere in a dry box. The vial was capped with a septum and removed from the dry box. *i*PrOH (3.0 mL) was added into the reaction vial via syringe, and the reaction solution was stirred at room temperature under an argon atmosphere for 24 h. Upon completion of the reaction, the vial was opened to air, and the reaction mixture was passed through a short pad of silica gel. The pad was then rinsed with 20:1 dichloromethane:methanol (20.0 mL). The solvent was removed under reduced pressure. The residue was purified by flash chromatography to afford the purified product.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

(1). For reviews, see:(a)Gilchrist TL; Moody CJ The chemistry of sulfilimines. Chem. Rev 1977, 77, 409;(b)Furukawa N; Oae S Sulfilimines. Synthetic applications and potential utilizations. Ind. Eng. Chem. Res 1981, 20, 260;(c)Koval IV Advances in the chemistry of sulfimides and related

compounds. Sulfur Rep. 1993, 14, 149;(d)Taylor PC Sulfimides (sulfilimines): Applications in stereoselective synthesis. Sulfur Rep. 1999, 21, 241;(e)Tian X; Song L; Hashmi ASK Alpha-imino gold carbene intermediates from readily accessible sulfilimines: Intermolecular access to structural diversity. Chem. Eur. J 2020, 26, 3197. [PubMed: 31793680] For recent examples, see: (f)Yoshida S; Yano T; Misawa Y; Sugimura Y; Igawa K; Shimizu S; Tomooka K; Hosoya T Direct thioamination of arynes via reaction with sulfilimines and migratory *N*-arylation. J. Am. Chem. Soc 2015, 137, 14071; [PubMed: 26521894] (g)Grange RL; Clizbe EA; Counsell EJ; Evans PA Enantioselective construction of *C*-chiral allylic sulfilimines *via* the iridium-catalyzed allylic amination with *S,S*-diphenylsulfilimine: Asymmetric synthesis of primary allylic amines. Chem. Sci 2015, 6, 777; [PubMed: 28936319] (h)Tian X; Song L; Rudolph M; Rominger F; Oeser T; Hashmi ASK Sulfilimines as versatile nitrene transfer reagents: Facile access to diverse azaheterocycles. Angew. Chem., Int. Ed 2019, 58, 3589;(i)Zhang ZX; Davies TQ; Willis MC Modular sulfondiimine synthesis using a stable sulfinylamine reagent. J. Am. Chem. Soc 2019, 141, 13022; [PubMed: 31398023] (j)Tian X; Song L; Hashmi ASK Synthesis of carbazoles and related heterocycles from sulfilimines by intramolecular C-H aminations. Angew. Chem., Int. Ed 2020, 59, 12342;(k)Xie X; Sun J [4+3]-cycloaddition reaction of sulfilimines with cyclobutenones: Access to benzazepinones. Org. Lett 2021, 23, 8921. [PubMed: 34723560]

(2). Otocka S; Kwiatkowska M; Madalinska L; Kielbasinski P Chiral organosulfur ligands/catalysts with a stereogenic sulfur atom: Applications in asymmetric synthesis. Chem. Rev 2017, 117, 4147. [PubMed: 28191933]

(3). (a)Milaeva ER; Shpakovsky DB; Maklakova IA; Rufanov KA; Neganova ME; Shevtsova EF; Churakov AV; Babkova VA; Babkov DA; Kosolapov VA; Spasov AA Novel diphenylsulfimide antioxidants containing 2,6-di-*tert*-butylphenol moieties. Russ. Chem. Bull 2018, 67, 2025; (b)Lücking U; Nguyen D; Von Bonin A; Von Ahsen O; Siemeister G; Jautelat R; Doecke W-D Sulphimides as protein kinase inhibitors. WO2007140957 A1, 2007.

(4). Vanacore R; Ham A-JL; Voehler M; Sanders CR; Conrads TP; Veenstra TD; Sharpless KB; Dawson PE; Hudson BG A sulfilimine bond identified in collagen IV. Science 2009, 325, 1230. [PubMed: 19729652]

(5). Fidler A; Vanacore R; Chetyrkin S; Pedchenko V; Bhave G; Yin V; Stothers C; Rose K; McDonald W; Clark T; Borza D-B; Steele R; Ivy M; Hudson J; Hudson B A unique covalent bond in basement membrane is a primordial innovation for tissue evolution. Proc. Natl. Acad. Sci. U. S. A 2014, 111, 331. [PubMed: 24344311]

(6). Lin S; Yang X; Jia S; Weeks AM; Hornsby M; Lee PS; Nichiporuk RV; Iavarone AT; Wells JA; Toste FD; Chang CJ Redox-based reagents for chemoselective methionine bioconjugation. Science 2017, 355, 597. [PubMed: 28183972]

(7). (a)Xu K; Luan D; Wang X; Hu B; Liu X; Kong F; Tang B An ultrasensitive cyclization-based fluorescent probe for imaging native HOBr in live cells and zebrafish. Angew. Chem., Int. Ed 2016, 55, 12751;(b)Luan D; Gao X; Kong F; Song X; Zheng A; Liu X; Xu K; Tang B Cyclic regulation of the sulfilimine bond in peptides and NC1 hexamers via the HOBr/H_2Se conjugated system. Anal. Chem 2018, 90, 9523. [PubMed: 29938494]

(8). (a)Koval IV Sulfides: Synthesis and properties. Russ. Chem. Rev 1994, 63, 323;(b)Claus PK; Rieder W; Hofbauer P; Vilsmaier E *N*-aryl sulfimides. Tetrahedron 1975, 31, 505.

(9). (a)Tamura Y; Sumoto K; Matsushima H; Taniguchi H; Ikeda M Reactions of *N*-substituted arylsulfilimines with acylating agents and with activated halobenzenes, alkynes, and alkenes. J. Org. Chem 1973, 38, 4324;(b)Abou-Gharbia M; Ketcha DM; Zacharias DE; Swern D Reactions of ketenes with sulfilimines. Synthetic routes to oxazolinones and indolinones. J. Org. Chem 1985, 50, 2224;(c)Claridge RP; Millar RW; Sandall JPB; Thompson C Preparation of *N*-aryl-*S,S*-diphenylsulfilimines by nucleophilic attack of *N*-lithio-*S,S*-diphenylsulfilimine on aromatic compounds. Tetrahedron 1999, 55, 10243;(d)Vlasova OG; Rakitin OA; Khmelnitski LI A simple synthesis of the heterocyclic *S,S*-diphenylsulfilimines. Org. Prep. Proced. Int 1994, 26, 331.

(10). (a)West MJ; Fyfe JWB; Vantourout JC; Watson AJB Mechanistic development and recent applications of the Chan-Lam amination. Chem. Rev 2019, 119, 12491; [PubMed: 31756093] (b)Chen JQ; Li JH; Dong ZB A review on the latest progress of Chan-Lam coupling reaction. Adv. Synth. Catal 2020, 362, 3311.

(11). Moessner C; Bolm C Cu(OAc)$_2$-catalyzed N-arylations of sulfoximines with aryl boronic acids. Org. Lett 2005, 7, 2667. [PubMed: 15957917]

(12). Bohmann RA; Bolm C Copper-catalyzed C-N cross-coupling of sulfondiimines with boronic acids. Org. Lett 2013, 15, 4277. [PubMed: 23937076]

(13). Ohata J; Zeng Y; Segatori L; Ball ZT A naturally encoded dipeptide handle for bioorthogonal Chan-Lam coupling. Angew. Chem., Int. Ed 2018, 57, 4015.

(14). (a)King AE; Brunold TC; Stahl SS Mechanistic study of copper-catalyzed aerobic oxidative coupling of arylboronic esters and methanol: Insights into an organometallic oxidase reaction. J. Am. Chem. Soc 2009, 131, 5044; [PubMed: 19309072] (b)Vantourout JC; Miras HN; Isidro-Llobet A; Sproules S; Watson AJ Spectroscopic studies of the Chan-Lam amination: A mechanism-inspired solution to boronic ester reactivity. J. Am. Chem. Soc 2017, 139, 4769. [PubMed: 28266843]

(15). Sies H; Berndt C; Jones DP Oxidative stress. Annu. Rev. Biochem 2017, 86, 715. [PubMed: 28441057]

(16). Rack J Electron transfer triggered sulfoxide isomerization in ruthenium and osmium complexes. Coord. Chem. Rev 2009, 253, 78.

(17). Alvarez-Paggi D; Hannibal L; Castro MA; Oviedo-Rouco S; Demicheli V; Tortora V; Tomasina F; Radi R; Murgida DH Multifunctional cytochrome c: Learning new tricks from an old dog. Chem. Rev 2017, 117, 13382. [PubMed: 29027792]

(18). (a)Wang C; Zhang H; Wells LA; Liu T; Meng T; Liu Q; Walsh PJ; Kozlowski MC; Jia T Autocatalytic photoredox Chan-Lam coupling of free diaryl sulfoximines with arylboronic acids. Nat. Commun 2021, 12, 932; [PubMed: 33568641] (b)Liang Q; Wells LA; Han K; Chen S; Kozlowski MC; Jia T Synthesis of sulfilimines enabled by copper-catalyzed S-arylation of sulfenamides. November 1, 2021. Research Square. https://www.researchsquare.com/article/rs-1037799/v1. (accessed 2021-11-11).

(19). Simbirtsev A; Kolobov A; Zabolotnych N; Pigareva N; Konusova V; Kotov A; Variouchina E; Bokovanov V; Vinogradova T; Vasilieva S; Tuthill C Biological activity of peptide SCV-07 against murine tuberculosis. Russ. J. Immunol 2003, 8, 11. [PubMed: 12717550]

(20). Ding W; Zhang J; Yao Z; Lu R; Wu D; Li G; Shen Z; Sun Y; Lin G; Wang C; Zhao M; Peng S The synthesis, distribution, and anti-hepatic cancer activity of YSL. Bioorg. Med. Chem. Lett 2004, 12, 4989.

(21). Kawakami A; Kayahara H; Tadasa K Taste evaluations of angiotensin I converting enzyme inhibitors, Leu-Lys-Tyr analogues. Biosci. Biotechnol. Biochem 1995, 59, 709. [PubMed: 7772837]

(22). Puig A; Antón JM; Mangues M A new decorin-like tetrapeptide for optimal organization of collagen fibres. Int. J. Cosmet. Sci 2008, 30, 97. [PubMed: 18377618]

(23). Ishihara Y; Oka M; Tsunakawa M; Tomita K; Hatori M; Yamamoto H; Kamei H; Miyaki T; Konishi M; Oki T Melanostatin, a new melanin synthesis inhibitor. Production, isolation, chemical properties, structure and biological activity. J. Antibiot 1991, 44, 25.

(24). Byun H-G; Kim S Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from alaska pollack (theragra chalcogramma) skin. Process Biochem. 2001, 36, 1155.

(25). Erspamer V; Melchiorri P; Falconieri-Erspamer G; Negri L; Corsi R; Severini C; Barra D; Simmaco M; Kreil G Deltorphins: A family of naturally occurring peptides with high affinity and selectivity for delta opioid binding sites. Proc. Natl. Acad. Sci. U. S. A 1989, 86, 5188. [PubMed: 2544892]

(26). Los GV; Encell LP; McDougall MG; Hartzell DD; Karassina N; Zimprich C; Wood MG; Learish R; Ohana RF; Urh M; Simpson D; Mendez J; Zimmerman K; Otto P; Vidugiris G; Zhu J; Darzins A; Klaubert DH; Bulleit RF; Wood KV Halotag: A novel protein labeling technology for cell imaging and protein analysis. ACS Chem. Biol 2008, 3, 373. [PubMed: 18533659]

(27). Elledge SK; Tran HL; Christian AH; Steri V; Hann B; Toste FD; Chang CJ; Wells JA Systematic identification of engineered methionines and oxaziridines for efficient, stable, and site-specific antibody bioconjugation. Proc. Natl. Acad. Sci. U. S. A 2020, 117, 5733. [PubMed: 32123103]

(28). According to ideal gas law (PV=nRT), even if entire headspace (6 mL from a 9 mL vial) was air, there would not be sufficient O_2 (18.5 mL air) to provide the observed yield for reaction of 87.0 mg (0.3 mmol) of substrate **3aa**.$_2$**3aa**

(29). Frisch MJ; Trucks GW; Schlegel HB; Scuseria GE; Robb MA; Cheeseman JR; Scalmani G; Barone V; Petersson GA; Nakatsuji H; Li X; Caricato M; Marenich AV; Bloino J; Janesko BG; Gomperts R; Mennucci B; Hratchian HP; Ortiz JV; Izmaylov AF; Sonnenberg JL; Williams; Ding F; Lipparini F; Egidi F; Goings J; Peng B; Petrone A; Henderson T; Ranasinghe D; Zakrzewski VG; Gao J; Rega N; Zheng G; Liang W; Hada M; Ehara M; Toyota K; Fukuda R; Hasegawa J; Ishida M; Nakajima T; Honda Y; Kitao O; Nakai H; Vreven T; Throssell K; Montgomery JA Jr.; Peralta JE; Ogliaro F; Bearpark MJ; Heyd JJ; Brothers EN; Kudin KN; Staroverov VN; Keith TA; Kobayashi R; Normand J; Raghavachari K; Rendell AP; Burant JC; Iyengar SS; Tomasi J; Cossi M; Millam JM; Klene M; Adamo C; Cammi R; Ochterski JW; Martin RL; Morokuma K; Farkas O; Foresman JB; Fox DJ Gaussian 16, Revision B.01. 2016.

(30). (a)Becke AD A new mixing of hartree-fock and local density-functional theories. J. Chem. Phys 1993, 98, 1372;(b)Becke AD Density-functional thermochemistry. III. The role of exact exchange. J. Chem. Phys 1993, 98, 5648;(c)Lee C; Yang W; Parr RG Development of the colle-salvetti correlation-energy formula into a functional of the electron density. Phys. Rev. B 1988, 37, 785.

(31). (a)Perdew JP Density-functional approximation for the correlation energy of the inhomogeneous electron gas. Phys. Rev. B 1986, 33, 8822;(b)Marenich AV; Cramer CJ; Truhlar DG Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. J. Phys. Chem. B 2009, 113, 6378. [PubMed: 19366259]

(32). Wahidur Rahaman SM; Matyjaszewski K; Poli R Cobalt(III) and copper(II) hydrides at the crossroad of catalysed chain transfer and catalysed radical termination: A DFT study. Polym. Chem 2016, 7, 1079.

(33). Yan L; Lu Y; Li X A density functional theory protocol for the calculation of redox potentials of copper complexes. Phys. Chem. Chem. Phys 2016, 18, 5529. [PubMed: 26863397]

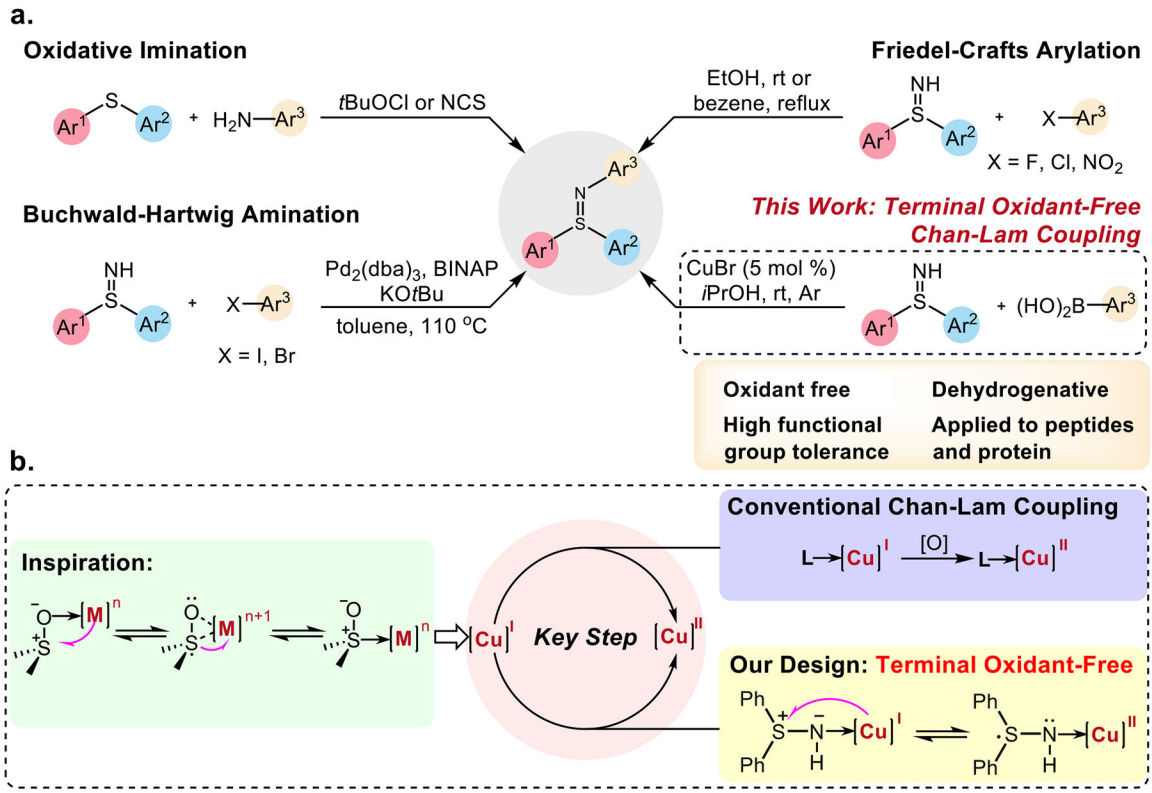


Figure 1. Conceptual Design of Terminal Oxidant-Free Process.

a, Preparative routes towards *N*-Ar diaryl sulfilimines. **b**, Design of Cu(I) to Cu(II) transformation via an electron-transfer pathway.

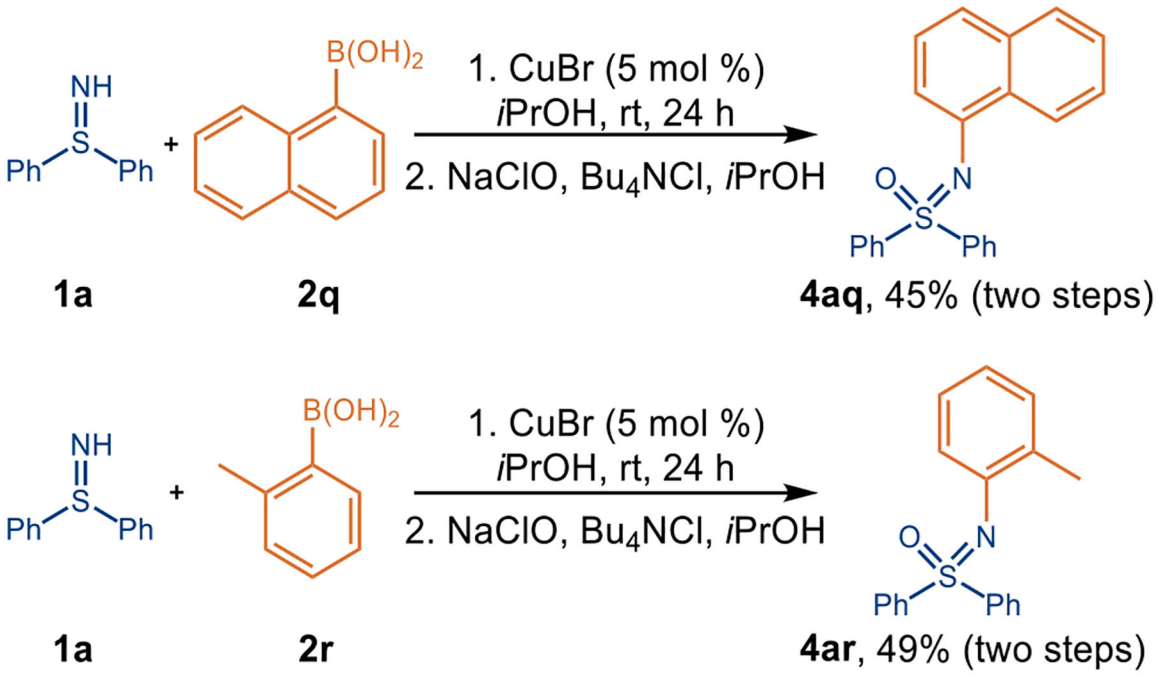


Figure 2.

Sequential Coupling/Oxidation Cascade to Prepare *N*-Aryl Sulfoximines Directly from Free Sulfilimines and Arylboronic Acids.

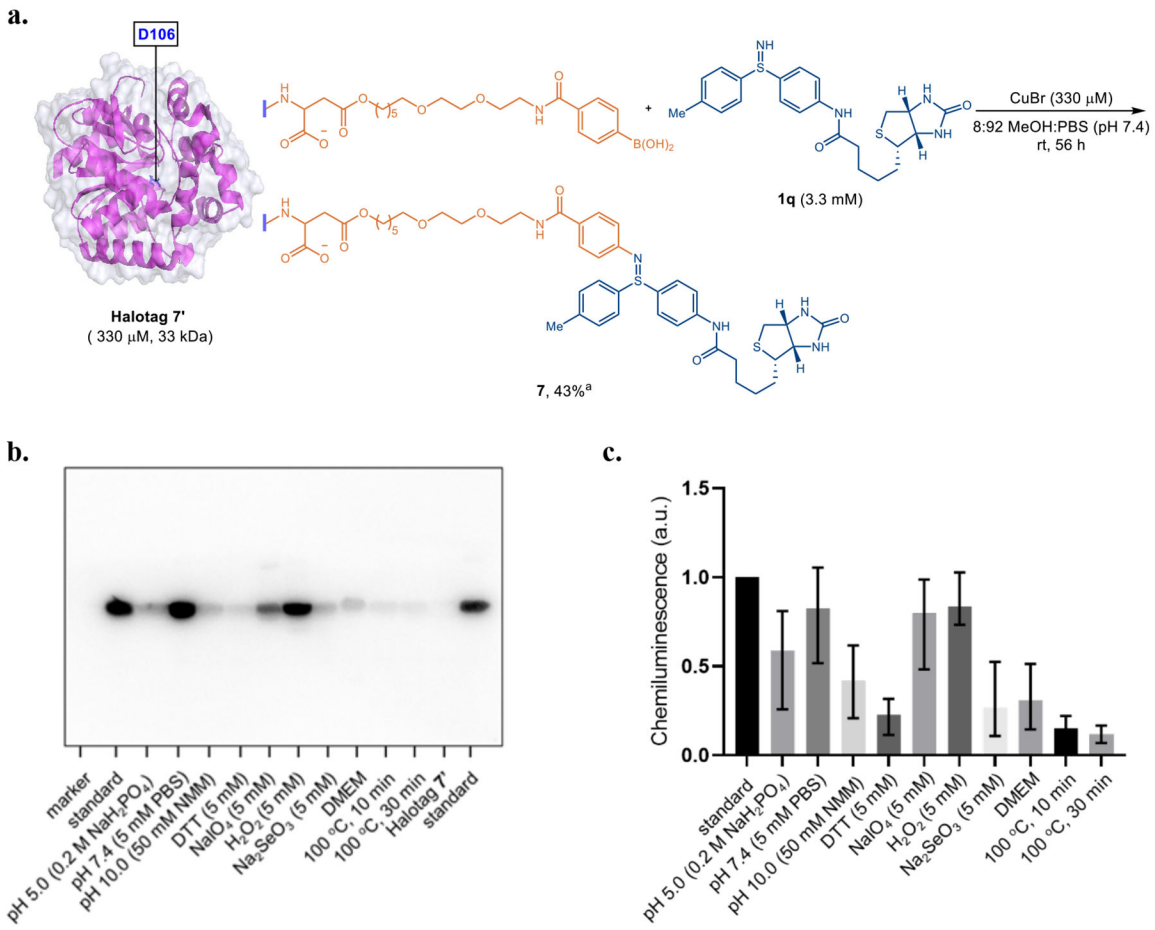


Figure 3. Cross Coupling Reaction of Halotag 7' with Biotin Group Containing *NH*-sulfilimine 1q.

a, Scheme for the reaction of **halotag 7'** with the biotin containing *NH*-sulfilimine **1q**. General reaction conditions: **halotag 7'** (330 μmol) treated with **1q** (10.0 equiv) using CuBr (1.0 equiv) in MeOH/PBS solvent (v/v = 8/92, pH 7.4) for 56 h at room temperature. **b**, Western blot image of stability experiments on purified protein **7** after incubating under the given conditions at room temperature for 12 h. **c**, Quantification of the western blot results with image J software. The untreated purified protein **7** was used as a standard (chemiluminescence intensity = 1.0 in Figure 3c). Average of two standard bands was used for each experiment.

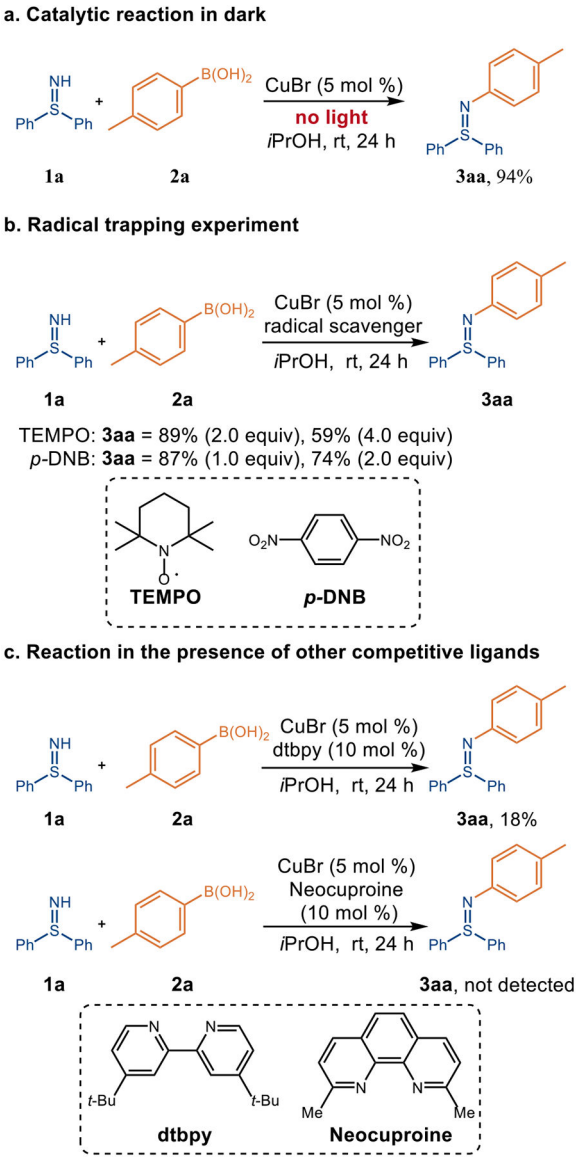


Figure 4.

Control Experiments of the Copper-Catalyzed Arylation of Sulfilimines.

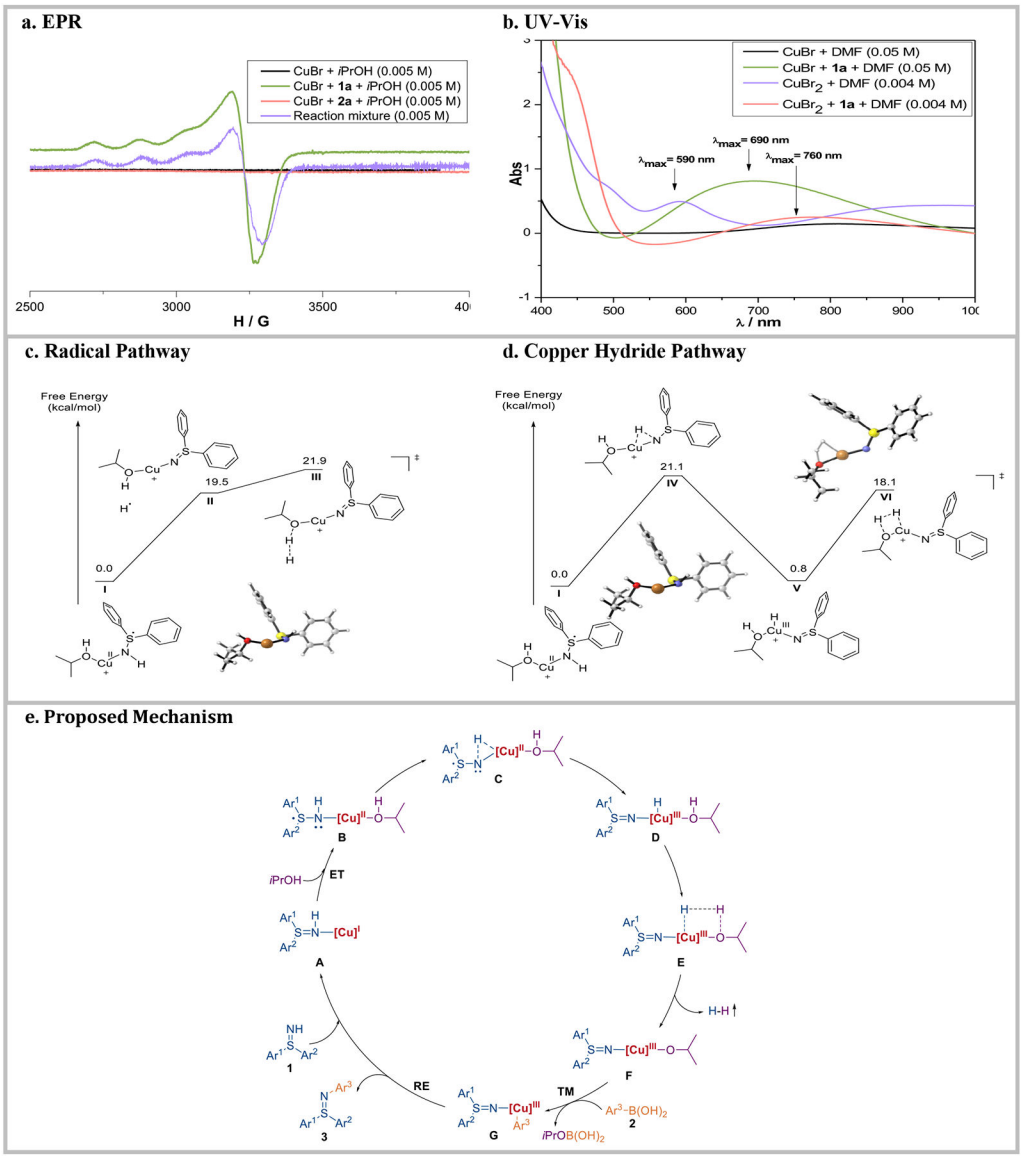
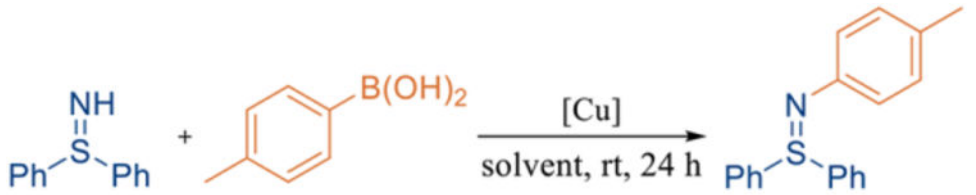


Figure 5. Mechanistic Studies.

a, EPR spectra. **b**, UV-Vis spectra. **c**, Energy profile for the formation of hydrogen gas through a radical mechanism. **d**, Energy profile for the formation of hydrogen gas through a copper hydride species. Free energy for both pathways were computed using SMD-2-propanol-BP86-d3/6-311G(d,p), Cu:SDD//BP86-d3/6-311G(d,p), Cu:SDD. **e**, Proposed mechanism of the terminal oxidant-free Chan-Lam coupling.

Table 1.

Optimization of the Copper-Catalyzed Cross-Coupling Reaction of 1a and 2a.[a]



1a **2a** **3aa**

entry	[Cu]/mol %	solvent	conc./M	assay yield[b]/%
1	$Cu(OAc)_2$/10	EtOH	0.3	11
2	$Cu(acac)_2$/10	EtOH	0.3	39
3	$CuCl_2$/10	EtOH	0.3	62
4	CuCl/10	EtOH	0.3	63
5	$CuBr_2$/10	EtOH	0.3	57
6	CuBr/10	EtOH	0.3	71
7	CuBr/10	MeOH	0.3	41
8	CuBr/10	*i*PrOH	0.3	86
9	CuBr/10	*t*BuOH	0.3	53
10	CuBr/10	*i*PrOH	0.2	77
11	CuBr/10	*i*PrOH	0.1	99
12[c]	CuBr/10	*i*PrOH	0.1	99
13[d]	CuBr/10	*i*PrOH	0.1	85
14[c]	CuBr/5	*i*PrOH	0.1	99(99[e])
15[c]	CuBr/2.5	*i*PrOH	0.1	75
16[c]	—	*i*PrOH	0.1	0
17[f]	CuBr/5	*i*PrOH	0.1	81

[a]Unless otherwise stated, reactions were carried out with **1a** (0.3 mmol), **2a** (2.3 equiv), copper source (10 mol %) in solvent at room temperature under an argon atmosphere for 24 h.

[b]Assay yields determined by ^{1}H NMR spectroscopy of unpurified reaction mixtures using 0.1 mmol (7.0 μL) of CH_2Br_2 as internal standard.

[c]**2a** (1.5 equiv).

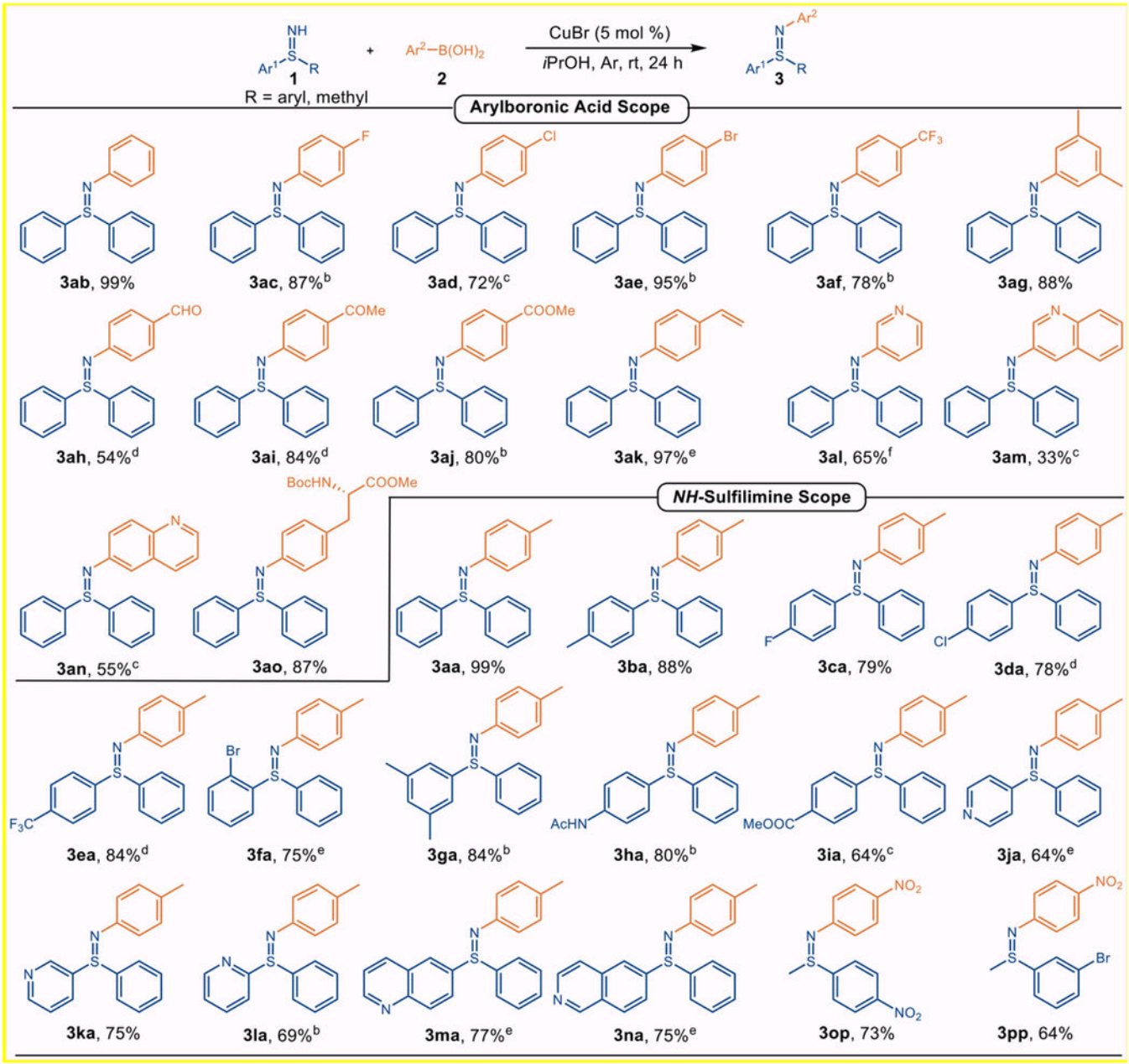
[d]**2a** (1.2 equiv).

[e]Isolated yield.

[f]Under air atmosphere.

Table 2.

Substrate Scope of Arylboronic Acids and Sulfilimines in Copper-Catalyzed Chan-Lam Coupling.[a]



Reaction conditions:

[a] **1** (0.3 mmol), **2** (1.5 equiv), 5 mol % CuBr in *i*PrOH (3.0 mL) under an argon atmosphere at room temperature for 24 h.

[b] 36 h.

[c] **2** (2.0 equiv), 48 h.

[d] 48 h.

[e] **2** (2.0 equiv), 36 h.

[f] **2** (2.0 equiv), 72 h.

Table 3.

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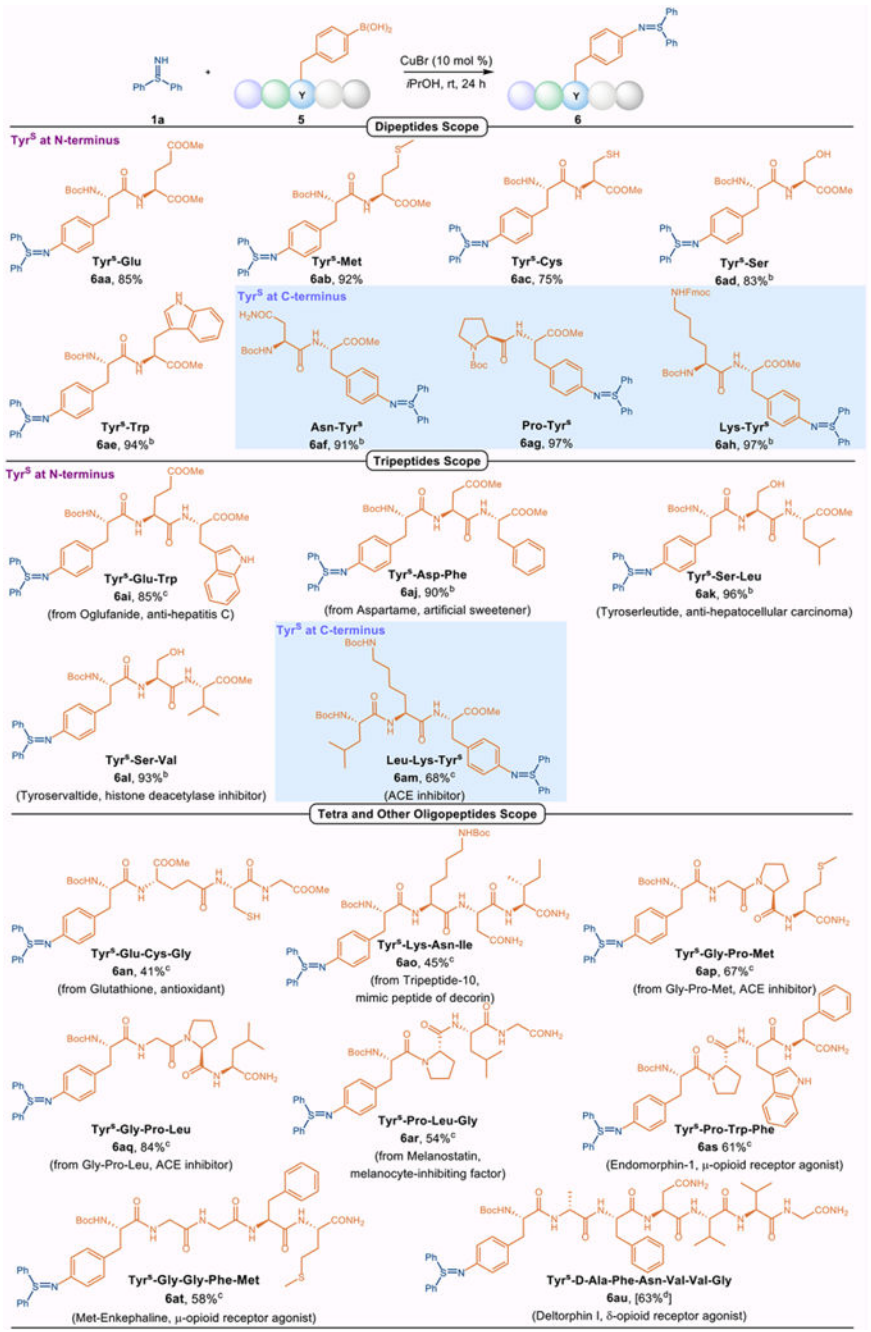
Scope of Peptides in Copper-Catalyzed Chan-Lam Coupling with 1a.[a]



Reaction conditions:

[a] **1a** (0.1 mmol), **5** (1.5 equiv), 10 mol % CuBr in *i*PrOH (1.0 mL) under an argon atmosphere at room temperature for 24 h.

[b] 48 h.

[c] **1a** (0.1 mmol), **5** (1.5 equiv), CuBr (10 mol %), MeOH (1.0 mL), 48 h.

[d] **1a** (0.05 mmol), **5** (1.5 equiv), CuBr (10 mol %), MeOH (1.0 mL), H_2O (1.0 mL), DMF (0.15 mL), 48 h. Yields provided in square brackets are reported as a % conversion determined from reverse-phase HPLC. Tyr^S: 4-diphenylsulfiliminyl Tyrosine.