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C–H Functionalization

Pd(0)-Catalyzed Direct C–H Functionalization of 2-*H*-4-Benzylidene Imidazolones: Friendly and Large-Scale Access to GFP and Kaede Protein Fluorophores

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Abstract: The first one-pot synthesis of N-substituted 2-*H*-4-benzylidene imidazolones and their subsequent palladium-catalyzed and copper-assisted direct C2–H arylation and alkenylation with aryl- and alkenylhalides are described. This innovative synthesis is step-economical, azide-free, high yielding, highly flexible in the introduction of a variety of electronically different groups, and can be operated on large-scale. Moreover, the method allows direct access to C2-arylated or alkenylated imidazolone-based green fluorescent protein (GFP) and Kaede protein fluorophores, including *ortho*-hydroxylated models.

The green fluorescent protein (GFP) is responsible for the fluorescence phenomenon in many deep-sea animals.[1] Since the major discovery of GFP, awarded by the Nobel Prize, several 4-benzylidene imidazolones have found widespread use in the design of fluorophores for biological studies, the most representative being the Kaede protein analogs and BODIPY-like Burgess fluorophores (Figure 1).

Arguably, the synthetic routes to C2-functionalized 4-benzylidene imidazolones, including C2-arylated and C2-alkenylated GFP and Kaede fluorophores, have been disclosed through two classes of condensation reactions (Scheme 1). The Erlenmeyer-like ring closure of *N*-arylated or *N*-acrylated amino acids was first reported to efficiently deliver the *N*-methyl 4-benzylidene oxazolinones, known as Erlenmeyer azlactones, which can be further converted to the imidazolones by treatment with an amine followed by condensation of the resulting diamide (Scheme 1 a).[2] Indifferently, the 2-methyl imidazolones were engaged in either oxidation/Wittig reaction or base-promoted condensation to aldehydes/dehydration sequences to produce various *N*-methylated Kaede protein fluorophores.[3] However, great limitations impact this classical route towards imidazolones, because the diamide condensation is often only suitable to furnish the N1-methylated series.

Noticing that the C2-heteroarylated 4-benzylidene imidazolones are unavailable from the precedent strategy, Burgess proposed alternatively the Staudinger-type ring closing of 2-azidoacetamides to 4-*H*-C2-heteroarylated imidazolones, which are then condensed with aldehydes (Scheme 1 b).[4] More re-

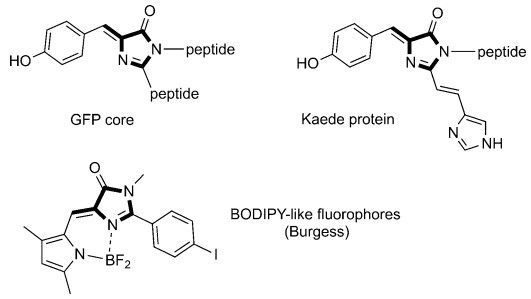
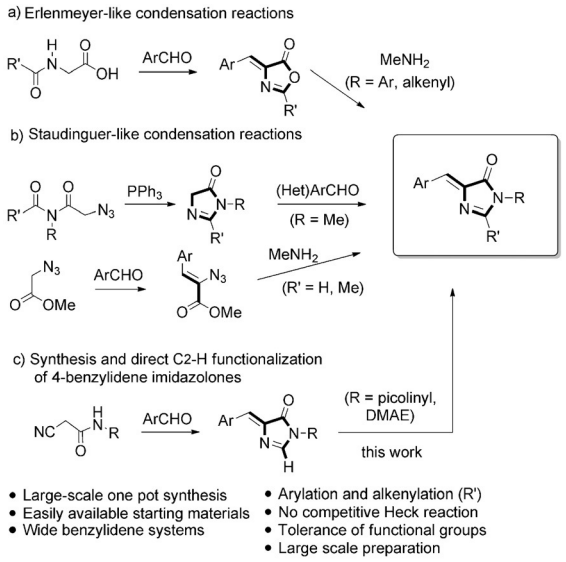


Figure 1. Main families of imidazolone-based fluorophores.



Scheme 1. Main synthetic routes towards GFP and kaede 4-benzylidene-based fluorophore analogs and our synthetic strategy.

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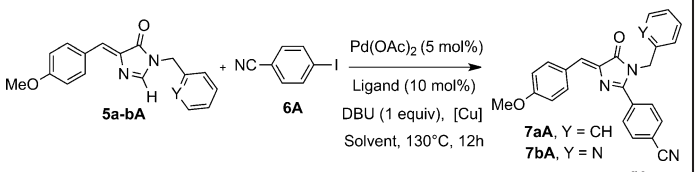
cently, Yampolsky group proposed an inversed synthetic strategy providing notably the 2-*H*-4-benzylidene imidazolones, albeit in a poor 22% yield over a four-step synthesis (Scheme 1b).[5] In this context, the development of innovative synthetic methods, which streamline the access to imidazolone-based fluorophores from readily available and safe starting materials (avoiding in particular the current use of hazardous azide precursors), would be of meaningful importance in material sciences.

Recently, the transition-metal-catalyzed C–H activation has emerged as a powerful synthetic tool to build and functionalize molecules.[6] Notably, most of the related achievements have been directed to main standard classes of heterocycles,[7] but their applications in organic materials remain sparse.[8] In the current context of promotion of this recent field towards biomedical applications, we recently demonstrated that 4,4′-dialkylimidazolones are valuable in the direct C–H functionalization methodology.[9] We report here our effort towards the synthesis and late-stage direct C2–H functionalization of 2-*H*-4-benzylidene imidazolones by addressing two major challenges (Scheme 1c): i) Friendly, flexible and large-scale access to key synthetic precursors through the development of an azide-free synthesis of 2-*H*-4-benzylidene imidazolones, including challenging *ortho*-hydroxylated models recently explored to display optimized quantum yields;[10] ii) The multi and selective C2–H functionalization of arylidene imidazolones using specifically nitrogen-chelating protective groups of the imidazolone ring, such as picolinyl and 2-*N*,*N*-dimethylaminoethyle (DMAE). The latter may be also used for a late-stage chemical modulation of the nitrogen appendage to increase solubility along with achieving bioconjugation to specific vector, such as radioisotope tagging.

With this plan in mind, we started to set up an innovative neat synthetic method to produce *N*-substituted 2-*H*-4-benzylidene imidazolones. The amido isocyanides **1a–c** were first quantitatively prepared by amidification of the commercially available methylisocyanoacetate with benzylamine, *N*-picolinylamine or *N*,*N*-dimethylethylenediamine.[11] A first attempt of condensation of *N*-picolinylamide **1b** with 4-methoxybenzaldehyde **2A** provided a mixture of 2-hydroxybenzyl and 2-benzylidene glycine intermediates **3bA** and **4bA** (Scheme 2). Fortunately, *N*,*O*-bis(trimethylsilyl)acetamide (BSA) was found highly efficient to cleanly achieve the expected ring-closing condensation of both the latter intermediates that afforded 2-*H*-4-benzylidene imidazolone **5bA** in fair 60% yield over the one-pot synthesis. Remarkably, the large-scale production of mono- and disubstituted *N*-picolinyl-4-benzylidene imidazolones **5bA–E** and **5bF** as well as the more sterically hindered 4-4′-fluorenylimidazolones **5bG** was also successfully achieved in a range of 51–91% isolated yields. Moreover, the procedure remained highly efficient to produce *N*,*N*-dimethylaminoethyl (DMAE) imidazolones **5cH–I** in large amounts and good yields.

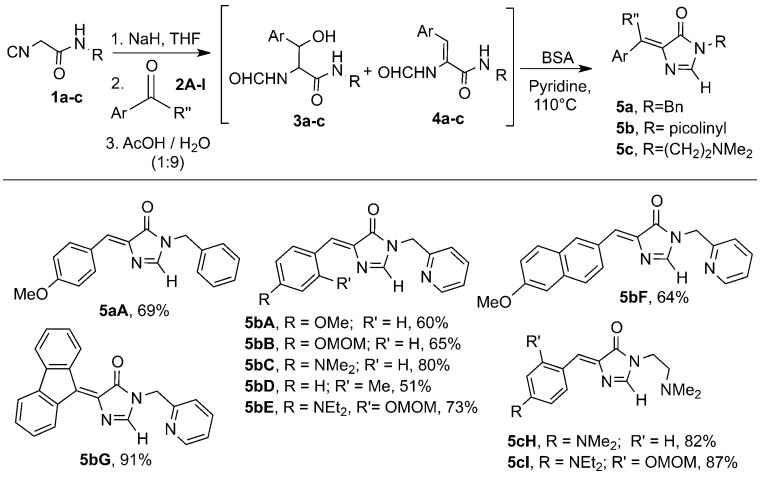
We started our study of the direct C2–H arylation by reacting *N*-benzylated and picolinyl 4-benzylidene imidazolones **5a–bA** with 4-iodobenzonitrile **6A** using $Pd(OAc)_2$ as catalyst and PPh_3 as ligand, under copper(I) and several carbonate, phosphate, and amine bases assistance.[12] In a broad set of experiments, no product resulting from a competitive Heck-type reaction of the double bond was identified, and first satisfactory result was obtained from **5bA**, when employing a combination of DBU/DMF, to afford the 2-arylated *N*-picolinyl benzylidene imidazolone **7bA** in a fair 78% yield (Table 1, entries 1 and 2). In this case, the picolinyl protective group may prevent the inherent difficulty of the side formation of ring-opening products resulting from the C2-metalated imidazolone, through a better stabilization of imidazolon-2-yl copper that is often suggested in several Cu(I)- and base-assisted Pd(0)-catalyzed direct C–H arylations of structurally related imidazoles.[7d–f] An optimized 86% yield of the production of **7bA** was then reached under CuBr·DMS co-catalysis (entry 3). We no-

Table 1. Optimized reaction conditions for direct C2–H arylation.[a]



Entry	Y	[Cu] (equiv)	Ligand	Solvent	Yield [%][b]
1	CH	CuI (1)	PPh_3	DMF	15
2	N	CuI(1)	PPh_3	DMF	78
3	**N**	**CuBr·DMS (1)**	**PPh_3**	**DMF**	**86 (78)[c]**
4	N	CuBr·DMS (0.5)	PPh_3	DMF	27
5	N	–	PPh_3	DMF	n.r.

[a] Reaction conditions: **5a–bA** (0.5 mmol), **6A** (1 equiv), $Pd(OAc)_2$ (5 mol%), [Cu] (*n* equiv), DBU (1 equiv). [b] Yield of isolated product. [c] Reaction scaled-up to 10 mmol of **5bA.**



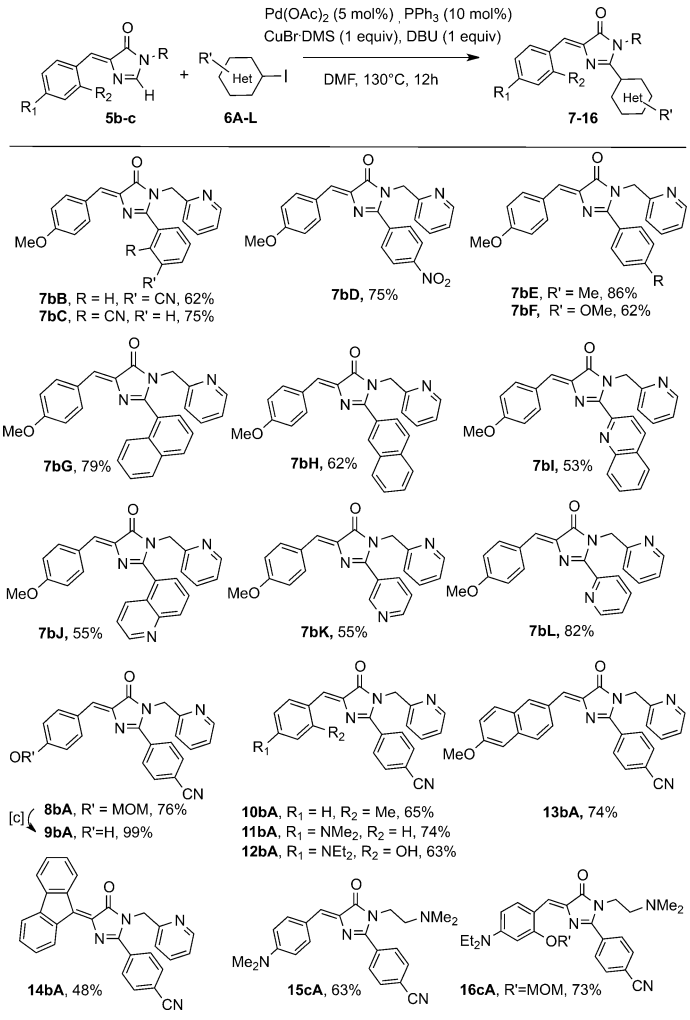
Scheme 2. Synthesis of 4-benzylidene imidazolones. [a] Reaction conditions: **1a–c** (10 mmol), **2A–F** (1 equiv), NaH (1.2 equiv) and then BSA (25 mmol). [b] Yield of isolated product.

ticed that a lower reaction temperature as well as a reduction of the amount of Cu(I) from 1 to 0.5 equivalent significantly disfavored the cross-coupling (entries 4–5). Therefore, a slight loss of performance was observed when the reaction was conducted using ten-fold amounts of 4-benzylidene imidazolone **5 bA** (entry 3).

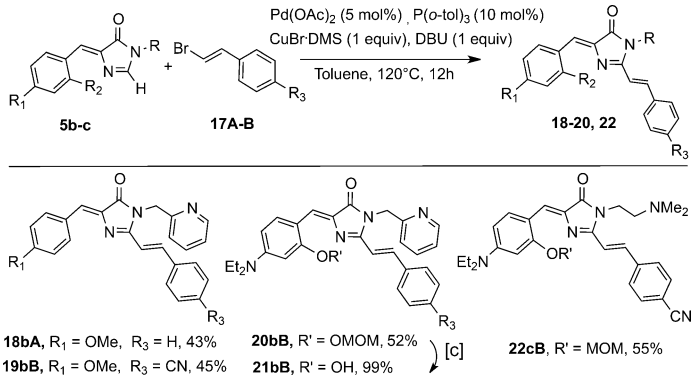
With the optimized conditions in hand, further investigations were focused on the direct C2–H arylation of 4-benzylidene imidazolone **5 bA** with various halogenoarenes **6** (Scheme 3). The first set of direct C2–H cross-couplings was successfully achieved using various electronically different iodoarenes **6 B–F** substituted by electron-withdrawing and -donating groups, such as cyano, nitro, methyl, and methoxy groups, indifferently located at *ortho*, *meta* or *para*-positions, as well as naphthyl halides **6 G–H**.

The expected 2-arylated 4-benzylidene imidazolones **7 bB–H** were thus obtained in a range of 62–86% yields. Remarkably, the procedure remained highly effective in the direct C2–H coupling of **5 bA** with various more challenging halogenoazines, such as 2- and 5-bromoquinolines, as well as 2- and 3-bromopyridines that gave the 2-azinyl-4-benzylidene imidazolones **7 I–L** in a range of 53–82% yields. After these successful results, we worked on the design of novel families of analogs of GFP fluorophores by achieving the direct C2–H coupling of a series of 4-benzylidene imidazolones variously substituted on the aromatic unit (Scheme 3). Invariably, the electronic and steric effects introduced on the arylidene system and the nature of *ortho*-directing groups had no influence on the success of the direct C2–H arylation of various 4-benzylidene imidazolones **5 b–c**, providing 2-arylated 4-benzylidene imidazolones **7 bA–13 bA** in fair yields.[13] To further demonstrate the versatility of the methodology, we focused on the preparation of challenging *ortho*-hydroxylated fluorophores, which are expected to display better quantum yields.[10] Pleasingly, a first assay of direct C2–H arylation of the adequate *N*-picolinyl-4-benzylidene imidazolone **5 bE** with 4-iodobenzonitrile **6 A** revealed immediately conclusive using the optimized procedure that gave the *N*-picolinyl-2-(4-cyanoaryl)-4,4′-(2-hydroxy-4-dimethylamino arylidene) imidazolones **12 bA** in 63% yield, the methoxymethyl (MOM) protection being subsequently removed during the isolation treatment.[13] Remarkably, the procedure also proved efficient when reacting the structurally related *N,N*-dimethylaminoethyl imidazolone **5 cI** with 4-iodobenzonitrile **6 A** to produce the corresponding imidazolones **16 cA** in 73% yield.

We next focused on the preparation of Kaede protein fluorophore analogs through an extension of the developed direct arylation methodology towards a direct C2–H alkenylation of the same substrates using halogenoalkenes as coupling partners (Scheme 4). Using strictly the optimized experimental conditions for C2–H arylation of 4-benzylidene imidazolones, the first reaction of **5 bA** with (*E*)-2-bromostyrene **17 A** led to production of the expected 2-styrenylimidazolone **18 bA** in 43% isolated yield.[12] To circumvent the degradation of the 2-bromostyrene reagent at high temperature in highly polar DMF solvent, the reaction was realized under less dissociative conditions using toluene as solvent. Owing to the additional screening of phosphines,[12] P(*o*-tol)$_3$ ligand was identified as the most efficient to achieve the direct C2–H alkenylation of **5 bA** with (*E*)-2-bromostyrene **17 A** and (*E*)-4-cyano 2-bromostyrene **17 B**, giving the expected (*E*)-2-vinylated 4-benzylidene imidazolones **18 bA** and **19 bB** in fair 43 and 45% isolated yields, respectively (Scheme 4). Interestingly, both reactions were fully selective at the C2–H site of the imidazolone ring and provided exclusively the (*E*)-isomer. The methodology was further successfully applied to the preparation of two novel analogues of Kaede protein fluorophores, the *N*- picolinyl **20 bB** and *N,N*-dimethylaminoethyl (DMAE) 4-benzylidene imidazolones **22 cB** flanked with an optimal two diametral *para*-disubstitution of aromatics with an electron-donor diethylamine and a nitrile acceptor as well as an *ortho*-H-bonding substitution of the arylidene system.



Scheme 3. Scope of (hetero)aryliodides.[a] Reaction conditions: **5 bA** (0.5 mmol) and **6 B–L** (0.5 mmol) in DMF (1.6 mL) under N_2 atmosphere. [b] Yield of isolated product. [c] HCl (10%) in methanol.



Scheme 4. Direct C2–H alkenylation. [a] Reaction conditions: [a] **5b–c** (0.5 mmol) and **17** (0.5 mmol) in toluene (1.6 mL) under N_2 atmosphere. [b] Yield of isolated product. [c] HCl (10%) in methanol.

At this stage, according to our initial objective of designing a novel class of GFP and Kaede protein-type fluorophores for applications in bioimaging, the reactivity of the picoline and DMAE groups towards alkylating agents was finally examined. Invariably, the nature of the substitution of the arylidene system, both *N*-picolinyl **7bD** and *N*-DMAE imidazolones **15cA** and **16cA** were successfully alkylated with dimethyl sulfate to afford the corresponding methylated fluorophore salts **25**–**27** in good yields, remarkably with both *N*,*N*-dimethylamino and free phenol functionality intact (Figure 2).The reactivity towards standardly used 1,3-propane sultone as alkylating agent[13] was also successfully achieved to deliver, for instance, the salt **28** in 52% isolated yield.

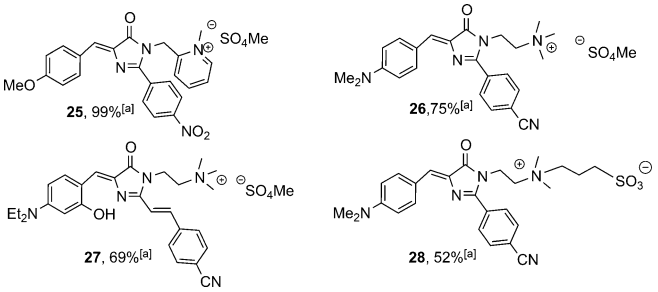


Figure 2. Preparation of salts using Me_2SO_4 or 1,3-propane sultone reagents. [a] Yield of isolated product.

The representative absorption and fluorescence spectra of novel GFP fluorophores are shown in Figure 3.[14] As expected, important Stokes shifts were observed with all selected analogs flanked with electron-donating and -withdrawing groups on both aromatic units. Nevertheless, GFP fluorophores bearing the most efficient electron-donating dimethylamino group display significant red shift (up to 627 nm for the emission spectra).

In summary, a novel one-pot azide-free access to *N*-substituted 2-*H*-4-benzylidene imidazolones, and their regioselective Pd(0)-catalyzed and Cu(I)-assisted direct C2–H arylation and alkenylation with various aryl- and alkenylhalides have been developed to build, on large-scale, a broad library of highly valuable GFP and Kaede protein-type imidazolone-based fluorophores. This straightforward synthetic route gives novel opportunities to achieve invariably the specific substitution of both arene units by electron-withdrawing and -donating groups as well as preparation of salts to modulate and optimize the fluorescence quantum yield and prepare water-soluble probes for biological studies. This work represents a rare application of the direct C–H functionalization methodology for the design of a broad library of organic materials. It paves the way for a further design of innovative imidazolone-based GFP and Kaede fluorophores.

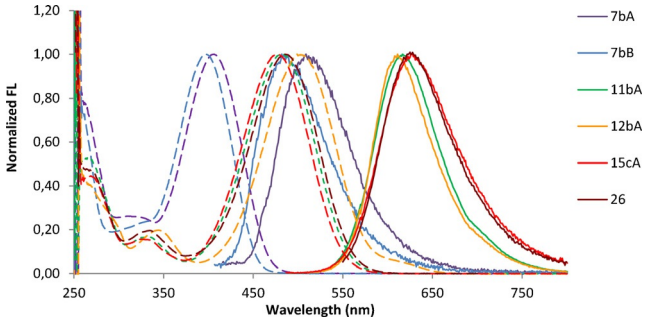


Figure 3. Normalized UV/Vis absorption spectra (dashed lines) and fluorescence spectra (solid lines) in DMSO of compounds **7bA**, **7bB**, **11bA**, **12bA**, **15cA** and **26**.

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[14] See the Supporting Information for all spectral data.

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