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COMMUNICATION

PCl_3-Mediated Synthesis of Green/Cyan Fluorescent Proteins Chromophore using Amino Acids

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Convenient synthesis involving aromatic amino acids, acetyl chlorides, and anilines in presence of phosphorous trichloride has been established for the rapid synthesis of functionalized imidazolinones. With all the starting materials which are commercially available, low cost and stable, the construction of the target products has been accomplished *via* tandem transformations involving a key C−N coupling, leading to the formation of two C(sp^2)−N, one C=N, and one C=C bonds.

Nitrogen containing heterocycles are of special interest in synthetic organic chemistry,[1] since they occur in a wide variety of natural products. Among them, imidazolinone is a fundamental non-aromatic naturally-occurring heterocycle that has been intensively used in the synthesis of functional materials and pharmaceuticals.[2] The imidazolinone substructures are found to act as the chromophores of the fluorescent proteins (FPs) in nature, for example, green fluorescent protein (GFP), cyan fluorescent protein (CFP), blue fluorescent protein (BFP) and for red kaede fluorescent protein (RFP) (Figure 1).[3a-c] Imidazolinones show various biological and pharmaceutical activities such as, angiotensin II receptor,[4] dual p38αMAPK and ERK1/2 inhibitors,[5] and monoamine oxidase (MAO) inhibitory.[6] They are also used as herbicides,[7] anticonvulsant agents,[8] anti-parkisonian,[9] and anti-inflammatory agents.[10] The omnipresence of 4-arylidene-5-imidazolinones and their intrinsic photochemical phenomena have made it intriguing synthetic targets and useful chemical models for investigating the mechanism of the fluorescence proteins.[11a-d] Till date numerous structural analogues of FPs have been reported, [12a-k] however a literature perusal revealed that only few synthetic routes have been utilized for the synthesis of highly functionalized imidazolinone that allow substitutions at 2-position upon a ring-closing condensation of α-amidoamides.[13a-b]

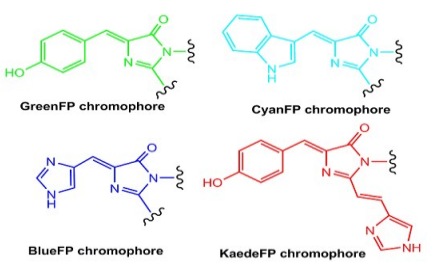
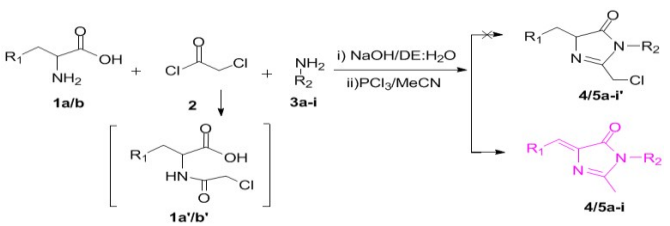


Figure 1. Imidazolinones containing flourescent protein in nature.

While each of these known protocols for imidazolinone synthesis has specific features and/or advantages, the design of new tandem reactions, using simpler starting materials to expand the structural diversity of the products, is still highly desirable and remains a challenge. In continuation of our interest in nitrogen containing heterocycles,[14a-b] herein we report a new phosphorous trichloride-catalyzed tandem approach for the synthesis of 4-arylidene-5-imidazolinones involving the assembly of *N*-chloroacetyl-amino acids (**1a'/b'**) and anilines (**3**), wherein (**1a'/b'**) are generated *in situ* by the direct use of the corresponding aromatic amino acids (**1**) and acetyl chlorides (**2**) in one pot (Scheme 1).



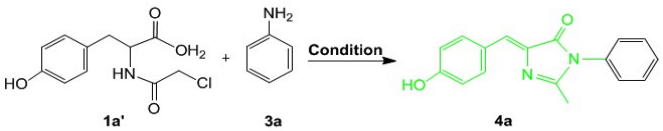
Scheme 1. Synthesis of 4-arylidene-5-imidazolinones in one-pot system

Originally, the reaction of L-tyrosine (**1a**), chloroacetyl chloride (**2**), and aniline (**3a**) were employed to probe the expected transformation. We then envisioned that cyclization of *N*-chloroacetyl-L-tyrosine **1a'** with aniline **3a** would give imidazolinone **4a'** in presence of an appropriate catalyst. But to our surprise, the final product was identified to be 4-arylidene-5-imidazolinone **4a**, a well known Green fluorescent protein chromophore using PCl_3 in acetonitrile and the expected imidazolinone **4a'** was not obtained (Scheme 1).

Then, we decided to investigate the solvent, catalysts loading and temperature effects on the efficiency of cyclization which could contribute eventually to higher conversion of imidazolinone. As shown in Table 1, comparison of different typical catalysts such as PCl_3, $POCl_3$ and $SOCl_2$ revealed that PCl_3 was the optimal catalyst (Table 1, entries 1−5). Increasing the amount of catalyst (2 mmol)

did not improve the yield (entry 1), whereas decreasing the amount of catalyst (0.5 mmol) decreased the yield (entry 2). The subsequent examination of reaction media of different polarity, including toluene, DMF, DCM, THF and ethanol demonstrated that a polar aprotic solvent was better favoured by the model reaction, and acetonitrile was among the best ones followed by THF (Table 1, entries 1,4−8). Further, variation of the reaction temperature proved that 50 °C was the favoured temperature (Table 1, entries 3 and 9). The reaction did not occur in the absence of mentioned catalysts even after 48 h stirring (entry 12). Finally, the optimized reaction conditions were obtained in the presence of 1 equiv of PCl_3 in MeCN at 50 °C for 3 h under ambient air (entry 1). It is noteworthy that only one regioselective imidazolinone is obtained by following this protocol.

Table 1. Optimization of 4-arylidene-5-imidazolinones synthesis[a]

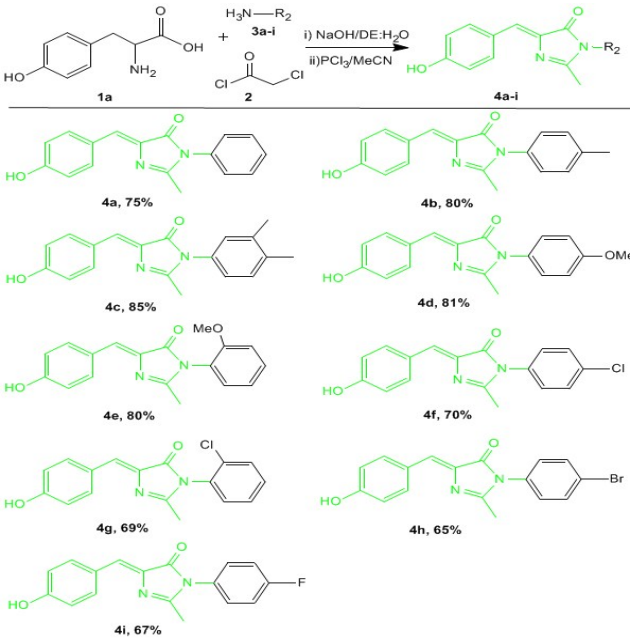


entry	solvent	catalyst (mmol)	temp (°C)	yield(%)[b]
	MeCN	PCl_3[2]	50	75
	MeCN	PCl_3[0.5]	50	70
	MeCN	PCl_3[1]	50	75
	MeCN	$POCl_3$[1]	50	25
	MeCN	$SOCl_2$[1]	50	50
	Toluene	PCl_3[1]	110	25
	DMF	PCl_3[1]	130	-
	DCM	PCl_3[1]	40	-
	THF	PCl_3[1]	80	70
	EtOH	PCl_3[1]	40	-
	MeCN	PCl_3[1]	70	75
	MeCN	No catalyst	50	-

[a]Reaction conditions: **1a'** (2 mmol), **2** (2 mmol) and solvent (2.5 mL) stirred at given temperature for 2-10 h (TLC). [b]Isolated yields.

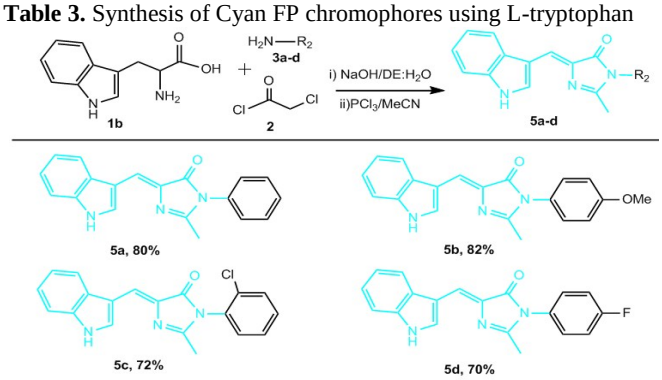
Under the optimized reaction conditions, we began to explore the scope of this annulation by employing various anilines (**3a−i**) to get different GFP chromophores (Table 2). Thus, a small library of GFP chromophores was established, in which anilines bearing electron-donating groups such as −Me and −OMe on the phenyl ring successfully provided the corresponding imidazolinone in good yields (**4b-e**), whereas halogen-substituted anilines gave moderate yields (**4f-i**). However, anilines containing electron withdrawing group like -NO_2, -COOH and –OH did not proceed to give the products even after reacting for 24 h. Simultaneously, we also focused our attention to the scope of another aromatic amino acid *viz* L-tryptophan to prove the general applicability of this protocol for the synthesis of imidazolinone. Thus, the reaction of L-tryptophan and chloroacetyl chloride yielded *N*-chloroacetyl-L-tryptophan (**1b'**), which was allowed to react with various anilines (**3a-d**) to afford the corresponding imidazolinones **5a−d**, which were again Cyan fluorescent protein chromophores (Table 3). This assured the broader scope of application of the present protocol toward imidazolinone synthesis. Meanwhile, investigations on this protocol for preparation of Blue FP chromophores using histamine are still going on in our laboratory.

Table 2. Synthesis of Green FP chromophores using L-tyrosine



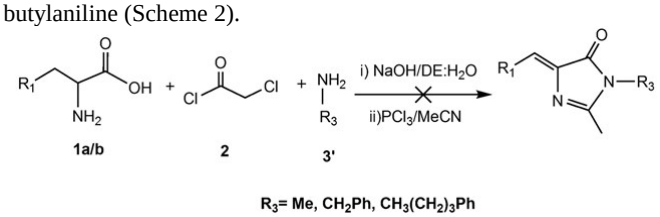
Optimal reaction conditions: **1a** (2.0 mmol), **2** (2.4 mmol) and **3** (2.0 mmol) using PCl_3 (1 mmol) in MeCN (2.5 mL) in 3 h.

Table 3. Synthesis of Cyan FP chromophores using L-tryptophan



Optimal reaction conditions: **1b** (2.0 mmol), **2** (0.2 mmol) and **3**(2.0 mmol) using PCl_3 (1.0 mmol) in MeCN (2.5 mL) in 3 h.

It is noteworthy that no imidazolinone was produced in the reaction involving an alkylamine, such as methyl amine, benzylamine or 4-butylaniline (Scheme 2).

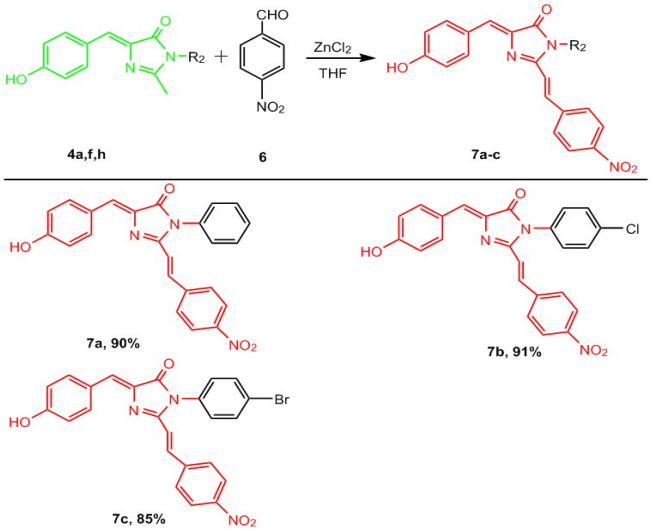


Scheme 2. Attempted synthesis with an aliphatic amine

Further, we intended to functionalize 2-methyl substituted imidazolinone **4** to get synthetic analogues of red Kaede FP chromophore **7** by the formation of the π-conjugated product on reaction with *p*-nitrobenzaldehyde. Following the reported literature,[11d, 13b-c] we tested for different Lewis acids such as $AlCl_3$, $FeCl_3$ and $ZnCl_2$ as catalyst in different reaction media. However, the best yields for our system was found using $AlCl_3$ (5 mol %) in THF

as solvent in excellent yields. Thus, **4a**, **4f**, and 4h were selected and successfully converted to the corresponding synthetic analogues of red Kaede FP chomophores **7a**, **7b**, and **7c** on condensation with *p*-nitrobenzaldehyde (1:1 ratio) in the presence of THF at 80 ^{o}C (Table 4).

Table 4. Synthesis of Red FP chromophores analogues



Reaction conditions: 2 (1.0 mmol), 6 (1.0 mmol) and $AlCl_3$ (5 mol %) in 2.0 ml of THF at 80 ^{o}C for 4 h.

In the ^{1}H NMR spectrum of **4a**, a singlet at δ 7.01 was observed, which could be assigned to the >C=C-H proton and singlet at 2.19 ppm for CH_3 linked to C-2 correlating well with other 4-arylidene-5-imidazolinones. In the ^{13}C NMR spectrum, the signals at δ 16.08 and 160.46 were due to –CH_3 linked to C-2 and tertiary C-2 linked to two N atoms, respectively, and the characteristic peak at δ 115.8 was assigned for allylic C linked to imidazolone. The relative configuration of the product was determined based on single-crystal X-ray diffraction of **4a**, figure 2. In compound **7a**, there was no methyl peak in ^{1}H NMR and ^{13}C NMR spectra, all the protons were in aromatic region in ^{1}H NMR. Analytical and spectral data further supported the structures of all the newly synthesized compounds.

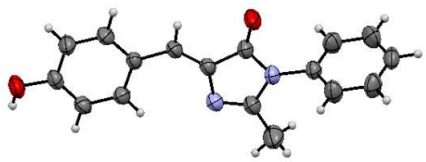
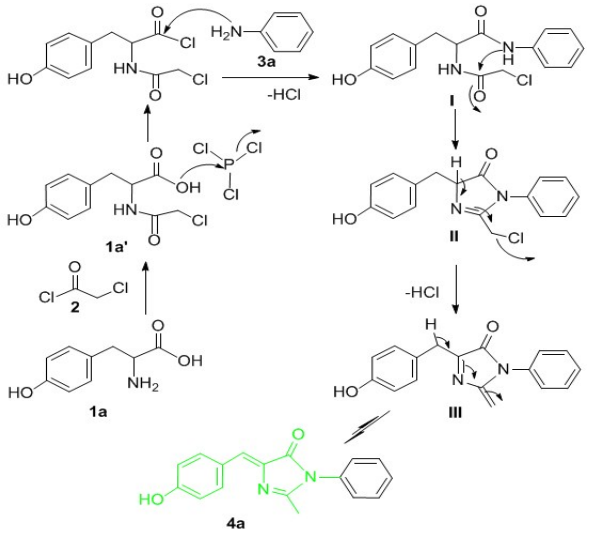


Figure 2. X-ray crystal structure of **4a** at 50 % thermal ellipsoids.

A possible mechanism is proposed in Scheme 3. The reaction of **1a** and chloroacetyl chloride **2** leading to the formation of *N*-chloroacetyl-L-tyrosine **1a'** in a basic solution, which undergoes amination with aniline **3a** initiated by PCl_3 to generate intermediate **I**. Intramolecular cyclization of the aniline NH with a nucleophilic attack at the carbonyl group followed by elimination of H_2O leads to the intermediate **II**. Then, tertiary H of C-4 participate in double bond extension leading to the elimination HCl to give intermediate **III**, which further rearrange to give more stable product **4a**.



Scheme 3. Proposed Mechanism of Imidazolinone synthesis

In summary, we have described the application of L-tyrosine and L-tryptophan to synthesis 4-arylidene-5-imidazolinones in a one-pot reaction. In these reactions, at least four different active sites were involved; one ring, one C=N bond, one C=C bond and two C-N bonds were constructed with all reactants efficiently utilized in the chemical transformation. To the best of our knowledge, no report on the use of L-tyrosine and L-tryptophan as starting materials for the synthesis of Green/Cyan FPs chromophore is known. Further, functionalization of methyl from G/C FPs chromophore with *p*-nitrobenzaldehde was successfully carried out giving synthetic analogues of red Kaede FP chromophores. Undoubtedly, this cycloannulation opens a new convenient and an effective way to construct the 4-arylidene-5-imidazolinones from readily available starting materials.

Experimental Section

Preparation of **4a**:

In a 25 mL round bottom flask, L-Tyrosine **1a** (2 mmol) was dissolved in diethyl ether/water (1:1 v/v) mixture (2 mL) in an ice bath (4 ^{o}C), and 2 mL of 4 M NaOH solution was added to mixture. Then, chloroacetyl chloride (2 mmol) dissolved in 1.0 mL of diethyl ether was added dropwise to the amino acid solution simultaneously with aqueous 4 M NaOH solution 2 mL over a 20-minute period under vigorous stirring. The aqueous layer was acidified to pH 1 with aqueous 4 M HCl and extracted with ethyl acetate. The combined organic layers were washed with saturated NaCl solution, dried with anhydrous Na_2SO_4, and concentrated under vacuum. To this solid product, aniline **3a** (2 mmol) in CH_3CN (2.5 mL) were taken. PCl_3 (1.0 mmol) was added and refluxed for 2 hour, where the reaction mixture turns yellow in colour as indicated by TLC. The reaction mixture was poured into ice-cold water and extracted with ethyl acetate (20 mL). The combined organic layer was washed with water for three times, dried over anhydrous Na_2SO_4 and organic layer was evaporated. The crude products were columned using

hexane/ethyl acetate (80:20) and were recrystallized from EtOH to afford the pure product **4a** in 75 % yield.

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Notes and references

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Electronic Supplementary Information (ESI) available: Supporting information includes analytical data and spectra. This material is available free of charge via the Internet at DOI: 10.1039/c000000x/

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TOC Content:

PCl_3-Mediated Synthesis of Green/Cyan Fluorescent Proteins Chromophore using Amino Acids

Thokchom Prasanta Singh and Raja Shunmugam*

An efficient synthesis of green and cyan fluorescent proteins chromophore from L-tyrosine and L-tryptophan using PCl_3 has been successfully developed.