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Lipase-Initiated Tandem Biginelli Reactions via In Situ-Formed Acetaldehydes in One Pot: Discovery of Single-Ring Deep Blue Luminogens

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Abstract: A facile approach for synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones was developed by a tandem multi-component reaction (MCR) in one pot. This approach involves two steps, lipase-catalyzed in situ generation of acetaldehyde and the Biginelli reaction in turns. Several control experiments were performed using acetaldehydes directly to explore the possible mechanism of this procedure. Moreover, owing to the distinct modularity and highly efficient features of the MCR, it assembles libraries of structurally diverse products (yields up to 98% under the optimized conditions in this paper) and provided an exceptional synthesis tool for the discovery of the minimal deep-blue luminogen in the solid state, namely, a single ring.

Keywords: enzyme catalysis; *Candida antarctica* Lipase B; tandem reaction; one-pot synthesis; fluorescence

Introduction

Enzyme catalysts, by allowing the development of green and efficient synthetic reactions, are increasingly becoming the best complements to the traditional chemocatalysts.[1] Among the enzymes, lipases (EC 3.1.1.3) normally catalyze hydrolytic cleavage of ester bonds in the aqueous media but also transesterification in the organic media.[2] The inherent advantages of lipases such as good stability, simple processing requirements, and mild reaction conditions greatly expand their application.[3] Of the lipases from different organisms, *Candida antarctica* lipase B (CALB) is a robust enzyme, especially in immobilized form (Novozyme 435), and can be readily expressed industrially in large quantities. Currently, it serves as one of the most frequently used lipases in both academic and industrial laboratories.[4] Additionally, numerous publications have demonstrated that CALB could exhibit promiscuity through catalyzing the generation of various carbon-carbon or carbon-heteroatom bonds.[5] In spite of continuous enthusiasm dedicated to this field, at the present stage, integrating a CALB-catalyzed process into a tandem reaction in one pot becomes fascinating and remains a considerable challenge.

Multi-component reactions (MCRs) have attracted sustained attention because they represent a powerful tool for the construction of complex molecular structures with evident advantages, such as simplified workup procedures, high overall yields and versatile product libraries. As one of the most useful MCRs, the Biginelli reaction enables straightforward access to privileged 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) and relevant heterocyclic scaffolds.[6] DHPM derivatives are well-known a class of biologically active molecules including calcium channel blockers, antitumor activity, HIV inhibitor, and interaction with sequence-selective DNA or bovine serum albumin, *etc*.[7] Up to now, the Biginelli reactions for synthesis of DHPMs have been reported mainly involving catalyst variations on the following types: Lewis acids, ionic liquids, Brønsted acids, biocatalysts, and organocatalysts.[6,8] However, these existing species suffer with certain drawbacks in terms of complicated procedures for preparations of catalysts, tedious reaction conditions, unsatisfactory yields, and environment pollution. In aspects of basic skeleton, almost all the DHPMs were obtained by using aromatic aldehydes as the substrates instead of acetaldehydes, because acetaldehydes are difficult to handle with low boiling points and tendency to polymerize.[6,8] Therefore, the in situ generation of acetaldehydes from the corresponding substrates under mild conditions is an ideal method for further reactions. Several groups (including ours) have developed some cascade strategies like this through elegant enzyme catalysis recently.[9]

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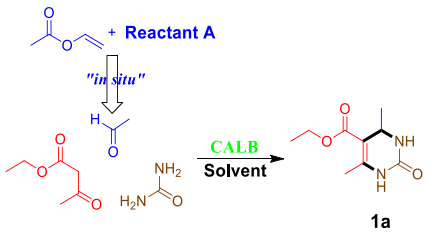
Herein, following the approach and continuous research towards enzyme catalysis, we wish to disclose a facile tandem MCR initiated by a lipase (CALB) for one-pot synthesis of DHPMs in the presence of water. This tandem procedure apparently consists of two steps: CALB-catalyzed generation of acetaldehydes from vinyl acetate and followed by the Biginelli reaction of in situ-formed acetaldehyde, urea and β-dicarbonyl compound. Some proof-of-principle experiments were designed using acetaldehydes directly to gain insights into the possible mechanism and the role of CALB, especially to the second-step reaction. More importantly, due to the modular feature of the MCR and high efficiency of this tandem procedure, it could easily assemble diverse product libraries by simply altering the starting materials and gave the yields from moderate to excellent. These structurally diverse molecules provided us an opportunity to screen their optical properties resulting in discovery of single-ring deep blue fluorogens in the solid state.

Results and Discussion

Multi-component reactions associated with tandem processes are networks of diversified reactions. Thus, finding the suitable parameters for these reaction systems are likely to be more difficult than for the stepwise processes. On the basis of our previous research, the condition optimization of the MCR was performed for the synthesis of **1a** using CALB as a biocatalyst to initiate the tandem reaction. Reaction medium has been recognized an important role in affecting the enzymatic reaction.[10] Accordingly, we began our research by exploring the effects of various organic solvents and **Reactant A** on the model reaction. It is reasonable that vinyl acetate reacts with **Reactant A** to form vinyl alcohol through transesterification in the first step, and then tautomerism of vinyl alcohol produces acetaldehyde. In this way, we merely limit the **Reactant A** to several alcohols. The results are summarized in Table 1 and clearly indicated that the solvent played an important role in realizing the tandem reaction. No reaction or only the trace amount of the desired product was generated when the reactants were incubated in the different pure organic solvents (entries 1-8, Table 1). On the contrary, we found that water could markedly promote the reaction. Therefore, followed condition parameters were evaluated in the "wet" alcohol media. The tandem process provided a better result in the wet *i*-PrOH, giving the product **1a** in 54% yield (entry 9, Table 1). An attempt to carry out the reaction in the aqueous medium led to the formation of trace product (entry 10, Table 1). Excess *i*-PrOH and vinyl acetate were utilized to produce acetaldehyde constantly due to acetaldehyde being a volatile liquid with a low boiling point. Actually, the mixture of water, *i*-PrOH and vinyl acetate was the true medium in this one-pot system.

Table 1. The influence of different solvents and **Reactant A** on the CALB-initiated tandem reaction.[a]



Entry	Solvent	Reactant A	Yield [%] [b]
1	THF	*i*-PrOH	<5
2	MeCN	*i*-PrOH	<5
3	*n*-Hexane	*i*-PrOH	<5
4	DMSO	*i*-PrOH	--
5	1,4-dioxane	*i*-PrOH	<5
6	CH_2Cl_2	*i*-PrOH	<5
7	Toluene	*i*-PrOH	--
8	*i*-PrOH	*i*-PrOH	<5
9	H_2O	EtOH	48
10	H_2O	*i*-PrOH	54
11	H_2O	*t*-BuOH	38
12	H_2O	H_2O	<5

[a] Conditions: urea (0.2 mmol), ethyl acetoacetate (0.4 mmol), vinyl acetate (0.2 mL), and CALB (10 mg) in different solvents (1.0 mL) and Reactant A (0.2 mL) at 50 °C for 3 d. [b] Determined by HPLC.

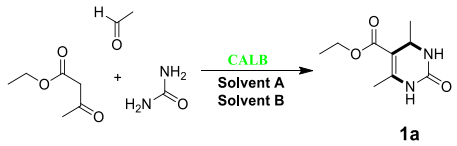
From the structure of **1a**, it contains an ester group which introduced through the β-dicarbonyl component. A question should be considered that whether CALB could catalyze transesterification between the ester group and alcohol to afford other types of products? Thus we performed the reaction using different alcohols and the results were listed below. It is found that the corresponding products via transesterification gave trace or no yield (entry 2 and 3, Table S1). Notably, when using ethanol as a substrate, the yield of the target product **1a** actually showed a decreased (entry 1, Table S1). Due to those results, we chose *i*-PrOH as a substrate and did not consider the effect of transesterification during the subsequent conditional optimization.

Although higher yields were obtained in the water-alcohol media, we were puzzled with the results in the pure organic solvents. In fact, CALB could catalyze transesterification between vinyl acetate and isopropanol to generate acetaldehyde in the absence of water, and some relevant literatures have been reported. Hence, we rationally considered that the failure to obtain the target product in the organic solvents may be ascribed to the second-step reaction (namely, Biginelli reaction). To demonstrate this, we directly utilized acetaldehyde as a substrate to incubate with urea and ethyl acetoacetate in the different solvents. Before the experiments, the amount of acetaldehyde was firstly calculated according to the amount of vinyl acetate in the tandem process (Scheme S1). When vinyl acetate (0.20 mL) was completely converted, the generation

amount of acetaldehyde was ~0.122 mL. Considering the loss of volatilization, the actual volume of acetaldehyde we took was 0.150 mL.

The experimental data are summarized in Table 2. It is obvious that the reactions barely proceed in all involved solvent systems. Especially, only trace product was detected in the water-alcohol mixture solvent (entry 2, Table 2), which is very close to the conditions of entry 10 in Table 1. Such a confusing but intriguing result promoted us to think what species make the second-step reaction proceed smoothly in the tandem process. Looking back at the first-step reaction, when there is water, hydrolysis of vinyl acetate by CALB could also generate acetaldehyde but acetic acid as a by-product. Therefore, we took into account that whether acetic acid has an effect on the Biginelli reaction. To prove the hypothesis, we calculated the amount of acetic acid at first when vinyl acetate (0.20 mL) was totally hydrolyzed (Scheme S2).

Table 2. The influence of different solvents on the Biginelli reaction.[a]



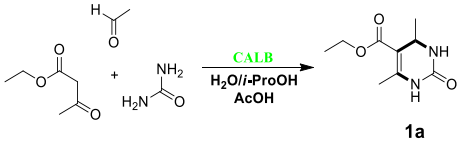
1a

Entry	Solvent A	Solvent B	Yield [%] [b]
1	H_2O	H_2O	<5
2	H_2O	*i*-PrOH	<5
3	H_2O	EtOH	<5
4	*i*-PrOH	*i*-PrOH	<5
5	EtOH	EtOH	<5
6	Toluene	*i*-PrOH	--
7	MeCN	*i*-PrOH	<5

[a] Conditions: urea (0.2 mmol), ethyl acetoacetate (0.4 mmol), acetaldehyde (0.15 mL), and CALB (10 mg) in different solvents (Solvent A: 1.0 mL, Solvent B: 0.2 mL) at 50 °C for 3 d. [b] Determined by HPLC.

Subsequently, we added different amount of acetic acid in the reaction system (based on the entry 2, Table 2) to examine its effect on the yield. The volume of acetic acid changes from 0 mL to the largest (0.125 mL). As shown in Table 3, the yields were significantly affected by the amount of acetic acid, and the highest yield was reached when the reaction system contained 0.125 mL of acetic acid. Besides, we found that the yields displayed a gradual increasing trend along with enhancing acetic acid.

Table 3. The influence of acetic acid on the Biginelli reaction.[a]



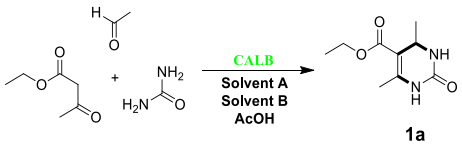
1a

Entry	V_{AcOH} [mL]	Yield [%] [b]
1	0	<5
2	0.002	<5
3	0.004	12
4	0.006	18
5	0.008	29
6	0.100	36
7	0.125	42

[a] Conditions: urea (0.2 mmol), ethyl acetoacetate (0.4 mmol), acetaldehyde (0.15 mL), *i*-ProOH (0.2 mL), acetic acid (0-0.125 mL) and CALB (10 mg) in deionized water (1.0 mL) at 50 °C for 3 d. [b] Determined by HPLC.

These results prompted us to further explore the influence of acetic acid on the second-step reaction in the other solvent systems. Keeping 0.125 mL of acetic acid in each reaction, we detected the yields of **1a** in the different solvents and the results are summarized in Table 4**.** It is clear that almost all of the reactions respectively give improved yields than those in Table 2 except in the pure water. By comparing the three sets of the experimental data (Table 2-4), we temporarily think that the second-step reaction may be related to acetic acid. However, we considered that if the role of acetic acid is a catalyst, in that way, the reaction has less relevant to its amount. But in truth, this judgment is inconsistent with the results of Table 3.

Table 4. The influence of acetic acid on the Biginelli reaction in different solvents.[a]



1a

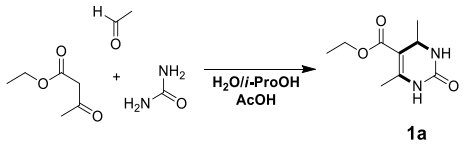
Entry	Solvent A	Solvent B	Yield [%] [b]
1	H_2O	H_2O	<5
2	H_2O	*i*-PrOH	42
3	*i*-PrOH	*i*-PrOH	35
4	EtOH	EtOH	49
5	MeCN	*i*-PrOH	25
6	Toluene	*i*-PrOH	10

[a] Conditions: urea (0.2 mmol), ethyl acetoacetate (0.4 mmol), acetaldehyde (0.15 mL), acetic acid (0.125 mL) and CALB (10 mg) in different solvents (Solvent A: 1.0 mL, Solvent B: 0.2 mL) at 50 °C for 3 d. [b] Determined by HPLC.

To better understand the role of acetic acid, we continued to perform some control experiments on the foundation. At first, we investigated the influence of the amount of acetic acid on the Biginelli reaction when CALB was absent (Table 5). The yields dropped slightly along with increasing the volume of acetic acid. It means that acetic acid could slightly inhibit the reaction. Additionally, in comparison with the reactions which contained CALB (Table 3), these cases gave higher yields under the corresponding

conditions, respectively. It seems to illustrate that CALB also has an inhibitory effect on the reaction. More importantly, we found that the highest yield was achieved when there is neither acetic acid nor CALB in the reaction system (entry 1, Table 5). It is a very interesting result.

Table 5. The influence of acetic acid on the Biginelli reaction without CALB.[a]

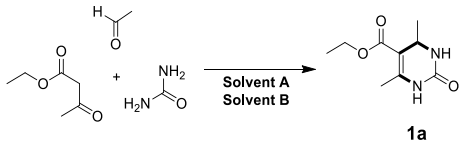


Entry	V_{AcOH} [mL]	Yield [%] [b]
1	0	70
2	0.002	63
3	0.004	57
4	0.006	57
5	0.008	55
6	0.100	54
7	0.125	50

[a] Conditions: urea (0.2 mmol), ethyl acetoacetate (0.4 mmol), acetaldehyde (0.15 mL), *i*-ProOH (0.2 mL), and acetic acid (0-0.125 mL) in deionized water (1.0 mL) at 50 °C for 3 d. [b] Determined by HPLC.

Following these experiments, we examined the influences of different solvents on this Biginelli reaction once again when both of acetic acid and CALB were absent. We shortened the reaction time to 1d. The results are summarized in Table 6. It is evident that the yields obtained from pure water or water-organic solvent mixtures are higher than those obtained from pure organic solvents. Thus, we think that water is a specific factor for the second-step reaction. Moreover, the process gave the highest yield in pure water (entry 1, Table 6) which is completely contrary to that contained CALB (entry 1, Table 2).

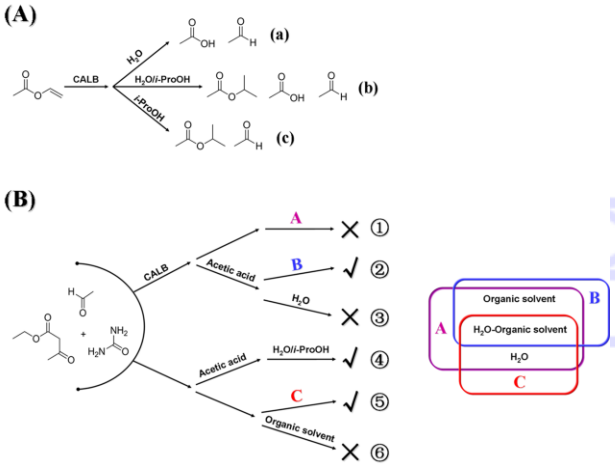
Table 6. The influence of different solvents on the Biginelli reaction without CALB and acetic acid.[a]



Entry	Solvent A	Solvent B	Yield [%] [b]
1	H_2O	H_2O	52
2	H_2O	*i*-PrOH	52
3	H_2O	MeCN	46
4	H_2O	THF	25
5	EtOH	EtOH	<5
6	MeCN	MeCN	<5
7	THF	THF	<5
8	Toluene	Toluene	<5

[a] Conditions: urea (0.2 mmol), ethyl acetoacetate (0.4 mmol) and acetaldehyde (0.15 mL) in different solvents (Solvent A: 1.0 mL, Solvent B: 0.2 mL) at 50 °C for 1 d. [b] Determined by HPLC.

As described above, we here conclude that (i) the second-step reaction, namely Biginelli reaction, can proceed smoothly in a "wet" media without CALB and acetic acid; (ii) both CALB and acetic acid could restrain the second-step reaction, however, CALB could completely restrain the reaction but acetic acid just partially restrains it; (iii) both CALB and acetic acid are in the reaction system, acetic acid can relieve the inhibitory effect of CALB making the reaction proceed (except for in the pure water). Furthermore, we roughly summed up our explorations of the second-step reaction into six routes (Scheme 1B). Although there are three routes in the first-step reaction, the generation of acetaldehyde needs to be catalyzed by CALB (Scheme 1A). Thus, water-*i*-PrOH mixture solvent is appropriate for the whole tandem reaction, namely, the route combination should be **(b)** + ②.



Scheme 1. Possible routes of (A) the first-step reaction and (B) the second-step reaction.

Although the mechanism of the CALB-initiated tandem reaction is complex and more detailed investigations are currently underway, the whole process is like a black box for us from a macro point of view. Therefore, we put our attention on improving the yield of the final product by optimizing its reaction conditions. Screening the amount and volume ratio of *i*-PrOH and vinyl acetate observed that 0.2/0.2 (mL/mL) was appropriate for the model reaction (Table S2-3). Moreover, it was very important to ascertain the optimal water content according to the above studies. The range of water content from 8 to 82% was examined and the results are illustrated in Figure 1. The product **1a** could be achieved in high yield of 73% at *ca.*26% water. Higher or lower water contents would lead to a sharp decline in yield values, as seen in the bell-shaped curves presented. Consequently, we selected the water content of 26% for the tandem reaction.

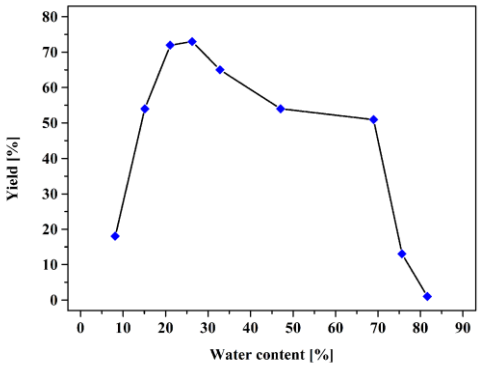


Figure 1. The influence of water content on the CALB-initiated tandem reaction. Conditions: urea (0.2 mmol), ethyl acetoacetate (0.4 mmol), vinyl acetate (0.2 mL), *i*-ProOH (0.2 mL), CALB (10 mg) and deionized water from 8% to 82% at 50 °C for 3 d. Yield determined by HPL.

Some other impact parameters including enzyme loading (Table 7), molar ratio of ethyl acetoacetate to urea (Table 8), and time course at different temperatures (Figure 2) were also investigated. It could be seen that the tandem reaction was unsuccessful in the absence of CALB because acetaldehyde was not formed in the first step (entry 1, Table 7). The outcomes also revealed 10 mg CALB is sufficient to push the reaction forward (entry 4, Table 7). Larger amounts of the catalysts lead to a slight decrease in yield. Besides, the molar ratio of ethyl acetoacetate/urea to 3:1 is a better choice (entry 4, Table 8). As displayed in Figure 2, the reaction had strong temperature dependence. The highest yield of 73% was achieved at 60 °C after 48 h, which indicated that the model reaction had reached the equilibrium at the time point. Indeed, the reaction can also afford almost the same yield by prolonging the time at 50 °C. Owing to the easy vaporization of acetaldehyde, higher temperatures were not performed.

Table 7. The influence of enzyme loading on the CALB-initiated tandem reaction. [a]

Entry	Enzyme loading [mg]	Yield [%] [b]
1	0	<5
2	2.5	68
3	5	69
4	10	72
5	20	66
6	50	63
7	100	65

[a] Conditions: urea (0.2 mmol), ethyl acetoacetate (0.4 mmol), vinyl acetate (0.2 mL), *i*-ProOH (0.2 mL) and CALB (0-100 mg) in deionized water (0.16 mL) at 50 °C for 3 d. [b] Determined by HPLC.

Table 8. The influence of mole ratio of **1** to **2** on the CALB-initiated tandem reaction. [a]

Entry	Ethyl acetoacetate : urea	Yield [%] [b]
1	1 : 1	53
2	1.5 : 1	61
3	2 : 1	71
4	3 : 1	73
5	5 : 1	66
6	10 : 1	59

[a] Conditions: urea (0.2 mmol), ethyl acetoacetate (0.2-2.0 mmol), vinyl acetate (0.2 mL), *i*-ProOH (0.2 mL) and CALB (10 mg) in deionized water (0.16 mL) at 50 °C for 3 d. [b] Determined by HPLC.

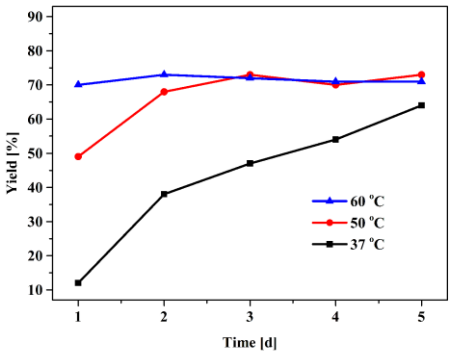


Figure 2. Time curves of the CALB-initiated tandem reaction at different temperatures. Conditions: urea (0.2 mmol), ethyl acetoacetate (0.6 mmol), vinyl acetate (0.2 mL), *i*-ProOH (0.2 mL) and CALB (10 mg) in deionized water (0.16 mL) at different temperature for 1-5 d. Yield was determined by HPLC.

With the optimal conditions in hand, a series of β-dicarbonyl compounds were first applied to check the generality and scope of the CALB-initiated tandem reaction. As shown in Scheme 2, the β-dicarbonyl compounds with linear substituent at R^1 provided better yields of the DHPMs products **1a-c** compared to the large ester groups (**1d**, **e**). Additionally, gradually enlarging in the size of the substituent R^2 led to a stepwise decrease in the yields as revealed in the production of compounds **1f-h**. The acetoacetanilide was also reactive to give **1i** in good yield. Many substrates and conditions were employed in this reaction to test the enantioselectivity, unfortunately, there is no obvious optical activity.

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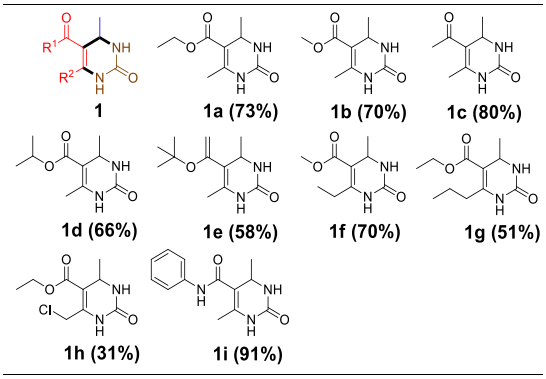
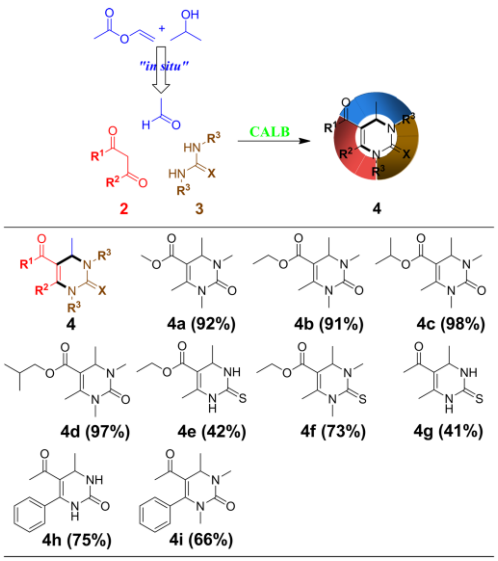


Scheme 2. Synthesis of **1** by CALB-initiated tandem Biginelli reactions under the optimal condition. Yields of the isolated products after chromatography on silica gel (calculated by urea).

Because of the modular characteristics of MCRs, it is facile to construct versatile product libraries from simple starting materials. The results described above motivated us to further expand the diversity-oriented library based on the DHMP core and get insight into the relationship of structure-photophysical behavior (Scheme 3). Firstly, we employed N,N′-dimethylurea in place of urea reacted with various β-keto esters and in situ-prepared acetaldehyde. To our delight, the MCRs gave rise to all of the corresponding products in excellent yields (**4a-d,** up to 98%) under the previously optimized reaction condition. Secondly, thiourea or its N,N′-dimethyl substituted derivatives was tested as a substrate to react with the other two. Generally, thioureas in the MCRs resulted in a sharp decline in yields, which maybe because of the difference in the electronegativity between oxygen and sulfur atoms (**4e-g**). Finally, to increase π-conjugated system of the DHPM core, an attempt to utilize benzoylacetone to carry out the one-pot Biginelli reaction. The substrate provided the desired products in satisfactory yields (**4h, i**).



Scheme 3. Scope of the tandem MCR for the synthesis of **4** under the optimal condition. Yields of the isolated products after chromatography on silica gel (calculated by urea).

Having established a preparative approach for libraries of structurally diverse DHPMs, we conducted a preliminary study on their optical properties in the solid state. We observed that all of the single-ring DHPMs exhibit similar emissions in blue light region. Due to introduction of a phenyl group onto the DHPM core, compound **4i** has the longest wavelength (λ_{em} = 495 nm) which clearly reflected the contribution of the extended π-conjugated system. Furthermore, we calculated the Commission Internationale de L'Eclairage (CIE) coordinates of all the DHPMs (Figure 3 and S1). In the National Television System Committee (NTSC) colour system, the blue emission should be defined as (CIE_x + CIE_y) value < 0.30, while that for deep blue should be further limited to CIE_y value < 0.08.[11] Deep blue emitters are critical for enriching the colour gamut space, reducing power consumption, and popularizing OLEDs.[12] It is intriguing, from the practical viewpoints, to develop concise and green methodologies to construct the deep-blue fluorogens in the solid state. Four compounds (**1e**, **f** and **4a**, **e**) located in the deep-blue region with little fluctuation in the CIE coordinates. Notably, **1f** and **4e** exhibited particular deep-blue colour with excellent CIE coordinates of (0.16, 0.07) and (0.15, 0.06), respectively, which is very close to the NTSC standard blue CIE coordinates of (0.14, 0.08). To our best of knowledge, this is the minimum size for deep blue emitter, namely, a single ring.

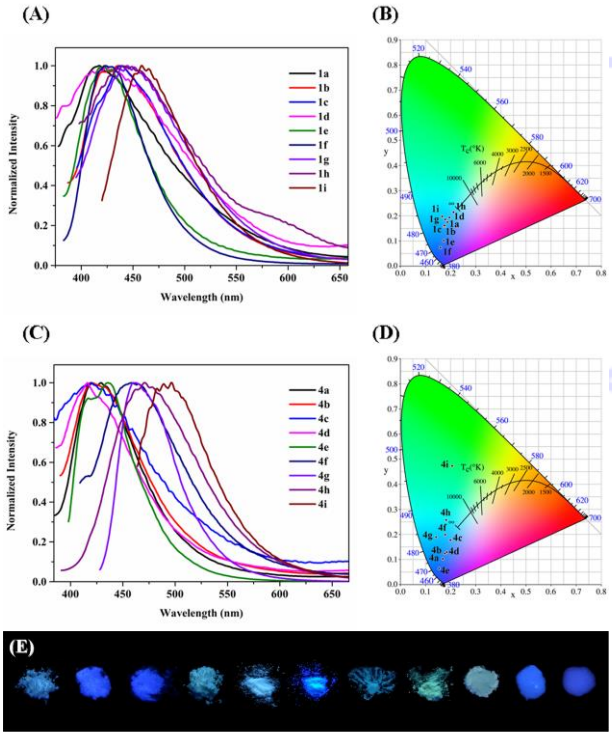


Figure 3. Solid-state fluorescence spectra of (A) **1** and (C) **4** in powder (**4a-d** were dispersed in Al_2O_3). The position of (B) **1** and (D) **4** plotted on CIE 1931 chromaticity diagrams. (E) Photographs of selected DHPMs under irradiation at 365 nm. From left to right: **1b**, **1e**, **1f**, **1g**, **1i**, **4e**, **4g**, **4h**, and **4i** in powder, **4a** and **4d** in oil.

Subsequently, density functional theory (DFT) calculations were performed to better understand the optical behavior and electronic structures of the DHPMs (Figure 4). The highest occupied molecular orbitals (HOMO) of the chemicals mainly populate on the C=C-N fragment of the DHPM cores, except for **4e** that is primarily localized over the sulfur atom, whereas their lowest unoccupied molecular orbitals (LUMO) are evident in the O=C-C=C framework, exhibiting a degree of charge transfer (CT) features. Such similar orbital distribution implied that the single-ring DHPMs have similar photophysical behaviors that are confirmed in the above. For the DHPMs **4h** and **4i**, it is note that their LUMO levels also distributed over the pendant phenyl groups. As a result, **4i** showed green fluorescence with CIE coordinates of (0.21, 0.47) due to the strong CT effect.

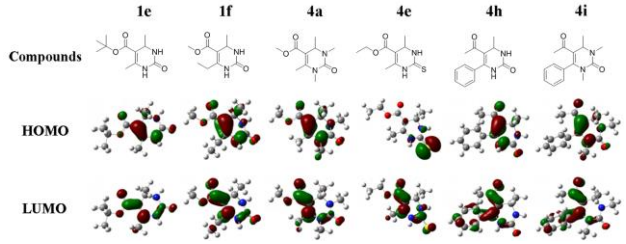


Figure 4. Frontier orbital of the selected compounds as calculated at the level of B3LYP/6-31G (d, p).

Conclusion

In the present work, we successfully developed the application of tandem reaction in one pot for synthesis of DHPMs using CALB as an economical and eco-friendly initiator. This tandem strategy actually combines two steps, in situ formation of acetaldehyde by lipase catalysis at first, and closely followed by a Biginelli reaction. Several preliminary studies indicated that collective effect of CALB, mixture solvent and acetic acid (a by-product from the first-step reaction) should be responsible for the second-step reaction. Under the optimal conditions, the target products were afforded in moderate to excellent yields with a broad range of substituent. Furthermore, this modular approach has led to the discovery of structurally diverse molecular libraries as luminogens in the solid state. A few of the compounds show deep-blue emissions which only contain a single ring. We also believe that the use of green biocatalysis will provide novel avenues for constructing a wide variety of new materials.

Experimental Section

Materials and Analytical Methods

All reagents were used without further purification unless otherwise noted. Novozyme 435 (immobilized lipase B from *Candida antarctica*, CALB) was purchased from Novozymes (Bagsvaerd, Denmark) and used as received. The NMR spectra were obtained on an Agilent 400-MR DD2 spectrometer. The ^{1}H NMR (400 MHz) chemical shifts were measured relative to DMSO-d_6 as the internal reference (DMSO-d_6: δ = 2.50 ppm). The ^{13}C NMR (100 MHz) chemical shifts were given using DMSO-d_6 as the internal standard (DMSO-d_6: δ = 39.52 ppm). HPLC experiments were performed on Waters instrument (Waters e2695, 2998) using a C18 column with MeOH/water = 60:40 (v/v), 0.8 mL min^{-1}, λ_{max}= 280 nm, and 35 °C. High resolution mass spectra (HR-MS) were obtained with a Waters-Q-TOF-Premier (ESI). Fluorescence emission spectra were obtained using F-7000 FL Spectrofluorophotometer (HITACHI).

Typical Procedure for CALB-Initiated Tandem Biginelli Reaction

Urea or thiourea derivative (0.2 mmol), β-dicarbonyl compound (0.6 mmol), vinyl acetate (0.2 mL), *i*-ProOH (0.2 mL), deionized water (0.16 mL) and CALB (10 mg) were charged in an Erlenmeyer flask and the reaction mixture was incubated at 200 rpm and 60 °C for 2 d. The reaction was completed by filtering the enzyme. The crude products were purified by silica gel column chromatography (200-300 mesh) with an eluent consisting of ethyl acetate-petroleum. Product-contained fractions were combined, concentrated, and dried to give respective product.

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FULL PAPER

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