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# Trypsin-catalyzed tandem reaction: One-pot synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones by in situ formed acetaldehyde

Zong-Bo Xie[a,b], Na Wang[a,*], Wan-Xia Wu[a], Zhang-Gao Le[b], Xiao-Qi Yu[a,*]

[a] *Key Laboratory of Green Chemistry and Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu 610064, China*
[b] *School of Chemistry, Biology and Material Science, East China Institute of Technology, Fuzhou 344000, China*[1]

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## ABSTRACT

A simple, mild, one-pot tandem method catalyzed by trypsin was developed for the synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones by the Biginelli reaction of urea, β-dicarbonyl compounds, and in situ-formed acetaldehyde. Trypsin was found to display dual promiscuous functions to catalyze transesterification and the Biginelli reaction in sequence.

## 1. Introduction

Multicomponent reactions (MCRs) are receiving increased attention because of their high synthesis efficiency, atom economy, molecular diversity, as well as extensive applications in combinatorial chemistry and diversity-oriented synthesis. The Biginelli reaction, originally described by Biginelli (Tron et al., 2011) in 1893 (Kappe, 2000), is one of the most useful multicomponent reactions that enables straightforward access to functionalized 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) and related heterocyclic compounds (Chen et al., 2006). DHPMs are currently important in natural and synthetic organic chemistry because of their diverse therapeutic and pharmacological properties (Gui et al., 2009; Ramos et al., 2012; Gore et al., 2011; Jain et al., 2007). Hence, the traditional Biginelli reaction for the synthesis of DHPMs is gaining renewed interest and has become a research hotspot over the past decade (Suresh and Sandhu, 2012). In addition, many improved procedures for the Biginelli reaction have been reported, mostly involving catalyst changes. Such catalyst variations can be summarized into the following types (Suresh and Sandhu, 2012): Brønsted acid (Kamble et al., 2010; Dilmaghani et al., 2012; Li et al., 2010), Lewis acid (Ramalingam and Kumar, 2009; Huang et al., 2005; Suzuki et al., 2006), ionic liquid (Fang et al., 2010; Hajipour et al., 2011; Yadav et al., 2008), organocatalyst (Lei et al., 2010; Wang et al., 2009; Wu et al., 2009), and biocatalyst (Li et al., 2011); however, most examples are acid catalysts. Given the drawbacks of existing catalysts (e.g., environmental pollution, toxicity, strongly acidic reaction conditions, unsatisfactory yields, and complicated operations), the search for novel, easily available, green catalysts is being actively pursued.

Biocatalysts are increasingly becoming the best alternatives to chemical catalysts because of their mild conditions, high selectivity, and few by-products. Enzyme promiscuity, an emerging area in the field of catalytic reactions, has provided a new direction to the application of biocatalysis (Humble and Berglund, 2011; Busto et al., 2010; Bornscheuer and Kazlauskas, 2004; Copley, 2003). Three major types of enzyme promiscuity has been considered, namely, condition promiscuity (enzymatic activities under various reaction conditions different from their natural ones), substrate promiscuity (enzymes with broad substrate specificity), and catalytic promiscuity (based on the ability of a single enzyme active site to catalyze several chemical transformations) (Humble and Berglund, 2011; Busto et al., 2010; Bornscheuer and Kazlauskas, 2004; Copley, 2003). Enzyme promiscuity, especially catalytic promiscuity, is widely used in multiple types of organic reactions such as aldol reaction (He et al., 2012; Li et al., 2008; Xie et al., 2013), Michael addition (Strohmeier et al., 2009; Cai et al., 2011), Mannich reaction (Li et al., 2009; He et al., 2010; Xue et al., 2012),

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* Corresponding authors. Tel.: +86 28 85415886; fax: +86 28 85415886.
*E-mail addresses:* zbxie@ecit.edu.cn (Z.-B. Xie), wnchem@scu.edu.cn (N. Wang), xqyu@scu.edu.cn (X.-Q. Yu).
[1] Tel.: +86 794 8258320; fax: +86 794 8258320.

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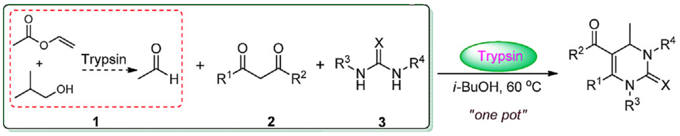


**Scheme 1.** Trypsin-catalyzed Biginelli reaction using an in situ-generated acetaldehyde.

and polymerization (Monsalve et al., 2010; Rustoy et al., 2007). To the best of our knowledge, only a few research groups (Kumar and Maurya, 2007, Zhang and co-workers, 2011, Shukla and co-workers, and Sharma et al., 2013) have reported on the biocatalytic Biginelli reaction using bakers' yeast, trypsin, lipase, and bovine serum albumin as biocatalysts, respectively; however, only aromatic aldehydes have been used yet.

More chemists are using aromatic aldehydes as substrate instead of acetaldehyde because it is generally difficult to handle and source directly given its low boiling point, easy polymerization and oxidation. Thus, indirectly preparing it in situ may be a better choice (Kumar et al., 2011; Perez-Sanchez and de Maria, 2012). Accordingly, as part of our continuous efforts toward biocatalytic promiscuity and green chemistry, we report for the first time an enzyme-catalyzed Biginelli reaction using in situ-prepared acetaldehyde. The reaction combines two catalytic activities of one enzyme in a one-pot strategy for a two-step cascade reaction (Scheme 1). Trypsin (a protease from porcine pancreas) displays dual functions, namely, substrate promiscuity and catalytic promiscuity, to catalyze the transesterification and Biginelli reaction in sequence.

## 2. Results and discussion

The reaction of vinyl acetate and urea with ethyl acetoacetate in isopropanol was chosen as a model reaction and performed at 50 °C to screen the enzymes. Several commercially available hydrolases were investigated, and the results are summarized in Table 1. Among these hydrolases, only a few can catalyze the tandem process to provide the corresponding product, and the best result of 27% yield was achieved using trypsin as the catalyst (Table 1, entry 6), consistent with the results of Zhang (Li et al., 2011). Lipase from *Mucor miehei* and lipase B from *Candida antarctica* (CAL-B), two immobilized lipases, also showed catalytic activities but gave inferior yield results of 17% and 6%, respectively (Table 1, entries 1 and2). However, five other candidates displayed almost no activity for the tandem reaction and only negligible products were observed (Table 1, entries 3–5 and 7–8). Notably, **4c** instead of **4b** was the major product when CAL-B was the catalyst used, indicating that another transesterification reaction probably occurred in this situation (Scheme S1). At the same time, some control experiments were designed to demonstrate that such a reaction was a trypsin-catalyzed tandem process. As shown in Table 1 (entries 9–11), no product was formed when the reactants were incubated with denatured trypsin or bovine serum albumin, or in the absence of enzyme. This finding ruled out the possibility that a similar amino acid distribution on the protein surface promoted the process. The control experiments also suggested that the tertiary structure of trypsin was necessary for the tandem process.

**Table 2**
Effect of solvent on the trypsin-catalyzed tandem reaction[a].

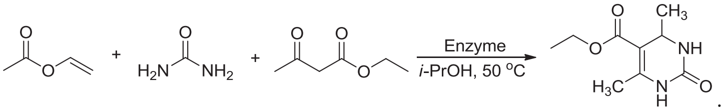
| Entry | Solvent | Yield[b] (%) |
|---|---|---|
| 1 | MeOH | 4 |
| 2 | EtOH | 11 |
| 3 | *n*-PrOH | 17 |
| 4 | *i*-PrOH | 26 |
| 5 | *i*-BuOH | 34 |
| 6 | Isopentanol | 26 |
| 7 | 1-Hexanol | 30 |
| 8 | MeCN | 6 |
| 9 | Toluene | 10 |
| 10 | $CHCl_3$ | 11 |
| 11 | Acetone | 8 |
| 12 | *n*-Hexane | 14 |
| 13 | Vinyl acetate | 8 |
| 14 | $H_2O$ | 7 |

[a] *Conditions*: urea (0.2 mmol), ethyl acetoacetate (0.24 mmol), vinyl acetate (0.2 mL), and trypsin (10 mg) in different solvents (0.8 mL) at 50 °C for 2 d.
[b] Yield was determined by HPLC (calculated by urea).

Some controlled experiments were conducted to demonstrate that either step of the two-step reaction was catalyzed by trypsin. First, in terms of the results of the controlled experiments (Table 1, entries 10 and 11), at least one of the two steps was catalyzed by enzyme. Then, after incubating vinyl acetate and *i*-PrOH with trypsin, acetaldehyde and isopropyl acetate were detected by GC/MS; however, neither was produced in the blank test. This result indicated that the generation of acetaldehyde in situ was an enzymatic reaction. Finally, acetaldehyde, urea, and ethyl acetoacetate were directly incubated with trypsin. Table S1 shows that trypsin exerted a crucial catalytic effect in the Biginelli reaction, although the controlled test also provided a few products (2% yield).

With the optimal catalyst determined, solvent selection was performed for the highest yield. As shown in Table 2, the yields

**Table 1**
Catalytic activities of different enzymes[a].



| Entry | Catalysts | Yield[b] (%) |
|---|---|---|
| 1 | Lipase B from *Candida antarctica* | 6 |
| 2 | Lipase from *Mucor miehei* | 17 |
| 3 | Lipase from *Rhizopus oryzae* | 0 |
| 4 | Lipase from *Candida rugosa* | 1 |
| 5 | Lipase from *Candida cylindracea* | 1 |
| 6 | Trypsin from porcine pancreas | 27 |
| 7 | Pepsin (porcine stomach mucosa) | 0 |
| 8 | Papain | 1 |
| 9 | Bovine serum albumin | 0 |
| 10 | Denatured trypsin[c] | 1 |
| 11 | No enzyme | 0 |

[a] *Conditions*: urea (0.2 mmol), ethyl acetoacetate (0.24 mmol), and enzyme (10 mg) in isopropanol (0.8 mL) and vinyl acetate (0.2 mL) at 50 °C for 2 d.
[b] Yield was determined by HPLC (calculated by urea).
[c] Pretreated with guanidine hydrochloride solution (6 mol/L).