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# BIGINELLI REACTION OF ALIPHATIC ALDEHYDES CATALYZED BY α-CHYMOTRYPSIN: ONE-POT BIOCATALYTIC SYNTHESIS OF DIHYDROPYRIMIDINONES

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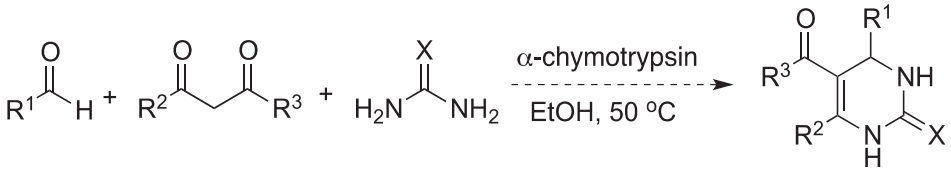
**Abstract** – A green and highly efficient procedure was developed for the one-pot, three-component synthesis of 3,4-dihydropyrimidin-2-(1*H*)-ones by the condensation of aliphatic aldehydes, β-keto esters, and urea. α-Chymotrypsin from porcine pancreas showed excellent catalytic activity in the reaction. The influences of solvents, temperature, and water content were investigated. The desired products were obtained in 85-96% yields. The methodology generates high yields with a wide range of substrates.

The Biginelli reaction, reported by Pietro Biginelli in 1893,[1] is a multicomponent reaction that involves the cyclocondensation of an aldehyde, β-keto ester, and urea or thiourea to form 3,4-dihydropyrimidinone (DHPM) derivatives. The DHPMs exhibit a wide range of biological activities, such as antiviral, anticancer, and antihypertensive, as well as calcium channel modulating activity.[2-6] As a consequence, the synthesis of DHPMs has attracted significant attention. The original Biginelli reactions were performed under strong acidic conditions, but the yields of the DHPMs were low.[7] In recent decades, more efficient conditions have been found for the Biginelli reaction, in which Lewis acids such as $NbCl_5$, $Hf(OTf)_3$, $Cu(NO_3)_2$, $FeCl_3$, $MoO_2Cl_2$, and $Ga(OTf)_3$ are used as catalysts.[8-13] Ultrasonication,[14] microwave exposure,[15] solid-phase,[16] and fluoro-phase[17]techniques to facilitate this synthesis have also become increasingly widespread. However, the above reports focus on the synthesis of DHPMs from aromatic aldehydes, because aliphatic aldehydes have problems such harsh conditions and expensive reagents. In addition, the Biginelli reaction of aliphatic aldehydes was more likely to have by-products, resulting in a lower yield.[18] Therefore, it is important for researchers to develop a more efficient and greener process to prepare DHPMs from aliphatic aldehydes using low cost, easily available, and easy to handle catalysts.

Enzyme protein sometimes works as organic catalysts other than showing original catalytic activity.[19-22] Then, some examples have been reported in the catalyst of enzymes under mild reaction conditions, such as aldol reactions,[23-27] Michael additions,[28-30] Mannich reactions,[31] Markovnikov reactions,[32] and Morita-Baylis-Hillman reactions.[33]

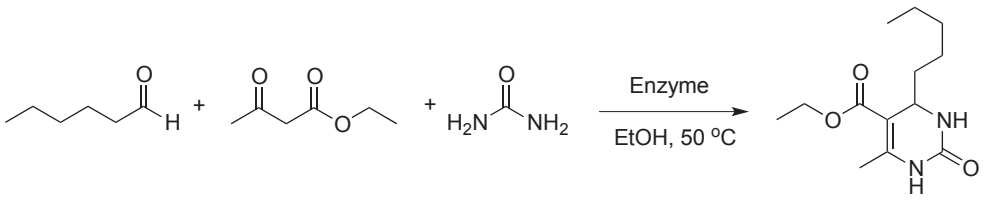
Herein, a series of DHPMs were synthesized via the α-chymotrypsin-catalyzed condensation reaction between aliphatic aldehydes, dicarbonyl compounds, and urea or thiourea in ethanol (Scheme 1). The method is suitable for a wide range of substrates, especially aliphatic aldehydes, is environmentally friendly and produces a high yield in comparison to the classical Biginelli procedure.




**Scheme 1.** Preparation of 3,4-dihydropyrimidin-2-(1*H*)-ones via Biginelli reaction

The catalytic activities of various enzymes were explored using 0.1 mmol *n*-hexanal, 0.15 mmol ethyl acetoacetate, and 0.15 mmol urea as a model reaction (Table 1). Trial reactions were conducted in order to optimize the yield of the DHPMs. Eleven commercially available enzymes were investigated. In the absence of enzyme, none of the target product was detectable after 108 h. In contrast, the reaction was distinctly accelerated in the presence of some of the enzymes. Different enzymes exhibited large differences in catalytic activity. For example, neither protease from *Bacillus licheniformis*, albumin from bovine serum, nor papain from papaya latex showed any detectable catalytic activity (Table 1, entries 2, 3, and 4). Other hydrolases did show catalytic activity. Among them, pancreatin from porcine pancreas and lipase B from *Candida antarctica* only provided low yields (Table 1, entries 5 and 6). Lipase from porcine pancreas and trypsin from porcine pancreas displayed moderate activity under the reaction conditions, and a 51-74% yield of the 3,4-dihydropyrimidin-2-(1*H*)-one could be isolated (Table 1, entries 7 and 8). However, Amano lipase from *Pseudomonas fluorescens*, Amano Lipase M from *Mucor javanicus*, and Amano Lipase A from *Aspergillus niger* displayed almost no activity and just trace target products were observed (Table 1, entries 9, 10, and 11). Fortunately, α-chymotrypsin showed significant catalytic activity, and the final yield of the desired 3,4-dihydropyrimidin-2-(1*H*)-one product was 91% after 108 h (Table 1, entry 12). In order to verify the necessity of the enzyme, some control experiments were performed. When α-chymotrypsin was deactivated, only a trace amount of the target product was detected, which suggests that the specific structure of the enzyme was necessary to carry out this biocatalytic reaction (Table 1, entry 13).

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




| Entry | Catalyst | Temperature (°C)[b] | Yield (%)[c] |
|---|---|---|---|
| 1 | None | - | trace |
| 2 | protease from *Bacillus licheniformis* | 37 | 0 |
| 3 | albumin from bovine serum | 39 | 0 |
| 4 | papain from papaya latex | 55 | 0 |
| 5 | pancreatin from porcine pancreas | 38 | 29 |
| 6 | lipase B from *Candida antarctica* | 37 | 31 |
| 7 | trypsin from porcine pancreas | 38.5 | 51 |
| 8 | lipase from porcine pancreas | 38 | 74 |
| 9 | Amano lipase from *Pseudomonas fluorescens* | 30 | < 5 |
| 10 | Amano Lipase M from *Mucor javanicus* | 40 | < 5 |
| 11 | Amano Lipase A from *Aspergillus niger* | 28 | < 5 |
| **12** | **α-chymotrypsin** | **38.5** | **91** |
| 13 | denatured α-chymotrypsin[d] | - | trace |

[a]Reaction conditions: *n*-hexanal (0.1 mmol), ethyl acetoacetate (0.15 mmol), urea (0.15 mmol), enzyme (20 mg), EtOH (2 mL), 50 °C, 108 h. [b]Optimum temperature of the enzyme. [c]Yields of pure products isolated by chromatography. [d]Pretreated with 8 M urea at 100 °C for 8 h.

The reaction medium and reaction temperature are considered to play important roles in enzyme-catalyzed reactions,[34-36] due to their effects on the rate of the reaction and the stability of the enzyme. Thus, organic solvents were examined, and it was found that the Biginelli multicomponent reaction could proceed in most common polar solvents, such as MeOH, EtOH, DMSO, MeCN, THF, and acetone (Table 2). At the same time, this reaction has good results in high polarity solvents. Among them, EtOH was the most suitable solvent, resulting in yields up to 91% (Table 2, entries 5 and 6). In solvents such as DMF, dioxane, and acetone, only a small amount of product was detected (Table 2, entries 12, 15, and 17). Since water can also affect the enzymatic reaction, water was used as the reaction medium, but the results were poor (Table 2, entry 8). Furthermore, when a small amount of water was added into EtOH, the yield decreased from 91 to 17% (Table 2, entries 5 and 18). Therefore, EtOH was chosen as the optimal solvent for the α-chymotrypsin-catalyzed Biginelli reaction. It was also found that the yield decreased significantly with a decrease in temperature (Table 2, entries 2-5). Only a small amount of product was detected at 30 °C (Table 2, entry 2), because the enzyme has lower activity in this environment. The yield also declined when the temperature reached 55 °C (Table 2, entries 6 and 7),

because the inactivation of enzyme at high temperature for a long time. Therefore, 50 °C was chosen as the optimal temperature.

**Table 2.** Effects of different solvents and temperatures on the model reaction[a]

| Entry | Solvent | Temperature (°C) | Yield (%)[b] |
|---|---|---|---|
| 1 | MeOH | 50 | 84 |
| 2 | EtOH | 30 | trace |
| 3 | EtOH | 37 | 13 |
| 4 | EtOH | 45 | 51 |
| **5** | **EtOH** | **50** | **91** |
| 6 | EtOH | 55 | 87 |
| 7 | EtOH | 60 | 61 |
| 8 | $H_2O$ | 50 | 0 |
| 9 | THF | 50 | 79 |
| 10 | $CH_2Cl_2$ | 50 | 0 |
| 11 | $CHCl_3$ | 50 | 0 |
| 12 | DMF | 50 | 34 |
| 13 | MeCN | 50 | 62 |
| 14 | *n*-hexane | 50 | 0 |
| 15 | dioxane | 50 | 23 |
| 16 | DMSO | 50 | 71 |
| 17 | acetone | 50 | 24 |
| 18 | EtOH + $H_2O$ (0.2 mL) | 50 | 17 |

[a]Reaction conditions: *n*-hexanal (0.1 mmol), ethyl acetoacetate (0.15 mmol), urea (0.15 mmol), α-chymotrypsin (20 mg), solvent (2 mL), 108 h. [b]Yield of pure products isolated by chromatography.

The effects of enzyme loading were also investigated, and the results showed that 10 mg/mL of enzyme loading was the optimal quantity for the model reaction between 0.1 mmol *n*-hexanal, 0.15 mmol ethyl acetoacetate and 0.15 mmol urea under employed conditions. Higher enzyme loading did not result in the increased yield, so 20 mg/mL was choosen as the optimal enzyme loading.

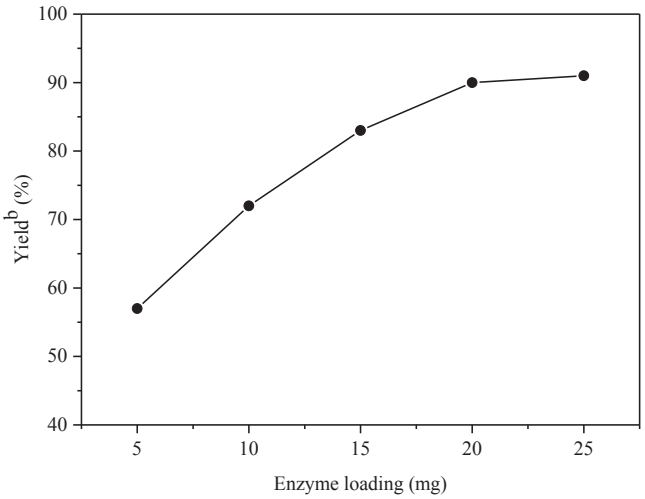



**Figure 1.** Effect of enzyme loading on the yield of reaction[a]

[a]All reactions were carried out using 0.1 mmol *n*-hexanal, 0.15 mmol ethyl acetoacetate and 0.15 mmol urea, EtOH (2 mL) and specified amount of α-chymotrypsin at 50 °C for 108 h. [b]Yields of pure products isolated by chromatography.

To optimize the experimental conditions, the effects of molar ratio of substrates were also examined (Table 3). It was found that the yields could be evidently affected by the molar ratio of substrates. When the amount of *n*-hexanal is increased, the yield is lowered. Conversely, the yield could reach a maximum when the amount of ethyl acetoacetate and urea is increased. Thus, the molar ratio of 1:1.5:1.5 (*n*-hexanal:ethyl acetoacetate:urea) was chosen as the optimal molar ratio (Table 3, entry 7).

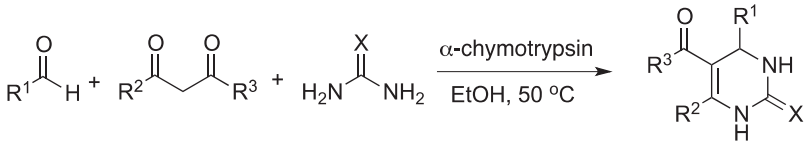
**Table 3.** Effect of molar ratio of substrates on the yield of reaction[a]

| Entry | *N*-Hexanal (mmol) | Ethyl Acetoacetate (mmol) | Urea (mmol) | Yield[b]/% |
|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 51 |
| 2 | 1.5 | 1 | 1 | 42 |
| 3 | 2 | 1 | 1 | 35 |
| 4 | 1 | 1.5 | 1 | 87 |
| 5 | 1 | 2 | 1 | 87 |
| 6 | 1 | 1 | 1.5 | 85 |
| 7 | 1 | 1.5 | 1.5 | 91 |
| 8 | 1 | 2 | 2 | 84 |

[a]All reactions were carried out using α-chymotrypsin (20 mg), EtOH (2 mL) and specified amounts of *N*-hexanal, ethyl acetoacetate and urea at 50 °C for 108 h. [b]Yields of pure products isolated by chromatograph.

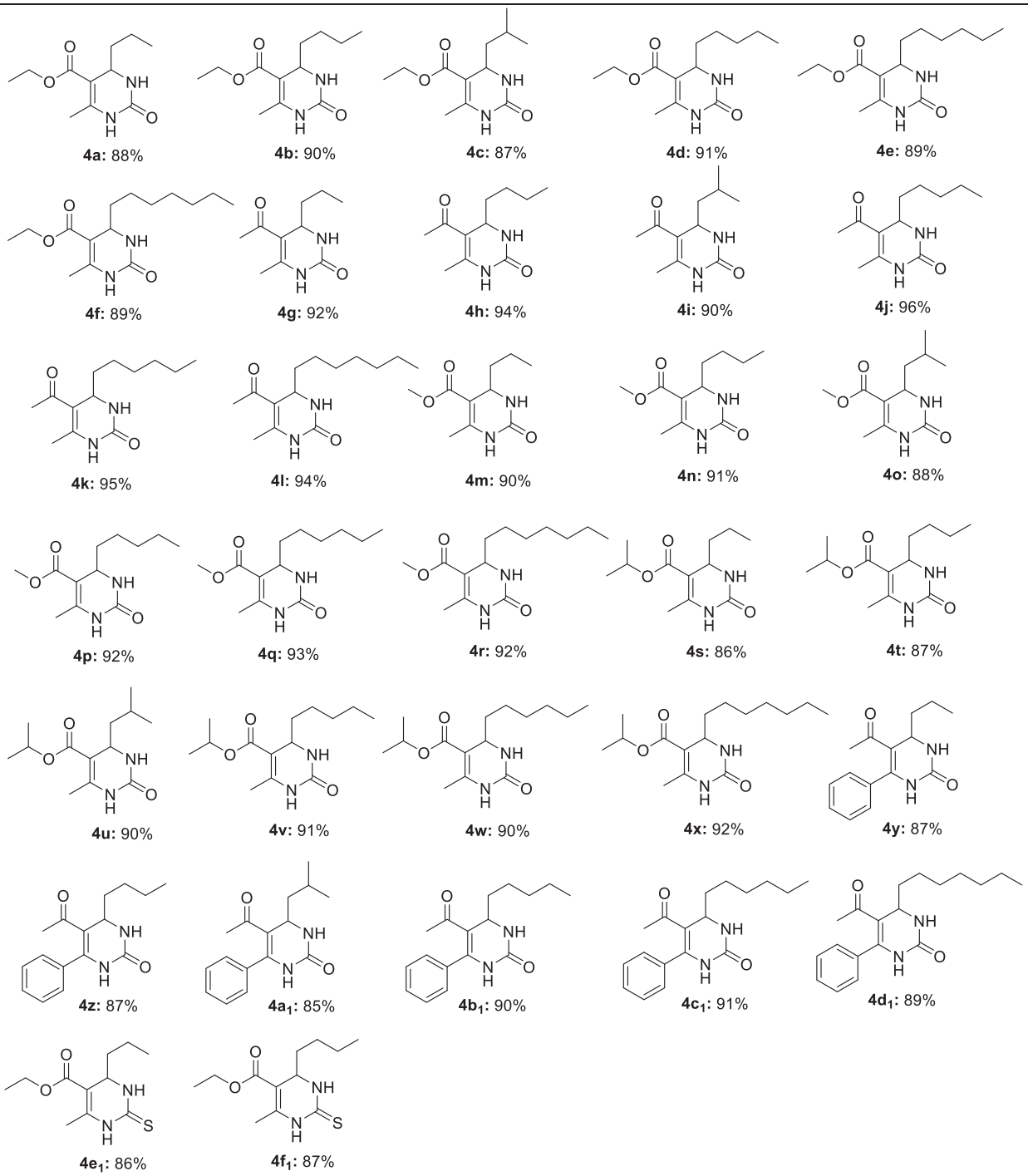
We applied the optimal conditions to other substrates and the results are shown in Table 4. A series of 3,4-dihydropyrimidin-2-(1*H*)-ones were synthesized via the α-chymotrypsin-catalyzed condensation reaction between an aliphatic aldehyde, dicarbonyl compound, and urea at 50 °C in ethanol (Table 4).

**Table 4.** α-Chymotrypsin catalyzed synthesis of 3,4-dihydropyrimidin-2-(1*H*)-ones[a]




**1(a-f)** **2(a-d)** **3(a-b)** **4(a-x): yield[b]**

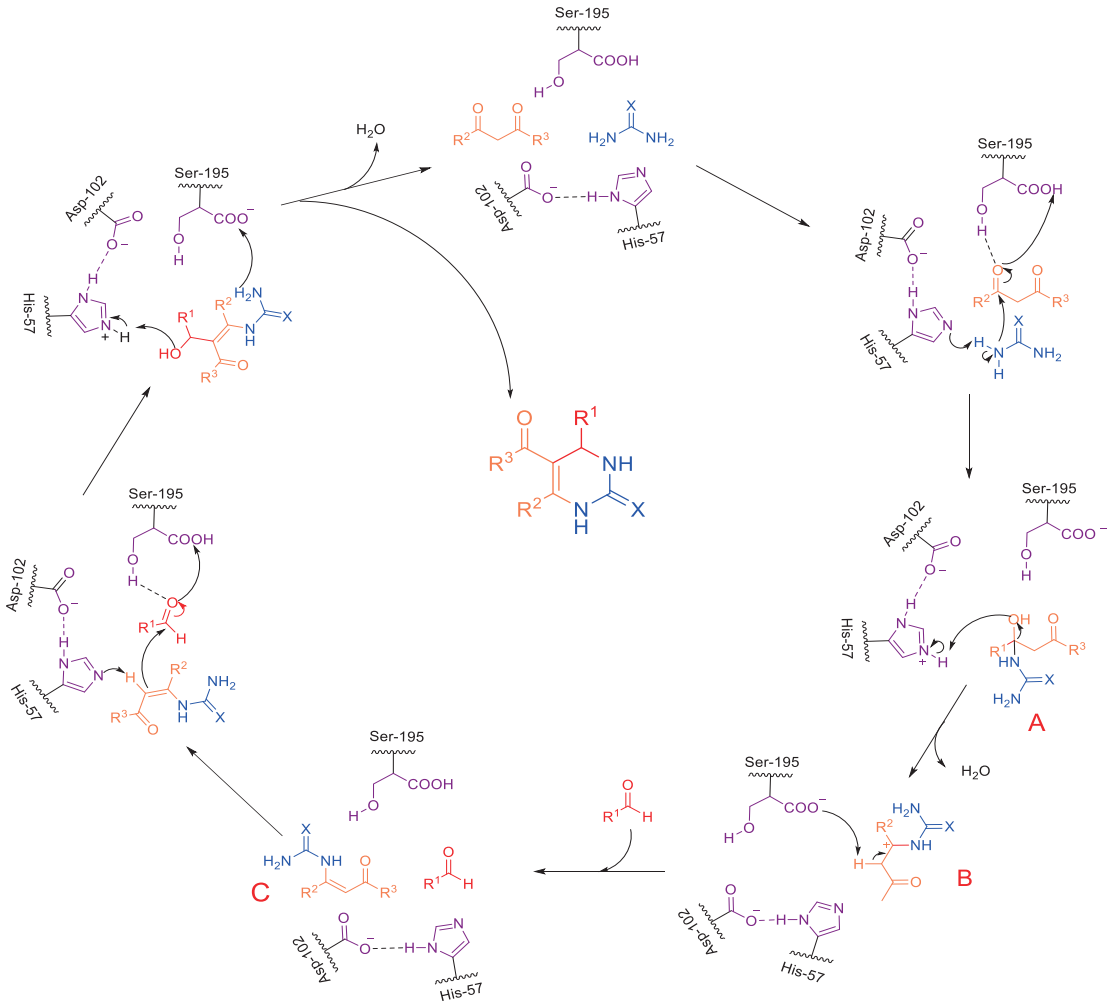
$R^1$= -$(CH_2)_2$Me, -$(CH_2)_3$Me, -$CH_2CH(Me)_2$, -$(CH_2)_4$Me, -$(CH_2)_5$Me, -$(CH_2)_6$Me;
$R^2$= -Me, -phenyl; $R^3$= -Me, -OMe, -$OCH_2$Me, -$OCH(Me)_2$; X= O, S




[a]Reaction conditions: aliphatic aldehydes (0.1 mmol), dicarbonyl compounds (0.15 mmol), urea or thiourea (0.15 mmol), α-chymotrypsin (20 mg), EtOH (2 mL), 108 h. [b]Yields of pure products isolated by chromatography.

A variety of aliphatic aldehydes, dicarbonyl compounds and urea or thiourea were compatible with the reaction conditions, and moderate to excellent yields were obtained (Table 4). In addition, benzoylacetone was also used as a substrate for this reaction, satisfactorily, corresponding 3,4-dihydropyrimidin-2(1*H*)-ones were obtained with 85-91% yields (Table 4, 4y-4d$_1$). Interestingly, when urea was replaced by thiourea, the similar results were obtained, which means that the substitution of oxygen by sulpfur in urea has no significant effect on the yield (Table 4, 4a-4b, 4e$_1$-4f$_1$). Furthermore, isobutyl acetoacetate also afforded the desired products in high yields (Table 4, 4s-4x).

Being part of the family of serine proteases, α-chymotrypsin is a polypeptide chain consisting of 245 amino acids and His-57, Asp-102 and Ser-195 constitute catalytic triad.[37-39] Combined with the information of this experiment, a possible mechanism of α-chymotrypsin-catalyzed Biginelli reaction was




**Scheme 2.** Probable mechanism of α-chymotrypsin catalyzed Biginelli reaction

proposed (Scheme 2). Initially, nucleophilic addition was taken place to form the intermediate imino ester, in which the proton from the urea maybe abstracted by His-57 and the carbonyl of ketone be effectively activated by Ser-195.[40,41] Then, intermediate **A** transformed into intermediate **B** by dehydration, and in

the presence of a His-57, intermediate **C** was formed from **B**. Last but not least, the target product was obtained through the similar catalytic mechanism.

In conclusion, this new activity of α-chymotrypsin not only provides a novel method for the synthesis of dihydropyrimidinones, but also provides an example of the versatility of enzymes as catalysts by broadening the application of α-chymotrypsin in organic synthesis. In addition, this method complies with the concepts of green chemistry, including mild reaction conditions and simple operation.

## EXPERIMENTAL

**General procedure for α-chymotrypsin-catalyzed synthesis of 3,4-dihydropyrimidin-2-(1*H*)-ones:** A mixture of aliphatic aldehyde **1** (0.1 mmol), β-keto ester **2** (0.15 mmol), urea or thiourea **3** (0.15 mmol), and α-chymotrypsin (20 mg) in 2 mL EtOH was incubated at 50 °C for 108 h. After completion of the reaction as indicated by TLC, the mixture was concentrated under reduced pressure to afford the crude product, which was then purified by column chromatography on silica gel (petroleum ether:EtOAc = 1:2) to give the pure product **4**. See Supporting Information for full experimental details.

**4a:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.95 (s, 1H), 7.35 (s, 1H), 4.52 – 3.71 (m, 3H), 2.16 (s, 3H), 1.44 – 1.25 (m, 4H), 1.19 (t, $J$ = 7.1 Hz, 3H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (126 MHz, DMSO-$d_6$): δ 165.92, 153.27, 148.77, 99.86, 59.52, 50.25, 18.17, 17.47, 14.69, 14.23.

**4b:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.95 (s, 1H), 7.34 (s, 1H), 4.12 – 3.98 (m, 3H), 2.15 (s, 3H), 1.46 – 1.20 (m, 6H), 1.18 (t, $J$ = 7.0 Hz, 3H), 0.84 (t, $J$ = 6.1 Hz, 3H); $^{13}$C NMR (126 MHz, DMSO-$d_6$): δ 165.48, 152.81, 148.35, 99.39, 59.07, 49.99, 36.52, 25.91, 21.95, 17.82, 14.26, 14.01.

**4c:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.98 (s, 1H), 7.45 (s, 1H), 4.10 – 3.98 (m, 3H), 2.15 (s, 3H), 1.74 – 1.64 (m, 1H), 1.36 (ddd, $J$ = 13.3, 9.7, 3.8 Hz, 1H), 1.18 (t, $J$ = 7.1 Hz, 3H), 1.08 (ddd, $J$ = 13.1, 9.9, 3.1 Hz, 1H), 0.85 (d, $J$ = 6.5 Hz, 6H); $^{13}$C NMR (126 MHz, DMSO-$d_6$): δ 165.32, 152.77, 148.28, 100.39, 59.09, 48.07, 46.04, 23.74, 22.84, 21.38, 17.66, 14.21.

**4d:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.95 (s, 1H), 7.35 (s, 1H), 4.13 – 3.93 (m, 3H), 2.15 (s, 3H), 1.43 – 1.32 (m, 2H), 1.31 – 1.21 (m, 6H), 1.18 (t, $J$ = 7.1 Hz, 3H), 0.84 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165.48, 152.80, 148.31, 99.41, 59.07, 50.03, 36.63, 31.01, 23.38, 22.12, 17.73, 14.29, 13.92.

**4e:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.94 (s, 1H), 7.33 (s, 1H), 4.06 (dtd, $J$ = 21.9, 7.1, 3.7 Hz, 3H), 2.15 (s, 3H), 1.46 – 1.33 (m, 2H), 1.26 (dd, $J$ = 48.8, 9.4 Hz, 7H), 1.18 (t, $J$ = 7.1 Hz, 3H), 0.85 (t, $J$ = 6.6 Hz, 3H); $^{13}$C NMR (126 MHz, DMSO-$d_6$): δ 165.45, 152.80, 148.34, 99.40, 59.06, 50.10, 36.68, 31.26, 28.47, 23.67, 22.07, 17.73, 14.25, 14.00.

**4f:** White solid, $^1$H NMR (600 MHz, DMSO-$d_6$): δ 8.93 (s, 1H), 7.30 (s, 1H), 4.13 – 3.87 (m, 1H), 3.59 (s,

3H), 2.15 (s, 3H), 1.42 – 1.33 (m, 2H), 1.32 – 1.20 (m, 12H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 165.92, 152.70, 148.42, 99.19, 50.68, 50.07, 36.66, 31.19, 28.76, 28.65, 23.62, 22.05, 17.71, 13.89.

**4g:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.96 (s, 1H), 7.44 (s, 1H), 4.10 (dd, $J$ = 7.4, 3.5 Hz, 1H), 2.19 (s, 3H), 2.17 (s, 3H), 1.49 – 1.28 (m, 2H), 1.24 (ddd, $J$ = 19.3, 9.9, 4.3 Hz, 2H), 0.85 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (126 MHz, DMSO-$d_6$): δ 194.57, 153.32, 147.91, 111.13, 50.47, 39.34, 30.69, 19.33, 17.68, 14.24.

**4h:** Yellow solid, $^1$H NMR (600 MHz, DMSO-$d_6$): δ 8.94 (s, 1H), 7.40 (s, 1H), 4.09 (dd, $J$ = 7.5, 3.5 Hz, 1H), 2.18 (s, 3H), 2.17 (s, 3H), 1.32 – 1.13 (m, 6H), 0.84 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 194.07, 159.49, 147.26, 110.68, 50.23, 36.25, 30.04, 26.04, 21.92, 18.72, 13.86.

**4i:** Yellow solid, $^1$H NMR (600 MHz, DMSO-$d_6$): δ 8.93 (s, 1H), 7.47 (s, 1H), 4.13 (dt, $J$ = 9.9, 3.3 Hz, 1H), 2.18 (s, 3H), 2.17 (s, 3H), 1.78 – 1.64 (m, 1H), 1.38 (ddd, $J$ = 13.6, 10.1, 3.7 Hz, 1H), 1.00 (ddd, $J$ = 13.2, 10.0, 3.0 Hz, 1H), 0.87 (dd, $J$ = 17.7, 6.6 Hz, 6H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 193.78, 152.71, 147.35, 111.45, 48.25, 45.42, 30.06, 23.75, 22.82, 21.20, 18.83.

**4j:** Yellow solid, $^1$H NMR (600 MHz, DMSO-$d_6$): δ 8.90 (s, 1H), 7.38 (s, 1H), 4.09 (dt, $J$ = 6.8, 3.2 Hz, 1H), 2.18 (s, 3H), 2.17 (s, 3H), 1.42 – 1.30 (m, 2H), 1.28 – 1.14 (m, 6H), 0.85 (t, $J$ = 7.1 Hz, 3H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 194.08, 152.76, 147.31, 110.70, 50.26, 36.53, 31.04, 30.15, 23.56, 22.06, 18.81, 13.84.

**4k:** Yellow solid, $^1$H NMR (600 MHz, DMSO-$d_6$): δ 8.90 (s, 1H), 7.37 (s, 1H), 4.19 – 3.99 (m, 1H), 2.18 (s, 3H), 2.17 (s, 3H), 1.42 – 1.28 (m, 2H), 1.30 – 1.17 (m, 8H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 194.07, 152.75, 147.31, 110.69, 50.26, 36.59, 31.26, 30.15, 28.50, 23.86, 21.99, 18.81, 13.91.

**4l:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.95 (s, 1H), 7.43 (s, 1H), 4.09 (d, $J$ = 3.6 Hz, 1H), 2.19 (s, 3H), 2.17 (s, 3H), 1.42 – 1.12 (m, 12H), 0.85 (t, $J$ = 6.5 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 194.57, 153.32, 147.88, 111.16, 50.72, 37.06, 31.72, 30.66, 29.32, 29.21, 24.42, 22.57, 19.33, 14.42.

**4m:** White solid, $^1$H NMR (600 MHz, DMSO-$d_6$): δ 8.94 (s, 1H), 7.31 (s, 1H), 4.04 (dt, $J$ = 6.8, 3.2 Hz, 1H), 3.60 (s, 3H), 2.15 (s, 3H), 1.43 – 1.32 (m, 2H), 1.32 – 1.16 (m, 2H), 0.84 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 165.92, 152.73, 148.44, 99.20, 50.69, 49.82, 39.05, 17.71, 16.94, 13.75.

**4n:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.98 (s, 1H), 7.35 (s, 1H), 4.09 – 3.91 (m, 1H), 3.59 (s, 3H), 2.15 (s, 3H), 1.43 – 1.32 (m, 2H), 1.28 – 1.10 (m, 4H), 0.84 (t, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (12 MHz, DMSO-$d_6$): δ 165.98, 152.78, 148.58, 99.18, 50.78, 50.11, 36.46, 25.90, 22.02, 17.79, 14.02.

**4o:** White solid, $^1$H NMR (600 MHz, DMSO-$d_6$): δ 8.95 (s, 1H), 7.40 (s, 1H), 4.27 – 3.87 (m, 1H), 3.60 (s, 3H), 2.15 (s, 3H), 1.75 – 1.61 (m, 1H), 1.37 (ddd, $J$ = 13.5, 9.4, 4.2 Hz, 1H), 1.09 (ddd, $J$ = 13.2, 9.5, 3.5 Hz, 1H), 0.85 (d, $J$ = 6.4 Hz, 6H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 165.80, 152.74, 148.27, 100.20,

50.68, 48.19, 45.91, 23.59, 22.84, 21.42, 17.69.

**4p:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.98 (s, 1H), 7.35 (s, 1H), 4.02 (dd, $J$ = 6.7, 3.5 Hz, 1H), 3.59 (s, 3H), 2.15 (s, 3H), 1.35 (ddd, $J$ = 18.2, 10.8, 6.9 Hz, 2H), 1.29 – 1.14 (m, 6H), 0.84 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165.97, 152.77, 148.53, 99.20, 50.78, 50.11, 36.70, 31.07, 23.37, 22.12, 17.79, 14.04.

**4q:** White solid, $^1$H NMR (600 MHz, DMSO-$d_6$): δ 8.93 (s, 1H), 7.30 (s, 1H), 4.27 – 3.93 (m, 1H), 3.59 (s, 3H), 2.04 (s, 3H), 1.45 – 1.32 (m, 2H), 1.30 – 1.15 (m, 8H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 165.92, 152.68, 148.44, 99.20, 50.68, 50.08, 36.69, 31.23, 28.47, 23.60, 22.00, 17.68, 13.92.

**4r:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.96 (s, 1H), 7.33 (s, 1H), 4.19 – 3.80 (m, 1H), 3.59 (s, 3H), 2.15 (s, 3H), 1.37 (dd, $J$ = 7.1, 4.8 Hz, 2H), 1.24 (d, $J$ = 11.1 Hz, 10H), 0.85 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 166.40, 153.22, 148.95, 99.65, 51.18, 50.55, 37.16, 31.70, 29.26, 29.14, 24.12, 22.56, 18.21, 14.43.

**4s:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.92 (s, 1H), 7.34 (s, 1H), 4.91 (dt, $J$ = 12.4, 6.2 Hz, 1H), 4.08 – 3.97 (m, 1H), 2.16 (s, 3H), 1.47 – 1.22 (m, 4H), 1.18 (dd, $J$ = 6.0, 4.4 Hz, 6H), 0.85 (t, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165.39, 153.30, 148.55, 100.16, 66.53, 50.15, 39.54, 22.29, 22.16, 18.11, 17.49, 14.20.

**4t:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.92 (s, 1H), 7.33 (s, 1H), 4.91 (dt, $J$ = 12.4, 6.2 Hz, 1H), 4.25 – 3.81 (m, 1H), 2.16 (s, 3H), 1.44 – 1.21 (m, 6H), 1.20 – 1.15 (m, 6H), 0.85 (t, $J$ = 5.9 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165.39, 153.29, 148.55, 100.13, 66.50, 50.42, 36.88, 26.42, 22.35, 22.28, 22.15, 18.11, 14.41.

**4u:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.95 (s, 1H), 7.44 (s, 1H), 5.33 – 4.66 (m, 1H), 4.04 (dt, $J$ = 9.5, 3.4 Hz, 1H), 2.16 (s, 3H), 1.77 – 1.64 (m, 1H), 1.37 (ddd, $J$ = 13.4, 9.7, 3.8 Hz, 1H), 1.18 (dd, $J$ = 6.1, 4.4 Hz, 6H), 1.07 (ddd, $J$ = 14.5, 8.8, 3.8 Hz, 1H), 0.86 (dd, $J$ = 6.5, 2.4 Hz, 6H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165.19, 153.25, 148.56, 100.97, 66.48, 48.58, 46.51, 24.25, 23.25, 22.29, 22.20, 21.79, 18.03.

**4v:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.92 (s, 1H), 7.33 (s, 1H), 4.91 (dt, $J$ = 12.5, 6.2 Hz, 1H), 4.03 – 3.87 (m, 1H), 2.16 (s, 3H), 1.42 – 1.34 (m, 2H), 1.31 – 1.23 (m, 6H), 1.17 (dd, $J$ = 11.6, 6.3 Hz, 6H), 0.85 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165.39, 153.28, 148.54, 100.15, 66.49, 50.45, 37.12, 31.43, 23.85, 22.48, 22.28, 22.15, 18.10, 14.32.

**4w:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.92 (s, 1H), 7.33 (s, 1H), 5.05 – 4.74 (m, 1H), 4.02 (s, 1H), 2.16 (s, 3H), 1.38 (s, 2H), 1.23 (s, 8H), 1.20 – 1.15 (m, 6H), 0.85 (t, $J$ = 6.2 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165.38, 153.28, 148.54, 100.14, 66.49, 50.45, 37.16, 31.66, 28.88, 24.13, 22.49, 22.28, 22.14, 18.10, 14.41.

**4x:** White solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.91 (s, 1H), 7.32 (s, 1H), 4.91 (dt, $J$ = 12.4, 6.2 Hz, 1H), 4.16 – 3.88 (m, 1H), 2.15 (s, 3H), 1.45 – 1.33 (m, 2H), 1.23 (s, 10H), 1.18 (dd, $J$ = 10.5, 4.6 Hz, 6H), 0.85 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 165.38, 153.27, 148.55, 100.14, 66.49, 50.46, 37.15, 31.67, 29.17, 29.08, 24.16, 22.57, 22.28, 22.14, 18.10, 14.42.

**4y:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 9.00 (s, 1H), 7.57 – 7.52 (m, 3H), 7.50 – 7.42 (m, 3H), 4.19 – 4.14 (m, 1H), 1.63 (s, 3H), 1.43 – 1.35 (m, 2H), 1.31 – 1.20 (m, 2H), 0.80 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 194.94, 153.26, 146.20, 141.57, 131.82, 129.06, 128.26, 110.22, 51.75, 18.84, 17.66, 14.23.

**4z:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.98 (s, 1H), 7.56 – 7.51 (m, 3H), 7.45 (dd, $J$ = 20.0, 12.4 Hz, 3H), 4.15 (dd, $J$ = 9.0, 5.5 Hz, 1H), 1.63 (s, 3H), 1.39 (dd, $J$ = 14.1, 7.8 Hz, 2H), 1.22 (m, 4H), 0.82 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 194.57, 152.89, 145.80, 141.17, 131.45, 128.68, 127.89, 109.83, 51.60, 36.89, 23.89, 22.11, 18.46, 14.02.

**4a$_1$:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 9.02 (s, 1H), 7.54 (dd, $J$ = 10.5, 4.3 Hz, 4H), 7.47 (dd, $J$ = 9.5, 5.4 Hz, 2H), 4.19 – 4.14 (m, 1H), 1.65 (s, 3H), 1.47 – 1.33 (m, 1H), 1.22 – 1.12 (m, 2H), 0.76 (dd, $J$ = 9.9, 6.6 Hz, 6H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 194.76, 153.24, 146.34, 141.56, 131.75, 129.03, 128.21, 110.98, 49.97, 46.61, 23.79, 23.38, 22.01, 18.85.

**4b$_1$:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.97 (s, 1H), 7.63 – 7.49 (m, 3H), 7.44 (dd, $J$ = 20.0, 12.4 Hz, 3H), 4.14 (dd, $J$ = 9.0, 5.5 Hz, 1H), 1.62 (s, 3H), 1.38 (dd, $J$ = 14.1, 7.8 Hz, 2H), 1.20 (m, 6H), 0.81 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 194.86, 153.18, 146.09, 141.46, 131.74, 128.97, 128.18, 110.12, 51.89, 37.18, 31.59, 24.18, 22.40, 18.75, 14.31.

**4c$_1$:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.99 (s, 1H), 7.57 – 7.52 (m, 3H), 7.46 (dd, $J$ = 20.0, 12.4 Hz, 3H), 4.16 (m, 1H), 1.64 (s, 3H), 1.40 (dd, $J$ = 14.1, 7.8 Hz, 2H), 1.22 – 1.12 (m, 8H), 0.83 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 194.93, 153.25, 146.16, 141.54, 131.82, 129.04, 128.25, 110.19, 51.96, 37.25, 31.66, 28.91, 24.25, 22.47, 18.82, 14.38.

**4d$_1$:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 9.01 (s, 1H), 7.54 (dd, $J$ = 6.8, 3.5 Hz, 3H), 7.46 (dd, $J$ = 16.6, 8.9 Hz, 3H), 4.16 (dd, $J$ = 8.9, 5.4 Hz, 1H), 1.64 (s, 3H), 1.40 (dd, $J$ = 13.9, 7.6 Hz, 2H), 1.24 (s, 10H), 0.83 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 194.91, 153.27, 146.11, 141.53, 131.81, 129.02, 128.25, 110.19, 51.97, 37.24, 31.65, 29.20, 29.08, 24.28, 22.53, 18.81, 14.40.

**4e$_1$:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 9.65 (s, 1H), 8.1 (s, 1H), 4.51 – 3.72 (m, 3H), 2.16 (s, 3H), 1.46 – 1.23 (m, 4H), 1.19 (t, $J$ = 7.1 Hz, 3H), 0.85 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-d$_6$): δ 174.35, 162.66, 136.288, 99.86, 59.52, 50.25, 18.17, 17.47, 14.69, 14.23.

**4f$_1$:** Yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$): δ 9.75 (s, 1H), 8.23 (s, 1H), 4.11 – 3.88 (m, 3H), 2.15 (s, 3H), 1.46 – 1.21 (m, 6H), 1.18 (t, $J$ = 7.0 Hz, 3H), 0.84 (t, $J$ = 6.1 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ 177.38, 160.83, 136.40, 99.39, 59.07, 49.99, 36.52, 25.91, 21.95, 17.82, 14.26, 14.01.

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