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Communication

Continuous-Flow Synthesis of β-Amino Acid Esters by Lipase-Catalyzed Michael Addition of Aromatic Amines

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Abstract: A continuous-flow procedure for the synthesis of β-amino acid esters has been developed via lipase-catalyzed Michael reaction of various aromatic amines with acrylates. Lipase TL IM from *Thermomyces lanuginosus* was first used to catalyze Michael addition reaction of aromatic amines. Compared with other methods, the salient features of this work include green reaction conditions (methanol as reaction medium), short residence time (30 min), readily available catalyst and a reaction process that is easy to control. This enzymatic synthesis of β-amino acid esters performed in continuous-flow microreactors is an innovation that provides a new strategy for the fast biotransformation of β-amino acid esters.

Keywords: enzymatic synthesis; β-amino acid esters; microreactor; aromatic amines; Michael addition

1. Introduction

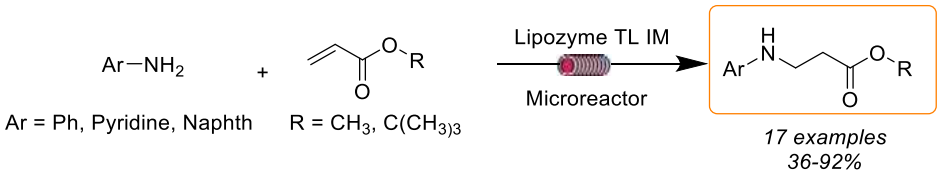
β-Amino acid ester derivatives have been widely found in biologically active molecules, many of which possess useful biological activities (e.g., anticancer, antiviral, antibacterial, antifungal, antipsychotics) [1–6]. β-Amino acid esters have also been used to construct peptidomimetic oligomers, which are of high interest in medicinal chemistry [7,8]. In particular, β-amino acid esters based on aromatic amines are important intermediates for the effective synthesis of urease inhibitors, which can be used in the treatment of *Helicobacter pylori* infection [9]. Due to these widespread applications in pharmacy and materials science, the development of synthesis methods of β-amino acid esters has become a hot topic in the chemical field.

Michael addition is one of the simplest and most effective strategies to prepare β-amino acid esters. Recently, Ahmed has reported the Michael addition of aromatic amines mediated by KOtBu [10]; HOTf (trifluoromethylsulfonic acid) was also used to catalyze the Michael addition of aromatic amines, and good yields were obtained within 4 h [11]. However, the use of acids and alkalis has brought challenges to the environment. In order to avoid such problems, various transition-metal catalysts have been developed and used to efficiently catalyze the Michael addition to synthesize β-amino acid esters [12–17]. Although desired results were obtained, the use of metal catalysts and expensive ligands are still shortcomings for the practical utility.

Enzymes are versatile catalysts of biological origin that can catalyze reactions with great specificity and high efficiency under the benign environmental conditions [18–20]. In the past few years, several works about the synthesis of β-amino esters by enzymatic Michael addition reaction were

reported [21–23]. However, there are few reports on the preparation of β-amino acid esters by the enzymatic Michael addition of aromatic amines. Bhanage reported that immobilized HMC:PFL (lipase from *Pseudomonas fluorescence* immobilized on hydroxypropyl methyl cellulose) could be used to catalyze the Michael addition of aromatic amines in toluene, although only 33%–40% yields could be obtained in 3 h [24]. Lipase CAL-B (lipase B from *Candida antarctica*) has also been used to catalyze the Michael addition of aromatic amines, but it requires a long reaction time (72 h) to achieve desired results [25]. Our attention is focused on finding a more efficient and green synthesis method of β-amino acid esters based on aromatic amines. In recent years, more and more reports have been published on enzymatic reactions in continuous-flow microreactors due to their advantages of improving process efficiency and material transfer [26–28]. The small size of the continuous-flow microreactors facilitates control of the reaction parameters, which can reduce waste generation and increase productivities [29–31].

In the interest of developing an efficient and green synthesis method for β-amino acid esters based on aromatic amines, we employed a continuous flow technology for the synthesis of β-amino acid esters. Lipase TL IM from *Thermomyces lanuginosus* was first used to catalyze the Michael addition reaction of aromatic amines with acrylates in continuous-flow microreactors, and 17 β-amino acid esters (3a–3r) were obtained with excellent yields in 30 min (Scheme 1). Effects of different reaction parameters including reaction solvent, temperature, enzyme, substrate ratio, residence time and aromatic amine structure on the reaction were studied.



Scheme 1. Synthesis of β-amino acid esters by enzymatic Michael reaction of aromatic amines in continuous-flow microreactors.

2. Results and Discussion

2.1. Effect of Reaction Media

The solvent plays an important role in enzymatic reaction by promoting the dissolution of the substrates and controlling the reaction chemo-selectivity [32]. Therefore, we first studied the effect of reaction media on β-amino acid ester synthesis in continuous-flow microreactors (Figure 1). We chose aniline (**1a**) and methyl acrylate (**2a**) as the model reaction; several solvents, including tert-amyl alcohol, acetonitrile, toluene, DCM (dichloromethane), ethanol and methanol, were tested. As shown in Figure 1, the reaction did not proceed in acetonitrile, and only 5.2% of the desired product was obtained in tert-amyl alcohol. Further solvent screening disclosed that methanol was the best solvent. Aromatic amines are a class of poor nucleophiles whose nucleophilicity is highly related to solvents. Research has shown that polar solvents can promote Michael addition of aromatic amines [33].

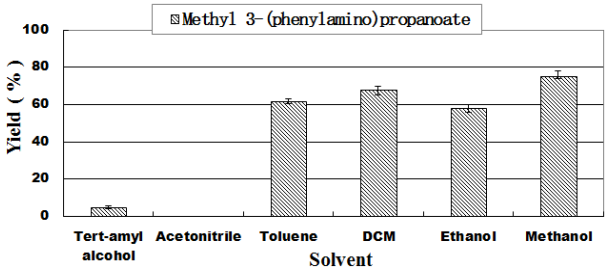


Figure 1. The effect of reaction media on enzymatic synthesis of β-amino acid esters in continuous-flow microreactors.

2.2. Effect of Reaction Temperature

Temperature plays significant functions, such as improving the interaction between the substrate and catalyst and affecting the stability of enzyme [34]. Therefore, we investigated the effect of reaction temperature on the synthesis of β-amino acid esters; the results are shown in Figure 2. We performed the reactions from 30 to 55 °C and found that the best yield (80.3%) can be achieved at 35 °C. After that, as the reaction temperature continued to increase, the yield was not improved. The higher temperature may cause a decrease in the selectivity of enzymatic reaction, which favors the double Michael product formation. Therefore, the optimal reaction temperature for the synthesis of β-amino acid esters is 35 °C.

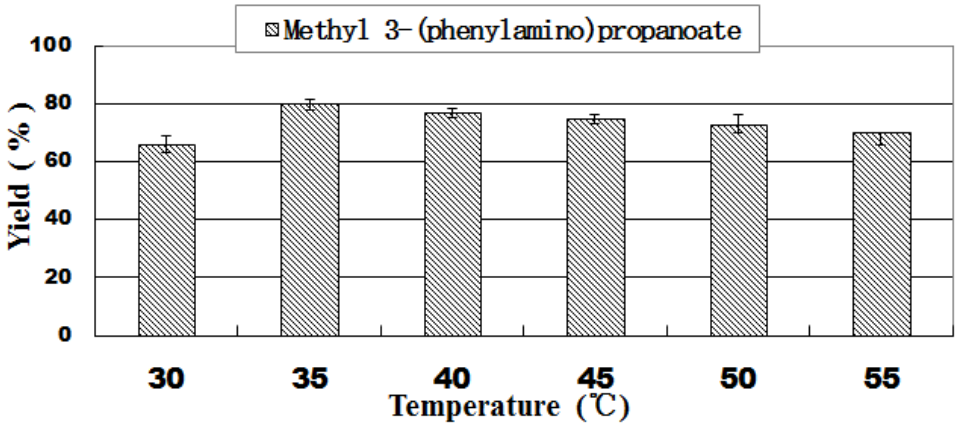


Figure 2. The effect of reaction temperature on enzymatic synthesis of β-amino acid esters in continuous-flow microreactors.

2.3. Catalyst Screening

We tested the efficiency of three enzymes in catalyzing β-amino acid ester synthesis (Figure 3). Further screening of enzymes showed that lipozyme TL IM was the best catalyst. In the absence of lipozyme TL IM, a group of blank tests was performed, and it was found that the reaction did not occur. This indicated that lipozyme TL IM played a key role in the synthesis of β-amino acid esters.

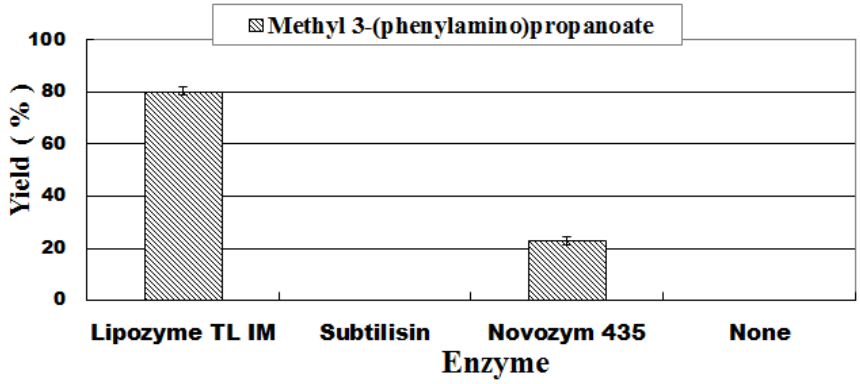


Figure 3. The catalyst screening on enzymatic synthesis of β-amino acid esters in continuous-flow microreactors.

2.4. Effect of Substrate Ratio

Another important factor that affects the synthesis of β-amino acid ester is the molar ratio of aniline to methyl acrylate. The influence of molar ratio (aniline/methyl acrylate) was investigated from 1:1 to 1:6. According to the Figure 4, the best reaction yield 80.3% was obtained when the molecular ratio of aniline (**1a**) and methyl acrylate (**2a**) reached 1:4. As the content of methyl acrylate continued to increase, the yield of target product **3a** was decreased. This occurred since a higher amount of

methyl acrylate favored the double Michael product, which reduced the yield of the target product **3a**. Therefore, we decided to choose aniline/methyl acrylate = 1:4 as the optimum substrate ratio.

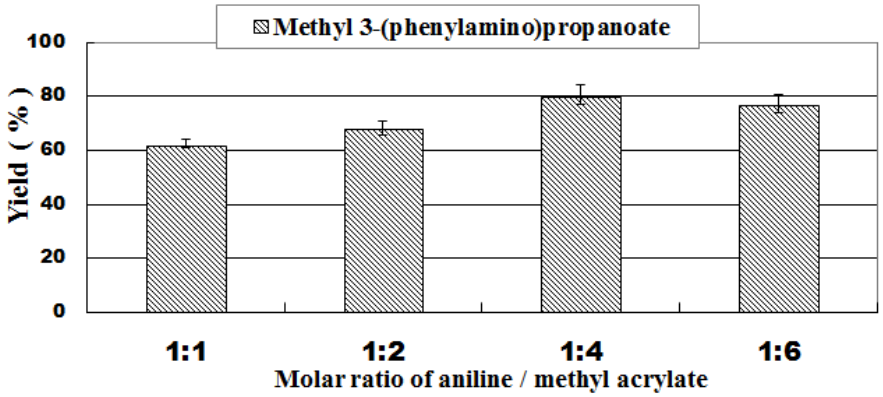


Figure 4. The effect of substrate ratio on enzymatic synthesis of β-amino acid esters in continuous-flow microreactors.

2.5. Effect of Residence Time

It is well known that prolonged reactions can promote the formation of impurities and reduce the selectivity of products. In addition, in the case of Michael reaction, the long-term reaction facilitates the accumulation of double Michael products [33]. In view of this, we performed the reaction from 20 to 40 min to investigate the effect of residence time/flow rate on the reaction; the results are shown in Figure 5. The best yield was reached in 30 min with a flow rate of 20.8 μL min^{-1}. As we continued to extend residence time, the yield of **3a** was decreased slightly. Detection found that with the increase of time, the target product underwent a second Michael addition reaction, which reduced the yield of the target product. Thus, we chose 30 min as the optimum residence time for the following experiment.

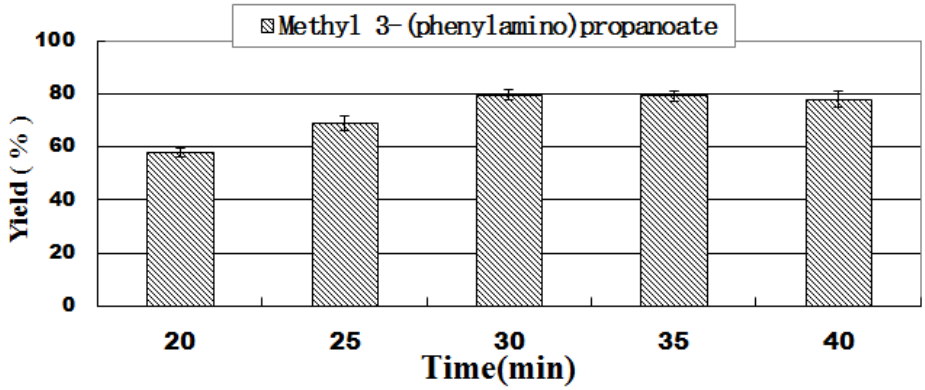


Figure 5. The effect of residence time on enzymatic synthesis of β-amino acid esters in continuous-flow microreactors.

2.6. The Effect of Aromatic Amine Structure on the Reaction

After identifying the optimum reaction conditions, we continued to investigate the effect of aromatic amine structure on the enzymatic β-amino acid ester synthesis reaction under continuous-flow microreactors. As shown in Figure 6, the reaction yields were 80.3%, 92.4%, 43.7% and 36.3% for aniline (**1a**), 4-toluidine (**1b**), 2-aminopyridine (**1g**) and 4-chloroaniline (**1e**), respectively. Experimental results showed that aromatic amines with electron-donating functional groups exhibited high reactivity in the enzymatic Michael addition reaction. The electron-donating groups increased the nucleophilicity of aniline, making the conjugated addition easier. In contrast, aromatic amines containing electron-withdrawing groups had lower yields in the enzymatic Michael addition reaction.

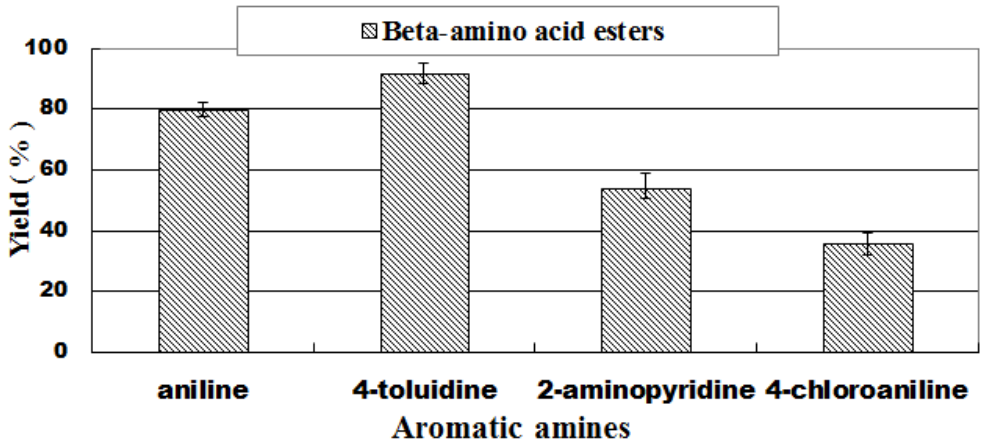
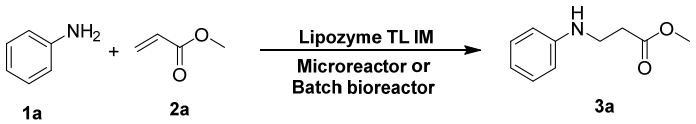


Figure 6. The effect of aromatic amine structure on enzymatic synthesis of β-amino acid esters in continuous-flow microreactors.

2.7. Enzymatic Synthesis of β-Amino Acid Ester in Continuous-Flow Microreactor and Batch Bioreactor

Due to high surface-to-volume ratio, better heat exchange and efficient mixing, continuous-flow microreactor technology has become increasingly popular as an alternative to conventional batch chemistry synthesis. In order to investigate the effects of enzymatic reactions in different reactors, we performed the enzymatic Michael addition of aniline to synthesize β-amino acid ester in the continuous-flow microreactor and in a batch bioreactor (Table 1). We detected the reaction process in the batch bioreactor and found that it took about 24 h to achieve the ideal yield (method B). However, in the continuous-flow microreactor, we could get better yield in 30 min (method A). Experiments showed that the continuous-flow microreactor can improve the efficiency of enzymatic synthesis of β-amino acid esters.

Table 1. Enzymatic Michael addition of aniline in the continuous-flow reactor or batch bioreactor. [a]



1a 2a 3a

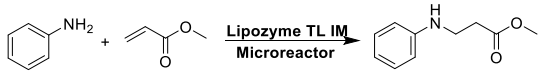
Entry	Method	Yield [b] (%)
1	A	80.3 ± 1.5
2	B	62.3 ± 2.5

[a] General reaction conditions: Method A: Continuous-flow microreactor, feed A. (5.0 mmol aniline in 10 mL methanol), feed B (20.0 mmol methyl acrylate in 10 mL methanol), residence time: 30 min, flow rate: 20.8 μL min−1, 35 °C, lipozyme TL IM 870 mg. Method B: Batch bioreactor, 5.0 mmol aniline, 20.0 mmol methyl acrylate and 20 mL methanol in a 50 mL conical flask, 200 r·min−1, lipozyme TL IM 870 mg, 35 °C, 24 h. [b] Yield of 3a. Yield: 100× (actual received quality/ideal calculated quality). The data are presented as average ± SD of triplicate experiments.

2.8. Combined Effects of Process Parameters

Lipase-catalyzed synthesis of β-amino acid ester by Michael addition between aniline and methyl acrylate in continuous-flow microreactors was studied, and the optimum conditions for the Michael addition were investigated. The results are shown in Tables 2 and 3.

Table 2. Solvent, temperature and enzyme screening for enzymatic synthesis of β-amino acid esters. [a]



1a 2a 3a

Entry	Catalyst	Solvent	T (°C)	Yield [b] (%)
1	Lipozyme TL IM	Tert-amyl alcohol	45	5.2 ± 0.7
2	Lipozyme TL IM	Acetonitrile	45	n.d.
3	Lipozyme TL IM	Toluene	45	62.0 ± 1.2
4	Lipozyme TL IM	DCM	45	68.3 ± 1.9
5	Lipozyme TL IM	Ethanol	45	58.2 ± 2.3
6	Lipozyme TL IM	Methanol	45	75.1 ± 0.9
7	Lipozyme TL IM	Methanol	30	66.5 ± 2.1
8	Lipozyme TL IM	Methanol	35	80.3 ± 1.5
9	Lipozyme TL IM	Methanol	40	77.4 ± 2.5
10	Lipozyme TL IM	Methanol	50	73.1 ± 2.6
11	Lipozyme TL IM	Methanol	55	70.0 ± 1.7
12	Subtilisin	Methanol	35	n.d.
13	Novozym 435	Methanol	35	23.2 ± 0.9
14	None	Methanol	35	n.d.

[a] Experimental conditions: feed A, 5.0 mmol aniline (**1a**) was dissolved in 10 mL solvent, feed B, 20.0 mmol methyl acrylate (**2a**) was dissolved in 10 mL solvent, enzyme 870 mg, 30 min. [b] Yield of **3a**. n.d. means no reaction was found. Yield: 100× (actual received quality/ideal calculated quality). The data are presented as average ± SD of triplicate experiments.

Table 3. Effects of substrate ratio and residence time on enzymatic synthesis of β-amino acid esters. [a]

Entry	n_{1a}:n_{2a}	Time/Min	Flow Rate/μL min^{-1}	Yield [b] (%)
1	1:1	30	20.8	62.6 ± 2.3
2	1:2	30	20.8	68.1 ± 1.6
3	1:4	30	20.8	80.3 ± 1.5
4	1:6	30	20.8	77.4 ± 2.8
5	1:4	20	31.2	58.7 ± 2.2
6	1:4	25	25.0	69.0 ± 0.9
7	1:4	35	17.8	80.1 ± 1.6
8	1:4	40	15.6	78.0 ± 1.2

[a] Experimental conditions: feed A, 5.0 mmol aniline (**1a**) was dissolved in 10 mL methanol, feed B, methyl acrylate (**2a**) was dissolved in 10 mL methanol, lipozyme TL IM 870 mg, 35 °C. [b] Yield of **3a**. Yield: 100× (actual received quality/ideal calculated quality). The data are presented as average ± SD of triplicate experiments.

For enzymatic reactions, the reaction medium and temperature are important influencing factors which affect the enzyme activity and process of the reaction. Preliminary experiments showed that the best reaction yield can be achieved when the reaction was catalyzed by lipozyme TL IM in methanol at 35 °C (Entry 8, Table 2). As we continued to increase the reaction temperature, the yield was not improved. Higher temperature may decrease the selectivity of enzymatic reactions, which increases the by-product formation. Residence time and substrate ratio are also important factors affecting the synthesis of β-amino acid esters. With the increase of residence time or methyl acrylate, the target product **3a** underwent a second Michael addition reaction, which reduced the yield of the target product.

2.9. The Scope and Limitation for the β-Amino Acid Ester Synthesis Methodology

Finally, we explored the scope and limitation of this continuous flow methodology for β-amino acid ester synthesis catalyzed by lipase TL IM from *Thermomyces lanuginosus*. Nine aromatic amines (aniline (**1a**), 4-toluidine (**1b**), 4-tert-butylaniline (**1c**), 4-methoxyaniline (**1d**), 4-chloroaniline (**1e**), 4-bromoaniline (**1f**), 2-aminopyridine (**1g**), 3,4-(methylenedioxy)aniline (**1h**) and 2-naphthylamine (**1i**))

and two acrylates (methyl acrylate (**2a**) and tert-butyl acrylate (**2b**)) were subjected to the optimal reaction conditions, using continuous-flow microreactors. Seventeen β-amino acid esters (**3a**–**3r**) were synthesized after 30 min residence time in excellent yields (Table 4).

Table 4. Continuous-flow synthesis of β-amino acid esters by enzymatic Michael addition of aromatic amines. [a]

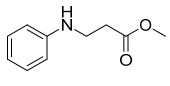
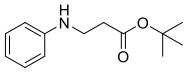
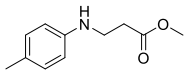
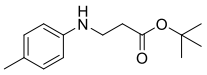
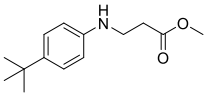
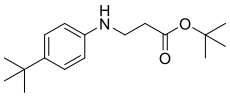
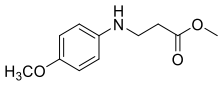
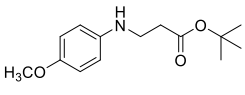
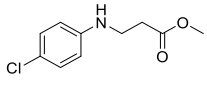
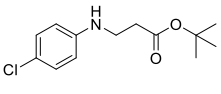
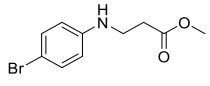
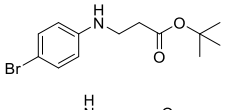
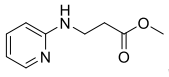
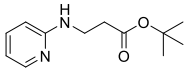
Entry	Aromatic Amine	Product	Yield [b] (%)
1	aniline (**1a**)	 **3a**	80.3 ± 1.5
2	aniline (**1a**)	 **3b**	75.1 ± 1.9
3	4-toluidine (**1b**)	 **3c**	92.4 ± 0.8
4	4-toluidine (**1b**)	 **3d**	81.2 ± 1.7
5	4-tert-butylaniline (**1c**)	 **3e**	83.6 ± 2.1
6	4-tert-butylaniline (**1c**)	 **3f**	87.7 ± 2.9
7	4-methoxyaniline (**1d**)	 **3g**	85.0 ± 2.7
8	4-methoxyaniline (**1d**)	 **3h**	82.1 ± 2.6
9	4-chloroaniline (**1e**)	 **3i**	36.3 ± 1.2
10	4-chloroaniline (**1e**)	 **3j**	48.5 ± 3.1
11	4-bromoaniline (**1f**)	 **3k**	42.4 ± 1.5
12	4-bromoaniline (**1f**)	 **3l**	47.3 ± 1.1
13	2-aminopyridine (**1g**)	 **3m**	43.7 ± 2.4
14	2-aminopyridine (**1g**)	 **3n**	trace

Table 4. *Cont.*

Entry	Aromatic Amine	Product	Yield [b] (%)
15	3,4-(methylenedioxy)aniline (**1h**)	**3o**	88.1 ± 2.2
16	3,4-(methylenedioxy)aniline (**1h**)	**3p**	85.4 ± 1.7
17	2-naphthylamine (**1i**)	**3q**	74.0 ± 1.4
18	2-naphthylamine (**1i**)	**3r**	72.1 ± 1.2

[a] Experimental conditions: feed A, 5.0 mmol aromatic amine was dissolved in 10 mL methanol, feed B, 20.0 mmol acrylic ester was dissolved in 10 mL methanol, lipozyme TL IM 870 mg, 30 min, 35 °C. [b] Yield of β-amino acid esters. Yield: 100× (actual received quality/ideal calculated quality). The data are presented as average ± SD of triplicate experiments.

The results showed that this continuous-flow enzymatic method has a broad applicability. Most aromatic amines showed good reactivity, except the halogen-substituted anilines and 2-aminopyridine (Entries 9–14, Table 4). The electronic effects of substrates have some impacts on additions. The introduction of electron-donating groups increased the nucleophilicity of aniline, making the conjugated addition easier, which was consistent with previous reports [25]. In addition, our studies have shown that Michael addition of aromatic amines catalyzed by lipase in continuous-flow microreactors was markedly influenced by the reaction parameters. The lipozyme TL IM catalyzed the Michael addition of aromatic amines with acrylates in methanol, which could effectively achieve the synthesis of β-amino acid esters. The selectivity of enzymatic reaction decreased with high reaction temperature or long residence time, which led to the increase of by-products. Compared with conventional batch bioreactor, continuous-flow microreactor improved the efficiency of enzymatic synthesis, which realized the rapid conversion of β-amino acid esters.

3. Materials and Methods

3.1. Materials

Unless otherwise stated, all chemicals were obtained from commercial sources and used without further purification. Lipozyme TL IM from *Thermomyces lanuginosus* was purchased from Novo Nordisk (Copenhagen, Denmark), and 4-tert-butylaniline, 4-methoxyaniline, 3,4-(methylenedioxy)aniline, 2-aminopyridine, 2-naphthylamine were purchased from Energy Chemical (Shanghai, China). Aniline, 4-toluidine, 4-chloroaniline, 4-bromoaniline, methyl acrylate and tert-butyl acrylate were purchased from Aladdin (Shanghai, China). Harvard Apparatus PHD 2000 syringe pumps were purchased from Harvard (Holliston, MA, USA). Bruker-ADNANCE III 500 MHz NMR spectrometer (Billerica, MA, USA) and Liquid Chromatography/Mass Spectrometer Detector (Agilent LC1290-MS6530, Santa Clara, CA, USA) were used in this study.

3.2. β-Amino Acid Ester Synthesis Operating Conditions

3.2.1. Experimental Setup

The equipment configuration that was used for synthesis of β-amino acid esters via lipase-catalyzed Michael reaction of aromatic amines with acrylates is described in Figure 7. The continuous-flow

microreactor was composed of a syringe pump (Harvard Apparatus PHD 2000), reactant injectors, Y-shaped mixer (Φ = 1.8 mm; M), microchannel reactor and product collector. Syringe pump was used to deliver reagents from reactant injectors to the Y-shaped mixer and then to the microchannel reactor. Reagent feed A (10 mL) with aromatic amines in methanol solution and reagent feed B (10 mL) with acrylates in methanol solution were fully mixed in the Y-shaped mixer. The microchannel reactor consists of a PFA reactor coil (inner diameter ID = 2.0 mm, length = 100 cm) filled with lipozyme TL IM (catalyst reactivity: 250 IUN g^{-1}). The microchannel reactor was immersed into a thermostatic apparatus (water bath) to control the temperature. The final solution was collected in a product collector connected to the microchannel reactor. The residence time was controlled by setting the flow rates of feed A and feed B.

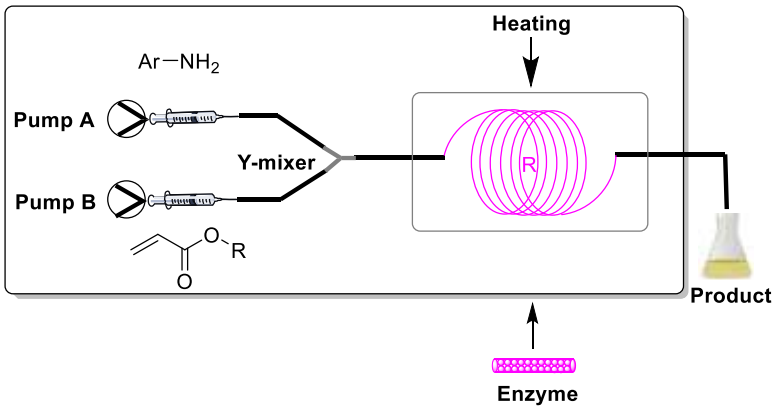


Figure 7. Experimental setup for the synthesis of β-amino acid esters.

3.2.2. General Procedure for β-Amino Acid Ester Synthesis in Continuous Flow Microreactor

Here, 5.0 mmol of aromatic amines were dissolved in 10 mL methanol (feed A, ~0.5 M), and 20.0 mmol acrylates were dissolved in 10 mL methanol (feed B, ~2.0 M). Lipozyme TL IM (870 mg) was added to the PFA reactor coil (inner diameter ID = 2.0 mm, length = 100 cm). Streams A and B were mixed together at a flow rate of 10.4 μL min^{-1} in a Y-mixer at 35 °C, and the resulting stream (20.8 μL min^{-1}) was connected to a sample vial which was used to collect the final mixture. The final mixture was then evaporated, and the residue was submitted to column chromatography on silica gel (200–300 mesh). The crude product was purified by silica gel flash chromatography with a petroleum ether/ethyl acetate gradient from 20:1 to 5:1. The purification was monitored by TLC. The fractions containing the main products were pooled, and the solvent was evaporated. The residue was analyzed by ^{1}H NMR, ^{13}C NMR and ESI-MS.

3.3. Analytical Methods

3.3.1. Thin-Layer Chromatography

Thin-layer chromatography analysis was conducted on silica gel plates with ethyl acetate/petroleum ether (1:5, by vol) as the eluent. Spots were detected by ultraviolet irradiation at 254 nm.

3.3.2. High-Performance Liquid Chromatography (HPLC)

The reaction was monitored by HPLC analysis using a Shim-Pack VP-ODS column (4.6 × 150 mm) and a UV detector (285 nm). Hexane/2-propanol solution 90/10 (*v*/*v*) was used as the mobile phase (flow rate: 1.0 mL min^{-1}).

3.3.3. Nuclear Magnetic Resonance (NMR) and Electrospray Ionization Mass Spectrometry (ESI/MS) Analysis

After purification of the synthesized products by column chromatography, the chemical structures of β-amino acid esters were determined by ^{1}H NMR, ^{13}C NMR and ESI-MS. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR spectra were acquired on a Bruker-ADNANCE III 500 MHz NMR spectrometer. The sample temperature was 22 °C and using $CDCl_3$ or DMSO-d_6 as solvent. ESI-MS was measured on a Liquid Chromatography/Mass Spectrometer Detector (Agilent LC1290-MS6530). Evaporated samples were dissolved in methanol. The injection volume was 10 μL. Reaction mixtures were injected and infused into the electrospray ion source at 0.2 mL min^{-1}. The spectrometer was operated in the positive ionization mode with the capillary voltage set to +3.5 kV. The sheath gas flow rate was 11 L min^{-1}, and the sheath gas temperature was 300 °C.

Methyl 3-(phenylamino)propanoate (**3a**). White solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (dd, *J* = 8.6, 7.3 Hz, 2H), 6.77 (tt, *J* = 7.3, 1.1 Hz, 1H), 6.67 (dd, *J* = 8.7, 1.1 Hz, 2H), 3.74 (s, 3H), 3.49 (t, *J* = 6.4 Hz, 2H), 2.66 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.83, 147.59, 129.35, 117.77, 113.09, 51.76, 39.47, 33.73. HRMS (ESI): calcd for $C_{10}H_{14}NO_2$ $[M + H]^+$: 180.1016, found: 180.1018.

Tert-butyl 3-(phenylamino)propanoate (**3b**). White powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (dd, *J* = 8.6, 7.3 Hz, 2H), 6.75 (tt, *J* = 7.3, 1.1 Hz, 1H), 6.65 (dd, *J* = 8.6, 1.2 Hz, 2H), 3.43 (t, *J* = 6.4 Hz, 2H), 2.55 (t, *J* = 6.4 Hz, 2H), 1.49 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.72, 147.73, 129.25, 117.60, 113.06, 80.83, 39.65, 35.08, 28.10. HRMS (ESI): calcd for $C_{14}H_{16}NO_2$ $[M + H]^+$: 222.1489, found: 222.1491.

Methyl 3-(p-tolylamino)propanoate (**3c**). White solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.84–6.96 (m, 2H), 6.48 (d, *J* = 8.4 Hz, 2H), 5.37 (t, *J* = 6.0 Hz, 1H), 3.61 (s, 3H), 3.24 (q, *J* = 6.6 Hz, 2H), 2.55 (t, *J* = 6.8 Hz, 2H), 2.14 (s, 3H); ^{13}C NMR **(**126 MHz, DMSO) δ 172.18, 146.17, 129.39, 124.31, 112.29, 51.36, 39.77, 33.56, 20.09. HRMS (ESI): calcd for $C_{11}H_{16}NO_2$ $[M + H]^+$: 194.1176, found: 194.1177.

Tert-butyl 3-(p-tolylamino)propanoate (**3d**). White solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (d, *J* = 7.8 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 2H), 3.44 (t, *J* = 6.4 Hz, 2H), 2.58 (t, *J* = 6.4 Hz, 2H), 2.33 (s, 3H), 1.54 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.59, 145.38, 129.58, 126.51, 113.12, 80.49, 39.85, 34.92, 27.93, 20.21. HRMS (ESI): calcd for $C_{14}H_{22}NO_2$ $[M + H]^+$: 236.1643, found: 236.1646.

Methyl 3-((p-(tert-butyl)phenyl)amino)propanoate (**3e**)**.** White powder**.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.91–7.24 (m, 2H), 6.66 – 6.18 (m, 2H), 5.41 (t, *J* = 6.0 Hz, 1H), 3.61 (s, 3H), 3.25 (q, *J* = 6.6 Hz, 2H), 2.56 (t, *J* = 6.8 Hz, 2H), 1.21 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 172.19, 146.03, 138.17, 125.53, 111.90, 51.36, 38.98, 33.61, 33.46, 31.49. HRMS (ESI): calcd for $C_{14}H_{22}NO_2$ $[M + H]^+$: 236.1643, found: 236.1645.

Tert-butyl 3-((p-(tert-butyl)phenyl)amino)propanoate (**3f**). Yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17–7.38 (m, 2H), 6.48–6.77 (m, 2H), 3.45 (t, *J* = 6.4 Hz, 2H), 2.58 (t, *J* = 6.4 Hz, 2H), 1.52 (s, 9H), 1.35 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.72, 145.36, 140.25, 125.95, 112.76, 80.66, 39.85, 35.18, 33.77, 31.50, 28.06. HRMS (ESI): calcd for $C_{17}H_{28}NO_2$ $[M + H]^+$: 278.2112, found: 278.2113.

Methyl 3-((p-methoxyphenyl)amino)propanoate (**3g**). Light yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.68–6.80 (m, 2H), 6.65 – 6.45 (m, 2H), 5.14 (t, *J* = 6.1 Hz, 1H), 3.65 (s, 3H), 3.63 (s, 3H), 3.26 (q, *J* = 6.6 Hz, 2H), 2.58 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (126 MHz, DMSO) δ 172.10, 150.86, 142.53, 114.47, 113.21, 55.09, 51.13, 39.54, 33.50. HRMS (ESI): calcd for $C_{11}H_{16}NO_3$ $[M + H]^+$: 210.1124, found: 210.1127.

Tert-butyl 3-((p-methoxyphenyl)amino)propanoate (**3h**). Yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.71–6.96 (m, 2H), 6.38–6.73 (m, 2H), 3.76 (s, 3H), 3.36 (t, *J* = 6.4 Hz, 2H), 2.52 (t, *J* = 6.4 Hz, 2H), 1.47 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.76, 152.29, 141.90, 114.84, 114.57, 55.69, 40.74, 35.06, 28.06. HRMS (ESI): calcd for $C_{14}H_{22}NO_3$ $[M + H]^+$: 252.1596, found: 252.1595.

Methyl 3-((4-chlorophenyl)amino)propanoate (**3i**). White powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06–7.19 (m, 2H), 6.50–6.58 (m, 2H), 3.71 (s, 3H), 3.42 (t, *J* = 6.4 Hz, 2H), 2.62 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR

(126 MHz, $CDCl_3$) δ 172.63, 146.10, 129.05, 122.16, 114.03, 51.75, 39.44, 33.46. HRMS (ESI): calcd for $C_{10}H_{13}ClNO_2$ $[M + H]^+$: 214.0628, found: 214.0630.

Tert-butyl 3-((4-chlorophenyl)amino)propanoate (**3j**). Yellow oil. ^1H NMR (500 MHz, $CDCl_3$) δ 6.98–7.20 (m, 2H), 6.35–6.66 (m, 2H), 3.38 (t, *J* = 6.3 Hz, 2H), 2.53 (t, *J* = 6.3 Hz, 2H), 1.47 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.61, 146.20, 129.12, 122.37, 114.28, 81.06, 39.91, 34.85, 28.12. HRMS (ESI): calcd for $C_{13}H_{19}ClNO_2$ $[M + H]^+$: 256.1096, found: 256.1098.

Methyl 3-((4-bromophenyl)amino)propanoate (**3k**). White powder. ^1H NMR (500 MHz, $CDCl_3$) δ 7.02–7.64 (m, 2H), 6.00–6.82 (m, 2H), 3.71 (s, 3H), 3.42 (t, *J* = 6.3 Hz, 2H), 2.62 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.61, 146.20, 129.12, 122.37, 114.28, 81.06, 39.91, 34.85, 28.12. HRMS (ESI): calcd for $C_{10}H_{13}BrNO_2$ $[M + H]^+$: 258.0124, found:258.0126.

Tert-butyl 3-((4-bromophenyl)amino)propanoate (**3l**). Clear oil. ^1H NMR (500 MHz, $CDCl_3$) δ 7.06–7.42 (m, 2H), 6.36–6.65 (m, 2H), 3.37 (t, *J* = 6.3 Hz, 2H), 2.53 (t, *J* = 6.3 Hz, 2H), 1.46 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.60, 146.63, 131.98, 114.74, 109.35, 81.07, 39.77, 34.82, 28.12. HRMS (ESI): calcd for $C_{13}H_{19}BrNO_2$ $[M + H]^+$: 300.0593, found:300.0595.

Methyl 3-(pyridin-2-ylamino)propanoate (**3m**)**.** White solid, ^1H NMR (500 MHz, $CDCl_3$) δ 8.04 (ddd, *J* = 5.1, 1.9, 0.9 Hz, 1H), 7.34 (ddd, *J* = 8.4, 7.1, 1.9 Hz, 1H), 6.51 (ddd, *J* = 7.1, 5.1, 1.0 Hz, 1H), 6.35 (dt, *J* = 8.4, 1.0 Hz, 1H), 5.04 (t, *J* = 6.3 Hz, 1H), 3.64 (s, 3H), 3.60 (q, *J* = 6.3 Hz, 2H), 2.61 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.85, 158.12, 147.85, 137.11, 112.76, 107.50, 51.52, 37.19, 33.87. HRMS (ESI): calcd for $C_9H_{13}N_2O_2$ $[M + H]^+$: 181.0972, found: 181.0975.

Methyl 3-(benzo[d][1,3]dioxol-5-ylamino)propanoate (**3o**). Light yellow solid. ^1H NMR (500 MHz, $CDCl_3$) δ 6.64 (d, *J* = 8.3 Hz, 1H), 6.25 (d, *J* = 2.3 Hz, 1H), 6.04 (dd, *J* = 8.3, 2.3 Hz, 1H), 5.82 (d, *J* = 0.8 Hz, 2H), 3.68 (d, *J* = 0.7 Hz, 3H), 3.35 (t, *J* = 6.4 Hz, 2H), 2.58 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.59, 148.19, 143.15, 139.62, 108.40, 104.51, 100.38, 96.10, 51.48, 40.24, 33.41. HRMS (ESI): calcd for $C_{11}H_{14}NO_4$ $[M + H]^+$: 224.0918, found: 224.0919.

Tert-butyl 3-(benzo[d][1,3]dioxol-5-ylamino)propanoate (**3p**). Light yellow oil. ^1H NMR (500 MHz, $CDCl_3$) δ 6.66 (d, *J* = 8.3 Hz, 1H), 6.27 (d, *J* = 2.4 Hz, 1H), 6.07 (dd, *J* = 8.3, 2.3 Hz, 1H), 5.86 (s, 2H), 3.33 (t, *J* = 6.3 Hz, 2H), 2.51 (t, *J* = 6.3 Hz, 2H), 1.47 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.73, 148.34, 143.48, 139.81, 108.59, 104.90, 100.55, 96.38, 80.84, 40.76, 34.96, 28.09. HRMS (ESI): calcd for $C_{14}H_{20}NO_4$ $[M + H]^+$: 266.1385, found: 266.1386.

Methyl 3-(naphthalen-2-ylamino)propanoate (**3q**). Pink powder**.** ^1H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.6 Hz, 1H), 7.66 (d, *J* = 5.8 Hz, 1H), 7.64 (d, *J* = 5.5 Hz, 1H), 7.39 (ddd, *J* = 8.2, 6.8, 1.3 Hz, 1H), 7.23 (ddd, *J* = 8.1, 6.8, 1.2 Hz, 1H), 6.90 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.85 (d, *J* = 2.3 Hz, 1H), 3.74 (s, 3H), 3.59 (t, *J* = 6.4 Hz, 2H), 2.72 (t, *J* = 6.3 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.85, 145.18, 135.11, 129.06, 127.67, 127.63, 126.37, 125.94, 122.16, 118.11, 104.66, 51.81, 39.41, 33.52. HRMS (ESI): calcd for $C_{14}H_{16}NO_2$ $[M + H]^+$: 230.1176, found: 230.1181.

Tert-butyl 3-(naphthalen-2-ylamino)propanoate (**3r**)**.** Pale violet powder. ^1H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, *J* =8.10 Hz, 1H), 7.66 (d, *J* =6.25 Hz, 1H), 7.64 (d, *J* =5..45 Hz, 1H), 7.37–7.40 (m, 1H), 7.21–7.24 (m, 1H), 6.92 (dd, *J* =8.8, 2.3 Hz, 1H), 6.87 (d, *J* =2.0 Hz, 1H), 3.53 (t, *J* =6.3 Hz, 2H), 2.63 (t, *J* =6.3 Hz, 2H), 1.48 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.72, 147.73, 129.25, 117.60, 113.06, 80.83, 39.65, 35.08, 28.10. HRMS (ESI): calcd for $C_{17}H_{22}NO_2$ $[M + H]^+$: 272.1645, found: 272.1649.

4. Conclusions

In summary, we have developed an effective and environmentally friendly methodology for the synthesis of β-amino acid esters based on aromatic amines catalyzed by lipase TL IM from *Thermomyces lanuginosus* under continuous-flow microreactors. Lipase TL IM from *Thermomyces lanuginosus* was first used to catalyze Michael addition reaction of aromatic amines with acrylates. We studied the effects of various reaction parameters including the reaction medium, reaction temperature, enzyme, substrate

molar ratio, residence time/flow rate and substrate structure on the reaction. Through this technique, 17 β-amino acid esters were rapidly synthesized. Compared with traditional methods, the salient features of this method include green reaction solvent (methanol), mild reaction condition (35 °C), short residence time (30 min) and high yield. These features make our methodology an attractive alternative to the current synthesis of β-amino acid esters. Our studies highlight the importance of selecting a reaction system for a specific biotransformation and show that enzymatic reactions can benefit greatly from the continuous-flow microreactor. Our results provide direction for the exploration of new enzyme catalysis processes.

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