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Promiscuous *Candida antarctica* lipase B-catalyzed synthesis of β-amino esters via aza-Michael addition of amines to acrylates

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ABSTRACT

An efficient protocol for the regioselective aza-Michael addition of amines with acrylates using CaL B as a biocatalyst at 60 °C has been developed. The reaction is applicable to a wide variety of primary and secondary amines with different acrylates to synthesize the corresponding β-amino esters with good yields. An alternative route for the synthesis of higher β-amino esters through the additional transesterification step is also studied and was found effective.

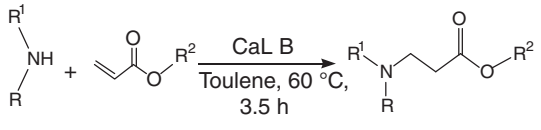
Biocatalysis has greatly gained importance in organic synthesis as it provides a high selectivity with fewer by-products, thus making it an environmentally benign alternative to chemical transformations.[1] Hydrolases, especially lipases are the dominating enzymes of the family being exploited for organic synthesis. Synthesis of β-amino esters has immense importance as β-amino acid moieties are biologically important molecules,[2] and also contribute as essential intermediates in the synthesis of β-lactams,[2b,c] and β-peptides.[2d] The Mannich reaction is the most common method used for the synthesis of β-amino esters.[3a–c] Also, Michael addition has served as one of the fundamental reactions in the organic synthesis of such important molecules. A variety of Lewis acids such as transition metals, lanthanide halides,[4a–c] triflates,[4d] and silica gel[4e] have been demonstrated as catalysts for the Michael addition reaction. Heterogeneous solid catalysts like [5a] $Cu(acac)_2$ immobilized in ionic liquid,[5b] quaternary ammonium salt/ionic liquid/H_2O,[5c] boric acid in H_2O,[5d] β-cyclodextrin in H_2O[5e], and clay[5f] have also been used for similar transformations.

Lipases are widely explored for esterification, transesterification, and aminolysis type of reactions to synthesize various important molecules related to pharmaceuticals, foods, and flavors.[6a,b] Apart from their natural behavior to catalyze the specified reactions, they are found to catalyze various unexpected reactions like C–C,[7a–c] C–N,[7d–g] C–S[7h] which shows their promiscuous behavior toward biocatalysis.[7i] Kitazume et al.[8a] were the first to reveal the ability of the hydrolytic enzymes to catalyze aza-Michael addition in a buffer solution of Na_2HPO_4 and KH_2PO_4. Biomacromolecules such as RNA, [8b] antibody,[8c]and baker's yeast[8d] were then reported to catalyze the aza-Michael addition reaction. In the recent decade, the promiscuity catalytic site has been discovered and studied for various unexpected reactions.[9a,b] Although some reports on *Candida antarctica* lipase B-catalyzed Michael addition in organic solvent exist, the area of β-amino esters synthesis using diethyl amine and acrylate yet remain unexplored, considering which we focused our attention toward β-amino esters synthesis.

In the present Letter, we report an efficient enzymatic protocol for the β-amino esters synthesis via aza-Michael addition of the primary and secondary amines to acrylates using CaL B as a biocatalyst (Scheme 1).

The catalyst exhibited remarkable activity and is applicable to a wide variety of primary and secondary amines with different acrylates to synthesize the corresponding β-amino esters.

In order to optimize the reaction conditions, the reaction of diethyl amine with methyl acrylate in the presence of CaL B was chosen as a model reaction[12] and the influence of various reaction parameters such as enzyme, solvent, temperature, time, catalyst loading, and molar ratio of acrylate to amine was studied. Commercially available lipases from various sources were screened for their catalytic activity toward aza-Michael addition reaction as shown in Table 1(entries 1–7). CaL B lipase (immobilized on acrylic resin from *C. antarctica* with ⩾10,000 U/g, recombinant, and expressed in *Aspergillus oryzae*) was found to be the most efficient lipase to catalyze the reaction while other lipases catalyzed the reaction with a low yield ranging from 10% to 36% of the desired



R, R^1 = H, alkyl
R^2 = methyl, ethyl, butyl

Scheme 1. CaL B-catalyzed aza-Michael addition of amine to acrylate.

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Table 1
Lipase screening for aza-Michael addition[a]

Entry	Lipase	Enzyme activity (U/g)	Yield[b] (%)
1	*Candida antarctica* lipase B	⩾10,000	58
2	Lipase PS 30	⩾30,000	10
3	Lipase AYS	⩾30,000	Traces
4	*Aspergillus niger*	⩾12,000	18
5	*Candida cylindracea*	⩾2,000	36
6	Hog pancreas	⩾15,000	20
7	*Rhizopus oryzae*	⩾30,000	Traces
8	No enzyme	—	Traces

[a] Reaction conditions: methyl acrylate (2 mmol), diethyl amine (1 mmol), toluene (3 ml), lipase (300 U), temperature (37 °C), time (3.5 h).
[b] Yield based on GC analysis.

product. It was observed that lipases studied led to a faster reaction when compared with the reaction carried out in the absence of a biocatalyst under the same reaction conditions.[7d,f,g]

An enzyme-catalyzed reaction in an organic solvent has numerous potential applications which suggested us to study the effect of solvents on enzyme activity.[10] Overall five solvents with log *P* value ranging from −1.1 to 2.5 were screened for the standard reaction, of which toluene was found to be the best solvent providing 89% yield of the desired product (Table 2, entry 5). CaL B was studied for its optimum temperature to obtain higher yields of the desired product as shown in Table 2 (entries 5–8). It was observed that the activity of enzyme increased as the temperature increased up to 60 °C (Table 2, entry 5), but at a higher temperature, i.e., 80 °C, the yield of the reaction had decreased to 75% (Table 2, entry 8) as the enzyme is believed to undergo a deactivation effect.

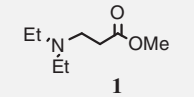
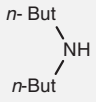
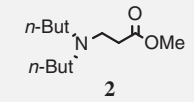
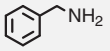
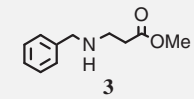
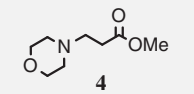
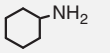
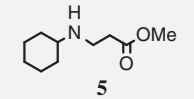
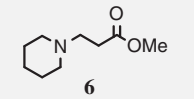
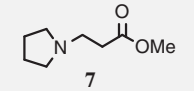
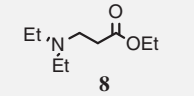
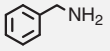
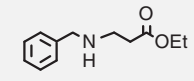
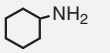
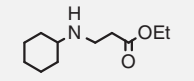
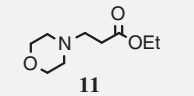
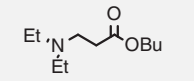
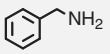
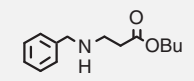
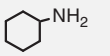
In an effort to evolve the best loading of a catalyst, reactions with various catalysts concentration were carried out (Table 2, entries 5, 9–12). The yield was observed to increase with the increase in the catalyst loading ranging from 10 to 50 mg, whereas 30 mg of CaL B was sufficient to catalyze the desired reaction under the present reaction parameters with 89% of yield (Table 2, entry 5). Further increase in enzyme concentration had no significant effect on the yields. For aza-Michael addition reaction, molar ratio of substrates has shown a promising effect on the yield of the desired product. The optimum yield of 58% was obtained with 2:1 ratio

Table 2
Optimization of reaction parameters for enzymatic aza-Michael addition reaction[a]

Entry	Solvent	Temp (°C)	Enzyme loading (mg)	Yield[b] (%)
Influence of solvent				
1	1,4-Dioxane	60	30	59
2	Tetrahydrofuran	60	30	78
3	Diisopropyl ether	60	30	78
4	Chloroform	60	30	81
5	Toluene	60	30	89
Influence of temperature				
6	Toluene	37	30	58
7	Toluene	45	30	69
8	Toluene	80	30	75
Influence of catalyst loading				
9	Toluene	60	10	45
10	Toluene	60	20	75
11	Toluene	60	40	91
12	Toluene	60	50	93
13[c]	Toluene	37	30	45
14[d]	Toluene	37	30	46
15	Toluene	37	—	Traces

[a] Reaction conditions: methyl acrylate (2 mmol), diethyl amine (1 mmol), toluene (3 ml), lipase (CaL B), time (3.5 h).
[b] Yields based on GC analysis.
[c] Molar ratio of methyl acrylate/diethyl amine (1:1).
[d] Time (2 h).

Table 3
CaL B-catalyzed aza-Michael addition reaction[a]

Entry	Amine	Acrylate	Product	Yield[b] (%)
1			 **1**	89 (87, 83,78)[d]
2			 **2**	51
3			 **3**	88
4			 **4**	90
5			 **5**	54
6			 **6**	65
7			 **7**	94
8			 **8**	82
9			 **9**	83
10			 **10**	90
11			 **11**	82
12			 **12**	54
13			 **13**	59
14			**14**	69
15			**15**	96
16[c]			**16**	31

[a] Reaction conditions: acrylate (2 mmol), amine (1 mmol), toluene (3 ml), CaL B (30 mg), temperature (60 °C), time (3.5 h).
[b] Yields based on GC analysis.
[c] Time (42 h).
[d] Recyclability study for three consecutive cycles.

of methyl acrylate to diethyl amine at 37 °C temperature (Table 2, entry 6) while it was low, that is, 45% for 1:1 molar ratio (Table 2, entry 13). During time study, the optimum yield was obtained in 3.5 h (Table 2, entry 5) whereas, lowering of time led to a decrease in yield (Table 2, entry 14). Thus optimized reaction conditions are solvent: toluene, temperature: 60 °C, time: 3.5 h, catalyst loading: 30 mg (300 U).

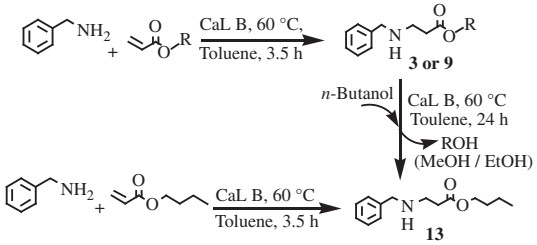
In order to test the generality and efficiency of this system, the optimized reaction conditions were then applied for aza-Michael addition of various primary and secondary aliphatic amines, with different acrylates (Table 3, entries 1–16). The reaction of diethyl amine with methyl acrylate (Table 3, entry 1) was smooth but as the chain length of the secondary amine increased, i.e., the reaction of dibutylamine with methyl acrylate (Table 3, entry 2) was hampered providing a comparatively low yield of 51%. A primary aliphatic amine such as benzylamine (Table 3, entry 3) also provided a good yield of the desired product. The alicyclic amines such as morpholine, cyclohexylamine, piperidine, and pyrrolidine reacted effectively to provide good yields of the desired β-amino esters (Table 3, entries 4–7). Other acrylates such as ethyl acrylate and butyl acrylate were studied for aza-Michael addition where the yield declined as the chain length increased from ethyl acrylate to butyl acrylate (Table 3, entries 8–15) except for morpholine where no significant effect of the chain length of the acrylate was observed on the yields of the product (Table 3, entries 11 and 15). The protocol was further extended to study aromatic amines as a substrate but the reaction was too sluggish. It was observed that an aromatic amine like aniline was not compatible as a substrate for this particular protocol as the yield was 31% with a long reaction time of 42 h (Table 3, entry 16).

Alternatively, the synthesis of **13** can be done by transesterification of **3** or **9** with *n*-butanol under the present conditions. This transesterification method suggests that several other alcohols can be used to synthesize the desired product starting with a single type of acrylate available. The reaction was conducted in two modes, tandem and sequential. In tandem, 1 mmol of *n*-butanol was added directly after the completion of the first step whereas in sequential, the **3** or **9** was isolated and to which 1 mmol of *n*-butanol was then added under the mentioned reaction conditions as shown in Scheme 2.

The yield was lower for the tandem type than for the sequential reaction mode as shown in Table 4 (entries 1–4). Also, the low yield of **13** may be due to alcohol, released as a by-product during the transesterification, and they are found to deactivate the enzymes.[6b]

During recyclability studies of the enzyme CaL B, it was observed that the enzyme worked efficiently with no significant decrease in yield during the first cycle whereas the yield declined up to 78% after completion of the third cycle (Table 3, entry 1). However, this slight decrease might be due to the handling loss of lipase during recycling.

In conclusion, we have developed an efficient protocol for the synthesis of aliphatic β-amino esters via aza-Michael addition between the primary and secondary amines with acrylates. The developed methodology is applicable for a variety of amines and acrylates providing good to excellent yield of the desired products. Also, alternative transesterification route was introduced to synthesize higher β-amino esters.



Scheme 2. Alternative route for synthesis of (**13**).

Table 4
Alternative route for synthesis of (**13**)[a]

Entry	Reaction Mode	Acrylate	Yield (%)[b] (**13**)
1	Tandem	Methyl acrylate	34
2	Stepwise	Methyl acrylate	58
3	Tandem	Ethyl acrylate	42
4	Stepwise	Ethyl acrylate	61

[a] Reaction conditions: acrylate (2 mmol), amine (1 mmol), toluene (3 ml), *n*-butanol (1 mmol), CaL B (30 mg), temperature (60 °C), time (3.5 h)/(24 h), respectively.
[b] Yield based on GC analysis.

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12. In a typical experimental procedure, to 3 ml of toluene, 2 mmol of methyl acrylate was added. The reaction was initiated by adding 30 mg of lipase which

was stirred at 37 °C for 5 min. Then 1 mmol of diethyl amine was added and stirred for 3.5 h at 37 °C or 60 °C as specified. The progress of the reaction was monitored by TLC analysis. After completion, the reaction mixture was filtered and was quantitatively analyzed using gas chromatography (Chemito 1000). The reaction mixture was then evaporated under vacuo. The residue obtained was purified with column chromatography (silica gel, mesh size 60–120) using chloroform/methanol (95:5) as an eluent to afford the pure products. All the products are well known [5a,11] and were compared with the authentic samples. The products are well characterized by ^{1}H and ^{13}C NMR spectra recorded on an NMR spectrometer (Varian-300) using TMS as internal standard and mass spectra by GC–MS (Shimadzu QP 2010) analysis.

Spectral data for selected products:

3-Diethylamino-propionic acid methyl ester (**1**): ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ = 1.13–1.18 (6H, t, J = 7.14 Hz), 2.66–2.71 (2H, t, J = 7.69 Hz), 2.79–2.86 (4H, q, J = 7.20 Hz), 3.03–3.08 (2H, t, J = 7.51 Hz), 3.69 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172, 51.1, 46.8, 45.9, 30, 11.3. MS (70 eV, EI): m/z (%): 159 (15) (M^+), 144 (25), 86 (100), 42 (20).

3-Benzylamino-propionic acid methyl ester (**3**): ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ = 1.94 (1H, s), 2.52–2.56 (2H, t, J = 6.41 Hz), 2.88–2.92 (2H, t, J = 6.59 Hz), 3.68 (3H, s), 3.80 (2H, s), 7.26–7.32 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 173.3, 140.1, 128.5, 128.2, 127.1, 53.8, 51.7, 44.5, 34.6. MS (70 eV, EI): m/z (%): 193 (15) (M^+), 120 (35), 106 (65), 91 (100), 65 (20), 42 (15).

3-Morpholin-4-yl-propionic acid methyl ester (**4**): ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ = 2.44–2.51 (6H, m), 2.66–2.71 (2H, t, J = 7.14 Hz), 3.69–3.71 (7H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.9, 66.9, 53.1, 53.4, 51.7, 31.9. MS (70 eV, EI): m/z (%): 173 (15) (M^+), 100 (100), 70 (15), 56 (40), 42 (30).

3-Benzylamino-propionic acid butyl ester (**13**): ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ = 0.89–0.94 (3H, t), 1.35–1.42 (2H, m),1.55–1.62 (2H, m), 1.86 (1H, s), 2.50–2.54 (2H, t, J = 6.41 Hz), 2.87–2.91 (2H, t, J = 6.41), 3.79 (2H, s), 4.05–4.10 (2H, t, J = 6.77), 7.23–7.32 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.9, 140.1, 128.4, 128, 126.9, 64.3, 53.8, 44.5, 34.7, 30.6, 19.1, 13.7. MS (70 eV, EI): m/z (%): 235 (10) (M^+), 144 (15), 120 (45), 106 (95), 91 (100), 65 (15).

3-Phenylamino-propionic acid methyl ester (**16**): ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ = 2.60 (2H, t, J = 6.42 Hz), 3.43 (2H, t, J = 6.42 Hz), 3.67 (3H, s), 6.62 (2H, d), 6.73 (1H, t), 7.16 (2H, t). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.8, 147.5, 129.2, 117.6, 112.9, 51.7, 39.3, 33.6. MS (70 eV, EI): m/z (%): 179 (25) (M^+), 106 (100), 77 (20), 65 (15), 51 (15).