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Enzyme-mediated domino synthesis of 2-alkylbenzimidazoles in solvent-free system: A green route to heterocyclic compound

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ABSTRACT

Solvent-free domino acylation/cyclization reactions between fatty acid esters and o-phenylenediamine mediated by immobilized lipase from *Mucor miehei* (MML) were found to be an efficient way in the synthesis of 2-alkylbenzimidazole. Compared with other substrates, methyl fatty acid esters with moderated chain length exhibited best activity with yield up to 95%. The mechanism of the domino process was clarified deeply through some detail experiments, including separation of intermediates. This efficient enzymatic domino process provides an attractive procedure for heterocyclic compound synthesis and could be regarded as a potential synthetic method in modern organic chemistry.

1. Introduction

The development of efficient synthesis of bioactive compounds in an ecologically and economically favorable way is a great challenge in modern chemistry [1]. Benzimidazole derivatives are important scaffolds, presenting in a variety of biologically active molecules, such as anti-hepatitis B virus agents and omeprazole of peptic antiulcer, *etc*. [2]. Among the approaches to synthesis of benzimidazoles [3], domino synthesis has received much attention in recent years, owing to the reduction of intermediate purification and isolation steps, which can minimize the waste and consumption of solvents [4]. However most domino reactions are promoted by conventional chemical catalysts, which are always not environmentally friendly [5]. As a mild and facile protocol in organic synthesis, biocatalysis, especially domino biocatalysis, presents a valuable alternative to conventional chemical methods due to its high selectivity and simplicity [6].

It is surprising that only a few enzymatic routes to heterocyclic compound such as benzimidazole have been reported. To the best of our knowledge, Renard and co-workers reported the one and only example of enzymatic synthesis of 2-alkylbenzimidazole [7]. However, moderate yield could be achieved only for long chain fatty acid. Additionally, the use of organic solvent and large consumption of lipase obviously enlarged the *E* factor of the enzymatic process [8]. Another critical problem which has not been solved is the unclear mechanism of the process. In the present work, we focused on a facile and useful enzymatic strategy for the synthesis of 2-alkybenzimidazoles by a enzymatic acylation/cyclization domino reactions between 1,2-arylenediamine and fatty acid esters (Scheme 1). A series of 2-alkybenzimidazoles with different chain length had been synthesized with moderate to good yields. The immobilized biocatalyst MML could be easily recycled and the activity did not decrease significantly after two cycles. In addition, a two-step mechanism was proposed based on the experiments, including the separation of intermediate a: Firstly, MML-mediated mono-acylation of 1,2-arylenediamine occurred, and sequentially the product b can be formed through cyclization promoted by a fatty acid, which produced from MML-catalyzed hydrolysis.

2. Experimental

2.1. Materials and methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DMX 400 or 600. Chemical shifts are given in δ relative to tetramethylsilane (TMS). HPLC was carried out using a Shimadzu organizer consisting of a LC-2010A$_{HT}$ Integrator, a UV/VIS Detector. Daicel Chiralpak OJ column and Welchrom-C18 (5 μm, 4.6 × 150 mm) column were used in the HPLC experiments. Electrospray ionization mass spectrometry (ESI-MS) experiments were performed on Bruker Daltonics Bio TOF mass spectrometer. MJL (lipase from *Mucor javanicus*, 10,000 U/mg, Aldrich); MML (lipase from *Mucor meihei*, 140 U/mg, Fluka), CRL (lipase from *Candida rugosa*, 700–1500 U/mg, Sigma); CAL-B (lipase acrylic resin from *Can-*

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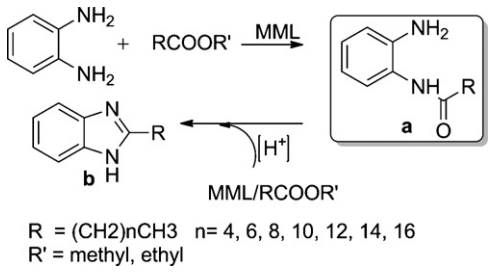


Scheme 1. Process of the enzyme-mediated domino synthesis of 2-alkylbenzimidazoles.

dida antarctica, 10,000 U/mg, Sigma); PCL (lipase from *Penicillium camemberti*, 30,000 U/mg, Fluka); BSNP (proteinase N from *Bacillus subtilis*, 6.67 U/mg, Sigma); and AMA (acylase I from *Aspergillus melleus*, 0.72 U/mg, Fluka).

2.2. A typical enzymatic procedure of synthesis

The reaction was initiated by adding 20 mg MML to a mixture of 20 mg (0.19 mmol) *o*-phenylenediamine and 1.11 mmol corresponding ester. The mixture was stirred at 50 °C for 60 h (formation of products was detected by TLC). Then ether was added and the residue was filtered off. After washing with NaOH aqueous solution and ethanol mixture (1N NaOH in an EtOH:H_2O (1:5, v/v) mixture) and water, the solution was finally dried on Na_2SO_4. A single product was prepared by silica gel chromatograph with an eluent consisting petroleum ether/ethyl acetate (from 5:1 to 2:1, v/v). Samples detected with OJ column were dissolved in the mixture of isopropanol and hexane and others were dissolved in the mixture of isopropanol and methanol. UV detection was carried out at 254 nm.

2.2.1. 2-Heptadecyl-benzimidazole

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.44 (m, 2H, ArH), 7.12 (m, 2H, ArH), 2.72 (t, J = 7.6 Hz, 2H, CH_2–Ar), 1.66 (m, 2H, CH_2–CH_2Ar), 1.18 (m, 28H, $(CH_2)_n$), 0.81 (t, J = 6.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.06, 136.54, 136.48, 122.85, 114.35, 31.94, 29.73, 29.69, 29.65, 29.59, 29.51, 29.44, 29.39, 29.28, 29.27, 28.12, 22.70, 14.12. ESI-MS: m/z = 357.3 $[M+H]^+$. IR (KBr, cm^{-1}): 3425.1, 2919.4, 2851.2, 1466.2, 1274.5, 726.4. Daicel Chiralpak OJ column, hexane/*i*-PrOH (85/15, v/v), 0.5 mL/min, 7.1 min.

2.2.2. N-(2-Aminophenyl)stearamide

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.53 (s, 1H, NHCO), 7.04 (d, J = 7.6 Hz, 1H, ArH), 6.97 (m, 1H, ArH), 6.67 (m, 2H, ArH), 3.76 (s, 2H, NH_2), 2.22 (t, J = 7.6 Hz, 2H, CH_2–CO), 1.61 (m, 2H, CH_2–CH_2CO), 1.23 (m, 28H, $(CH_2)_n$), 0.81 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.18 (–C=O), 140.93, 127.12, 125.43, 124.37, 119.41, 118.07, 36.95, 31.94, 29.72, 29.68, 29.54, 29.41 29.38, 29.34, 25.88, 22.70, 14.13. ESI-MS: m/z = 397.3 $[M+Na]^+$, 413.2 $[M+K]^+$. IR (KBr, cm^{-1}): 3413.4, 3382.4, 3273.6, 2920.0, 2850.7, 1646.13, 1532.5, 1460.2, 1261.0, 740.8.

2.2.3. 2-Pentadecyl-benzimidazole

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.46 (m, 2H, ArH), 7.22 (m, 2H, ArH), 2.83 (t, J = 7.6 Hz, 2H, CH_2–Ar), 1.701 (m, 2H, CH_2–CH_2Ar), 1.30 (m, 24H, $(CH_2)_n$), 0.90 (t, J = 6.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.05, 137.42, 122.52, 114.48, 34.87, 31.94, 29.71, 29.67, 29.64, 29.54, 29.49, 29.38, 29.30, 28.79, 28.14, 25.18, 22.70, 14.13. ESI-MS: m/z = 345.3 $[M-H]^-$. IR (KBr, cm^{-1}): 3429.4, 2919.5, 2849.8, 1464.6, 1417.7, 1271.1, 750.0, 722.4. Daicel Chiralpak OJ column, hexane/*i*-PrOH (85/15, v/v), 0.5 mL/min, 7.2 min.

2.2.4. N-(2-Aminophenyl)palmitamide

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28 (s, 1H, NHCO), 7.20 (m, 1H, ArH), 7.08 (m, 1H, ArH), 6.82 (m, 2H, ArH), 2.41 (t, J = 7.2 Hz, 2H, CH_2–CO), 1.75 (m, 2H, CH_2–CH_2CO), 1.23 (m, 24H, $(CH_2)_n$), 0.90 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.96, 140.69, 127.11, 125.24, 124.51, 119.64, 118.26, 37.10, 31.91, 29.68, 29.64, 29.60,29.48, 29.44, 29.34, 29.30, 29.25,29.10, 25.87, 24.78, 22.67, 14.08. ESI-MS: m/z = 329.3 $[M+H]^+$. IR (KBr, cm^{-1}): 3399.7, 3331.3, 3265.5, 2919.7, 2850.4, 1642.9, 1534.9, 1462.7, 1254.7, 766.8, 718.3.

2.2.5. 2-Tridecyl-benzimidazole

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.31 (m, 2H, ArH), 7.12 (m, 2H, ArH), 2.28 (t, J = 7.2 Hz, 2H, CH_2–Ar), 1.64 (m, 2H, CH_2–CH_2Ar), 1.21 (m, 20H, $(CH_2)_n$), 0.81 (t, J = 6.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.22, 122.14, 31.29, 29.67, 29.65, 29.61, 29.49, 29.41, 29.38, 29.36, 29.34, 28.28, 22.69, 14.12. ESI-MS: m/z = 301.2 $[M+H]^+$. IR (KBr, cm^{-1}): 3442.8, 2922.3, 2852.0, 1460.6, 761.1, 717.8. Daicel Chiralpak OJ column, hexane/*i*-PrOH (85/15, v/v), 0.5 mL/min, 7.3 min.

2.2.6. N-(2-Aminophenyl)tetradecanamide

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.49 (s, 1H, NHCO), 7.06 (m, 1H, ArH), 6.95 (m, 1H, ArH), 6.68 (m, 2H, ArH), 4.01 (s, 2H, NH_2), 2.26 (t, J = 7.6 Hz, 2H, CH_2–CO), 1.61 (m, 2H, CH_2–CH_2CO), 1.19 (m, 20H, $(CH_2)_n$), 0.81 (t, J = 6.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.29 (–C=O), 140.88, 127.15, 125.47, 124.32, 119.44, 118.07, 36.97, 31.93, 29.70, 29.67, 29.53, 29.40, 29.37, 29.33, 25.89, 22.70, 14.13. ESI-MS: m/z = 341.2 $[M+Na]^+$. IR (KBr, cm^{-1}): 3397.4, 3330.1, 3263.7, 2919.3, 2850.0, 1643.2, 1535.2, 1467.5, 1453.4, 1258.7, 766.5, 717.8.

2.2.7. 2-Undecyl-benzimidazole

^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.97 (s, 1H, NH), 7.53 (m, 2H, ArH), 7.21 (m, 2H, ArH), 2.78 (m, 2H, CH_2–Ar), 1.73 (m, 2H, CH_2–CH_2Ar), 1.25 (m, 16H, $(CH_2)_n$), 0.88 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.93, 136.39, 122.88, 114.34, 35.05, 31.90, 29.63, 29.52, 29.37, 29.30, 29.22, 28.03, 25.23, 22.67, 14.08. ESI-MS: m/z = 273.2 $[M+H]^+$. IR (KBr, cm^{-1}): 3424.0, 2923.1, 2852.1, 1458.2, 1413.7, 1273.2, 749.2, 723.8. Welchrom-C18 column (5 μm, 4.6 × 150 mm), MeOH/H_2O (90/10, v/v), 0.8 mL/min, 7.7 min.

2.2.8. N-(2-Aminophenyl)dodecanamide

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.21 (s, 1H, NHCO), 7.17 (d, J 8.0, 1H, ArH), 7.05 (t, J = 7.2 Hz, 1H, ArH), 6.80 (d, J = 6.4 Hz, 2H, ArH), 3.91 (s, 2H, Ar–NH_2), 2.38 (t, J = 7.2 Hz, 2H, CH_2–CO), 1.73 (d, J = 6.4 Hz, 2H, CH_2–CH_2–CO), 1.26 (m, 16H, $(CH_2)_n$), 0.88 (t, J = 6.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.96, 140.84, 127.13, 125.26, 124.41, 119.52, 118.21, 37.08, 31.91, 30.93, 29.61, 29.50, 29.37, 29.34, 29.32, 25.88, 22.69, 14.12. ESI-MS: m/z = 289.3 $[M-H]^-$. IR (KBr, cm^{-1}): 3397.3, 3330.4, 3265.1, 2920.3, 2850.7, 1644.1, 1535.1, 1503.0, 1467.3, 1454.2, 1257.4, 767.3, 717.2

2.2.9. 2-Nonyl-benzimidazole

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.45 (m, 2H, ArH), 7.12 (m, 2H, ArH), 2.76 (t, J = 7.8 Hz, 2H, CH_2–Ar), 1.70 (t, J = 6.6 Hz, 2H, CH_2–CH_2Ar), 1.23 (m, 12H, $(CH_2)_n$), 0.80 (t, J = 10.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.19, 137.47, 137.37, 122.51, 114.45, 31.87, 29.69, 29.48, 29.39, 29.25, 28.79, 28.17, 22.63, 14.08. ESI-MS: m/z = 245.1 $[M+H]^+$. IR (KBr, cm^{-1}): 3428.5, 2925.2, 2853.9, 1453.3, 1270.2, 748.0. Welchrom-C18 column (5 μm, 4.6 × 150 mm), MeOH/H_2O (73/27, v/v), 0.8 mL/min, 26.0 min.

2.2.10. N-(2-Aminophenyl)decanamide

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.43 (s, 1H, NHCO), 7.19 (m, 1H, ArH), 7.06 (t, J = 7.2 Hz, 1H, ArH), 6.80 (m, 2H, ArH), 4.30 (s, 2H, Ar–NH_2), 2.37 (t, J = 7.2 Hz, 2H, CH_2–CO), 1.73 (m, 2H,

CH_2–CH_2–CO), 1.28 (m, 12H, $(CH_2)_n$), 0.88 (t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.11(–C=O), 140.70,127.10, 125.35, 124.49, 119.59, 118.19, 37.03, 34.16, 25.86, 22.63, 14.05. ESI-MS: m/z = 261.1 $[M-H]^-$. IR (KBr, cm^{-1}): 3397.3, 3330.7, 3262.8, 2956.4, 2922.6, 2852.0, 1643.1, 1534.1, 1501.2, 1458.9, 1259.6, 767.3, 717.0.

2.2.11. 2-Heptyl-benzimidazole

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.53 (t, J 2.8, 2H, ArH), 7.19 (m, 2H, ArH), 2.87 (d, J = 4.0 Hz, 2H, CH_2–Ar), 1.75 (t, J = 6.8 Hz, 2H,CH_2–CH_2Ar), 1.18 (m, 8H, $(CH_2)_n$), 0.80 (t, J = 6.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.34, 122.12, 31.66, 29.40, 29.33, 28.98, 28.32, 22.58, 14.03. ESI-MS: m/z = 217.0 $[M+H]^+$. IR (KBr, cm^{-1}): 3417.5, 2958.7, 2927.0, 2856.4, 1455.6, 1419.1, 1272.7, 745.6. Welchrom-C18 column (5 μm, 4.6 × 150 mm), MeOH/H_2O (90/10, v/v), 0.8 mL/min, 4.0 min.

2.2.12. N-(2-Aminophenyl)octanamide

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.15 (s, 1H, NHCO), 7.10 (d, J = 7.6 Hz, 1H, Ar), 6.98 (t, J = 7.6 Hz, 1H, ArH), 6.71 (d, J = 7.2 Hz, 2H, ArH), 3.86 (s, 2H, Ar–NH_2), 2.31 (t, J = 7.6 Hz, 2H, CH_2–CO), 1.66 (t, J = 6.4 Hz, 2H, CH_2–CH_2–CO), 1.22 (m, 8H, $(CH_2)_n$), 0.81 (t, J = 6.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.97, 140.84, 127.16, 125.26, 124.40, 119.55, 118.23, 37.08, 31.69, 29.27, 29.03, 25.87, 22.61, 14.07. ESI-MS: m/z = 257.0 $[M+Na]^+$. IR (KBr, cm^{-1}): 3397.8, 3330.9, 3267.3, 2956.3, 2924.3, 2853.0, 1642.8, 1534.0, 1499.9, 1452.4, 1255.1, 766.0, 716.0.

2.2.13. 2-Pentyl-benzimidazole

^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.57 (s, 1H, NH), 7.53 (t, J = 3.6 Hz, 2H, ArH), 7.19 (t, J = 3.6 Hz, 2H, ArH), 2.88 (t, J = 7.6 Hz, 2H, CH_2–Ar), 1.81 (m, 2H, CH_2–CH_2Ar), 1.25 (t, J = 2.8 Hz, 4H, $(CH_2)_n$), 0.80 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.69, 122.05, 31.50, 29.35, 28.11, 22.35, 13.87. ESI-MS: m/z = 188.9 $[M+H]^+$. IR (KBr, cm^{-1}): 3426.9, 2951.7, 2928.3, 2864.4, 1453.6, 1419.4, 1268.0, 747.2. Welchrom-C18 column (5 μm, 4.6 × 150 mm), MeOH/H_2O (70/30, v/v), 0.8 mL/min, 6.9 min.

2.2.14. N-(2-Aminophenyl)hexanamide

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.19 (s, 1H, NHCO), 7.09 (d, J = 8.0 Hz, 1H, ArH), 6.97 (m, 1H, ArH), 6.720 (m, 2H, ArH), 3.81 (s, 2H, Ar–NH_2), 2.32 (t, J = 7.6 Hz, 2H, CH_2–CO), 1.67 (t, J = 7.2 Hz, 2H, CH_2–CH_2–CO), 1.31 (m, 4H, $(CH_2)_n$), 0.85 (t, J = 6.4 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.03, 140.78, 127.17, 125.29, 124.41, 122.17, 119.60, 118.24, 37.04, 31.46, 25.55, 22.42, 13.95. ESI-MS: m/z = 229.0 $[M+Na]^+$. IR (KBr, cm^{-1}): 3410.3, 3274.6, 2957.4, 2929.8, 2863.2, 1642.3, 1532.7, 1458.1, 1299.8, 747.9, 716.2.

3. Results and discussion

3.1. Selection of biocatalysts

The enzyme source is one of the main factors influencing enzymatic reactions [9]. Methyl fatty acid esters with moderate chain length were chosen for testing at 50 °C, and wide range of enzyme were checked (Table 1). Results indicated that the MML, which is widely used in esterification and acylation [10], was the best catalyst among those enzymes (entry 2). Poor yields were obtained for other hydrolases such as lipase acrylic resin from *Candida antarctica* B (CAL-B), Amano lipase M from *Mucor javanius* (MJL) and lipase from *Candida rugosa* (CRL). The reactions in the absence of enzyme and incubated with denatured MML (pre-treated with urea at 100 °C for 24 h) led to no product after 24 h. All the results reveal that the reaction activity depends on the special spatial conformation of these lipases.

Table 1
Synthesis of 2-undecyl-benzimidazole with different enzymes[a].

Entry	Catalyst	Yield (%)
1	No enzyme	–
2	MML	25.7
3	CAL-B	6.3
4	MJL	6.0
5	CRL	3.2
6	PCL	–
7	BSNP	–
8	AMA	–
9	Denatured[b]	–

[a] *Reaction conditions*: lipase 20 mg, o-phenylenediamine 20 mg, methyl dodecanoate: diamine = 6:1 (mol), *n*-hexane 1 mL, 50 °C, 24 h.
[b] Pretreated with urea at 100 °C for 8 h.

3.2. Effect of reaction media

Reaction media is an important factor in the enzymatic reaction since a given lipase may show distinct activities in different media [11]. Some conventional organic solvents with different log *P* values were screened for the enzymatic synthesis and the results were shown in Table 2. The alkane solvents such as *n*-hexane (yield up to 25.7% in 24 h) and cyclohexane were more effective than the hydrophilic solvents such as THF, dioxane and acetone. Remarkably, this domino reaction gave the best result under solvent-free condition, providing yield up to 37.8% after 24 h. Compared with the reactions employing organic solvents, the solvent-free domino reaction permitted cost saving, high efficiency and less *E* factor [12]. Therefore solvent-free condition was chosen for all the subsequent studies.

3.3. Effect of acyl donors

Further experiments were focused on the influence of different acyl donors in activity. The alcohol part of esters was firstly investigated (Table 3). For the same chain length of fatty acid, methyl decanoate exhibited obviously higher activity than both ethyl decanoate and butyl decanoate (entries 2, 3 and 4), due to the steric effect and the feasibility of releasing alcohols from the system. The results can be explained by the principle that alcohol with lower boiling point was generally easier to be released from a reaction system. The methyl decanoate (71.3%) got higher yield than acid (64.1%) after 60 h, which obviously showing the reaction was accelerated owing to the formation of MeOH. While vinyl ester as a widely used acyl donor was also studied, none of the desired product was observed after 24 h (entry 5). It might be caused by the acetaldehyde formed in the active site quickly reacted with amine in the system, leading to a hindrance to the acylation process.

Table 2
Solvent effect on the yield of 2-undecyl-benzimidazole[a].

Entry	Solvent	Log *P*[b]	Yield (%)[c]
1	*n*-Heptane	4.00	12.4
2	*n*-Hexane	3.90	25.7
3	Cyclohexane	3.40	23.2
4	*n*-Pentane	3.39	21.8
5	Toluene	2.60	2.5
6	CH_2Cl_2	0.93	–
7	THF	0.49	–
8	1,4-Dioxane	−0.50	–
9	Acetone	−0.23	–
10	None	–	37.8

[a] *Reaction conditions*: MML 20 mg, *o*-phenylenediamine 20 mg, methyl dodecanoate:*o*-phenylenediamine = 6:1 (mol), solvent 1 mL, 50 °C, 24 h.
[b] According to the literature.
[c] Determined by HPLC.

Table 3
Synthesis of 2-alkybenzimidazoles with different decanoate[a].

Entry	Ester	Time (h)[b]	Yield (%)[c]
1	Capric acid	24	46.2
2	Methyl decanoate	24	20.7
3	Ethyl decanoate	24	12.8
4	Butyl decanoate	24	9.8
5	Vinyl decanoate	24	C. M.[b]
6	Capric acid	60	64.1
7	Methyl decanoate	60	71.3
8	Ethyl decanoate	60	34.4
9	Butyl decanoate	60	28.7

[a] *Reaction conditions*: MML 20 mg, o-phenylenediamine 20 mg, ester: o-phenylenediamine = 6:1 (mol), 50 °C.
[b] C.M.: complicated mixture, did not detect the aim compound by TLC.
[c] Determined by HPLC.

Various fatty acid esters with alkyl chain length from two to eighteen carbon atoms were incubated with *o*-phenylenediamine under the optimized condition. As shown in Fig. 1, the esters with moderate aliphatic chain (entries 6, 8, 10 and 12) were more fitted, and the best result was the methyl dodecanoate (entry 10). Esters with too short lipophilic group (R) were naturally unsuitable to the active site of enzyme and reflected this on low yields (esters with less than six carbon atoms were almost unreactive). Compared to the literature previously reported by Renard, most of the substrates, especially the short chain substrates (entries 4–8), can get higher yields efficiently with lower amount of enzyme [7]. Like the results in Table 3, all the methyl esters were more active than the ethyl esters.

3.4. Reaction mechanism of enzymatic synthesis of 2-alkylbenzimidazole

However, how did the cyclization achieve after the acylation was finished in the first step was still unclear, was it caused by a catalytic promiscuity of lipase or just reacted spontaneously? To gain further insight into the mechanism of the reaction, we separated the intermediate (a) from the enzymatic reaction system. Structure analysis (^{1}H NMR, ^{13}C NMR, MS and IR) revealed that it was the mono-amide. Controlled experiments were performed to get more information about the mechanism using intermediate (a) as substrate: Benzimidazole product was detected in the presence of fatty acid, whether the lipase was added or not. Interestingly, no product was detected only in the presence of lipase. In addition, the sample incubated with both lipase and fatty acid did not show any difference to the one with only acid added. It was clearly demonstrated that the acid accelerated the cyclization and the lipase did not catalyze the cyclization directly. But where did the acid come from? Since the lipase could efficiently hydrolyze ester into acid and alcohol [13], further study was focused on the ester systems. It can be observed that excess amount of ester could not cyclize the mono-amide without lipase. In contrast benzimidazole product was successfully investigated when reaction was carried out with both ester and lipase. As expected, when the hydrolysis of

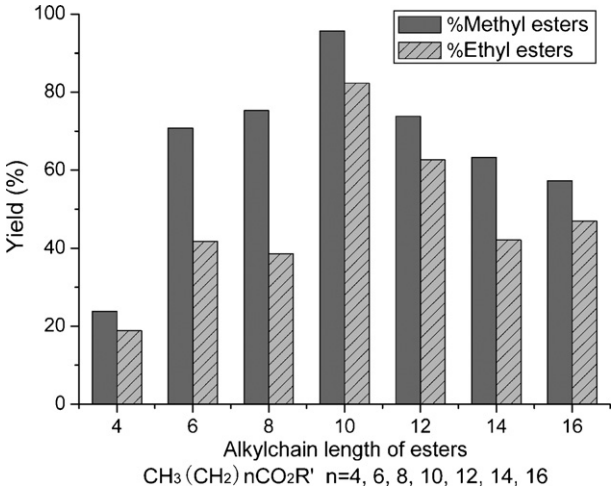
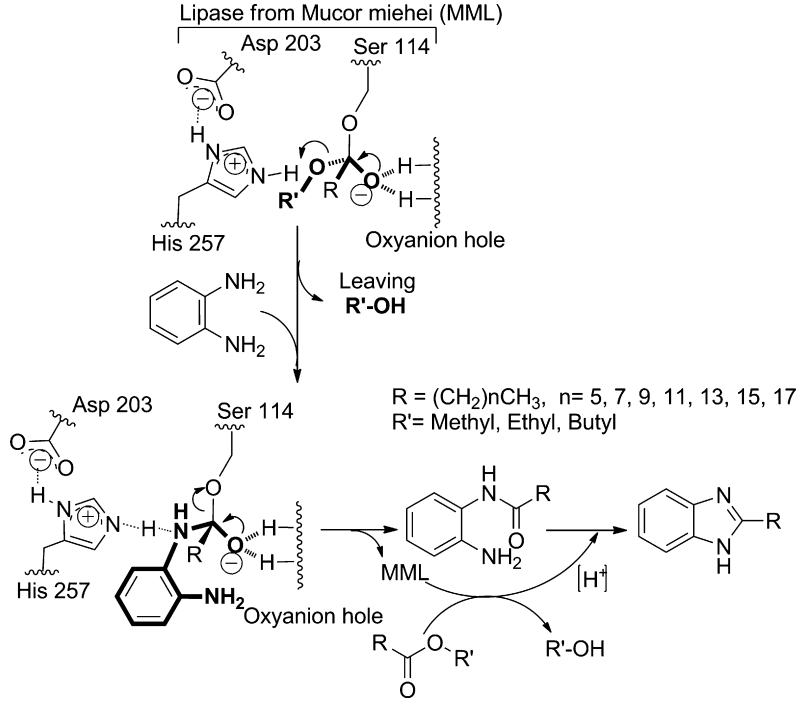


Fig. 1. Effect of chain length on the yield of 2-alkylbenzimidazoles, MML 20 mg, ester: o-phenylenediamine = 6:1 (mol), 50 °C, 60 h, yields were determined by HPLC.



Scheme 2. Proposed mechanism of the acylation/cyclization domino synthesis of 2-alkylbenzimidazole.

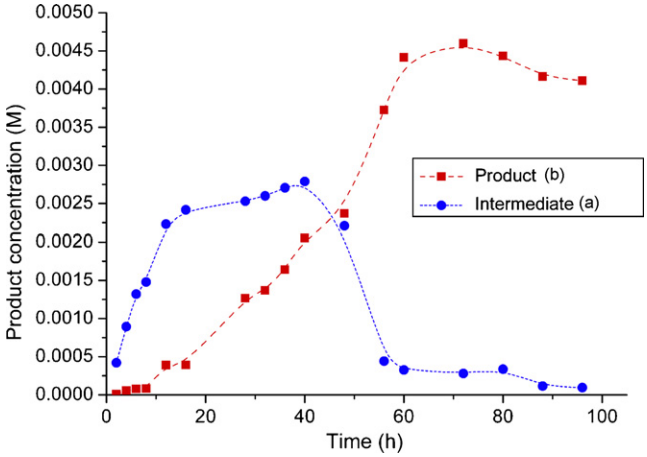


Fig. 2. Progress curves of synthesis of 2-heptadecal-benzimidazole, 50 °C.

ester was inhibited by molecular sieves, no 2-alkybenzimidazole can be detected by TLC and HPLC. Additionally, a signal of fatty acid can be found on the NMR spectrum. All of these results proved that in the domino process the acylation was the first step, and then the weak acid, which was provided by the hydrolyzation of corresponding fatty acid esters, efficiently improved the cyclization of mono-amide to the 2-alkybenzimidazole in the second step (Scheme 2).

The progress of this reaction was inspected by measuring the concentration of intermediate (a) and benzimidazole (b) with HPLC. As shown in Fig. 2, with the increasing concentration of acid in the enzymatic system, the growth rate of benzimidazole (b) was quite slow at the beginning and then accelerated. Meanwhile, the concentration of intermediate (a) increased to the maximum at about 40 h and then gradually decreased to the very low concentration at about 60 h, while the benzimidazole (b) reached the max concentration.

The 2-alkylbenzimidazoles were good donors in Michael addition. They could be easily added to methyl propiolate without any catalyst at room temperature. The potential use of the final product in organic synthesis is still in progress.

4. Conclusions

In conclusion, a facile biocatalysis to 2-alkybenzimidazole has been developed. This lipase-mediated acylation/cyclization domino reaction is carried out in solvent-free condition with less enzyme loaded. Much higher yields are achieved for most of the substrates in shorter reaction time, and a series of substrates are tested. Methyl fatty acid esters with moderated chain length exhibit the best reactivity. Meanwhile, the mechanism has been clarified by detail experiments. This efficient domino process provides an attractive procedure for heterocyclic compound synthesis and may be a potential synthetic method for green organic chemistry.

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