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Candida antarctica lipase B-catalyzed synthesis of acetamides using [BMIm(PF_6)] as a reaction medium

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ABSTRACT

An efficient protocol has been developed for synthesis of acetamides using *Candida antarctica* lipase B (CaL B) in [BMIm(PF_6)] as a greener reaction medium. The reaction is applicable to a wide variety of aliphatic esters/acetic acid and amines providing excellent yields of corresponding acetamide. The catalyst exhibits remarkable activity and is reusable for up to four consecutive cycles.

Biocatalysis in non-aqueous medium replacing the aqueous ones has been widely discussed for more than two decades. However, use of the ionic liquids (ILs) as a reaction medium for biocatalysis has attracted great attention in recent years, as the processes are green and ILs have several advantages compared to conventional reaction media.[1–4] Room temperature ionic liquids (RTILs) are organic salts, which are liquids at room temperature. Their unique properties such as non-volatility, non-flammability, excellent chemical and thermal stability, high polarity, and recyclability, have made them a promising replacement for traditional volatile organic solvents.[5,6] By varying the cations and anions, the physico-chemical properties of ILs such as hydrophobicity, density, viscosity, melting point, polarity, and solvent properties may be tailored in many ways and thus can be made compatible with various biocatalytic processes.[6,7]

Klibanov[8] studied the utility of enzymes in organic solvents and reported the non-aqueous ammonolysis reaction of methyl butyrate with butylamine using porcine pancreatic lipase. Though the use of organic solvents for enzymatic transformation played an important role, their use has raised a lot of questions regarding the environmental hazards, safety, and health issues.[9] When using organic solvents at industrial scale, their post treatment in terms of evaporation and recycling becomes more expensive and involves tedious procedures. Also for many biocatalytic processes, substrates and products have low solubilities in aqueous medium, which require an alternative non-conventional medium such as ionic liquids. Lipase-catalyzed reactions in ILs have been found to be advantageous over those in conventional reaction medium, accompanied by increased activity, enantioselectivity and stability.[10–12] After the first successful report by Erbeldinger et al.[13] on thermolysin-catalyzed synthesis of *Z*-aspartame in [BMIm(PF_6)]/H_2O, the work from Sheldon and co-workers was the second publication to demonstrate the potential use of ILs for enzymatic catalysis and they were the first to use lipases.[14]

An exhaustive research work related to esterification and transesterification catalyzed by lipase has been done but less attention subsists toward amidation reactions. Though some reports on *Candida antarctica* lipase-catalyzed amidation in organic solvent exist,[15] the area of amidation reaction using esters/acetic acid as acyl donors remains unexplored. Considering this issue, we have focused our attention toward the synthesis of acetamides. Acetamides, being biologically active, have been widely used as building blocks for pharmaceutical drugs,[16] clinical applications,[17] enzyme inhibitors,[18] and herbicides.[19]

We, herein, report an efficient protocol for the synthesis of acetamides from esters and acetic acid as acyl donors with aliphatic amines in [BMIm(PF_6)] as a reaction medium using CaL B as a biocatalyst. The catalyst showed remarkable activity and is applicable to a wide variety of aliphatic esters/acetic acid and amines to yield the corresponding acetamides (Scheme 1).

In order to optimize the reaction conditions, reaction of benzyl acetate with benzyl amine in presence of CaL B was chosen as a model reaction.[22] The type of *C. antarctica* lipase B enzyme used

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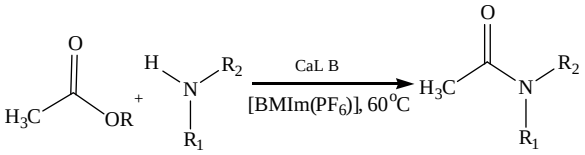


R= Benzyl, vinyl, ethyl, butyl, H.
R_1, R_2 = H, alkyl

Scheme 1. CaL B-catalyzed synthesis of acetamides in [BMIm(PF_6)].

was lipase immobilized on acrylic resin from *C. antarctica* with ⩾10,000 U/g, recombinant, and expressed in *Aspergillus oryzae*. Hence, the amount of enzyme equivalent units used for this study is 200 U. The influence of several parameters such as solvent, temperature, catalyst loading, time, and mole ratio of substrates has been studied (Table 1, entries 1–13).

Lipase-catalyzed reactions are markedly influenced by the reaction media. Recent studies,[4,10,11] have also shown that the activity, stability, and selectivity of the enzymes are considerably enhanced by changing the reaction medium. As compared with organic solvents and ionic liquids, high activity was observed with [BMIm(PF_6)] (Table 1, entries 1–4). Low yield with [BMIm(BF_4)] may be because it is prone to desorb water from the enzyme surface and thus decrease the enzyme activity.[20] Also the half-life period of [BMIm(PF_6)] is greater than that of the other two ILs. The effect of temperature on the activity of CaL B for acetamide synthesis was studied in [BMIm(PF_6)] by varying temperatures ranging from 30 °C to 80 °C. It was observed that with increase in temperature the yield of acetamide increases initially (Table 1, entries 5–7). However, at higher temperature, i.e., 80 °C the yield decreases (Table 1, entry 7), reflecting that 60 °C is the optimum temperature. In an effort to determine the optimum concentration of the catalyst, various amounts of catalyst loadings were deliberated as shown in Table 1 (entries 8 and 9). The optimum results were observed with respect to yield 94% (Table 1, entry 3), demonstrating that further increase in the catalyst concentration has no significant effect on the yield of acetamides. A remarkable increase in yield, that is, 94% (Table 1, entry 3) was obtained when molar ratio of benzyl acetate/benzyl amine (1.2:1) was used whereas the equimolar concentration of substrates (1:1) yielded 81% of acetamides (Table 1, entry 11). The control reaction experiments were also carried out in absence of the enzyme keeping other reaction parameters constant, which demonstrated that only the enzyme CaL B is responsible for the respective transformations (Table 1, entries 12 and 13). Hence, the optimum reaction parameters were: ionic liquid [BMIm(PF_6)] 0.5 ml, temperature 60 °C, CaL B loading 20 mg, and time 24 h. Optimized reaction conditions were subsequently applied for amidation of various esters or acetic acid with different aliphatic and alicyclic amines providing moderate to good yields of the desired products (Table 2, entries 1–19).

Table 1
Effect of reaction parameters on amidation reaction catalyzed by CaL B[a]

Entry	Solvent	Temp (°C)	Catalyst loading (mg)	Yield[b] (%)
Influence of solvent				
1	[BMIm(BF_4)]	60	20	76
2	[BMIm(NTF_2)]	60	20	80
3	[BMIm(PF_6)]	60	20	94
4	Di-iso propyl ether	60	20	66
Influence of temperature				
5	[BMIm(PF_6)]	30	20	51
6	[BMIm(PF_6)]	45	20	62
7	[BMIm(PF_6)]	80	20	81
Influence of catalyst loading				
8	[BMIm(PF_6)]	60	10	70
9	[BMIm(PF_6)]	60	30	95
10[c]	[BMIm(PF_6)]	60	20	76
11[d]	[BMIm(PF_6)]	60	20	81
12[e]	[BMIm(PF_6)]	60	—	0
13[e]	[BMIm(BF_4)]	60	—	0

[a] Reaction conditions: Benzyl acetate (0.3 mmol), benzyl amine (0.25 mmol), solvent (0.5 ml), catalyst (CaL B lipase), molecular sieves 4 Å (35 mg), time = 24 h.
[b] Yields based on GC analysis.
[c] Time = 18 h.
[d] Benzyl acetate/benzyl amine molar ratio is 1:1.
[e] Without catalyst.

It was observed that CaL B has the potential to catalyze the reactions of four different esters as acyl donors with a comparative high yield with butyl acetate (Table 2, entries 1–4). On the other hand, CaL B was effective as catalyst only with aliphatic amines. The reaction was smooth for simple aliphatic amines whereas yield was very poor for the alicyclic amines. *n*-Butylamine and *n*-hexylamine were found to react smoothly with benzyl acetate providing good yields of the desired acetamides (Table 2, entries 5 and 6) whereas with cyclohexylamine the yield had decreased (Table 2, entry 7). The system also permits the reaction of benzyl acetate with secondary amines such as piperidine with moderate yields of the corresponding acetamide (Table 2, entry 8). The yield decreases with butylacetate as we move down from *n*-hexylamine to cyclohexylamine (Table 2, entries 10–12). Vinyl acetate is the most commonly used acyl donor for lipase-catalyzed acylation or esterification reactions.[3] Vinyl acetate has been observed to be an efficient donor with benzyl amine as well as with long chain aliphatic dodecamine for acetamide-synthesis producing moderate to good yields (Table 2, entries 4 and 13). Also ethyl acetate served as a good acyl donor producing corresponding acetamide (Table 2, entry 14).

Carboxylic acid has scarcely been used as acyl donor as it is supposed that the spontaneous formation of unreactive salt with amine would prevent the formation of the acyl–enzyme complex. However, the salt formation is reversible reaction. Montet et al.[24] demonstrated that the amide bond was synthesized by lipase in hexane, though with a very low reaction rate of 60% conversion of amine in 12 days. Also it is noteworthy to mention that while using aliphatic acids[25] with racemic 2-ethylhexyl amine, no amidation reaction had occurred in organic solvents, i.e., diisopropyl ether, tetrahydrofuran, and acetonitrile using CaL B and hence it was carried out in bulk (without solvent) at 90 °C under vacuum. In contrary, the use of acetic acid as an acyl donor has been found to be effective for acetamide-synthesis in ionic liquid [BMIm(PF_6)]. For these particular transformations, water was produced instead of alcohols and it had no adverse effects on the enzyme activity. Acetic acid as acyl donor reacted smoothly with the aliphatic amines producing the good yields of the corresponding acetamides (Table 2, entries 15 and 16). However, as like with esters, the acetic acid when reacted with secondary amines such as morpholine and pyridine gave lower yields of 40% and 62%, respectively.(Table 2, entries 17 and 18).

The aromatic amines such as aniline were not compatible as substrates for this particular protocol (Table 2, entries 9 and 19). Basicity is one of the key factors differentiating the reactivity of aliphatic amines and aromatic amine, for example, aniline. It is well known that aromatic amines are weak bases due to the aromaticity. We think that it may be contributing to the results obtained in present work. In case of acetic acid as an acyl donor in the reaction with aniline, a low yield of 20% was obtained as compared with benzyl acetate wherein the reaction did not proceed. Again this may be due to the steric hindrance to N lone pair between these two moieties.

Table 2
Reactions of esters/acetic acid with amines catalyzed by CaL B in [BMIm(PF_6)][a]

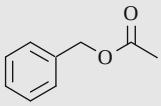
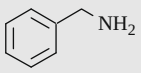
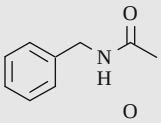
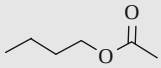
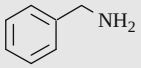
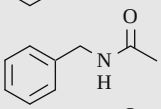
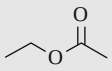
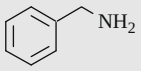
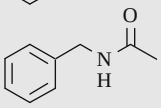
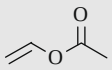
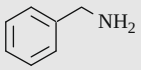
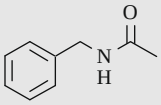
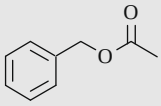
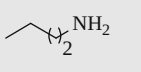
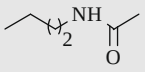
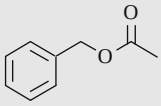
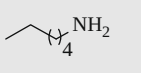
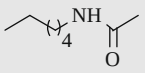
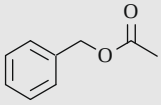
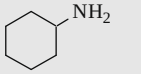
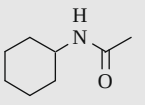
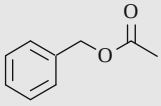
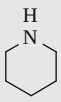
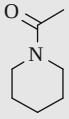
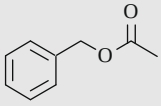
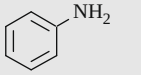
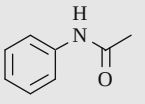
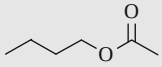
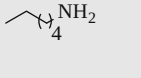
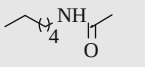
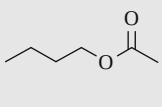
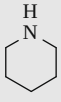
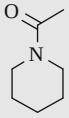
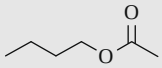
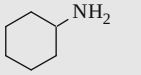
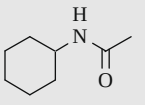
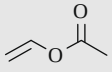
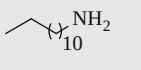
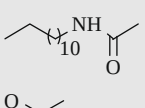
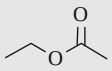
No.	Ester/Acid	Amine	Product	Yield [b] (%)
1				94 (90)
2				97 (92)
3				82 (77)
4				64 (61)
5				87 (82)
6				87 (84)
7				32 (27)
8				61 (57)
9				0
10				91 (88)
11				64 (59)
12				35 (28)
13				95 (90)
14				44 (39)
15				96 (91)

Table 2 (*continued*)

No.	Ester/Acid	Amine	Product	Yield [b] (%)
16				84 (79)
17				40 (35)
18				62 (57)
19				20 (14)

[a] Reaction conditions: Acetate/acetic acid (0.3 mmol), amine (0.25 mmol), [BMIm(PF_6)] (0.5 ml), CaL B (20 mg), molecular sieves 4 Å (35 mg), time = 24 h.
[b] Yields based on GC analysis, the yields given in the parentheses are the isolated yields.

The reaction mechanism involves covalently linked acyl–enzyme intermediate that deacylates via nucleophilic attack of amine. Generally one of the possible reasons for lower yield with alicyclic amines is that lipase, being a serine hydrolase, depends on the accessibility of the nucleophile to attack and cleave the acyl–serine bond inside the cavity of the active site. Lipase usually discriminates against certain acyl chain length, degrees of unsaturation and location of double bond in the chain. Any of these factors could affect the interaction between the acyl chain and the active site. Also, the release of smaller alcohols may cause deactivation of the enzyme to some extent; similar results were observed with esterification using goat pregastric lipase where the rate of formation of ethyl butyrate was found to be lower than that of butyl butyrate.[21a] Hence, the yield was more for butyl acetate than for other acetates. Also, the alicyclic amines find it difficult to approach the catalytic site of the enzyme due to their size and thus produce a poor yield.[21b] In addition, cyclic structures could produce steric hindrance thus reducing the product yield.

In order to make the biocatalytic processes economical at large scale, the recyclability of the enzyme in ionic liquids has to be taken into consideration.[23] During this study, enzyme–ionic liquid reaction system was recycled up to four cycles. There was no significant decrease during first cycle whereas the yield declined up to 65% after the completion of the fourth cycle as shown in Figure

Figure 1. Reaction conditions: Benzyl amine (0.25 mmol), benzyl acetate (0.3 mmol), 0.5 ml of [BMIm(PF_6)], 20 mg CaL B, 35 mg molecular sieves 4 Å at temp = 60 °C for time = 24 h.

1. This decrease in yield might be due to handling loss of the enzyme during recycles or inactivation of the enzyme as the cycles are increased.

In conclusion, we have studied the behavior of CaL B in ionic liquid for acetamide-synthesis. The ionic liquid has influenced the stability and yield of the acetamides with considerable recyclability. These advantages thus suggest that ionic liquid would be used as green alternative reaction medium for acetamide-synthesis.

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References and notes

1. Sheldon, R. A.; Lau, R. M.; Sorgedrager, M. J.; Rantwijka, F. V.; Seddonb, K. R. *Green Chem.* **2002**, *4*, 147–151.
2. Seddon, K. R. *J. Chem. Technol. Biotechnol.* **1997**, *68*, 351–356.
3. (a) Seongsoon, P.; Kazlauskas, R. J. *Curr. Opin. Biotechnol.* **2003**, *14*, 432–437; (b) Seongsoon, P.; Kazlauskas, R. J. *J. Org. Chem.* **2001**, *66*, 8395–8401; (c) Davis, B. G.; Boyer, V. *Nat. Prod. Rep.* **2001**, *18*, 618–640; (d) Xu, Q.; Geng, X.; Chen, P. *Tetrahedron Lett.* **2008**, *49*, 6440–6441.
4. Rantwijk, F. V.; Lau, R. M.; Sheldon, R. A. *Trends Biotechnol.* **2003**, *21*, 131–138.
5. Yang, Z.; Pan, W. *Enzyme Microb. Technol.* **2005**, *37*, 19–28.
6. Huddleston, J. G.; Visser, A. E.; Reichert, W. M.; Willauer, H. D.; Broker, G. A.; Rogers, R. D. *Green Chem.* **2001**, *3*, 156–164.
7. (a) Dzyuba, S. V.; Bartsch, R. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 148–150; (b) Holbrey, J. D.; Seddon, K. R. *J. Chem. Soc., Dalton. Trans* **1999**, *13*, 2133–2139.
8. Klibanov, A. M. *Nature* **2001**, *409*, 241–246.
9. Blanchard, L. A.; Hancu, D.; Beckman, E. J.; Brennecke, J. F. *Nature* **1999**, *399*, 28–29.
10. Kim, K. W.; Song, B.; Choi, M. Y.; Kim, M. J. *Org. Lett.* **2001**, *3*, 1507–1509.
11. Lozano, P.; Diego, T. D.; Carrie, D.; Vaultier, M.; Iborra1, J. L. *Biotechnol. Lett.* **2001**, *23*, 1529–1533.
12. Gordon, C. M. *Appl. Catal. A* **2001**, *222*, 101–117.
13. Erbeldinger, M.; Mesiano, A. J.; Russell, A. J. *Biotechnol. Prog.* **2000**, *16*, 1129–1131.
14. Lau, R. M.; Rantwijk, F. V.; Seddon, K. R.; Sheldon, R. A. *Org. Lett.* **2000**, *2*, 4189–4191.
15. (a) Garcia, M. J.; Rebolledo, F.; Gotor, V. *Tetrahedron* **1994**, *23*, 6935–6940; (b) Astorga, C.; Rebolledo, F.; Gotor, V. *J. Chem. Soc., Perkin Trans. 1* **1994**, 829–832; (c) Castillo, E.; Gavilan, A. T.; Severiano, P .; Arturo, N.; Munguia, A. L. *Food Chem.* **2007**, *100*, 1202–1208; (d) Tufvesson, P.; Annerling, A.; Kaul, R. H.; Adlercreutz, D. *Biotechnol. Bioeng.* **2007**, *97*, 447–453; (e) Adamczyk, M.; Grote, J. *Tetrahedron Lett.* **1996**, *37*, 7913–7916; (f) Adamczyk, M.; Grote, J. *Tetrahedron: Asymmetry* **1997**, *8*, 2099–2100; (g) Gotor-Fernández, V.; Busto, E.; Gotor, V. *Adv. Synth. Catal.* **2006**, *348*, 797–812.
16. (a) Rivera, D. G.; Wessjohann, L. A. *Molecules* **2007**, *12*, 1890–1899; (b) Isoherranen, N.; Yagen, B.; Soback, S.; Roeder, M.; Schurig, V.; Bialer, M. *Epilepsia* **2001**, *42*, 825–830.
17. (a) Kelly, N. M.; Falkiner, F. R.; Keane, C. T. *J. Clin. Microbiol.* **1983**, *17*, 159; (b) Dennes, T. J.; Hunt, G. C.; Schwarzbauer, J. E.; Schwartz, J. *J. Am. Chem. Soc.* **2007**, *129*, 93–97.
18. Paul, G.; Barry, P.; Lydia, S.; Henriette, W.; Jac, W. Bacterial Enzyme Inhibitors, Patent Pub.no. WO/2003/048114.
19. Jeffrey, A.; Bunting, F.; Simmons, W.; Wax, L. M. *Weed Technol.* **2003**, *17*, 291–296.
20. Persson, M.; Bornscheuer, U. T. *J. Mol. Catal.: B Enzym.* **2003**, *22*, 21–27.
21. (a) Lai, D. T.; O'Connor, C. J. *J. Mol. Catal.: B Enzym.* **1999**, *6*, 411–420; (b) Ema, T.; Maeno, S.; Takaya, Y.; Sakai, T.; Utaka, M. *J. Org. Chem.* **1996**, *61*, 8610–8616.
22. In a typical experimental procedure, to 0.5 ml of ionic liquid, 0.3 mmol of benzyl acetate and 0.25 mmol of benzyl amine were added. In order to maintain the water activity of enzyme 70 mg/ml of molecular sieves 4 Å was added. The reaction was initiated by adding 20 mg of the enzyme and reaction mixture was stirred at 60 °C for 24 h. After completion, 2–3 ml of di-isopropyl ether was added and vigorously shaken to extract all the reactants and products in the ether phase. The extraction procedure was repeated for about 3–4 times. The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The residue obtained was purified using column chromatography (silica gel, mesh size 60–120) using pet ether/ethyl acetate (90:10) as eluent to afford the pure products. The products were known and were compared with authentic samples. The products are characterized by ^{1}H NMR (JEOL FT-NMR, Model-AL300), GC–MS (Shimadzu QP 2010), FT-IR (Perkin Elmer).
Spectral data for selected products:
Benzyl acetamide, Table 2, *entry 1*: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.99 (s, 3H), 4.34 (s, 2H), 6.75 (br s, 1H), 7.24 (s, 5H) ppm. MS (70 eV, EI): *m*/*z* (%): 149 (75) (M^+), 106 (100), 91 (35). IR (KBr): ν = 1650 (CO), 3287 (NH).
n-Hexyl acetamide, Table 2, *entry 6*: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.88 (s, 3H), 1.28 (s, 6H), 1.43–1.49 (t, 2H), 1.97 (s, 3H), 3.19–3.25 (q, 2H), 5.90 (br s, 1H) ppm. MS (70 eV, EI): *m*/*z* (%): 143 (20) (M^+), 128 (15), 72 (90), 43 (100). IR (KBr): ν = 1652 (CO), 3274 (NH).
Cyclohexyl acetamide, Table 2, *entry 7*: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.09–1.68 (m, 10H), 1.95 (s, 3H), 3.48 (s, 1H), 5.43 (br s, 1H) ppm. MS (70 eV, EI): *m*/*z* (%): 141 (20) (M^+), 98 (15), 60 (100), 43 (50). IR (KBr): ν = 1637 (CO), 3291 (NH).
Morpholine acetamide, Table 2, *entry 14*: ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.10 (s, 3H), 3.45–3.70 (m, 8H) ppm. MS (70 eV, EI): *m*/*z* (%): 129 (35) (M^+), 114 (20), 86 (50), 57 (100), 43 (85). IR (KBr): ν = 1630 (CO).
23. For recyclability study, after complete extraction procedure, the reaction medium containing CaL B and molecular sieves 4 Å was kept in desiccator for 24–48 h. Then the molecular sieves 4 Å were removed very carefully. The fresh reactants were added and the reaction was carried out at 60 °C for 24 h. After completion of the reaction, extraction was done and the same procedure was followed for consecutive recycling.
24. Montet, D.; Pina, M.; Graille, J.; Renard, G.; Grimaud, J. *Fat. Sci. Technol.* **1989**, *91*, 14–18.
25. Prasad, K. A.; Husain, M.; Singh, K. B.; Gupta, R. K.; Manchanda, V. K.; Olsend, C. E.; Parmar, V. S. *Tetrahedron Lett.* **2005**, *46*, 4511–4514.