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Lipase-catalyzed enantioselective reaction of amines with carboxylic acids under reduced pressure in non-solvent system and in ionic liquids

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Abstract—Lipase-catalyzed enantioselective acylation of 1-phenylethylamine and 2-phenyl-1-propylamine was performed by reacting the amines with carboxylic acids in a non-solvent system or in ionic liquids as reaction media. The reaction equilibrium was shifted toward amide synthesis by the removal of formed water under reduced pressure.

Lipase-catalyzed acylation is one of the currently employed methods for the resolution of primary amines. Unlike the alcohols, for which vinyl esters are the most common acylating agents, less reactive compounds are required for the much more nucleophilic amines to avoid spontaneous non-enzymatic reactions. Simple esters or carbonates are usually employed.[1] Carboxylic acids have been scarcely used as acyl donors as it was supposed that the spontaneous formation of an unreactive salt with the amine would prevent the formation of the acyl-enzyme complex. However, the salt formation is a reversible reaction with the salt being in equilibrium with some amounts of free acid and amine. This small amount of acid is available to the enzyme, and Montet et al.[2] demonstrated that the amide bond was synthesized by lipase in hexane, though with a very low reaction rate: 60% conversion of amine in 12 days. The acylation reaction is also a reversible reaction with the amide and water on the synthetic side of the equilibrium. In this study, we tried to develop an effective method for enantioselective acylation of primary amines with carboxylic acids catalyzed by lipase in a non-solvent system or in ionic liquids as reaction media. The reaction equilibrium was displaced in favor of amide formation by the continuous removal of the by-product water under low pressure (Scheme 1). Two primary amines: 1-phenylethylamine (**1**) with the chiral center at the α-carbon atom, and 2-phenyl-1-propylamine (**2**) with the chiral center at the β-carbon atom were used as substrates in the reactions catalyzed by immobilized *Candida antarctica* lipase B (CALB).

The immobilized CALB catalyst (Chirazyme L-2, c.-f, C2, Lyo.) (EC 3.1.1.1.) was purchased from Roche Diagnostics (Mannheim, Germany). The experiments in non-solvent system were carried out as follows: the lipase (23 mg) was added to a mixture of amine (1 mmol) and acid (1 mmol) under stirring at a specified temperature and 5 mm Hg vacuum was applied to the system. The reaction was stopped by neutralizing the unreacted acid with NaOH solution (2 N) followed by extraction of amine and amide with diethyl ether. The experiments in ionic solvents were carried out with the same amounts as above by adding the lipase to a mixture of amine and acid in ionic solvent (1.5 mL) and connecting the system to a vacuum pump. The reaction was stopped by the extraction of the reaction mixture with diethyl ether. The amine and amide were extracted separately according to Gonzalez-Sabin et al.[3] The enantiomeric compositions of the residual amine and the formed amide were determined by HPLC on a Chiralpak AD-H (Dicel Chemical Industries, Tokyo, Japan) column eluted with a mixture of hexane/2-propanol/diethylamine with a volumetric ratio of 95:5:0.05. The

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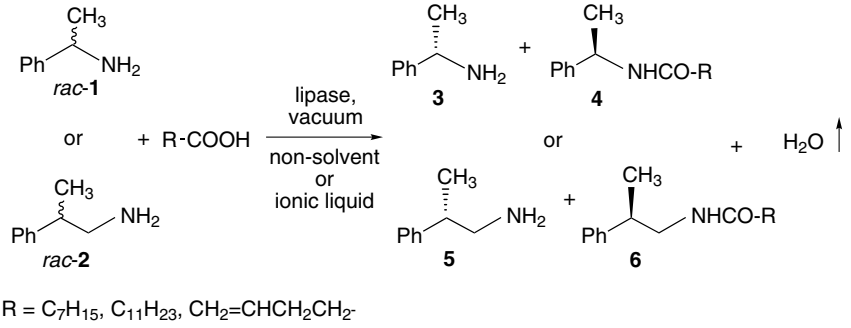

Scheme 1. Lipase-catalyzed acylation of primary amines.

(*R*)-enantiomer was preferentially acylated for amine **1** while for amine **2**, it was the (*S*)-enantiomer. The absolute configuration of amine **5** was determined by comparison with the retention times of the pure enantiomer standards on chiral HPLC. The enantiomeric excess of amine **1** was determined after its transformation into the corresponding acetamide (acetic anhydride, pyridine). The (*S*)-configuration of amine **3** isolated from the reaction mixture and transformed in acetamide was determined after comparison of the retention times with the enantiomerically pure (*R*)- and (*S*)-acetamides of **1** prepared chemically from pure enantiomers of **1**. The absolute configuration of the formed amides **4** and **6** was determined similarly using chemically prepared amide enantiomer standards (carboxylic acid, DCC, DMAP in dichloromethane).

First, the feasibility of a non-solvent system was investigated. The reactions with equimolar amounts of amine **1** and octanoic acid (Table 1, entry 1), and dodecanoic acid (entry 2) were performed. For both, the reaction mixtures were very viscous and the reaction temperature had to be raised to 55 °C in order to achieve an efficient stirring in the system. Although a much slower reaction was expected, the rate and enantioselectivity for the reaction with octanoic acid were close to that obtained in a previous study, which used ethyl octanoate as acyl donor in a non-solvent system under reduced pressure.[4] An explanation might be that the negative effect on the reaction rate produced by the formation of the salt with the amine was compensated by the higher specificity of the enzyme for the acid than for the corresponding ethyl ester.[5] Dodecanoic acid was a better acyl donor: the reaction was faster and highly enantioselective. A similar effect was observed for these acids in our previous study on enantioselective esterification of secondary alcohols with free fatty acids in non-solvent system.[5] The pent-4-enoyl group is an amine protecting group that is readily cleaved under mild conditions[6] and therefore, it is worth investigating the use of 4-pentenoic acid as acyl donor for enzymatic acylation of amines. The reaction with 4-pentenoic acid was very sluggish for amine **1** (entry 5), but proceeded with a moderate rate for amine **2** (entry 7). Amine **2** can access more easily the active site of the enzyme due to the remote stereogenic center. Therefore, the reaction is faster, but the enantioselectivity is much lower than for **1**.

Ionic liquids are increasingly used as reaction media in organic syntheses as they offer a wide range of advantages over classical organic solvents.[7] Recently, enzymatic transformations were also performed successfully and in many cases, with remarkably improved results in ionic liquids.[8] Lau et al.[8a] reported the first examples of lipase-catalyzed reactions performed in ionic liquids in the absence of water. First asymmetric enzymatic reactions in ionic liquids were reported almost at the same time by Itoh et al.[8b] and Schöfer et al.[8c] Both studies dealt with kinetic resolution of secondary alcohols by acylation with vinyl acetate and demonstrated that the enzyme suspended in the ionic liquids could be reused efficiently several times. In a further development of the

Table 1. Lipase-catalyzed enantioselective acylation of primary amines with carboxylic acids

Entry	Amine	Acid	Solvent	Temperature (°C)	Initial rate (μmol/h mg)[a]	Reaction time (h)	Conversion amine (%)	Ee_S (%)[b]	Ee_P (%)[c]	*E*
1	**1**	Octanoic	None	55	0.57	24	31.6	44.8	97.1	106
2	**1**	Dodecanoic	None	55	0.88	19	44.5	80.3	>99.0	>500
3	**1**	Dodecanoic	[bmim]PF_6	55	1.67	19	48.9	95.9	>99.0	>500
4	**1**	Dodecanoic	[emim]BF_4	55	0.38	24	20.7	26.2	>99.0	>500
5	**1**	4-Pentenoic	None	30	0.005	24	0.6	0.6	>99.0	>500
6	**1**	4-Pentenoic	[bmim]PF_6	30	0.07	24	8.0	8.7	>99.0	>500
7	**2**	4-Pentenoic	None	30	0.31	24	38.2	36.9	59.8	6
8	**2**	4-Pentenoic	[bmim]PF_6	30	1.92	5	48.1	24.5	48.1	2
9	**2**	4-Pentenoic	[emim]BF_4	30	0.71	5	17.8	5.3	25.7	2

[a] Amount of amine (μmol) esterified per hour and milligram of immobilized enzyme.
[b] Enantiomeric excess of unreacted amine.
[c] Enantiomeric excess of resulting amide.

ionic liquid system, Itoh et al.[8d] used reduced pressure to remove resulting methanol from the enantioselective transesterification of 5-phenyl-1-penten-3-ol with methyl esters and improved the reaction rates and yields. The reasons for investigating the use of ionic liquids as reaction media for our system are the following: (1) they have no detectable vapor pressure and therefore can be used under reduced pressure, and (2) they are very good solvents for a wide range of organic compounds. Ionic liquids have the ability to dissolve compounds with relatively different polarities (in this work: the substrates and the resulting amide) so that they can improve the properties of the reaction mixture and alleviate the diffusional limitations of a very viscous reaction mixture, as in our case. In an attempt to improve the reaction performance, two ionic liquids with different properties, the hydrophobic 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6) and the hydrophilic 1-ethyl-3-methylimidazolium tetrafluoroborate ([emim]BF_4), were tested as reaction media. The initial rate for the reaction of amine **1** with dodecanoic acid doubled in [bmim]PF_6 (entry 3), but reduced to half in [emim]BF_4 (entry 4). The rate was raised more than an order by performing the reaction of **1** with 4-pentenoic acid in [bmim]PF_6 (entry 6). The enantioselectivity remained very high for the reactions of **1** in both ionic liquids. Different results were obtained for the reactions of amine **2** with 4-pentenoic acid. In both ionic liquids, the initial reaction rate increased (entries 8 and 9) (with a more remarkable effect for [bmim]PF_6), but the enantioselectivity decreased to less than half of the value in non-solvent system.

To our knowledge, the results presented in this study demonstrated for the first time the amide synthesis in non-solvent system by the direct lipase-catalyzed reaction of amines with carboxylic acids and its use for kinetic resolution of primary amines. This system is the enzymatic equivalent to the general procedure for amide synthesis involving the heating of a mixture of amine and acid at about 200 °C.[9] The present enzymatic procedure offers some important advantages over the chemical one for the application in industrial processes. The reaction temperatures are significantly reduced and as a result its applications can be extended to thermally labile compounds. In addition, the enantioselectivity of lipase allows the kinetic resolution of chiral substrates. In comparison to other lipase-catalyzed systems used to date, the reaction rates, conversions and enantioselectivities are comparable to the reactions employing other more expensive acyl donors such as esters or carbonates.[10] Better reaction rates were achieved by the use of ionic liquids as reaction media. We intend to further improve the performances of the system by expanding this study to a larger variety of ionic liquids. Finding the best reaction medium is quite a complicated and tedious task as its performances depend on the nature of each substrate and expected product and in many cases, the most performing reaction medium is very different even for similar compounds.[10] This work is made easier by the fact that the ionic liquid properties (polarity, viscosity, density, solubility, etc.) can be finely or drastically modified according to the one's purpose by changing the anion or the components of the cation.[7,8,10]

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