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Enzymatic resolution of *sec*-butylamine

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Abstract—Resolution of (±)-*sec*-butylamine by *Candida antarctica* lipase provided a very low enantiomeric excess of the residual amine when either ethyl or vinyl butyrate was used as the acylating agent. The enantiomeric excess was increased by using ethyl esters of long chain fatty acids. The rate of the reaction was increased by using methyl *t*-butyl ether as a solvent. (*S*)-*sec*-Butylamine of very high enantiomeric excess was obtained by *C. antarctica* lipase catalyzed acylation with ethyl decanoate in methyl *t*-butyl ether.

1. Introduction

Chiral amines are important intermediates for many bulk drug substances. There are many reports on the resolution of amines by enzymes.[1–6] Though proteases[1] and lipases[2–6] have been used for the resolution, the lipase from *Candida antarctica* was shown to be the most promising for the resolution of primary amines.[2–6] The enzyme was shown to be especially good for the resolution of arylalkylamines, for example, α-phenylethylamine, but the enantioselectivity was reported to be lower for shorter chain amines. The enantiospecificity of the resolution could be enhanced by changing solvents. However, enzyme catalyzed acylation with *C. antarctica* lipase gave only an enantiomeric excess (ee) of 70% for the short chain aliphatic amine *sec*-butylamine.[6] This ee is too low for synthetic purposes. Herein we report a process for obtaining *sec*-butylamine in high ee by an enzyme catalyzed acylation.

2. Results and discussion

Lipases and proteases were evaluated for the resolution of (±)-*sec*-butylamine using either heptane or decane as solvent and vinyl butyrate or ethyl butyrate as the acylating agent (Scheme 1). In many cases, there was significant enzyme catalyzed amide formation as observed by reduction in the amount of amine. Only five enzymes showed a selectivity in the initial screening (Table 1), but the *E*-values[7] were low (<10) even in the best cases.

Enzymatic hydrolysis of the butyryl amide of (±)-*sec*-butylamine was also investigated. Except for a few cases, there was very little hydrolysis with these enzymes. Novozym 435 from *C. antarctica* showed the most promising results with about 41% conversion and about 21% ee for the (*R*)-amine over 4 days.

Esters are true substrates for the lipases. Since the few successful enzymes showed very low enantioselectivity (Table 1) with either ethyl or vinyl butyrate as the acylating agent, the next set of experiments sought to improve the enantioselectivity of Novozym 435, ChiroCLEC BL and Acylase by changing the ester. In the first trial, esters of acetic acid (ethyl acetate, propyl acetate, butyl acetate, vinyl acetate, isopropenyl acetate, etc.), esters of butyric acid (ethyl butyrate, propyl butyrate, butyl butyrate, etc.) and three esters of longer chain acids (vinyl dodecanoate, ethyl decanoate and ethyl oleate) were investigated in heptane. ChiroCLEC BL and Acylase did not show significant improvement worthy of further consideration. Novozym 435 showed significantly better selectivity with increasing number of carbon atoms in the acid chain. With Novozym 435, ethyl decanoate gave an ee of 90.6% for the (*S*)-amine with 40% residual amine after 7 days. The ee of the residual (*S*)-*sec*-butylamine for the ethyl butyrate and ethyl oleate reactions were 49.1% and 70.9%, respectively, after 7 days. Although the *E*-value increased when increasing the chain length, the reaction rate slowed down.

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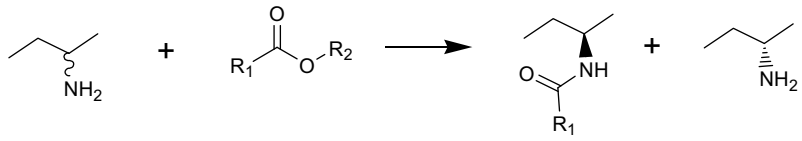

Scheme 1.

Table 1. Result of initial screening of enzymes for the resolution of (±)-*sec*-butylamine

Enzyme	Source	Vendor	Acylating agent	Enzyme to amine ratio	Time (days)	Conversion (%)	Ee (%) (*S*)-amine	*E*-Value
Acylase 30000	*Aspergillus* sp.	Amano	Vinyl butyrate	25	1	53	6.2	1
Novozym 435	*Candida antarctica*	Novo	Ethyl butyrate	14	1	15	3.3	2
			Ethyl butyrate	50	7	55	74.6	9
Peptidase R	*Rhizopus hangchow*	Amano	Vinyl butyrate	14	1	51	16.5	2
Prozyme 6	*Aspergillus melleus*	Amano	Vinyl butyrate	14	1	34	21.4	3
ChiroCLEC BL	*Bacillus globigii*	Altus	Vinyl butyrate	14	1	50	29.7	2

A second set of resolutions of (±)-*sec*-butylamine was performed in heptane using Novozym 435 with ethyl esters of fatty acids of increasing carbon chain length. Aliphatic acids with even numbers of carbons from 2 to 18 (acetate, butyrate, hexanoate, octanoate, decanoate, dodecanoate, tetradecanoate, hexadecanoate and octadecanoate), and one unsaturated (*cis*-9-ocatadecanoate) were investigated. The non-enzymatic acylation was not significant. There was some reduction in the amount of residual amine even with no acylating agent and no enzyme after extended periods of time, most probably due to oxidation and/or some evaporation of low boiling *sec*-butylamine. Therefore, although the conversion and residual amine information could be qualitatively used for comparison, their absolute values were somewhat less quantitative. There are some interesting patterns in the results for this series of esters (Fig. 1). Ethyl octanoate (C8) showed some discrepancies from the general trend perhaps due to some impurity. The initial rate (as measured by conversion after 1 day) decreased when increasing the carbon chain length of the acid. The rate change was significant from C2 to C6 but was much less pronounced for the longer chains. The ee of the residual amine was almost negligible (ee ~ 0) for C2 (acetate). The ee increased with increasing chain length from C2 to C4 to C6. Thereafter the ee of the residual amine showed only a slight improvement up to C18 (saturated). For acid carbon chains $\geqslant 6$ the ee was $\geqslant 95\%$ with conversions of 50–60% (residual amine 40–50%) after 7 days. The highest ee of 98.8% was achieved with ethyl octadecanoate (C18) after 7 days. With the only unsaturated ester ethyl *cis*-9-ocatadecanoate (C18, *cis* double bond at 9), the ee and conversion were less than the corresponding saturated ester (ethyl octadecanoate). *E*-Values showed the general trend of increasing with increasing chain length of the acid. The increase was highest from 2 to 6 and only slightly changed above C6. Since the ee was within a narrow range from ethyl decanoate to ethyl octadecanoate while the residual amine was slightly higher with ethyl decanoate, ethyl decanoate was used for further optimization of ee, yield and reaction rate.

Solvents have been reported to have significant effect on enzymes in general[1] and, in particular, the enzyme from *C. antarctica*.[4] Various solvents were examined with

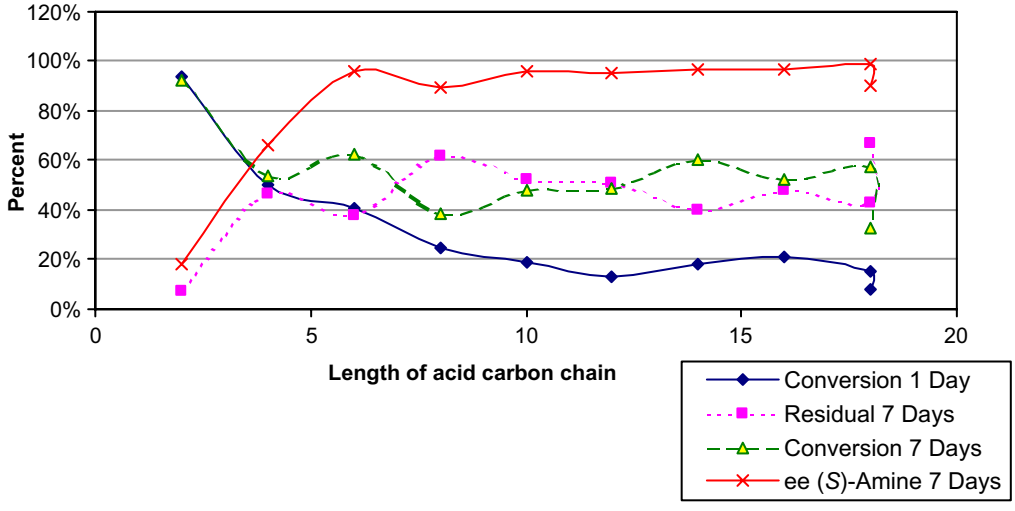

Figure 1. Novozym 435 catalyzed resolution of (±)-*sec*-butylamine with ethyl esters of fatty acids of different chain length.

Novozym 435 in combination with various ethyl esters. Considering the yield, ee and reaction time, ethyl decanoate showed the best results (Table 2). The reaction proceeded faster in MTBE and in acetonitrile when compared to heptane. Acetonitrile, MTBE and heptane were further evaluated with ethyl decanoate at lower enzyme amounts and higher substrate concentration. Again, both acetonitrile and MTBE showed faster conversions compared to heptane (Table 3). In order to facilitate the isolation process, the water immiscible solvent MTBE was preferable over acetonitrile.

Table 2. Novozym 435 catalyzed resolution of (±)-*sec*-butylamine with ethyl decanoate in various solvents

Solvent	Time (days)	Ee (%) (*S*)-amine
Acetonitrile	1	91.2
	4	99.6
Cyclohexane	1	16.4
	4	95.1
Toluene	1	16.0
	4	75.9
Methyl-*t*-butyl ether	1	71.7
	4	98.0
2-Methyl-2-pentanol	1	4.9
	4	19.1
Ethyl caprate	1	42.3
	4	78.1

Table 3. Novozym 435 catalyzed resolution of (±)-*sec*-butylamine with ethyl decanoate in various solvents at high substrate concentration

Solvent	Ee of (*S*)-*sec*-butylamine after	
	1 Day	6 Days
Acetonitrile	61.7%	95.3%
Methyl-*t*-butyl ether	94.4%	99.2%
MTBE–heptane (1:1)	68.7%	98.3%
Heptane	9.5%	27.2%

The trend seen with many even numbered (C2–C18) acids was also seen with the two odd numbered (C7 and C11) acids studied (Table 4). Two glyceryl esters (the natural substrate for lipase) also showed the same preference providing the remaining (*S*)-amine. However, the reaction was much slower and the ee of the remaining (*S*)-amine with the glyceryl esters was much lower when compared to the ethyl esters at similar time points.

A small scale preparative resolution of (±)-*sec*-butylamine by Novozym 435 catalyzed acylation with ethyl decanoate in MTBE was carried out. The ee of the remaining (*S*)-amine reached 99.5% after 1 day. The enzyme recovered from the reaction mixture by filtration retained activity and could be reused. (*S*)-*sec*-Butylamine with an ee of 99.8% was isolated from the reaction mixture as the phosphate salt in 50% yield [theoretical maximum yield of (*S*)-amine as 100%]. From the organic phase remaining after extraction of the amine, *N*-(*R*)-*sec*-butyl decanamide with an ee of 53% was isolated. This ee corresponds to a mixture of the (*R*)-amide (97.7% yield) and (*S*)-amide (29.8% yield). The material balance of the (*S*)-enantiomer (amine plus amide) was 84% and the material balance

Table 4. Novozym 435 catalyzed resolution of (±)-*sec*-butylamine

Ester	Ester amount	Enzyme/amine ratio	Time (days)	Ee (%) (*S*)-amine
Trilaurin	50 mg	20	1	0.5
			5	47.6
Monostearin	50 mg	20	1	3.8
			5	7.5
Ethyl heptanoate	20 μL	42	1	5.7
			5	40.6
Ethyl undecanoate	20 μL	42	1	15.9
			5	91.1

of the (*R*)-enantiomer was 98%. The low yield of the residual (*S*)-amine of high ee and low ee value of the (*R*)-amide probably resulted from a combination of some non-enzymatic amidation and still not extremely high (though significantly improved by the long chain ester) enantioselectivity of the enzyme.

3. Conclusion

This work demonstrates that the variation of the substrate can dramatically change the enantiospecificity of the enzymatic reaction. The enantioselectivity of the acylation of *sec*-butylamine catalyzed by the lipase from *C. antarctica* was increased by using esters of long chain fatty acids. The rate of the reaction was improved by changing the solvent. A combination of these variables provided a satisfactory process for making (*S*)-*sec*-butylamine with high enantiomeric excess.

4. Experimental

Chemicals and enzymes were purchased from commercial sources. Novozym 435 is a product of Novozyme Corporation.

4.1. Analytical method: FMOC derivatization

HPLC analysis was performed by converting the *sec*-butylamine to its 9-fluorenylmethoxycarbonyl (FMOC) derivative. To acetonitrile (1 mL), a solution (20 μL) of amine (0.1 μL) in heptane was added. Solutions of FMOC chloride in acetonitrile (100 μL, 5 mg/mL) and 50 mM borate buffer pH 7.7 (100 μL) were added. The reaction was conducted by gentle mixing in a rotating mixer for 30 min. The solution was filtered through a 0.2 μm filter prior to HPLC.

In experiments with long chain fatty esters, direct use of the reaction mixture caused some problems in the HPLC. In these cases, the amine was extracted from the reaction mixture before derivatization. The reaction mixture (100 μL) was extracted with 200 μL HCl (1 M). A portion of the acid extract (100 μL) was mixed with 100 μL NaOH (1 M) and acetonitrile (1 mL) added. FMOC chloride solution (100 μL) was then added followed by 100 μL of sodium borate solution (1 M) pH 7.7. The FMOC reaction was carried out for

30 min. The reaction mixture was filtered and analyzed by HPLC.

4.2. Analytical method: HPLC analysis of FMOC derivative

For the determination of the extent of conversion, HPLC was done on a YMC pack Pro C18, 150 × 4.6 mm, 3 μm (Waters) column at a temperature of 40 °C using a gradient of solvent A (0.05% TFA in water–methanol 80:20) to solvent B (0.05% TFA in acetonitrile–methanol 80:20) over 20 min at a flow rate of 1 mL/min and detection by UV at 220 nm. The blank reaction (no amine) showed peaks at 13.9, 17.0 and 18.5 min. The FMOC derivative of *sec*-butylamine showed a strong peak at 17.8 min in addition to the blank peaks. For the analysis of resolution with long chain esters, reversed phase HPLC was done in the same system, except with a gradient of 50–100% solvent B over 20 min where the FMOC derivative of the residual amine eluted at 9.8 min.

The enantiomeric composition was determined by analysis on a Chiralcel OD-RH (150 × 4.6 mm, 5 μ, Chiral Technologies Inc.) column at room temperature using an isocratic mixture of 12% solvent A and 88% solvent B at a flow rate of 0.5 mL/min for a total time of 15 min and detection by UV at 220 nm. The blank (no amine) showed a peak at 7.1 min. The FMOC (*R*)-amine peak was at 9.8 min, while that of the (*S*)-amine was at 11.2 min. The (*R*)- and (*S*)-amine derivative peaks showed baseline separation with an area ratio of 50.04:49.96 for the (±)-*sec*-butylamine.

4.3. General method of enzymatic resolution of (±)-*sec*-butylamine by acylation

To separate small vials containing 50 mg of each enzyme were added solutions of (±)-*sec*-butylamine (5 μL = 3.62 mg) in 1 mL of solvent (e.g., heptane). Acylating agent (e.g., ethyl butyrate 20 μL) was added to each vial. A blank contained no acylating agent and no enzyme. The chemical control contained only acylating agent but no enzyme. The vials were closed and placed in the wells of a multiwell plate and shaken at 700 rpm at 25 °C in Thermomixer R shaker. After various times, a small amount (20 μL) of solution was withdrawn from each vial for FMOC derivative formation and HPLC analysis as described above.

4.4. Resolution of (±)-*sec*-butylamine via Novozym 435 catalyzed amide formation with different esters

(±)-*sec*-Butylamine (50 μL) was dissolved in heptane (30 mL) to make a 1.67 μL/mL (1.2 mg/mL) solution. To separate small vials, 50 mg of Novozym 435 enzyme was added. The amine solution (1 mL) and acylating agent (20 μL) were added to each vial. The vials were closed and placed in a multiwell plate, which was placed in a Thermomixer R shaker at 500 rpm at 25 °C. After 1, 2, 3, 4 and 7 days, samples (100 μL) were withdrawn from each vial for analysis. The sample was extracted with 200 μL HCl (1 M). The amine was extracted in the aqueous acid layer and was converted to the FMOC derivative and analyzed by HPLC.

4.5. Resolution of (±)-*sec*-butylamine via Novozym 435 catalyzed amide formation with ethyl decanoate in different solvents

Solutions of (±)-*sec*-butylamine were prepared in different solvents at a concentration of 8.35 μL/mL or 5 mg/mL. To each of the separate small vials, 100 mg of Novozym 435 enzyme were added. The 5 mg/mL amine solution (1 mL) and ethyl decanoate (50 μL) were added to each tube. The vials were closed and placed in a multiwell plate, which was placed in a Thermomixer R shaker at 500 rpm at 25 °C. After 1, 2 and 6 days, samples (100 μL) were withdrawn from each vial, extracted with acid and analyzed.

4.6. Resolution of (±)-*sec*-butylamine via Novozym 435 catalyzed amide formation with ethyl esters of odd number fatty acids or glyceryl esters as acylating agents

Solutions of (±)-*sec*-butylamine were prepared in heptane at concentrations of 5 or 1.2 mg/mL. To two separate vials, 100 mg Novozym 435 enzyme was added. To these two vials, trilaurin (50 mg) or monostearin (50 mg) was added followed by the 5 mg/mL amine solution (1 mL). The 1.2 mg/mL amine solution (1 mL) was added to other two vials each containing 50 mg Novozym 435 enzyme. Ethyl heptanoate (20 μL) or ethyl undecanoate (20 μL) was added to these vials. The vials were closed, placed in a multiwell plate and shaken on a Thermomixer R shaker at 500 rpm at 25 °C. After 1 and 5 days, samples (100 μL) were withdrawn from each vial. The samples were extracted with acid, derivatized and analyzed.

4.7. Preparative resolution of (±)-*sec*-butylamine by Novozym 435 catalyzed amide formation with ethyl decanoate as acylating agent in MTBE

Novozym 435 (50 g) was added to MTBE (250 mL) in a 1 L, round-bottom flask. (±)-*sec*-Butylamine (5 g) and ethyl decanoate (50 mL) were added, followed by an additional 250 mL of MTBE (total 500 mL of MTBE). The flask was closed and stirred at room temperature with a magnetic stirrer. After 1 day, 0.5 mL of the reaction mixture was withdrawn and analyzed. HPLC showed an ee of 99.5% for the *S*-amine. The stirring was stopped. The enzyme was filtered off from the MTBE solution. The enzyme was washed with more MTBE and kept in the cold for future use.

The residual amine was extracted from the MTBE solution with dilute phosphoric acid (pH 2.9–4.4). Concentration of the aqueous extract gave 3.4 g of the largely crystalline phosphate salt of (*S*)-*sec*-butylamine, ee 99.7%. Methanol-insoluble impurities were removed (the amine phosphate was freely soluble in methanol) and the resulting material (3.26 g) recrystallized from ethanol to give 2.68 g of (*S*)-*sec*-butylamine phosphate, ee 99.8%, 45.8% yield. A second crop, 0.25 g, 4.3% yield, ee 99.8%, was obtained from ethanol. The mother liquor

residue, 0.22 g, purity 89.9% relative to the first crop, had an ee of 99.7%. The useful yield of the (*S*)-*sec*-butylamine salt (first and second crops) was 50.1%.

The organic phase remaining after extraction of the amine was extracted with aqueous sodium bicarbonate to remove capric acid. Silica gel chromatography of the remaining organic phase gave 9.92 g of (*R*)-*sec*-butyl decanamide, ee 53%.

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