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Note

Amidation of amines with esters catalyzed by *Candida antarctica* lipase (CAL)

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Candida antarctica lipase (CAL) is used to convert amines and esters into amides**,** especially as a chiral resoluting catalyst for the enantioselective amidation of racemic esters or racemic amines in an effort to demonstrate the ability of lipase to differentiate between enantiomeric substrates. It is a very useful synthetic method for amides. The effects of substrate modification on the yield and enantiomeric excess (*ee*) are studied. The *R*-enantiomer will react faster for racemic mixture of amine in which the amino group attaches directly to the chiral carbon, and the same for racemic mixture of ester in which the carbonyl group attaches directly to the chiral carbon. The enantioselectivity of CAL is complicated for the amidation of racemic mixture of amine with racemic mixture of ester.

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Intense research efforts have been devoted to enzymatic reaction over the past years, especially in synthetic organic chemistry to produce enantiomerically pure molecules. The enzymatic reaction can be carried out in both aqueous and anhydrous organic solutions[1-3]. Many lipases and esterases show striking similarities in their stereoselectivity[4-6], and empirical rules have been formulated to predict preferred stereochemistry for various types of reactions. Lipases have attracted much attention as they are relatively in-expensive and show broad substrate tolerance, often with high enantiomeric selectivity[7-12].

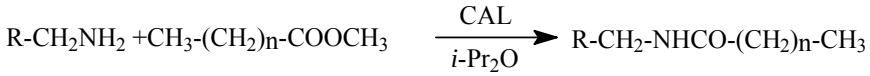
In this paper, we will report our results of *Candida antarctica* lipase (CAL) catalyzed amidation of amines and esters in organic solvent, especially for racemic amines or racemic esters in an effort to demonstrate the ability of lipase to differentiate between enantiomeric substrates.

The studies on four types of the enzymatic reaction of amines with esters catalyzed by CAL were caused at (i) for the amidation of achiral amines and achiral esters, the high yields are obtained, (ii) the *R*-enantiomer will react faster for racemic mixture of amine in which the amino group attaches directly to the chiral carbon, (iii) the *R*-enantiomer will react faster for racemic mixture of ester in which the carbonyl group attaches directly to the chiral carbon, (iv) the enantioselectivity of CAL is complicated for the amidation of racemic mixture of amine with racemic mixture of ester, this needs further study.

Results and Discussion

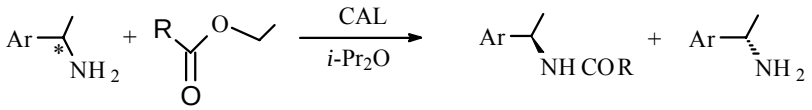
Four types of the enzymatic reaction of amines with esters catalyzed by CAL, using diisopropylether as reacting solvent were carried out. The results are shown in **Scheme I-IV** and **Tables I-IV**.

It is demonstrated that CAL is a particularly attractive biocatalyst for the amidation of achiral



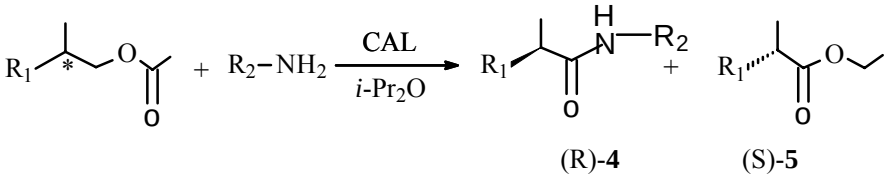
n=5-10 **1**

Scheme I

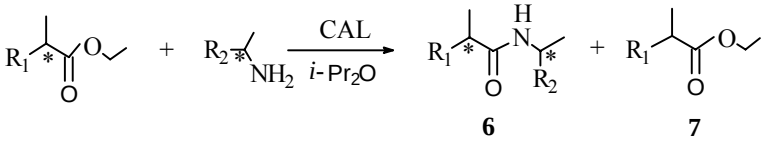


$R=(CH_2)_nCH_3$, n=0,2,4,10 (R)-**2** (S)-**3**

Scheme II



Scheme III



Scheme IV

amines and achiral esters. The corresponding amides were obtained in good yields (**Table I**).

In the studies of the enzymatic amidation of racemic mixture of amines with achiral esters; the experiments showed that enantioselectivity of CAL to amines in which the amino group attaches directly to the chiral carbon containing phenyl (Ph), naphthyl (Np) or benzyl (Bz), *R*-enantiomer reacts faster. The enantioselectivities are different according to the modification of the substitute Ar, and optically active amides with high enantiomeric excess (*ee*) have been obtained (**Table II**). And the empirical rule is the same with that of secondary alcohol.

Next, The amidation of achiral amine with racemic mixture of esters catalyzed by CAL was observed. The result showed that the enzymatic reaction of racemic mixture of esters with different achiral amines in which the carbonyl group attaches directly to the chiral carbon, *R*-enantiomer reacts faster. The enantioselectivity is not very high, and it varies with R_1 and R_2 (**Table III)**.

Next the amidation of chiral amines and chiral esters catalyzed by CAL was carried out. The racemic mixture of 2-phenylpropyl ethyl ester or 2-(4-isobutylphenyl) propyl ethyl ester was employed to react with the racemic mixture of 3-phenyl-2-butylamine or 3-methyl-2-pentylamine respectively. We controlled the conversion at 30%. Since the product amides were complicated, we recovered the esters and measured the enantiomeric excess (*ee*) by HPLC (**Table IV)**.

Table I — Amidation of achiral amine and achiral ester catalyzed by CAL

Compd	R	*n*	Yield (%)
a	Ph	10	96
b	CH_2COOEt	10	82
c	CH_2CN	10	99

Table II — Enantioselectivity of CAL toward chiral amine

Compd	Ar	n	Reaction time/days	*R*-form		*S*-form [b]		E[d]
				Yield (%)	*ee*[a] /(%)	Yield (%)	*ee*[a] /(%)	
a	Ph	0	5	46	98	49	98	458
b	Ph	4	5	40	99	43	99	1057
c	Np	4	5	42	99	45	77	466
d	Bz	4	30 hr	43	99	41	91	67
e	Np	10	5	37	99	52	37	286

[a] Measured by HPLC equipped with a Chiralcel OG;
[b] The absolute configurations are decided by comparing the $[\alpha]_D$ of recovery amines with that of literatures[13];
[c] Converted to lauramide by chemical method, and then measured by HPLC (*ee*: enantiomeric excess);
[d] Enantioselectivity E [14]

$$E = \ln[1-c(1+ee_s)]/\ln[1-c(1-ee_p)]$$

$$c = ee_s/(ee_s+ee_p)$$

ee_s – *ee*% of amines ee_p – *ee*% of amides

Table III — Enantioselectivity of CAL toward chiral ester

Compd	Ester R_1	Amine R_2	Reaction time/hr	*R*-form yield/(%)	*R*-form *ee*[a] /(%)	*S*-form[b] yield/(%)	*S*-form[b] *ee*[c]/(%)	E[d]
a	Ph	$PhCH_2$	75	36	36	44	52	3
b	Ph	$NC(CH_2)_2$	83	38	78	46	49	14
c	Ph	$CH_3(CH_2)$	122	19	57	38	46	6
d	$(CH_3)_2CHCH_2$	$PhCH_2$	75	28	30	40	32	2
e	$(CH_3)_2CHCH_2Ph$	$NC(CH_2)_2$	96	31	81	28	69	25
f	$(CH_3)_2CHCH_2Ph$	$CH_3(CH_2)$	170	17	89	38	32	46

[a] Measured by HPLC equipped with a Chiralcel OD; [b] The absolute configurations are decided by comparing the $[\alpha]_D$ of recovery esters with that of literatures[13; c d] Same as **Table II**.

Table IV — Enantioselectivity of CAL toward chiral ester and chiral amine

Compd	Ester R_1	Amine R_2	Reaction time (hr)	Product amide Yield (%)	Recovered ester Yield (%)	Recovered ester *ee*[a]. (%)
a	Ph	$Ph(CH_3)CH$	79	27	60	71
b	Ph	$CH_3CH_2(CH_3)CH$	114	39	55	41
c	$(CH_3)_2CHCH_2Ph$	$Ph(CH_3)CH$	100	22	60	26
d	$(CH_3)_2CHCH_2Ph$	$CH_3CH_2(CH_3)CH$	83	33	55	42

[a] Same as **Table III**

In conclusion, we have investigated the amidation of amines with esters using CAL as a biocatalyst. Our research indicates the method is efficient and has its exclusive characteristics. That is, high yields of amides for the achiral synthesis from amines and esters; high selectivity for the amidation of chiral amines with achiral esters in which the amino group attaches directly to the chiral carbon, in contrast, adequate selectivity for the amidation of chiral esters with achiral amines in which the carbonyl group attaches directly to the chiral carbon, the *R*-enantiomer will react faster for both of them; but the enantioselectivity of CAL is complicated for the amidation of racemic mixture of amines with racemic mixture of esters, it still needs further study.

Materials and Methods

The ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution with TMS as internal standard on a Varian Mercury 200 and Varian Innova 500. Mass spectra were obtained with Japan Electron AX-505HA model mass spectrometer with electron impact source. IR spectra were determined on a Horiba FT-710 Infrared Spectrophotometer. The enantiomeric excess (*ee*) is measured by Hitachi – 6000 high pressure chromatography (Chiralcel OG, OD 4.6 mm I.D.×250 mm stainless steel column, Hexane: *i*-Pr_2O = 9:1, flow 0.5 mL/min, 254 nm). The yields were determined by Hitachi 263-50 Gas chromatography.

AR grade chemicals: 2-phenylpropyl acetate, 2-(4-isobutylphenyl)-propyl acetate; 1-phenylethylamine, 1-benzylethylamine, 1-α-naphthylethylamine, 3-phenyl-2-butylamine, 3-methyl-2-pentylamine; acyl donors; acyl acceptors; solvent; CAL (Lipase from *Candida antarctica*) Novozyme 435 Novo Nordis Co, Ltd.

The substrate amine, ester, organic solvent and CAL enzyme were placed in a small cylinder bottle, substrate-amine; 1:1 (mole/mole), substrate-enzyme; 1:1 (w/w), the bottle was placed in a water-bath and shaking was carried out at 30°C (150 rpm), TLC (chloroform-hexane; 9:1; 4:1) and HPLC were used to trace the change of the enzymatic reaction. When the conversion reached 50% the reaction was stopped, chromatographed over silica gel to get the product. The product amides and recovery amines or esters were characterized by IR, ^1H NMR, ^{13}C NMR and MS studies. Elemental analysis was performed on a Perkin-Elmer 240.

Experimental Section

Preparation of *N*-benzyllaurylamide 1a. CAL (0.51 g) was added to the solution of benzylamine

(0.51g, 1.7 mmoles) and lauryl acetate (1.02 g, 4.7 mmole) in dry *i*-Pr_2O (10 mL). The reaction mixture was shaken at 30°C. After 5 hr the contents were filtered, washed with $CHCl_3$ and concentrated *in vacuo*, then chromatographed over silica gel with chloroform-hexane as eluent (4:1), yield 96%; Anal. Calcd for $C_{19}H_{31}NO$: C, 78.82; H, 10.81; N, 4.84. Found: C, 78.80; H, 10.82; N, 4.86%; IR (KBr): 3250, 1641 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ 14.13, 22.69, 25.78, 29.24-29.62, 25.90, 35.80, 175.00, 57.40, 141.10, 128.20, 128.20, 126.70; ^1H NMR ($CDCl_3$): δ 0.88 (3H,t), 1.51 (2H, sext), 1.21 (16H, m), 1.41 (2H, t), 3.85 (2H, d), 7.15-7.33 (5H,m); Mass: m/z 289 [M^+].

Preparation of (*R*)-*N*-(1-benzylethyl)capryl-amide 2d. To the solution of 1-benzylethylamine (0.52 g; 3.80 mmoles) and capryl acetate (0.55 g, 3.80 mmoles) in dry *i*-Pr_2O (10 mL), CAL (0.52 g) was added. After 30 hr the contents were filtered, washed with $CHCl_3$ and concentrated *in vacuo*, then chromatographed over silica gel with chloroform-hexane as eluent (6:1).The white crystals were obtained, yield 0.37g (43%); $[\alpha]_D^{23}$ + 41.56°C, 0.38% in MeOH), Anal. Calcd for $C_{15}H_{23}NO$: C, 77.37; H, 9.96; N, 5.97. Found: C, 77.25; H, 9.87; N, 6.01%; IR (KBr): 3281, 1642 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, t), 1.11 (3H, d), 1.20-1.32 (4H, m), 1.58 (2H, sext), 2.11 (2H, t), 2.66-2.92 (2H, m), 4.28 (1H, sext), 5.46 (1H, br-s), 7.16-7.32 (5H, m); ^{13}C NMR ($CDCl_3$): δ 13.94, 20.08, 22.41, 25.46, 31.39, 36.93, 42.48, 45.88, 126.42, 128.35, 129.45, 138.04, 172.49; Mass: m/z 233 [M^+].

Recovery of (*S*)-1-benzylethylamine 3d. The yellow liquid amine was recovered from above procedure, yield 41%, Anal. Calcd for $C_9H_{13}N$: C, 80.13; H, 9.75; N, 10.12. Found: C, 80.00; H, 9.63; N, 10.37%; IR (KBr): 3129, 3289, 2927, 2869, 3046, 1594, 777 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.11 (3H, d), 1.36 (3H, br-s), 2.45-2.76 (2H, m), 3.14 (1H, sext), 7.16-7.34 (5H, m); ^{13}C NMR ($CDCl_3$): δ 23.52, 46.71, 48.53, 126.21, 128.42, 129.26, 139.67; Mass: m/z 135 [M^+].

(*R*)-*N*-Benzyl-2-phenylpropylamide 4a. To the solution of benzylamine (0.50 g, (4.64 mmoles) and 2-phenylpropyl acetate (0.83 g, 4.64 mmoles) in dry *i*-Pr_2O (10 mL), CAL (0.50g) was added. After 75 hr the mixture was filtered, washed with $CHCl_3$, concentrated *in vacuo* and chromatographed over silica gel with chloroform-hexane as eluent (9:1), yield 36%. Anal. Calcd for $C_{16}H_{17}NO$: C, 80.30; H, 7.17; N, 5.85. Found: C, 80.29; H, 7.18; N, 5.87%; IR (KBr): 3250, 1625 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ 126.50-143.41, 57.70, 175.70, 51.50, 18.50, 125.80-144.11; ^1H NMR ($CDCl_3$): δ 7.08-7.13 (5H,m), 4.00 (1H, q), 1.48 (3H, d), 5.89 (1H, br-s), 4.83 (2H, d), 7.15-7.20 (5H, m); Mass: m/z 239 [M^+].

Recovery of (*S*)-2-phenylpropyl acetate 5a. The ester was recovered from above procedure; yield 44%; Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.16; H, 7.88. Found: C, 74.17; H, 7.89%; IR (KBr)): 1740, 1100 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ 125.80-144.10, 50.50, 18.50, 176.00, 57.50, 12.50; ^1H NMR ($CDCl_3$): δ 718-7.30 (5H, m), 4.05 (1H, q), 4.05 (2H, q), 1.21 (3H, t); Mass: m/z 178 [M^+].

***N*-(3-Phenyl-2-butyl)-2-phenylpropylamide 6a**. To the solution of 3-phenyl-2-butylamine (0.57 g, 3.8 mmoles) and 2-phenylpropyl acetate (0.68 g, 3.8 mmoles) in dry *i*-Pr_2O (10 mL), CAL (0.57 g) was added. The contents were filtered after 79 hr, washed with $CHCl_3$, concentrated *in vacuo* and chromato-graphed over silica gel with chloroform-hexane as eluent (9:1), yield 27%; Anal. Calcd for $C_{19}H_{23}NO$: C, 81.08; H, 8.25; N, 4.98. Found: C, 81.06; H, 8.24; N, 5.88%; IR (KBr): 1625, 3350 cm^{-1}; ^{13}C NMR ($CDCl_3$): δ 127.90-140.20, 125.80, 48.70, 17.90, 175.40, 55.50, 47.50, 16.80, 125.70-148.70; ^1H NMR ($CDCl_3$): δ 7.18-7.30 (5H, m), 1.48(3H, d), 4.01(1H, q), 5.96 (1H, br-s), 2.74 (1H, m), 0.86 (3H, d), 2.66 (1H, m), 7.08-7.13 (5H, m); Mass: m/z 281 [M^+].

Acknowledgement

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