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# Lipase-catalyzed kinetic resolutions of racemic β- and γ-thiolactones

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**Abstract**

Several racemic β- and γ-thiolactones were synthesized and kinetic resolutions of them were executed using lipases. While a lipase from *Pseudomonas cepacia* (PCL) showed the highest enantioselectivity for (*S*)-form (>99% $ee_S$ at 53% conversion, $E > 100$) in the kinetic resolution of racemic α-methyl-β-propiothiolactone (*rac*-MPTL), it showed no hydrolysis activity in the kinetic resolution of α-benzyl-α-methyl-β-propiothiolactone (*rac*-BMPTL), suggesting that the changes in the size of alkyl group from *rac*-MPTL to *rac*-BMPTL leads to lower hydrolysis activity and enantioselectivity. In contrast, racemic γ-butyrothiolactones were hydrolyzed by several lipases with low enantioselectivity, whereas a lipase from *Candida antarctica* (CAL) showed moderate enantioselectivity for (*S*)-form (>99% $ee_S$ at 76% conversion, $E = 11$) in the kinetic resolution of racemic α-methyl-γ-butyrothiolactone (*rac*-MBTL). Computer-aided molecular modeling was also performed to investigate the enantioselectivites and activities of PCL toward β-propiothiolactones. The computer modeling results suggest that the alkyl side chains of β-propiothiolactones and γ-butyrothiolactones interact with amino acid residues around *hydrophobic crevice*, which affects the activity of PCL.


*Keywords:* Lipase; Thiolactone; Kinetic resolution; Enantioselective; Computer modeling

## 1. Introduction

Owing to high enantio- and regioselectivity, lipases have been extensively studied for hydrolysis, esterification, alcoholysis, and transesterification reactions [1–5]. They have been applied to synthesis of peptides [6], acylation of carbohydrates [7], modification of triglycerides [8], and preparation of enantiopure compounds [9]. In particular, enantiopure compounds have gained great interest in pharmaceutical industry for their desirable biological activities [10].

Optically active lactones or thiolactones are useful chiral intermediates in organic synthesis and flavor components of fruits and wines, pheromones and useful bio-functional synthons. Various chemical and biological methods for preparation of enantiopure lactones or thiolactones have been investigated [11–14]. Among them, lipase-catalyzed enantioselective hydrolysis can become a commercially useful and clean technology.

While many studies on the lipase-catalyzed asymmetric resolution of lactones have been reported until now [15,16], the study on the lipase-catalyzed kinetic resolution of thiolactones has been lacking despite optically active thiolactones are especially useful in pharmaceutical industry [17]. For example, α-methyl-β-propiothiolactone is the chiral intermediate of captopril [9]. The β-thiolactone analog of a prostaglandin has less hypotensive activity than $PGA_2$ [18]. Thiolactone ring structures are also found in thiolactonic antibiotics such as thiolactomycin [19] and intermediates of sugar thiolactones [20].

In this paper, racemic β- and γ-thiolactones were chemically synthesized and kinetic resolutions of β- and γ-thiolactones by lipase-catalyzed hydrolysis were investigated (Fig. 1). Computer-aided molecular modeling was applied to investigate the effects of substrate structure on the lipase activity and its enantioselectivity [21,22].

## 2. Materials and methods

### 2.1. Enzymes and chemicals

Lipase from *Pseudomonas cepacia* (PCL) and lipase from *Candida rugosa* (CRL) were purchased from Amano Phar-

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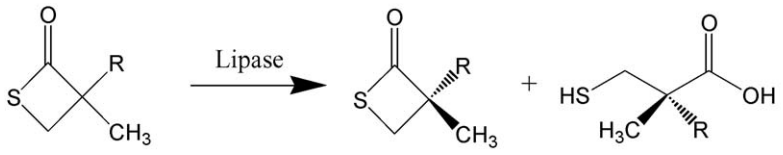
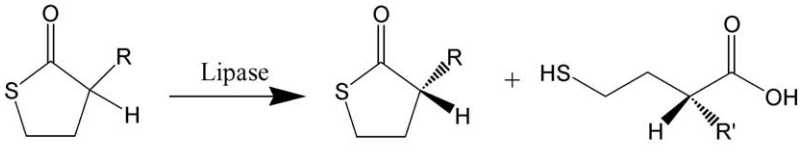



| | R | Compound |
|---|---|---|
| 1 | H | α-methyl-β-propiothiolactone (MPTL) |
| 2 | $C_2H_5$ | α-ethyl-α-methyl-β-propiothiolactone (EMPTL) |
| 3 | $C_6H_5$ | α-benzyl-α-methyl-β-propiothiolactone (BMPTL) |




| | R' | Compound |
|---|---|---|
| 4 | $CH_3$ | α-methyl-γ-butyrothiolactone (MBTL) |
| 5 | $C_2H_5$ | α-ethyl-γ-butyrothiolactone (EBTL) |
| 6 | $C_6H_5$ | α-benzyl-γ-butyrothiolactone (BBTL) |

Fig. 1. Lipase-catalyzed hydrolysis of β-propiothiolactones and γ-butyrothiolactones.

maceutical Company (Nagoya, Japan). Lipase from *Candida antarctica* (CAL) was gifts from Novo Nordisk (Bagsvaerd, Denmark). Porcine pancreas lipase (PPL) was purchased from Sigma (St. Louis, MO). All these lipases were used for the hydrolysis of β-and γ-thiolactones without further purification.

Chemical reagents were purchased from variant suppliers. Thioacetic acid, methacrylic acid, methyl chloroformate, and γ-butyrothiolactone were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO). All the other reagents and solvents used were obtained commercially and were of analytical grade.

### 2.2. Synthesis of β-and γ-thiolactones

*rac*-MPTL (**1**) was synthesized from β-acetylmercaptoisobutyric acid which was obtained by 1,4-addition of thioacetic acid to methacrylic acid [9]. $^1$H NMR of **1** $\delta$ 1.37 (d, 3H, $J$ = 7.1, CHC$\underline{H}_3$), 2.67 (dd, 1H, $J$ = 3.8, 8.4, C$\underline{H}_2$S), 3.18 (dd, 1H, $J$ = 7.5, 8.4, C$\underline{H}_2$S), 4.25 (m, 1H, C$\underline{H}$CH$_3$); $^{13}$C NMR $\delta$ 15.8 (C$\underline{H}_3$), 23.8 (C$\underline{H}_2$), 65.7 (C$\underline{H}$), 195.0 (C$\underline{O}$S). Racemic α-alkyl-α-methyl-β-propiothiolactones were prepared from *rac*-MPTL and alkyliodide or alkylbromide. To a magnetically stirred solution of lithium hexamethyldisilazanide (LHMDS, 4.0 mmole) in tetrahydrofuran (THF) at −78 °C in an acetone-dry ice bath was added *rac*-MPTL (3.0 mmole) in THF (5.0 ml). After stirring for 30 min, an alkyliodide or alkylbromide (5.0 mmole) in THF (5.0 ml) at −78 °C was added dropwise via a syringe over 2 min. The reaction mixture was stirred at the same temperature for 1 h and then allowed to warm to 0 °C. After addition of saturated $NH_4Cl$ solution, the resulting mixture was extracted with diethyl ether (2 × 10 ml). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated. Dry-column flash chromatography with *n*-hexane and slowly increasing amounts of ethylacetate yielded a product. $^1$H NMR of **2** $\delta$ 1.01 (t, 3H, $J$ = 7.5, CH$_2$C$\underline{H}_3$), 1.37 (s, 3H, CC$\underline{H}_3$), 1.71 (m, 2H, C$\underline{H}_2$CH$_3$), 2.70 (d, 1H, $J$ = 8.4, C$\underline{H}_2$S), 2.89 (d, 1H, $J$ = 8.4, C$\underline{H}_2$S); $^{13}$C NMR $\delta$ 8.4 (CH$_2$C$\underline{H}_3$), 21.5 (C$\underline{H}_3$), 28.6 (C$\underline{H}_2$S), 29.5 (C$\underline{H}_2$CH$_3$), 76.3 (C(CH$_3$)Et), 198.9 (C$\underline{O}$S). $^1$H NMR of **3** $\delta$ 1.41 (s, 3H, $J$ = 7.5, CC$\underline{H}_3$), 2.62 (d, 1H, $J$ = 8.5, C$\underline{H}_2$S), 2.70 (d, 1H, $J$ = 13.7, C$\underline{H}_2$Ph), 3.00 (d, 1H, $J$ = 8.5, C$\underline{H}_2$S), 3.13 (d, 1H, J = 13.7, C$\underline{H}_2$Ph), 7.17–7.34 (m, 5H, C$_6\underline{H}_5$); $^{13}$C NMR $\delta$ 22.5 (C$\underline{H}_3$), 28.0 (C$\underline{H}_2$S), 42.1 (C$\underline{H}_2$Ph), 75.7 (C(CH$_3$)Bn), 126.9 (C$\underline{H}$=), 128.3 (C$\underline{H}$=), 130.0 (C$\underline{H}$=), 135.8 (C=), 198.7 (C$\underline{O}$S).

Racemic α-alkyl-γ-butyrothiolactones were prepared from γ-butyrothiolactone and alkyliodide or alkylbromide by the same procedure as the preparation of racemic α-alkyl-α-methyl-β-propiothiolactones. $^1$H NMR of **4** $\delta$ 1.19 (d, 3H, $J$ = 6.4, CHC$\underline{H}_3$), 1.88 (m, 1H, C$\underline{H}$CH$_3$), 2.50 (m, 2H, C$\underline{H}_2$), 3.29 (m, 2H, C$\underline{H}_2$S); $^{13}$C NMR $\delta$ 14.9 (C$\underline{H}_3$), 30.4 (C$\underline{H}_2$), 34.1 (C$\underline{H}_2$S), 46.8 (C$\underline{H}$), 210.3 (C$\underline{O}$S). $^1$H NMR of **5** $\delta$ 0.98 (t, 3H, $J$ = 7.5, CH$_2$C$\underline{H}_3$), 1.44 (m, 1H, C$\underline{H}$CH$_2$), 1.89 (m, 2H, C$\underline{H}_2$CH$_3$), 2.39 (m, 1H, C$\underline{H}_2$), 2.45 (m, 1H, C$\underline{H}_2$), 3.29 (m, 2H, C$\underline{H}_2$S); $^{13}$C NMR $\delta$ 12.0 (CH$_2$C$\underline{H}_3$), 23.2 (C$\underline{H}_2$CH$_3$), 30.5 (C$\underline{H}_2$), 31.5 (C$\underline{H}_2$S), 53.3 (C$\underline{H}$CH$_2$), 210.7 (C$\underline{O}$S). $^1$H NMR of **6** 1.92 (m, 1H, C$\underline{H}$CH$_2$), 2.31 (m, 1H, C$\underline{H}_2$), 2.59 (d, 1H, $J$ = 13.7, C$\underline{H}_2$Ph),

2.61 (d, 1H, $J$ = 13.7, C$\underline{H}_2$Ph), 2.73 (m, 1H, C$\underline{H}_2$), 3.23 (m, 2H, C$\underline{H}_2$S), 7.16–7.32 (m, 5H, C$_6\underline{H}_5$); $^{13}$C NMR 30.2 (C$\underline{H}_2$), 31.4 (C$\underline{H}_2$Ph), 36.0 (C$\underline{H}_2$S), 53.6 (C$\underline{H}$), 126.7 (C$\underline{H}$=), 128.1 (C$\underline{H}$=), 129.7 (C$\underline{H}$=), 135.5 (C$\underline{}$=), 208.7 (C$\underline{}$OS).

### 2.3. Hydrolysis of β- and γ-thiolactones by lipase

All the reactions were conducted in screw-capped glass vials in a shaking incubator at 37 °C. The shaking speed was 250 rpm. In a typical experiment, 50 μmole of *rac*-MPTL, 10 μl of phosphate buffer (100 mM, pH 7.4) and 10 mg of lipase were mixed in 1 ml solvent. Samples were removed for HPLC or GC analysis at different time intervals.

The hydrolysis was monitored by HPLC using chiral column (Daicel Chiralpak AS, Tokyo, Japan) capable of separating (*R*)- and (*S*)-isomers of β-propiothiolactones without derivatization. The mobile phase was hexane at a flow rate of 0.5 ml min$^{-1}$. UV detection at 210 nm was used for quantification at the ambient temperature. The hydrolysis was also monitored by GC using chiral column (Macherey-Nagel Lipodex E, Düren, Germany) capable of separating (*R*)- and (*S*)-isomers of γ-butyrothiolactones without derivatization. *n*-Dodecane was used as internal standard. *E*-values were calculated based on the enantiomeric excess of the substrate (ee$_S$) and the conversion (*c*) according to Chen and Sih [23].

### 2.4. Computer molecular modeling

Computer molecular modeling was performed by previously published protocols [21]. The X-ray structure of the open conformation of PCL (PDB entry 2lip [24]) was obtained from the Protein Data Bank (PDB). Structural models of the substrates were created using SYBYL 6.7 (Tripos, St. Louis, MO) and docked into the binding site mimicking the first tetrahedral intermediate which is the rate-limiting step of ester hydrolysis in lipase. The charge distribution at the catalytic residues S87, H286, and the substrate was modified as calculated by the semi-empirical method, MNDO94/PM3 [25], according to Holzwarth et al. [26].

## 3. Results and discussion

### 3.1. Hydrolysis of β-propiothiolactones

Kinetic resolution of *rac*-MPTL by lipase was reported in the previous paper [9]. Commercial lipases were tested for their ability to catalyze the enantioselective hydrolysis of *rac*-MPTL in cyclohexane containing 1% (v/v) 100 mM phosphate buffer. PCL showed the highest enantioselectivity ($E$ > 100) and 47% of (*R*)-MPTL having above 99% ee$_S$ was yielded. To investigate the substrate specificity and enantioselectivity of PCL toward β-propiothiolactones, racemic α-ethyl-α-methyl-β-propiothiolactone (*rac*-EMPTL, **2**) and racemic α-benzyl-α-methyl-β-propiothiolactone (*rac*-BMPTL, **3**) were prepared as colorless oil by the protocol as shown in Materials and Methods. The hydrolysis activity and enantioselectivity of PCL differed significantly according to the size of alkyl group of the substrates (Table 1). PCL showed high activity and enantioselectivity for *rac*-MPTL, whereas it showed low activity and enantioselectivity for *rac*-EMPTL (23% conversion, 22% ee$_S$). It showed no hydrolysis activity in the kinetic resolution of *rac*-BMPTL. It is assumed that the benzyl group of the substrate is too large to be adapted at the active site of the PCL.

Table 1
PCL-catalyzed hydrolysis of racemic β-propiothiolactones in cyclohexane containing 1% (v/v) 100 mM phosphate buffer (pH 7.4) at 37 °C for 24 h[a]

| β-Propiothiolactones | $c$ (%) | ee$_S$ (%) | $E$ |
|---|---|---|---|
| **1** | 53 | >99 (*R*) | >100 |
| **2** | 23 | 22 (*R*) | 8 |
| **3** | n.d. | n.d. | n.d. |

n.d.: not detected.
[a] 50 mM β-propiothiolactones and 10 mg of lipase/ml of reaction medium were used.

The substrate binding site of PCL consists of four binding pockets [21,24]. One is small oxyanion hole which consists of two residues (L17 and Q88). These residues donate their backbone amide protons to stabilize the substrate in the transition state. The *hydrophobic crevice* is composed of L17, P113, S117, F119, V123, L164, L167, and V267, the *hydrophobic dent* is composed of L248, T251, V266, and L287, and the *cavity* is composed of T18, V26, L27, Y29, F146, P243, S244, I290, Q292, and L293 [21]. Computer-aided molecular modeling of transition state analogues bound to the PCL was carried out to investigate its substrate specificity. After molecular dynamics simulation the alkyl side chain of the thiolactones were oriented toward the *hydrophobic crevice* (data not shown). The formation of the essential hydrogen bonds was due to the correct placement of H286 imidazole which could be monitored by measuring the distance between the N$_\varepsilon$2 of the imidazole ring in the active site H286 and O$_\gamma$ of the active site S87 (N$_\varepsilon$-O$_\gamma$) and S of the thiolactone ring of the substrate (N$_\varepsilon$-S), and between the N$_\delta$1 of the imidazole ring in the active site H286 and the C$_\gamma$ of the active site D264 (N$_\delta$-C$_\gamma$), respectively. In the case of (*S*)-MPTL, the distance of N$_\varepsilon$ (H286)-O$_\gamma$ (S87), N$_\varepsilon$ (H286)-S (substrate), and N$_\delta$ (H286)-C$_\gamma$ (D264) were 2.2 Å, 2.5 Å, and 2.6 Å, respectively. This result showed the essential hydrogen bonds could be formed. However, in the case of (*R*)-MPTL, the distance of N$_\varepsilon$ (H286)-O$_\gamma$ (S87), N$_\varepsilon$ (H286)-S (substrate), and N$_\delta$ (H286)-C$_\gamma$ (D264) were 2.0 Å, 3.6 Å, and 4.3 Å, respectively. For (*R*)-MPTL, sterical hindrance and electrostatic repulsion occurred between the alkyl chain of the thiolactone and residues in the binding site of the lipase. The substrate was moved toward H286. This change in local geometry and electrostatics influenced the position of H286. Hence, the imidazole ring of H286 was moved out of its favorable position and the hydrogen bond between N$_\delta$ (H286)-C$_\gamma$ (D264) was broken (Fig. 2A). For BMPTL, sterical hindrance and electrostatic repulsion occurred between the benzyl chain and residues in the binding site of the PCL. For (*S*)-BMPTL, the distance of N$_\varepsilon$ (H286)-O$_\gamma$ (S87), N$_\varepsilon$ (H286)-S (substrate), and N$_\delta$ (H286)-C$_\gamma$ (D264) were 2.4 Å, 4.5 Å, and 5.3 Å, respectively. The distance of N$_\varepsilon$ (H286)-O$_\gamma$ (S87), N$_\varepsilon$ (H286)-S (substrate), and N$_\delta$ (H286)-C$_\gamma$ (D264) for (*R*)-BMPTL

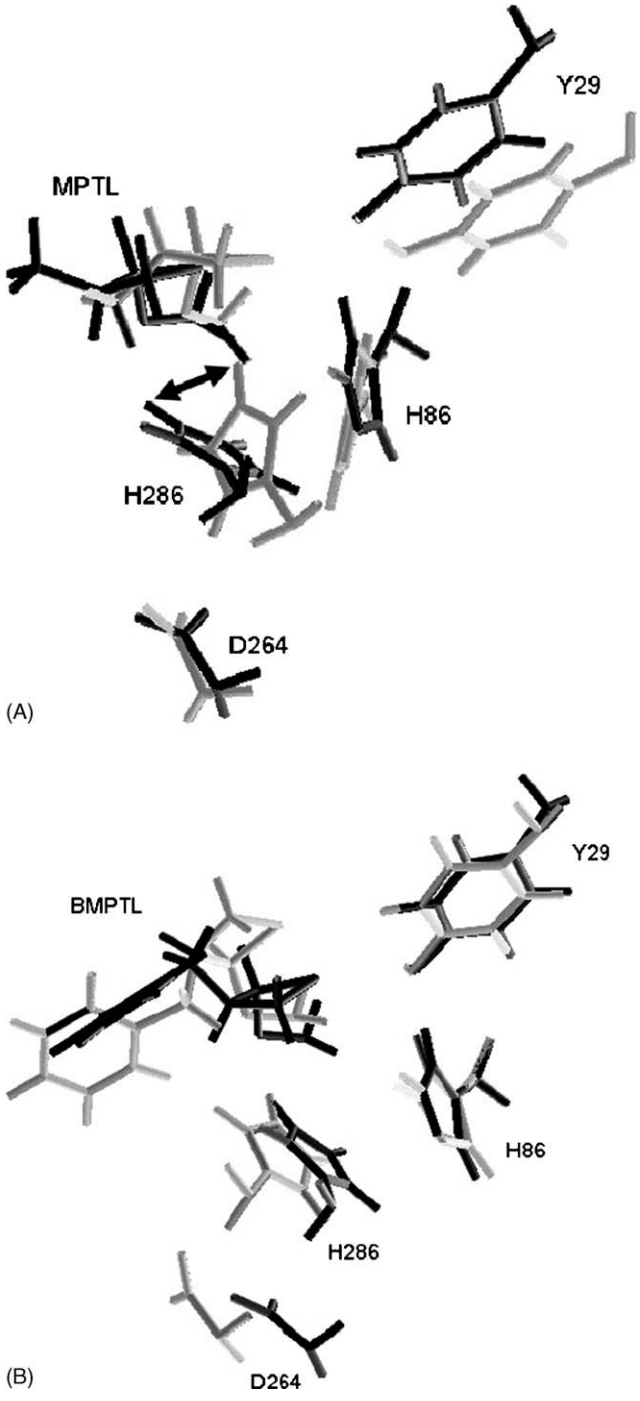



Fig. 2. The superimposed average structures of (*R*)- (black) and (*S*)-MPTL (gray) complexes (A) and (*R*)- (black) and (*S*)-BMPTL (gray) complexes (B). Panel A: In the case of (*S*)-MPTL, the residues of the catalytic triad (S87, D264, and H286) are displayed as well as H86 which supposedly stabilized the position of H286. For (*R*)-MPTL, the side chain of H286 was moved out of its favorable position and no longer stabilized. The hydrogen bond between $N_\delta$ (H286) and $C_\gamma$ (D264) was broken. Panel B: The side chains of H286 of in both (*R*)- and (*S*)-BMPTL were moved out of its favorable position and no longer stabilized. The hydrogen bond between $N_\delta$ (H286) and $C_\gamma$ (D264) and the hydrogen bond between $N_\varepsilon$ (H286) and S (substrate) were broken.

were 2.2 Å, 3.8 Å, and 4.4 Å, respectively (Fig. 2B). The hydrogen bond between $N_\delta$ (H286) and $C_\gamma$ (D264) and the hydrogen bond between $N_\varepsilon$ (H286) and S (substrate) were broken during molecular dynamics simulation. These results agreed well with no hydrolysis activity of PCL for *rac*-BMPTL.

Enzelberger et al. [16] examined lipase-catalyzed resolution of γ- and δ-lactones, and the enantioselectivity of PCL was investigated elsewhere by computer-aided molecular modeling [21]. PCL showed (*R*)-specific enantioselectivity for γ- and δ-lactones and showed high enantioselectivity for δ-lactones with a long alkyl side chain. The chiral centers of γ- and δ-lactones in the previous paper are located by the oxygen of the lactone ring, but the chiral centers of β-propiothiolactones and γ-butyrothiolactones are located by the carbonyl group of the thiolactone. This explains that PCL showed (*R*)-specific enantioselectivity for the γ- and δ-lactones, but (*S*)-specific enantioselectivity for the β-propiothiolactones and γ-butyrothiolactones. The alkyl side chains of the γ- and δ-lactones are oriented toward the *hydrophobic dent* or *cavity*. For δ-dodecalactone and δ-undecalactone, the alkyl side chains ($C_6H_{13}$ and $C_5H_{11}$, respectively) are oriented toward *cavity* exposed to solvent media. Therefore, the long alkyl side chains are placed in the active binding pocket of PCL without any severe steric hindrance and PCL showed high activity and enantioselectivity for δ-dodecalactone ($E > 100$) and δ-undecalactone ($E > 100$). However, the alkyl side chains of β-propiothiolactones and γ-butyrothiolactones are oriented toward *hydrophobic crevice* having smaller space than its *cavity*. Therefore, it is agreeable that PCL appears to show activity only for β-propiothiolactones and γ-butyrothiolactones with a short alkyl chain.

### 3.2. Hydrolysis of γ-butyrothiolactones

Several commercial lipases were tested for their ability to catalyze the enantioselective hydrolysis of racemic α-methyl-γ-butyrothiolactone (*rac*-MBTL) in cyclohexane containing 1% (v/v) 100 mM phosphate buffer. In contrast to the kinetic resolution of *rac*-MPTL, we observed lower enantioselectivities of the lipases towards the *rac*-MBTL (Table 2). PCL showed medium enantioselectivity ($E = 8$) but the hydrolysis activity of PCL was low (8% conversion). Therefore the low hydrolysis activity of PCL is the critical drawback in the preparation of enantiopure MBTL. In the case of CAL, the hydrolysis activity and enantioselectivity were higher than PCL (70% conversion, >99% $ee_S$).

We also investigated the kinetic resolution of racemic γ-butyrothiolactones using CAL and PCL (Table 3). CAL showed

Table 2
Lipases-catalyzed hydrolysis of *rac*-MBTL in cyclohexane containing 1% (v/v) 100 mM phosphate buffer (pH 7.4) at 37 °C for 24 h[a]

| Lipases | *c* (%) | $ee_S$ (%) | *E* |
|---|---|---|---|
| *Pseudomonas cylindracea* | 8 | 6 (*R*) | 8 |
| *Candida rugosa* | 10 | 7 (*R*) | 5 |
| *Candida antarctica* | 76 | >99 (*R*) | 11 |
| *Porcine pancrease* | 26 | 4 (*R*) | 1 |

[a] 50 mM *rac*-MBTL and 10 mg of lipase/ml of reaction medium were used.

Table 3
CAL and PCL-catalyzed hydrolysis of racemic γ-butyrothiolactones in cyclohexane containing 1% (v/v) 100 mM phosphate buffer (pH 7.4) at 37 °C for 24 h[a]

| γ-Butyrothiolactones | $c$ (%) | | $ee_S$ (%) | | $E$ | |
|---|---|---|---|---|---|---|
| | CAL | PCL | CAL | PCL | CAL | PCL |
| **4** | 76 | 8 | >99 (*R*) | 6 (*R*) | 11 | 8 |
| **5** | 41 | n.d. | 24 (*R*) | n.d. | 3 | n.d. |
| **6** | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

n.d.: not detected.
[a] 50 mM β-propiothiolactones and 10 mg of lipase/ml of reaction medium were used.

the hydrolysis activity for racemic α-ethyl-γ-butyrothiolactone (*rac*-EBTL), but the reaction rate and enantioselectivity decreased compared with those for *rac*-MBTL (41% conversion, 24% $ee_S$). CAL has no hydrolysis activity for *rac*-BBTL. However, PCL showed no hydrolysis activity for *rac*-EBTL and *rac*-BBTL. This substrate specificity of CAL and PCL seems to be caused by the binding pocket of the lipases to be unacceptable for the benzyl group of the substrate.

## 4. Conclusion

This study provides new additional information to the synthetic utility of lipases for thiolactone hydrolysis. It has been shown that lipases in organic solvents can act as practical, stereoselective catalysts for kinetic resolution of β-propiothiolactones and γ-butyrothiolactones. The rule derived by Kazlaukas et al. [27] for the lipase catalyzed kinetic resolution of secondary alcohols can not be used for thiolactones, because the stereocenter lies in a different position. However, it seems that a correlation between the size of the side alkyl group and the enantioselectivity exists. PCL showed the highest enantioselectivity for the hydrolysis of *rac*-MPTL. We also investigated the kinetic resolution of other β-propiothiolactones using PCL. The activity and enantioselectivity of PCL vary significantly with variations in the substrate structure. As the size of another α-substituted side group of β-propiothiolactones increases, the hydrolysis activity and enantioselectivity of PCL decrease. Moreover PCL showed no hydrolysis activity in the resolution of *rac*-BMPTL. Molecular modeling was used to identify and explain the activity and enantioselectivity of PCL for β-propiothiolactones.

In the case of γ-butyrothiolactones, kinetic resolution by lipase-catalyzed was less efficient for the preparation of enantiopure γ-butyrothiolactones. CAL showed the highest enantioselectivity in the resolution of γ-butyrothiolactones. Highest enantioselectivity (>99% $ee_S$ at 76% conversion, $E = 11$) was found for *rac*-MBTL. *rac*-BBTL was not hydrolyzed by CAL. CAL also showed lower activity and enantioselectivity for the γ-butyrothiolactones with a large α-substituted side group.

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