A 2-Propanol Treatment Increases the Enantioselectivity of *Candida rugosa* Lipase toward Esters of Chiral Carboxylic Acids

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Crude lipase from *Candida rugosa* (CRL) is a poorly to moderately enantioselective catalyst for the hydrolysis of esters of 2-substituted carboxylic acids such as 2-arylpropanoic acids ($E = 4-10$) and 2-(aryloxy)propanoic acids ($E = 2-17$). Previous workers converted CRL into a high enantioselectivity form using a four-step purification procedure that included an organic solvent treatment. In this paper we report a simple 2-propanol treatment that converts crude CRL to the high enantioselectivity form. Dissolving crude commercial CRL in 50% 2-propanol followed by dialysis to remove 2-propanol increased the total activity by a factor of 1.2–1.6 and the enantioselectivity toward seven carboxylic acids by a factor of 2.3 to >25. We demonstrated synthetic use of this 2-propanol-treated CRL by resolving 10 g of methyl 2-(4-chlorophenoxy)propionate (**3**-methyl ester), yielding 2.8 g of product acid (93.1% ee *R*, a serum cholesterol-lowering compound) and 4.7 g of recovered ester (94.4% ee *S*), corresponding to an enantiomeric ratio of 100 for the resolution. Recent X-ray crystal structures identified two conformational forms of CRL—open and closed. We suggest that the 2-propanol treatment may increase the activity and enantioselectivity by converting the closed form of CRL to the open form.

Introduction

The most common limitation of enantioselective synthetic methods, both chemical and enzymic, is insufficient enantioselectivity. For example, crude lipase from *Candida rugosa*[1] (CRL) efficiently resolves many chiral cyclic and bicyclic secondary alcohols[2] but, in general, is not sufficiently enantioselective toward chiral carboxylic acids for synthetic use. Increasing enantioselectivity of catalysts such as CRL is a current research goal.

One approach to increasing the enantioselectivity of CRL is to change the reaction conditions by changing the solvent,[3] temperature, or pH or by carrying out the reaction in a microemulsion.[4] Another approach is to introduce additives such as (*S*)-2-amino-4-(methylthio)-1-butanol,[5] $CaCl_2$,[6] Triton X-100 (a surfactant),[7] or dextromethorphan.[8] Kinetic analysis showed that dextromethorphan increased the enantioselectivity by inhibiting the hydrolysis of the slow-reacting enantiomer.

In other cases, chemists increased the enantioselectivity of CRL by covalent modification. Linking the ϵ-amino group of lysine residues to a solid support,[9] nitration of tyrosyl residues,[10] or cross-linking of crystals of CRL all increased the enantioselectivity of CRL toward 2-arylpropanoates.[11] Chemists do not know how these changes increase enantioselectivity on a molecular level.

An unusual strategy for increasing the enantioselectivity of CRL is to change its conformation using an organic solvent treatment.[12] Wu *et al.* isolated two hydrolases from crude CRL that differed in their enantioselectivity toward chiral carboxylic acids. They converted the low enantioselectivity hydrolase into the high enantioselectivity hydrolase by adding sodium deoxycholate and precipitating the enzyme with ethanol and ethyl ether. They suggested that the two hydrolases differed only in their conformation and that the organic solvent treatment changed the conformation to the high enantioselectivity form. Allenmark and Ohlsson confirmed these results for three arylpropionic acids.[13]

Unfortunately for the synthetic chemist, this procedure involved four purification steps including a cation exchange column and is, therefore, too tedious and expensive for synthetic use. Direct treatment of the crude lipase with deoxycholate followed by ethanol–ethyl ether only partially increased the enantioselectivity.[12]

In this paper we report a simple organic solvent treatment for crude CRL that increases its activity and enantioselectivity toward carboxylic acids. Like the more complex procedure above, this 2-propanol treatment increases the enantioselectivity of CRL toward 2-(aryloxy)- and 2-arylpropanoates but is simpler and yields synthetically useful amounts of enzyme. We used this 2-propanol-treated CRL to resolve 2-(4-chlorophenoxy)propanoic acid (**3**), whose (*R*)-enantiomer lowers cholesterol levels and prevents platelet aggregation.

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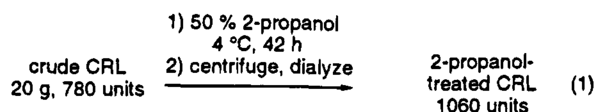
Table 1. 2-Propanol Treatment of *C. rugosa* Lipase

fraction	lipase activity[a] (U)	protein (mg)[b]	specific activity[a] (U/mg)	yield (%)
crude	780	800	1.0	100
2-propanol-treated[c]	1060	94	11	136

[a] Activity was measured using *p*-nitrophenyl acetate (PNPA) as the substrate (50 mM PNPA in 10 mM phosphate buffer pH 7.5, 25 °C). U = μmol of PNPA hydrolyzed per min. [b] Protein concentration was measured using a dye-binding assay from Bio-Rad. [c] The data refer to the supernatant. The resuspended precipitate contained 210 units of activity (PNPA assay) and a specific activity of 3 units/mg.

Results

2-Propanol Treatment of CRL. One gram of crude commercial lipase powder (Sigma) contained 2–4 wt % protein and 40–50 units of hydrolytic activity, measured with *p*-nitrophenyl acetate (PNPA) as the substrate, corresponding to a specific activity of ~1 unit/mg of protein. Twenty grams of this powder (780 units total) was dissolved in 50% 2-propanol, and the solution was stirred at 4 °C for 42 h, Table 1. A small amount of precipitate containing 210 units of activity and a specific activity of 3 units/mg of protein[14] was removed by centrifugation and discarded. The supernatant was dialyzed to remove 2-propanol and concentrated to 100 mL by ultrafiltration. This solution showed a specific activity of 11 units/mg of protein, indicating an 11-fold purification, and also a total activity of 1060 units (136% yield), indicating an activation of the lipase (eq 1). The



overall yield of hydrolytic activity was typically 120–160%. This solution, called 2-propanol-treated CRL, was stable for at least 3 months at 4 °C in the presence of 0.02 wt/vol % sodium azide as a preservative. This 2-propanol treatment is similar to the one used by Rubin *et al.*[15] to purify the CRL for crystallization. However, we extended the time of stirring the enzyme in 2-propanol–water from overnight to 42–48 h because this modification yielded a larger activation.

Enantioselectivity of 2-Propanol-Treated CRL. Our 2-propanol treatment increased the enantioselectivity of CRL toward carboxylic acid esters in the same manner as a multistep purification used by others.[12,13] We measured the enantioselectivity (enantiomeric ratio, E^{16}) of 2-propanol-treated CRL toward esters of seven different carboxylic acids and compared them to the reported enantioselectivities of crude CRL and CRL purified by a multistep procedure, Table 2. For all seven examples, the 2-propanol-treated CRL showed 2.3 to >25 times higher enantioselectivity than crude CRL. For all five examples where the enantioselectivities of both 2-propanol-treated and multistep-purified CRL were measured, both showed similar enantioselectivities, suggesting that both procedures modified the enzyme in a similar manner.

(14) The pellet was resuspended in buffer (50 mM MES, pH 6), centrifuged at 10000*g* for 10 min, and the specific activity of the supernatant was measured.
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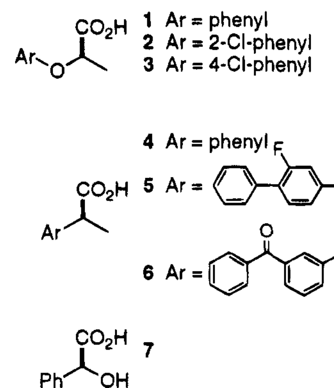
Scheme 1



All three enzyme preparations—crude CRL, 2-propanol-treated CRL, and CRL purified by a multistep procedure—showed the same stereochemical preference. Although the *R*/*S* designation of the fast-reacting enantiomer varied for the different acids, the shape of the fast-reacting enantiomer remained constant. An empirical rule based on the size of the substituents at the stereocenter predicts this enantiopreference, Figure 1.[17] Although this rule is not reliable for crude CRL (ref 17 lists exceptions), it correctly predicts which enantiomer reacts faster for multistep-purified CRL (16 examples in ref 17) and 2-propanol-treated CRL (seven examples in this paper). As researchers test a wider range of carboxylic acids with 2-propanol-treated CRL, we will learn how general this rule will be.

2-Propanol-treated CRL was less enantioselective than crude CRL toward two esters containing stereocenters in the alcohol portion of the ester. It was slightly less enantioselective than crude CRL in the hydrolysis of (±)-menthyl acetate ($E_{IPA} = 20$; $E_{crude} = 23$) and significantly less enantioselective in the hydrolysis of (±)-(2-acetoxy-1-naphthyl)methylphenylphosphine oxide[18] ($E_{IPA} = 3$, $E_{crude} = 81$). Thus, although the 2-propanol treatment increased enantioselectivity for all seven carboxylic acids tested, it decreased enantioselectivity toward esters of chiral alcohols.

The changes in enantioselectivity reported here are not caused by changes in the reaction conditions. All resolutions reported in this paper for both crude and 2-propanol-treated CRL occurred in water with the substrate dissolved in a small amount of ethyl ether. None of the reaction mixtures contained 2-propanol. The changes induced by the 2-propanol treatment persisted in water after the 2-propanol was removed by dialysis.

Although Baskar Rao found that addition of 2 wt % Triton X-100 to the reaction mixture increased the enantioselectivity of crude CRL toward chrysanthemic acid,[7] when we added 2 wt % Triton X-100 to the reaction mixture containing **3**-methyl ester, we measured only a small change in enantioselectivity of untreated CRL (from $E = 2.7$ to 4.6) and no change in the intial reaction rate.

Synthetic Use of 2-Propanol-Treated CRL. To demonstrate the synthetic usefulness of this 2-propanol-treated CRL, we resolved 10 g of **3**-methyl ester. Only (*R*)-**3** lowers serum cholesterol levels and prevents plate-

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Table 2. Enantioselectivity of Crude and Partially Purified *C. rugosa* Lipase toward Esters of 1–7

substrate	crude $E^{a,b}$	multistep purification $E^{a,b}$	2-propanol treatment					
			time[c] (h)	ee_s^d (%)	ee_p^d (%)	c^e (%)	SP[f]	E[a]
1-methyl ester	4[g]		0.5	53	77	41	*R*	13
2-methyl ester	2	14	1.0	54	73	43	*R*	11
3-methyl ester	2.3–17[h]	>100	0.6	77	98	44	*R*	>100
4-chloroethyl ester	10	>100	2.6	73[i]	96	43	*S*	>100
5-chloroethyl ester	10	>100	18.6	70	97	42	*S*	>100
6-chloroethyl ester	4	>100	1.8	38	99	28	*S*	>100
7-methyl ester	7–21[j]		22[k]	58	93	38	*S*	49

[a] Enantiomeric ratio as defined in ref 16. [b] Enantioselectivities for crude CRL and for CRL purified by the multistep procedure are from ref 12 unless otherwise noted. [c] Time required to reach 30–40% conversion starting with 0.32–0.56 mmol of substrate and 56 units (PNPA assay) of 2-propanol-treated CRL. [d] Determined by HPLC using a Chiralpak AD column. [e] Extent of conversion. [f] Stereochemical preference; the absolute configuration of the enantiomer that reacts faster. [g] In water containing 20% DMSO, ref 26. [h] Reference 12 reported an enantioselectivity of 17; however, we measured an enantioselectivity of 2.3 using their reaction conditions. Using the conditions described in the Experimental Section (pH stat, second phase of ethyl ether), we measured an enantioselectivity of 1.5 favoring the opposite enantiomer. These inconsistencies underscore the importance of using solvent treated CRL for predictable enantiopreference. [i] Determined by ^{1}H NMR in the presence of Eu(hfc)$_3$ chiral shift reagent. [j] References 11 and 17. [k] Due to slow reaction, we used 169 units (PNPA assay) of lipase, three times the usual amount.

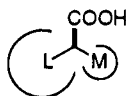
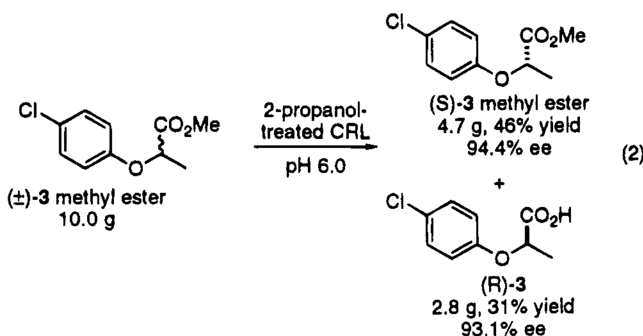


Figure 1. An empirical rule which summarizes the observed enantiopreference of hydrolysis reactions catalyzed by purified CRL or 2-propanol-treated CRL (L = a large substituent, e.g. Ar or OAr; M = a medium substituent, e.g. methyl or OH). Reference 17 summarizes the 16 examples for purified CRL; Table 2 summarizes the seven examples for 2-propanol-treated CRL. Note that this rule is not reliable for crude CRL.

let aggregation, while (*S*)-**3** causes a side effect—muscle irritability and spasms—by inhibiting the chloride channel in muscles.[19] The (*R*)-enantiomer of **3** and related 2-(aryloxy)propanoic acids are also herbicides, while the (*S*)-enantiomers are inactive.[20]

We stopped the hydrolysis at 50% conversion and isolated 4.7 g of the the starting ester (94.4% ee *S*) and 2.8 g of the product acid (93.1% ee *R*), which corresponds to an enantioselectivity of 100 (eq 2). The 2-propanol



treatment described above yields enough lipase to resolve ~200 g of **3**-methyl ester.

Molecular Basis for the Change in Enantioselectivity. We considered three possible mechanisms by which the 2-propanol treatment could change the enantioselectivity and specific activity of crude CRL: (1) it may remove contaminating hydrolases with lower, or opposite, enantioselectivity, (2) it may remove non-protein contaminants that inhibit the lipase and lower its enantioselectivity, or (3) it may change the conformation of the lipase, thereby activating the lipase and changing its enantioselectivity. We favor the last explanation but cannot conclusively rule out other possibilities.

Previous workers purified at least two hydrolases from crude CRL,[21] so the 2-propanol treatment may remove one of these hydrolases. However, we found that crude and 2-propanol-treated CRL contained the same proteins. Both electrophoresis under denaturing conditions (SDS-PAGE) and isoelectric focusing showed one major protein band for both crude and the 2-propanol-treated CRL (M_r 60–62 kD, pI = 4.1). Isoelectric focusing further showed several minor bands, but the relative amounts were similar in both the crude and the 2-propanol-treated CRL. Thus, we found no evidence that 2-propanol treatment removed contaminating proteins from crude CRL.

One possible non-protein contaminant in the crude lipase is a fatty acid. To test whether the 2-propanol treatment increased enantioselectivity by removing a fatty acid, we tried to reverse the increase in enantioselectivity by adding dodecanoic acid. The enantioselectivity of 2-propanol-treated CRL toward **3**-methyl ester did not change after addition of 8 mM dodecanoic acid (E > 100). Dialysis of crude CRL to remove low molecular weight contaminants also did not change its enantioselectivity toward **3**-methyl ester. These results suggest that the 2-propanol treatment does not remove fatty acids or other low molecular weight contaminants from the crude enzyme.

Recent crystal structures show two conformations of CRL that differ mainly in the orientation of a helical surface loop.[22] In one conformation, called "closed", the surface loop partially covered the hydrophobic crevice containing the active site, while in the other conformation, called "open", the surface loop has moved to uncover this crevice. To test whether the 2-propanol treatment

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increased enantioselectivity by changing CRL from the closed to the open conformation, we tried to lower the enantioselectivity by using conditions that should favor the closed form. Heating 2-propanol-treated CRL to 40 °C for 24 h in aqueous buffer decreased its enantioselectivity toward **3a**-methyl ester from >100 (ee_p = 97.5% at 44% conv) to 32 (ee_p = 91.2% at 30.8% conversion). This decrease is consistent with the notion that 2-propanol–water favors the more hydrophobic open conformation while pure water favors the less hydrophobic closed conformation. Heating presumably converts some of the open conformation to the closed, thereby decreasing enantioselectivity. A 2-propanol treatment of this heated sample increased the enantioselectivity to 56 (ee_p = 94.6% at 32.2% conversion), less than the original 2-propanol treatment, but consistent with a partial reopening of the lid. These changes in enantioselectivity are consistent with the open and closed conformations, but we cannot rule out other unknown conformational changes.

Interfacial activation experiments are also consistent with open and closed conformations for 2-propanol-treated CRL and crude CRL, respectively. Neither crude CRL, dialyzed CRL, nor 2-propanol-treated CRL showed significant interfacial activation using ethyl butyrate as a substrate. The rate of hydrolysis, monitored by pH stat, when the ethyl butyrate concentratation was 300 mM (drops of undissolved ester were clearly visible) was only slightly faster than the rate of hydrolysis when the ethyl butyrate concentration was 15 mM (completely dissolved): 2.1 times faster for crude CRL, 2.7 times faster for dialyzed CRL, 1.7 times faster for 2-propanol-treated CRL. While we did not expect 2-propanol-treated CRL, the open conformation, to show interfacial activation because the lid is already open, we were surprised by the absence of interfacial activation for the crude CRL, which presumably exists in the closed form. The lack of interfacial activation shows that the active site is accessible to both soluble and insoluble subtrates, suggesting that the closed conformation (expected to predominate in untreated CRL in the absence of an interface) is catalytically-active. Again, these experiment are consistent with the open and closed conformations but do not rule out other possibilities.

Discussion

The 2-propanol treatment described in this paper increased both the specific activity and the enantioselectivity of CRL toward 2-substituted carboxylic acids. The increase in enantioselectivity is the same as that reported by Wu *et al.*[12] using a longer, four-step procedure. In addition to being simpler, the 2-propanol treatment is also a more efficient purification. It gave an 11-fold increase in specific activity while Wu *et al.*'s procedure gave only a 2.4-fold increase. The 2-propanol treatment also activated CRL, since we recovered more units than we started with.

We demonstrated the synthetic use of 2-propanol-treated CRL in the resolution of **3**-methyl ester, an (aryloxy)propanoic acid. This resolution rivals the best of previously reported routes[23] and may be useful for the resolution of other carboxylic acids. For example, 2-propanol-treated CRL might be used for preparative-scale resolution of arylpropanoic acids, a class of non-steroidal antiinflammatory drugs, such as **4**–**6**. The (*S*)-arylpropanoic acids relieve pain and reduce fever and inflammation more effectively than the (*R*)-enantiomers.[24] Other routes, both chemical and enzymic, to pure enantiomers of arylpropanoic acids already exist.[25]

Other researchers have reported increased enantioselectivity of crude CRL under conditions that involve treating aqueous solutions of CRL with an organic solvent. For example, Hedstöm *et al.*[4] found that esterification of ibuprofen in a microemulstion of water in isooctane stabilized by bis(2-ethylhexyl) sulfosuccinate showed excellent enantioselectivity (E > 150), while similar reactions in only water or isooctane were less enantioselective E = 1.3 and 3.0). Cambou and Klibanov[23a] reported high enantioselectivity (E > 100) in the hydrolysis of **3**-methyl ester in concentrated solutions (~50 wt %), while other groups[12,26] reported lower enantioselectivities (E = 10.5–17) in more dilute solutions (<12 wt %). The organic solvents in these examples may cause changes similar to the 2-propanol treatment. The other additives and covalent modifications summarized in the Introduction may also cause changes similar to the 2-propanol treatment.

Although we have not ruled out other possibilities, we currently believe that 2-propanol treatment converts the closed form of CRL, present in the crude samples, to the open form. The decrease in enantioselectivity upon heating water and the lack of interfacial activation for the 2-propanol-treated CRL are both consistent with this suggestion. Three additional observations also support this suggestion. First, our 2-propanol treatment followed the same purification procedure that gave the crystalline open form of CRL.[15] Second, the activation and higher specific activity of the 2-propanol-treated lipase are consistent with a more accessible active site in the open form. Third, organic solvents accelerate the cis–trans isomerization of prolyl amides.[27] The X-ray crystal structures show that opening the lid requires a cis to trans isomerization of a prolyl amide link at Ser 91-Pro 92, so the role of 2-propanol may be to accelerate this isomerization. 2-Propanol may also create a hydrophobic environment that favors exposing the hydrophobic binding site in the "open" conformation. The crystallization conditions that yielded the open form of CRL contained 40% (v/v) of an alcohol, 2-methyl-2,4-pentanediol.[15,28]

Two further questions arise from the notion that the two conformations are the open and closed forms: (1) How can the closed form of CRL be catalytically active?

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and (2) How can the open conformation persist for months at 4 °C? The X-ray crystal structures suggest an explanation for both of these questions. Crystallographers identified two structural features involved in interfacial activation of lipases: the lid, or flap, of the lipase and the orientation of one of the oxyanion-stabilizing residues. In the inactive conformations of lipase from *Rhizomucor miehei* and human pancreatic lipase, the lid covers the active site and one oxyanion stabilizing residue orients incorrectly.[29] In the active conformation, this lid opens, exposing the active site. In addition, this opening of the lid also places the oxyanion-stabilizing residue into the catalytic orientation. Cutinase and ACE, which show no interfacial activation, lack a lid and contain a preformed oxyanion hole. The X-ray crystal structures show an intermediate case for CRL. Although the lid adopts two possible orientations (open and closed), the oxyanion-stabilizing residues orient correctly in both conformations. Thus, the closed conformation may be catalytically competent if substrate can reach the active site. It is reasonable to suggest that small substrates may reach this active site because the lid in CRL does not cover the active site crevice completely and also because the lid may be flexible.

The X-ray crystal structures also suggest how an open conformation can persist for months in aqueous solution at 4 °C. Closing the lid requires (1) isomerization of a prolyl amide link at Ser 91-Pro 92 from trans to the less stable cis, (2) breaking seven hydrogen bonds between the lid and protein including the carbohydrate attached at Asn 351, and (3) changing the secondary structure of the lid. These requirements may slow the closing of the lid. Although a prolyl amide link isomerizes on a time scale of minutes at 25 °C in peptides and a few proteins,[30] most protein structures fix the prolyl amide conformation as either cis or trans through other interactions. In CRL, hydrogen bonds and the secondary structure in the lid can stabilize the open conformation, thereby slowing the closing of the lid.

A comparison of the open and closed structures of CRL in the region that binds carboxylic acids reveals **1**–**4** Å differences in the orientations of the side chains. These differences may account for the differences in enantioselectivity, but a firm answer will require more detailed analysis. Other researchers previously suggested that the lid in lipases can influence its enantioselectivity. Covalent modification of the arginine side chain in the lid of lipases from *Humicola lanuginosa* and from *R. miehei* decreased their enantioselectivity toward esters of 2-methyldecanoic acid.[31]

Experimental Section

General. (±)-Ketoprofen, (±)-flurbiprofen, 2-(*N*-morpholino)ethanesulfonic acid (MES), and lipase from *C. rugosa* (L-1754, listed as *C. cylindracea*[1]) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). **7**-Methyl ester (Aldrich) was purified by flash chromatography on silica gel before use. Protein concentrations were measured using a dye-binding assay (Bio-Rad, Missisauga, ON) with bovine serum albumin (Sigma) as the standard. Enzyme activity was measured at pH 7.5 and 25 °C using *p*-nitrophenyl acetate as the substrate as previously described.[17,32] Crude CRL showed 30 U/g solid (1 U/mg prot) with this assay. U = μmol of ester hydrolyzed per minute.

2-Propanol Treatment of *C. rugosa* Lipase. Crude *C. rugosa* lipase (20 g, 780 units with PNPA assay) was dissolved in MES buffer (100 mL, 50 mM, pH 6.0, 4 °C) by stirring for 30 min. 2-Propanol (100 mL) was added dropwise over 40 min at 4 °C. This translucent solution became cloudy after stirring at 4 °C for 46 h. The precipitate was removed by centrifuging at 3000 rpm for 30 min at 4 °C. The supernatant was dialyzed against deionized distilled water (3 × 4 L) and concentrated to 116 mL by ultrafiltration (Amicon PM-10 filter) under N_2: 1290 U with PNPA assay, 165% yield. This solution retained full activity for at least 3 months when stored at 4 °C with 0.02 wt/vol % sodium azide as preservative.

Esters of Carboxylic Acids 1–6 have been prepared previously.[8a,11] We prepared the methyl esters using Brook and Chan's method[33] and the chloroethyl esters using the DCC method.[34] Reference 13 includes ^{1}H-NMR data for **4**-2-chloroethyl ester. The ^{1}H-NMR ($CDCl_3$, 200 MHz) data for the others are listed here: **1**-methyl ester: 84% yield, δ 7.32–6.85 (m, 5H), 4.78 (q, 1 H, J = 6.8 Hz), 3.76 (s, 3H), 1.63 (d, 3H, J = 6.9 Hz). **2**-methyl ester: 68% yield, δ 7.4–6.8 (m, 4H), 4.78 (q, 1H, J = 6.8 Hz), 3.77 (s, 3H), 1.69 (d, 3H, J = 6.8 Hz). **3**-methyl ester: 67% yield, δ 7.26–6.78 (m, 4H), 4.72 (q, 1 H, J = 6.8 Hz), 3.76 (s, 3 H), 1.62 (d, 3 H, J = 6.8 Hz). **5**-2-chloroethyl ester: 71% yield, δ 7.57–7.11 (m, 8H), 4.37 (m, 2H), 3.82 (q, 1H, J = 7.1 Hz), 3.67 (t, 2H, J = 5.7 Hz), 1.57 (d, 3H, J = 7.2 Hz). **6**-2-chloroethyl ester: 72% yield, δ 7.84–7.41 (m, 9H), 4.34 (m, 2H), 3.86 (q, 1H, J = 7.2 Hz), 3.64 (t, 2H, J = 5.7 Hz), 1.57 (d, 3H, J = 7.2 Hz).

Enantioselectivity of CRL toward Esters of 1–7. (±)-Carboxylic acid ester (100 mg) dissolved in 2 mL of ether was added to a solution of 2-propanol-treated *C. rugosa* lipase (5 mL, 56 units with PNPA assay) and MES buffer (5 mL, 50 mM, pH 6.0). The progress of the reaction was monitored by pH stat which controlled the addition of 0.1 N NaOH. When 30–40% conversion was reached, the reaction was worked up as decribed below for the preparative scale resolution. The enantiomeric purities of the acid and ester were measured as described below and the enantiomeric ratio, E,[16] calculated using $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$ and $c = ee_s/(ee_s + ee_p)$ where ee_s represents the enantiomeric excess of remaining substrate and ee_p represents enantiomeric excess of product.

Enantiomeric Purity. The enantiomers of the acids **1**–**7** were separated by HPLC using a column containing a modified amylose stationary phase (Chiralpak AD, 0.46 × 25 cm column, Daicel Chemical Industries Ltd., Fort Lee, NJ) as described previously by Okamoto *et al.* for **1** and **4**–**6**.[35] Acids **1**–**5** were eluted with 95/5/1 hexanes/2-propanol/CF_3COOH, 0.5 mL/min. This solvent mixture also separated the enantiomers of both **7**-methyl ester and **7** in a single injection using a flow rate of 1 mL/min. Acid **6** was eluted with 80/20/1 hexanes/2-propanol/CF_3COOH, 0.5 mL/min. The areas of the peaks were measured either by electronic integration or by cut and weigh when electronic integration gave unequal areas for the racemic acid. **1**: $k'_S = 1.46$; $k'_R = 2.10$; $\alpha = 1.44$; R = 4.70. **2**: $k'_S = 1.32$; $k'_R = 1.65$; $\alpha = 1.25$; $R = 2.67$. **3**: $k'_S = 2.13$; $k'_R = 2.80$; $\alpha = 1.31$; $R = 3.78$. **4**: $k'_R = 1.51$; $k'_S = 1.66$; $\alpha = 1.10$; $R = 1.16$. **5**: $k'_R = 1.80$; $k'_S = 2.69$; $\alpha = 1.49$; $R = 5.28$. **6**: $k'_R = 0.98$; $k'_S = 1.21$; $\alpha = 1.23$; $R = 1.98$. **7**-methyl ester: $k'_S = 2.57$; $k'_R = 2.79$; $\alpha = 1.09$; $R = 1.50$. **7**: $k'_S =$

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7.16; k'_R = 8.63; α = 1.21; R = 3.50. Esters of **1–3** and **5–6** were hydrolyzed to the acids using NaOH (1.5 equiv) and separated as described above. The enantiomeric purity of **4**-2-chloroethyl ester was measured by ^{1}H-NMR using Eu(hfc)$_3$ chiral shift reagent. Absolute configurations were established by comparison with an authentic sample for **7**, by comparing to the reported relative retention times of the enantiomers for **1,4–6**,[35] and by comparing the relative positions of the ^{1}H-NMR resonances in the presence of Eu(hfc)$_3$ for **2** and **3**.[12]

Preparative Resolution of (±)-3-Methyl Ester. 2-Propanol-treated *C. rugosa* lipase (6 mL, 67 units with PNPA assay) was added to a suspension of (±)-**3**-methyl ester (10.0 g, 47 mmol) in MES buffer (100 mL, 50 mM, pH 6.0). The progress of the hydrolysis reaction was monitored by pH stat which controlled the addition of 0.50 N NaOH. When the hydrolysis reached ~50% conversion, the reaction mixture was adjusted to pH 2 using 1 N HCl and extracted with ether (3 × 100 mL). The combined ether extracts were dried with $MgSO_4$ and concentrated by rotary evaporation. The residue was dissolved in a mixture of hexanes (100 mL) and saturated $NaHCO_3$ (100 mL), and the layers were separated. The hexanes layer was washed with saturated $NaHCO_3$ (50 mL), dried with K_2CO_3, and concentrated in vacuo, giving (*S*)-**3**-methyl ester, 4.7 g, 46% yield, a maximum yield of 50% is possible in a kinetic resolution, 94.4% ee. The aqueous layer was washed with hexanes (50 mL), acidified to pH 2, extracted with ether (3 × 100 mL), dried with $MgSO_4$, and concentrated in vacuo, giving (*R*)-**3**, 2.8 g, 31% yield, 93.1% ee.

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