Enantiopreference of Lipase from *Pseudomonas cepacia* toward Primary Alcohols

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We propose an empirical rule that predicts which enantiomer of a primary alcohol reacts faster in reactions catalyzed by lipase from *Pseudomonas cepacia* (PCL). This rule, based on the size of the substituents at the stereocenter, shows an 89% reliability (correct for 54 of 61 examples). This rule is not reliable for primary alcohols that have an oxygen atom attached to the stereocenter; we excluded these alcohols from the tally above. Surprisingly, the sense of enantiopreference of PCL toward primary alcohols is opposite to its enantiopreference toward secondary alcohols. That is, the OH of secondary alcohols and the CH_2OH of primary alcohols point in opposite directions. We suggest, however, that this opposite orientation does not imply a different position of the substituents in the active site of the lipase. Instead, PCL accommodates the extra CH_2 in primary alcohols as a kink between the stereocenter and the oxygen which allows a similar position of the alcohol oxygen in both. We tried to increase the enantioselectivity of PCL toward primary alcohols by increasing the difference in the size of the substituents but did not find a consistent increase in enantioselectivity. We suggest that high enantioselectivity toward primary alcohols requires not only a significant difference in the size of the substituents, but also control of the conformation along the C(1)–C(2) bond.

Introduction

Organic chemists have embraced lipases and esterases as enantioselective catalysts for synthetic applications because they combine broad substrate specificity with high enantioselectivity.[1] One current goal of organic chemists is to map the specificity of these enzymes. This mapping identifies both efficiently resolved substrates and the structural features important for their enantiorecognition, allowing chemists to more rationally design resolutions.

Previous mapping of the specificity of lipase from *Pseudomonas cepacia*[2] (PCL, Amano Lipase P) established a simple rule that predicts its enantiopreference toward secondary alcohols, Figure 1a.[3–6] This rule predicts which enantiomer reacts faster based on the sizes of the substituents at the stereocenter. The same rule holds for ten other hydrolases whose specificities have been mapped: lipase from *Candida rugosa*,[3] lipase from *Pseudomonas* sp.,[7] lipase from *P. aeruginosa*,[8] *Rhizomucor miehei*,[9] lipase from *Arthrobacter* sp.,[10] porcine pancreatic lipase,[11] pancreatic cholesterol esterase,[3] *Mucor* esterase,[11] cultures of *Rhizopus nigricans*,[12] and cultures of *B. subtilus* var. *Niger*.[13] This rule suggests that these hydrolases distinguish between enantiomers based on the size of the substituents. Consistent with this suggestion, researchers have increased the enantioselectivity of lipase-catalyzed reactions of secondary alcohols by increasing the difference in size of the two substituents.[3,5,14]

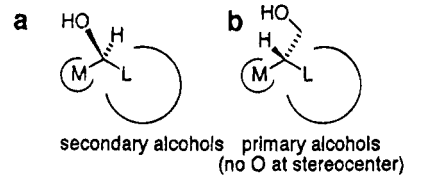

Figure 1. Empirical rules that summarize the enantiopreference of *P. cepacia* lipase (PCL) toward chiral alcohols. (a) Shape of the favored enantiomer of secondary alcohols. M represents a medium substituent, e.g. CH_3, while L represents a large substituent, e.g., Ph. (b) Shape of the favored enantiomer of primary alcohols. This rule for primary alcohols is reliable only when the stereocenter lacks an oxygen atom. Note that PCL shows an opposite enantiopreference toward primary and secondary alcohols.

Recent X-ray crystal structures of transition state analogs bound to lipase from *Candida rugosa* showed that this rule is a good description of the alcohol binding

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(1) Review: Faber, K. *Biotransformations in Organic Chemistry*, 2nd ed; Springer-Verlag, New York, 1995.

(2) Amano Pharmaceuticals Co. sells several different types of lipase from *P. cepacia*: lipase PS, lipase P, LPL-80, LPL-200S, and SAM-II. All of these types come from the same microorganism but differ in the purification method and the types of stabilizers used. Lipase P or PS is the industrial grade which contains diatomaceous earth, dextran, and $CaCl_2$. LPL-80 and LPL-200S are diagnostic grades that contain glycine. SAM-II differs from lipase P or PS only in the purification method. This type is also sold by Fluka under the name of lipase from *Pseudomonas fluorescens*, SAM-2, because this microorganism was only recently reclassified as *Pseudomonas cepacia*. We thank Dr. Yoshihiko Hirose (Amano Pharmaceuticals Co.) for explaining these differences.

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pocket.[15] This lipase contains a large hydrophobic binding pocket that binds the larger substituent of a secondary alcohol and a smaller pocket that binds the medium substituent. Conserved structural elements create this binding crevice, especially the pocket for the medium substituent. For this reason, other lipases probably contain similar alcohol binding sites.

Several groups have tried to extend this rule for secondary alcohols to include primary alcohols, but these efforts have been only partially successful. Naemura *et al.* used a rule based on the size of the substituents to account for the enantiopreference of lipase from *Pseudomonas* sp. toward two primary alcohols,[16] and Carrea *et al.*[17] used a similar rule to account for the enantiopreference of PCL toward nine primary alcohols. However, Xie *et al.*[18] proposed an enantiomeric rule for the enantiopreference of PCL toward two primary alcohols without an oxygen at the stereocenter and another rule based on two primary alcohols with an oxygen at the stereocenter.[19] Researchers encountered similar difficulties with porcine pancreatic lipase (PPL) and also proposed enantiomeric rules.[20]

In this paper, we examine the reported enantiopreference of PCL and propose an empirical rule that summarizes its enantiopreference toward primary alcohols. Although the rule is opposite to the one for secondary alcohols, we suggest that the large and medium substituents adopt a similar position in both. We also attempt, unsuccessfully, to increase the enantioselectivity of PCL toward primary alcohols by increasing the difference in size of the two substituents at the stereocenter.

Results and Discussion

Enantiopreference of PCL toward Primary Alcohols. Following the suggestion of Xie *et al.*,[18] we divided the primary alcohols into those with an oxygen at the stereocenter and those without an oxygen at the stereocenter. Table 1 and Chart 1 summarize the reported enantioselectivities of PCL toward primary alcohols that lack an oxygen at the stereocenter. The enantiomers shown in Chart 1 are those predicted to react faster by the empirical rule in Figure 1b: the CH_2OH points into the page and the larger substituent lies on the right. The notation "(ent)" in Table 1 marks those examples where PCL favored the enantiomer opposite to the one predicted. This list includes only primary alcohols with a tertiary stereocenter, that is, $R^1R^2CHCH_2OH$. The list further includes only reactions catalyzed by PCL from Amano (P, P30, PS, LPL-80, LPL-200S) and SAM-2 from Fluka,[2] and only those substrates for which the enantiomeric ratio,[21] E, was >2. The list includes esterifications and transesterifications of primary alcohols as well as hydrolyses of esters of primary alcohols.

The rule in Figure 1b predicts the absolute configuration of the favored enantiomer for 49 out of 54 substrates, 91% accuracy. We will discuss seven more examples in this paper, five of which follow the rule giving an accuracy of 54 out of 61 or 89%. The examples include acyclic, cyclic, and bicyclic alcohols with a wide range of functional groups in the substituents. Approximately 30% of the substrates (16 of the 54) showed excellent enantioselectivities of $E > 50$. The five exceptions to the empirical rule are 2-methyl-1,3-propanediol (structure **1** where $R = CH_2OH$), two aziridines, structure **9**, and two cyclohexenone derivatives, structure **18**. In spite of these exceptions, the empirical rule is reliable for most primary alcohols without an oxygen at the stereocenter. The rule in Figure 1b is similar to the rule proposed by Xie *et al.*[18] for two primary alcohols. Although our rule is opposite to the one suggested by Carrea *et al.*,[17] we excluded their examples because they all contained an oxygen at the stereocenter.

Table 2 and Chart 2 summarize the reported enantioselectivities of PCL toward primary alcohols that have an oxygen at the stereocenter. All structures show the enantiomer predicted to react faster, while the notation "(ent)" marks the exceptions in Table 2. The large number of exceptions shows that the empirical rule is not reliable for this group of substrates. The empirical rule predicts the enantiopreference for only 10 of the 27 examples, corresponding to 37% accuracy which is similar to that expected by chance. We excluded substrate **25** ($R = CH_3$) from the tally because both substituents are similar in size. Approximately 30% of the substrates (8 of 27) showed excellent enantioselectivities ($E > 50$); all of these are exceptions to the empirical rule. Thus, the empirical rule in Figure 1b is not reliable when the primary alcohols have an oxygen at the stereocenter. An opposite rule would be slightly more reliable, but still only a slight improvement over guessing.

A similar division of primary alcohols into two groups, those with and without oxygens at the stereocenter, may also resolve the dilemma of enantiomeric rules in the case of PPL. Our preliminary survey found 41 examples of PPL-catalyzed resolutions of primary alcohols without an oxygen at the stereocenter. Twenty-seven examples fit the rule in Figure 1b and four did not; we excluded ten substrates because the sizes of the substituents were too similar. Thus, the reliability was 27/31 or 87% for those with substituents that differed in size. This degree of reliability is similar to that for PCL. We also found 10 examples of PPL-catalyzed resolutions of primary alcohols which have an oxygen at the stereocenter. Three examples fit the rule and six did not; we excluded one substrate. Thus, as with PCL, the rule is not reliable for primary alcohols that have an oxygen at the stereocenter.

Do the Medium and Large Substituents of Primary and Secondary Alcohols Bind to the Same Regions of PCL? We propose two hypotheses to explain how PCL can have an opposite enantiopreference for primary and secondary alcohols, Figure 2a. Hypothesis 1 proposes that the large and medium substituents of primary alcohols bind in the same L and M pockets as

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(18) Xie, Z.-F.; Suemune, H.; Sakai, K. *Tetrahedron: Asymmetry* **1993**, *4*, 973–980. Earlier reports (see reference 4) from this group indicate that PFL is Amano lipase P.

(19) Only two of the three examples supported this suggestion. The original reference for the third example, structure **21** in reference 18, indicated that PCL prefers the enantiomer opposite to the one drawn. Other researchers also noted difficulties in extending the rule for secondary alcohols to primary alcohols. Ferraboschi, P.; Casati, S.; Degrandi, S.; Grisenti, P.; Santaniello, E. *Biocatalysis* **1994**, *10*, 279–288.

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Table 1. Enantioselectivity of Lipase from *Pseudomonas cepacia* toward Primary Alcohols without an Oxygen at the Stereocenter[a]

structure	R or Ar	E^b	ref	structure	R or Ar	E^b	ref
1	$CH_2CH_2CH_3$	5.9	*c*	**5**	$CH_2(CH_2)_2CH_3$	7.3	*c*
1	$CH_2(CH_2)_2CH_3$	8.7	*c*	**6**	2,4-Cl_2-C_6H_3	~10	*s*
1	$CH_2(CH_2)_4CH_3$	5.7	*c*	**6**	*N*-Cbz-piperid-4-yl	~7	*t*
1	$CH_2(CH_2)_6CH_3$	9.9, >20	*c,d*	**6**	NHC(O)-1-naphthyl	~25	*u*
1	$CH_2CH_2CH(CH_3)_2$	11	*c*	**6**	$CH_2CH(OEt)_2$	>50	*v*
1	*trans*-CH_2CH=$CHCH_3$	13	*c*	**6**	$(CH_2)_3CHMe(CH_2)_3i$-Pr	13	*w*
1	CH_2CH=$CHCH_2$	9.7	*c*	**7**	3,4-OCH_2O-C_6H_3	>~50	*x*
1	CH_2Ph	20	*e*	**7**	3,4-$(MeO)_2$-C_6H_3	>~50	*x*
1	CH_2(2-thiophene)	>24–>50	*f*	**7**	3,4,5-$(MeO)_3$-C_6H_2	20	*x*
1	CH_2CH_2SPh	21	*g*	**7**	4-Ph-C_6H_4	>~50	*x*
1	$CH_2CH_2SO_2Ph$	21	*g*	**7**	Ph	>30–>50	*j,x,y*
1	$CH_2OC(O)Pr$	~28	*h*	**7**	1-naphthyl	13, >~50	*x, y*
1	NHC(O)OEt	8.6	*i*	**8**		11	*z*
1	CH_2OH	4 (ent)	*j*	**9**	$C(O)OCH_2Ph$	~40 (ent)	*aa*
1	CH_2OAc	2–>50	*k–m*	**9**	tosyl	>~50 (ent)	*aa*
1	CH_2CH_2OH	~4	*n*	**10**		5	*bb*
1	$CH_2CH_2OCH_2Ph$	~20	*n*	**11**		~13	*cc*
1	CH_2O-TBDMS	>50	*m*	**12**		>50	*dd,ee*
1	CH_2O-TBDPS	>50	*k,m*	**13**		~4	*dd*
1	CH_2OCH_2Ph	12–35	*m*	**14**		~3	*ff*
1	$CH_2OC(O)Ph$	9–35	*m*	**15**		14	*gg*
1	$CH_2CH(CH_3)CH_2OH$	>~50	*j*	**16**		4	*hh*
2		2–>40	*o*	**17**		>50	*ii*
3	4-MeO-C_6H_4	7.5–40	*p*	**18**	CH_3	14 (ent)	*ii*
4	Ph	3.3	*q*	**18**	CH_2OCH_3	>50 (ent)	*ii*
5	CH_2OAc	~16	*r*	**19**		>50	*jj*
5	NHC(O)OEt	8.9	*i*	**20**		~2	*kk*

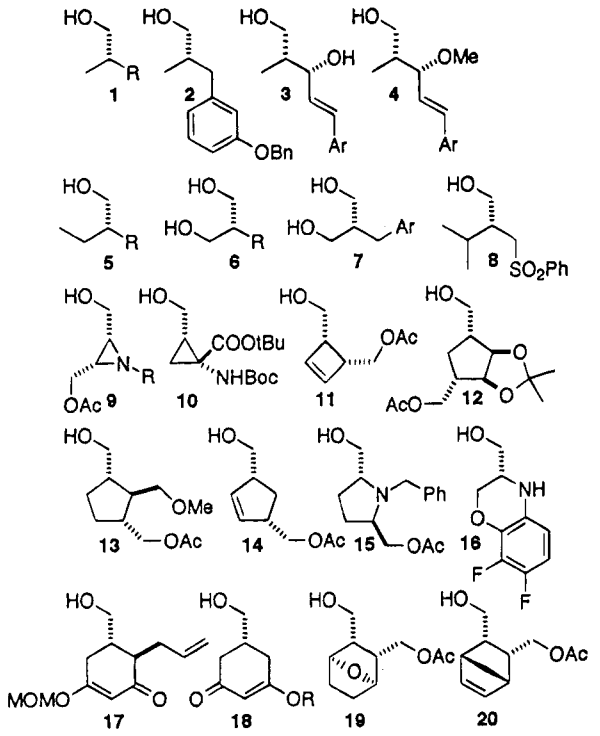
[a] All reactions refer to the hydrolysis of the acetate ester in aqueous solution at room temperature, using lipase from *Pseudomonas cepacia* as defined in reference 2, unless otherwise noted. When another ester was hydrolyzed, it is identified; when the alcohol was esterified, the acylating reagent is identified. Only examples that give $E > 2$ are listed. The structures are shown in Chart 1. [b] E, the enantioselectivity, is calculated as in reference 21, except in the case of asymmetric syntheses where it is the ratio of the preferred enantiomer to the least preferred enantiomer. [c] Vinyl acetate: Barth, S.; Effenberger, F. *Tetrahedron: Asymmetry* **1993**, *4*, 823–833. [d] Vinyl acetate, PFL (Fluka): Ferraboschi, P.; Grisenti, P. Manzocchi, A.; Santaniello, E. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1159–1161. [e] Vinyl acetate: Delinck, D. L.; Margolin, A. L. *Tetrahedron Lett.* **1990**, *31*, 6797–6798. [f] Vinyl acetate: Nordin, O.; Hedenström, E.; Högberg, H. E. *Tetrahedron: Asymmetry* **1994**, *5*, 785–788. Bracher, F.; Papke, T. *Tetrahedron: Asymmetry* **1994**, *5*, 1653–1656. [g] Vinyl acetate, PFL (Fluka): Ferraboschi, P.; Grisenti, P.; Manzocchi, A.; Santaniello, E. *J. Org. Chem.* **1990**, *55*, 6214–6216. [h] Butyrate: Wirz, B.; Schmid, R.; Walther, W. *Biocatalysis* **1990**, *3*, 159–167. [i] Francalanci, F.; Cesti, P.; Cabri, W.; Bianchi, D.; Martinengo, T.; Foà, M. *J. Org. Chem.* **1987**, *52*, 5079–5082. [j] Vinyl acetate: Tsuji, K.; Terao, Y.; Achiwa, K. *Tetrahedron Lett.* **1989**, *30*, 6189–6192. [k] Vinyl acetate, PFL (Fluka): Santaniello, E.; Ferraboschi, P.; Grisenti, P. *Tetrahedron Lett.* **1990**, *31*, 5657–5660. TBDPS = *tert*-butyldiphenylsilyl. [l] Xie, Z.-F.; Suemune, H.; Sakai, K. *Tetrahedron: Asymmetry* **1993**, *4*, 973–980. [m] Vinyl acetate, PFL (Fluka): Grisenti, P.; Ferraboschi, P.; Manzocchi, A.; Santaniello, E. *Tetrahedron* **1992**, *48*, 3827–3834. [n] Vinyl acetate, PFL (Fluka): Grisenti, P.; Ferraboschi, P.; Casati, S.; Santaniello, E. *Tetrahedron: Asymmetry* **1993**, *4*, 997–1006. [o] Ethyl acetate: Bianchi, D.; Cesti, P.; Golini, P.; Spezia, S.; Filippini, L.; Garavaglia, C.; Mirenna, L. *J. Agric. Food Chem.* **1992**, *40*, 1989–1992. [p] Isopropenyl acetate: Akita, H; Umezawa, I.; Nozawa, M.; Nagumo, S. *Tetrahedron: Asymmetry* **1993**, *4*, 757–760. [q] Isopropenyl acetate: Akita, H.; Nozawa, M.; Umezawa, I.; Nagumo, S. *Biocatalysis* **1994**, *9*, 79–87. [r] Gaucher, A.; Ollivier, J.; Marguerite, J.; Paugam, R.; Salaün, J. *Can. J. Chem.* **1994**, *72*, 1312–1327. [s] Ethyl acetate: Bianchi, D.; Cesti, P.; Spezia, S.; Garavaglia, C.; Mirenna, L. *J. Agric. Food Chem.* **1991**, *39*, 197–201. [t] PFL (Fluka): Guanti, G.; Banfi, L.; Brusco, S.; Narisano, E. *Tetrahedron: Asymmetry* **1994**, *5*, 537–540. [u] Vinyl butyrate: Terradas, F.; Teston-Henry, M.; Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1993**, *115*, 390–396. [v] Vinyl acetate: Ohsawa, K.; Shiozawa, T.; Achiwa, K.; Terao, Y. *Chem. Pharm. Bull.* **1993**, *41*, 1906–1909. [w] Takabe, K.; Sawada, H.; Satani, T.; Yamada, T.; Katagiri, T.; Yoda, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 157–160. [x] Vinyl acetate: Itoh, T.; Chika, J.; Takagi, Y.; Nishiyama, S. *J. Org. Chem.* **1993**, *58*, 5717–5723. [y] Vinyl acetate: Atsuumi, S.; Nakano, M.; Koike, Y.; Tanaka, S.; Ohkubo, M.; Yonezawa, T.; Funabashi, H.; Hashimoto, J.; Morishima, H. *Tetrahedron Lett.* **1990**, *31*, 1601–1604. [z] Chloroacetate: Guevel, R.; Paquette, L. A. *Tetrahedron: Asymmetry* **1993**, *4*, 947–956. [aa] Transesterification with 1-butanol: Fuji, K.; Kawabata, T.; Kiryu, Y.; Sugiura, Y.; Taga, T.; Miwa, Y. *Tetrahedron Lett.* **1990**, *31*, 6663–6666. [bb] Vinyl acetate: Burgess, K.; Ho, K.-K. *J. Org. Chem.* **1992**, *57*, 5931–5936. [cc] Harvey, I.; Crout, D. H. G. *Tetrahedron: Asymmetry* **1993**, *4*, 807–812. [dd] Tanaka, M.; Yoshioka, M.; Sakai, K. *Tetrahedron: Asymmetry* **1993**, *4*, 981–996. [ee] PFL (Fluka): Mohar, B.; Stimac, A.; Kobe, J. *Tetrahedron: Asymmetry* **1994**, *5*, 863–878. [ff] PFL (Fluka): Mekrami, M.; Sicsic, S. *Tetrahedron: Asymmetry* **1992**, *3*, 431–436. [gg] In phosphate buffer containing 20% DMSO: Kawanami, Y.; Moriya, H.; Goto, Y. *Chem. Lett.* **1994**, 1161–1162; vinyl acetate: Sibi, M. P.; Lu, J. *Tetrahedron Lett.* **1994**, *35*, 4915–4918. [hh] Sakano, K.; Yokohama, S.; Hayakawa, I.; Atarashi, S.; Kadoys, S. *Agric. Biol. Chem.* **1987**, *51*, 1265–1270. [ii] Vinyl acetate: Miyaoka, H.; Sagawa, S.; Inoue, T;. Nagaoka, H.; Yamada, Y. *Chem. Pharm. Bull.* **1994**, *42*, 405–407. [jj] Patel, R. N.; Liu, M.; Banerjee, A.; Szarka, L. J. *Appl. Microbiol. Biotechnol.* **1992**, *37*, 180–183. [kk] Vinyl acetate: Murata, M.; Ikoma, S.; Achiwa, K. *Chem. Pharm. Bull.* **1990**, *38*, 2329–2331.

the substituents of secondary alcohols. The CH_2OH group must point to the back to place the oxygen in a position similar to that for secondary alcohols. Thus, the opposite enantiopreference for primary and secondary alcohols would stem from accommodating the extra CH_2 in primary alcohols as a kink between the O and the stereocenter.

Hypothesis 2 proposes that the substituents of primary and secondary alcohols bind to different regions of PCL. In particular, both the large and medium substituents of primary alcohols bind in the L pocket, while a hydrogen binds in the M pocket. According to this hypothesis, PCL accommodates the added CH_2 in a primary alcohol as a turn that places the stereocenter of primary alcohols into the L pocket. Stereoselectivity within the L pocket would determine the enantioselectivity of PCL toward primary alcohols. Vanmiddlesworth and Sih[22] proposed that stereoselectivity within the L pocket of a reductase in yeast can influence the diastereoselectivity of reduction reactions.

To distinguish between these two hypotheses, we measured the stereoselectivity of the L pocket in PCL

Chart 1. Structures for Table 1 and Figure 4



and compared it to the enantioselectivity of PCL toward primary alcohols, Figure 2b. Hypothesis 1 predicts no relationship between the stereoselectivity of the L pocket and the enantioselectivity of PCL toward primary alcohols. Hypothesis 2 predicts that the stereoselectivity within the L pocket is the same as the enantioselectivity of PCL toward primary alcohols. As detailed below, we found that the stereoselectivity of the L pocket of PCL was significantly lower that the enantioselectivity of PCL toward primary alcohols; thus, we favor hypothesis 1.

To measure the stereoselectivity of the L pocket in PCL, we measured the stereoselectivity of PCL toward acetates of **35a** and **35b**, Scheme 1. Binding these secondary alcohols to PCL as suggested by the rule in Figure 1a places the β-stereocenter into the L pocket.[23] Thus, the stereoselectivity of PCL toward the β-stereocenter of **35a** and **35b** corresponds to the stereoselectivity of the L pocket.

Scheme 1 summarizes the experimental results for the PCL-catalyzed hydrolysis of the acetate esters of **35a** and **35b**. We prepared alcohols **35a** and **35b** and their acetates as mixtures of diastereomers following literature procedures. After hydrolysis, we determined the relative amounts of each isomer using gas chromatography of the (*S*)-acetyl lactic acid derivatives for **35a** and of the free alcohols for **35b**. To identify the diastereomers, we prepared authentic samples of pure *erythro*-**35a**, and *threo*-**35b** using literature procedures. We confirmed this assignment for **35b** by comparing the ^{1}H-NMR spectrum of the mixture to the ^{1}H-NMR of a known (4:1) mixture of *erythro*- and *threo*-**35b**.[24]

The absolute configurations of the preferred *erythro*- and *threo*-**35a** were (2*R*,3*R*) and (2*R*,3*S*), respectively, based on the negative rotations of the tosyl derivatives. The preferred enantiomer of *threo*-**35b** was (2*R*,3*S*), as determined by chemical correlation to the ketone derivative, (3*S*)-3-methyl-4-phenyl-2-butanone. The rule for secondary alcohols, Figure 1a, predicted the favored configuration at the secondary alcohol for all three of these substrates.[23] On this basis, we assigned the (2*R*,3*R*) configuration to the favored enantiomer for *erythro*-**35b**.

To calculate the α- and β-selectivities, the stereoselectivities at the α and β stereocenters, we used the approach developed by Sih to measure the enantioselectivity of an enzyme.[25] Equation 1 relates the selectivity of the enzyme to experimentally measured quantities: A_0 and B_0 are the initial amounts of each isomer in the starting material and A and B are the amounts remaining after hydrolysis.

$$\text{selectivity} = \frac{\ln(A/A_0)}{\ln(B/B_0)} \qquad (1)$$

We calculated the α-selectivity either from the relative amounts of the (2*R*,3*S*) and (2*S*,3*S*) isomers, $\alpha > 32$ for **35a**, $\alpha > 68$ for **35b**, or from the relative amounts of the (2*R*,3*R*) and (2*S*,3*R*) isomers, $\alpha > 100$ for **35a**, $\alpha > 55$ for **35b**. The α-selectivity was high for each pair of isomers, and the favored enantiomer was the one predicted by the secondary alcohol rule. These results suggest that the **35a**-acetate and the **35b**-acetate bound to PCL in the same manner as other secondary alcohols and that the β-stereocenters bound in the L pocket.

We calculated the β-selectivity from the selectivity between the favored 2*R* isomers, (2*R*,3*S*) and (2*R*,3*R*), to be $\beta = 1.4$ for **35a** and $\beta = 1.8$ for **35b**. We did not calculate the β-selectivity from the pair of minor isomers because these "incorrect" secondary alcohols may bind to PCL in a manner that does not place the β-stereocenter in the L pocket.

To interpret these results, we also measured the enantioselectivity of PCL toward the corresponding secondary alcohols **36a** and **36b**, and the corresponding primary alcohols **37a** and **37b** (**37b** is the same as **1** where R = CH_2Ph, Table 1), Table 3. As expected, PCL favored the (*R*)-enantiomer of secondary alcohols **36a** and **36b** with high selectivity, $E = 54$ and 80, respectively. Also as expected, PCL favored the (*S*)-enantiomer of primary alcohols **37a** and **37b**. The enantioselectivity was low toward **37a**, $E = 2.3$, and moderate toward **37b**, $E = 16$. These enantioselectivities are consistent with those measured previously by others for the corresponding esterification reaction, $E = 1.3$ for **37a**[26] and $E = 20$ for **37b** (Table 1, structure **1** where R = CH_2Ph).

A summary of these selectivities, Figure 2c, shows a low selectivity in the L pocket of PCL (1.4 and 1.8),[27]

(22) Vanmiddlesworth, F.; Sih, C. J. *Biocatalysis* **1987**, *1*, 117–127; see also Shieh, W. R.; Sih, C. J. *Tetrahedron: Asymmetry* **1993**, *4*, 1259–1269.

(23) Our earlier survey (reference 3) showed that this rule shows a 98% reliability (63 of 64 substrates) for PCL-catalyzed reactions of secondary alcohols.

(24) Gammill, R. B.; Nash, S. A.; Bell, L. T.; Watt, W.; Mizsak, S. A.; Scahill, T. A.; Sobieray, D. *Tetrahedron Lett.* **1990**, *31*, 5303–5306. We thank Dr. Gammill for sending us a copy of his ^{1}H-NMR spectrum.

(25) According to this approach, the relative rate of reaction of two competing substrates, v_A/v_B, depends on the concentration of the two substrates, A and B, and on the ratio of the specificity constants, V/K, for the two substrates. The ratio of the specificity constants measures the inherent selectivity of the enzyme for a pair of competing substrates. Integration of the equation yields eq 1 in the text. See reference 21.

$$\frac{v_A}{v_B} = \frac{V_A/K_A}{V_B/K_B} \times \frac{[A]}{[B]}$$

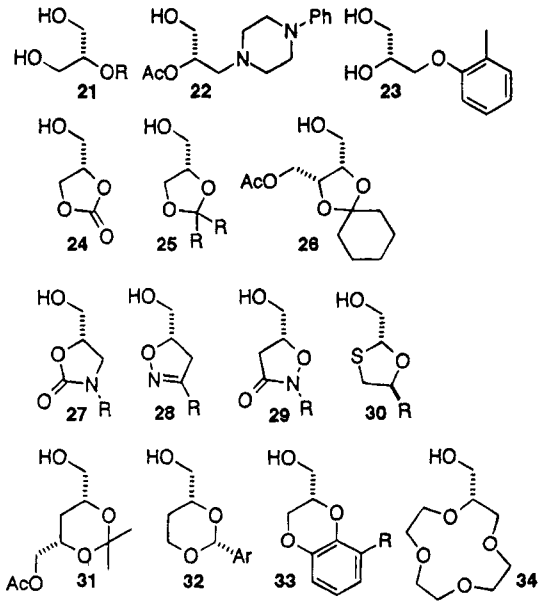
(26) Bianchi, D.; Cesti, P.; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531–5534.

Table 2. Enantioselectivity of Lipase from *Pseudomonas cepacia* toward Primary Alcohols with an Oxygen at the Stereocenter[a]

structure	R or Ar	E^b	ref	structure	R or Ar	E^b	ref
21	CH_3	~6 (ent)	*c*	**27**	$CH(CH_3)_2$	31->50 (ent)	*i,m*
21	CH_2CH_3	~20 (ent)	*c*	**28**	Br	6->50 (ent)	*n-p*
21	CH_2Ph	~20-28 (ent)	*c,d*	**28**	OCH_3	14 (ent)	*o*
22		>50 (ent)	*e*	**28**	OCH_2Ph	4 (ent)	*o*
23		4	*f*	**28**	CH_2OH	8 (ent)	*o*
24		15	*g*	**29**	CH_3	3	*o,p*
25	CH_3	4-9	*h-j*	**29**	Ph	41 (ent)	*o, p*
25	CH_2CH_3	2-6	*i,j*	**30**	1-(5-fluorocytosine)	12	*q*
25	Ph	3-8	*i-k*	**31**		~50 (ent)	*r*
25	$-(CH_2)_5-$	3	*j*	**32**	Ph	5->50 (ent)	*s*
25	$CH(CH_3)_2$	23	*i*	**32**	4-OMe-C_6H_4	21->50 (ent)	*s*
25	CH_2Ph	9	*i*	**33**	H	8-16 (ent)	*t,u*
26		>50 (ent)	*l*	**33**	OCH_3	28 (ent)	*t*
27	$C(CH_3)_3$	>50 (ent)	*m*	**34**		3 (ent)	*v*

[a] All reactions refer to the hydrolysis of the acetate ester in aqueous solution at room temperature, using lipase from *Pseudomonas cepacia* as defined in reference 2, unless otherwise noted. When another ester was hydrolyzed, it is identified; when the alcohol was esterified, the acylating reagent is identified. Only examples that give $E > 2$ are listed. The structures are shown in Chart 2. [b] E, the enantioselectivity, is calculated as in reference 21, except in the case of meso compounds where it is the ratio of the favored to unfavored enantiomers. [c] Vinyl acetate or phenyl acetate: Terao, Y.; Murata, M.; Achiwa, K.; Nishio, T.; Akamtsu, M.; Kamimura, M. *Tetrahedron Lett.* **1988**, *29*, 5173-5176; vinyl acetate: Murata, M.; Terao, Y.; Achiwa, K.; Nishio, T.; Seto, K. *Chem. Pharm. Bull.* **1989**, *37*, 2670-2672. [d] Breitgoff, D.; Laumen, K.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 1523-1524; vinyl stearate: Baba, N.; Yoneda, K.; Tahara, S.; Iwasa, J.; Kaneko, T.; Matsuo, M. *J. Chem. Soc., Chem. Commun.* **1990**, 1281-1282; vinyl stearate: Baba, N.; Tahara, S.; Yoneda, K.; Iwasa, J. *Chem. Express* **1991**, *6*, 423-426; Wirz, B.; Schmid, R.; Foricher, J. *Tetrahedron: Asymmetry* **1992**, *3*, 137-142. [e] Transesterification with 1-propanol as nucleophile: Bianchi, D.; Bosetti, A.; Cesti, P.; Golini, P. *Tetrahedron Lett.* **1992**, *33*, 3231-3234. [f] Vinyl acetate: Theil, F.; Weidner, J.; Ballschuh, S.; Kunath, A.; Schick, H. *J. Org. Chem.* **1994**, *59*, 388-393. [g] Pallavicini, M.; Valoti, E.; Villa, L.; Piccolo, O. *J. Org. Chem.* **1994**, *59*, 1751-1754. [h] Benzoate hydrolysis in 25% DMSO: Bosetti, A.; Bianchi, D.; Cesti, P.; Golini, P. *Biocatalysis* **1994**, *9*, 71-77. [i] Succinic anhydride: Terao, Y.; Tsuji, K.; Murata, M.; Achiwa, K.; Nishio, T.; Watanabe, N.; Seto, K. *Chem. Pharm. Bull.* **1989**, *37*, 1653-1655. [j] Butanoate: Partali, V.; Melbye, A. G.; Alvik, T.; Anthonsen, T. *Tetrahedron: Asymmetry* **1992**, *3*, 65-72. [k] Bellemare, M.-J.; Kazlauskas, R. J., unpublished results. [l] In a water/isopropyl ether emulsion: Gais, H.-J.; Hemmerle, H.; Kossek, S. *Synthesis* **1992**, 169-173. [m] Acetic anhydride: Bianchi, D.; Cesti, P.; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531-5534. [n] Trifluoroethyl butanoate: Secundo, F.; Riva, S.; Carrea, G. *Tetrahedron: Asymmetry* **1992**, *3*, 267-280. [o] Butyrate: Carrea, G.; De Amici, M.; De Micheli, C.; Liverani, P.; Carnielli, M.; Riva, S. *Tetrahedron: Asymmetry* **1993**, *4*, 1063-1072. [p] Butyrate: De Amici, M.; Magri, P.; De Micheli, C.; Cateni, F.; Bovara, R.; Carrea, G.; Riva, S.; Casalone, G. *J. Org. Chem.* **1992**, *57*, 2825-2829. [q] Butyrate: Hoong, L. K.; Strange, L. E.; Liotta, D. C.; Koszalka, G. W.; Burns, C. L.; Schinazi, R. F. *J. Org. Chem.* **1992**, *57*, 5563-5565. [r] Xie, Z.-F.; Suemune, H.; Sakai, K. *Tetrahedron: Asymmetry* **1993**, *4*, 973-980. [s] Vinyl acetate: Herradón, B. *Tetrahedron: Asymmetry* **1992**, *3*, 209-212; *J. Org. Chem.* **1994**, *59*, 2891-2893; Herradón, B.; Valverde, S. *Tetrahedron: Asymmetry* **1994**, *5*, 1479-1500. [t] Acetic anhydride: Ennis, M. D.; Old, D. W. *Tetrahedron Lett.* **1992**, *33*, 6283-6286. These authors also resolved a similar compound, flesinoxan, but its absolute configuration was tentatively assigned: *Tetrahedron Lett.* **1992**, *33*, 6287-6290. [u] Vinyl acetate: Mauleón, D.; Lobato, C.; Carganico, G. *J. Heterocycl. Chem.* **1994**, *31*, 57-59. [v] Tsukube, H.; Betchaku, A.; Hiyama, Y.; Itoh, T. *J. Chem. Soc., Chem. Commun.* **1992**, 1751-1752; *J. Org. Chem.* **1994**, *59*, 7014-7018. We chose the enantiomer shown because a CH_2 is larger than an oxygen.

Chart 2. Structures for Table 2 and Figure 4



while the enantioselectivity toward primary alcohols can be either low or moderate (2.3 and 16). This different selectivity is inconsistent with hypothesis 2 and thus favors hypothesis 1. However, we caution that the addition of a methyl group at the α carbon may inhibit optimal binding of the "primary alcohol portion" within the large pocket and therefore yield an incorrect measure of the selectivity of the L pocket. With this caution in mind, our results nevertheless favor hypothesis 1. Thus, we propose that the medium and large substituent of primary alcohols bind in the same M and L pockets as the substituents of a secondary alcohol. The CH_2-kink between the stereocenter and the oxygen causes the reverse orientation of the OH and the CH_2-OH in Figure 1.

Further support for the notion that PCL accommodates the extra CH_2 of primary alcohols as a kink comes from an overlay of models of primary and secondary alcohols, Figure 3. We overlaid minimized structures of the fast- and slow-reacting enantiomers of a primary alcohol acetate, (*S*)- and (*R*)-2-methyl-3-phenylpropyl acetate, onto the fast-reacting enantiomer of the corresponding secondary alcohol acetate, (*R*)-2-acetoxy-1-phenylpropane. In spite of their opposite configurations, the fast-reacting enantiomers overlay more closely at the reaction center, especially at the alcohol oxygen and at the carbonyl group of the ester. This overlay supports the notion that the medium and large substituents of both primary and secondary alcohols can bind

(27) Barco *et al.* found that the PCL-catalyzed acetylation of the secondary alcohol (*R*)-*N*-benzoyl-3-phenylisoserine methyl ester showed high selectivity for the anti isomer (Barco, A.; Benetti, S.; Derisi, C.; Pollini, G. P.; Romagnoli, R.; Zanirato, V. *Tetrahedron Lett.* **1994**, *35*, 9289-9292). This diastereoselectivity shows that, in some cases, PCL can show high β-selectivity. Nevertheless, the β -selectivity for the simpler structures such as **36a** and **36b** is low.

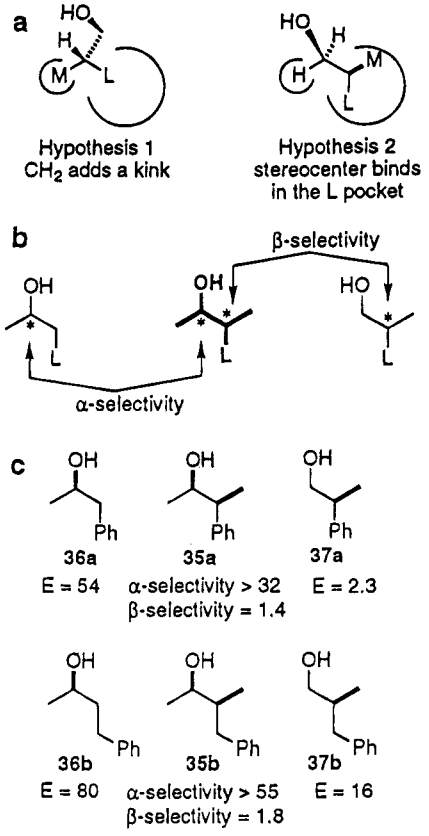
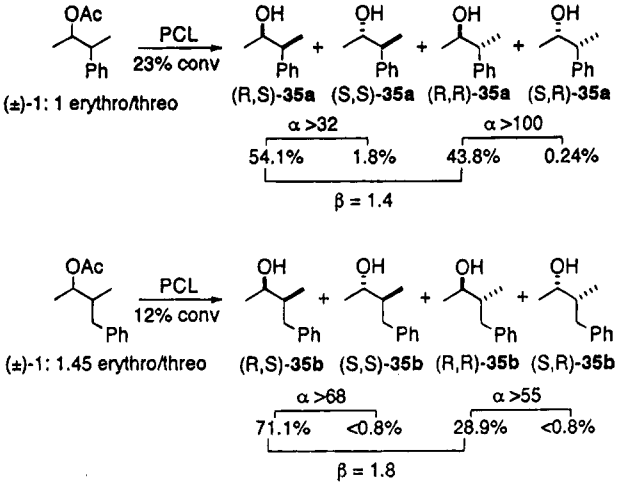


Figure 2. Orientation of the medium and large substituents of primary alcohols in the active site of PCL. (a) Two possible orientations of the medium and large substituents can account for the opposite enantiopreference of PCL toward primary and secondary alcohols. In hypothesis 1, PCL accommodates the extra CH_2 group in primary alcohols as a kink between the stereocenter and the oxygen. The CH_2 group points into the plane of the paper to place the oxygen in a position similar to that in secondary alcohols. In hypothesis 2, PCL accommodates the extra CH_2 group in primary alcohols as a turn that places both the medium and large substituents into the L pocket. According to this hypothesis, enantioselectivity toward primary alcohols comes from details within the L pocket. (b) Measuring the stereoselectivity of the L pocket using a substrate with two stereocenters. Hypothesis 2 predicts that stereoselectivity within the L pocket (β-selectivity) will be similar to the enantioselectivity of PCL toward the corresponding primary alcohol. Hypothesis 1 predicts no relationship between the two selectivities. (c) For two examples, the stereoselectivity within the L pocket was lower than the enantioselectivity for the corresponding primary alcohols. These experimental results favor hypothesis 1. Experimental data for the selectivities are in Scheme 1 and Table 3.

in the same M and L pockets, yet show opposite enantiopreference. Other researchers have also noted a reversal in enantiopreference when a CH_2 group is inserted between the stereocenter and the oxygen of other alcohols. For example, the enantiopreference of the Katsuki–Sharpless epoxidation reverses for allylic and homoallylic alcohols.[28]

This explanation is also consistent with the structure of the active site of lipases and their likely mechanism. Researchers believe that the histidine of the catalytic triad protonates the oxygen of the leaving alcohol.[15] For this reason the alcohol oxygen must adopt a similar position in both structures. Furthermore, modeling

Scheme 1



suggests that the CH_2OH of a primary alcohol would disrupt the orientation of the catalytic histidine if it pointed in the same direction as the OH of a secondary alcohol.

Increasing Enantioselectivity by Increasing the Size of the Large Substituent. The empirical rule suggests that PCL uses the sizes of the substituents to distinguish between enantiomers. Researchers might enhance enantioselectivity by increasing the difference in size of the substituents. Indeed, this strategy was successful for secondary alcohols.[3,5,14] However, we demonstrate below that this strategy was rarely successful for primary alcohols.

The first group are primary alcohols without an oxygen at the stereocenter, Figure 4a and Table 3. The first example, **37a** vs **38**, showed reversed enantioselectivity when we increase the size of the large substituent. PCL-catalyzed hydrolysis of the acetate ester of **37a**, 2-phenylpropyl acetate (L = Ph, M = CH_3), showed low enantioselectivity, E = 2.3, favoring the enantiomer predicted by the empirical rule. Replacing the phenyl with the larger naphthyl group in **38** increased the enantioselectivity to E = 10, but also reversed the enantiopreference. Taking into account the reversal, the enantioselectivity decreased by a factor of 23.

The next example showed a slight decrease in enantioselectivity when we increased the size of the large substituent. PCL-catalyzed hydrolysis of the acetate ester of **39** showed an enantioselectivity of 9. Replacing the acetyl protective group with the larger phthalimido group (compound **40**) decreased the enantioselectivity by a factor of 1.3 to $E = 7$. For both **39** and **40**, PCL favored the enantiomer predicted by the empirical rule.

The third example showed a modest increase in enantioselectivity when we increased the size of one substituent. PCL-catalyzed hydrolysis of racemic *cis*-1-acetoxymethyl-2-(hydroxymethyl)cyclohexane, **41**-acetate, showed low enantioselectivity, E = 1.4, in favor of the (1*R*,2*S*)-enantiomer of the substrate, which is opposite to the one predicted by the empirical rule. Increasing the size of the CH_2OH substituent to CH_2OAc (**42**-acetate) or CH_2OBz (**43**-acetate) increased the enantioselectivity to E = 2 and E = 2.4. The favored enantiomer for the products, *cis*-1-(acetoxymethyl)-2-(hydroxymethyl)cyclohexane and *cis*-1-[(benzoyloxy)methyl]-2-(hydroxymethyl)cyclohexane, was (1*R*,2*S*), as predicted by the empirical rule. Taking into account the reversal, the enantiose-

(28) Rossiter, B. E.; Sharpless, K. B. *J. Org. Chem.* **1984**, *49*, 3707–3711.

Table 3. Hydrolysis of Acetates of Primary and Secondary Alcohols Catalyzed by Lipase from *Pseudomonas cepacia*

structure	% conv	% ee (product-OH)	abs config (product-OH)	*E*
36a			*R*	54[a]
36b	34	96[b]	*R*[c]	80
37a	14	38[d]	*S*[e]	2.3
37b	44	79[f]	*S*[g]	16
38	40	71[h]	*R*[i]	10
39	47	60[d] (SM)[j]	*S* (SM)[k]	9
40	43	63[l]	*R*[m]	7
41	49	12[d] (SM)[j]	1*S*,2*R* (SM)[n]	1.4
42	46	34[d]	1*R*,2*S*[o]	2.0
43	41	23[g](SM)[j]	1*R*,2*S* (SM)[p]	2.4
44	85	84[q]	nd[r]	12

[a] Bianchi, D.; Cesti, P.; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531−5534. Nishio, T.; Kamimura, M.; Murata, M.; Terao, Y.; Achiwa, K. *J. Biochem. Tokyo* **1989**, *105*, 510−512: Esterification with acetic anhydride in organic solvent. [b] Determined by GC of the (*S*)-acetyl lactate derivative using a OV-1701 column. [c] $[\alpha]_D = -17.8$ (1.8, $CHCl_3$) (lit. (*R*)-(−): Hayashi, T.; Okamoto, Y.; Kabeta, K.; Hagihara, T.; Kumada, M. *J. Org. Chem.* **1984**, *49*, 4224−4226). [d] Determined by GC using a Chiraldex G-TA column. [e] $[\alpha]_D = -10.4$ (neat) (lit. (*R*)-(−) and (*S*)-(+): Bianchi, D; Cesti, P.; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531−5534. Bernstein, H.; Whitemore, C. *J. Am. Chem. Soc.* **1939**, *61*, 1326). [f] Determined by GC of the trifluoroacetate derivative using a Chiraldex G-TA column. [g] The product from the PCL-catalyzed esterification of **37a** was identified as the (*S*)-enantiomer (Delinck, D. L.; Margolin, A. L. *Tetrahedron Lett.* **1990**, *31*, 6797−6798). [h] Determined by HPLC using a Chiralcel OD column. [i] $[\alpha]_D = -12.1$ (0.43, C_6H_6), $[\alpha]_D = -19.1$ (0.43, $CHCl_3$) (lit. (*R*)-(−): Menicagli, R.; Piccolo, O.; Lardicci, L.; Wis, M. L. *Tetrahedron* **1979**, *35*, 1301−1306. Sonnet, P. E.; Heath, R. R. *J. Chromatogr.* **1985**, *321*, 127−136). [j] Determined for the unreacted acetyl ester. [k] Determined by comparison of the gas chromatogram (Chiraldex G-TA) of the unreacted acetyl ester with that of the acetyl ester of (*S*)-(+)-**39** obtained from authentic (*S*)-(+)-2-amino-1-butanol. [l] Determined by ^1H NMR of the acetyl ester derivative in the presence of (+)-Eu(hfc)$_3$. [m] Determined by comparison of the ^1H NMR spectrum (in the presence of (+)-Eu(hfc)$_3$) of the acetyl ester of the product with that of the actyl ester of (*S*)-(+)-**40** obtained from authentic (*S*)-(+)-2-amino-1-butanol. [n] Determined by comparison of the gas chromatogram (Chiraldex G-TA) of the unreacted acetyl ester with that of monoacetate **42** obtained from the PCL-catalyzed hydrolysis of the diacetate. [o] $[\alpha]_D = -9.04$ (3.54, $CHCl_3$) (lit. (1*R*,2*S*)-(−): Alder, U.; Breitgoff, D.; Klein, P.; Laumen, K. E.; Schneider, M. P. *Tetrahedron Lett.* **1989**, *30*, 1793−1796. Laumen, K.; Schneider, M. *Terahedron Lett.* **1985**, *26*, 2073−2076). [p] Determined by comparison of the ^1H NMR spectrum (in the presence of (+)-Eu(hfc)$_3$) of *cis*-(1*R*,2*S*)-1-(acetoxymethyl)-2-[(benzoyloxy)methyl]cyclohexane, obtained by benzoylation of monoacetate (1*R*,2*S*)-**42**, to the spectrum of the remaining starting material. The preferred product is therefore *cis*-(1*R*,2*S*)-1-[(benzoyloxy)methyl]-2-(hydroxymethyl)cyclohexane. [q] Determined by ^1H NMR of the Mosher ester. [r] The absolute configuration was not determined.

lectivity increased by modest factors of 2.8 and 3.4, respectively.

The last example in Figure 4a comes from the literature and represents a dramatic increase in enantioselectivity. Tsuji *et al.*[29] reported a low enantioselectivity for the esterification of alcohol **1** where R = CH_2OH, $E = 4$, in favor of the *pro-R* hydroxyl group. The empirical rule predicts the opposite enantiopreference (M = CH_3, L = CH_2OH). When Tsuji *et al.* replaced the methyl with a larger substituent, CH_2Ph (compound **7** where Ar = Ph in Table 1), the enantioselectivity increased to $E > 30$. Since the CH_2OH is now the medium substituent and CH_2Ph is the large substituent, the enantiopreference now agrees with the empirical rule. Other workers also

(29) Tsuji, K.; Terao, Y.; Achiwa, K. *Tetrahedron Lett.* **1989**, *30*, 6189−6192.

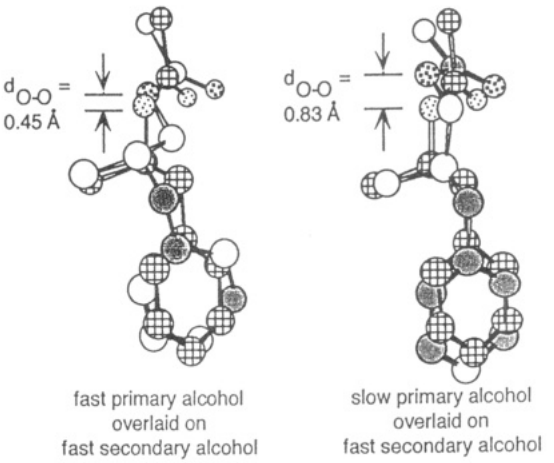


Figure 3. Overlay of the minimized structures of the fast-reacting (*S*)- and slow-reacting (*R*)-enantiomers of the primary alcohol acetate, 2-methyl-3-phenylpropyl acetate (gray carbons) onto the fast-reacting (*R*)-enantiomer of the secondary alcohol acetate, 2-acetoxy-1-phenylpropane (crosshatched carbons). All oxygen atoms are speckled. In spite of the opposite configuration, the (*S*)-primary alcohol acetate mimics the shape of (*R*)-secondary alcohol acetate better than does the (*R*)-primary alcohol acetate. Note the closer overlap of the alcohol oxygens and the carbonyl groups of the acetates. Models were minimized and overlaid using Chem 3-D. Hydrogen atoms are hidden for clarity.

reported increased enantioselectivity when they replaced the methyl by five other CH_2-aryl groups, see Table 1. This last example is the only one where increasing the size of the substituent dramatically increased the enantioselectivity.

For primary alcohols that contain an oxygen at the stereocenter, increasing the size of the large substituent also did not consistently increase the enantioselectivity, Figure 4b. The hydrolysis of the diacetate of **21** (R = CH_2Ph) showed an enantioselectivity of 20−24 (see Table 2). When we increased the size of the substituent to R = $CHPh_2$ (acetyl ester of **44**), the enantioselectivity decreased by a factor of 2 to $E = 12$, Table 3. The second example in Figure 4b comes from the literature. The enantioselectivity of PCL toward the glycerol acetal, substructure **25**, changed little as researchers replaced the methyl with ethyl, phenyl, benzyl, or a cyclohexyl acetal; E ranged from 3 to 9, Table 2. An isopropyl substituent increased enantioselectivity, $E = 23$. Carrea *et al.* also noted that changes in the size of the substituent did not correlate with changes in enantioselectivity for substructures **28** and **29**.[17]

To summarize the effect on enantioselectivity of increasing the difference in the size of the substituents, we found no consistent behavior. Increasing the difference in size may increase, decrease, or have no effect on enantioselectivity. We believe the flexibility of primary alcohols accounts for these observations. Flexibility along the C(1)−C(2) bond allows both enantiomers of primary alcohols to adopt conformations with similar positions of the large and medium substituents, Figure 5. For one enantiomer, a partially eclipsed orientation along the C(1)−C(2) bond orients the large substituent upward and the medium substituent downward. For the other enantiomer, a staggered orientation along the C(1)−C(2) bond also orients the large substituent upward and the medium substituent downward. To distinguish between enantiomers of primary alcohols, PCL must not only distinguish between the large and medium substit-

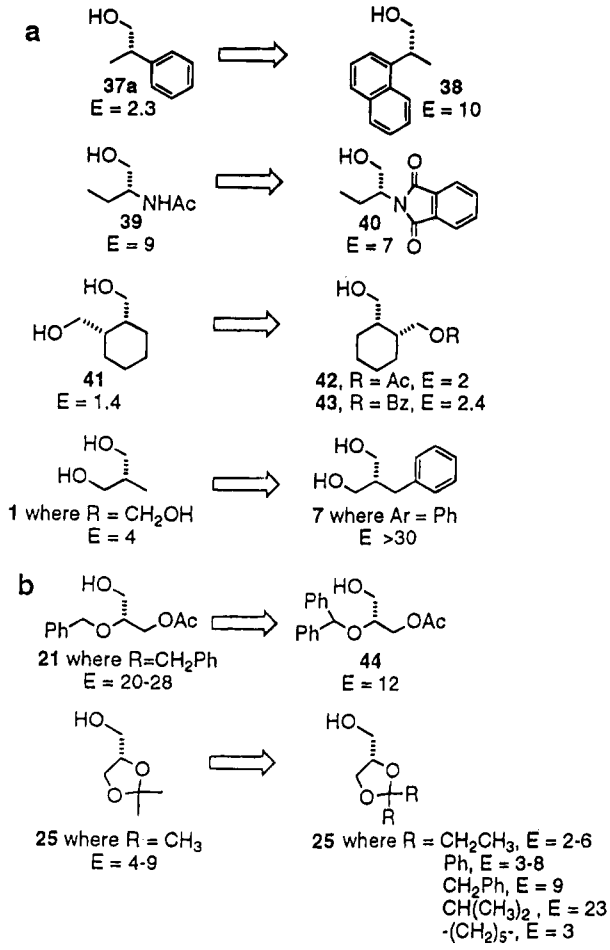


Figure 4. Changes in the enantioselectivity of PCL-catalyzed hydrolyses as the size of one substituent was increased. (a) Four examples where the primary alcohol does not have an oxygen at the stereocenter. The first example showed a reversal in enantioselectivity, the second a small decrease, the third a small increase, and the fourth a large increase. Data for the first three examples are in Table 3; the fourth comes from the literature. (b) Two examples where the primary alcohol has an oxygen at the stereocenter. The first example showed a modest decrease in enantioselectivity, the second example, taken from the literature, showed only small changes in enantioselectivity, except where R = $CH(CH_3)_2$.

uents but also discriminate between the two possible reactive conformations. This explanation may also explain why we needed to exclude primary alcohols with an oxygen at the stereocenter. The oxygen at the stereocenter stabilizes a gauche orientation of the oxygen at the stereocenter and the alcohol oxygen due to the gauche effect.[30] This stabilization may change the favored orientation along the C(1)–C(2) bond and thereby change the enantiopreference.

Experimental Section

General. Lipase from *P. cepacia* (PS30 and LPL-200S) was purchased from Amano International Enzyme Co. (Troy, VI). Unless otherwise noted, organic starting materials were purchased from Aldrich Chemical Co. 4-Phenyl-2-butanol was purchased from Janssen Chimica and *cis*-2,3-epoxybutane from Lancaster. 2-(1-Naphthyl)-1-propanol was prepared from 1-napthylacetic acid as described by Sonnet and Heath.[31] *N,O*-Diacetyl-2-amino-1-butanol, **39**, was prepared by a known method.[32]

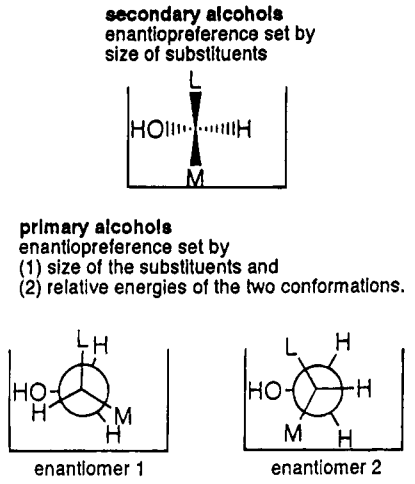


Figure 5. Discrimination between enantiomers of primary alcohols is more difficult than discrimination between enantiomers of secondary alcohols. Flexibility along the C(1)–C(2) bond of primary alcohols allows both enantiomers to adopt conformations with similar orientations of medium and large substituents. For example, enantiomer 1 adopts an upward orientation of the large substituent and a downward orientation of the medium substituent in the eclipsed conformation. Enantiomer 2 adopts a similar orientation in the staggered conformation. To distinguish between enantiomers of primary alcohols, PCL must distinguish between different conformations along the C(1)–C(2) bond in addition to distinguishing between the substituents. The conformations above are only an example. We do not know the conformation of primary alcohols in the active site of PCL.

(±)-*threo/erythro*-3-Phenyl-2-butanol, 35a, was prepared by the nucleophilic addition of methyl magnesium iodide to 2-phenylpropionaldehyde, as described by Overberger *et al.*[33] The ^{1}H NMR of the product agreed with that reported previously.[34] A portion of the product (1 g) was purified by medium pressure chromatography (200 g silica gel, 60% pentane/37% chloroform/3% ethyl acetate, 2.1 min/20 mL fraction). Pure threo alcohol (66.7 mg) and pure erythro alcohol (50.3 mg) were obtained, as well as mixed fractions. Mixed fractions were combined to form a sample (~400 mg) of 50/50 threo/erythro. The threo and erythro isomers can be separated by TLC when eluted three times with 60% pentane/37% chloroform/3% ethyl acetate: on a 7.5 cm plate, R_f = 0.49 (threo) and R_f = 0.40 (erythro). The diastereomers were identified by comparison of the R_f's and ^{1}H NMR of the mixture to that of the pure erythro alcohol prepared as described below.

(±)-*erythro*-3-Phenyl-2-butanol, (±)-*erythro*-35a, was prepared by reaction of phenyllithium with *trans*-2,3-epoxybutane according to a literature procedure.[35] The only change in the procedure was the use of a stock solution of phenyllithium (1.8 M in 70: 30 cyclohexane/ether) instead of preparing the reagent *in situ*. This method was not used for large scale preparation due to low yields (34%). R_f = 0.40 (60% pentane/37% chloroform/3% ethyl acetate). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.17–7.35 (m, 5), 3.88 (apparent quintet, 1, J_{app} ~ 6.2 Hz), 2.73 (apparent quintet, 1, J_{app} ~ 6.7 Hz), 1.55 (br s, 1), 1.32 and 1.08 (two d, 3 + 3, J = 6.6 Hz and J = 5.8 Hz).

Acetyl Esters. Acetic anhydride (1.5 equiv), DMAP (0.05 equiv), and anhydrous sodium carbonate (1.5 equiv) were added to a stirred solution of alcohol in ethyl acetate overnight at ambient temperature. The reaction mixture was then

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diluted with ethyl acetate and washed with water and brine, and the organic extracts were dried ($MgSO_4$) and evaporated to afford the pure acetyl ester.

(±)-*threo*-2-Acetoxy-3-phenylbutane, Acetyl Ester of *threo*-35a has been previously prepared.[36] ^1H NMR ($CDCl_3$, 200 MHz) δ 7.18–7.34 (m, 5, aromatic), 5.09 (apparent quintet, 1, J_{app} = 7 Hz), 2.93 (apparent quintet, 1, J_{app} = 7 Hz), 1.92 (s, 3), 1.28 (d, 3, $J_{H4,H3}$ = 7.3 Hz), 1.16 (d, 3, $J_{H1,H2}$ = 6.6 Hz).

(±)-*erythro*-2-Acetoxy-3-phenylbutane, Acetyl Ester of *erythro*-35a has been previously prepared.[36] ^1H NMR ($CDCl_3$, 200 MHz) δ 7.16–7.34 (m, 5), 5.03 (dq, 1, $J_{H2,H1}$ = 6.2 Hz), 2.85 (apparent quintet, 1, J_{app} = 7.3 Hz), 2.06 (s, 3), 1.28 (d, 3, $J_{H4,H3}$ = 7.3 Hz), 1.05 (d, 3, $J_{H1,H2}$ = 6.2 Hz).

(±)-*threo/erythro*-3-Methyl-4-phenyl-2-butanol, (±)-*threo/erythro*-35b, was prepared in three steps following a literature procedure.[33,37] Reduction of α-methyl-*trans*-cinnamaldehyde with lithium aluminum hydride yielded 3-phenyl-2-methyl-1-propanol, (±)-**37b**. Swern oxidation followed by the addition of methyl magnesium iodide yielded (±)-*threo/erythro*-**35b**. R_f = 0.26 (4:1 cyclohexane/ethyl acetate). The ^1H NMR of this diasteromeric mixture agrees with that reported in the literature.[38]

(±)-*threo*-3-Methyl-4-phenyl-2-butanol, (±)-*threo*-35b.[39] A solution of benzyl bromide (2.5 mL, 21.0 mmol) in ether (15 mL) was slowly added to magnesium turnings (1 g, 41.1 mmol) under nitrogen. The reaction flask was kept in an ice bath until complete addition, and then the reaction mixture was stirred at room temperature for 1 h. The flask was again cooled, and *cis*-2,3-epoxybutane (0.8 mL, 9.2 mmol) in ether (5 mL) was added dropwise. After complete addition, the mixture was refluxed for 2 h. Excess Grignard was quenched by the careful addition of saturated NH_4Cl. Water was added, and the slurry was filtered into a separatory funnel, rinsing with ethyl acetate. The filtrate was washed with 0.5 N HCl, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, filtered, and evaporated *in vacuo*. Purification by flash column chromatography (9:1 pentane/ethyl acetate) yielded the threo alcohol (125 mg, 8%): ^1H NMR ($CDCl_3$, 200 MHz) δ 7.17–7.33 (m, 5), 3.70 (br apparent quintet, 1, $J_{app} \sim 6$ Hz), 2.88 (dd, 1, $^2J_{H4-H4'}$ = 13.3 Hz, J_{H4-H3} = 4.8 Hz), 2.35 (dd, 1, $^2J_{H4-H4'}$ = 13.3 Hz, $J_{H4'-H3}$ = 9.3 Hz), 1.83 (m, 1), 1.37 (br s, 1), 1.21 (d, 3, J_{H1-H2} = 6.3 Hz), 0.83 (d, 3, J_{H3-CH_3-3} = 6.8 Hz).

(±)-*threo/erythro*-2-Acetoxy-3-methyl-4-phenylbutane, Acetyl Ester of (±)-*threo/erythro*-35b, has been previously prepared.[40] ^1H NMR ($CDCl_3$, 250 MHz) (resonances of the two diastereomers overlap except for the OAc and CH_3-3 resonances) δ 7.09–7.33 (m, 10), 4.76–4.95 (m, 2), 2.72–2.89 (m, 2), 2.20–2.43 (m, 2), 1.82–2.05 (m, 2), 2.05 (s, 3) 2.03 (s, 3), 1.22 (d, 6, $J_{H1,H2}$ = 6.3 Hz), 0.88 (d, 3, $J_{CH_3-3,H3}$ = 6.9 Hz), 0.83 (d, 3, $J_{CH_3-3,H3}$ = 6.8 Hz).

(±)-*threo*-2-Acetoxy-3-methyl-4-phenylbutane, Acetyl Ester of (±)-*threo*-35b, has been previously prepared.[40] ^1H NMR ($CDCl_3$, 250 MHz) δ 7.12–7.29 (m, 5), 4.82 (apparent quintet, 1, $J_{H2,H1}$ = 6.3 Hz, $J_{H2,H3}$ = 6.3 Hz), 2.80 (dd, 1, $^2J_{H4,H4'}$ = −13.4 Hz, $J_{H4,H3}$ = 4.7 Hz), 2.28 (dd, 1, $^2J_{H4',H4}$ = −13.4 Hz, $J_{H4',H3}$ = 9.6 Hz), 2.01 (s, 3), 1.89–2.10 (m, 1), 1.20 (d, 3, $J_{H1,H2}$ = 6.3 Hz), 0.81 (d, 3, $J_{CH_3-3,H3}$ = 6.8 Hz).

(±)-2-Phthalimido-1-butanol, 40, was prepared using a standard method for the protection of amino acids.[41] Phthalic anhydride (1.5 g, 10.1 mmol) and triethylamine (0.6 mL, 4.2 mmol) were added to a solution of (±)-2-amino-1-butanol (500 mg, 5.6 mmol) in toluene (10 mL) in a reaction vessel equipped with a condenser and Dean–Stark trap. After refluxing overnight, the reaction mixture was cooled to room temperature and extracted twice with ethyl acetate (30 + 20 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (2 × 50 mL), H_2O (50 mL), and brine (50 mL) and dried over anhydrous $NaSO_4$. The crude residue obtained upon evaporation of the solvent was purified by flash column chromatography (7:3 pentane/ethyl acetate) yielding 2-phthalimido-1-butanol as an oil (723 mg, 59%): R_f = 0.20 (7:3 pentane/ethyl acetate); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.79–7.90 (m, 2), 7.68–7.79 (m, 2), 4.29 (m, 1), 4.08 (m, 1), 3.89 (m, 1), 2.73 (br d, 1), 1.92 (apparent decatet, 2), 0.94 (t, 3, J = 7.4 Hz).

1-Acetoxy-2-phthalimidobutane, Acetyl Ester of 40. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.79–7.90 (m, 2), 7.68–7.79 (m, 2), 4.31–4.58 (overlapping m, 3), 1.71–2.22 (overlapping m, 2), 1.97 (s, 3), 0.92 (t, 3, J = 7.4 Hz). Exact mass 261.0999 ($C_{14}H_{15}O_4N$ requires 261.1001, −0.8 ppm error).

***cis*-1-(Acetoxymethyl)-2-[(benzoyloxy)methyl]cyclohexane, Acetyl Ester of 43.** 4-(dimethylamino)pyridine (5.4 mg, 0.044 mmol) and benzoic acid (108 mg, 0.88 mmol) were added to a solution of *cis*-1-(acetoxymethyl)-2-(hydroxymethyl)-cyclohexane (83 mg, 0.44 mmol) in dichloromethane (5 mL). The mixture was cooled in a cold water bath, and dicyclohexylcarbodiimide (100 mg, 0.48 mmol) was added. After stirring at room temperature for 3 days, the solvent was removed by rotary evaporation. The residue, taken up in ethyl ether, was filtered, and the filtrate was washed with 0.5 N HCl, saturated aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The crude product was purified by flash column chromatography (gradient from 9:1 to 1:1 pentane/ethyl acetate), yielding *cis*-1-(acetoxymethyl)-2-[(benzoyloxy)methyl]cyclohexane as an oil (68 mg, 53%): R_f = 0.68 (7:3 pentane/ethyl acetate); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.98–8.08 (m, 2), 7.38- 7.62 (m, 3), 4.31 and 4.14 (two d, 2 + 2, J = 7.1 Hz), 2.06–2.32 (m, 2), 1.99 (s, 3), 1.33–1.72 (m, 8). Exact mass 290.1511 ($C_{17}H_{22}O_4$ requires 290.1518, −2.4 ppm error).

2-*O*-(Diphenylmethyl)glycerol Diacetate, Acetyl Ester of 44. *tert*-Butyldimethylsilyl chloride (10 g, 66 mmol) was slowly added to a stirred solution of 1,3-dihydroxyacetone dimer (3 g, 17 mmol) and imidazole (9 g, 133 mmol) in dimethylformamide (22 mL) under nitrogen. The mixture was cooled in a cold water bath until the initial exothermic reaction was over. After stirring at room temperature for 3 h, the reaction mixture was poured into H_2O (800 mL) and extracted with ethyl ether (2 × 700 mL). Each ether extract was washed with H_2O (2 × 800 mL) and dried over $MgSO_4$. The combined ether extracts were filtered and evaporated *in vacuo*, yielding 1,3-bis(*tert*-butyldimethylsiloxy)acetone as a clear colorless oil, 10.45 g (98%): ^1H NMR ($CDCl_3$, 200 MHz) δ 4.41 (s, 4), 0.91 (s, 18), 0.08 (s, 12). Sodium borohydride (237 mg, 6.26 mmol) was added to a stirred solution of the 1,3-bis(*tert*-butyldimethylsilyloxy)acetone (2 g, 6.28 mmol) in dry methanol (40 mL) at 4 °C under nitrogen. After stirring for 30 min, the reaction mixture was quenched with 5% aqueous CH_3COOH (15 mL) and H_2O (150 mL). The mixture was extracted with ethyl acetate (800 mL), and the organic phase was washed with 5% aqueous CH_3COOH (2 × 200 mL), saturated aqueous $NaHCO_3$ (2 × 250 mL), H_2O (200 mL), and brine (300 mL) and dried over $MgSO_4$. The solvent was evaporated to yield the alcohol as a clear colorless oil, 1.98 g (98.5%): R_f = 0.38 (9:1 hexane/ethyl acetate). Diphenyldiazomethane[42] (333 mg, 1.71 mmol) was added to a stirred solution of the alcohol (250 mg, 0.78 mmol) in dry acetonitrile (10 mL). After refluxing overnight, additional diphenyldiazomethane (150 mg, 0.77 mmol) was added and the mixture was again refluxed overnight. The reaction mixture was cooled in a refrigerator for 3 h, and the filtrate obtained after filtration of the resulting white precipitate was concentrated *in vacuo*. The crude oil was purified by flash column chromatography (98:2 hexane/ethyl acetate), yielding 1,3-di-*O*-(*tert*-butyldimethylsilyl)-2-*O*-(diphenylmethyl)glycerol (340 mg, 90%): R_f = 0.65 (9: 1 hexane/ethyl acetate). ^1H NMR ($CDCl_3$, 200 MHz) δ 7.21–7.39 (m, 10), 5.77 (s, 1), 3.49–3.78 (m, 5), 0.87 (s, 18), 0.01 (s, 12). Tetrabutylammonium fluoride (1 N in THF, 0.80 mL, 0.80 mmol) was

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added to a stirred solution of the pure 1,3-di-*O*-(*tert*-butyldimethylsilyl)-2-*O*-(diphenylmethyl)glycerol (130 mg, 0.27 mmol) in THF (3 mL). After stirring for 1 h at room temperature, the reaction mixture was extracted with ethyl acetate (90 mL), washed with H_2O (2 × 5 mL) and brine (2 × 40 mL), and dried over $MgSO_4$. Evaporation of the solvent yielded the crude 1,3-diol (156 mg): R_f = 0.18 (1: 1 hexane/ethyl acetate). Anhydrous sodium carbonate (84 mg, 0.80 mmol), 4-(dimethylamino)-pyridine (3.3 mg, 0.027 mmol), and acetic anhydride (0.075 mL, 0.80 mmol) were added to a solution of the crude diol (150 mg) in ethyl acetate (2 mL). The mixture was stirred at room temperature for 18 h and then extracted with ethyl acetate (50 mL). The organic phase was washed with H_2O (4 × 10 mL) and brine (2 × 10 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude residue was purified by flash column chromatography (9:1 hexane/ethyl acetate) to afford 2-*O*-(diphenylmethyl)glycerol diacetate as an oil (70 mg, 77% over two steps). R_f = 0.37 (7:3 hexanes/ethyl acetate). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.22–7.38 (m, 10), 5.61 (s, 1), 4.21 (m, 4, J_{AB} = 16.5 Hz), 3.86 (m, 1, J_{AX} = 11.6 Hz, J_{BX} = 5.1 Hz), 2.02 (s, 6). Exact mass 342.1471 ($C_{20}H_{22}O_5$ requires 342.1467, 1.1 ppm error).

General Procedure for PCL-Catalyzed Hydrolyses. A rapidly stirred suspension of substrate dissolved in a small amount of ether and phosphate buffer (10 mM, pH 7) containing lipase from *P. cepacia* was maintained at pH 7.0 by automatic titration with NaOH (0.1 N) using a Radiometer RTS 822 pHstat. The reaction was stopped at the desired conversion, and the mixture was extracted once with 9:1 ether/ethanol and three times with ether. The combined organic extracts were washed with saturated aqueous $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The starting esters and product alcohols were separated by flash chromatography.

PCL-Catalyzed Hydrolysis of (±)-*threo,erythro*-2-Acetoxy-3-phenylbutane, Acetyl Ester of 35a. As a pHstat was not available for this hydrolysis reaction, a series of 25 mL-Erlenmeyer flasks containing 2-acetoxy-3-phenylbutane (16 mg, 0.083 mmol, a racemic 1:1 mixture of threo/erythro isomers), ethyl ether (0.3 mL), sodium phosphate buffer (7 mL, 0.1 M, pH 7), and lipase from *P. cepacia* (40 mg) were shaken in an incubator at 29–32 °C. After 2–3 h, the contents of one flask were transferred to a test tube and extracted with ether (5 × 2 mL), mixing with a vortex mixer. Persistent emulsions were broken by centrifugation. The ethereal extracts were dried over Na_2SO_4, filtered, and then analyzed by GC to determine the conversion. The extract was then concentrated to 2 mL. The acetyl lactate derivative was subsequently made to determine the optical and diastereomeric purities of the product alcohols by gas chromatography.

PCL-Catalyzed Hydrolyses of (±)-1-Acetoxy-2-phenylpropane, Acetyl Ester of 37a. Test tubes containing (±)-1-acetoxy-2-phenylpropane (20 mg, 0.112 mmol), ether (0.1 mL), sodium phosphate buffer (0.5 mL, 0.1 M, pH 7), and PCL (2 mg) were shaken in an incubator at 29–32 °C. At various time intervals, the entire contents of one test tube were extracted with ether (4 × 2 mL) using a vortex mixer to mix the two phases. Persistent emulsions were broken by centrifugation. The combined ether extracts were analyzed by GC.

Trifluoroacetyl Esters.[43] Trifluoroacetic anhydride (0.2 mL) was added to a solution of alcohol (1–5 mg) in dichloromethane (0.5 mL). Esterification was complete after 30 min and solvent and excess reagent were evaporated in a stream of nitrogen. The residue was dissolved in ethyl ether for analysis by gas chromatography.

Enantiomeric Purity. Gas Chromatography of Acetyl Lactate Derivatives.[44] (*S*)-*O*-Acetyllactic acid chloride (4 drops) was added to the alcohol (10–15 mg) dissolved in anhydrous ether (2 mL). The mixture was cooled in a cold water bath (5 °C), and pyridine (3 drops) was added. The mixture was stirred for 10–45 min and then stirred at room temperature for 1–3 h. The reaction mixture was washed three times with 0.5 N HCl, twice with saturated aqueous $NaHCO_3$, H_2O, and brine, and dried over Na_2SO_4. This ethereal solution of (*S*)-*O*-acetyllactoyl esters was analyzed by GC using an SE30 or OV-1701 capillary column. The enantiomeric excess values obtained were corrected to account for the optical purity of the derivatizing agent (97.5% ee). ((*S*)-*O*-Acetyllactoyl)-4-phenyl-2-butanol: OV-1701 column, 150 °C, α = 1.06, 21.0 min (*R*), 22.2 min (*S*). *threo*-((*S*)-*O*-Acetyllactoyl)-3-phenyl-2-butanol: SE30 column, 10 min at 160 °C then gradient from 160–220 °C at 3 °C/min, α = 1.03, 22.0 min (2*R*,3*S*), 22.6 min (2*S*,3*R*). *erythro*-((*S*)-*O*-Acetyllactoyl)-3-phenyl-2-butanol: SE30 column, 10 min at 160 °C then gradient from 160–220 °C at 3 °C/min, α = 1.02, 22.8 min (2*R*,3*R*), 23.3 min (2*S*,3*S*).

Enantiomeric Purity. Gas Chromatography Using a Chiral Stationary Phase. The alcohol, acetyl ester, or trifluoroacetyl ester was dissolved in ether or ethyl acetate and analyzed by GC using a Chiraldex G-TA30 capillary column (Astec, Inc., Whippany, NJ). 1-Acetoxy-2-phenylpropane: 90 °C, α = 1.03, 36.0 (*S*), 37.0 min (*R*). 2-Phenylpropanol: 90 °C, α = 1.06, 39.3 min (*S*), 41.6 min (*R*). 2-Methyl-3-phenylpropanol: 90 °C, α = 1.01, 46.4 min (*S*), 47.3 min (*R*). 1-(Trifluoroacetoxy)-2-methyl3-phenyl-propane: 70 °C, α = 1.03, 49.9 min (*R*), 51.2 min (*S*). *erythro*-3-methyl-4-phenyl-2-butanol: 100 °C, α = 1.02, 28.9 min (2*S*,3*S*), 29.6 min (2*R*,3*R*). *threo*-3-methyl-4-phenyl-2-butanol: 100 °C, α = 1.02, 31.6 min (2*S*,3*R*), 32.4 min (2*R*,3*S*). *threo*- and *erythro*-2-acetoxy-3-methyl-4-phenylbutane: 100 °C, 37.5 min (2*S*,3*S*), 38.3 min (2*S*,3*R*), 40.5 min (2*R*,3*S* and 2*R*,3*R*). *N*,*O*-Diacetyl-2-amino-1-butanol: 120 °C, α = 1.03, 33.9 min (*S*), 34.9 min (*R*).

Enantiomeric Purity. ^{1}H-NMR. (1) The racemic acetyl ester was dissolved in an NMR tube, and the ^{1}H NMR spectrum was obtained using a 200 MHz spectrometer. Solid tris[[3-(heptafluoropropyl)hydroxymethylene]-(+)-camphorato]-europium(III), (+)-Eu(hfc)$_3$, was added portionwise until base-line separation of the acetate signals was obtained. The number of equivalents of shift reagent necessary to obtain base-line separation of peaks was then added to the sample for which the enantiomeric purity was to be determined. (2) The alcohol was treated with Mosher's acid chloride using a standard procedure[45] and the resulting ester was analyzed by 500 MHz ^{1}H NMR spectroscopy.

Enantiomeric Purity. HPLC Using a Chiral Stationary Phase. A sample of the alcohol and acetyl ester dissolved in the eluting solvent was analyzed by HPLC using a Chiralcel OD column. 2-(1-Naphthyl)-1-propanol (99: 1 hexane/2-propanol, 0.5 mL/min, α = 1.26, 18.4 min (*S*), 23.2 min (*R*).

Absolute Configurations. 35a: The tosyl derivatives of the separated threo and erythro alcohols were prepared as previously described and their optical rotary powers were compared to literature values. Erythro tosylate: $[\alpha]_D = -14.8$ (5.64, benzene), lit.[36] (*R*,*R*): $[\alpha]_D = -17.41$. Threo tosylate: $[\alpha]_D = -12.05$ (4.73, benzene), lit.[36] (*R*,*S*): $[\alpha]_D = -16.89$.

35b: The alcohol obtained from the PCL-catalyzed hydrolysis of *threo*-2-acetoxy-3-methyl-4-phenylbutane was oxidized as follows. Jones reagent (~0.1 mL) was added dropwise to the alcohol (18 mg) in acetone (5 mL) at 0 °C until the orange color persisted. After stirring for 30 min, 2-propanol was added until the mixture was green. After an additional 10 min, sodium hydroxide (1 M) was added until pH 7 was reached. The mixture was filtered and the solvent was evaporated *in vacuo* from the filtrate. The residue was dissolved in ethyl acetate, washed twice with both water and brine, and dried over $MgSO_4$. The crude product obtained upon evaporation of the solvent was purified on a preparative TLC plate (95:5 pentane/ethyl acetate), R_f = 0.26. The ketone obtained was identified as the *S* enantiomer, $[\alpha]_D$ +4.9 (*c* 4.1,

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ethanol), lit.[46] *S*-(+), therefore since the starting material was the acetyl ester of *threo*-**35b**, the preferred product is the (2*R*,3*S*) isomer. By comparison of the GC chromatogram (Chiraldex-GTA 30) of the product obtained from hydrolysis of the threo ester to the chromatogram of the products obtained from the hydrolysis of the racemic threo/erythro mixture, it was determined that the preferred product of the latter hydrolysis was also the (2*R*,3*S*) isomer.

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