A Rule To Predict Which Enantiomer of a Secondary Alcohol Reacts Faster in Reactions Catalyzed by Cholesterol Esterase, Lipase from *Pseudomonas cepacia*, and Lipase from *Candida rugosa*[1]

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The enantioselectivity of the title enzymes for more than 130 esters of secondary alcohols is correlated by a rule based on the sizes of the substituents at the stereocenter. This rule predicts which enantiomer of a racemic secondary alcohol reacts faster for 14 of 15 substrates of cholesterol esterase (CE), 63 of 64 substrates of lipase from *Pseudomonas cepacia* (PCL), and 51 of 55 cyclic substrates of lipase from *Candida rugosa* (CRL). The enantioselectivity of CRL for acyclic secondary alcohols is not reliably predicted by this rule. This rule implies that the most efficiently resolved substrates are those having substituents which differ significantly in size. This hypothesis was used to design syntheses of two chiral synthons: esters of (*R*)-lactic acid and (*S*)-(−)-4-acetoxy-2-cyclohexen-1-one, **70**. As predicted, the acetate group of the methyl ester of lactyl acetate was hydrolyzed by PCL with low enantioselectivity because the two substituents, CH_3 and $C(O)OCH_3$, are similar in size. To improve the enantioselectivity, the methyl ester was replaced by a *tert*-butyl ester. The acetate group of the *tert*-butyl ester of lactyl acetate was hydrolyzed with high enantioselectivity ($E > 50$). Enantiomerically pure (*R*)-(+)-*tert*-butyl lactate (>98% ee, 6.4 g) was prepared by kinetic resolution. For the second example, low enantioselectivity ($E < 3$) was observed in the hydrolysis of *cis*-1,4-diacetoxycyclohex-2-ene, a meso substrate where the two substituents, CH_2CH_2 and CH=CH, are similar in size. To improve enantioselectivity, the size of the CH=CH substituent was increased by addition of Br_2. The new substrate was hydrolyzed with high enantioselectivity ($E > 65$) using either CE or CRL. Enantiomerically pure **70** (98% ee) was obtained after removal of the bromines with zinc and oxidation with CrO_3/pyridine.

Microorganism- and enzyme-catalyzed syntheses and resolutions are among the best methods for the preparation of enantiomerically pure compounds.[2] Enantioselective hydrolyses and transesterifications are especially useful because they are efficient, can be carried out on a large scale, and apply to a wide range of substrates. Unfortunately, there is little X-ray structural information available for the esterases and lipases that are used for these preparations;[3] thus, the appropriate enzyme is usually chosen by screening.

In order to streamline screening, substrate models have been developed using substrate selectivity data. Some models attempt to define the shape and hydrophobic character of the active site and are used to predict which new substrates will be transformed by the enzyme and whether the reaction will be enantioselective. In most cases, this type of model is only accurate for substrates that are similar to those already tested. For example, a model for PLE[4] predicts its selectivity with α,β-substituted carboxylic acids,[5] and a model for CRL predicts its selectivity for bicyclo[2.2.1]heptanols and bicyclo[2.2.2]octanols.[6] In some cases, more general models which define the sizes of hydrophobic pockets near the active site have been developed. These can be used to predict reactivity for a wider range of substrates.[7]

The simplest models for enzyme selectivity, more accurately referred to as rules, predict only which enantiomer reacts faster, usually based on either the size or hydrophobicity of the substituents at the stereocenter. The earliest example of such a rule is Prelog's rule which predicts the enantioselectivity of the reduction of ketones by the yeast *Culvaria lunata* based on the size of the two substituents at the carbonyl (Figure 1).[8] The advantage of this rule is that it applies to a wider range of substrates, but the disadvantage is that there are exceptions to this rule. Nevertheless, this rule is sufficiently reliable to be used for the determination of absolute configurations[9] and has been used to redesign substrates in order to improve the enantioselectivity.[10]

A rule similar to Prelog's rule has been proposed for a hydrolysis catalyzed by a yeast. For 47 esters of racemic secondary alcohols, a rule based on the sizes of the substituents at the stereocenter predicts which enantiomer is hydrolyzed faster in cultures of the yeast *Rhizopus nigrigans*.[11] This rule has been used to determine the ab-

(1) *Candida rugosa* was previously classified as *Candida cylindracea* and many workers still use the older name (*Catalogue of Fungi/Yeasts*, 17th ed.; American Type Culture Collection: Washington, 1987). Lipase from *Pseudomonas cepacia* is Amano Lipase P, PS, or P30. The microbe from which this enzyme is prepared was previously classified as *Pseudomonas fluorescens*, but now has been reclassified as *P. cepacia* (Amano Pharmaceutical Co., Nagoya, Japan, personal communication). This paper was presented in part at the Chemical Institute of Canada Congress, Halifax, NS, July 1990, Abstract 881 and the American Chemical Society National Meeting, Washington, DC, August 1990, Abstract ORG 226.

(2) Recent reviews: Wong, C.-H. *Science* **1989**, *244*, 1145-1152. Sih, C. J.; Wu, S.-H. *Top. Stereochem.* **1989**, *19*, 63-125. Gramatica, P. *Chim. Oggi* **1989**, *7*, 9-15.

(3) First high resolution x-ray crystal structures of lipases: Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature* **1990**, *343*, 767-770. Winkler, F. K.; D'Arcy, A.; Humziker, W. *Nature* **1990**, *343*, 771-774.

(4) Abbreviations for enzymes used in this paper: ACE, acetylcholinesterase from electric eel; CE, bovine cholesterol esterase; CRL, lipase from *Candida rugosa*; MML, lipase from *Mucor meihei*; PCL, Amano P = lipase from *Pseudomonas cepacia*; PLE, porcine liver esterase.

(5) Mohr, P.; Waespe-Sarcevic, N.; Tamm, C.; Gawronska, K.; Gawronski, J. K. *Helv. Chim. Acta* **1983**, *66*, 2501-2511.

(6) Oberhauser, Th.; Faber, K.; Griengl, H. *Tetrahedron* **1989**, *45*, 1679-1682.

(7) For example, Toone, E. J.; Werth, M. J.; Jones, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 4946-4952.

(8) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119-130. This rule has also been extended to reductions catalyzed by baker's yeast, *Saccharomyces cerevisiae*.

(9) Fiaud, J. C. In *Stereochemistry: Fundamentals and Methods*; Kagan, H. B., Ed., George Thieme: Stuttgart, 1977; Vol. 2, pp 95-126.

(10) Review: VanMiddlesworth, F.; Sih, C. J. *Biocatalysis* **1987**, *1*, 117-127.

(11) Ziffer, H.; Kawai, K.; Kasai, M.; Imuta, M.; Froussios, C. *J. Org. Chem.* **1983**, *48*, 3017-3021. Kasai, M.; Kawai, K.; Imuta, M.; Ziffer, H. *J. Org. Chem.* **1984**, *49*, 675-679. Charton, M.; Ziffer, H. *J. Org. Chem.* **1987**, *52*, 2400-2403.

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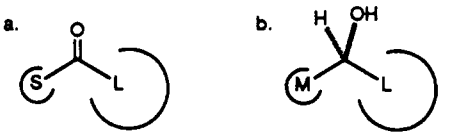

Figure 1. (a) Prelog's rule predicts that the yeast *Culvaria lunata* preferentially adds H_2 to the front side of ketones having the shape indicated. (b) An extension of Prelog's rule to hydrolases. For esters of secondary alcohols, the enantiomer shown reacts faster with CE, PCL, and CRL than the other enantiomer.

solute configuration of secondary alcohols.[12] However, this microorganism has not been used for large scale synthesis.

Similar rules have been proposed for two commercially available lipases: *Mucor meihei*[13] (MML) and *Arthrobacter*.[14] It is not yet clear how useful these rules will be because they are based on only six substrates for the *Mucor* enzyme and only two substrates for the *Arthrobacter* enzyme. A "two-site model" that has been recently proposed for cyclic substrates of PCL also appears similar to the rule discussed herein.[15]

This paper proposes a rule based on the sizes of the substituents for three hydrolases (Figure 1). This rule is supported by 14 out of 15 substrates for CE, 63 out of 64 substrates for PCL, and 51 out of 55 cyclic substrates for CRL and has an accuracy of ≥93%. This rule is the first attempt to predict the enantioselectivity of CE. For PCL, this rule is similar to the "two-site model" for cyclic substrates,[14] but the evidence presented in this paper shows that this rule accounts for the enantioselectivity of acyclic secondary alcohols as well. For CRL, this rule is the first general rule and complements the model for bicyclic substrates.[6]

Besides correlating a large amount of experimental data, this rule also suggests a strategy for improving the efficiency of resolutions catalyzed by these enzymes: secondary alcohols having substituents which differ significantly in size should be more efficiently resolved than secondary alcohols having substituents which are similar in size. This hypothesis was used to design the preparations of enantiomerically pure *tert*-butyl lactate and (*S*)-(−)-4-acetoxy-2-cyclohexen-1-one.

Lactate esters are used as chiral starting materials and chiral auxiliaries. For example, lactate esters were used in the preparation of (*S*)-2-arylpropionic acids, a class of nonsteroidal antiinflammatory drugs,[16] α-*N*-hydroxy amino acids,[17] chiral enolates,[18] and chiral auxiliaries for an enantioselective Diels–Alder reaction.[19] Polylactides containing interpenetrating networks of poly-(*R*)-lactide and poly-(*S*)-lactide are stronger than those containing racemic chains.[20]

Although enantiomerically pure (*S*)-(+)-lactic acid is readily available, the unnatural enantiomer, (*R*)-(−), remains expensive. Enantiomerically pure (*R*)-lactic acid can be prepared by D-lactate dehydrogenase catalyzed reduction of pyruvate,[21] fermentation, or microbial destruction of the *S* enantiomer.[22] The resolution of lactate esters using PCL described in this paper yields material of high enantiomeric purity and is inexpensive and simple to carry out. An enzyme-catalyzed resolution of lactate esters has been reported previously using an enzyme from a similar microorganism.[23]

The second example is an enantioselective synthesis of (*S*)-(−)-4-acetoxy-2-cyclohexen-1-one, a new compound that should also be useful for synthesis. The analogous (4*S*)-*tert*-butyldimethylsiloxy derivative has been prepared in six steps from quinic acid[24] and has been used for the synthesis of the cholesterol-lowering drugs ML 236A and compactin.[25] The procedure described below for the acetate derivative is simpler. Since the analogous cyclopentenone, 4-acetoxy-2-cyclopenten-1-one, has been widely used in synthesis,[26] the six-membered analog should also find many uses.

Results

A Survey of Enantioselectivity for Esters of Secondary Alcohols. Pancreatic CE catalyzed the hydrolysis of the acetates of the secondary alcohols listed in Table I. The measured values of enantiomeric excess and percent conversion were used to calculate the enantioselectivity, *E*, which indicates the degree to which the enzyme prefers one enantiomer over the other.[27] Several examples from the literature are also included in Table I. Chart I indicates the structure of the fast-reacting enantiomer. The chart and tables are arranged so that the larger group is always on the right side as predicted by the rule in Figure 1b. CE showed no enantioselectivity toward **40**, but for the other 14 examples the rule correctly predicts which enantiomer reacts faster. The overall accuracy of the rule for CE is 14 of 15 substrates or 93%; the single exception, the *tert*-butyl ester of lactyl acetate, will be introduced and discussed below.

Enantioselectivity data for PCL was gathered from the literature (Table II). This list includes all secondary alcohols prepared using lipase P from Amano Pharmaceutical. Reactions using other lipases from *Pseudomonas* (e.g. AK, K-10, or SAM-II) are not included. Patent literature is also not included. The reaction conditions used for the examples listed in Table II include both hydrolyses in aqueous solution as well as transesterifications and esterifications in organic solvents. The structures of the substrates in Table II include acyclic secondary alcohols and cyclic secondary alcohols in rings ranging from four- to seven-membered. For 63 of the 64 substrates, the rule in Figure 1 correctly predicts the fast-reacting enantiomer. The single exception, indicated by "(ent)" in the enantioselectivity column, was one of five substrates which

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(13) Roberts, S. M. *Phil. Trans. R. Soc. Lond. B* **1989**, *324*, 557–587.
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(26) Harre, M.; Raddatz, P.; Walenta, R.; Winterfeldt, E. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 480–492.
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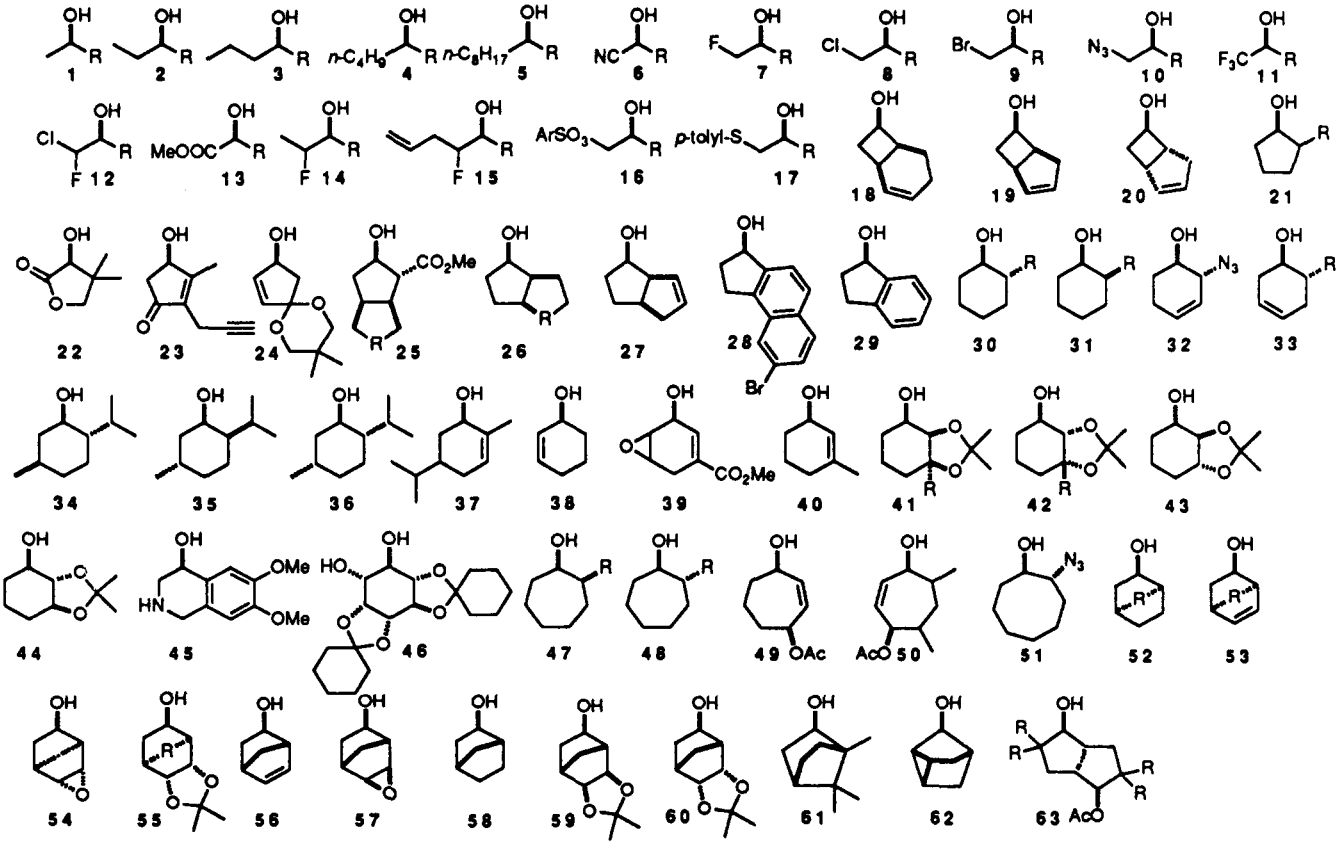
Table I. Enantioselectivity of Bovine Pancreatic Cholesterol Esterase for Acetates of Secondary Alcohols

structure	R =	initial rate[a] (units/mg)	convn (%)	enantiomeric excess (%)[b]	E, enantioselectivity
1	Ph	0.31	43	69	8.8[c]
1	n-C_6H_{13}	0.36	46	35	2.6[d]
1	$(CH_2)_2CH{=}C(CH_3)_2$	0.50	46	45	4.3[e]
2	Ph	0.12	34	6.2	4.6[f]
28		0.02	-	-	2.9[g]
29		0.56	29	65	6.3[d]
30	CH_3	0.32	50	94	>100[h,i]
30	OAc	0.20	44	56	10[j]
30	OH	0.59	48	59	6.8[j]
31	CH_3	0.59	53	46	4.4[h,k]
34		0.21	44	96	>100[d]
39	cis	0.04	-	-	14[l]
39	trans	0.07	-	-	37[l]
40		0.60	50	0	1
46		-	-	-	37[m]

[a] For hydrolysis of the corresponding acetate ester unless otherwise noted. Unit = μmol of ester hydrolyzed/min. [b] The enantiomeric purity of the product alcohol was determined by derivatizing with isoproyl isocyanate and separating the enantiomers by capillary GC using an XE-60-(*S*)-valine-(*S*)-α-phenylethylamide column (Chromopak). [c] The product alcohol showed $[\alpha]_D$ (obsd) = +40.5° (*c* 1.2, hexane) indicating the *R* configuration: lit. *S*-(−), Klyne, W.; Buckingham, J. *Atlas of Stereochemistry*; Chapman and Hall: London, 1974; p 26. [d] Absolute configuration was determined by comparison to an authentic sample. [e] The product alcohol showed $[\alpha]_D$ (obsd) = −5.3° (*c* 2.2, hexane) indicating the *R* configuration: lit. *S*-(+), $[\alpha]_D$ = +16.1° (neat): Jacques, J.; Gros, C.; Bourcier, S. *Absolute Configuration of 6000 Selected Compounds with One Asymmetric Carbon Atom*; G. Thieme: Stuttgart, 1977. Levene, P. A.; Haller, H. L. *J. Biol. Chem.* **1929**, *83*, 177–183. [f] The product alcohol showed a positive rotation (hexane) indicating the *R* configuration: lit. *S*, $[\alpha]_D$ = −39° (neat): MacLeod, R.; Welch, F. J.; Mosher, H. S. *J. Am. Chem. Soc.* **1960**, *82*, 876–880. [g] Kazlauskas, R. J. *J. Am. Chem. Soc.* **1989**, *111*, 4953–4959. [h] The enantiomeric purity was determined by formation of the Mosher's ester and integration of the ^{1}H NMR signals for the ring CH_3 groups: Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549. [i] The product alcohol showed $[\alpha]_D$ (obsd) = −8.7° (*c* 1.2, CH_2Cl_2) indicating the *R* configuration: lit. *R*-(−), Beard, C.; Djerassi, C.; Elliott, T.; Tao, R. C. C. *J. Am. Chem. Soc.* **1962**, *84*, 874–875. [j] Hydrolysis was caried out in aqueous solution saturated with sodium chloride. The % ee refers to unreacted starting material and was determined by HPLC of the benzoate derivative on a Chiralpak OT (Daicel, New York): Caron, G.; Kazlauskas, R. J., unpublished results. [k] The product alcohol showed $[\alpha]_D$ (obsd) = −34° (*c* 1.6, hexane) indicating the *R* configuration: lit. *R*-(−), Beard, C.; Djerassi, C.; Elliott, T.; Tao, R. C. C. *J. Am. Chem. Soc.* **1962**, *84*, 874–875. [l] As the butyrate ester at 0–5 °C: Pawlak, J. L.; Berchtold, G. A. *J. Org. Chem.* **1987**, *52*, 1765–1771. [m] Liu, Y.-C.; Chen, C.-S. *Tetrahedron Lett.* **1989**, *30*, 1617–1620.

Chart I. Structures for Tables I–III



showed low enantioselectivity, $E \leq 3$. Thus, for PCL the rule predicts which enantiomer reacts faster with 98% accuracy.

Enantioselectivity data for CRL was also gathered from the literature (Table III). The *Candida* list includes results using enzyme either from Sigma Chemical Co. or

Table II. Enantioselectivity of Lipase from *Pseudomonas cepacia* (Amano P) for Esters of Secondary Alcohols

structure	R =	E	ref	structure	R =	E	ref
1	Ph, CH_2Ph	>50	*a*	12	Ph, CH_2Ph, CH_2CH_2Ph	23 to >50	*k*
1	*n*-C_6H_{13}, *n*-$C_{10}H_{21}$	~12	*a*	13	$CH(CH_3)$-1-(2,6-dithiane)	>50	*l*
1	CH_2CN	29	*b*	13	*syn*-CH(Ar)(SAr')	>50	*m*
1	CH_2COOEt	~8	*c*	21	*cis*-COOEt, OAc; *trans*-COOEt, OAc	>50	*n*
1	1-(2,6-dithiane), 1-(2,5-dithiolane), CH_2-1-(2,6-dithiane), CH_2-1-(2,5-dithiolane)	>50	*d*	21	*trans*-N_3	>50	*o*
				23		>50	*h*
1	CH_2-1(2,6-dioxane)	2	*d*	24		>50	*p*
2	Ph	>50	*a*	25	C=O, C=CH_2, HC=CH	>50	*n*
2	$CH(N_3)C_2H_5$ (threo, erythro)	>50	*e*	25	1-(2,5-dioxolane)	3	*n*
3	$CH(N_3)CH_2CH_2CH_3$ (erythro)	>50	*e*	26	C=O, C=CH_2, 1-(2,5-dioxolane)	>50	*q*
4	CH_2N_3	2	*e*	27		>50	*r*
4	CH_2OSO_2Ar	25	*f*	30	COOEt, OAc, CN, C_6H_5	>50	*n, o, s*
5	*syn*-$CH(Cl)CH_2COOEt$	50	*g*	30	OH	17	*t*
6	*p*-C_6H_4OPh	>50	*h*	31	OAc	8	*n*
6	Ph, CH_2CH_2Ph, CH=CHPh	18 to >50	*b*	31	COOEt	>50	*n*
8	Ph, 3,4-$(MeO)_2C_6H_3$	>50	*i*	33	OAc	>50	*u*
8	CH_2OSO_2Ar	24	*f*	45		17	*v*
9	2-naphthyl, 4-BrC_6H_4, 4-$MeOC_6H_4$	>50	*i*	47	OAc	1	*n*
9	1-(3-bromo-5-isoxazolyl)	20	*j*	47	CO_2Me	>50	*n*
10	Ph	>50	*e*	48	CO_2Me,OAc	>50	*n*
10	*n*-C_6H_{13}	2 (ent)	*e*				

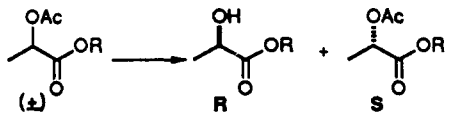
[a] Bianchi, D.; Cesti, P.; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531–5534. Esterification with propionic anhydride in benzene: Nishio, T.; Kamimura, M.; Murata, M.; Terao, Y.; Achiwa, K. *J. Biochem. Tokyo* **1989**, *105*, 510–512. Esterification with vinyl acetate, neat solution. [b] Itoh, T.; Tagaki, Y. *Chem. Lett.* **1989**, 1505–1506. Hydrolysis of β-methylthio- or β-phenylthioacetyl ester. [c] Sugai, T.; Ohta, H. *Agric. Biol. Chem.* **1989**, *53*, 2009–2010. Esterification with vinyl butanoate, 65 °C. [d] Bianchi, D.; Cesti, P.; Golini, P. *Tetrahedron* **1989**, *45*, 869–876. [e] Foelsche, E.; Hickel, A.; Hönig, H.; Seufer-Wasserthal, P. *J. Org. Chem.* **1990**, *55*, 1749–1753. Hydrolysis of butyrates. [f] Chen, C.-S.; Liu, Y.-C. *Tetrahedron Lett.* **1989**, *30*, 7165–7168. Transesterification of the butyrate in hexane. [g] Tsuboi, S.; Sakamoto, J.; Sakai, T.; Utaka, M. *Chem. Lett.* **1989**, 1427–1428. [h] Hirohara, H.; Mitsuda, S.; Ando, E.; Komaki, R. In *Biocatalysis in Organic Syntheses*; Tramper, J., van der Plas, H. C., Linko, P., Eds.; Elsevier: Amsterdam, 1985; pp 119–134. [i] Hiritake, J.; Inagaki, M.; Nishioka, T.; Oda, J. *J. Org. Chem.* **1988**, *53*, 6130–6133. Esterification with isopropenyl acetate isopropyl ether. [j] Di Aminica, M.; De Micheli, C.; Carrea, G.; Spezia, S. *J. Org. Chem.* **1989**, *54*, 2646–2650. Esterification with trifluoroethyl octanoate in 9:1 hexane/benzene. [k] Yamazaki, T.; Ichikawa, S.; Kitazume, T. *J. Chem. Soc., Chem. Commun.* **1989**, 253–255. [l] Suemune, H.; Mizuhara, Y.; Akita, H.; Oishi, T.; Sakai, K. *Chem. Pharm. Bull.* **1987**, *35*, 3112–3118. [m] Akita, H.; Enoki, Y.; Yamada, H.; Oishi, T. *Chem. Pharm. Bull.* **1989**, *37*, 2876–2878. Hydrolysis in water-saturated benzene, enzyme was immobilized on Celite. [n] Xie, Z.-F.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1987**, 838–839. Xie, Z.-F.; Suemune, H.; Nakamura, I.; Sakai, K. *Chem. Pharm. Bull.* **1987**, *35*, 4454–4459. Xie, Z.-F.; Nakamura, I.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1988**, 966–967. [o] Hönig, H.; Seufer-Wasserthal, P.; Fülöp, F. *J. Chem. Soc., Perkin Trans. 1* **1989**, 2341–2345. Hydrolysis of butyrate ester. [p] Washausen, P.; Grebe, H.; Kieslich, K.; Winterfeldt, E. *Tetrahedron Lett.* **1989**, *30*, 3777–3778. [q] Xie, Z-F.; Suemune, H.; Sakai, K. *Tetrhedron: Asymmetry* **1990**, *1*, 395–402. [r] Klempier, N.; Faber, K.; Griengl, H. *Synthesis* **1989**, 933–934. [s] Schwartz, A.; Madan, P.; Whitesell, J. K.; Lawrence, R. M. *Org. Synth.* **1990**, *69*, 1–9. [t] Caron, G.; Kazlauskas, R. J., unpublished results. [u] Suemune, H.; Hizuka, M.; Kamashita, T.; Sakai, K. *Chem. Pharm. Bull.* **1989**, *37*, 1379–1381. [v] Hoshino, O.; Itoh, K.; Umezawa, B.; Akita, H.; Oishi, T. *Tetrahedron Lett.* **1988**, *29*, 567–568.

from Meito Sangyo (Lipase MY or OF-360); however, examples where the absolute configuration of the product is uncertain were not included,[28] nor were examples from patents. The reaction conditions again include both hydrolyses in aqueous solution as well as transesterifications and esterifications in organic solvents.

For acyclic substrates of CRL the rule in Figure 1 does *not* reliably predict which enantiomer reacts faster. Only for about half, 14 of the 31 acyclic substrates, is the fast-reacting enantiomer predicted correctly; this proportion is close to that expected for random guesses. A large fraction of the acyclic substrates (12 of 31) showed low enantioselectivity, $E \leq 3$. Thus, the rule is not useful for acyclic substrates of CRL. Generalizations about this rule given below do *not* include acyclic substrates of CRL, but do include acyclic substrates of CE and PCL.

For cyclic substrates of CRL the rule reliably predicts which enantiomer reacts faster. These substrates include secondary alcohols in four- to eight-membered rings as well as secondary alcohols in bicyclic substrates. The rule predicts correctly for 51 of the 55 substrates where CRL was enantioselective, an accuracy of 93%. Three exceptions, indicated by "(ent)" as before, were among the 13 cyclic substrates which showed low enantioselectivity, $E \leq 3$; one exception, **22**, showed moderate enantioselectivity, $E = 8$.[29]

Scheme I. Kinetic Resolution of Esters of Lactyl Acetate



Thus, the rule predicts which enantiomer of a secondary alcohol reacts faster for ≥93% of both the cyclic and acyclic substrates of CE and PCL and the cyclic substrates of CRL. None of the substrates that react contrary to the rule show enantioselectivity greater than eight and therefore none of the exceptions are synthetically useful.

This generalization implies that a substrate having substituents which differ significantly in size should be resolved with higher enantioselectivity than a substrate where the two substituents are similar in size. The data in Tables I–III suggests that this generalization is valid;

(28) Chênevert, R.; Desjardins, M.; Gagnon, R. *Chem. Lett.* **1990**, 33–34. Abramowicz, D. A.; Keese, C. R. *Biotechnol. Bioengineer.* **1989**, *33*, 146–156. Takano, S.; Inomata, K.; Ogasawara, K. *J. Chem. Soc., Chem. Commun.* **1989**, 271–272.

(29) It was difficult to decide which side was larger for **37**, **38**, **50**, and **62**. Compounds **37** and **50** contain a small substituent near the carbinol carbon on one side and a larger substituent further away on the opposite side. Compound **37** was drawn as shown arbitrarily. For **50**, the larger acetoxy substituent is three carbons away from the carbinol carbon and is judged to be less important than the CH_3 at the 2-position. The unsaturated portion of **38** and the cyclopropyl portion of **62** were judged to be smaller using three-dimensional models. Several other bicyclic structures appear symmetrical as drawn, but three-dimensional models clearly show the right side to be larger.

Table III. Enantioselectivity of Lipase from *Candida rugosa* for Acetates of Secondary Alcohols

structure	R	E	ref	structure	R	E	ref
1	C_2H_5	34	a	33	N_3	>50	o
1	n-C_6H_{13}	1.6	b	34		~10 to >50	q, s
1	2-furyl	5	c	35		>50	q
1	$CH_2(OCH_3)_2$	2.5	d	36		27	q
2	$CH(N_3)C_2H_5$ (threo, erythro)	12	e	37	trans	1	t
3	*erythro*-$CH(N_3)CH_2CH_2CH_3$	18	e	37	cis	25	t
4	CH_2N_3	2 (ent)	e	38		1.2	t
6	Ph	8 (ent)	f	39	cis, trans	6	u
6	p-C_6H_4OPh	12 (ent)	g	40		3.4	d
7	CH_2S-p-tolyl	1.3 (ent)	h	41	H, CH_3	>50	v
8	Ph	2	f	42	H	2	v
10	Ph	13	e	42	CH_3	26	v
10	n-C_6H_{13}	2	e	43		>50	v
10	t-C_4H_9	>50 (ent)	e	44		>50	v
11	CH=CHPh (E, Z)	>50 (ent)	i	45		20	w
11	$CH{=}CH(CH_2)_5CH_3$(E, Z)	~16 (ent)	i	48	N_3	30	o
12	Ph	6	j	49		2.5	x
12	CH_2Ph, CH_2CH_2Ph	~3 (ent)	j	50	cis, trans	>50	x
13	Ph	>50 (ent)	f	51		>50	o
13	*anti*-$CH(CH_3)$-3-indolyl	3-40	k	52	CH_2	11	y
14	CH_2S-p-tolyl (syn, anti)	1.3 (ent)	h	52	CHCOOR', $CHOCH_2Ph$	3-10	y
15	CH_2S-p-tolyl (syn, anti)	1.2-2.1 (ent)	h	53	CH_2	35	y
16	Ph	>50 (ent)	l	53	$CHCO_2Me$, $C(OMe)_2$	~10	y
17	$CFPhCH_3$ (syn, anti)	6.5	h	53	O	>50	y
18		17	m	54		>50	y
19		>40	n	55	CH_2	22	y
20		1.2 (ent)	n	55	O	>50	y
21	*trans*-N_3	1.4	o	56		1	y
22		8 (ent)	f	57		1.4 (ent)	y
27		>50	p	58		1.5	y
30	CH_3, C_2H_5, i-C_3H_7, t-C_4H_9	>50	q	59		4	y
30	CN, NO_2, N_3	40 to >50	o	60		1.8	y
30	OAc, OH	2, 1 (ent)	r	61		>10	z
31	CH_3, i-C_3H_7	24	q	62		10	aa
32		20	o	63	H, CH_3	15	bb

[a] Cambou, B.; Klibanov, A. M. *Biotechnol. Bioeng.* **1984**, *26*, 1449–1454. [b] Gerlach, D.; Missel, C.; Schreier, P. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 315–318. Esterification with n-$C_{11}H_{23}COOH$ in heptane. [c] Drueckhammer, D. G.; Barbas, C. F., III; Nozaki, K.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 1607–1611. [d] Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205. Acetylation with vinyl acetate. [e] Foelsche, E.; Hickel, A.; Hönig, H.; Seufer-Wasserthal, P. *J. Org. Chem.* **1990**, *55*, 1749–1753. Hydrolysis of butyrates. [f] Bevinakatti, H. S.; Banerji, A. A.; Newadkar, R. V. *J. Org. Chem.* **1989**, *54*, 2453–2455. Bevinakatti, H. S.; Newadkar, R. V. *Biotechnol. Lett.* **1989**, *11*, 785–788. Transesterification with 1-butanol in isopropyl ether. [g] Hirohara, H.; Mitsuda, S.; Ando, E.; Komaki, R. In *Biocatalysis in Organic Synthesis*; Tramper, J., van der Plas, H. C., Linko, P., Eds.; Elsevier: Amsterdam, 1985; 119–134. [h] Bucciarelli, M.; Forni, A.; Moretti, I.; Prati, F.; Torre, G.; Resnati, G.; Bravo, P. *Tetrahedron* **1989**, *45*, 7505–7514. [i] Kitazume, T.; Lin, J. T.; Yamazaki, T. *J. Fluorine Chem.* **1989**, *43*, 177–187. [j] Yamazaki, T.; Ichikawa, S.; Kitazume, T. *J. Chem. Soc., Chem. commun.* **1989**, 253–255. [k] Akita, H.; Enoki, Y.; Yamada, H.; Oishi, T. *Chem. Pharm. Bull.* **1989**, *37*, 2876–2878. Hydrolysis in water-saturated benzene, enzyme was immobilized on Celite. [l] Chen, C.-S.; Liu, Y.-C. *Tetrahedron Lett.* **1989**, *30*, 7165–7168. Transesterification of the butyrate ester in hexane. [m] Cotterill, I. C.; Finch, H.; Reynolds, D. P.; Roberts, S. M.; Rzepa, H. S.; Short, K. M.; A. M. Z.; Wallis, C. J.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1988**, 470–472. [n] Cotterill, I. C.; MacFarlane, E. L. A.; Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 3387–3389. [o] Hönig, H.; Seufer-Wasserthal, P.; Fülöp, F. *J. Chem. Soc., Perkin Trans. 1* **1989**, 2341–2345. Faber, K.; Hönig, H.; Seufer-Wasserthal, P. *Tetrahedron Lett.* **1988**, *29*, 1903–1904. Hydrolysis of butyrate ester. [p] Klempier, N.; Faber, K.; Griengl, H. *Synthesis* **1989**, 933–934. [q] Legrand, G.; Secchi, M.; Buono, G.; Baratti, J.; Triantaphylides, C. *Tetrahedron Lett.* **1985**, *26*, 1857–1860. Esterification in hexane or heptane with n-$C_{11}H_{23}COOH$. [r] Caron, G.; Kazlauskas, R. J., unpublished results. [s] Koshino, S.; Sonomoto, K.; Tanaka, A.; Fukui, S. *J. Biotechnol.* **1985**, *2*, 47–57. Esterification with 5-phenylpentanoic acid in water-saturated isooctane. [t] Oritani, T.; Yamashita, K. *Agric. Biol. Chem.* **1980**, *44*, 2637–2642. [u] Pawlak, J. L.; Berchtold, G. A. *J. Org. Chem.* **1987**, *52*, 1765–1771. Hydrolysis of butyrate ester. [v] Dumortier, L.; Van der Eycken, J.; Vandewalle, M. *Tetrahedron Lett.* **1989**, *30*, 3201–3204. [w] Hoshino, O.; Itoh, K.; Umezawa, B.; Akita, H.; Oishi, T *Tetrahedron Lett.* **1988**, *29*, 567–568. Hydrolysis in water-saturated isooctane. [x] Pearson, A. J.; Lai, Y.-S.; Lu, W.; Pinkerton, A. A. *J. Org. Chem.* **1989**, *54*, 3882–3893. [y] Eichberger, G.; Penn, G.; Faber, K.; Griengl, H. *Tetrahedron Lett.* **1986**, *27*, 2843–2844. Oberhauser, T.; Bodenteich, M.; Faber, K.; Penn, G.; Griengl, H. *Tetrahedron* **1987**, *43*, 3931–2944. Saf, R.; Faber, K.; Penn, G.; Griengl, H. *Tetrahedron* **1988**, *44*, 389–392. Königsberger, K.; Faber, K.; Marschner, C.; Penn, G.; Baumgartner, P.; Griengl, H. *Tetrahedron* **1989**, *45*, 673–680. [z] Sonomoto, K.; Tanaka, A. *Ann. N. Y. Acad. Sci.* **1988**, *542*, 235–239. Esterification with 5-phenylpentanoic acid in water-saturated isooctane. [aa] Hirose, Y.; Anzai, M.; Saitoh, M.; Naemura, K.; Chikamatsu, H. *Chem. Lett.* **1989**, 1939–1942. [bb] Naemura, K.; Matsumura, T.; Komatsu, M.; Hirose, Y.; Chikamatsu, H. *J. Chem. Soc., Chem. Commun.* **1988**, 239–241.

however, it is difficult to test this hypothesis quantitatively. Nevertheless, we used this hypothesis to redesign substrates that could be efficiently resolved by these enzymes.

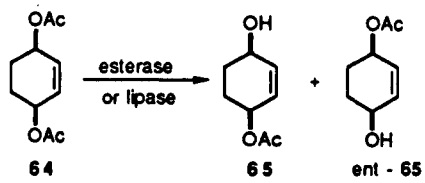
Kinetic Resolution of Lactate Esters. To resolve lactate esters we increased the size of the ester group until we found a substrate that was hydrolyzed with high enantioselectivity (Scheme I, Table IV). Lactyl acetate, R = H, was not a substrate for the three enzymes tested: activity <0.002 units/mg. The methyl ester of lactyl acetate, R = CH_3, was hydrolyzed with the *R* enantiomer reacting faster, but with very low enantioselectivity: the remaining starting material showed <32% ee at ~45% conversion. Accurate determination of the enantioselectivity was complicated by competing hydrolysis of the methyl ester which accounted for 55–80% of the disappearance of the starting material. The *tert*-butyl ester of lactyl acetate, R = *tert*-butyl, still showed low enantioselectivity with CE and CRL, but showed excellent enantioselectivity with PCL, $E > 50$. The lack of any increase in enantioselectivity for CRL is not surprising because the rule is not reliable for acyclic substrates of CRL. The unexpected result was that the enantioselectivity of CE was opposite of that predicted by the rule. The reason for this reversal is not known; this is the first substrate of CE

Table IV. Enantioselectivity of the Hydrolysis of Esters of Lactyl Acetate

	methyl ester				*tert*-butyl ester				
enzyme	rate[a]	% C[b]	% ee[c]	(R/S)[d]	rate[a]	% C[b]	% ee[c]	(R/S)[d]	E[e]
CE	0.18	42	8	R	0.06	32	21	S	3
CRL	0.30	49	32	R	0.01	40	26	R	4.4
PCL	0.01	40	24	R	0.35	50	89	R	51
PCL[f]					0.35	47	>98[g]	R	>200

[a] Initial activity of the enzyme in units/mg solid. Units = μmol of ester hydrolyzed/min. [b] The conversion refers to the amount of starting material consumed. This value was determined by ^{1}H NMR after extraction of the reaction mixture with ethyl ether. [c] Enantiomeric excess of the recovered starting material was determined by ^{1}H NMR in the presence of Eu(hfc)$_3$. [d] The absolute configuration of the preferred enantiomer was determined from the rotation of the recovered starting material; *R*-(−), Ito, S.; Kasai, M.; Ziffer, H.; Silverton, J. V. *Can. J. Chem.* 1987, *65*, 574–582. [e] Enantioselectivity, see ref 27 for details. [f] Hydrolysis of esters of lactyl butyrate. [g] Enantiomeric excess of the product.

Scheme II. Enzyme-Catalyzed Hydrolysis of 64 Showed Poor Enantioselectivity



that does not fit the rule in Figure 1. Even with this exception, the accuracy of the rule remains high: 14/15 substrates, 93%. The increase in enantioselectivity with PCL is consistent with the rule; indeed another lipase from *Pseudomonas* sp. (lipase K-10 from Amano) has been reported to show excellent enantioselectivity when R = *tert*-butyl.[23] Thus, increasing the size of the R group in Scheme I resulted in a substrate which was more efficiently resolved.

A preparative-scale resolution of *tert*-butyl lactate was carried out using the butyrate instead of the acetate to simplify separation of the product alcohol and unreacted butyrate by fractional distillation. This change from acetate to butyrate also resulted in a further increase in enantioselectivity of PCL to >200. Hydrolysis of racemic *tert*-butyl ester of lactyl butyrate (50 g) yielded the unnatural enantiomer, (*R*)-(+)-*tert*-butyl lactate (6.4 g), with >98% ee after distillation.

Enantioselective Synthesis of (*S*)-(−)-4-Acetoxy-2-cyclohexen-1-one (70). The five-membered 4-acetoxy-2-cyclopenten-1-one is a useful chiral starting material that can be prepared by an enzyme-catalyzed hydrolysis.[30] Acetylcholinesterase selectively hydrolyzes the *R* acetate in *cis*-1,4-diacetoxy-2-cyclopentene and the resulting olefinic alcohol is oxidized to the enone. An attempt to prepare the corresponding six-membered compound by an analogous enzyme-catalyzed synthesis was not successful because the acetate was removed with only low to moderate selectivity (0–72% ee, Scheme II, Table V).

The rule suggests a possible reason for this difficulty. In the cyclopentene case, the substituents at the stereocenter—CH_2CHOAc and CH=CH—differ in size and can be distinguished by the enzyme; however, in the cyclohexene case, the substituents—CH=CH and CH_2CH_2—are too similar in size to be distinguished by the enzyme. To increase the selectivity, bromine was added across the double bond of **64** to increase the difference in size of the substituents (Scheme III).

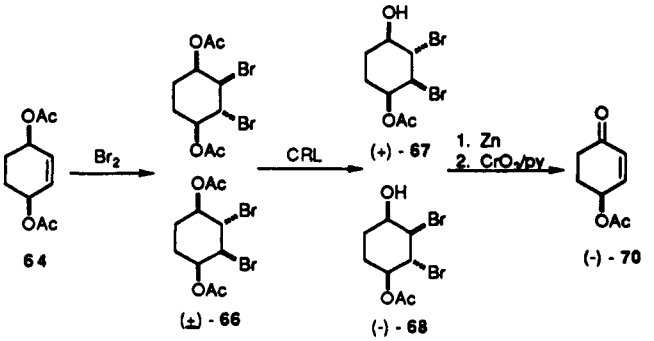
The highest yields for the addition of bromine to **64** were obtained using reaction conditions that favor free radical intermediates (CS_2, −78 °C, *hν*). Addition of bromine under conditions which favor ionic intermediates (polar solvents, dark) resulted in a mixture of products which may have resulted from intramolecular attack of an acetate on the bromonium ion intermediate. The addition of bromine to **64** yielded the trans-dibromide, **66**, identified from coupling constant of 9.3 Hz for the ^{1}H NMR signals for the hydrogens at CHBr (δ 4.36, 4.27), indicating an axial–axial arrangement.[31] For the cis-dibromide a coupling

Table V. Enantioselective Hydrolysis of *cis*-1,4-Diacetoxycyclohexane Derivatives

enzyme[a]	substrate	rate[b]	product	% ee[c]
CE	**64**	0.6	**65**	32
CRL	**64**	4.1[d]	ent-**65**	41
PCL	**64**	0.03	ent-**65**	72
PLE	**64**	18.2[d]	**65**	62
ACE	**64**	18	**65** + ent-**65**	0
MML	**64**	0.0004	ent-**65**	14
CE	(±)-**66**	3.9	(+)-**67**, (−)-**68**	>97, >97
CRL	(±)-**66**	0.4[d]	(+)-**67**, (−)-**68**	>97, >97
PLE	(±)-**66**	4.2[d]	(+)-**67**, (−)-**68**	>97, >97
PCL	(±)-**66**	0.002	(+)-**67**, **68** + diol	14[e]
ACE	(±)-**66**	0.57	(+)-**67**, (−)-**68** + diol[f]	85,[e] >95[g]
MML	(±)-**66**	0.00005	(+)-**67**, **68** + diol[h]	87[e]

[a] ACE = acetylcholinesterase from electric eel, MML = lipase from *Mucor meihei*. [b] Initial activity of the enzyme in units/mg solid. Unit = μmol of ester hydrolyzed/min. [c] Determined by ^{1}H NMR in the presence of Eu(hfc)$_3$. For the hydrolysis of (±)-**66**, the enantiomeric purity was determined for both **67** and **68** after separation by flash chromatography. The ^{1}H NMR signals for the acetyl methyl group of the two enantiomers of **67** or of **68** are separated by 0.4 ppm in the presence of approximately 1.5 equiv of Eu(hfc)$_3$. With this excellent separation even 1.5 mol % of the other enantiomer can be detected as shown by a deliberate addition of racemate to an enantiomerically pure sample. [d] In units/mg protein. [e] For **67**. [f] The ratio of isolated **68**:**67**:diol was approximately 1:4:8 after 0.9 equiv of base had been consumed. [g] For **68**. [h] After 0.7 equiv of base had been consumed, the major product was diol. Only traces of **68** were observed.

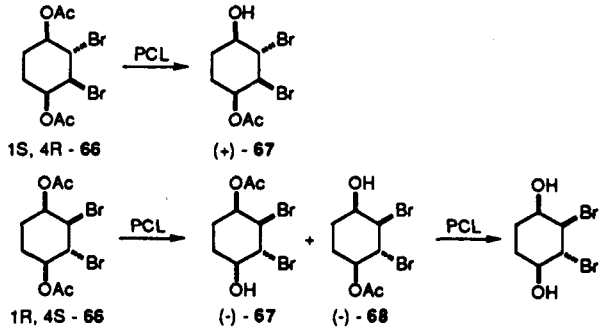
Scheme III. Preparation of 70 via the Dibromide Derivative



(30) Deardorff, D. R.; Matthews, A. J.; McMeekin, D. S.; Craney, C. L. *Tetrahedron Lett.* **1986**, *27*, 1255–1256. Wang, Y.-F.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1984**, *106*, 3695–3696.

(31) Cooper, J. W. *Spectroscopic Techniques for Organic Chemists*; Wiley: New York, 1980; p 81.

Scheme IV. Hydrolysis of Racemic 66 Catalyzed by PCL



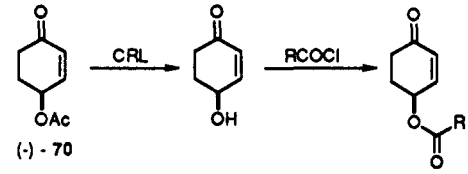
constant of 2–3 Hz would be expected. This trans addition of bromine converted, **64**, a meso compound, into a pair of enantiomers, (±)-**66**. The rule predicts selective hydrolysis of the *R* acetate in both enantiomers yielding the two enantiomerically pure diastereomers: **67** and **68**. Note that the two *R* acetates that are predicted to be hydrolyzed are diastereotopic: one *R* acetate is cis to an adjacent bromine whereas the other *R* acetate is trans.

With CE, CRL, or PLE as catalysts, the hydrolysis of (±)-**66** slowed and stopped after half of the acetates had been hydrolyzed. Analysis of the reaction mixture by TLC showed no remaining starting material, equal amounts of **67** and **68**, and traces of diol which resulted from hydrolysis of both acetates. The two products were enantiomerically pure as shown by ^{1}H NMR in the presence of Eu(hfc)$_3$ (Table V). The opposite sign of rotation of (+)-**67** and (−)-**68** is presumably caused by the differing orientation of the adjacent bromine. Thus, for these three enzymes the hydrolysis of (±)-**66** proceeded as predicted by the rule.

When PCL was used as the catalyst, the reaction did not slow appreciably after half of the acetates had been hydrolyzed. The products isolated from a reaction stopped after half of the acetates had been hydrolyzed were unreacted starting material, (+)-**67** having low enantiomeric excess, a small amount of **68**, and a small amount of diol. Several other enzymes were also screened as possible catalysts, Table V. MML and ACE[4] showed results similar to PCL. Formation of these products can be accounted for by the different reactions of the two enantiomers of **66** as shown in Scheme IV. For (1*S*,4*R*)-**66**, removal of the *R* acetate is predicted by the rule. This acetate is also the more chemically reactive one because it is oriented trans to the adjacent bromine. Hydrolysis occurs as predicted and yields (+)-**67**. For the other enantiomer, (1*R*,4*S*)-**66**, removal of the *R* acetate is also predicted by the rule; however, this acetate is the less chemically reactive one because it is oriented cis to the adjacent bromine. Hydrolysis of both acetates is observed: hydrolysis of the *R* acetate yields (−)-**68**, hydrolysis of the *S* acetate yields (−)-**67**. This last product accounts for the low enantiomeric excess of the isolated **67**. Hydrolysis of both acetates yields the diol. Thus, the rule only partly accounts for the PCL-catalyzed hydrolysis of (±)-**66** due to the differences in chemical reactivity of the acetates caused by the differing orientation of the adjacent bromine. The rule in Figure 1 is too simple to include such effects.

Of the three enzymes which showed excellent selectivity, CRL was chosen for the preparative-scale reaction because it is the least expensive on a unit basis. Hydrolysis of 26 g of (±)-**66** catalyzed by CRL yielded 20 g of enantiomerically pure (+)-**67** and (−)-**68** in 82% yield as cocrystalline diastereomers. To complete the synthesis of **70**, the bromines were removed using zinc dust (82–90% yield) and the resulting olefinic alcohol was oxidized to the enone, **70**, with chromium trioxide/pyridine (84% yield). Other

Scheme V. Preparation of Derivatives of 70



methods of oxidation either gave lower yields (MnO_2, 62%) or gave side products (Swern, PCC/NaOAc, DMSO/Ac_2O, DMSO/Ac_2O/py/CF_3COOH).

The enantiomeric purity of **70** was 98% as shown by ^{1}H and ^{19}F NMR of the Mosher's ester derivative. This derivative was prepared by removal of the acetyl group either by CRL-catalyzed or base-promoted hydrolysis followed by reaction with the acid chloride as shown in Scheme V. As a control, a racemic sample of **70** was also hydrolyzed using CRL and derivatized with the Mosher's acid chloride.[32] For the racemic sample, the diastereomers could be distinguished by ^{1}H NMR (two well-separated multiplets for the proton at the carbinol carbon centered at δ 6.85) or by ^{19}F NMR (two multiplets centered at δ −71.95). The ester derived from enantiomerically pure **70** showed ~1% of the minor diastereomer by either method corresponding to 98% ee. Deliberate addition of material derived from racemic **70** confirmed that the small peaks were due to the other diastereomer.

Determination of the Absolute Configuration of 70. The absolute configuration of **70** was established to be (*S*)-(−) using the exiton chirality method.[33] The acetyl group of **70** was replaced with a benzoyl group as shown in Scheme V. The acetyl group was removed by an enzyme-catalyzed hydrolysis[31] and the product alcohol was treated with benzoyl chloride. The circular dichroism spectrum of the resulting benzoate showed a split Cotton effect, negative at 227 nm ($\Delta\epsilon$ = −5.7) and positive at 192 nm ($\Delta\epsilon$ = +4.0). This splitting indicates a left-handed screw sense between the benzoate and the enone chromophores, i.e. *S*. This assignment of absolute configuration is consistent with the expected enantioselectivity of the three enzymes and with previous assignments for a cyclopentenone[34] and a substituted cyclohexenone.[35]

Discussion

The major advantage of this rule is its simplicity. It is straightforward to use and correlates a large amount of experimental data because it applies to a wide range of substrates. It further suggests a strategy for improving the efficiency of resolutions: to increase the difference in size of the substituents at the stereocenter. Two tests of this strategy were successful because an efficiently resolved substrate/enzyme combination was found for both examples. The strategy of adding a large group to one side of a molecule resulted in a substrate that was efficiently

(32) The enzymic hydrolysis of **70** showed no enantiospecificity; nevertheless, the hydrolysis was continued to completion to assure that no enhancement of enantiomeric purity occurred at this step. Consistent with this notion, the Mosher's ester derived from racemic **70** using CRL-catalyzed hydrolysis showed equal amounts of each diastereomer. The enzyme-catalyzed reaction was preferred because the reaction showed none of the side products observed by TLC during base-promoted hydrolysis.

(33) Harada, H.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exiton Coupling in Organic Stereochemistry*; University Science Books: New York, 1983.

(34) Tanaka, T. Kurozumi, S. Toru, T.; Miura, S. Kobayashi, M.; Ishimoto, S. *Tetrahedron* **1976**, *32*, 1713–1718.

(35) Koreeda, M.; Weiss, G.; Nakanishi, K. *J. Am. Chem. Soc.* **1973**, *95*, 239–240.

resolved by a least one of the enzymes. Thus, screening a substrate with a large group as one of the substituents at the stereocenter appears to be more reliable than screening a substrate having substituents of similar size at the stereocenter.

The major disadvantage of this rule is that it does not account for subtleties in the selectivities of these enzymes. For example, it does not rationalize why only PCL and not the other two enzymes showed increased enantioselectivity for the *tert*-butyl ester of lactyl acetate as compared to the methyl ester. The rule also does not predict the effect of additional stereocenters, for example, the cis vs trans oriented bromine at C_2 of **66**. The sensitivity of PCL to this orientation rendered this enzyme unsuitable for the preparation of **70**, whereas the other two enzymes which were not sensitive to this orientation were suitable.

In spite of this disadvantage, a general rule such as that proposed in Figure 1 may be the most reasonable way to describe the active site of these enzymes. Structural data suggests that large conformational changes are required before the substrate can bind to the active site in pancreatic lipase and lipase from *Mucor meihei*.[3] Due to this flexibility it may never be possible to define an exact size and shape for the substrate binding region of lipases because this region may change for each substrate. Consistent with this notion are reports that the enantioselectivity and conformation of CRL change upon treatment with bile salt and organic solvent[36] or with dextromethorphan.[37]

A second reason to use a general rule is that these enzymes may not be homogeneous. Sequencing of the gene for CRL showed several nonidentical DNA sequences which code for this enzyme,[38] thus it is likely that the commercial enzymes are a mixture of isozymes. The enantioselectivities of isozymes of PLE were similar, but not identical;[39] a similar situation may hold for the isozymes of CRL. This heterogeneity may frustrate attempts to precisely define the size and shape of the active site, thus a general rule may be the most accurate way of describing the commercial catalyst.

A third reason for using rules and models is that even when the X-ray crystal structure of an enzyme is known, models are often used to predict enantioselectivity because they are simpler to use. For example, a high resolution X-ray crystal structure has been determined for alcohol dehydrogenase from horse liver, yet a cubic space model is usually used to predict its enantioselectivity.[40] Further, it remains difficult to predict which binding interactions are most important in an enzyme-substrate complex, thus it may remain difficult to predict enantioselectivity for an untested substrate even when the X-ray crystal structure is known. For example, the origin of the high selectivity for transfer of the *pro*-4*S* hydrogen of NADH catalyzed by lactate dehydrogenase ($>10^8$:1) is difficult to explain from the known crystal structure.[41] These rules and models may be used, along with X-ray crystal structures and molecular modeling, to determine which interactions are most important in determining the enantioselectivity of these enzymes.

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Experimental Section

General. Cholesterol esterase (bovine, 0.2 units/mg solid with 0.1 M cholesterol acetate in ethyl ether as substrate) was purchased from Genzyme Corp., Boston, MA. Lipase from *C. rugosa* (L-1754, 0.2 units/mg solid using olive oil), porcine liver esterase (E-3126, 240 units/mg protein using ethyl butyrate), and acetyl cholinesterase (electric eel, C-3389, 28 units/mg solid with acetyl choline) were purchased from Sigma Chemical Co., St. Louis, MO. Lipase from *P. cepacia* (lipase P30, 0.06 units/mg solid using olive oil) and lipase from *M. meihei* (lipase MAP-10, 0.05 units/mg solid using olive oil) were purchased from Amano International Enzyme Co., Troy, VI. Activated MnO_2 (Aldrich) was heated at 120 °C for 24 h before use. Zinc dust (60 g) was activated by stirring for 1 min with 2% aqueous HCl. The dust was collected by vacuum filtration and washed with 120 mL of the following: 1 × 2% HCl, 2 × 95% ethanol, 1 × anhydrous ethyl ether. Elemental analyses were done by Guelph Laboratories, ON.

Enzyme-Catalyzed Hydrolyses. A rapidly stirred suspension of substrate (1 mmol) in phosphate buffer (10 mM, 10 mL) containing enzyme (0.2–50 mg) was maintained at pH 7.0 by automatic titration with NaOH (0.1 N) using a Radiometer RTS 822 pHstat. Crystalline substrates were first dissolved in ethyl ether (10 mL). Sodium taurocholate (30 mg) was added to hydrolyses where CE was used as the catalyst. The rate of consumption of sodium hydroxide over the first 5% of the reaction was used to calculate the initial rates listed in Tables I, IV, and V. The reaction was stopped after the consumption of base indicated 20–50% conversion and the mixture was extracted four times with ethyl acetate. The combined extracts were washed with saturated aqueous sodium bicarbonate, water, and brine, dried over magnesium sulfate, and concentrated. The starting ester and alcohol were separated by flash chromatography, and the enantiomeric purity was determined.

Determination of Enantiomeric Purity by Gas Chromatography. Alcohol (4 μL), isopropyl isocyanate (300 μL), and dichloromethane (300 μL) were heated at 100 °C for 1.5 h in a tightly sealed glass vial. The completeness of the reaction was checked by TLC. Solvent and excess reagent were evaporated in a stream of nitrogen, and the residue was diluted to 1 mL with dichloromethane and analyzed by gas chromatography using an XE-60-(*S*)-valine-(*S*)-α-phenylethylamide capillary column.

Determination of Enantiomeric Purity by ^{1}H NMR. A 5-mg sample of the ester was dissolved in 0.5 mL of $CDCl_3$ in an NMR tube, and the ^{1}H NMR spectrum was obtained using a Varian XL-200 NMR spectrometer. Solid tris[(3-heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), Eu(hfc)$_3$, was added in four portions, and the spectra were obtained. A total of more than 1.3 equiv of shift reagent was added to each sample.

Acetyl Esters. Two to three equivalents of acetyl chloride was added dropwise to a stirred solution of alcohol in pyridine. Solid alcohols were dissolved in a mixture of ethyl ether and pyridine. Acetylation was complete after 10 min to 2 h as shown by TLC. The reaction mixture was washed twice with 10% sodium bicarbonate and once with water. If only one layer formed upon washing, the product was extracted into ether. The organic layer was dried with magnesium sulfate and concentrated by rotary evaporation. The esters were purified either by distillation or by flash chromatography.

(±)-Lactyl acetate was prepared using a literature procedure,[42] but substituting toluene for benzene. A mixture of racemic lactic acid (120 mL of 85% purity, 1.1 mol), glacial acetic acid (640 mL, 11.2 mol), toluene (80 mL), and concd sulfuric acid (0.40 mL) was refluxed with the continuous removal of distillate with a Dean–Stark trap until a ^{1}H NMR spectra of the distillate showed that no more water was present. Approximately 1 L of solution was removed during 40 h; acetic acid (~600 mL) and toluene (~100 mL) were periodically added to the reaction to replace what was removed. The reaction mixture was neutralized with sodium acetate (1.6 g) and distilled under vacuum yielding 86 g (59%): bp 35–37 °C (0.2 Torr) [lit.[43] bp 127 °C (11 Torr)]; ^{1}H NMR

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($CDCl_3$, 200 MHz) δ 10.6 (s, 1), 5.10 (q, 1, J = 7.1 Hz), 2.14 (s, 3), 1.53 (d, 3, J = 7.1 Hz).

(±)-Methyl Ester of Lactyl Acetate. A suspension of potassium methoxide (3.8 g, 54 mmol) in dry ethyl ether (100 mL) containing (±)-2-acetoxypropionyl chloride[44] (7.3 g, 50 mmol) was stirred for 72 h. The reaction mixture was washed twice with saturated sodium bicarbonate solution, the organic layer was collected, and the aqueous phase was extracted with ether (2 × 200 mL). The combined organic layers were dried with anhydrous magnesium sulfate, concentrated by rotary evaporation, and distilled, giving 1.6 g (22%): bp 60–64 °C (~4 Torr) [lit.[45] bp 64 °C (9.8 Torr)]; ^{1}H NMR (neat, 60 MHz) δ 4.8 (q, 1, J = 7 Hz), 3.5 (s, 3), 1.8 (s, 3), 1.2 (d, 3, J = 7 Hz).

(±)-*tert*-Butyl Ester of Lactyl Acetate. A two-phase mixture of (±)-lactyl acetate (66 g, 0.5 mol), liquified isobutylene (120 mL, 1.5 mol), ethyl ether (85 mL), and concd sulfuric acid (4 mL) in a 500-mL pressure bottle was sealed with a rubber stopper wired securely like the cork of a champagne bottle and stirred for 7 h at room temperature until a single phase formed. The bottle was chilled in an ice–salt water bath or dry ice–acetone bath and opened, and the contents were slowly added to a saturated phosphate buffer (300 mL, pH 7). The pH of the buffer was maintained between 7 and 8 throughout the addition with a concd sodium hyroxide solution. The combined ether extracts were dried over anhydrous potassium carbonate and filtered into a round-bottomed flask that had been washed with a sodium hydroxide solution and rinsed with water to ensure the removal of trace acid. The ether and excess isobutylene were evaporated under vacuum and the resulting clear, slightly yellow oil was distilled giving 81 g (86%): bp 95–100 °C (~1 Torr); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.64 (q, 1, J = 7.1 Hz), 1.84 (s, 3), 1.21 (s, 9), 1.18 (d, 3, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 169.4, 169.3 (C=O), 81.0 ($C(CH_3)_3$), 68.5 (CH), 27.3 ($C(CH_3)_3$), 20.0 (CH_3C=O), 16.3 (CH_3CH).

(±)-*tert*-Butyl Ester of Lactyl Butyrate. Butyryl chloride (244 mL, 2.36 mol) was added dropwise to stirred lactic acid (125 g of 85% purity, 1.18 mol). After addition was complete, the mixture was stirred overnight at room temperature. A ^{1}H NMR spectrum of the reaction mixture indicated no remaining lactic acid. Excess butyryl chloride and butyric acid were removed by vacuum distillation leaving crude lactyl butyrate, 156 g (82%). A portion of this material (125 g, 0.78 mol) was treated with isobutylene (187 mL, 2.34 mol) as described above for the acetate derivative. Distillation yielded 51 g (36%): bp 94 °C (~2 Torr); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.95 (q, 1, J = 7 Hz), 2.35 (t, 2, J = 7 Hz), 1.68 (m, 2), 1.45 (s + d, 9 + 3), 0.95 (t, 3, J = 7 Hz); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 173.6, 170.7 (C=O), 82.2 ($C(CH_3)_3$), 69.2 (CH), 36.1 ($OC(O)CH_2$), 28.1 ($C(CH_3)_3$), 18.5 ($OC(O)CH_2CH_2$), 17.1 (CH_3CH), 13.8 ($CH_2CH_2CH_3$).

(*R*)-(+)-*tert*-Butyl Lactate. A suspension of racemic *tert*-butyl ester of lactyl butyrate (50 g, 0.23 mol) in phosphate buffer (400 mL, 0.1 M, pH 7.0) containing PCL (1.0 g) was stirred at room temperature. The pH was maintained between 6.9 and 7.1 by automatic addition of NaOH (0.5 M). After 22 h, 215 mL of base had been added, indicating 47% conversion. The suspension was saturated with sodium chloride and extracted with ethyl ether (4 × 750 mL). The combined extracts were dried over magnesium sulfate and concentrated by rotary evaporation to yield an oil, 42 g. Distillation yielded (*R*)-(+)-*tert*-butyl lactate, 6.4 g (38% of theoretical yield): bp 51–54 °C (4 Torr), [lit.[46] bp 45–47 °C (9 Torr)]; oil which solidifies, mp 35–37.5 °C; $[\alpha]_D$ = +7.98 (*c* 1.7 CH_2Cl_2) [lit.[45] $[\alpha]^{20}_D$ = +9.48 (neat, *l* = 1)]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.62 (q, 1, J = 7 Hz), 2.9 (s, br), 1.49 (s, 9), 1.38 (d, 3, J = 7 Hz); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 175.8 (C=O), 82.6 ($C(CH_3)_3$), 67.2 (CH), 28.2 ($C(CH_3)_3$), 20.7 (CH_3CH); >98% ee by ^{1}H NMR with Eu(hfc)$_3$ on the acetyl derivative. The limit of detection was determined by deliberate addition of racemic *tert*-butyl ester of lactyl acetate to the NMR tube.

***cis*-1,4-Diacetoxy-2-cyclohexene, 64,** was prepared using Bäckvall's method[47] with the following changes. The acetic acid solution was heated to dissolve the palladium diacetate, then cooled prior to the addition of the other reagents. After the reaction completed, the solution was filtered through Whatman no. 41 paper on a Büchner funnel prior to extraction with pentane to minimize the formation of an emulsion.

(±)-1α,4α-Diacetoxy-2β,3α-dibromocyclohexane, 66. A solution of **64** (5.0 g, 25 mmol) in CS_2 (120 mL) was cooled to −78 °C in an acetone/dry ice bath and irradiated with a Phillips 150-W reflector flood lamp placed 35 cm from the reaction mixture. A solution of Br_2 in CS_2 (3.9 M, 8.8 mL, 34 mmol) was added to the stirred reaction mixture in one portion. After 50 min, TLC showed the presence of a small amount of starting material; however, longer reaction time did not result in its disappearance. The reaction mixture was diluted with cold chloroform (−20 °C, 700 mL) and subsequently washed with saturated aqueous Na_2SO_3 (2 × 70 mL), water (70 mL), and brine (2 × 80 mL). The reaction mixture must remain cold until after the first washing with Na_2SO_3. The organic phase was dried over magnesium sulfate and concentrated by rotary evaporation. Recrystallization (ether/hexanes) yielded white crystals, 7.35 g (81%): mp 71.5–72 °C; R_f = 0.3 (4:1 hexanes/ethyl acetate); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.32 (m, 1, H4), 5.01 (ddd, 1, H1), 4.36 (dd, 1, $J_{1,2}$ = 9.0 Hz, $J_{2,3}$ = 9.3 Hz, H2), 4.27 (dd, 1, $J_{3,4}$ = 2.7 Hz, H3), 2.15 (s, 3, CH_3), 2.11 (s, 3, CH_3), 2.20–2.00 and 1.70–1.85 (2 m, 4, H5, H5′, H6, H6′); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 169.7, 169.6 (C=O); 73.9, 70.7 (CHOAc), 54.1 (CHBr), 26.1, 25.3 (CH_2), 21.0, 20.9 (CH_3); IR (Nujol mull) 1751, 1731, 1257, 1231, 1024 cm^{-1}; MS (CI, NH_3) m/z 374 (M + NH_4^+, 46); exact mass 373.96018 ($C_{10}H_{18}Br_2NO_4$ requires 373.96026, 2 ppm error). Anal. Calcd for $C_{10}H_{14}Br_2O_4$: C, 33.55; H, 3.94. Found: C, 33.14; H, 3.84.

(1*S*)-(+)-1α-Acetoxy-2α,3β-dibromo-4α-hydroxycyclohexane, (+)-67, and (1*S*)-(−)-1α-Acetoxy-2β,3α-dibromo-4α-hydroxycyclohexane, (−)-68. Lipase from *C. rugosa* (15 g) was added to a stirred mixture of dibromo diacetate, (±)-**66** (26 g, 73 mmol), aqueous phosphate buffer (260 mL, 0.1 M, pH 7.00), and ethyl ether (10 mL). Aliquots of a 0.5 M NaOH solution were added as required to maintain the pH of the mixture between 6.95 and 7.05. After 3 days a total of 1 equiv (73 mmol) of base had been added. The mixture was extracted with ethyl acetate (4 × 750 mL), and the combined extracts were washed with saturated aqueous sodium bicarbonate (600 mL), and brine (600 mL). The organic phase was dried over magnesium sulfate and concentrated by rotary evaporation. Recrystallization of the crude residue from 115 mL of CH_2Cl_2/hexanes (35:65 v/v) yielded cocrystalline diastereomers **67** and **68**, 18.7 g (82%): mp 97–104 °C. A sample of the diastereomers was separated by flash chromatography (4:1 hexanes/ethyl acetate). (+)-**67**: mp 125.5 °C; R_f = 0.34 (3:2 hexanes/ethyl acetate); >97% ee by ^{1}H NMR with Eu(hfc)$_3$; $[\alpha]_D$ = +141° (*c* 1.6, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.31 (m, 1, H1), 4.29 (dd, 1, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 8.4 Hz, H3), 4.19 (dd, 1, $J_{1,2}$ = 2.6 Hz, H2), 3.79 (m, 1, H4), 2.66 (br, 1, OH), 2.15 (s, 3, OAc), 1.97–2.17 (m, 2, H5, H6), 1.65–1.88 (m, 2, H5′ and H6′); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 169.6 (C=O), 74.4 (COAc), 71.6 (COH), 63.0 (CHBrCHOH), 54.6 (CHBrCHOAc), 27.0, 26.6 (CH_2), 20.9 (CH_3); IR (Nujol mull) 3409 (br), 1728, 1260, 1072 cm^{-1}. Anal. Calcd for $C_8H_{12}Br_2O_3$: C, 30.41; H, 3.83. Found: C, 30.03; H, 3.54. (−)-**68**: mp 122 °C; R_f = 0.48 (3:2 hexanes/ethyl acetate); >97% ee by ^{1}H NMR with Eu(hfc)$_3$; $[\alpha]_D$ = −125° (*c* 1.6, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.97 (ddd, 1, H1), 4.37 (dd, 1, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 8.4 Hz, H2), 4.29 (dd, 1, $J_{3,4}$ = 2.0 Hz, H3), 4.15 (ddd, 1, H4), 2.45 (br, 1, OH), 2.07–2.22 (m, 1, H5), 2.11 (s, 3, OAc), 1.88–2.02 (m, 2, H6 and H6′), 1.58–1.81 (m, 1, H5′); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 169.8 (C=O), 74.6 (COAc), 69.5 (COH), 61.6 (CHBrCOH), 54.8 (CHBrCOAc), 27.6, 24.8 (CH_2), 21.0 (CH_3); IR (Nujol mull) 3429 (br), 1717, 1257, 1034 cm^{-1}. Anal. Calcd for $C_8H_{12}Br_2O_3$: C, 30.41; H, 3.83. Found: C, 30.29; H, 3.52.

(1*S*)-(−)-*cis*-1-Acetoxy-4-hydroxy-2-cyclohexene, 69. Activated zinc dust (31 g, 470 mmol) was added to a mixture of the dibromides **67** and **68** (19.4 g, 61.4 mmol) dissolved in absolute ethanol (300 mL). The suspension was heated and allowed to reflux for 10 min. After cooling of the mixture in a cold water

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bath, pyridine (30 mL) was added. The mixture was filtered, and the filtrate was concentrated by rotary evaporation. The residue was dissolved in ethyl acetate (120 mL) and washed with saturated aqueous sodium bicarbonate (2 × 120 mL) and brine (120 mL). The ethyl acetate solution was dried over magnesium sulfate and concentrated by rotary evaporation, yielding a light yellow oil, 7.9 g (82%).[48] Vacuum distillation gave 6.0 g (62%): bp 75–77 °C (0.05 Torr); R_f = 0.26 (3:2 hexanes/ethyl acetate); $[\alpha]_D$ = −100° (c 1.3, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 5.98 (dd, 1, $J_{1,2}$ = 2.8 Hz, $J_{2,3}$ = 10.1 Hz, H2), 5.80 (dd, 1, $J_{3,4}$ = 3.4 Hz, H3), 5.19 (m, 1, H1), 4.18 (m, 1, H4), 2.15 (br, 1, OH), 2.06 (s, 3, CH_3), 1.70–1.96 (m, 4, H5, H5′, H6, H6′); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 170.7 (C=O), 134.8, 127.8 (*CH*=*CH*), 67.2 (*C*OAc), 65.3 (*C*OH), 28.1 (CH_2COAc), 24.9 (CH_2COH), 21.2 (CH_3); IR (neat) 3372 (br), 3415 (br), 1736, 1245, 1037 cm^{-1}; MS (CI, NH_3) *m/z* 174 (M + NH_4^+, 63%); exact mass 174.11306 ($C_8H_{16}NO_3$ requires 174.11302, 0.2 ppm error).

(*S*)-(−)-4-Acetoxy-2-cyclohexen-1-one, (−)-70. Chromium trioxide (16.3 g, 163 mmol) was added to a stirred solution of dry pyridine (26.3 mL, 326 mmol) in dry methylene chloride (380 mL) under nitrogen. After 30 min of stirring at room temperature, the olefinic alcohol **69** (4.24 g, 27.1 mmol) in dry methylene chloride (10 mL) was added to the dark reddish-brown solution. A black tarry substance precipitated after a few minutes. The flask was stoppered with a drying tube, and the mixture was stirred for 24 h. The methylene chloride solution was decanted, and the residue was extracted with alternating portions of ethyl ether and saturated aqueous sodium bicarbonate (2 × 150 mL, 1 × 250 mL each). All extracts were combined with the methylene chloride solution and shaken. The aqueous phase was removed and extracted once with ethyl ether (1000 mL). The organic extracts were washed with saturated aqueous sodium bicarbonate (4 × 250 mL), 2% sulfuric acid (4 × 250 mL), saturated sodium bicarbonate (200 mL), and brine (2 × 200 mL). The resulting organic phase was dried over magnesium sulfate and concentrated by rotary evaporation yielding an oil, 3.52 g (84%): R_f = 0.41 (3:2 hexanes/ethyl acetate); $[\alpha]_D$ = −137° (c 1.6, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 6.85 (ddd, 1, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 2.8 Hz, $J_{3,5}$ = −1.4 Hz, H3), 6.06 (ddd, 1, $J_{2,4}$ = −1.9 Hz, $J_{2,6}$ = −0.9 Hz, H2), 5.57 (dddd, 1, $J_{4,5}$ = 4.8 Hz, $J_{4,5'}$ = 8.7 Hz, H4), 2.28–2.70 and 1.99–2.19 (2 m, 3 + 1, H5, H5′, H6, H6′), 2.12 (s, 3, CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 197.7 (C=O), 170.2 ($OCOCH_3$), 147.5 (*C*HCOAc), 130.8 (*C*HC=O), 67.7 (*C*OAc), 34.9 (CH_2C=O), 28.6 (CH_2COAc), 20.9 (CH_3); IR (neat) 1741, 1686, 1372, 1236, 1037 cm^{-1}. MS (CI, NH_3) *m/z* 172 (M + NH_4^+, 100), 155 (M + H^+, 10); exact mass 155.07075 ($C_8H_{11}O_3$ requires 155.07082, 0.4 ppm error).

Enantiomeric Purity of 70. Acetylcholinesterase (4 mg) was added to a stirred suspension of acetoxy ketone **70** (322 mg, 2.09 mmol) in aqueous phosphate buffer (20 mL, 10 mM, pH 7.13). The pH was maintained at 7.13 by automatic addition of NaOH (0.10 N). After 27 h only 0.4 mmol of base had been consumed, thus additional enzyme (CRL, 300 mg) was added. After an additional 41 h a total of 2.1 mmol of base had been added. The reaction mixture was extracted with ethyl acetate (3 × 150 mL), and the combined extracts were washed with saturated aqueous bicarbonate (20 mL), water (20 mL), and brine (2 × 20 mL). The organic phase was dried over magnesium sulfate and concentrated by rotary evaporation yielding crude alcohol, 150 mg (64%): R_f = 0.07 (3:2 hexanes/ethyl acetate). This alcohol was treated with Mosher's acid chloride using a standard procedure.[49] The resulting ester was purified by column chromatography on silica gel eluted with 9:1 hexanes/ethyl acetate; R_f = 0.41 (3:2 hexanes/ethyl acetate). A racemic sample of **70** was also treated in the same manner.

Absolute Configuration of 70. Acetyl cholinesterase (3.6 mg, 0.57 units) was added to a stirred mixture of acetoxy ketone **70** (300 mg, 1.95 mmol) and aqueous phosphate buffer (20 mL, 10 mM, pH 7). Aliquots of a 0.107 N NaOH solution were added automatically to maintain the pH of the mixture at 7.01. Due to the slow rate of the hydrolysis, a large amount (700 mg) of CRL was added in three portions over a period of 6 days. The reaction was stopped at 93% conversion. The reaction mixture was extracted with ethyl acetate (3 × 200 mL). Each organic extract was washed with saturated aqueous sodium bicarbonate (5 mL), water (2 × 5 mL), and brine (2 × 5 mL). The extracts were combined, dried over magnesium sulfate, and concentrated by rotary evaporation, yielding 169 mg (77%); R_f = 0.07 (3:2 hexanes/ethyl acetate). Without further purification of the alcohol, the benzoate derivative was prepared. Benzoyl chloride (340 μL, 2.92 mmol) was added to a solution of alcohol (164 mg 1.46 mmol) in pyridine (3 mL, 37 mmol). The mixture was stirred at room temperature for 70 min at which time TLC analysis showed no remaining alcohol. The reaction mixture was added to a separatory funnel containing 0.5 M H_2SO_4 (74 mL) and ethyl ether (50 mL). After vigorous shaking, additional ethyl ether (150 mL) was added. The aqueous phase was discarded, and the organic phase was washed with saturated aqueous sodium bicarbonate (20 mL) and brine (2 × 20 mL), dried over magnesium sulfate, and concentrated by rotary evaporation. Purification by flash chromatography (85:15 hexanes/ethyl acetate) yielded the benzoate as an oil, 184 mg (58%): R_f = 0.46 (3:2 hexanes/ethyl acetate); $[\alpha]_D$ = −197° (c 1.9, CH_2Cl_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.41–8.09 (m, 5, aromatic), 6.98 (ddd, 1, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 2.8 Hz, $J_{3,5}$ = −1.3 Hz, H3), 6.11 (ddd, 1, $J_{2,4}$ = −1.9 Hz, $J_{2,6}$ = −0.8 Hz), 5.82 (m, 1, H4), 2.40–2.77 and 2.15–2.34 (2 m, 3 + 1, H5, H5′, H6, H6′); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 197.8 (C=O), 165.7 (OCOPh), 147.6 (*C*HCOBz), 133.4 (*p*-CH), 131.0 (*C*HC=O), 129.7, 128.5 (*o*- and *m*-CH), 129.5 (*C*COOR), 68.2 (*C*OBz), 35.0 (CH_2C=O), 28.8 (CH_2COBz); UV (CH_3OH) 228 nm (ε 14 200 M^{-1} cm^{-1}), 271 nm (ε 554 M^{-1} cm^{-1}); IR (neat) 3050, 2960, 1722, 1683, 1452, 1270, 1113, 710 cm^{-1}; MS (CI, NH_3) *m/z* 217 (M + H^+, 100); exact mass 217.08646 ($C_{13}H_{13}O_3$ requires 217.08647, 0.0 ppm error). The CD spectrum was obtained using a 4.4 × 10^{-5} M solution of the benzoate in CH_3OH in a 0.1-cm cell using a JASCO 500C spectropolarimeter. A total of 10 scans were made from 250 to 185 nm.

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(48) This material showed a trace of a less polar impurity by TLC. This crude material can also be used for the next step without distillation.

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