Enzyme Reactions in Apolar Solvent. 5. The Effect of Adjacent Unsaturation on the PPL-Catalyzed Kinetic Resolution of Secondary Alcohols

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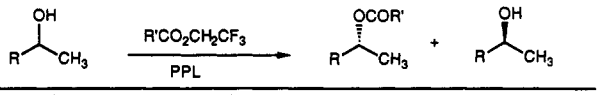
The effect of adjacent unsaturation on the enzyme-catalyzed kinetic resolution of secondary alcohols is studied for a series of allylic, homoallylic, propargylic, homopropargylic, and phenyl-substituted 2-alkanols, using porcine pancreatic lipase (PPL) in anhydrous Et_2O. Excellent enantioselectivity (high E value) was observed for α-phenethyl alcohol (3), propargylic alcohols (8 and 11), and (E)-allylic alcohols (9 and 12), but (Z)-allylic alcohols (10 and 13) showed poor selectivity. Enantioselectivity was also low for both (E)- and (Z)-homoallylic alcohols (15 and 16), homopropargylic alcohol (14), 1-phenyl-2-propanol (6), and 4-phenyl-2-butanol (7). The enhanced enantioselectivity observed for (E)-allylic alcohols was exploited in the synthesis of the enantiomers of both components of the aggregation pheromone of the lesser grain borer, *Rhyzopertha dominica* (F.). The magnitude of the enantiomeric ratio (E value) can be dramatically affected by the accuracy of the values of ee_s and ee_p used in the calculation, especially when E is large. Variation in the value of E with the optical purity of the chiral derivatizing agent used to determine ee_s and ee_p is illustrated.

The use of enzymes in organic synthesis is especially attractive to synthetic chemists due to the remarkable regio- and stereoselectivity of enzyme-catalyzed reactions.[1] While the ability of enzymes to function in organic solvents has long been known,[2] it was not until the work of Klibanov et al.[3] that there developed an intense interest in enzyme-catalyzed reactions in anhydrous apolar solvents. Lipases (triglycerol acylhydrolases, EC 3.1.1.3) are particularly suited for the resolution of secondary alcohols, since these enzymes exhibit enhanced stability and enantioselectivity in organic solvents while accepting a broad range of substrates.[4] For these reasons porcine pancreatic lipase (PPL), which is available as an inexpensive crude preparation, has found many practical applications. We have previously reported on the regioselectivity[5] and enantioselectivity[6] of PPL-catalyzed transesterification reactions in apolar solvents. Earlier, we were successful in the resolution of straight-chain and branched 2-alkanols, which are of limited synthetic utility. To prepare more synthetically useful alcohols and as a means of probing the structural requirements of the enzyme(s) substrate binding site, we have investigated the kinetic resolution of a series of allylic and propargylic 2-alkanols and their homo analogues. In this paper we report on the effect of adjacent unsaturation on the kinetic resolution of secondary alcohols.

Results and Discussion

1. Resolution of Allylic, Homoallylic, and Aromatic 2-Alkanols. Transesterification using PPL in ether with trifluoroethyl butyrate (TFEB) as the acylating agent was efficient at room temperature (Tables I and II). Reactions were stopped by filtration and the enantiomeric ratio (E) of the transesterifications was determined from the degree of conversion and the enantiomeric excess (ee) of reactants and products.[7,8] The effect of reverse reaction was minimized by using a high concentration of acylating agent. Under these conditions most of the enzyme will be in the acyl–enzyme form and the concentration of the free enzyme necessary for reverse reaction insignificant. No attempt was made to distinguish between values of E greater than 100 as E becomes increasingly sensitive to small errors in measurement of ee.

Table I. Resolution of Phenyl- and Cyclohexyl-Substituted 2-Alkanols[a]



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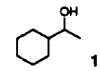
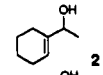
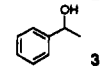
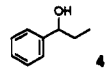
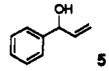
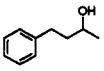
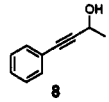
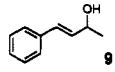
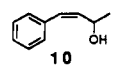
1	 1	16.0	0.360	0.532	0.947	62
2		89.25 a	0.293	0.398	0.960	71
3	 2	15.75	0.347	0.509	0.958	76
4	 3	31.0	n/d	0.383[b]	> 0.97[b]	>100
5		89.25[a]	0.405	0.658[b]	0.967[b]	>100
6		18.0	n/d	0.785[c]	>0.95[c]	>100
7	 4	18.25	n/d	0.784[c]	>0.95[c]	>100
8	 5	18.25	n/d	0.433[c]	0.90-0.95[c]	n/d
9	 6	30.5	0.108	0.112	0.929	29
10		89.25[a]	0.231	0.270	0.898	24
11	 7	30.5	0.383	0.526	0.848	20.5
12	 8	48.25	0.320	0.442	0.940	51
13	 9	28.0	0.468	0.846	0.963	>100
14	 10	167.0	0.144	0.068	0.404	2.5

[a] Resolutions were carried in Et_2O (5 mL), alcohol (0.3–0.4 M), trifluoroethyl butyrate (0.9M), and PPL (1.0 g), except where noted. a = reaction was run in hexanes. b = ee determined using Mosher reagent. c = ee determined on a Chirasil Dex (8) GC column.

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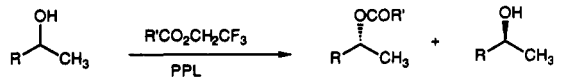
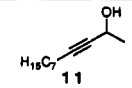
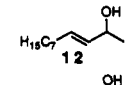
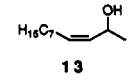
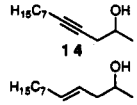
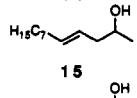
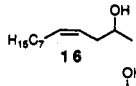
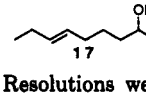
(1) (a) Ohno, M.; Otsuka, M. *Org. React.* **1989**, *37*, 1. (b) Yamada, H.; Shimizu, S. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 622. (c) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (d) Whitesides, G. M.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617.

0022-3263/92/1957-3231$03.00/0

Table II. Resolution of Allylic and Propargylic 2-Alkanols and Their Homo Derivatives[a]



entry	substrate	time (h)	convrsn	ee_s	ee_p	E
1		57.0	0.282	0.384	0.972	>100
2	11	46.5	0.308	0.434	0.973	>100
3		17.3	0.477	0.894	0.980	>100
4	12	28.0	0.488	0.915	0.960	>100
5		28.0	0.086	0.064	0.684	5.7
6	13	167.0	0.318	0.292	0.626	5.7
7		46.5	0.297	0.341	0.809	13
8	14	31.5	0.224	0.237[a]	0.823	13
9		8.3	0.315	0.377	0.819	14
10	15	15.3	0.364	0.465	0.811	15
11		8.3	0.492	0.813[a]	0.838	28
12	16	15.3	0.507	0.852	0.829	30
13		23.0	0.445	0.754	0.942	75
14	17	15.6	0.366	0.551	0.955	76

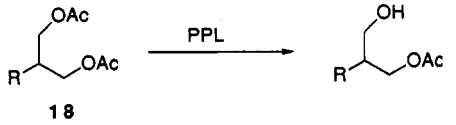
[a] Resolutions were carried out in Et_2O (5 mL), using alcohol (0.25–0.35 M), trifluoroethyl butyrate (0.9 M), and PPL (1.0 g). a = average of two determinations.

Resolutions for a series of phenyl- and cyclohexyl-substituted 2-alkanols are presented in Table I. Resolutions of 1-cyclohexylethanol (1), α-phenylethyl alcohol (3), and 1-phenyl-2-propanol (6) have been reported previously[6b] but have been included for the sake of completeness. Comparison of the data for 1-cyclohexylethanol (1) and 1-cyclohexenylethanol (2) indicates that the presence of the double bond has little effect on the selectivity of the enzyme or the speed of the reaction.

For phenyl-substituted alkanols, rigidity adjacent to the chiral center appears to be important in determining enantioselectivity. While excellent selectivity ($E > 100$) is observed for α-phenethyl alcohol (3), insertion of one or two methylene groups between the chiral center and the phenyl ring (as for 6 and 7) results in a dramatic decrease in enantioselectivity (E = 20–30). The presence of a nonadjacent phenyl group also has a deleterious effect on selectivity. Compounds 6 and 7 are also much poorer substrates than analogous straight-chain or branched alcohols such as 2-hexanol or 5-methyl-2-hexanol.[6b] In comparison to compound 7, if rigidity is introduced into the butyl chain via a conjugated alkene or alkyne, enantioselectivity is restored. A modest increase in selectivity is observed for 4-phenyl-3-butyn-2-ol (8) (E = 40–50), while a selectivity similar to that for 3 ($E > 100$) is observed for (*E*)-4-phenyl-3-buten-3-ol (9). This remarkable selectivity disappears upon changing the double-bond geometry since (*Z*)-4-phenyl-3-buten-2-ol (10) reacts more slowly and shows essentially no enantioselectivity under the same conditions. While 3 and 4 show similar behavior under the reaction conditions, introduction of a double bond on the side of the carbinyl carbon opposite to the phenyl ring, as in 5, results in a decrease in enantioselectivity.

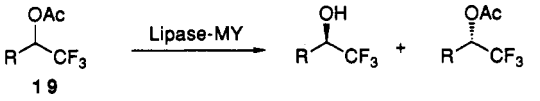
The effect of rigidity and cis/trans isomerism adjacent to the chiral center is also observed for a series of straight-chain allylic and propargylic 2-alkanols and their homo analogues (Table II). Excellent enantioselectivity ($E > 100$) is observed for propargylic (11) and (*E*)-allylic alcohols (12), although 11 has a significantly slower rate of reaction. In contrast, the (*Z*)-allylic alcohol (13) is a very poor substrate, reacting very slowly and exhibiting poor selectivity (E = 6).[9] In the case of alkynol (11) and its homo analogue (14), similar reactivities are observed, but the enantioselectivity of 14 is significantly less (E = 12–13) than that of 11.[10] A similar decrease in enantioselectivity is observed for the (*E*)-homoallylic alcohol (15) (E = 14–15), but surprisingly for the corresponding (*Z*)-homoallylic alcohol (16) faster reaction and slightly higher selectivity (E = 28–30) are observed.[11] Remote unsaturation has little effect on enzyme selectivity and the observed E value for 6-nonen-2-ol (17) is similar to that of the saturated alcohol.[12]

Scheme I



18

Scheme II



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a R = $Ph(CH_2)_2$ **e** R = $PhCH_2$
b R = (E)-PhCH=CH **f** R = PrC≡C
c R = (Z)-PhCH=CH **g** R = BuC≡C
d R = Ph **h** R = C_8H_{17}

(2) Neidelman, S. L. In *Biocatalysis*; Abramowicz, D. A., Ed.; Van Nostrand Reinhold: New York, 1990; p 12.

(3) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192.

(4) (a) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114. (b) Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695. (c) Sih, C. J.; Wu, S.-H. In *Topics in Stereochemistry*; Eliel, E. L., Wilen, S. H., Eds.; Wiley-Interscience: New York, 1989; Vol. 19, pp 63–125.

(5) For the PPL-catalyzed acylation of primary alcohols in the presence of secondary alcohols, see: Oehlschlager, A. C.; Ramaswamy, S.; Morgan, B. *Tetrahedron Lett.* **1990**, *31*, 165.

(6) For the PPL-catalyzed kinetic resolution of straight-chain and branched 2-alkanols, see: (a) Stokes, T. M.; Oehlschlager, A. C. *Tetrahedron Lett.* **1987**, *28*, 2091. (b) Morgan, B.; Oehlschlager, A. C.; Stokes, T. M. *Tetrahedron* **1991**, *47*, 1611.

(7) The enantiomeric excess of unreacted starting materials and the products (after reductive deacylation or hydrolysis) was determined by derivatization with acetyl (*S*)-lactyl chloride, followed by GLC analysis. See ref 36.

(8) The enantiomeric ratio (E) is a measure of the enzyme discrimination between two competing enantiomers and is the ratio of the rate constants for the fast and slow enantiomers. The enantiomeric ratio (E values) was calculated from

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

where $c = ee_s/(ee_s + ee_p)$. The listed E values are averages calculated from both ee_s and ee_p. See: (a) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294. (b) Chen, C.-S.; Wu, S.-H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812.

(9) Kinetic resolution of acyclic (*Z*)-allylic alcohols using the Sharpless epoxidation also shows poor selectivity, $E \approx 20$ for (*Z*)-3-penten-2-ol. For cyclohexenylethanol (2) the selectivities of the PPL-catalyzed transesterification (E = 70–80) and the Sharpless epoxidation (E = 60–80) are similar. See: Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, T.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237. Optically enriched (*Z*)-allylic alcohols may be accessed via Lindlar reduction of the corresponding 2-alkynols, which are efficiently resolved by PPL.

(10) A similar lack of selectivity was observed for the transesterification of 4-heptyn-2-ol under similar conditions: ee_s = 0.297, ee_p = 0.861, calcd conversion = 0.256, and enantiomeric ratio (E) = 18.

(11) The Sharpless epoxidation of homoallylic alcohols proceeds in modest yields with low ee (<60%). See: Rossiter, B. E.; Sharpless, K. B. *J. Org. Chem.* **1984**, *49*, 3707.

(12) Remote unsaturation has been previously reported to have little effect on enantioselectivity, e.g., ethyl 9-hydroxy-2-decenoate. See ref 6b.

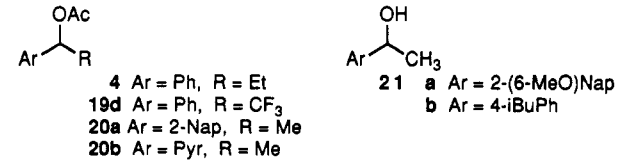
Our observations on the effect of a proximate π system are similar to those observed by Guanti et al. for the PPL-catalyzed monohydrolysis of 2-substituted 1,3-diacetoxypropanes (18) (Scheme I).[13] As in our case, these authors observed that alkynyl and (*E*)-akenyl groups adjacent to the chiral center led to improvement in both the yield and enantioselectivity of the PPL-catalyzed monohydrolysis, compared to the saturated analogues. (*Z*)-Alkenyl substituents resulted in both a decreased and reversed enantioselectivity. In the present cases it is always the *R* enantiomer that reacts preferentially.

Similar observations have been made by Schreier et al. for the esterification of unsaturated and aromatic alcohols using a crude porcine pancreatic carboxylesterase (Sigma) in hexane at 70 °C.[14] These investigators found similar differences in enantioselectivity and reactivity between (*E*)- and (*Z*)-allylic alcohols and between (*E*)-4-phenyl-3-buten-2-ol (9) and 4-phenyl-2-butanol (7). Their values of ee_p and ee_s yield enantiomeric ratios (*E*) somewhat less than we observed for 3-penten-2-ol (25) (vide infra), 1-phenyl-1-propanol (4), and 1-phenyl-2-propen-1-ol (5). High enantioselectivities have also been observed for a number of alkynols and 1-phenyl-2-propen-1-ol (5) using lipase *Pseudomonas* AK.[15]

In an analagous study, Kitazume et al.[16] examined the hydrolysis of a series of propargylic and phenyl-substituted trifluoromethyl alkanol acetates, using lipase-MY (*Candida cylindracea*) (Scheme II). In this work it was the more flexible 4-phenyl-1,1,1-trifluorobutan-2-ol (**19a**) that was the best substrate (*E* = 230), while introduction of unsaturation into the chain led to a decrease in selectivity for both the corresponding (*E*)-**19b** and (*Z*)-**19c** isomers (*E* = 48 and 30, respectively). Furthermore, selectivity increased as the separation of the phenyl group and the chiral center increased, e.g., **19d** (*E* = 5) and **19e** (*E* = 80). Trifluoromethyl propargylic acetates (**19f** and **19g**) and to a lesser extent the saturated acetate (**19h**) were poor substrates, while in present study the reverse generally holds. The complimentary relationship of the two systems is further emphasized by the observation that lipase-MY preferentially hydrolyzes the (*R*)-trifluoroacetates, while PPL esterifies the corresponding non-fluorinated (*R*)-alcohol.[17]

Klibanov et al. have also observed high selectivity in the transesterification of α-phenethyl alcohol (3) using PPL in ether (*E* = 90–120)[18] but only moderate selectivity (*E* = 36) when using *Candida cylindracea* absorbed on Chromosorb with tributyrin as acylating agent and solvent.[19] Somewhat better selectivities were reported by Sonnett[20] using a lipase from *Mucor meihi* for 1-cyclohexylethanol (1) and the propargylic alcohol dodec-3-yn-2-ol (*E* > 50 in both cases). Bianchi et al. have observed excellent enantioselectivity for the acylation of both α-phenethyl alcohol (3) and 1-phenyl-2-propanol (6) (*E* > 100 in both cases) with propionic anhydride using lipase amano P from *Pseudomonas fluorescens* adsorbed on Celite,[21] although they report that adsorbed PPL showed

Scheme III



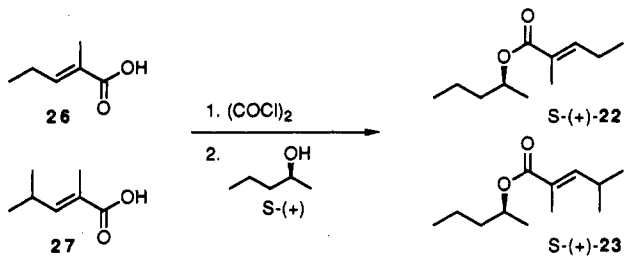
poor activity toward **3**.[22] Schneider et al.[23] examined the hydrolysis of a series of aromatic acetates using a lipase from *Pseudomonas* sp. (amano lipase SAM-2). Excellent selectivities (*E* > 1000) were reported for most substrates, e.g., α-phenethyl alcohol (3), 1-phenyl-1-propanol (4), and 1-naphthylalcohol (**20a**) (Scheme III). Somewhat lower but still outstanding selectivities (*E* > 100) were obtained for α-phenyltrifluoroethanol (**19d**), 1-pyridylethanol (**20b**), and 1-phenyl-2-propanol (6). Wong et al. have reported the enantiomeric ratios for the transesterification of a naphthylethanol (**21a**) and 1-(4-isobutylphenyl)ethanol (**21b**) (*E* = 78 and 73, respectively) using LPL-amano (*Pseudomonas* sp.) immobilized on XAD-8, with vinyl acetate as acyl donor.[24]

There have been a number of attempts to devise active-site models to explain and predict the enantioselectivity of enzymes. These models range in sophistication from simple two-site binding models involving a larger and a smaller binding pocket,[25] to multiple binding sites,[26] and three dimensional cubic space models.[27] Presuming that it is a single (iso)enzyme in the crude PPL extract that is responsible for activity,[28] the observed enantioselectivity of PPL cannot be easily explained by a simple two-site binding model based solely on the size of the substituents attached to the carbinyl carbon.

2. Synthesis of Chiral Dominicalure. The aggregation pheromone of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae), has been identified as a mixture of (*S*)-(+)-2′-pentyl (*E*)-2-methyl-2-pentenoate (**22**) and (*S*)-(+)-2′-pentyl (*E*)-2,4-dimethyl-2-pentenoate (**23**).[29] In the original paper enantiomers of both pheromone components were prepared in eight steps from glutamic acid, while a recent synthesis of both components has been carried out in 30% overall yield from crotonaldehyde, using a Sharpless kinetic resolution to introduce the *S* chiral center in 90% enantiomeric excess.[30]

(13) Guanti, G.; Banfi, L.; Narisano, E. *Tetrahedron Asymm.* **1990**, *1*, 721.
(14) Lutz, D.; Guldner, A.; Thums, R.; Schreier, P. *Tetrahedron Asymm.* **1990**, *1*, 783.
(15) Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1990**, *112*, 7434.
(16) Kitazume, T. *J. Org. Chem.* **1987**, *52*, 3211.
(17) The product from the lipase-MY hydrolysis is formally designated *R* because of the priority of the trifluoromethyl group, but is analogous to (*S*)-2-alkanol.
(18) Kirchener, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072.
(19) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687.
(20) Sonnet, P. E. *J. Org. Chem.* **1987**, *52*, 3477.
(21) Bianchi, D.; Cesti, P.; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531. The acylation of 2-octanol and 2-dodecanol under the same conditions resulted in poor enantioselectivity. We have also experienced poor selectivity in the acylation of 2-octanol, 2-dodecanol, and ethyl 9-hydroxydecenoate using PPL and butyric anhydride.
(22) Their observed poor activity may be due to a low enzyme/substrate ratio. These workers used 80 mg of PPL per g of substrate, whereas the present work employs 1 g of PPL per 200 mg of substrate, a 60-fold difference.
(23) Schneider, M. *J. Chem. Soc., Chem. Commun.* **1988**, 598.
(24) Hsu, S.-H.; Wu, S.-S.; Wang, Y.-F.; Wong, C.-H. *Tetrahedron Lett.* **1990**, *31*, 6403.
(25) (a) For subtilisin, see: Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1991**, *113*, 3166. (b) For cholesterol esterase, lipase *Pseudomonas cepacia* (amano lipase P, PS, or P-30), and lipase *Candida rugosa* (formerly *C. cylindracea*), see: Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656. (c) For *Pseudomonas fluorescens* lipase (now *Pseudomonas cepacia*), see: Xie, Z.-F.; Suemune, H.; Sakai, K. *Tetrahedron Asymm.* **1990**, *1*, 395.
(26) For Lipase A6 (*Aspergillus* sp.), see: Itoh, T.; Kuroda, K.; Tomosada, M.; Takasi, Y. *J. Org. Chem.* **1991**, *56*, 797.
(27) (a) For horse liver alcohol dehydrogenase, see: Jones, J. B.; Jakovac, I. J. *Can. J. Chem.* **1982**, *60*, 19. (b) For pig liver esterase, see: Toone, E. J.; Worth, M.; Jones, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 4948.
(28) For a comparison of enantioselectivities using crude and fractionated PPL, see: (a) Ramos Tombo, G. M.; Schar, H.-P.; Fernandez i Busquets, X.; Ghisalba, O. *Tetrahedron Lett.* **1986**, *27*, 5707. (b) Hemmerle, H.; Gais, H.-J. *Tetrahedron Lett.* **1987**, *28*, 3471.
(29) Williams, H. J.; Silverstein, R. M.; Burkholder, W. E.; Khorramshah, A. *J. Chem. Ecol.* **1981**, *7*, 759.

Scheme IV



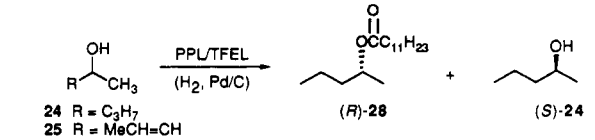
We have previously shown that the resolution of 2-pentanol (**24**) proceeds with moderate selectivity.[31] Thus, stirring 2-pentanol (13.0 g, 0.15 mol), trifluoroethyl laurate (50.1 g, 0.18 mol), and PPL (13 g) in ether for 45.5 h yielded unreacted starting material (ee_s = 0.589) and the laurate ester (ee_p = 0.923), corresponding to a calculated conversion of 39% and an enantiomeric ratio of 45. Subjecting the unreacted starting material (6.4 g, 73.1 mmol) to a second resolution yielded (*S*)-(+)-pentanol (ee_s = 0.959).

The enhanced enantioselectivity of PPL toward (*E*)-allylic 2-alkanols versus the saturated alcohol was exploited for the preparation of both enantiomers of 2-pentanol in high optical purity. Stirring a mixture of (*E*)-3-penten-2-ol (**25**), trifluoroethyl laurate, and PPL for 45.5 h, followed by hydrogenation[32] and reductive deacylation, yielded (*S*)-(+)-2-pentanol (ee_s = 0.747) and (*R*)-(−)-2-pentanol (ee_p = 0.980). While this represents a calculated conversion of 43% and an enantiomeric ratio (*E*) > 100, the optical purity of the desired (*S*)-(+)-enantiomer was too low to be synthetically useful. Both enantiomers of **25** were obtained in high optical purity by allowing the reaction to proceed to higher conversion (~50%). Under these conditions the enantiomeric excesses of the unreacted (*S*)-(+)-isomer and the product (*R*)-(−)-isomer were 0.942 and 0.949, respectively.

The required carboxyl-containing portion of **22**, (*E*)-2-methyl-2-pentenoic acid (**26**), was prepared by the Wittig reaction of propionaldehyde and (carbethoxyethylidene)-triphenylphosphorane[33] in refluxing CH_2Cl_2, followed by hydrolysis in aqueous NaOH/iPrOH.[34] The required carboxyl-containing portion of **23**, (*E*)-2,4-dimethyl-2-pentenoic acid (**27**), was prepared similarly from isobutyraldehyde (Scheme IV). Treatment of the neat acid (**26** or **27**) with oxalyl chloride, followed by reaction with neat (*S*)-(+)- or (*R*)-(−)-pentanol, furnished the enantiomers of both pheromone components in modest yield.[30,35]

3. Variation of Enantiomeric Ratio with the Optical Purity of the Chiral Derivatizing Agent. Caution must be exercised when employing acetyl (*S*)-lactyl chloride (ASLA-Cl) for the determination of the enantiomeric excess of alcohols via GC analysis of the corresponding diastereomeric mixtures. To ensure accurate results, the formation of the diastereomers (i) must proceed to completion to avoid possible kinetic enrichment of one diastereomer[36] and (ii) the chiral derivatizing reagent must be 100% enantiomerically pure.[37,38] ASLA-Cl should be prepared using the most optically pure form of L-(+)-lactic acid available.[39] A sample of ASLA-Cl which was prepared from less pure lactic acid[40] gave consistently lower

Table III. Variation in Observed Enantiomeric Excess as a Function of the Optical Purity of the Chiral Derivatizing Reagent



run	(*S*)-24 ee_s	(*R*)-28 ee_p	convrsn	*E*
1[a]	0.747[c]	0.980[c]	0.433	216
	0.731[d]	0.956[d]	0.433	99
2[b]	0.909[a]	0.959[a]	n/d	n/d
	0.886[d]	0.936[d]	n/d	n/d

[a] Resolution of (*E*)-(±)-3-penten-2-ol (**25**) followed by hydrogenation. [b] Resolution of optically enriched 2-pentanol (**24**) (ee = 0.588). [c] Enantiomer excess determined using ASLA-Cl prepared from Sigma lactic acid. [d] Enantiomeric excess determined using ASLA-Cl prepared from Aldrich lactic acid.

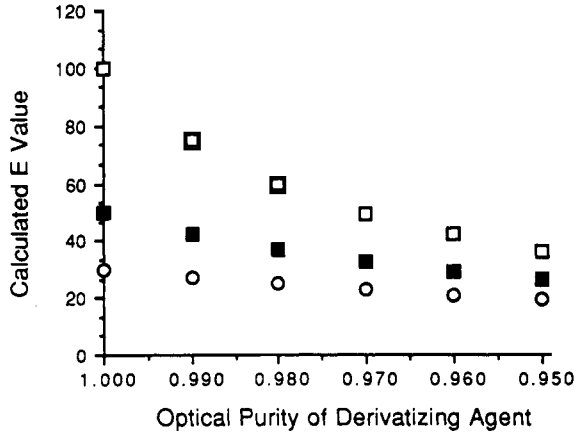


Figure 1. Variation of enantiomeric ratio (*E*) with optical purity of the chiral derivatizing agent. (□) E_{actual} = 100, ee_p = 0.970, ee_s = 0.420, *c* = 0.302. (■) E_{actual} = 49, ee_p = 0.943, ee_s = 0.385, *c* = 0.290. (○) E_{actual} = 30, ee_p = 0.906, ee_s = 0.401, *c* = 0.307. (E_{actual}, ee_p, and ee_s are the values when the optical purity of the chiral derivatizing agent is 100%).

(30) Liu, L.-Y.; Lin, G.-Q. *J. Chem. Ecol.* **1990**, *16*, 1921.

(31) On a 1-mmol scale at a calculated conversion of 31%, the acylated product was 94.3% ee, while the unreacted starting material was 42.8% ee. This represents an enantiomeric ratio (*E*) of 52. See ref 6b.

(32) Unlike the derivatives of 2-pentanol (**24**), the ASLA derivatives of (*E*)-3-penten-2-ol (**25**) did not resolve on GC. Thus reaction aliquots were hydrogenated prior to GC analysis to determine the course of the reaction.

(33) Isler, V. O.; Gutmann, H.; Moutavon, M.; Ruegg, R.; Ryser, G.; Zeller, P. *Helv. Chim. Acta* **1957**, *40*, 1242.

(34) In our hands **25** was obtained as a 16:1 *E*:*Z* mixture as shown by GC analysis. Poorer *E*:*Z* ratios were obtained using ethyl (diethylphosphono)propionate with NaH (5:1 *E*:*Z*), aqueous K_2CO_3 under phase-transfer conditions (5:1 *E*:*Z*), or BuLi (1:1 *E*:*Z*).

(35) The modest yields are presumably due to the volatility of the product esters and of the intermediate acid chlorides. Failure to completely remove excess oxalyl chloride results in contamination with the corresponding dipentyl oxalate.

(36) Slessor et al. report only minimal kinetic resolution for racemic alcohols from incomplete derivatization with ASLA-Cl. Slessor, K. N.; King, G. G. S.; Miller, D. R.; Winston, M. L.; Cutforth, T. L. *J. Chem. Ecol.* **1985**, *11*, 1659.

(37) For a discussion of the enantiomeric purity of commercial (+)- or (−)-2-methoxy-2-phenyl-3,3,3-trifluoropropionic acid (Mosher's acid), see: König, W. A.; Nippe, K.-S.; Mishnick, P. *Tetrahedron Lett.* **1990**, *47*, 6867.

(38) If the enantiomeric purity is less than 100% but the value is known accurately, then it is possible to correct measured ee's and calculate an actual ee. This might be an attractive alternative since it is generally easier to determine the enantiomeric purity of a relatively impure derivatizing agent rather than a highly pure one where the concentration of the minor optical isomer may be approaching the limits of detection. The problem with this approach is that if a number of determinations of ee are made, the standard deviations of the corrected *E* values are disproportionately larger than for the uncorrected *E* values, and this disparity increases as the optical purity of the reagent decreases. Furthermore, because of the logarithmic nature of the equations defining *E*, a determination that is the average of a group of uncorrected determinations is not necessarily the average when corrected.

(39) L-(+)-Lactic acid from Sigma Chemical Company (L-1750) is recommended.

(40) L-Lactic acid from Aldrich Chemical Company (cat. no. 19,925-7, lot no. 04815AP).

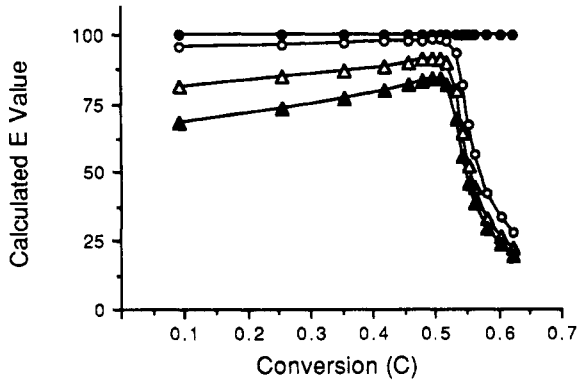


Figure 2. Effect of optical purity of the chiral derivatizing agent on the enantiomeric ratio (E = 100) as a function of conversion (C). Optical purity of derivatizing agent: (●) 1.000; (○) 0.995; (△) 0.990; (▲) 0.980.

ee values, as shown in Table III for the ee determination of 2-pentanol (**24**) and (*E*)-3-penten-2-ol (**25**).

The effect of chiral dilution on the value of the enantiomeric ratio (E) is perhaps not readily apparent from the form of the equation used to calculate this parameter.[8a] Optical impurity of the derivatizing agent will lower the observed values for both ee_s and ee_p but will leave the value of the calculated conversion (c) unaffected. From a plot of the optical purity of the chiral derivatizing agent vs calculated E values, it is seen that chiral impurity of the reagent has a profound effect upon the calculated E value when E is large (Figure 1). For example, a decrease in value from 100 to 61 is the consequence of using a derivitizing agent which is 98% optically pure. Under the same circumstances an E value of 30 will decrease only to 23. Using the data in Table III (run 2), the calculated value for 3-penten-2-ol (**25**) would decrease from 220 to 100 using the less pure ASLA-Cl.[41]

Normally the enantiomeric ratio (E) is independent of conversion as well as substrate and enzyme concentrations, providing the reaction is irreversible and there is only one enzyme species available to the competing enantiomers.[8,42] However, as shown in Figure 2, when the chiral derivatizing agent used to determine ee_s is optically impure, the value of E is not constant with respect to conversion.[43] In this case E increases slowly with increasing conversion, reaching a maximum and then dropping off sharply. The position of the maximum with respect to conversion is independent of the optical purity of the chiral derivatizing agent but dependent on the actual enantiomeric ratio. As the actual enantiomeric ratio increases, the maximum is displaced further along the conversion axis.

Experimental Section

General Methods. Porcine pancreatic lipase (Type II) was obtained from Sigma Chemical Company and was used as received. The listed activity was 16 units per mg of solid using olive oil at pH 7.7 and an incubation time of 30 min. 1-Cyclohexylethanol (**1**), α-phenethyl alcohol (**3**), 1-phenyl-2-propanol (**6**), and 2-pentanol (**24**) were commercially available and used as received. 4-Phenylbutan-2-ol (**7**), (*E*)-4-phenylbut-3-en-2-ol (**9**), and 1-cyclohexenylethanol (**2**) were prepared by reduction of the corresponding ketone. 4-Phenylbut-3-yn-2-ol (**8**) was prepared by the reaction of lithium phenylacetylide with acetaldehyde. Subsequent reduction with P-2 nickel catalyst furnished (*Z*)-4-phenylbut-3-en-2-ol (**10**). Undec-3-yn-2-ol (**11**) and dodec-4-yn-2-ol (**14**) were prepared by condensation of the 1-lithio-1-nonyne with acetaldehyde and propylene oxide, respectively.[44] Reduction with $LiAlH_4$ furnished the corresponding (*E*)-allylic alcohols (**12** and **15**), while P-2 nickel reduction furnished the (*Z*)-allylic alcohols (**13** and **16**). (*E*)-3-Penten-2-ol (**25**) was prepared by addition of MeLi to crotonaldehyde. (*E*)-6-Nonen-2-ol (**17**) was generously provided by Desiree Vanderwel. 2,2,2-Trifluoroethyl butyrate (bp 109–111 °C) was prepared from butyryl chloride and trifluoroethanol, or from refluxing butyric anhydride and trifluoroethanol in the presence of *p*-TsOH. Reagent-grade anhydrous ether was used as received for resolution experiments.

Gas chromatography was performed using a J/W fused silcia DB-1 capillary glass column (15 m × 0.25 mm). Reactions were monitored using the following temperature program: initial temperature, 60 °C for 1 min; rate, 20 deg/min; final temperature, 250 °C for 2 min. The enantiomeric excesses of optically enriched alcohols were determined by derivatization with acetyl (*S*)-lactyl chloride,[36] and GC analysis of the resultant diastereoisomeric mixture used the following run program: initial temperature, 70 °C; rate 5 deg/min; final temperature 250 °C for 5 min.

Lipase-Mediated Resolution of Alcohols. General Procedure. A mixture of the alcohol (1–2 mmol), trifluoroethyl butyrate (3 equiv), and PPL (Sigma Type II) (1.0 g) in Et_2O (5 mL) was stirred at room temperature. Reactions were stopped (14–167 h) by filtration through a bed of Celite to remove the enzyme and the solvent was removed in vacuo. The unreacted alcohol was separated from the butyrate by column chromatography, eluting with 5–30% EtOAc/hexanes and combining the relevant fractions. Hydrolysis (6 M NaOH) or reduction of the butyrate furnished the product alcohol. In most cases enantiomeric excesses of the unreacted and product alcohols were determined by derivatization with acetyl (*S*)-lactyl chloride followed by GC analysis of the diastereomers. The order of elution of the (*S*)-lactate esters was assumed to be constant with the (*S*)-lactate esters of the (*R*)-enantiomers eluting prior to the esters of the (*S*)-enantiomers. This elution order had previously been established for optically active sulcatol[6a] and 9-HDA.[45] The (*S*)-lactate esters of α-phenethyl alcohol (**3**) and (*Z*)- and (*E*)-4-phenylbut-3-en-2-ol (**9** and **10**) did not resolve on a 15-m DB-1 GC column. The phenylbutenols were hydrogenated to 4-phenylbutan-2-ol (**7**), the (*S*)-lactate ester of which did resolve, and the enantiomeric excess of α-phenethyl alcohol was determined using the Mosher derivative.[46]

(*R*)-(−)-2-Pentanol (24). 2-Pentanol (12.9 g, 0.15 mol) and TFEL (50.1 g, 0.18 mol) were dissolved in freshly distilled Et_2O (240 mL), PPL (13.0 g) was added, and the mixture was stirred at room temperature. After 45.5 h the reaction mixture was filtered through a Celite pad to remove enzyme and the filtrate distilled to remove most of the Et_2O solvent (bath temperature 60–80 °C). The residue was distilled in vacuo (0.01 mmHg) (bath temperature 60–70 °C), with collection of the distillate in a receiver cooled in a dry ice/acetone bath. The distillate was redistilled at atmospheric pressure, collecting a fraction boiling at 100–120 °C (6.5 g, 49.6%; ee_s = 0.589).

The pot residue contained pentyl laurate (**28**) and TFEL. It was dissolved in freshly distilled Et_2O (200 mL) and cooled in an ice bath, and $LiAlH_4$ (2 g, 53 mmol) was added in portions. The reaction mixture was heated to reflux for 2 h, more $LiAlH_4$ (1 g, 27 mmol) was added, and the reaction mixture was heated for a further 1 h. The reaction was quenched by the careful addition of wet Na_2SO_4 and filtered through a Celite pad which was washed with Et_2O (100 mL), and the filtrate was distilled at atmospheric pressure to remove Et_2O. The residue was distilled in vacuo (0.5 mmHg) (bath temperature 60–160 °C), with collection of the distillate in a receiver cooled in a dry ice/acetone bath. The distillate was redistilled at atmospheric pressure, collecting a fraction boiling at 104–118 °C (2.63 g, 20%; ee_p =

(41) A comparison of the accuracy of three methods of determining E values has recently been published. See: van Tol, J. B.; Jongejan, J. A.; Geerlof, A.; Duine, J. A. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 255.

(42) Stokes, T. M.; Wilson, I. B. *Biochemistry* **1972**, *11*, 1061.

(43) The quantitative expression used was the same as derived previously,[8a] except that $ee_{p(apparent)}$ was substituted for ee_p, where $ee_{p(apparent)} = ee_{p(actual)} \times ee$ (chiral derivatizing agent). The $ee_{p(actual)}$ was calculated using the parametric representations described in ref 8a and was used to calculate points for graphing.

(44) Yamaguchi, M.; Hirao, I. *Tetrahdron Lett.* **1983**, *24*, 391.

(45) Kandil, A. A.; Slessor, K. N. *Can. J. Chem.* **1983**, *61*, 1166.

(46) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

0.909[47]): $[\alpha]^{24}_D$ = −15.76° (c = 4.815, Et_2O) (lit.[29] $[\alpha]^{25}_D$ = −13.2° (c = 0.72, EtOH)).

(S)-(+)-2-Pentanol (24). 2-Pentanol (6.45 g, 73.1 mmol; ee_s = 0.589) and TFEL (29.4 g, 104 mmol) were dissolved in freshly distilled Et_2O (100 mL), PPL (10.0 g) was added, and the mixture was stirred at room temperature for 47.6 h.

The reaction was filtered through Celite and the Et_2O removed by distillation. The residue was distilled in vacuo (0.5 mmHg) (bath temperature 100–130 °C), with collection of the distillate in a receiver cooled in dry ice/actone. The crude distillate was redistilled at atmospheric pressure, collecting a fraction boiling at 107–123 °C (4.50 g, 69%; ee_s = 0.959): $[\alpha]^{25}_D$ = +14.82° (c = 3.535, Et_2O) (lit.[29] $[\alpha]^{25}_D$ = +13.1 (c = 0.55, EtOH)).

(S)-(+)-2-Pentanol (24). Resolution of (E)-(±)-3-Penten-2-ol (25) (High Conversion). A mixture of (E)-3-penten-2-ol (9.33 g, 0.11 mol), TFEL (41.44 g, 0.15 mol), and PPL (20 g) was stirred in freshly distilled Et_2O (250 mL). After 78 h the reaction was filtered through a Celite pad which was then washed with Et_2O (4 × 25 mL). The filtrate was concentrated to ~100 mL and hydrogenated in a Parr apparatus for 20.75 h (40 psi H_2; 5% Pd/C). After being filtered through a Celite pad, the solvent was removed by distillation at atmospheric pressure. The residue was heated under vacuum (0.05 mmHg; bath temperature 90 °C), with collection of the distillate in a receiver cooled in a dry ice/acetone bath. The crude (S)-(+)-2-pentanol was redistilled at atmospheric pressure with collection of the fraction boiling at 112–118 °C (2.27 g, 24.3%; ee_s = 0.942) (The product contained ~10% of 2-pentanone, presumably formed due to the presence of O_2 during the hydrogenation.): $[\alpha]^{25}_D$ = +17.12° (c = 4.135, Et_2O).

(R)-(−)-2-Pentanol (24). Resolution of (E)-(±)-3-Penten-2-ol (25) (Low Conversion). A mixture of (E)-3-penten-2-ol (8.07 g, 93.7 mmol), TFEL (26.14 g, 92.6 mmol), and PPL (13 g) was stirred in freshly distilled Et_2O (250 mL) for 45.5 h. Hydrogenation and removal of 2-pentanol was carried out as described for (S)-(+)-2-pentanol. The stillpot residue was dissolved in dry Et_2O (100 mL) and cooled in an ice bath, and $LiAlH_4$ (1.5 g, 40 mmol) was added in portions. After heating to reflux for 2 h, additional $LiAlH_4$ (1.0 g, 26 mmol) was added and the solution was heated for a further 1.5 h. The reaction mixture was cooled in an ice bath and the reaction was quenched by the addition of damp Na_2SO_4. The white granular precipitate was removed and the solvent distilled at atmospheric pressure. The residue was distilled under vacuum (0.01 mmHg; bath temperature 70–80 °C), with collection in a cooled receiver (dry ice/acetone). The distillate was dried ($MgSO_4$), filtered, and redistilled, with collection of the fraction distilling at 110–120 °C (2.72 g, 33.7%; ee_p = 0.980): $[\alpha]^{25}_D$ = −19.12° (c = 2.950, Et_2O).

(S)-(+)-2′-Pentyl (E)-2-Methyl-2-pentenoate (22). Neat (E)-2-methyl-2-pentenoic acid (26) (1.04 g, 9.12 mmol) was cooled in an ice bath and $(COCl)_2$ (1.0 mL, 11.5 mmol) added. The mixture was stirred at room temperature for 1.25 h and then excess $(COCl)_2$ removed in vacuo. The residue was cooled in an ice bath, neat (S)-(+)-2-pentanol (24) (0.75 mL, 6.93 mmol; ee = 0.959) was added in one portion, and the reaction was stirred at room temperature for 21.5 h. The reaction was partitioned between water (25 mL) and Et_2O (25 mL), the layers were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 25 mL) and saturated aqueous NaCl (25 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by SiO_2 chromatography (40 g), using 5% EtOAc/hexanes as the eluant to yield a colorless liquid (0.61 g, 47.6%): $[\alpha]^{25.5}_D$ = +35.65 (c = 12.21, Et_2O) (lit.[29] $[\alpha]^{25}_D$ = +32.1° (c = 0.156, Et_2O)).

(R)-(−)-2′-Pentyl (E)-2-Methyl-2-pentenoate (22). This compound was prepared as described above from 26 (1.03 g, 9.07 mmol) and (R)-(−)-2-pentanol (0.75 mL, 6.93 mmol; ee = 0.980) (0.81 g, 63.6%): $[\alpha]^{24.5}_D$ = −35.98 (c = 8.795, Et_2O).

(S)-(+)-2′-Pentyl (E)-2,4-Dimethyl-2-pentenoate (23). This compound was prepared as described above from 27[33] (1.16 g, 9.03 mmol) and (S)-(+)-2-pentanol (0.75 mL, 6.93 mmol; ee = 0.959) (0.75 g, 54.7%): $[\alpha]^{22.5}_D$ = +33.59° (c = 6.365, Et_2O) (lit.[29] $[\alpha]^{25}_D$ = +32.3° (c = 0.777, Et_2O)).

(R)-(−)-2′-Pentyl (E)-2,4-Dimethyl-2-pentenoate (23). This compound was prepared as described above from 27 (1.07 g, 8.32 mmol) and (R)-(−)-2-pentanol (0.75 mL, 6.93 mmol; ee = 0.949) (0.57 g, 41.6%): $[\alpha]^{22}_D$ = −32.42° (c = 6.150, Et_2O).

Registry No. (±)-1, 104713-60-2; (R)-1, 140390-59-6; (S)-1, 3113-98-2; (±)-2, 79646-40-5; (R)-2, 140390-60-9; (S)-2, 76888-38-5; (±)-3, 98-85-1; (R)-3, 89378-61-0; (S)-3, 1445-91-6; (±)-4, 93-54-9; (E)-4, 140390-61-0; (S)-4, 613-87-6; (±)-5, 42273-76-7; (R)-5, 140390-62-1; (S)-5, 104713-12-4; (±)-6, 698-87-3; (R)-6, 118295-00-4; (S)-6, 1517-68-6; (±)-7, 2344-70-9; (R)-7, 140390-63-2; (S)-7, 22148-86-3; (±)-8, 104597-03-7; (R)-8, 140390-64-3; (S)-8, 81555-86-4; (±)-9, 84519-62-0; (R)-9, 124219-14-3; (S)-9, 81176-43-4; (±)-10, 104974-60-9; (R)-10, 140390-65-4; (S)-10, 140461-14-9; (±)-11, 131101-36-5; (R)-11, 140390-66-5; (S)-11, 140461-15-0; (±)-12, 140461-12-7; (R)-12, 140390-67-6; (S)-12, 95586-07-5; (±)-13, 140461-13-8; (R)-13, 140390-68-7; (S)-13, 140461-16-1; (±)-14, 135469-87-3; (R)-14, 140390-69-8; (S)-14, 140461-17-2; (±)-15, 140390-56-3; (R)-15, 140390-70-1; (S)-15, 140461-18-3; (±)-16, 140390-57-4; (R)-16, 140390-71-2; (S)-16, 140461-19-4; (±)-17, 140390-58-5; (R)-17, 140390-72-3; (S)-17, 140461-20-7; (S)-(+)-22, 80510-15-2; (R)-(−)-22, 140461-21-8; (S)-(+)-23, 80510-16-3; (R)-(−)-23, 140461-22-9; (±)-24, 60102-79-6; (R)-(−)-24, 31087-44-2; (S)-(+)-24, 26184-62-3; 28, 140390-73-4; TFEL, 70253-78-0; lipase, 9001-62-1; (±)-2-pentanal, 6032-29-7.

(47) An aliquot of the final reaction mixture was analyzed. From the values of ee_s (0.589) and ee_p (0.923) the calculated conversion was 0.390, and the enantiomeric ratio (E) was 46, in agreement with the previously reported value (ref 6b). The lower ee of the isolated product probably reflects incomplete separation of unreacted starting material or chemical esterification during the isolation.