Resolution of Binaphthols and Spirobiindanols Using Cholesterol Esterase[1]

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Abstract: Cholesterol esterase (EC 3.1.1.13) catalyzes the hydrolysis of steroid, binaphthol, and spirobiindanol esters. The hydrolysis of binaphthol and spirobiindanol esters is enantiospecific and can be used to resolve these materials. Simple, synthetic-scale (200 g) procedures are detailed for the resolution of [1,1′-binaphthalene]-2,2′-diol (**1**) and 2,2′,3,3′-tetrahydro-3,3,3′,3′-tetramethyl-1,1′-spirobi[1*H*-indene]-6,6′-diol (**6**). Resolution of **1** involved hydrolysis of the dipentanoate ester catalyzed by crude, inexpensive enzyme (bovine pancreas acetone powder) and yielded each enantiomer in >60% of theoretical yield with ≥99% enantiomeric purity. Similar resolution of **6** by hydrolysis of the dihexanoate ester yielded each enantiomer in >50% of theoretical yield with >95% enantiomeric purity. These resolutions involve two enzymic reactions: hydrolysis of the diester to the monoester followed by hydrolysis of the monoester to the diol. A theoretical analysis of such two-step resolutions suggests that two-step resolutions can yield products with higher enantiomeric purity than can single-step resolutions.

Consider using pancreas as a catalyst. It is available, is inexpensive, and contains a variety of digestive enzymes. If one of these enzymes catalyzes a selective reaction and the other enzymes do not interfere, then pancreas could be a useful catalyst. This paper reports that pancreas can be used to resolve binaphthols and spirobiindanols.

The pancreatic enzyme responsible for these resolutions is cholesterol esterase (CE).[2] Purified CE has been used to resolve 2-bromo alcohols[3] and intermediates for the synthesis of chorismic and shikimic acids.[4] This work examines the substrate specificity of CE and shows that CE also resolves binaphthols and spirobiindanols. However, purified CE is expensive for synthetic use, so crude preparations—even ground-up pancreas—were examined as possible catalysts. The most convenient catalyst is pancreas acetone powder (defatted, ground-up pancreas) because it is commercially available, is inexpensive, and contains CE activity.

The interest in enantiomerically pure [1,1′-binaphthalene]-2,2′-diol (**1**) and its derivatives stems from their usefulness as chiral auxiliaries.[5] For example, chiral reducing agents for carbonyl compounds base on aluminum hydrides[6] or a ruthenium-H_2 species,[7] chiral Lewis acid catalysts for cyclization of unsaturated aldehydes[8] and for the hetero-Diels-Alder reaction,[9] and chiral crown ethers for complexation of amino acids[10] have all incorporated a binaphthol moiety.

The best current preparation of enantiomerically pure **1** involves fractional crystallization of the diastereomic cinchonine salts of **1** cyclic phosphate ester.[11] This method is laborious but is preferred to other methods because it can be carried out on a synthetic scale and yields material of high enantiomeric purity. The current standards of enantioselective organic synthesis require >99% ee for a chiral auxiliary.

Other methods for resolution of **1** include chromatography using a chiral stationary phase,[12] enantiospecific hydrolyses using microorganisms[13] or enzymes,[14] and fractional crystallizations of chiral sulfoxide,[15] copper-amphetamine,[16a] or tartrate amide[16b] complexes or of the 2-aminobutanol salt of **1** cyclic phosphate.[17] Enantioselective syntheses have also been reported.[18] Chromatography using a chiral stationary phase is useful on an analytical scale in the laboratory and possibly in industrial applications where the use of large preparatory columns can be optimized by engineering. However, chiral columns have limited usefulness on a laboratory synthetic scale (100–200 g). The copper-amphetamine method for resolution of **1** involves kilogram quantities of a controlled substance, amphetamine, which renders this method unsuitable for most laboratories. The disadvantages of the other methods are that they have not been demonstrated on a synthetic scale are difficult or expensive to carry out or that they yield material of insufficient enantiomeric purity.

The CE method described in this paper meets the requirements of simplicity, ability to run on a synthetic scale, and ≥99% ee product. Further, it is preferable to the cinchonine method because it is simpler to run and involves fewer manipulations.

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Spirobiindanols, like binaphthols, are chiral due to axial dissymmetry. Enantiomerically pure **6** has been prepared previously in five steps after resolution of the corresponding diacid by fractional crystallization of the dibrucine salt.[19] The substituted spirobiindanol **8** has been resolved by fractional crystallization of diastereomeric carbamate derivatives.[19] Resolution of these compounds using CE is substantially simpler and may lead to the use of these compounds as chiral auxiliaries. Because the hydroxyl groups are further apart in the spirobiindanols (~7.4 Å) than in binaphthols (~4.1 Å), the spirobiindanols cannot chelate a single metal center. Nevertheless, they may be useful auxiliaries in bimetallic systems and in building chiral cavities.

Results

Use of CE in Water–Ethyl Ether Emulsions. Purified pancreatic CE (bovine or porcine) catalyzed the hydrolysis of binaphthol, spirobiindanol, and steroid esters, Tables I and II. These water-insoluble substrates were hydrolyzed in emulsions of ethyl ether and phosphate buffer containing taurocholate, a bile salt which activates CE, vide infra, and aids formation of an emulsion. The initial rates of hydrolysis, as determined by pHstat or HPLC, were similar for both cholesterol and the unnatural substrates. Comparable rates were also observed with toluene or methyl isobutyl ketone as the organic phase. No hydrolysis (relative rate <0.01) was observed with methylene chloride.

Cholesterol esterase was less active toward cholesterol acetate emulsified in ethyl ether–water than toward cholesterol acetate emulsified in water with a surfactant: V_{max} = 0.5 vs 2.0 units/mg for a partly purified sample of CE. The apparent K_m was also higher in the water–ether emulsions: 200 mM for cholesterol acetate in the ether phase vs 0.5 mM for water-soluble phenyl acetates.[20] This lower activity and increased K_m are probably due to reduced availability of substrate which partitions into the ether. In spite of the lower activity, water–ether emulsions were used to survey the substrate specificity of CE because these conditions mimic conditions used for synthesis.

Required Purity of CE. Pancreatic CE, not an impurity, hydrolyzes binaphthol and spirobiindanol esters because the highest activity (2× partially purified CE) was observed with a purified sample of bovine CE. This sample was >90% pure by gel filtration and migrated as a single band on SDS–polyacrylamide gel electrophoresis with an apparent molecular weight of 59K, consistent with the reported molecular weight of 63K–67K.[21] Partially purified bovine CE showed additional proteins migrating with apparent molecular weights of 43K, 26K, and 15K. Cholesterol esterase is activated by taurocholate, a bile salt that induces association of monomers into more active dimers[22] and hexamers[23] and aids formation of an emulsion.[20] Omission of taurocholate in a hydrolysis of **6** dipentanoate reduced the rate of hydrolysis by a factor of 2, consistent with the notion that hydrolytic activity is due to CE. Competitive hydrolysis of a 1:1 mixture of cholesterol acetate and binaphthol acetate proceeded with the same overall rate as a single compound and yielded equal amounts of each alcohol, consistent with the notion that the same catalyst was responsible for both hydrolyses. Neither porcine liver esterase nor purified pancreatic lipase catalyzed the hydrolysis of **6** dibutanoate (<0.01 unit/mg). Crude porcine pancreatic lipase did show traces of CE activity (~0.002 unit/mg) probably due to impurities of CE.

Crude extracts of pancreas also catalyzed the hydrolysis of binaphthol and spirobiindanol esters. Both homogenized porcine pancreas and bovine pancreas acetone powder catalyzed hydrolysis of **1** diacetate with enantiospecificities identical with that of purified CE (>95% ee *S*). Pancreas acetone powder catalyzed hydrolysis of **6** diacetate, **6** dibutanoate, and **6** dihexanoate with identical enantiospecificities as purified CE (75% ee *R*, 70% ee *R*, and 95% ee *R* at low conversion). These crude preparations had lower specific activity than purified CE: ~2 units/g of dry weight for porcine pancreas and ~14 units/g of solid for pancreas acetone powder. Pancreas acetone powder is the least expensive source of CE; $50 worth (100 g) was enough to resolve 200 g of **1**, vide infra.

The stability of CE activity in bovine pancreas acetone powder was lower than that in purified CE. The crude material had a half-life of ~2 days (phosphate buffer, 0.1 M, pH 7, 25 °C, 5 mM taurocholate, 0.1% sodium azide) while the purified material showed <10% loss after 5 days under the same conditions. The lowered stability was presumably due to degradation by trypsin because addition of trypsin inhibitors (benzamidine, 2 mM, or soybean trypsin inhibitor, 0.25 mg/mL) to pancreas acetone powder increased the stability (~10% loss after 5 days).

Specificity of CE. Cholesterol esterase catalyzed the hydrolysis of straight-chain carboxylic acid esters of the binaphthol **1** and the octahydrobinaphthol **3**, with rates similar to that of cholesterol acetate. The preferred enantiomer in each case had the *S* configuration, on the basis of comparison with authentic samples. On the other hand, esters of the bromobinaphthols **2** and **4** were not substrates for CE.

The butanoate of the benzindanol **5** was hydrolyzed with moderate enantiospecificity for the *R* enantiomer, on the basis of optical rotation.

Shorter chain carboxylic acid esters of the spirobiindanol **6** were hydrolyzed faster, but with lower overall enantiospecificity, than were longer esters of **6**. The substituted spirobiindanols **7** and **8** were hydrolyzed more slowly, but with enantiospecificities similar to that of the parent. The absolute configurations of the preferred enantiomers of **6**, **7**, and **8** were *R* on the basis of optical rotation.

The diacetate of the spirobenzopyranol 9 was hydrolyzed with moderate enantiospecificity.

Acetate esters of several steroids were also substrates for CE, Table II. Cholestanol and cholesterol acetate both have a 3β configuration and were good substrates. But esters of 3α-cholesterol were not substrates.[2] The opposite behavior was observed in the coprostonol series: coprostan-3β-ol acetate was not a substrate[24] while coprostan-3α-ol acetate was a good substrate. In all cases, esters in an equatorial position were good substrates while those in an axial position were not.

Enantiomeric Ratio for Each Step. In order to compare the enantiospecificity of CE for different substrates, the enantiomeric ratio *E* was determined. This quantity defines the discrimination between two enantiomers by the enzyme. For a simple, single-step reaction this can be calculated from the experimentally determined values of percent enantiomeric excess at a known extent of conversion with eq 1 or 2.[25] Here ee(S) represents the enantiomeric excess of the recovered substrate fraction and ee(P) represents the enantiomeric excess of the product fraction.

$$E = \ln [(1 - c)(1 - \text{ee(S)})]/\ln [(1 - c)(1 + \text{ee(S)})] \quad (1)$$

$$E = \ln [1 - c(1 + \text{ee(P)})]/\ln [1 - c(1 - \text{ee(P)})] \quad (2)$$

The hydrolysis of diesters to diols is not a simple, single-step reaction but involves two sequential hydrolyses where each step can exhibit enantioselectivity, eq 3 and 4. In order to apply eq

$$\text{diester} \xrightarrow{E_1} \text{monoester} \quad (3)$$

$$\text{monoester} \xrightarrow{E_2} \text{diol} \quad (4)$$

1 and 2 to these reactions, each hydrolysis was treated separately.

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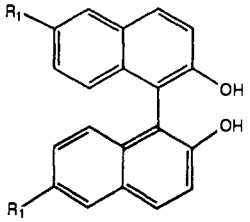
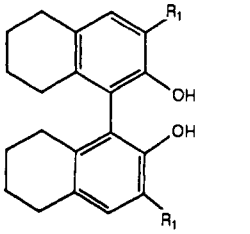
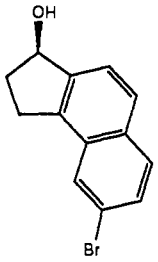
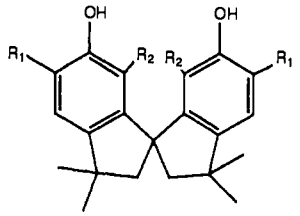
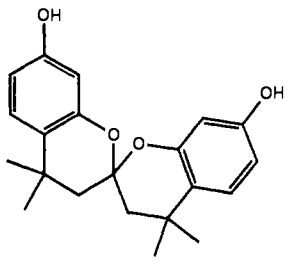
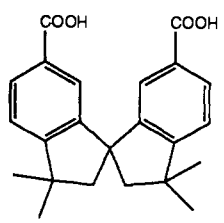
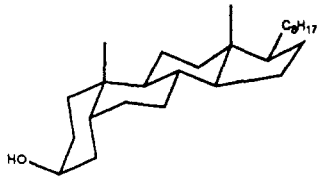
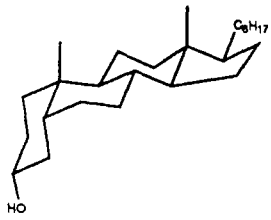
Table I. Substrate Specificity of Cholesterol Esterase

substrate			relative	% ee of		
structure	no.	ester	rate[a]	diol[b]	E_1[c]	E_2[c]
	**1**, R_1 = H	diacetate	1.1	>95 *S*		3.5 ± 0.1 *S*
		dipropanoate	1.1	>95 *S*	nd	5.2 ± 0.3 *S*
		dibutanoate	0.5	>95 *S*	nd	3.5 ± 0.4 *S*
		dipentanoate	1.1	>95 *S*	>400 *S*[d]	4.9 ± 0.2 *S*
		dihexanoate	0.5	>95 *S*	nd	nd
		diheptanoate	0.3	>95 *S*	nd	nd
		dioctanoate	0.4	>95 *S*	nd	nd
	2, R_1 = Br	diacetate	<0.03[e]			
		dibutanoate	<0.001			
	**3**, R_1 = H	diacetate	0.4[f]	nd	nd	nd
		dipentanoate	0.2	>90 *S*[g]	nd	nd
	4, R_1 = Br	dipentanoate	<0.004			
	**5**	butyrate	0.12[f]	39 *R*[h]	2.9 *R*	
	**6**, R_1 = R_2 = H	diacetate	1.0	61 *R*	nd	1.7 ± 0.2 *S*
		dipropanoate	0.8[i]	nd	nd	nd
		dibutanoate	0.6	61 *R*	nd	1.6 ± 0.1 *S*
		bis(2-methylpropanoate)	0.08	37 *R*	nd	nd
		dipentanoate	1.3	79 *R*	nd	1.1 ± 0.1 *S*
		dihexanoate	0.3	90 *R*	9.4 ± 2.2 *R*	1.2 ± 0.1 *S*
		diheptanoate	0.2	88 *R*	nd	nd
		dioctanoate	0.1	85 *R*	nd	nd
		dinonanoate	0.07	90 *R*	nd	nd
	7, R_1 = CH_5, R_2 = H	dihexanoate	0.1	>80[j]	nd	nd
	8, R_1 = CH_3, R_2 = Br	dibutanoate	0.007	91 *R*[k]	nd	nd
	**9**	diacetate	1.0	43[l]	2.6 ± 0.6[m]	nd
	**10**	diethyl	<0.001			

[a] Rate of hydrolysis (relative to cholesterol acetate) was measured with a pHstat, which measured the amount of base (0.1 N NaOH) required to maintain the pH at 7.00 for an emulsion of ester (1.0 mmol) dissolved in ethyl ether (10 mL) and aqueous buffer (10 mL of 10 mM phosphate, pH 7, containing 30 mg of sodium taurocholate monohydrate). The specific activity of cholesterol esterase from bovine pancreas (Genzyme lot. no. 3846) under these conditions was 0.14 units/mg with cholesterol acetate. nd = not determined. unit = μmol of ester hydrolyzed/min at 25 °C. [b] Enantiomeric purity of the product after ~15% hydrolysis. [c] E_1 = enantiomeric ratio for the first hydrolysis (diester to monoester); E_2 = enantiomeric ratio for the second hydrolysis (monoester to diol). [d] Based on a limit of >99% ee for the combined monoester and diol at 40% conversion. [e] Poor solubility limits the concentration of ester in the ether to 14 mM. The rate of hydrolysis of **1** diacetate is ~4 times slower at 14 mM than at 100 mM. The limit of 0.03 was calculated on the basis of this slower rate. [f] The concentration of the ester in the ether phase was 50 mM. [g] Determined by ^{1}H NMR using Eu((+)-hfc)$_3$. [h] Enantiomeric purity of alcohol was calculated on the basis of a measured value of 26% ee *S* for the ester recovered after 40% conversion. [i] Substrate was not completely dissolved in the ether. [j] The diastereomeric camphanate diesters are incompletely resolved on HPLC. The order of elution suggests that the preferred enantiomer is the same as that with the unsubstituted spirobiindane. [k] Enantiomeric purity was determined from the optical rotation of the diol isolated by TLC after 58% hydrolysis. [l] Enantiomeric purity of combined diol and monoester after 31% hydrolysis. [m] Average of the values determined from the enantiomeric purity of the diester fraction and the diol/monoester fraction.

Table II. Steroid Specificity of Cholesterol Esterase

structure	no.	relative rate[a]
	**11**	1.0
	**12**	1.5
	**13**	<0.0005[b]
	**14**	1.2[b]

[a] Hydrolysis of acetate ester determined as in Table I, footnote *a*. [b] The concentration of the ester in the ether phase was 55 mM.

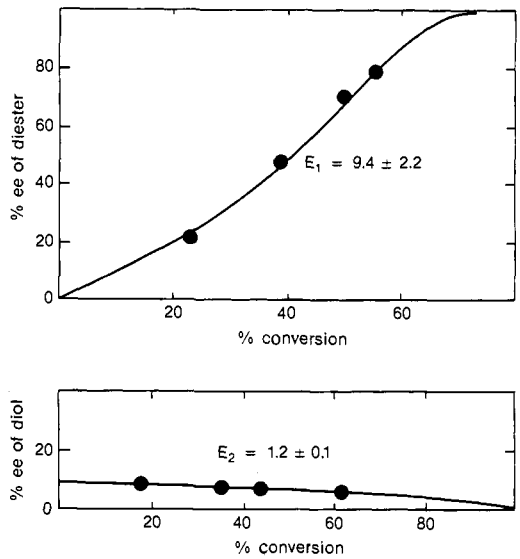
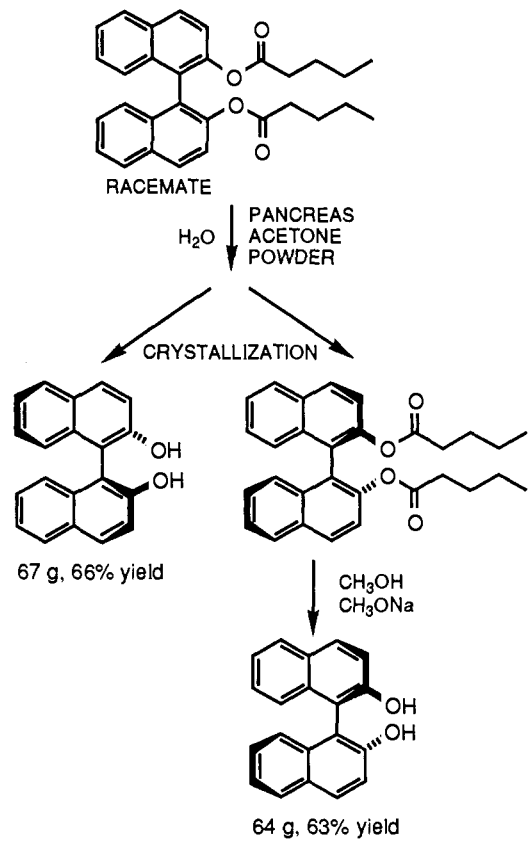


Figure 1. Determination of enantiomeric ratios, E_1 and E_2, for hydrolysis of **6** dihexanoate. Top panel shows the determination of E_1 by measuring the % ee of the unreacted **6** dihexanoate as a function of the percent of starting diester consumed. Hydrolysis of the *R* enantiomer was favored. The line shown was calculated with eq 1, where $E = 9.4$. Bottom panel shows the determination of E_2 by measuring the % ee of **6** during a hydrolysis of **6** monohexanoate as a function of the percent of starting monoester consumed. Hydrolysis of the *S* enantiomer was favored. The line shown was calculated with eq 2, where $E = 1.2$.

The disappearance of diester is influenced only by the first hydrolysis, eq 3. Therefore, the value of E_1 was determined by measuring the percent enantiomers excess of the recovered diester and applying eq 1, see Table I and Figure 1. Alternatively, the enantiomeric purity of the combined monoester and diol can be determined, and eq 2 can be used. This alternate approach was used to set a lower limit for E_1 during the hydrolysis of **1** dipentanoate. The second step, hydrolysis of the monoester to diol, eq 4, is a simple, single-step reaction when racemic monoester is used as the substrate. Either eq 1 or 2 was applied in a straightforward manner to determine E_2, Table I and Figure 1.

Scheme I. Resolution of Binaphthol Using Cholesterol Esterase



The enantiomeric ratios show that CE is specific for the *S* enantiomer in both steps of the hydrolysis of **1** diesters with the enantiospecificity of the first hydrolysis being considerably larger than that of the second. On the other hand, CE prefers the *R* enantiomer of **6** diesters in the first step but the *S* enantiomer in the second step. The enantiospecificity is higher in the first step; thus, the overall reaction yields an excess of the *R* enantiomeric.

Synthetic Use. Crude cholesterol esterase (bovine pancreas acetone powder) was used to separate the enantiomers of **1** on a 200-g scale as outlined in Scheme I. The dipentanoyl ester was chosen for the synthetic-scale resolution because it is a good substrate and differs sufficiently from binaphthol to simplify separation after hydrolysis. Hydrolysis slowed and stopped when approximately half of the **1** dipentanoate had been hydrolyzed. The binaphthol and diester were separated by crystallization, yielding 67 g (66% of theory) of (*S*)-**1** having an enantiomeric purity of >99.95% ee and after hydrolysis of the diesters 64 g, 63% of theory, of (*R*)-**1** having 98.8% ee.

High enantiomeric excess was expected for the *S* enantiomer due to the high degree of specificity of CE for this enantiomer. The unexpected result was that the *R* enantiomer could also be isolated in high enantiomeric excess. After enzymic reaction a maximum of 90% ee *R* was expected for the unhydrolyzed diester because 55% of the original diester remained. Apparently, the two crystallizations involved in isolation (first as the dipentanoate, second as binaphthol) raised the enantiomeric purity to 99%.

Similarly, the spirobiindanol **6** was resolved on a synthetic scale (200 g) by hydrolyzing the dihexanoate with partially purified CE. This ester was chosen because it gave the best combination of enantioselectivity, rate of reaction, and ease of separation of diol and diester. This hydrolysis was less enantioselective than the hydrolysis of **1** dipentanoate. Therefore, the enantiomeric purity of the diol was lower: 82% ee *R* after 35% hydrolysis. The enantiomeric excess of the diol was increased to ~90% by selective precipitation of racemic diol in CH_2Cl_2. Racemic **6** forms high-melting (213.5–216.5 °C), slightly soluble crystals (32 mM,

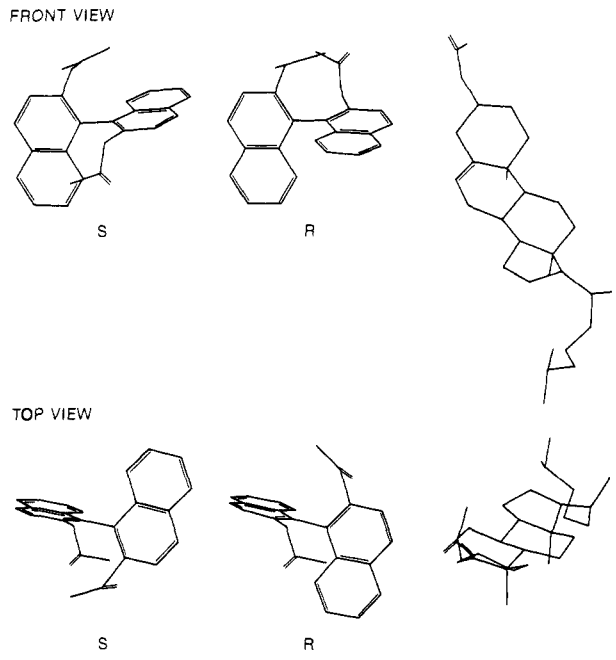


Figure 2. A comparison of the structure of cholesterol acetate with the structures of the two enantiomers of **1** diacetate. In the front view the A and B rings of cholesterol have been aligned with one of the naphthyl rings to emphasize their similarity. The top view emphasizes the twist of each structure. Both (*S*)-**1** and cholesterol show a twist in the same direction.

CH_2Cl_2; 15 mM, toluene; 25 °C) while the enantiomerically pure material forms low-melting (125–127 °C), very soluble crystals (1900 mM, CH_2Cl_2; 760 mM, toluene; 25 °C).

Discussion

Cholesterol esterase, like other digestive enzymes, is useful for synthesis because it catalyzes the hydrolysis of a broad range of esters. The specificity of CE complements the specificity of lipase, other digestive enzyme from pancreas. Cholesterol esterase catalyzes the hydrolysis of esters of secondary alcohols while lipase is specific for esters of primary alcohols.

It is surprising that CE, whose presumed function is to hydrolyze steroid esters, can also hydrolyze binaphthol and spirobiindanol esters. To rationalize the enantiospecificity of CE for (*S*)-**1**, the three-dimensional structures of **1** diacetates were compared with the structure of cholesterol acetate, Figure 2. In the front view the three structures look somewhat similar as the A and B rings of cholesterol are lined up parallel to one of the naphthols in binaphthol. The carbonyl carbon of each ester could adopt a similar orientation and be attacked by the serine –OH. In the top view, all three structures look different and lopsided. It is satisfying to find that both cholesterol acetate and the preferred enantiomer of **1** diacetate are lopsided in the same direction. Similar comparisons of the structures of other substrates are being used to map the active site of cholesterol esterase.[26]

Potential Advantage of Multistep Resolutions. A disadvantage of enzymes with a broad substrate specificity is that they often show imperfect enantiospecificity. One approach to products with enhanced enantiomeric purity is the coupling of an enantioselective synthesis to a resolution.[27,28] Another approach that is suggested by the resolutions described above is to use multistep resolutions.

The resolution of binaphthol involves two sequential hydrolyses each favoring the *S* enantiomer. Scheme II shows how the second hydrolysis can enhance the enantioselectivity of the first. A quantitative comparison of the enantiomeric purity of the product obtained from a single-step resolution and from a two-step resolution is given in Figure 3 for the special case where the enantiospecificity of each step is the same. These calculations show that for an enzyme of a given enantiospecificity the two-step resolution gives higher enantiomeric purity than a single-step resolution. For example, an enzyme having an enantiospecificity of $E > 9$ yields product with >90% ee at 40% conversion during a two-step resolution, while a single-step resolution requires an enzyme with an enantiospecificity of $E > 35$ for the same purity of product. Thus, substrates which require two enzymic reactions during resolutions can yield product of higher enantiomeric purity than similar resolutions involving only a single reaction.

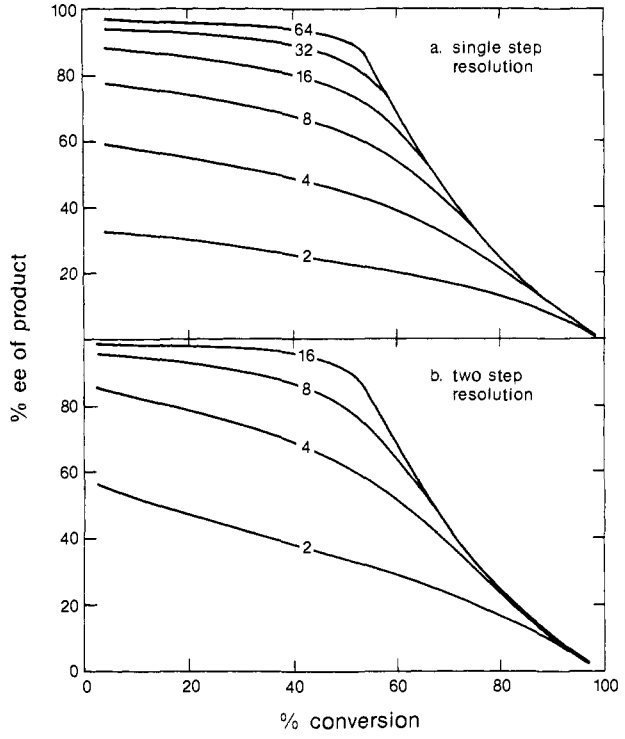
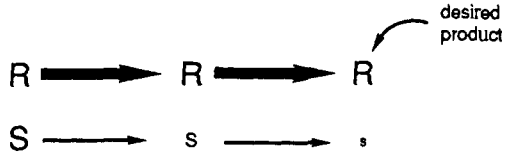


Figure 3. Theoretical curves which compare the enantiomeric purity of the product as a function of conversion for a single-step (panel A) and a two-step resolution (panel B). The different lines represent enzymes with different enantiospecificities and show that for a given enantiospecificity the two-step resolution yields higher purity product. Both % ee and % conversion refer to the final product. Equations used to calculate these curves are given in the Supplementary Material.

Scheme II. Multistep Resolutions Can Enhance Enantiomeric Purity



The high enantiomeric purity of **1** resolved with CE may in part be due to this phenomenon since both steps prefer the *S* enantiomer. Unfortunately, it is difficult to test this hypothesis because the specificity of the first step is so high that it is experimentally difficult to determine whether the second step affects enantiomeric purity. For spirobiindanol, resolution is probably worsened by the two-step process because the two steps have opposite enantiospecificity. Quantitative analysis indicated that the enantiomeric purity of **6** during a resolution was slightly higher than expected on the basis of the measured values of E_1 and E_2. This suggests that the irreversible two-step model may be an oversimplification for this reaction. Nevertheless, the suggested strategy of using multistep resolutions to enhance the enantiomeric purities appears theoretically valid and may find use in other kinetic resolutions.

Experimental Section

General. Chemicals were purchased from Aldrich Chemical Co. and enzymes from Sigma Chemical Co., unless otherwise noted. Cholestan-

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3β-ol, cholestane-3β-acetate, coprostan-3α-ol, and coprostan-3β-ol were purchased from Pfaltz and Bauer (Waterbury, CT). Bovine cholesterol esterase was purchased from Genzyme Corp. (Boston, MA). Porcine cholesterol esterase was purchased from Worthington Diagnostics (Freehold, NJ). Fresh porcine pancreas was a gift from the State University of New York Agricultural and Technical College, Cobleskill, NY. Rotations were determined at the sodium D line on a Perkin-Elmer Model 241 polarimeter. Melting points were determined in open capillaries with a Hoover melting point apparatus and are uncorrected. Microanalysis was performed by Galbraith Microanalysis (Knoxville, TN) or GE-CRD's Material Characterization Laboratory. Purity of cholesterol esterase was established by SDS gel electrophoresis using a 10–20% gradient polyacrylamide gel (Integrated Separation Systems, Hyde Park, MA) stained with Coomassie Blue and also by gel filtration using a Bio-Sil TSK-125 column (Bio-Rad, Inc., Richmond, CA). Substrates and hydrolysis products were separated by HPLC on a reverse-phase (C-18) column eluted with a gradient of water–acetonitrile containing 0.06% acetic acid, flow rate 1–2 mL/min. Peaks were detected by absorbance at 254 nm (4-nm band width).

The structures in Figure 2 were minimized and drawn with the program BIOGRAF (BioDesign, Inc., Pasadena, CA). The minimized structure for cholesterol agrees with the structure determined by X-ray diffraction.[29]

Alcohols. Literature methods were used to prepare the following: (**2**) 6,6′-dibromo[1,1′-binaphthalene]-2,2′-diol;[30] (**3**) 5,5′,6,6′,7,7′,8,8′-octahydro[1,1′-binaphthalene]-2,2′-diol;[31,32] (**4**) 3,3′-dibromo-5,5′,6,6′,7,7′,8,8′-octahydro[1,1′-binaphthalene]-2,2′-diol[31] (racemic: mp 166.5–170 °C); (**5**) 8-bromo-2,3-dibromo-1*H*-benz[*e*]inden-3-ol;[33] (**6**) 2,2′,3,3′-tetrahydro-3,3,3′,3′-tetramethyl-1,1′-spirobi[1*H*-indene]-6,6′-diol;[34] (**7**) 2,2′,3,3′-tetrahydro3,3,3′,3′,5,5′-hexamethyl-1,1′-spirobi[1*H*-indene]-6,6′-diol;[34] (**8**) 7,7′-dibromo-2,2′,3,3′-tetrahydro-3,3,3′,3′,5,5′-hexamethyl-1,1′-spirobi[1*H*-indene]-6,6′-diol[19] (racemic: mp 223–227 °C). A sample of **9** (3,3′,4,4′-tetrahydro-4,4,4′,4′-tetramethyl-2,2′-spirobi[2*H*-1-benzopyran]-7,7′-diol was purchased from Somitomo Chemical Co. (Japan).

Diesters. Alcohols dissolved in ethyl ether were treated with 1.1 equiv of triethylamine and 1.1 equiv of the appropriate acid chloride. When reaction was complete as shown by TLC on silica gel eluted with 4:1 cyclohexane–ethyl acetate, the solution was washed twice with an equal volume of aqueous potassium bicarbonate (1 M) and once with water, dried over magnesium sulfate, concentrated, and recrystallized from ethyl ether. **1** diacetate: mp 104–107 °C; M^+ 370. Anal. Calcd for $C_{24}H_{18}O_4$: C, 77.82; H, 4.90. Found: C, 77.60; H, 5.05. **1** dipropanoate: mp 101.5–103.5 °C; M^+ 398. Anal. Calcd for $C_{26}H_{22}O_4$: C, 78.37; H, 5.56. Found: C, 78.07; H, 5.77. Other diesters of **1** were oils that were purified by chromatography on silica gel eluted with petroleum ether–ethyl acetate (4:1) and characterized by ^1H and ^{13}C NMR. **1** dibutanoate: M^+ 426. **1** dipentanoate (oil which solidifies): mp 55–60 °C; M^+ 454. Anal. Calcd for $C_{30}H_{30}O_4$: C, 79.27; H, 6.65. Found: C, 79.22; H, 6.78. **1** dihexanoate: M^+ 482. **1** diheptanoate: M^+ 510. **1** dioctanoate: M^+ 538. **2** diacetate recrystallized from heptane: mp 180.0–181.5 °C; M^+ 526. Anal. Calcd for $C_{24}H_{16}Br_2O_4$: C, 54.57; H, 3.05. Found: C, 54.73; H, 3.14. **2** dibutanoate recrystallized from pentane: mp 92–94 °C; M^+ 582. Anal. Calcd for $C_{28}H_{24}Br_2O_4$: C, 57.56; H, 4.14. Found: C, 57.93; H, 4.24. The diacetate of **3** was purified by chromatography on silica gel eluted with methylene chloride and recrystallized from heptane: mp 109.5–111.5 °C; M^+ 378. Anal. Calcd for $C_{24}H_{26}O_4$: C, 76.17; H, 6.92. Found: C, 76.49; H, 6.83. The dipentanoate of **3**, oil: M^+ 462. The dipentanoate of **4** was recrystallized from ethyl ether–petroleum ether: mp 108–109.5 °C; M^+ 618. Anal. Calcd for $C_{30}H_{38}Br_2O_4$: C, 57.89; H, 6.15. Found: C, 58.25; H, 5.76. The butanoate of **5**, oil: M^+ 332.

Diesters of the spirobiindanol **6** were recrystallized from ethyl ether–petroleum ether (acetate through pentanoate) or petroleum ether (hexanoate through decanoate). Diacetate of **6**: mp 166–167 °C; M^+ 392. Anal. Calcd for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19. Found: C, 76.49; H, 7.20. Dipropanoate of **6**: mp 175–176 °C; M^+ 420. Anal. Calcd for $C_{27}H_{32}O_4$: C, 77.11; H, 7.67. Found: C, 77.24; H, 7.68. Dibutanoate of **6**: mp 160–161 °C; M^+ 448. Anal. Calcd for $C_{29}H_{36}O_4$: C, 77.65; H, 8.09. Found: C, 77.93; H, 8.07. Bis(2-methylpropanoate) of **6**: mp 205.5–206.5 °C; M^+ 448. Anal. Calcd for $C_{29}H_{36}O_4$: C, 77.65; H, 8.09. Found: C, 77.44; H, 8.14. Dipentanoate of **6**: mp 116–120 °C; M^+ 476. Anal. Calcd for $C_{31}H_{40}O_4$: C, 78.12; H, 8.46. Found: C, 78.18; H, 8.49. Dihexanoate of **6**: mp 113–114 °C; M^+ 504. Anal. Calcd for $C_{33}H_{44}O_4$: C, 78.53, H, 8.79. Found: C, 78.79; H, 8.73. Diheptanoate of **6**: mp 79–80 °C; M^+ 532. Anal. Calcd for $C_{35}H_{48}O_4$: C, 78.91; H, 9.08. Found: C, 79.01; H, 9.04. Dioctanoate of **6**: mp 74–75 °C; M^+ 560. Anal. Calcd for $C_{37}H_{52}O_4$: C, 79.24; H, 9.35. Found: C, 79.22; H, 9.42. Dinonanoate of **6**: mp 68–70 °C; M^+ 588. Anal. Calcd for $C_{39}H_{56}O_4$: C, 79.55; H, 9.59. Found: C, 79.32; H, 9.60. Didecanoate of **6**: mp 77–79 °C; M^+ 616. Anal. Calcd for $C_{41}H_{60}O_4$: C, 79.82; H, 9.80. Found: C, 79.56; H, 9.71. Dihexanoate of **7**: mp 152–154 °C; M^+ 532. Anal. Calcd for $C_{35}H_{48}O_4$: C, 78.91; H, 9.08. Found: C, 78.78; H, 9.16. Dibutanoate of **8**: mp 132–134 °C; M^+ 632. Anal. Calcd for $C_{31}H_{38}Br_2O_4$: C, 58.87; H, 6.04. Found: C, 58.54; H, 6.19. Diacetate of **9**: mp 192–194 °C; M^+ 424. Anal. Calcd for $C_{25}H_{28}O_6$: C, 70.74; H, 6.65. Found: C, 71.08; H, 6.67. The diethyl ester of **10** was prepared by refluxing overnight the diacid in absolute ethanol containing several drops of concentrated H_2SO_4: mp 172–174 °C; M^+ 420. Anal. Calcd for $C_{27}H_{32}O_4$: C, 77.11; H, 7.67. Found: C, 76.98; H, 7.86.

Monoesters were prepared with 0.5 equiv of acid chloride and were purified by chromatography on silica gel eluted with methylene chloride and recrystallized from ethyl ether. **1** acetate, oil which solidifies: mp 107–113 °C; M^+ 328. Anal. Calcd for $C_{22}H_{16}O_3$: C, 80.47; H, 4.91. Found: C, 80.80; H, 4.86. **1** propanoate: mp 145.0–146.5 °C; M^+ 342. Anal. Calcd for $C_{23}H_{18}O_3$: C, 80.68; H, 5.30. Found: C, 80.91; H, 5.13. **1** butanoate: mp 134.5–136.5 °C; M^+ 356. Anal. Calcd for $C_{24}H_{20}O_3$: C, 80.88; H, 5.66. Found: C, 80.77; H, 5.72. **1** pentanoate, oil which solidifies: mp 73–80 °C; M^+ 370. Anal. Calcd for $C_{25}H_{22}O_3$: C, 81.06; H, 5.99. Found: C, 81.00; H, 6.16. **6** acetate (from Et_2O–heptane): mp 175–176 °C; M^+ 350. Anal. Calcd for $C_{23}H_{26}O_3$: C, 78.83; H, 7.48. Found: C, 78.46; H, 7.53. **6** butanoate (E_2O–heptane): mp 128–130 °C; M^+ 378. Anal. Calcd for $C_{25}H_{30}O_3$: C, 79.33; H, 7.99. Found: C, 79.21; H, 8.23. **6** pentanoate, oil: M^+ 392. **6** hexanoate, oil: M^+ 406.

Assay of Cholesterol Esterase with Emulsified Cholesterol Acetate. Cholesterol esterase was assayed according to the method recommended by Genzyme Corp., which uses cholesterol acetate (0.7 mM) emulsified in aqueous solution with poly(oxyethylene) 9-lauryl ether. Typical activity of commercial samples of cholesterol esterase was 1–2 units/mg at 25 °C.

Assay of Cholesterol Esterase with Cholesterol Acetate Dissolved in Ethyl Ether. Cholesterol acetate (1.0 mmol) dissolved in ethyl ether (10 mL) was added to an aqueous phosphate buffer (10 mL of 10 mM, pH 7.00) containing sodium taurocholate (5.4 mM) and cholesterol esterase (5–20 mg of protein). The mixture was stirred rapidly, and the amount of sodium hydroxide (0.1 N) required to maintain the pH at 7.00 was measured as a function of time with a Radiometer RTS 822 pHstat. Typical activity of commercial samples of cholesterol esterase was 0.1–0.2 unit/mg at 25 °C.

Survey of Substrates for Cholesterol Esterase. Substrate (1.0 mmol) was substituted for cholesterol acetate in the two-phase assay above. Rates of hydrolysis determined by the amount of base required to maintain a constant pH were confirmed by HPLC. For esters of shorter, water-soluble acids, the rates measured by both HPLC and pHstat agree to within 5%. However, base consumption during the later states of hydrolysis of longer chain esters was less than expected presumably because a fraction of the released acid partitioned into the ether phase and was not neutralized.

Hydrolysis of 1 Diacetate Using Porcine Pancreas Homogenate. Porcine pancreas homogenate (10 mL) was stirred with sodium taurocholate (30 mg) and **1** diacetate (380 mg, 1 mmol) in ethyl ether (10 mL). Homogenate was prepared by blending 10 g of porcine pancreas (3.8 g of dry weight) with 10 mL of phosphate buffer (0.1 M, pH 7). The rate of hydrolysis, determined by HPLC, was ~4 μmol of ester/min (>95% ee *S*), which corresponds to a specific activity of ~2 units/g of dry weight of pancreas.

Stability of Cholesterol Esterase. Samples of cholesterol esterase were dissolved in phosphate buffer (0.1 M, pH 7) containing taurocholate (5 mM) and sodium azide (0.1%) to give an activity of 1–2 units/mL. The decrease in activity as a function of time at 25 °C was monitored with the emulsified cholesterol acetate assay. Trypsin inhibitor benzamidine (2 mM) or soybean trypsin inhibitor (0.25 mg/mL) enhanced the stability of cholesterol esterase activity in bovine pancreas acetone powder, while 3-phenylpropionic acid (2 mM) or N^{α}-benzoyl-D,L-arginine (1 mM) had no effect.

Extent of Conversion. The extent of hydrolysis was determined by HPLC eluting with a 0–100% gradient of water–acetonitrile over 15 min. Relative response factors were determined for each series of alcohol and

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ester: **1**, 1.00; **1** propanoate, 1.13; **1** dipropanoate, 1.01; **1** butanoate, 1.13; **1** dipentanoate, 1.02; **3**, 1.00; **3** diacetate, 1.20; **6**, 1.00; **6** acetate, 1.50; **6** diacetate, 1.92; **6** butanoate, 1.44; **6** dibutanoate, 1.99; **6** dihexanoate, 1.93; **7**, 1.00; **7** diacetate, 1.85; **8**, 1.00; **8** dibutanoate, 1.31; **9**, 1.00; **9** diacetate, 1.59. Where crystalline samples were not available, the relative absorbance of a homologue was used in the calculations.

Enantiomeric Purity. Enantiomers of **1** and **1** monoesters were separated in an HPLC column containing ionically bonded *N*-(3,5-dinitrobenzoyl)-D-phenylglycine (Regis Chemical Co., Morton Grove, IL) eluted with hexane containing 5 vol % 2-propanol.[12b] To determine enantiomeric purities >99% ee, chromatograms of unknown and unknown with 0.1% deliberately added racemate were compared. Saponification of the unreacted **5** butanoate after 40% hydrolysis yielded a sample of **5** which showed $[\alpha]_D = -16°$, which corresponds to 26% ee.[35] A sample of **8** isolated after 48% hydrolysis of **8** dibutanoate showed $[\alpha]^{25}_D = -99.2°$ (*c* 0.12, dioxane) [lit.[19] $[\alpha]^{25}_D = -108.9°$ (dioxane)], corresponding to 91% ee. Enantiomeric purity of **6** was determined by separation of diastereomeric camphanate derivatives by HPLC. To a sample of **6** (2 μmol, 50 μL of a 0.04 M solution) was added (1*R*)-(−)-camphanic acid chloride (20 μmol, 100 μL of a 0.2 M solution in CH_2Cl_2) and triethylamine (36 μmol, 5 μL). After 10 min at room temperature this mixture was diluted with acetonitrile and the diastereomers were separated by HPLC eluting with a gradient of 70–100% acetonitrile over 20 min. The extinction coefficients of the two diastereomers were assumed to be equal because racemic material gave equal peak areas to within integration errors (2%). To determine the enantiomeric purity of **6** dihexanoate, samples were isolated by preparative TLC (silica gel eluted with methylene chloride), hydrolyzed to the diol with base, and derivatized with camphanic acid chloride as above.

Enantiomeric purities of all other compounds were determined by ^1H NMR using Eu((+)-hfc)$_3$ as the chiral shift reagent. In a typical procedure a sample (20 mg) of the alcohol was isolated by preparative thin-layer chromatography (silica gel eluted with CH_2Cl_2) and acetylated with excess acetyl chloride (30 μL) and triethylamine (56 μL) in ethyl ether (1.0 mL) for 15 min. After washing with aqueous $KHCO_3$ and drying over $MgSO_4$, the ether was evaporated, and a portion of the residue (~7 mg) was dissolved in 0.6 mL of C_6D_6. Shift reagent dissolved in C_6D_6 (100 mg/mL) was added in 50-μL portions until separation of the acetyl methyl resonances was observed, typically 200–300 μL. Base-line separation was not achieved; areas were estimated by cut and weigh.

Absolute Configurations. The preferred enantiomer of **1** was *S* since it exhibited a (−) rotation $[[\alpha]^{25}_D = -34.2°$ (*c* 0.2, THF); lit.[9] $[\alpha]^{25}_D = -33.3°$ (*c* 1.1, THF)] and comigrated with authentic (*S*)-(−)-**1** on a chiral HPLC column. Analysis by ^1H NMR in the presence of Eu((+)-hfc)$_3$ indicated that the preferred enantiomer of **3** was also *S* by comparison with authentic (*S*)-**3** prepared from (*S*)-**1**. Saponification of the unreacted **5** butanoate (isolated by TLC) yielded **5** having a (−) rotation, indicating that the *R*-(+) enantiomer had been selectively hydrolyzed.[35] The preferred enantiomer of **6** showed a (+) rotation $[[\alpha]^{25}_D = +35.3°$ (*c* 0.35, MeOH)] which has been assigned the *R* configuration on the basis of chemical correlations.[19,36] The absolute configuration of **7** was also probably *R* since the order of elution of the diastereomeric camphanates was the same as that for **6**. The preferred enantiomer of **8** (isolated by TLC eluting with toluene) showed a (−) rotation which indicates the *R* configuration.[36,37]

Large-Scale Resolutions. (1) Resolution of [1,1′-Binaphthalene]-2,2′-diol (1). Pentanoyl chloride (185 mL, 1.56 mol) was added over 15 min to a suspension of [1,1′-binaphthalene]-2,2′-diol (203 g, 0.71 mol) in ethyl ether (2 L) containing triethylamine (215 mL, 1.54 mol). After being stirred for an additional hour, the mixture was washed twice with aqueous sodium bicarbonate (2 L of 1 M) and once with water (2 L). Analysis by reverse-phase HPLC showed no detectable binaphthol and only 0.2% monopentanoate. For high enantiomeric purity of product it is important that no unreacted binaphthol remain at this point. The solution was diluted to 4 L with ethyl ether and stirred with phosphate buffer (pH 7.5, 0.1 M, 4 L) containing sodium taurocholate (12 g of crude material from ox bile, Sigma) to form an emulsion. Bovine pancreas acetone powder (100 g) was added, and the pH of the aqueous phase was measured several times a day and readjusted to 7.2 ± 0.2 with sodium hydroxide (1 M, 500 mL was required). The hydrolysis stopped after 65 h; HPLC showed 43.0% diol, 4.4% monopentanoate, and 54.8% dipentanoate. The mixture was strained through cheesecloth into a separatory funnel, ethanol (400 mL) was added, and the two phases were allowed to settle for 4 h. The brown aqueous phase was discarded, and the remaining emulsion in the yellow ether phase was broken up by addition of magnesium sulfate (180 g). The ether phase was dried over additional magnesium sulfate, filtered, and concentrated to 500 mL. Toluene (200 mL) was added and the solution cooled to 4 °C overnight. The white crystals which formed were collected by filtration and washed with cold toluene. This first crop (50.4 g, 97% binaphthol) and three additional crops of lower purity were combined (a total of 85 g of binaphthol contaminated with diester and monoester) and recrystallized from toluene (500 mL) to yield (*S*)-(−)-[1,1′-binaphthalene]-2,2′-diol (67.4 g, 66% yield; 99.5% diol, >99.9% ee *S* containing 0.5% monopentanoate).

Toluene in the filtrate removed by rotary evaporation and hexane (200 mL) was added. After cooling to 4 °C, **1** dipentanoate crystallized and was collected by filtration (91 g, 98.4% purity). Chromatography of filtrate on silica gel (1 kg) eluted with methylene chloride yielded an additional 2.3 g of diol and 24.8 g of crystalline dipentanoate. Sodium methoxide (0.15 mol) was added to the combined dipentanoate samples (115.8 g) dissolved in methanol (1 L). After 2 h at room temperature the solution was neutralized with concentrated HCl (~10 mL), and ethyl ether (1 L), toluene (0.5 L), and phosphate buffer (1 L of 0.1 M, pH 7) were added. The organic layer was separated, washed once with water (1 L), dried over magnesium sulfate, and concentrated to 200 mL. The white crystals which separated were collected by filtration, washed with cold toluene, and dried to yield (*R*)-(+)-[1,1′-binaphthalene]-2,2′-diol (63.7 g, 63% yield; >99% diol, 98.8% ee *R*).

(2) Resolution of 6. Cholesterol esterase (10.0 g of solid containing 8.3 g of protein obtained from Genzyme Corp., Boston, MA; cat. no. 1061, lot no. B08615) was added to a 12-L flask containing **6** dihexanoate (333 g, 0.66 mol), ethyl ether (4 L), sodium taurocholate monohydrate (1.8 g), and aqueous buffer (0.60 L of 0.1 M potassium phosphate, pH 7.5). The resulting two-phase mixture was stirred at room temperature for 6.1 days. The pH of the aqueous phase was maintained at 7.1 ± 0.3 by addition of aqueous NaOH (1 N). Analysis of the ethyl ether phase by HPLC indicated 56% diol (68% ee *R*), 1% hexanoate, and 43% dihexanoate. The reaction was stopped by separating the two phases in a separatory funnel. The enzyme remained in the aqueous phase, which was washed once with ethyl ether (1 L) and reused for a second reaction. The combined ethyl ether extracts were concentrated to 1.5 L and washed three times with a equal volume of aqueous potassium bicarbonate (1 M) and twice with an equal volume of distilled water. The ether phase was dried over $MgSO_4$ and concentrated under vacuum to a white solid. Methylene chloride (1 L) was added to the residue and the resulting suspension stirred for 1 h. The white undissolved solid was removed by filtration. This material was mostly racemic **6** (31.5 g, 9% ee). The filtrate was chromatographed in two portions on silica gel (1.5 kg of 60–200 mesh packed in a 12 cm diameter column), elution being with methylene chloride to recover **6** dihexanoate and with ethyl ether to recover **6**. The diol was recrystallized from petroleum ether–ethyl ether: 55.6 g of 95% ee *R*; 55% yield. The hexanoate fractions were evaporated to dryness and redissolved in ethyl ether (500 mL). Ethanol (200 mL) and KOH (200 mL of a 50 wt % solution) were added, and the resulting gel was allowed to sit overnight. An additional 1 L of ethyl ether was added, and the solution was neutralized to pH 7 with concentrated HCl. The two phases were separated, and the ether phase was washed three times with potassium bicarbonate (1 M) and twice with distilled water. The solution was dried over $MgSO_4$ and the diol recrystallized from ethyl ether–petroleum ether: 40.0 g of >95% ee *S*; 40% yield. An additional 40.0 g of 73% ee *S* was also isolated.

The enzyme solution recovered from this reaction was used for a second reaction on the same scale, which was stopped when 38% of the ester groups had been hydrolyzed: 64.4 g of >95% ee *R*, 64% yield, $[\alpha]^{25}_D = +35.3°$ (*c* 0.35, MeOH), and mp 125.0–127.5 °C; 51.6 g of >95% ee *S*, 52% yield, $[\alpha]^{25}_D = -34.1°$ (*c* 0.34, MeOH), and mp 124.0–127.0 °C.

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Supplementary Material Available: Comparison of a resolution consisting of a single enantiospecific step with a resolution consisting of two enantiospecific steps (3 pages). Ordering information is given on any current masthead page.

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