with 0.37 unit of transcarboxylase in 20 μL containing KP_i (250 mM, pH 7.0) and DTT (10 mM) at 25 °C. After 1 min the incubation was centrifuged through two spun-dry DE52 (1 mL wet) beds in sequence in the cold within 10 min. The ^{14}C radioactivity and enzyme activity of the second filtrate were compared. This ratio was determined as a function of the MMCoA concentration used to label the enzyme: 0.05, 0.1, and 0.25 mM. The limit, determined graphically, was 1280 cpm/unit of activity; i.e. 0.275 nmol of biotin could be carboxylated by MMCoA per unit of activity. This value is ~40% greater than the highest value reported by Wood et al.[10] on the basis of the counts of labeled biotin incorporated and the highest enzyme activity that was obtained in the same assay. The difference can be attributed to loss during storage of the assay rate, which depends on the coordinated function of the two half-reactions, without loss of capacity for the MMCoA/propionyl CoA half-reaction.[8]

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Marked Dependence of Enzyme Prochiral Selectivity on the Solvent

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Abstract: Prochiral selectivity of various hydrolytic enzymes (lipases and proteases) in organic solvents was investigated in transformations involving a 2-substituted 1,3-propanediol or its diester. In two instances, a significant dependence of enzyme prochiral selectivity on the solvent was found: transesterification of diol **1** with vinyl butyrate catalyzed by *Aspergillus oryzae* protease in anhydrous solvents and hydrolysis of diester **3** catalyzed by *Pseudomonas* sp. lipase in hydrated organic solvents (monoester **2** was a product in both reactions). The latter process, where the *pro-S* selectivity of the enzyme varied from around 3 in some solvents to greater than 30 in others, was examined in more detail. A mechanistic model was proposed that predicted an inverse correlation between lipase's prochiral selectivity and solvent hydrophobicity, as well as particular effects of substrate structure variation and an additive on the prochiral selectivity; all these predictions were confirmed experimentally. Subtilisin Carlsberg lacked appreciable prochiral selectivity in either transesterification or hydrolysis reactions regardless of the solvent; this was rationalized by means of interactive computer modeling based on the X-ray crystal structure of this serine protease.

Introduction

The ability of enzymes to catalyze useful synthetic transformations in neat organic solvents is now beyond doubt.[1] Perhaps the most exciting and significant development in this emerging area is a recent discovery that enzyme specificity, in particular enantioselectivity[2] and regioselectivity,[3] can be profoundly affected simply by switching from one organic solvent to another. This paves the way to altering specificity of a given enzyme at will and provides a valuable alternative to enzyme screening.

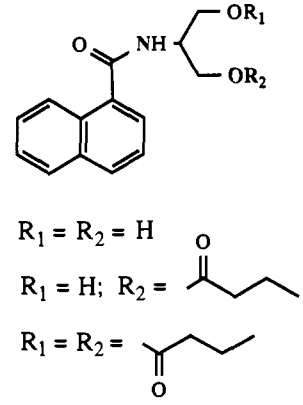
Among other biocatalytic asymmetric processes in non-aqueous media,[1] prochiral conversions catalyzed by hydrolases have been profitably utilized.[4] In the present study, we demonstrate that prochiral selectivity of enzymes also can be controlled by the solvent and that this dependence can be mechanistically rationalized.

Results and Discussion

In order to test the possibility of affecting enzyme prochiral selectivity by the solvent, we synthesized the prochiral diol **1**. The bulky and hydrophobic naphthoyl moiety was deliberately introduced into the C-2 position of the diol to allow it to serve as an orienting anchor in the active center of an enzyme. Initially, we investigated the kinetics of the transesterification reaction between **1** and vinyl butyrate[5] catalyzed by different hydrolytic enzymes in anhydrous tetrahydrofuran. The progress of the transesterification was followed by HPLC using a chiral column in order to separate enantiomers of the monobutyryl ester **2**.

Chromatogram *a* in Figure 1 corresponds to chemically prepared, racemic **2**. One can see two twin peaks; since a chiral HPLC column was employed, they must represent *R* and *S* enantiomers of **2**. It was not known at that point which peak

Chart I



corresponded to what enantiomer; therefore, for the time being, we shall refer to them simply as "first" and "second" peaks.

[†] A Swiss National Science Foundation/Foundation for the 450th Anniversary of Lausanne University Postdoctoral Fellow.

[‡] A Bourse Lavoisier/Rhône-Poulenc (France) Postdoctoral Fellow.

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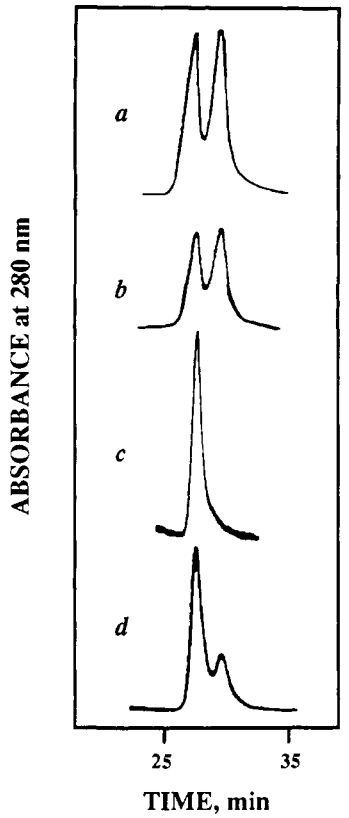

Figure 1. Relevant portions of chiral HPLC chromatograms of **2**: (*a*) chemically synthesized, racemic **2**; (*b*, *c*, and *d*) **2** formed as a result of the transesterification reaction between **1** and vinyl butyrate in tetrahydrofuran catalyzed by subtilisin Carlsberg, *Pseudomonas* sp. lipase, and *Aspergillus oryzae* protease, respectively. For experimental details, see footnote *a* to Table I and the Materials and Methods section.

HPLC chromatograms *b*, *c*, and *d* in Figure 1 represent the monobutyryl ester **2** peaks formed during the initial stages (<10% conversion) of the transesterification catalyzed by three representative hydrolytic enzymes—subtilisin Carlsberg, *Pseudomonas* sp. lipase, and *Aspergillus oryzae* protease—in anhydrous tetrahydrofuran.[6] Even a superficial inspection of these chromatograms reveals that the first enzyme exhibits virtually no prochiral selectivity (the areas of the first and second peaks are the same), the second enzyme displays a remarkable prochiral selectivity (the second peak is altogether absent), and the third enzyme shows a modest prochiral selectivity (the first peak is significantly larger than the second).

Using the same methodology, we measured the kinetics of the acylation of **1** into **2** catalyzed by 15 different proteases and lipases in tetrahydrofuran.[7] Table I depicts the initial rates of accumulation of both enantiomers of **2**, as well as resultant prochiral selectivity (the ratio of the initial rates) for each enzyme. It is seen that depending on the enzyme, the prochiral selectivity varies

Table I. Prochiral Selectivity of Different Proteases and Lipases in the Transesterification between Diol **1** and Vinyl Butyrate in Anhydrous Tetrahydrofuran[a]

enzyme	initial rate,[b] $\mu M \cdot h^{-1} \cdot mg^{-1}$		
	first enantiomer[c]	second enantiomer[c]	prochiral selectivity[d]
subtilisin Carlsberg	1.6	1.6	1.0
Pseudomonas sp. lipase	120	n.d.[e]	>27
Aspergillus oryzae protease	0.51	0.22	2.3
Penicillium roqueforti lipase	0.048	0.020	2.4
Chromobacterium viscosum lipase	4.0	0.51	7.8
Pseudomonas lipoprotein lipase	400	16	25
Mucor sp. lipase	2.6	0.36	7.2
Rhizopus arrhizus lipase	0.053	0.015	3.5
Rhizopus oryzae lipase	0.099	0.014	7.1
Bacillus subtilis protease	0.045	0.008	5.6
Mucor javanicus lipase	0.67	0.086	7.8
Aspergillus niger lipase	1.3	0.26	5.0
porcine pancreatic lipase	1.1	n.d.[e]	>27
Rhizopus sp. protease	0.026	0.010	2.6
Rhizopus javanicus lipase	0.041	0.008	5.1

[a] Enzyme powders (10 mg for subtilisin Carlsberg, 5 mg for *Pseudomonas* sp. lipase and lipoprotein lipase, and 100 mg for all others) were placed in 1 mL of anhydrous tetrahydrofuran containing 10 mM each **1** and vinyl butyrate. Following a 5-s sonication, the reaction mixtures were shaken at 45 °C and 300 rpm; periodically, aliquots were withdrawn and assayed by HPLC as described in the Materials and Methods section. All enzymes were used as supplied by the manufacturers. [b] Measured on the basis of 4 to 6 experimental points (all at less than 10% conversion). The instrument was precalibrated with chemically prepared, racemic **2**. In the units, "mg" refers to the weight of the enzyme. [c] First and second refer to the peaks in Figure 1. See text for details. [d] Defined as the ratio of the rate of accumulation of the enantiomer of **2** represented by the first peak in Figure 1 to that for the second peak in the same figure. [e] Below the sensitivity limit of our assay.

Table II. Prochiral Selectivity of Three Different Hydrolases in the Transesterification between Diol **1** and Vinyl Butyrate as a Function of the Solvent[a]

solvent	prochiral selectivity[b]		
	subtilisin Carlsberg	*Pseudomonas* sp. lipase	*Aspergillus oryzae* protease
tetrahydrofuran	1.1	>27	2.3
pyridine			3.2
dimethylformamide			2.2
nitrobenzene	1.1		4.1
cyclohexanone			3.2
dioxane	0.65	>27	2.0
3-octanone			2.4
xylene/pyridine (9:1 (v/v))			2.1
acetonitrile	0.80	>27	1.2

[a] Enzyme powders (3 mg for subtilisin Carlsberg, 5 mg for *Pseudomonas* sp. lipase, and 100 mg for *Aspergillus oryzae* protease) were placed in 1 mL of anhydrous organic solvents containing 10 mM each **1** and vinyl butyrate. Following a 5-s sonication, the reaction mixtures were shaken at 45 °C and 300 rpm; periodically, aliquots were withdrawn and assayed by HPLC as described in the Materials and Methods section. All enzymes were used as supplied by the manufacturers except for subtilisin which was lyophilized from a buffered aqueous solution, pH 7.8, as described in ref 2f (such a lyophilization greatly increases the subtilisin activity in anhydrous solvents: Zaks, A.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 3194-3201). [b] See footnote *d* to Table I.

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(5) An activated and irreversible acylating agent: Degueil-Castang, M.; DeJeso, B.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953-954.

(6) These three hydrolases have been used previously for mechanistic studies of the effect of the solvent on enzyme selectivity.[2a,b,f,i,j,l,m] Tetrahydrofuran was selected because it, in contrast to most other organic solvents, readily dissolved **1**.

(7) All initial rates were determined at less than 10% conversion of **1**. At such low conversions, no formation of diester **3** was detected for any enzyme.

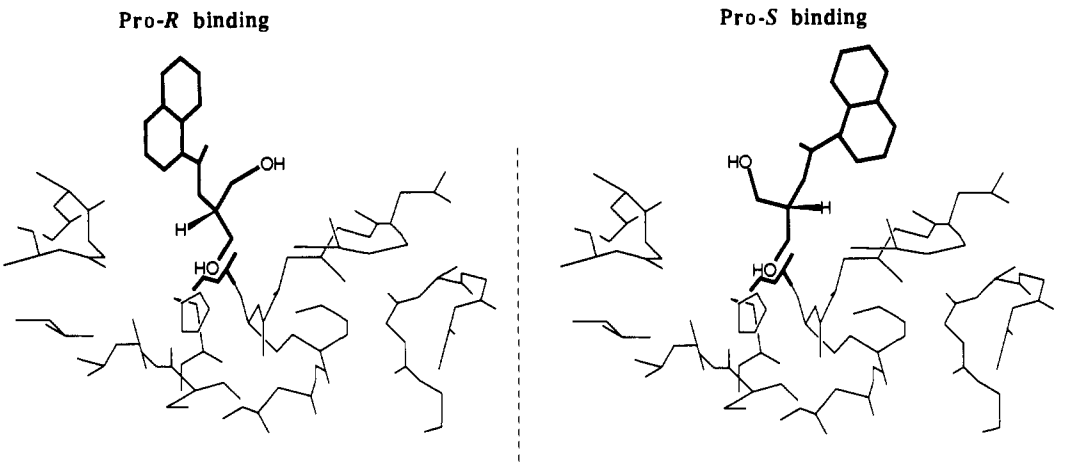

Figure 2. The computer-generated structures of the active center region of butyrylsubtilisin containing **1** bound in either the *pro-R* (left) or the *pro-S* (right) position. The diol nucleophile molecule and the butyryl moiety of the acyl-enzyme are shown in bold lines. An OH group of **1** is always correctly oriented for a nucleophilic attack on the butyryl's carbonyl leading to deacylation. See text and the Materials and Methods section for details.

from negligible to overwhelming (greater than 27, which is the sensitivity limit of this HPLC analysis).

Next, for the three hydrolases shown in Figure 1 (which exemplify enzymes with low, moderate, and high prochiral selectivities) we examined the dependence of prochiral selectivity in the butyrylation of **1** on the solvent. It is seen in Table II that for subtilisin Carlsberg, prochiral selectivity fluctuated around unity in all the solvents tested, while for the lipase it was absolute within the sensitivity limits of our analysis. For *Aspergillus oryzae* protease, we found prochiral selectivity to be significantly affected by the solvent, although the magnitude of this effect (a 3.4-fold range) was deemed to be insufficient to warrant a more detailed investigation.

We were rather surprised that subtilisin's prochiral selectivity only weakly depended on the reaction medium (Table II) because *enantioselectivity* of this enzyme was found to be a strong function of the solvent.[2a,b,f,m] In order to rationalize this observation, we employed the known X-ray crystal structure of subtilisin Carlsberg[8] and interactive computer modeling to dock[2m] **1** into the active center of butyrylsubtilisin in two positions—*pro-R* and *pro-S*. In both instances, one of the diol's hydroxyl groups was fitted into the location believed to be occupied by the water molecule conducting the nucleophilic attack on the ester carbonyl of the acyl-enzyme (as in indoleacryloyl-α-chymotrypsin[9]). We made an assumption that the structure of the acyl-enzyme and the direction of nucleophilic attack in anhydrous solvents are the same as in water. This assumption is supported by the growing body of evidence that the structure and mechanism of action of serine proteases in organic solvents are indistinguishable from those in water.[10] The torsion angle between the hydroxyl oxygen and the adjacent carbon of **1** was rotated to find an acceptable fit of the nucleophile (both in *pro-R* and *pro-S* positions) against butyrylsubtilisin. Other torsion angles within the diol were also rotated to optimize this fit (i.e., overlapping van der Waals radii were avoided), but for each stereochemical position of **1** the hydroxyl group was not moved or rotated from its location facing the ester carbonyl of butyrylsubtilisin to preserve the reactive nature of the complex.

Figure 2 depicts the relevant portions of the derived structures of the reactive complexes between butyrylsubtilisin and **1** in the *pro-R* (left) and *pro-S* (right) positions. One can see that although the orientations of the diol substrate in the enzyme active center are clearly distinct in the two positions, in both cases the molecule of **1** freely extends through the spacious opening in subtilisin to reach the enzyme surface. Therefore, there is no compelling reason to expect that the *pro-R* orientation will be significantly favored over the *pro-S* or vice versa, and, consequently, to expect dissimilar reactivities. Hence the computer modeling data (Figure 2) explain why subtilisin shows little prochiral selectivity in the reaction between **1** and vinyl butyrate (Table II).

A severe experimental limitation of the transesterification examined is that **1** is nearly insoluble in hydrophobic solvents (which is why they are absent in Table II), thus greatly narrowing the range of reaction media that could be tested for their effect on prochiral selectivity. In order to overcome this obstacle, we decided to switch to another, albeit related, transformation. Namely, we chemically dibutyrylated **1** and then set out to investigate enzymatic hydrolysis of diester **3** in organic solvents.

Prior to measuring enzyme kinetics in this reaction, we again employed the computer modeling approach to predict whether subtilisin Carlsberg would exhibit prochiral selectivity in hydrolysis of **3**. Using the same methodology as above, we constructed reactive subtilisin·**3** complexes in *pro-S* and *pro-R* positions. As seen in Figure 3, in neither orientation does the substrate molecule encounter appreciable steric hindrances. Hence one would expect no significant prochiral selectivity from subtilisin Carlsberg in the hydrolysis of **3** into **2**. Moreover, since the degree and manner of exposure of the substrate to the solvent appear similar in *pro-S* and *pro-R* positions (Figure 3), as they were when the prochiral molecule acted as a nucleophile instead of an ester (Figure 2), one would anticipate no major dependence of prochiral selectivity on the solvent either. These visual perceptions were confirmed by modeling-based calculations that showed that the differences in solvent-accessible areas in *pro-R* and *pro-S* positions of **1** and **3** in the enzyme active center varied by less than 15% and 6%, respectively.

Inspection of the subtilisin data in Table III indeed shows that the prochiral selectivity varies in a narrow range from 0.6 to 1.1 in the solvents tested (varied from hydrophilic acetonitrile to hydrophobic carbon tetrachloride), thus testifying again to the usefulness and predictive power of the computer modeling analysis.

A striking feature of Table III is a pronounced solvent dependence of prochiral selectivity of *Pseudomonas* sp. lipase—it changes from less than three in carbon tetrachloride to more than thirty in acetonitrile and nitrobenzene. Note that this dependence is not due to different water contents in some of the solvents listed in the table.[11] First, for most of the solvents the water content was 1%, and yet the prochiral selectivity varied widely (e.g., from

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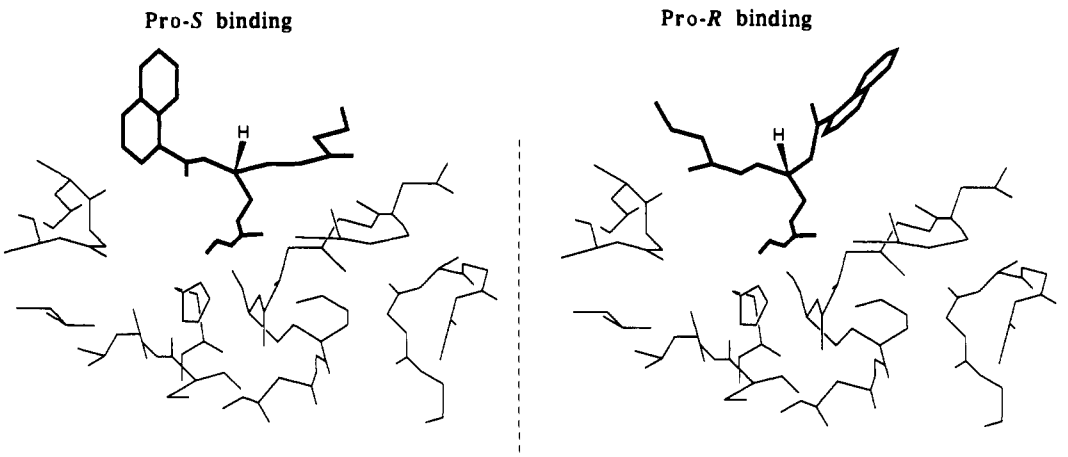

Figure 3. The computer-generated structures of the active center region of subtilisin Carlsberg containing **3** bound in either the *pro-S* (left) or the *pro-R* (right) position. The diester substrate molecule is shown in bold lines. The hydroxyl group of the Ser head nucleophile of the enzyme is always correctly oriented for a nucleophilic attack on one of the ester carbonyls of **3**. See text and the Materials and Methods section for details. Note that the modes of substrate binding depicted in this figure are analogous to those in Figure 2, yet the structures shown in the two figures yield products of opposite chirality. This is because, according to the Cahn–Ingold–Prelog rule, in the hydrolysis the priority of the reactive moiety decreases, while in the transesterification (Figure 2) the priority increases.

<5 for entries 12–14 to >30 for entry 1). Second, for dioxane and toluene the prochiral selectivity of the lipase was found to be the same at 1% and 0.03% as at 0.01% and 0.003%, respectively.

Prior to rationalizing the data in the last column of Table III, we decided to determine the absolute configuration of **2** formed by *Pseudomonas* sp. lipase from **3** in all organic solvents tested (albeit in some of them with much higher fidelity than in others, Table III). To this end, the lipase was used to preparatively synthesize the individual enantiomers of **2** represented by the two peaks in Figure 1. The first one was prepared by enzymatically acylating **1** with vinyl butyrate in anhydrous acetonitrile, while the second one was formed by enzymatically hydrolyzing **3** in the same solvent containing 1% water. Following isolation and purification, the resultant enantiomers were oxidized with the Jones reagent in acetone (leading to the oxidation of the hydroxyl group of **2** into carboxyl) and then refluxed in acidic ethanol (which leads to the simultaneous deacylation of the ester moiety and esterification of the carboxyl group; this conversion results in a formal inversion of the stereochemical configuration at C-2). The two products obtained were the enantiomers of *N*-(1-naphthoyl)serine ethyl ester. To establish their stereochemistry, we chemically acylated commercially available, authentic (*R*) and (*S*) serine ethyl esters with 1-naphthoyl chloride. Comparison of the enzymatically and chemically prepared *N*-(1-naphthoyl)serine ethyl esters by HPLC revealed that the first peak in Figure 1 corresponds to (*R*)-**2** and the second to (*S*)-**2**.

Why does the *pro-S* selectivity of *Pseudomonas* sp. lipase in the hydrolysis reaction markedly change upon transition from one organic solvent to another (Table III)? We thought it would be instructive to ascertain whether the level of prochiral selectivity correlated with a physicochemical characteristic of the solvent. Enantioselectivities,[2a-j] as well as reactivities,[12] of several hydrolases were found to be a function of solvent hydrophobicity; thus this parameter seemed a reasonable candidate. Figure 4A depicts a plot of lipase's *pro-S* selectivity (represented by the logarithm of the ratio of the initial rates of the formation of (*S*) and (*R*) enantiomers of **2**) versus the solvent hydrophobicity (represented by the logarithm of the solvent partition coefficient *P* between 1-octanol and water[13]). One can see a discernable trend of lower prochiral selectivity in more hydrophobic solvents. A quantitatively clearer correlation ($r = 0.98$) in these coordinates was observed when various binary mixtures of two extreme solvents, acetone and carbon tetrachloride, were employed as the reaction media (Figure 4B).

Note that another solvent parameter frequently invoked in non-aqueous enzymology, its polarity,[2f,14] does not appear to be

Table III. The Effect of the Solvent on Prochiral Selectivity of Subtilisin Carlsberg and *Pseudomonas* sp. lipase in the Hydrolysis of **3** into **2**[a]

		prochiral selectivity[c]	
entry	solvent[b]	subtilisin Carlsberg[d]	*Pseudomonas* sp. lipase[e]
1	acetonitrile	0.67	>30
2	nitrobenzene		>30
3	acetone		18
4	cyclohexanone		18
5	butanone		16
6	2-pentanone		16
7	chloroform		9.9
8	tetrahydrofuran	1.1	9.1
9	2-hexanone		8.8
10	dioxane		5.4
11	*tert*-butyl acetate		5.3
12	*tert*-butyl alcohol		4.9
13	*tert*-amyl alcohol		4.8
14	triethylamine		4.7
15	toluene		3.5
16	benzene		3.2
17	carbon tetrachloride	0.56	2.6

[a] Enzyme powders (for exact amounts, which varied from solvent to solvent in order to afford conveniently measured reaction rates, see footnotes *d* and *e* below) were placed in 1 mL of hydrated (for water contents, see footnote *b* below) organic solvents containing 10 mM **3**. Following a 5-s sonication, the reaction mixtures were shaken at 45 °C and 300 rpm; periodically, aliquots were withdrawn and assayed by HPLC as described in the Materials and Methods section. Enzymes were treated as described in footnote *a* to Table II. Only monohydrolysis (i.e., conversion of **3** into **2**, as opposed to **1**) was observed during the initial rate measurements herein. [b] Water content was 0.09% (here and hereafter (v/v)) for entries 2 and 7, 0.65% for entry 11, 0.03% for entries 15 and 16, 0.01% for entry 17, and 1% for all other solvents. Less than 1% of water was used when 1% exceeded the solubility limit of water in a given solvent. All such water contents approximately equaled to three quarters of the saturation level (full saturation of a solvent with water often led to a transformation of enzyme powders into unmanageable glue-like fibrils). [c] Defined as the ratio of the rate of accumulation of the enantiomer of **2** represented by the second peak in Figure 1 to that for the first peak in the same figure. [d] 20 mg/mL in acetonitrile and carbon tetrachloride and 100 mg/mL in tetrahydrofuran. [e] 5 mg/mL in triethylamine, *tert*-butyl alcohol, and *tert*-amyl alcohol; 10 mg/mL in *tert*-butyl acetate and carbon tetrachloride; 20 mg/mL in all other solvents.

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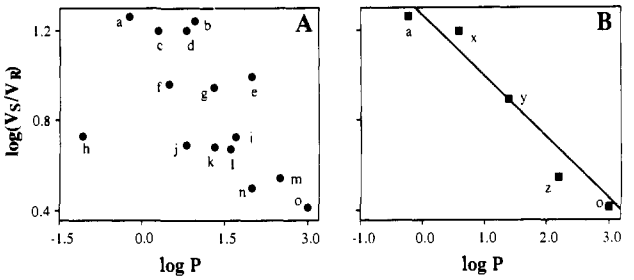

Figure 4. Prochiral selectivity of *Pseudomonas* sp. lipase in the hydrolysis of **3** into **2** as a function of the hydrophobicity of the solvent. Solvent hydrophobicity values, defined as log *P* where *P* is the partition coefficient for a given solvent between 1-octanol and water, were taken from ref 13. Solvents: (a) acetone, (b) cyclohexanone, (c) butanone, (d) 2-pentanone, (e) chloroform, (f) tetrahydrofuran, (g) 2-hexanone, (h) dioxane, (i) *tert*-butyl acetate, (j) *tert*-butyl alcohol, (k) *tert*-amyl alcohol, (l) triethylamine, (m) toluene, (n) benzene, and (o) carbon tetrachloride (A); in B, a and o are the same as in A, and x, y, and z correspond to the following binary mixtures of acetone and carbon tetrachloride, respectively, 75:25, 50:50, and 25:75. Experimental points for acetonitrile and nitrobenzene are not shown because the prochiral selectivity in these solvents was too high to measure. The water content in the solvents was the same as indicated in footnote *b* to Table III; for x, y, and z it was 1% (v/v). For other experimental conditions, see the footnotes to Table III and the Materials and Methods section.

relevant to this case: we found that prochiral selectivities of the lipase in the hydrolysis of **3** into **2** in two isomeric (and hence possessing the same log *P*[13]) solvents, polar *o*-difluorobenzene and apolar *p*-difluorobenzene, were similar (4.3 and 3.4, respectively).

In contrast to subtilisin Carlsberg, the three-dimensional structure of *Pseudomonas* sp. lipase is not yet known. Therefore any mechanistic model of the dependence of the enzyme prochiral selectivity on the solvent has to be speculative. We propose the following hypothesis (Figure 5) to explain this phenomenon: **3** can bind to the active center of the lipase in two distinct modes, stereoselective and nonstereoselective (modes I and II, respectively). In the latter mode, the diester substrate may be oriented in equally probable *pro-S* and *pro-R* positions. Note that all the complexes depicted in Figure 5 are reactive, i.e., the serine head nucleophile of the enzyme[15] is aligned in a manner conducive to a nucleophilic attack on an ester carbonyl. Another distinctive feature of our model is that the enzyme possesses a hydrophobic binding pocket in its active center[15] and that this pocket is occupied by the naphthyl moiety of **3** only in the stereoselective mode of binding (in the nonstereoselective one this moiety is exposed to the solvent, Figure 5).

Analysis of the model presented readily explains the observed inverse relationship between prochiral selectivity and solvent hydrophobicity (Figure 4). In hydrophilic solvents, it is thermodynamically more advantageous for the hydrophobic naphthyl moiety to partition into the hydrophobic binding pocket of the enzyme (as in mode I) than to be exposed to the solvent (as in mode II). In hydrophobic solvents, this advantage vanishes. Therefore, hydrophilic solvents (low log *P*) should favor the stereoselective mode of binding, while hydrophobic ones should exert no substantial stereobias.

The model depicted in Figure 5 affords two additional predictions than can be tested experimentally. First, it predicts that if the naphthyl moiety in **3** is replaced with a much less hydrophobic group, then the prochiral selectivity of the enzyme should drop drastically in hydrophilic solvents (since partitioning into the lipase's hydrophobic pocket will no longer yield a significant thermodynamic gain) and hence the prochiral selectivity will cease to be strongly solvent-dependent. This prediction was verified with a diester substrate where propyl was substituted for naphthyl at the C-2 position. With this substrate, prochiral selectivity of *Pseudomonas* sp. lipase (under the same conditions as in Table III) was just 2.0 in acetonitrile and 1.4 in carbon tetrachloride (compare with >30 and 2.6 for **3**).

Another prediction afforded by our model involves the influence of a hydrophobic additive. Imagine that we add an analog of the naphthyl moiety in **3**, e.g., 1-naphthol, to the reactive system. It follows from Figure 5 that this additive, which is capable of binding into the hydrophobic pocket, will compete with **3** for binding to the enzyme in mode I but not in II. Consequently, one would expect that naphthol will shift the equilibrium between the two

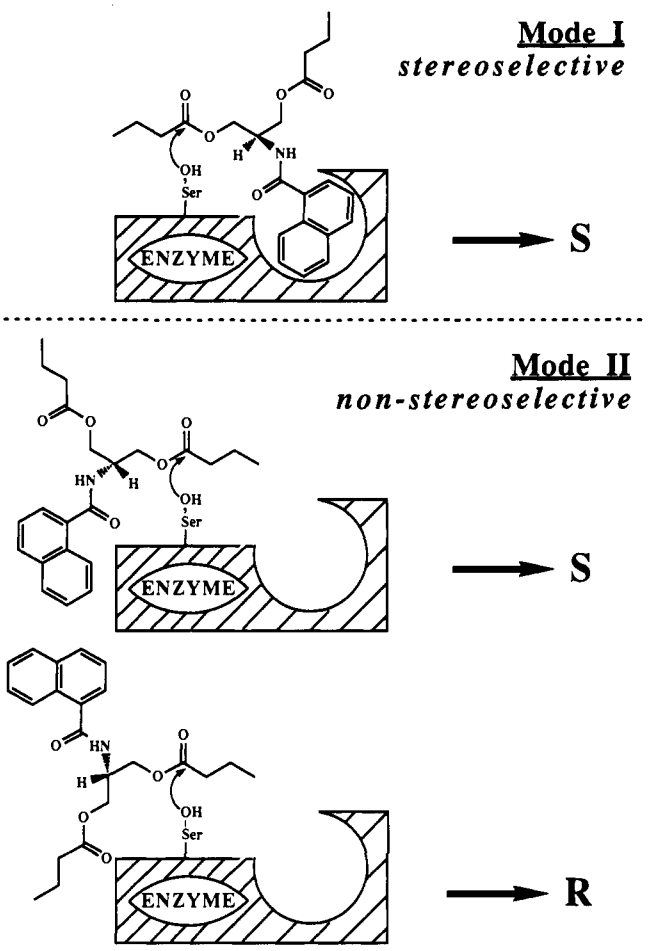

Figure 5. Schematic representation of the modes of binding of **3** to *Pseudomonas* sp. lipase. The unfinished circle denotes a hydrophobic binding pocket in the active center, and Ser-OH refers to the head nucleophile of the enzyme which conducts a nucleophilic attack (shown by curved arrows) on one of the ester's carbonyls. See text for details.

Table IV. The Effect of 1-Naphthol on Prochiral Selectivity of *Pseudomonas* sp. lipase in the Hydrolysis of **3** into **2** in Acetonitrile[a]

concentration of 1-naphthol, M	initial rate, v(μM·h^{-1}·mg^{-1}) formation of *S* enantiomer	formation of *R* enantiomer	prochiral selectivity, v_S/v_R
0	5.3	<0.18[b]	>30
0.1	3.6	0.35	10
1.0	1.2	0.42	2.9

[a] Lipase powder (20 mg) was placed in 1 mL of acetonitrile containing 1% water, 10 mM **3**, and the indicated concentration of 1-naphthol. Following a 5-s sonication, the reaction mixtures were shaken at 45 °C and 300 rpm; periodically, aliquots were withdrawn and assayed by HPLC as described in the Materials and Methods section. Only monohydrolysis (i.e., conversion of **3** into **2**) was observed during the initial rate measurements herein. [b] The sensitivity limit of our assay.

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(15) By analogy with other lipases whose structure has been determined by X-ray crystallography: Blow, D. *Nature* **1990**, *343*, 694–695. Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature* **1990**, *343*, 767–770. Winkler, F. K.; D'Arcy, A.; Hunziker, W. *Nature* **1990**, *343*, 771–774. Schrag, J. D.; Li, Y.; Wu, S.; Cygler, M. *Nature* **1991**, *351*, 761–764. Derewenda, U.; Brzozowski, A. M.; Lawson, D. M.; Derewenda, Z. S. *Biochemistry* **1992**, *31*, 1532–1541.

modes toward the latter, thereby lowering v_S, increasing v_R, and hence decreasing v_S/v_R (i.e., prochiral selectivity). Inspection of Table IV reveals that all of these effects were indeed observed. For example, addition of 1 M 1-naphthol to acetonitrile depresses the rate of the enzymatic formation of the *S* enantiomer of **2** more than 6-fold, increases the rate of the production of (*R*)-**2** from a nondetectable to a readily measurable level, and leads to a more than 10-fold drop in prochiral selectivity.

In closing, the results of this study demonstrate that prochiral selectivity of an enzyme can be greatly affected by the solvent. The discovered dependence can be rationalized in terms of a simple structural model which, although by no means proven, correctly predicts the effects of solvent hydrophobicity, substrate variation, and a selected additive on the enzyme's prochiral selectivity. Thus this work constitutes yet another step toward enzyme selectivity "to order". In addition, the phenomenon of prochiral selectivity controlled by the solvent, if general, should prove useful in further enhancing the synthetic potential of enzymes in organic solvents.[1]

Materials and Methods

Enzymes. Subtilisin Carlsberg (serine protease from *Bacillus licheniformis*), *Aspergillus oryzae* protease, and porcine pancreatic lipase were purchased from Sigma Chemical Co. The concentration of the competent active centers in subtilisin, determined by spectrophotometric titration with *N-trans*-cinnamoylimidazole,[16] was 54%. *Pseudomonas* sp. lipase, *Pseudomonas* lipoprotein lipase, *Mucor* sp. lipase, *Rhizopus oryzae* lipase, *Bacillus subtilis* protease, *Mucor javanicus* lipase, *Aspergillus niger* lipase, and *Rhizopus* sp. protease were obtained from Amano International Enzyme Co. *Penicillium roqueforti*, *Rhizopus arrhizus*, and *Rhizopus javanicus* lipases were purchased from Biocatalysts Ltd. *Chromobacterium viscosum* lipase was a gift from FinnSugar Biochemicals (now Cultor). All enzymes were used directly as supplied by the manufacturers, except for subtilisin which in some instances (when specifically stated) was "pH-adjusted" by lyophilization from a buffered aqueous solution (see footnote *a* to Table II).

Chemicals and Solvents. All commercially obtained chemicals used in this work (analytical grade or purer) were from Aldrich Chemical Co., except for vinyl butyrate (99+% pure by gas chromatography) which was from American Tokyo Kasei Co. Organic solvents were either purchased in the anhydrous form (in Aldrich Sure/Seal bottles, water content below 0.005%) or dehydrated by shaking with 3-Å molecular sieves (Linde) to bring the water content[17] below 0.01%.

Kinetic Measurements. Whether in the transesterification between **1** and vinyl butyrate or in the hydrolysis of **3** (for experimental conditions, see footnote *a* to Tables I–IV), the concentration of **2** was determined by HPLC using the Regis Pirkle ionic D-phenylglycine chiral column. The flow rate was 1 mL/min, different binary mixtures of hexane and 2-propanol (9:1 for the hydrolysis of **3** and 85:15 for the transesterification of **1**; hereafter, these mobile phases are referred to as A and B, respectively) were used as mobile phases, and the reactants were followed by UV absorbance at 280 nm. For each reaction studied, 20-μL aliquots of the reaction mixture were withdrawn and subjected to centrifugation to separate the enzyme powder, and then 5-μL aliquots of the supernatant were injected into the HPLC column.

Individual enantiomers of 2-(butyrylamino)-3-hydroxypropyl butyrate could not be separated by HPLC as described above. Therefore, we employed a precolumn derivatization with (*R*)-(1-naphthyl)ethyl isocyanate (by analogy with (*R*)-phenylethyl isocyanate[18]). After certain time periods, the reaction mixture was centrifuged to remove the enzyme, and a 100-μL sample of the supernatant was evaporated under vacuum. To the residue, 100 μL of the 1% derivatizing agent in toluene was added, the mixture was placed in an ampule, the toluene was evaporated under vacuum, and the ampule was sealed and heated at 120 °C overnight. After being cooled to room temperature, the ampule was opened, 20 μL of MeOH was added, and 5 μL of the reaction mixture was injected into the HPLC column (94:6 hexane/2-propanol as a mobile phase).

Computer Modeling. All interactive computer modeling experiments were performed on an Evans and Sutherland PS300 workstation using the program Frodo (Version 6.0).[19] Data points for subtilisin Carlsberg[8b] and for its complex with the inhibitor eglin *c*[8a] were obtained from the Brookhaven Protein Data Bank. Molecular models of substrate molecules were constructed and subjected to energy minimization through the program Quanta on the Silicon Graphics system, and the resultant data points were reformatted for use on the PS300 Frodo system.

All manipulations (rotations and translations) of the enzyme and substrate molecules were performed in real time on the PS300 Frodo system, including the docking (movement) of the diester substrate into the enzyme's active center to produce a model of the reactive, noncovalent substrate–enzyme complex and the docking of the diol substrate into the acyl-enzyme's active center to produce a model of the reactive, noncovalent substrate–acyl-enzyme complex.

Docking (which essentially is a constraint satisfaction problem) of nucleophilic substrates such as **1** necessitated the construction of an acyl-enzyme (butyrylsubtilisin) model on the PS300 system. A model of butyric acid was constructed using Quanta, the hydroxyl group deleted, and the remainder covalently attached to the serine 221 residue in the active site of subtilisin in a way analogous to indoleacryloyl-α-chymotrypsin.[9] As described in the text, in order to construct a model of either *pro-R* or *pro-S* binding modes of **1**, one of the diol's hydroxyl groups was fitted into the position occupied by the water molecule conducting the nucleophilic attack on the ester carbonyl. The torsion angles within **1** were rotated to optimize the fit of the diol against the acyl-enzyme, but the substrate's reactive hydroxyl group was not moved or rotated from its position facing the ester carbonyl of butyrylsubtilisin in order to preserve the reactive nature of the complex. To find the second mode of binding of the diol (which would lead to the product with chirality opposite to that of the first case), the other hydroxyl of **1** was chosen to be fixed in the active site of butyrylsubtilisin, and substrate torsion angles were again rotated for fit optimization as described above.

The diester was docked into the active site of the free enzyme similarly to the diol (see text). Eglin *c* was used as a guide such that one of the ester moieties of **3** was overlapped with the active site portion of the inhibitor and then the latter was deleted. This reactive portion of **3** was fixed, and the torsion angles of the remaining portion of the substrate were rotated in order to optimize the fit. The binding mode leading to the product of opposite chirality was determined by choosing the other butyryl group to be the reactive portion of the substrate.

Water-accessible surface areas were calculated using the program Frodo. The "surf" command was utilized to display a space-filling model of the substrate in the enzyme active site. The computer then requested information regarding the context of the substrate and a clear space limit. Displaying this space filling model in the context of the whole enzyme and setting a clear space limit to 3.0 Å (the approximate diameter of a water molecule) allowed us to display only those portions of the substrate which were water-accessible. In essence, the computer rolled a hypothetical ball with a diameter of 3.0 Å over the substrate surface and displayed only those portions of the latter where the ball did not impinge on the enzyme surface. The computer then outputted quantitative data on the water-accessible surface area of the substrate.

(*R*)- and (*S*)-*N*-(1-Naphthoyl)serine Ethyl Ester (used for the synthesis of **1** and for absolute structure determinations). One gram (5.9 mmol) of (*R*) or (*S*) serine ethyl ester was dissolved in 50 mL of dry pyridine. To this solution, stirred at 25 °C, 0.9 mL (5.9 mmol) of 1-naphthoyl chloride was added every 30 min in 0.05-mL portions. The stirring was continued overnight at 25 °C. The solution was then concentrated by evaporation to a 10-mL volume and poured into 50 mL of 10% HCl at 0 °C. The product was extracted with four 100-mL portions of methylene chloride. The combined organic layers were washed with 100 mL of a saturated solution of $NaHCO_3$, 100 mL of water, and 100 mL of brine and then dried over $MgSO_4$. The organic solvent was removed by rotatory evaporation, and the residue was purified by silica gel column (32 × 2 cm) chromatography; the mobile phase was a mixture of hexane and ethyl acetate (1:1 (v/v)). The yield of *N*-(1-naphthoyl)-serine ethyl ester was 1.25 g (77% of theoretical). Mp 92–93 °C; $[\alpha]^{25}_D$ −2.84° (*c* 2.1, $CHCl_3$) for the (*R*) enantiomer, $[\alpha]^{25}_D$ +3.04° (*c* 2.3, $CHCl_3$) for the (*S*) enantiomer. ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 8.4–7.4 (7 H, m), 6.96 (1 H, d, *J* = 7.0 Hz), 4.97 (1 H, ddd, *J* = 7.0, 3.6, 3.6 Hz), 4.31 (2 H, q, *J* = 7.2 Hz), 4.16 (1 H, dd, *J* = 3.6, 11.3 Hz), 4.08 (1 H, dd, *J* = 3.6, 11.3 Hz), 1.35 (3 H, t, *J* = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ 170.3, 169.8, 133.7, 133.4, 131.0, 130.1, 128.3, 127.2, 126.4, 125.5, 125.3, 124.6, 63.4, 62.0, 55.2, 14.1. Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.90; H, 5.92; N, 4.88; O, 22.30. Found for the (*R*) enantiomer: C, 66.92; H, 5.89; N, 4.72; O, 22.14. For the (*S*) enantiomer: C, 66.80; H, 5.84; N, 4.75; O, 21.91. Typical retention times for the HPLC (mobile phase A) were 41 min for the (*R*) enantiomer and 43 min for the (*S*) enantiomer.

2-(1-Naphthoylamino)-1,3-propanediol (1). In a 100-mL, three-necked, round-bottom flask equipped with a condensor, a drop funnel, and a magnetic stirrer, to 1.43 g (5 mmol) of *N*-(1-naphthoyl)serine ethyl ester dissolved in 20 mL of *tert*-butyl alcohol was added 4.75 g (12.5

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mmol) of $NaBH_4$. The mixture was refluxed, and 4 mL of MeOH was added dropwise over 1 h; the heating and stirring were continued for 1 h. The mixture then was cooled to room temperature, and 20 mL of cold water was added to quench the reaction. The product was extracted with four 100-mL portions of ethyl acetate, and the organic phase was washed and dried as described above. After the solvent was removed by rotary evaporation, the residue was purified by crystallization in hexane containing small amounts of ethyl acetate and MeOH. The yield of **1** was 8.50 mg (70% of theoretical). Mp 109–111 °C. ^1H NMR (CD_3OD, TMS as an internal standard) δ 8.3–7.4 (7 H, m), 7.68 (1 H, d, J = 8.7 Hz), 4.28 (1 H, ddd, J = 8.7, 5.7, 5.7 Hz), 3.81 (1 H, dd, J = 5.7, 11.3 Hz), 3.74 (1 H, dd, J = 5.7, 11.3 Hz). ^{13}C NMR (CD_3OD) δ 173.6, 170.0, 136.7, 136.6, 136.0, 132.3, 130.2, 129.6, 128.8, 128.3, 127.3, 126.8, 63.1, 56.1. Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.57; H, 6.12; N, 5.72; O, 19.59. Found: C, 66.33; H, 6.38; N, 5.31; O, 20.98. A typical retention time for the HPLC (mobile phase B) was 57 min.

2-(1-Naphthoylamino)trimethylene Dibutyrate (3). Fifty milligrams (0.2 mmol) of **1** was dissolved in 5 mL of dry pyridine. To the resultant solution, stirred at 25 °C, 0.16 mL (15 mmol) of butyryl chloride was added every 30 min in 0.02-mL portions. The stirring was continued for 1 h, and then the mixture was poured into 25 mL of 10% HCl at 0 °C. The product was extracted, washed, and dried as **1** above, except that all volumes were halved. After removal of the solvent by rotary evaporation, the residue was purified by TLC on a silica gel plate with a mixture of hexane and ethyl acetate (1:1 (v/v)) as a mobile phase. The area at the RF value of 0.46 was collected, and the silica gel was washed with ethyl acetate. After evaporation of the solvent, the yield of **3** was 57 mg (74% of theoretical). Mp 63–64 °C. ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 8.5–7.5 (7 H, m), 6.31 (1 H, d, J = 9.0 Hz), 4.7 (1 H, ddd, J = 9.0, 5.5, 5.5 Hz), 4.45 (2 H, dd, J = 5.5, 11.4 Hz), 4.22 (2 H, dd, J = 5.5, 11.4 Hz), 2.33 (4 H, t, J = 7.4 Hz), 1.66 (4 H, tq, J = 7.4 Hz), 0.94 (6 H, t, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$) δ 173.4, 169.8, 134.0, 133.8, 130.9, 130.1, 128.4, 127.2, 126.5, 125.2, 125.0, 124.7, 62.9, 48.2, 36.0, 18.4, 13.6. Anal. Calcd for $C_{22}H_{27}NO_5$: C, 68.57; H, 7.01; N, 3.64; O, 20.78. Found: C, 68.32; H, 6.82; N, 3.40; O, 20.47. A typical retention time for the HPLC (mobile phase A) was 35 min.

3-Hydroxy-2-(1-naphthoylamino)propyl butyrate (2) (a racemate used to calibrate the HPLC) was prepared from **1** similarly to **3** except that only 0.09 mL (0.91 mmol) of butyryl chloride was added. The reaction was followed by gas chromatography (10-m HP-5 capillary column coated with 5% phenylsilicone/95% methylsilicone gum) and stopped when 95% of the diol was transformed. The product was purified by TLC on a silica gel plate with a mixture of hexane and ethyl acetate (1:3 (v/v)) as a mobile phase. The area at the RF value of 0.37 was collected, and the silica gel was washed with ethyl acetate. After evaporation of the solvent, the yield of racemic **2** was 28 mg (44% of theoretical). Mp 82–83 °C. ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 8.3–7.5 (7 H, m), 6.64 (1 H, d, J = 8.0 Hz), 4.5 (1 H, tddd, J = 5.8, 8.0, 4.2, 4.2 Hz), 4.34 (2 H, d, J = 5.8 Hz), 3.82 (1 H, dd, J = 4.2, 11.6 Hz), 3.73 (H, dd, J = 4.2, 11.6 Hz), 2.32 (2 H, t, J = 7.4 Hz), 1.64 (2 H, tq, J = 7.4, 7.4 Hz), 0.93 (3 H, t, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$) δ 174.1, 169.9, 133.9, 133.7, 130.8, 130.0, 128.3, 127.1, 126.4, 125.2, 125.1, 124.6, 62.6, 61.8, 50.8, 36.0, 18.3, 13.6. Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.57; H, 6.67; N, 4.44; O, 20.32. Found: C, 67.57; H, 6.79; N, 4.25; O, 20.83. Typical retention times for the HPLC were 50 and 27 min for the (*R*) enantiomer and 56 and 29 min for the (*S*) enantiomer (mobile phases A and B, respectively).

2-(Butyrylamino)trimethylene Dibutyrate. Serinol hydrochloride (300 mg, 2.4 mmol) was dissolved in 25 mL of dry pyridine. To this solution, stirred at 25 °C, 2.5 mL (24 mmol) of butyryl chloride was added dropwise over 2 h, and the stirring was continued overnight. The solution, concentrated by evaporation of the solvent to a 10-mL volume, was subsequently poured into 50 mL of 10% HCl at 0 °C. The product was extracted, washed, dried, and purified as *N*-(1-naphthoyl)serine ethyl ester above. After evaporation of the solvent, the yield of the product was 548 mg (77% of theoretical). Mp 37–38 °C. ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 5.76 (1 H, d, J = 8.3 Hz), 4.48 (1 H, ddd, J = 8.3, 5.4, 5.4 Hz), 4.32 (2 H, dd, J = 5.4, 11.3 Hz), 4.01 (2 H, dd, J = 5.4, 11.3 Hz), 2.31 (4 H, t, J = 7.4 Hz), 2.16 (2 H, t, J = 7.3 Hz), 1.65 (6 H, tq, J = 7.3, 7.3 Hz), 0.95 (9 H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$) δ 173.4, 172.7, 62.7, 47.6, 38.6, 35.0, 19.0, 18.4, 13.6. Anal. Calcd for $C_{15}H_{27}NO_5$: C, 59.80; H, 8.97; N, 4.65; O, 26.58. Found: C, 59.67, H, 8.97; N, 4.58; O, 26.10.

2-(Butyrylamino)-3-hydroxypropyl Butyrate (a racemate used to calibrate the HPLC). One hundred milligrams (0.78 mmol) of serinol hydrochloride was dissolved in 5 mL of dry pyridine. To this solution, stirred at 25 °C, 0.2 mL (1.9 mmol) of butyryl chloride was added every 30 min in 0.04-mL portions. The stirring was continued overnight at 25 °C. The resultant solution was poured into 25 mL of 10% HCl at 0 °C. The product was extracted with four 50-mL portions of ethyl acetate, and the combined organic layers were washed and dried as **3** above. After removing the solvent by rotary evaporation, the residue was purified by TLC as **2** above. The yield of the oily product was 38 mg (21% of theoretical). ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 6.17 (1 H, s large), 4.2–4.5 (3 H, m), 3.73 (1 H, dd, J = 3.3, 11.2 Hz), 3.60 (1 H, dd, J = 3.3, 11.2 Hz), 2.85 (1 H, s large), 2.33 (2 H, t, J = 7.3 Hz), 2.21 (2 H, t, J = 7.3 Hz), 1.66 (4 H, tq, J = 7.3, 7.3 Hz), 0.96 (6 H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$) δ 174.0, 173.8, 62.5, 61.4, 50.2, 38.4, 35.9, 18.9, 18.2, 13.4. Anal. Calcd for $C_{11}H_{21}NO_4$: C, 57.14; H, 9.09; N, 6.06; O, 27.71. Found: C, 56.91; H, 9.24; N, 8.85; O, 28.08.

Enzymatically Prepared (*R*)-2. In a 30-mL screw-cap scintillation vial, 39.2 mg (0.16 mmol) of **1** was dissolved in 8 mL of anhydrous acetonitrile containing 182 mg (1.6 mmol) of vinyl butyrate. Then 800 mg of *Pseudomonas* sp. lipase was added and, following a 5-s sonication, the vial was shaken at 45 °C and 300 rpm. When all the diol was acylated (in 30 min, judged by gas chromatography), the mixture was filtrated on a glass filter funnel. The solid enzyme was washed with 10 mL of acetonitrile to recover all the **2**. The subsequent workup was the same as described above for chemically synthesized racemic **2**. The yield of (*R*)-**2** was 28 mg (56% of theoretical, ee >95% by HPLC). Mp 60–65 °C, $[\alpha]_D^{25}$ +1.26° (*c* 2.8, $CHCl_3$). ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 8.3–7.5 (7 H, m), 6.64 (1 H, d, J = 8.0 Hz), 4.5 (1 H, tddd, J = 5.8, 8.0, 4.2, 4.2 Hz), 4.34 (2 H, d, J = 5.8 Hz), 3.82 (1 H, dd, J = 4.2, 11.6 Hz), 3.73 (H, dd, J = 4.2, 11.6 Hz), 2.32 (2 H, t, J = 7.4 Hz), 1.64 (2 H, tq, J = 7.4, 7.4 Hz), 0.93 (3 H, t, J = 7.4 Hz). Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.57; H, 6.67; N, 4.44; O, 20.32. Found: C, 67.77; H, 6.71; N, 4.24; O, 20.71. A typical retention time for the HPLC (mobile phase A) was 50 min.

Enzymatically Prepared (*S*)-2. In a 30-mL screw-cap scintillation vial, 38.5 mg (0.10 mmol) of **3** was dissolved in 5 mL of acetonitrile containing 1% H_2O. Then 500 mg of *Pseudomonas* sp. lipase was added and, following a 5-s sonication, the vial was shaken at 45 °C and 300 rpm. After 24 h, the enzyme was removed by filtration. The workup was the same as for the (*R*) enantiomer above. The yield of (*S*)-**2** was 14 mg (44% of theoretical, ee = 99% by HPLC). Mp 68–69 °C, $[\alpha]_D^{25}$ −1.46° (*c* 1.4, $CHCl_3$). ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 8.3–7.5 (7 H, m), 6.58 (1 H, d, J = 8.0 Hz), 4.5 (1 H, tddd, J = 5.9, 8.0, 4.1, 4.1 Hz), 4.36 (2 H, d, J = 5.8 Hz), 3.81 (1 H, dd, J = 4.1, 11.8 Hz), 3.76 (H, dd, J = 4.1, 11.8 Hz), 3.0 (1 H, s large), 2.33 (2 H, t, J = 7.5 Hz), 1.65 (2 H, tq, J = 7.5, 7.5 Hz), 0.94 (3 H, t, J = 7.5 Hz). Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.57; H, 6.67; N, 4.44; O, 20.32. Found: C, 67.99; H, 6.84; N, 4.26; O, 20.83. A typical retention time for the HPLC (mobile phase A) was 56 min.

(*R*)- and (*S*)-*N*-(1-Naphthoyl)-*O*-butyrylserine. Either (*R*)-**2** or (*S*)-**2** prepared above was dissolved in 3 mL of acetone, and 5 drops of the Jones reagent (chromic oxide in concentrated sulfuric acid)[20] were slowly added with stirring at 0 °C. The resultant brown solution was stirred for 1 h at room temperature, and then 2-propanol was added dropwise to destroy the excess of the oxidant (a green precipitate appeared). The mixture was dissolved in 10 mL of water, and the product was extracted with four 25-mL portions of ethyl acetate. The organic solution was washed with two 25-mL portions of brine and dried over $MgSO_4$. After evaporation of the solvent, the product was used without further purification in the next step. ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 8.3–7.5 (7 H, m), 6.92 (1 H, d, J = 7.8 Hz), 5.18 (1 H, ddd, J = 5.8, 3.6, 3.6 Hz), 4.65 (1 H, dd, J = 3.6, 10.9 Hz), 4.60 (H, dd, J = 3.6, 10.9 Hz), 2.33 (2 H, t, J = 7.4 Hz), 1.61 (2 H, tq, J = 7.4 Hz), 0.90 (3 H, t, J = 7.4 Hz).

(*R*)- and (*S*)-*N*-(1-Naphthoyl)serine Ethyl Ester. Either enantiomer prepared in the preceding paragraph was dissolved in 3 mL of absolute EtOH and then 0.05 mL of H_2SO_4 was added. The resultant solution was refluxed for 5 h before cooling to room temperature and then poured into 20 mL of cold water and extracted with four 25-mL portion of ethyl acetate. The organic solution was washed with two 25-mL portions of brine and dried over $MgSO_4$. After evaporation of the solvent by rotary evaporation, without further purification, the products were compared by HPLC with their counterparts prepared chemically from (*R*) or (*S*) serine ethyl esters (as described before). ^1H NMR ($CDCl_3$, TMS as an internal standard) δ 8.4–7.4 (7 H, m), 6.96 (1 H, d, J = 7.0 Hz), 4.97 (1 H, ddd, J = 7.0, 3.6, 3.6 Hz), 4.31 (2 H, q, J = 7.2 Hz), 4.16 (1 H, dd, J = 3.6, 11.3 Hz), 4.08 (1 H, dd, J = 3.6, 11.3 Hz); 1.35 (3 H, t, J = 7.2 Hz). Typical retention times for the HPLC (mobile phase A) were 41 and 43 min for the (*R*) and (*S*) enantiomers, respectively.

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