Chemoenzymatic Synthesis of the C-13 Side Chain of Taxol: Optically-Active 3-Hydroxy-4-phenyl β-Lactam Derivatives

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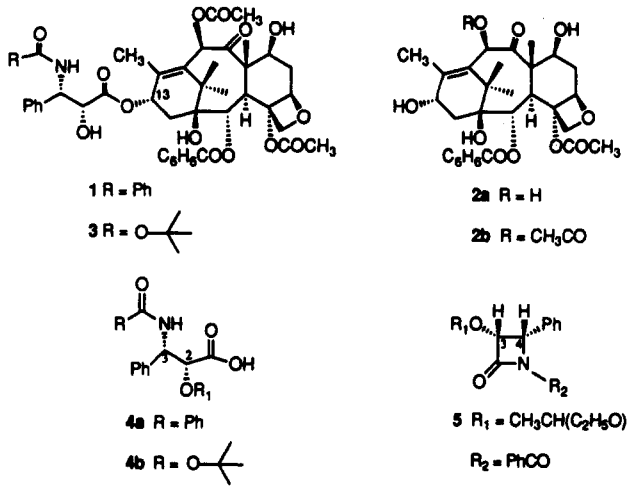
Received October 8, 1992

Enantiomerically-pure 3-hydroxy-4-phenyl β-lactam derivatives have been successfully prepared via enantioselective hydrolyses and transesterifications of racemic esters and alcohols respectively catalyzed by bacterial lipases. These lipases also catalyzed highly enantioselective cleavage of the β-lactam ring of (±)-10 to yield derivatives of (2*R*,3*S*)-phenylisoserine in high enantiomeric excess. The resolved enantiomers are important intermediates in the synthesis of the C-13 side chain of taxol.

In 1971, Wani et al.[1] isolated taxol (1), a unique diterpene from the bark of the Western Yew (*Taxus brevifolia*). Taxol has now undergone several clinical trails and has exhibited activity against malignant melanoma and carcinoma of the ovary including cisplatin-refractory ovarian cancer.[2] More recently, there are reports[3] suggesting that taxol may also be effective in the treatment of breast and lung cancer. Taxol inhibits cell division at the G_2–M interface by inducing tubulin polymerization to form stable nonfunctional microtubules, thus disrupting the tubulin–microtubule equilibrium.[4]

Currently, taxol is obtained from the bark of the Pacific yew. Unfortunately, the concentration of taxol in the bark is relatively low and a laborious purification process is needed to secure taxol in pure form. It is estimated that about 20 000 pounds of the yew's bark—the equivalent of 2000 to 4000 trees—are needed to produce 1 kg of taxol.[5] Approximately 38 000 trees will be needed this year to conduct the limited clinical trials. Since there is no assurance that the rate of utilization will remain constant in the future, many environmentalists have raised concerns about the potential mass destruction of yews. Hence if and when taxol receives FDA approval for use in treating certain cancers, a more efficient means of obtaining taxol is necessary.

Although a vast array of synthetic strategies have been developed for the construction of the taxane skeleton,[6] to date only moderate success has been achieved in the total synthesis of naturally occurring taxanes. To overcome this supply problem, the French group turned to semisynthesis.[7] They found an abundant ornamental bush, *T. baccata*, whose (renewable) needles contain high concentrations of 10-deacetylbaccatin III[8] (**2a**) (1 g/kg), which was successfully transformed into **1** or the analog, taxotere (**3**), via reaction with a derivative of *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine, **4a** or **4b**, respectively.[7] Alternatively, **1** may be prepared by direct coupling of **2b** with a suitably protected derivative of (3*R*,4*S*)-3-hydroxy-4-aryl β-lactam (**5**).[9]



Since the C-13 side-chain moiety *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine (**4a**) is essential for the potent antitumor activity of taxol, there is a need for developing efficient processes[10] for the preparation of **4a** or **5** in their enantiopure forms.

In recent years, there has been an increasing interest in the use of hydrolytic enzymes to produce optically-active compounds. In particular, lipases have been widely used

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(9) Holton, R. A. Method for preparation of Taxol. Eur. Pat. Appl. 1990, 0400-971 A2, pp 1–18.

(10) For nonenzymatic syntheses of **4a**, see: (a) Denis, J. N.; Greene, A. E.; Serra, A. A.; Luche, M. J. *J. Org. Chem.* **1986**, *51*, 46–50. (b) Denis, J. N.; Correa, A.; Greene, A. E. *Ibid.* **1990**, *55*, 1957–1959. (c) Ojima, I.; Habas, I.; Zhao, M.; Georg, G. I.; Jayasinghe, L. R. *Ibid.* **1991**, *56*, 1681–1683. (d) Denis, J. N.; Correa, A.; Greene, A. E. *Ibid.* **1991**, *56*, 6939–6942.

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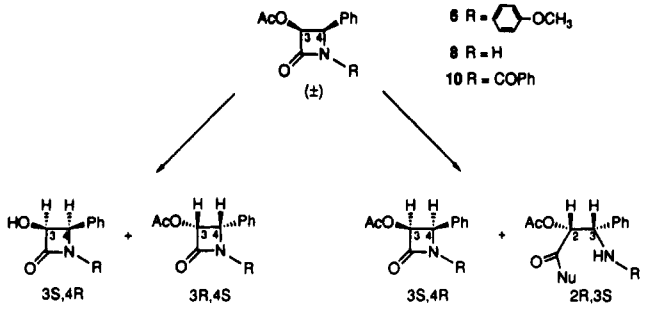
Scheme I. Possible Modes of Enzymatic Cleavage



for the kinetic resolution of racemic alcohols and carboxylic esters.[11] Their commercial availability and relative stability make them an attractive class of catalysts for effecting industrial-scale kinetic resolutions.

This paper describes the application of lipases for the preparation of optically active 3-hydroxy-4-aryl β-lactam derivatives. The selection of the substrates **6**, **8**, and **10** was based on their ready accessibility[12] and their utility in the enantiospecific synthesis of **1**. The high stereochemical specificity and broad substrate specificity of the lipases have allowed the successful preparation of the requisite intermediates for the C-13 amino acid side chain of taxol. The experimental procedures of biocatalytic enantioselective hydrolyses in aqueous media and transesterification in organic solvents are reported herein.

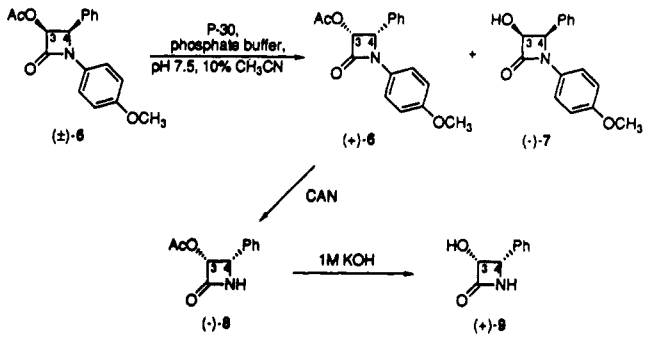
Results

An inspection of the substrates **6**, **8**, and **10** reveals that there are two sites where lipases could effect the kinetic resolution by catalyzing the enantioselective cleavage of either the ester at C-3 or the β-lactam ring (Scheme I). The stereochemical preference of the reaction would be dependent on the lipase used.

Our initial experiments were designed to find the most suitable lipase for catalyzing the proposed enantioselective cleavages. Of the seven commercial lipases examined on (±)-**6** in phosphate buffer (pH 7.5), three of these (P-30, K-10, and PL) exhibited high enantioselectivity ($E = >50$) in cleaving the acetoxy ester at C-3, but unfortunately, the rate of the reaction was slow as evidenced by the low extent of conversion and the time of incubation (Table I).

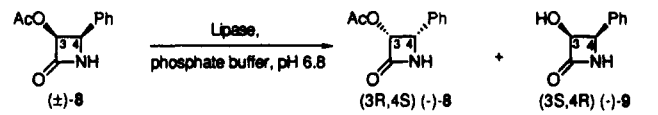
A common approach for improving the biocatalytic reaction rates of water-insoluble substrates is the use of cosolvents.[13] The effect of various organic cosolvents on the rate of enantioselective hydrolysis of (±)-**6** by the AP lipase is shown in Table II. The most striking observation was that obtained with 10% CH_3CN, which markedly improved the reaction rate and the enantioselectivity of the reaction from an E value[14] of 2 to >100. The results in Table III demonstrate that the beneficial effects of CH_3CN may be extended to the other lipases, suggesting that this stimulatory effect may be of more general occurrence.

The stereochemical preference of the P-30 lipase catalyzed hydrolysis of (±)-**6** was determined by establishing the absolute configuration of the remaining substrate, (+)-**6**, to be (3*R*,4*S*) via the following series of reactions:



Oxidation of (+)-**6** afforded (−)-**8** which upon hydrolysis gave (+)-**9**, whose physical constants were in agreement with the reported values of (3*R*,4*S*)-(+)-**9**,[10c] mp 187–188°, $[\alpha]^{20}_D$ +198.8° (*c* 1.0, CH_3OH). This series of experiments confirm that the P-30 lipase preferentially hydrolyzed the (3*S*,4*R*) enantiomer of **6**. Since the optical rotations of the remaining substrate fraction derived from all the lipase-catalyzed hydrolysis of (±)-**6** are positive, one may conclude that all of these lipases possess the same (3*S*,4*R*) stereochemical preference.

Next we examined the enantioselective properties of three *Pseudomonas* lipases (P-30, AK, and K-10) on the substrate (±)-**8** and the results are tabulated in Table IV. While both P-30 and AK catalyzed the hydrolysis of the acetoxy ester of **8** rapidly and with a high degree of enantioselectivity, in contrast the rate of hydrolysis of the acetoxy ester in **8** by K-10 was slow even with 10% CH_3CN as cosolvent. All three enzymes have the same (3*S*,4*R*) stereochemical preference as indicated by the negative sign of optical rotation of the product, **9**.[10c]



The introduction of the *N*-benzoyl substituent in **8** causes the β-lactam ring to be rather unstable in aqueous media. For example, the β-lactam ring of **10** was readily hydrolyzed nonenzymatically in pH 7.5 phosphate buffer. Hence, a more suitable medium is required for conducting the biocatalytic transformation. Table V summarizes the results of the experiments using immobilized P-30 enzyme on Celite in various organic solvents at 50 °C, keeping water to a minimum (1 equiv). Under these conditions, it is apparent that the most suitable solvents were *tert*-butyl methyl ether (*t*-BuOMe) and isopropyl ether; good reaction rates and enantioselectivity were achieved in these environments. Although high enantioselectivity was attained in benzene and dichloroethane, the rates of ester hydrolysis were simply too slow to be useful in these media.

To optimize this biocatalytic system, we examined the water content of the reaction medium (Table VI). While the enantioselectivity of the hydrolytic reaction remained unchanged (E = 100) as the water content is increased, a marked improvement in the reaction rate was noted by raising the water content to 10 molar equiv. The results of the effect of temperature on this biocatalytic reaction

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Table I. Lipase-Catalyzed Enantioselective Hydrolysis of (±)-6

		remaining ester (+)-6			product (−)-7				
lipase	time (h)	yield (%)[a]	$[\alpha]^{26}_D$ (deg)[b]	ee_S (%)[c]	yield (%)	$[\alpha]^{26}_D$ (deg)[b]	ee_P (%)[c]	c (%)[d]	E[e]
P-30	24	81	+1.5	17	13	−189	99.5	14	>100
K-10	50	70	+4.0	33	24	−178	99.5	25	>100
AP	50	76	+0.1	5	16	−26	20	19	2
AK	89	82	+0.1	5	5	−167	80	6	10
PL	149	57	+2.3	46	26	−171	94	33	54
CCL	288	84		5	2	−163	73		7
PPL	240	54			6	−16	17		1

[a] Isolated yield. [b] (c 1.0, $CHCl_3$). [c] Determined by NMR [Eu(hfc)$_3$]. [d] Conversion (c) = $ee_s/(ee_s + ee_p)$. [e] Enantiomeric ratio (E) = $\ln[(1 - c)(1 + ee_P)]/\ln[(1 - c)(1 - ee_P)]$.

Table II. Effect of Cosolvents on Lipase AP-Catalyzed Hydrolysis of (±)-6

		remaining ester (+)-6			product (−)-7				
organic cosolvent	time (h)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee_S (%)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee_P (%)	c (%)	E
none	18	76	+0.1	5	18	−26	20	19	2
CH_3CN	45	55	+5.0	82	45	−173	97	46	>100
THF	21	77	+2.0	39	21	−179	95	29	53
$(CH_3)_2C{=}O$	45	54	+7.0	97	45	−162	80	55	36
DMF	30	70	+3.0	41	30	−129	85	33	19
C_2H_5OH	22	75	+2.0	23	22	−137	79	23	10
DMSO	15	76	+0.3	5	16	−59	26	16	2

Table III. Effect of CH_3CN on the Lipase-Catalyzed Hydrolysis of (±)-6

		remaining ester (+)-6			product (−)-7				
lipase	time (h)	yield (%)[a]	$[\alpha]^{26}_D$ (deg)	ee_s (%)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee_P (%)	c (%)	E
P-30	23	47	+8.0	99.5	46	−167	98	51	>100
K-10	29	44	+8.0	95	39	−170	99.5	49	>100
AP	29	55	+5.0	82	44	−173	97	46	>100
AK	89	67	+3.0	54	28	−174	99	35	>100
PL	109	80	+0.5	9	8	−179	99	8	>100
CCL	288	60	+0.3	9	7	−177	99.5	8	>100
PPL	240	85		5	3	−163	97	5	77

[a] Isolated yield.

Table IV. Lipase-Catalyzed Enantioselective Hydrolysis of (±)-8

		remaining ester (−)-8			product (−)-9				
lipase	time (h)	yield (%)[a]	$[\alpha]^{27}_D$ (deg)[b]	ee_S (%)[c]	yield (%)[a]	$[\alpha]^{27}_D$ (deg)[d]	ee_P (%)[c]	c (%)	E
P-30 (40 mg)	96	39	−51	96	21	−164	98	49	>100
AK (20 mg)	69	50	−31	67	31	−193	100	40	>100
OF-360 (40 mg)	31	66	−13	35	25	−172	97	26	50
FAP (40 mg)	78	52	−26	62	25	−188	99	40	52
K-10 (40 mg)	96							<10	

[a] Isolated yield. [b] (c 1.0, $CHCl_3$). [c] Determined by NMR [Eu(hfc)$_3$]. [d] Taken in CH_3OH.

Table V. Influence of Organic Solvents on the P-30 Lipase-Catalyzed Hydrolysis of (±)-10

		remaining ester (+)-10			product (−)-11				
lipase	time (h)	yield (%)[a]	$[\alpha]^{26}_D$ (deg)[b]	ee_S (%)[c]	yield (%)[a]	$[\alpha]^{26}_D$ (deg)[b]	ee_P (%)[c]	c (%)	E
t-BuOMe	48	70	+51	41	25	−277	99	29	>100
i-Pr_2O	49	52	+58	44	27	−284	99	31	>100
$C_6H_5CH_3$	264	56	+20	17	14	−243	82	17	12
$ClCH_2CH_2Cl$	336	55	+18	5	5		93	5	43
C_6H_{12}	360	58	+33	29	6	−280	99	23	>100
C_6H_{14}	384	56	+12	9	3	−183	66	12	5

[a] Isolated yield. [b] (c 1.0, $CHCl_3$). [c] Determined by NMR [Eu(hfc)$_3$].

are shown in Table VII The enantiomeric ratio (E) remained unperturbed at 50 °C but the reaction rate was markedly enhanced. The stability of the immobilized P-30 lipase was investigated and the data are shown in Table VIII. A small loss of enzyme activity was observed as indicated by the increase in the length of time required for completing the hydrolytic reaction after each successive cycle. Nevertheless, the economic efficiency of this biocatalytic system is indicated by the feasibility of recycling the enzyme three times with only a modicum loss of enzyme activity.

Since the conformation of the enzyme may be altered in organic solvent, it is necessary to confirm the stereochemical preference of the hydrolytic reaction. This was achieved by transforming the remaining substrate (+)-**10** into *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine methyl ester, (−)-**12**, $[\alpha]^{29}_D$ = −48.1° (c 1.0, CH_3OH), mp 182–184 °C, whose physical constants agreed nicely with the reported values[10a,b] ($[\alpha]^{24}_D$ = −48° (c 1.0, CH_3OH) mp 184–185 °C). This observation affirms that the absolute configuration of the remaining substrate, (+)-**10**, is 3*R*,4*S*. Hence the P-30 enzyme retains its stereochemical preference in

Table VI. Effect of Water Concentration on the P-30-Catalyzed Hydrolysis of (±)-10 in *tert*-Butyl Methyl Ether

		remaining ester (+)-10			product (−)-11				
water (molar equiv)	time (h)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_S$ (%)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_P$ (%)	c (%)	E
10	48	45	+137	98	43	−305	99.5	50	>100
5	68	44	+130	96	35	−282	99	49	>100
1	187	51	+70	56	30	−287	99	36	>100

Table VII. Effect of Temperature on Lipase P-30-Catalyzed Hydrolysis of (±)-10

		remaining ester (+)-10			product (−)-11				
temp (°C)	time (h)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_S$ (%)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_P$ (%)	c (%)	E
50	48	45	+137	98	43	−305	99.5	50	>100
40	62	45	+134	99	38	−284	99	50	>100
30	92	54	+93	70	35	−287	99	41	>100
20	94	67	+48	33	18	−278	95	26	57

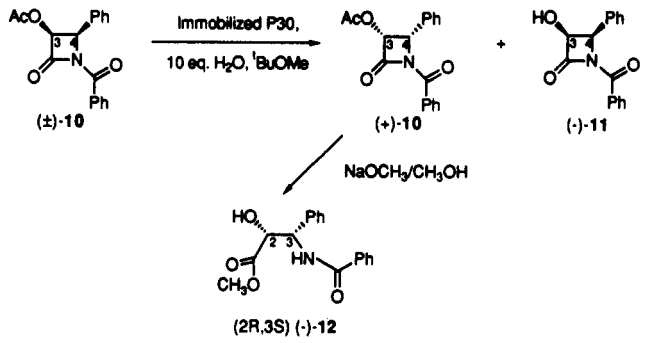
Table VIII. Recycling of Immobilized P-30 Lipase

		remaining ester (+)-10			product (−)-11				
cycle no.	time (h)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_S$ (%)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_P$ (%)	c (%)	E
1	48	45	+137	98	43	−305	99.5	50	>100
2	66	48	+135	98	50	−286	99	50	>100
3	121	46	+112	86	37	−285	99	47	>100
4	144	47	+132	94	44	−270	99	49	>100

Table IX. Enzyme-Catalyzed Enantioselective Cleavage of the β-Lactam Ring of (±)-10

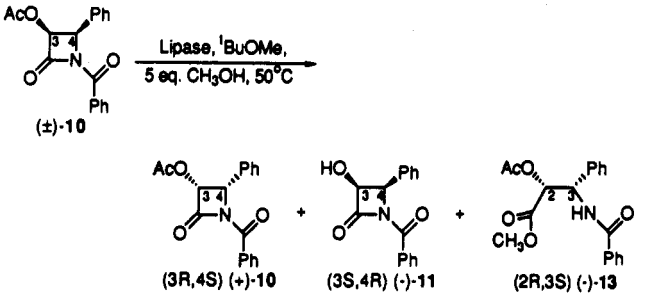
		remaining ester (+)-10			product (−)-11			product (−)-13		
lipase	time (h)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_S$ (%)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_P$ (%)	yield (%)	$[\alpha]^{26}_D$ (deg)	ee$_P$ (%)
P-30	90	19	+140	95	18	−280	100	19	−46	100
AK	48	4			35	−303	100	42	−50	100
OF-360	168	47		racemic	5	−193	68	14	−41	83
K-10	144	41	+94	87	24	−294	96	6	−54	100
Penicillinase *E. coli* 205 (0.2 mg)	144	60		racemic	trace			24		racemic
E. cloacae (0.3 mg)	216	63		racemic	trace			14	(2*R*,3*S*)	16

cleaving the ester of the 3*S*,4*R* enantiomer of (±)-10 in organic media.



When (±)-10 was exposed to the P-30 lipase in the presence of 5 equiv of CH_3OH as the nucleophile (instead of H_2O) in *t*-BuOMe, two products were formed. These were characterized as (3*S*,4*R*)-11 and (−)-13 by comparison with the chemical and physical properties of known samples.[10a,b] This finding demonstrates that this lipase catalyzed the cleavage of the β-lactam ring with a high degree of enantioselectivity. The results in Table IX show that several lipases besides P-30 are also capable of catalyzing this enantioselective ring opening reaction with different reaction rates but in each case the enantioselectivity was high. Moreover, the stereochemical preference of all the lipases was the same in that the β-lactam ring of the (3*R*,4*S*)-enantiomer was preferentially cleaved.

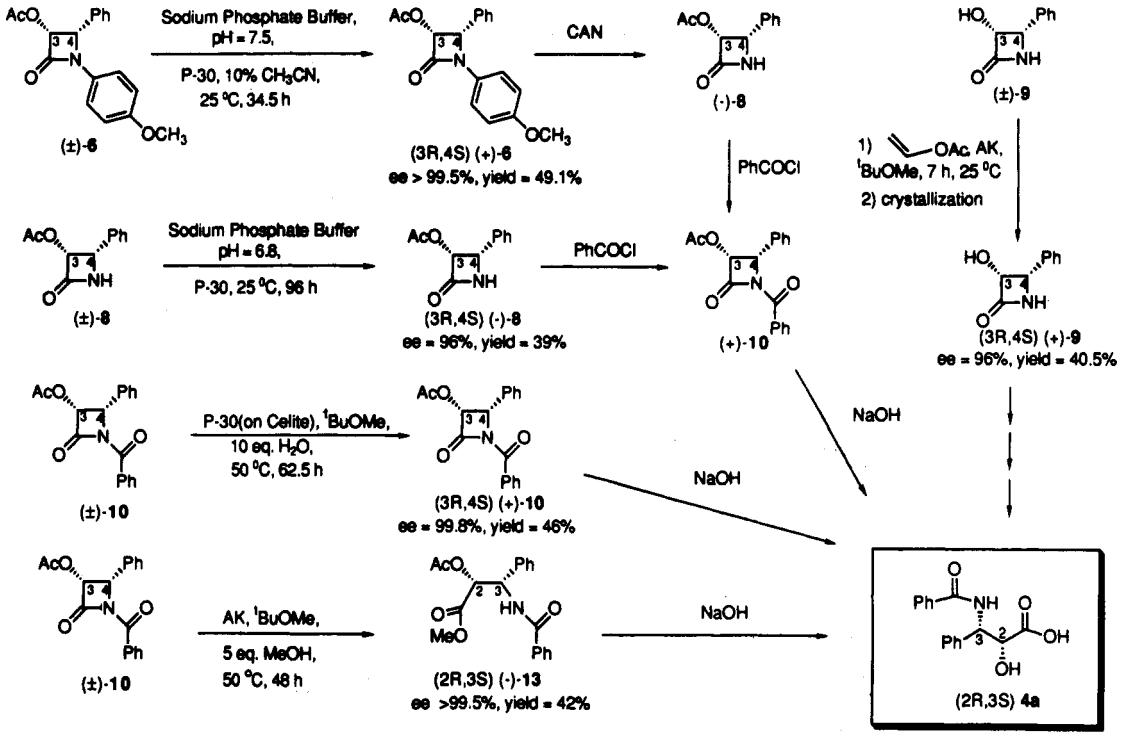
The best lipase was that of AK, which afforded (−)-13 (ee = 100%) in 34% yield after 48 h.



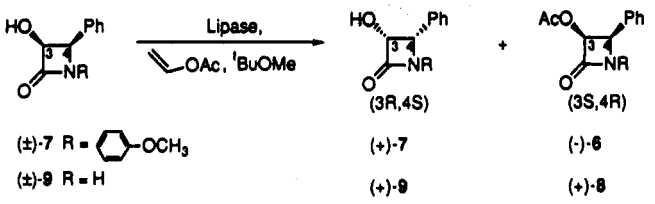
We then examined the action of two commercial penicillinases under these same solvolytic conditions. It is interesting to note that although both penicillinases from *Escherichia coli* 205 and *Enterobacter cloacae* afforded 13 after a prolonged period of incubation, the enantiomeric excess of the product (13) was very low (Table IX). According to the principle of microscopic reversibility,[15] reversible reactions must proceed via the same transition states. Since the conformations of lipases do not appear to change markedly in nonpolar organic media,[11b] we anticipated that the faster reacting enantiomer (e.g., 3*S*,4*R*) in the hydrolytic direction will be preferentially acylated under transesterification reaction conditions. In accord with this prediction, the (3*S*,4*R*)-

(15) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman & Co.: New York, 1985; p 89.

Scheme II. Summary of Lipase-Catalyzed Kinetic Resolutions



enantiomer of (±)-7 was indeed preferentially acylated by the P-30 lipase in *t*-BuOMe using vinyl acetate as the acyl donor. Although the reaction rate was slow under these conditions, the enantioselectivity was very high (E = >100). On the other hand, the reaction rate was rapid using the substrate (±)-9. After only 7 h, a 51% conversion to the acylated product was achieved, with high degrees of enantioselectivity (E = >100). It is noteworthy that the product and remaining substrate may be conveniently separated by direct crystallization from CH_3OH and $CHCl_3$, respectively.



Discussion

The required optically-active intermediates for the synthesis of the side chain of taxol have been successfully prepared via lipase-catalyzed kinetic resolution procedures. These results are summarized in Scheme II.

The most suitable lipases for these transformations are the *Pseudomonas* lipases such as P-30 and AK, which were highly enantioselective and catalyze the reaction with reasonable reaction rates. For water-insoluble substrates in aqueous media, addition of a cosolvent appeared to improve the reaction rate and with some lipases such as AP, CCL, and AK the enantioselectivity was also markedly improved. The function of the cosolvent is not well understood. However, since the conformation of the lipase is affected by the presence of cosolvent, it is not surprising that the enantioselectivity expressed as the E value is altered.

One prominent feature of the lipases is their remarkable stability in organic solvents[16] such as *t*-BuOMe, even at 50 °C, provided that only a small quantity of water is present (<0.4%). The conformational mobility of the enzyme becomes more restricted when very small quantities of water are present. This allows one to conduct enantioselective hydrolytic reactions in organic solvent. This reaction medium is suited not only for water-insoluble substrates but also the enantioselectivity of the reaction may often be improved by systematically manipulating the organic solvent medium with different log P values[17] and dielectric constants.[18]

In the presence of a small quantity of water, the reaction rate in organic solvent is considerably slower than that in aqueous phosphate buffer. However, this obstacle may be compensated for by raising the temperature up to 50 °C. In turn, the stability of the enzyme may be extended by immobilizing the lipase on a solid support such as Celite. This allows one to recycle the enzyme several times and reduces the cost of the catalyst.

It is noteworthy that the reaction pathway was markedly influenced by the nucleophile used in the reaction medium. When water was used as the nucleophile, only cleavage of the C-3 acetoxy ester of (±)-10 was observed. On the other hand, when methanol was substituted for water, methanolysis of the β-lactam ring was observed by the *Pseudomonas* lipases P-30 and AK. In fact both the reaction rate and enantioselectivity were considerably better than the pencillinases, enzymes which are known to cleave the β-lactam ring of penicillins. This observation demonstrates the possibility of expanding the substrate specificity of lipases. To our knowledge, this constitutes the first report of a lipase catalyzing the cleavage of a

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β-lactam ring. As a matter of fact, lipases are generally unsuitable for cleaving amide bonds because of their slow reaction rates. This observation demonstrates clearly the different reaction pathway of hydrolysis versus methanolysis. Moreover, lipases P-30 and AK preferentially hydrolyzed the acetoxy ester of the (3*S*,4*R*)-enantiomer of **10**. In contrast under solvolytic conditions, these enzymes preferentially cleaved the β-lactam ring of the (3*R*,4*S*)-enantiomer of **10**, presumably via the formation of an acyl–enzyme intermediate. Hence, baccatin III (**2b**) was substituted for methanol as the nucleophile hoping that these lipases transfer the phenylisoserine moiety directly onto the C-13 hydroxyl of **2b**. Unfortunately, no coupling adduct was detected after much experimentation. It is possible that the size of the acyl acceptor (**2b**) may be too large to be optimally accommodated by the enzymes, because of a lack of conformational mobility under a dehydrated environment.

It is not surprising that the *Pseudomonas* lipases are the most suitable enzymes for catalyzing these biocatalytic transformations, for they have been widely employed for the kinetic resolutions of secondary alcohols.[19a] These lipases can assume a variety of conformations in solution to accommodate a wide variety of substrates (induced fit). The flexible nature of these enzymes in solution makes the prediction of stereochemical preference and enantioselectivity of new artificial substrates rather difficult. However, several crude two-dimensional working models[19] of the P-30 lipase have been advanced and some of these have proven to be useful in predicting the stereochemical preference of hydrolysis of a variety of esters of secondary alcohols. Unfortunately, the three-dimensional structure of the P-30 lipase has not yet been deduced by X-ray crystallographic analysis.

Since the Japanese workers[20] have clearly shown that the stereochemical behavior of the *Pseudomonas* lipases are sensitive to the ester functionality, it seems to us that a cubic lattice model could more simply represent the stereochemical requirements of the P-30 lipase. According to this model, the β-lactam substrates (**6**, **8**, and **10**) are preferentially oriented in the active site of the lipase as shown in Figure 1. The acetoxy ester resides in the upper right quadrant with the hydrogen on C-3 on the *y*-axis as the small group (S). The phenyl attached to C-4 in the large group (L) and the carbonyl function of C-2 as the medium sized group (M). Thus, we envisaged that the P-30 lipase should preferentially cleave the acetoxy esters of the (3*S*,4*R*)-enantiomer of the substrates (**6**, **8**, and **10**), a prediction that coincides nicely with the experimental results, lending further support of such a model for predicting the binding mode of this enzyme.

One of the outstanding features of lipases is their stability in nonpolar organic solvents (e.g., log *P* values around 2–3). This property allows one to conduct transesterification reactions in organic solvents to furnish the versatility of securing the optically-active ester or alcohol of either configuration. The observation that both stereochemical preference and high enantioselectivity were retained via hydrolysis in aqueous medium and transesterification in organic medium strongly suggests that the conformation of this lipase is not drastically altered in these different media, in accord with the principle of microscopic reversibility.[21]

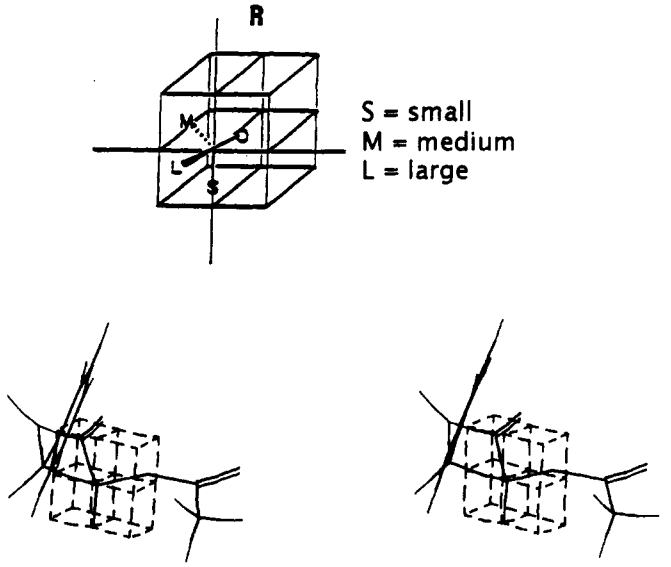

Figure 1. Spectroscopic view of the cubic lattice model of P-30 using the (3*R*,4*S*)-configuration of the substrate (−)-**8**.

Thus far, only one report has appeared pertaining to the use of enzymes for the preparation of the C-13 side chain of taxol. Hönig et al.[22] have described the lipase-catalyzed enantioselective hydrolysis of 3-azido-2-(butanoyloxy)-3-phenylpropionic esters. Although high enantioselectivity was achieved with the P-30 lipase, numerous steps are needed to transform 3(*S*)-azido-2(R)-(butanoyloxy)-3-phenylpropionic acid methyl ester into a suitable intermediate of (2*R*,3*S*)-phenylisoserine for coupling to baccatin III.

The optically active intermediate **9** was prepared by Holton via the formation of the diastereomeric ester (Mosher's) followed by chromatographic separation. Unfortunately, no yield was given. In contrast, **9** may be conveniently prepared via lipase-catalyzed enantioselective transesterification and isolated by direct crystallization without chromatographic separation.

Our results clearly illustrate the versatility of the lipases. All the requisite intermediates have been obtained in their optically-pure forms which provides the medicinal chemist with the choice of the most suitable intermediate(s) for synthetic applications.

Experimental Section

Materials. The following lipases were purchased from the Amano Co.: *P. cepacia* (P-30); *Pseudomonas* sp. (AK and K-10); and *Aspergillis niger* (AP). The lipase of *Alcaligenes* sp. (PL) was a product of Meito Sangyo Co. Porcine pancreatic lipase (PPL) and the lipase of *Candida cylindracea* (CCL) were products of Sigma Chemical Co. All other chemicals and solvents were of the highest quality grade available.

^{1}H NMR spectra were recorded on a WM-200 spectrometer in deuteriochloroform with tetramethylsilane as the internal standard. Optical rotations were measured with a Perkin-Elmer Model 241C polarimeter in the indicated solvents. Thin-layer chromatography (TLC) was performed on glass plates coated

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with a 0.25-mm thickness of silica gel 60F-254 (Mackerey-Nagel). Flash column chromatography was performed with Baker silica gel (40 μm). All solvents were glass distilled prior to use. The combined organic solvent extracts were dried over $MgSO_4$, filtered, and evaporated to dryness under reduced pressure.

Computer Graphics. The molecule was minimized using the Maximin2 program of Sybyl 5.5 on an Evans & Sutherland PS390 graphics display running under VMS 5.4 on a Microvax II. The minimizer used the Powell derivative technique with convergence determined by the total gradient. The minimized structure was plotted using Alchemy II.

Enantioselective Hydrolysis of (±)-6 in Aqueous Buffer. General Procedure. To 80 mg of (±)-6 suspended in 4 mL of 0.2 M sodium phosphate buffer (pH 7.5) was added 80 mg of a crude lipase, and the mixture was stirred vigorously with a magnetic stirrer at 25 °C until approximately 50% of the substrate was transformed into product (monitored by TLC). If cosolvent (10%) was used, only 3.6 mL of 0.2 M sodium phosphate buffer was employed and 0.4 mL of an organic cosolvent was added. The reaction was terminated by extraction of the mixture with ethyl acetate three times (3 × 25 mL). TLC analysis of the residue using ethyl acetate–hexane (1:1) as the mobile phase revealed that the remaining substrate and product have R_f values of 0.58 and 0.28, respectively. The mixture was separated by silica gel flash chromatography using ethyl acetate–hexane (1:3 and 1:1) as the mobile phase.

Optical rotation of the remaining substrate 6 and the product 7 was measured in chloroform. The enantiomeric excess (ee) of the remaining ester 6 was determined by ^1H NMR ($CDCl_3$, 200 MHz) analysis of the methoxy group in the presence of the chiral shift reagent Eu(hfc)$_3$. The product (alcohol) 7 was first acetylated with acetic anhydride/pyridine in the presence of a catalytic quantity of DMAP to convert it into the ester 6 and the ee was measured by the same method. The results are tabulated in Tables I, II, and III.

The enantiomeric ratio (E value) was calculated from

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]}$$

where $c = \frac{ee_s}{ee_s + ee_p}$ (see ref 14).

Absolute Configuration of (±)-6. (a) To 280 mg of (±)-6 suspended in 12.6 mL of 0.2 M sodium phosphate buffer (pH 7.5) and 1.4 mL of acetonitrile was added 280 mg of P-30 lipase, and the mixture was vigorously stirred at 25 °C. After 35 h, the reaction was terminated by extraction of the mixture with ethyl acetate three times (3 × 50 mL). The residue was dissolved in a mixture of ethyl acetate–hexane (1:3) and the mixture was separated using silica gel flash column chromatography (ethyl acetate:hexane, 1:3 and 1:1, was used as the mobile phase) to yield 0.137 g of (+)-6, $[\alpha]^{26}_D = +7.0°$ (c 1.0, $CHCl_3$) (ee = 0.99) and 0.118 g of (−)-7 (ee = 0.99).

(b) Transformation of (+)-6 into (−)-8. To a solution of 0.13 g of (+)-*cis*-1-(*p*-methoxyphenyl)-3-acetoxy-4-phenylazetidin-2-one (6) in 5 mL of acetonitrile at −10 °C was slowly added a solution of ceric ammonium nitrate in 3 mL of water over a 30-min period. The mixture was stirred for 30 min at −10 °C and diluted with 5 mL of ether. The aqueous layer was extracted with two 5-mL portions of ether, and the combined organic layer was washed successively with water, saturated sodium bisulfite, and saturated aqueous sodium bicarbonate solutions. Crystallization of the solid residue (80 mg) from acetone–hexane gave (−)-*cis*-3(*R*)-acetoxy-4(*S*)-phenylazetidin-2-one (8) (62 mg, 68%), mp 151–153 °C, $[\alpha]^{28}_D = -44.5°$ (c 1.0, CH_3Cl_3).

(c) Conversion of (−)-8 into (+)-*cis*-3(*R*)-Hydroxy-4(*S*)-phenylazetidin-2-one (9). To a mixture of 2 mL of THF and 3 mL of 1 M aqueous potassium hydroxide solution at 0 °C was added a solution of 50 mg of (−)-*cis*-3-acetoxy-4-phenylazetidin-2-one (8) in 3 mL of THF over a 30-min period. The solution was stirred at 0 °C for 45 min and 1 mL of water and 1 mL of saturated sodium bicarbonate were added. The mixture was extracted with three 4-mL portions of ethyl acetate and the residue consisted of 35 mg of (+)-9, mp 187–188 °C, $[\alpha]^{28}_D = +178°$ (c 1.0, CH_3OH); lit.[10c] mp 187–188 °C, $[\alpha]^{24}_D = +198.8°$ (c 1.0, CH_3OH).

Enzymatic Kinetic Resolution of (±)-*cis*-3-Acetoxy-4-phenylazetidin-2-one ((±)-8). A suspension consisting of 60 mg of (±)-8 in 4 mL of 0.2 M sodium phosphate buffer (pH 6.8) was added either to 20 or 40 mg of lipase (as indicated). The reaction mixture was stirred with a magnetic stirrer at 25 °C until about 50% conversion was attained. The progress of the reaction was monitored by TLC using the solvent system ethyl acetate–hexane–ethanol (1:1:0.2). The R_f value of (±)-8 was 0.5 and the R_f value of the alcohol (−)-9 was 0.25.

The reaction mixture was extracted with ethyl acetate (3 × 25 mL). The product and the remaining substrate were separated by silica gel flash column chromatography using the solvent mixture ethyl acetate–hexane (2:1) and pure ethyl acetate as eluent.

The ee of the remaining substrate 8 was determined by ^1H NMR ($CDCl_3$, 200 MHz) analysis of the acetyl group in the presence of the chiral shift reagent, Eu(hfc)$_3$. The product (−)-9 was first converted to the diacetyl derivative by reaction with acetic anhydride–pyridine (1:1) containing a trace of 4-(dimethylamino)pyridine, and the ee was then determined in a similar manner. The results of these enzymatic transformations are summarized in Table IV.

Enantioselective Hydrolysis of (±)-*cis*-1-Benzoyl-3-acetoxy-4-phenylazetidin-2-one (10) Catalyzed by Immobilized P-30 Lipase. (a) Immobilization of P-30 Lipase on Celite. The commercial *P. cepacia* P-30 (Amano) lipase (2 g) was dissolved in 4 mL of deionized distilled water and 2 g of Celite was added. After thorough mixing by stirring, the mixture was lyophilized under high vacuum for 20 h (−78 → 25 °C) using a cryolizer freezer-drying apparatus to give 3.91 g of a solid powder, which was used for the subsequent experiments.

(b) Effect of Organic Solvent. The reaction mixture contained 80 mg of (±)-10 and 160 mg of immobilized P-30 in 4 mL of the organic solvent containing 4.2 μL of H_2O (1.0 equiv). The mixture was incubated at 50 °C with stirring and the progress of the reaction was monitored by TLC using ethyl acetate–hexane (1:1) as the mobile phase. The R_f values for the remaining substrate 10 and the product 11 were 0.66 and 0.48, respectively.

At the indicated times, the immobilized P-30 lipase was separated from the reaction mixture by filtration and washed with ethyl acetate. The residue consisting of the remaining substrate 10 and the product 11 was separated by silica gel flash column chromatography using hexane–ethyl acetate (5:1 and 4:1) as the mobile phase.

Optical rotations were determined in chloroform and the ee of the remaining substrate 10 was determined by ^1H NMR ($CDCl_3$) analysis of the acetyl groups in the presence of the chiral shift reagent Eu(hfc)$_3$. The product 11 was first converted to 10 by treatment with acetic anhydride–pyridine and a trace of 4-(dimethylamino)pyridine (DMAP). The results are tabulated in Table V.

(c) Effect of Water. The reaction mixture contained 100 mg of (±)-10 and 200 mg of immobilized P-30 lipase in 5 mL of *tert*-butyl methyl ether and varying quantities of water as indicated (Table VI). The mixture was incubated at 50 °C with stirring until approximately 50% conversion was attained. At the indicated times, the reaction was terminated. The isolation and characterization procedures are the same as described above. The results are shown in Table VI.

(d) Effect of Temperature. The reaction mixture contained 100 mg of (±)-10 and 200 mg of immobilized P-30 lipase in 5 mL of *tert*-butyl methyl ether and 52 μL of water (10 equiv). The contents were stirred and incubated at the indicated temperature (Table VII) until about 50% conversion was attained. The isolation and characterization procedures are the same as described above. The results are given in Table VII.

(e) Recycling the Immobilized P-30 Lipase. The reaction mixture contained 100 mg of (±)-10 and 200 mg of immobilized P-30 lipase in 5 mL of *tert*-butyl methyl ether and 52 μL of water (10 equiv). The mixture was incubated at 50 °C with stirring for the indicated times; the immobilized P-30 lipase was removed by filtration and reused and the process was repeated (total of 4 cycles). In the third cycle, the amount of immobilized enzyme was reduced to 190 mg and substrate to 95 mg. In the fourth cycle, the amount of Celite P-30 was 180 mg and substrate was 90 mg. In these cases, the quantity of *tert*-butyl methyl ether

Table X. Lipase-Catalyzed Enantioselective Transesterification of (±)-7 and (±)-9

			remaining substrate			product				
substrate	acyl donor (3 equiv)[a]	time (h)	yield (%)[b]	$[\alpha]^{26}_D$ (deg)[c]	ee$_S$ (%)	yield (%)[b]	$[\alpha]^{26}_D$ (deg)[c]	ee$_P$ (%)	c	E
(±)-7[d]	IPA	96	75	+54	20	13	−8	100	17	99
(±)-7[d]	VA	96	26	+164	87	36	−9	100	47	>100
(±)-9[d]	IPA	61	60	+69	43	22	+50	97	31	70
(±)-9[d]	VA	24	41	+200	100	38	+48	99	51	>100
(±)-9[d]	VA	19[f]	49	+108	59	29	+45	96	38	96
(±)-9[e]	IPA	24	51	+175	80	37	+51	96	45	>100
(±)-9[e]	VA	7	32	+206	100	41	+48	96	51	>100
(±)-9[e]	VA	5′	37	+175	97	34	+45	100	50	>100

[a] IPA, isopropenyl acetate; VA, vinyl acetate. [b] Isolated yield. [c] Optical rotation ($CHCl_3$). [d] P-30 lipase. [e] AK lipase. [f] 40 °C.

and water was reduced proportionately. The results are shown in Table VIII.

Absolute Configuration of (+)-*cis*-1-Benzoyl-3-acetoxy-4-phenylazetidin-2-one (10). A sample of 100 mg of (+)-10, obtained from a large-scale incubation of immobilized P-30 lipase with (±)-10, was treated with sodium methoxide–methanol (66.5 mg of Na in 5 mL of methanol) at 25 °C for 1 h. The reaction mixture was acidified with 1 N HCl and extracted with ethyl acetate (3 × 25 mL). The organic residue was purified by chromatography over a silica gel column using ethyl acetate–chloroform–hexane (2:3:3) as the mobile phase to yield 79 mg of *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine methyl ester ((−)-12), mp 182–184 °C, $[\alpha]^{28}_D = -48.1°$ (*c* 1.0, CH_3OH); lit.[10a,b] mp 184–185 °C, $[\alpha]^{24}_D = -48°$ (*c* 1.0, CH_3OH).

Enantioselective Cleavage of the β-Lactam Ring of (±)-10. The reaction mixture contained 60 mg of (±)-10, 5 equiv (1 mmol) of methanol, and 20 mg of enzyme in 3 mL of *tert*-butyl methyl ether. The mixture was incubated at the indicated temperatures (27 or 50 °C) with stirring. The progress of the reaction was monitored by TLC using the solvent system chloroform–ethyl acetate–hexane (3:2:3) as the mobile phase. The R_f values of 10, 13, and 11 were 0.60, 0.30, and 0.25, respectively. At the indicated times, the reaction was terminated by filtration. The residue was chromatographed over a silica gel column and the products 10, 13, and 11 were separated using the solvent system hexane–chloroform–ethyl acetate (3:3:2). The enantiomeric excesses (ee) of the remaining substrate 10 and the product 13 were determined by ^1H NMR analysis in the presence of Eu(hfc)$_3$. The product 11 was first converted into 10 using acetic anhydride–pyridine and a catalytic amount of DMAP and analyzed in a similar manner. The results are shown in Table IX. Optical rotations of 10 and 11 were measured in chloroform and the product 13 in methanol. The absolute configuration of (−)-13 was established by its conversion into (−)-12 and 4a.

Enantioselective Transesterification of (±)-*cis*-3-Hydroxy-4-phenylazetidin-2-one (9). The reaction mixture contained 33 mg of (±)-9, 3 equiv of acylating agent (vinyl acetate or isopropenyl acetate), and 20 mg of enzyme in 3 mL of *tert*-butyl methyl ether. The mixture was incubated at 27 °C (unless otherwise stated) with stirring and the progress of the reaction was monitored by TLC (ethyl acetate–hexane–ethanol, 1:1:0.2). When the reaction had reached about 50% conversion, the enzyme was removed by filtration and washed with ethyl acetate. The residue was chromatographed over a silica gel column. Elution of the column with hexane–ethyl acetate (1:1) afforded (+)-8 followed by ethyl acetate to yield the remaining substrate, 9. The ee was analyzed as previously described. The results are shown in Table X.

Acknowledgment. This work was supported in part by the National Institutes of Health, Grant no. GM33149. R.B. was a recipient of a Fulbright Fellowship. We thank Dr. Matt Suffness of NCI for a sample of baccatin III, and Mr. Gary Girdaukas for the computer graphic analysis.