Enzymatic Asymmetric Synthesis of α-Amino Acids. Enantioselective Cleavage of 4-Substituted Oxazolin-5-ones and Thiazolin-5-ones

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A general enzymatic asymmetric synthesis of L-α-amino acids has been developed. This method entails the use of the *Pseudomonas cepacia* lipase (P-30) to catalyze the enantioselective methanolysis of a variety of 4-substituted 2-phenyloxazolin-5-one derivatives in a nonpolar organic solvent to furnish optically-active *N*-benzoyl-L-α-amino acid methyl esters (ee = 66–98%), which in turn is subjected to a protease-catalyzed kinetic resolution yielding enantiomerically-pure *N*-benzoyl-L-α-amino acids. This synergistic coupling of two enzymes allows the ready preparation of L-α-amino acids of high enantiopurity in yields greater than 50%, an inherent advantage over conventional resolution procedures. Two proteases were found to catalyze the enantioselective hydrolysis of a variety of 4-substituted 2-phenylthiazolin-5-one derivatives to give *N*-(thiobenzoyl)-L-α-amino acids of high optical purity.

Optically-active natural and unnatural α-amino acids are important precursors for the synthesis of pharmaceuticals, agrochemicals, and food ingredients.[1] They also have been amply utilized in synthetic studies as chiral auxiliaries, reagents, and catalysts for asymmetric syntheses.[2] Because of their widespread use, intensive research efforts have been directed toward the development of new and improved stereoselective syntheses of α-amino acids.[3] Of particular interest are catalytic methods that possess high degrees of enantioselective control.

A wide variety of asymmetric syntheses have been developed for the preparation of optically-active α-amino acids. However, the diverse nature of the side chains of α-amino acids restricts the applicability of every method thus far developed. Consequently, the development of new and more versatile methodology for their syntheses continues to be a challenging topic to the organic chemist.

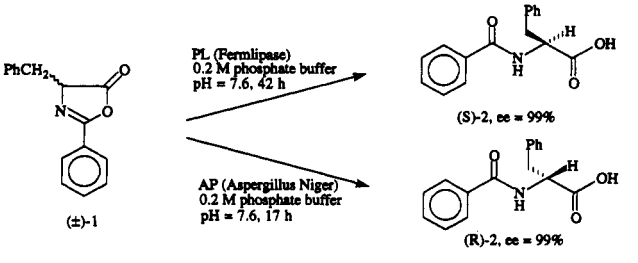
The 5(4*H*)-oxazolones have been extensively investigated because of their transient formation, which accounts for much of the racemization occurring during peptide synthesis.[4] In contrast, little attention has thus far been paid to the study of the possibilities of asymmetric induction during ring opening of 5(4*H*)-oxazolones by chiral nucleophiles.[5]

The enantioselective hydrolysis of 5(4*H*)-oxazolone derivatives, catalyzed by proteolytic enzymes and carboxyesterase, had been investigated by several groups.[6] However, as the rates of enzyme-catalyzed ring opening are not considerably faster than the rates of competing nonenzymatic hydrolysis, the reactions were conducted in dilute solution with stoichiometric amounts of enzyme and products of modest optical purity were obtained.

In a preliminary paper,[7] we disclosed our results on the lipase-catalyzed enantioselective hydrolysis of a series of 4-substituted 2-phenyloxazolin-5-ones to furnish optically-active *N*-benzoyl-α-amino acids. Since then, we have explored further the scope of this biocatalytic methodology. We now report our strategy and progress directed at the development of lipase-catalyzed enantioselective ring clevage of 5(4*H*)-oxazolones and 5(4*H*)-thiazolones into a general procedure for the preparation of optically-active L-α-amino acids.

Results

Enantioselective Hydrolysis of 4-Substituted 2-Phenyloxazolin-5-ones in Aqueous Medium. In our earlier work,[7] we discovered two lipases that are uniquely suited for catalyzing the enantioselective cleavage of 4-benzyl-2-phenyloxazolin-5-one (1). The lipase of *Aspergillus niger* (AP) catalyzed highly enantioselective hydrolysis of (±)-1 into (*R*)-2 (ee = >99%) in aqueous phosphate buffer, pH 7.4, whereas porcine pancreatic lipase (PL) has an opposite preferred chirality in converting (±)-1 into (*S*)-2 (ee = >99%).



These results show that under the conditions of enzymatic catalyses, the rate of equilibration (k_{rac}) of the two

(1) Kleeman, A.; Leuchtenberger, W.; Hoppe, B.; Tanner, H. In *Ullmann's Encyclopedia of Industrial Chemistry*; VCH: Weinheim, 1985; Vol. A2, pp 57–97.
(2) O'Donnell, M. J., Ed. α-Amino Acid Synthesis (Tetrahedron Symposium-in-print). *Tetrahedron* **1988**, *44*, 5253–5614.
(3) Williams, R. M. *Synthesis of Optically-Active α-Amino Acids*, 1st ed.; Pergamon Press: Oxford, England, New York, 1989.
(4) Benoiton, N. L. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1983; pp 217–264.
(5) The aminolysis of oxazolones by amino acid esters had been reported by: (a) Weygang, F.; Steglich, W.; Barocio de la Lama, X. *Tetrahedron Suppl.* **1966**, *8*(1), 9–13. (b) Steglich, W.; Frauendorfer, E.; Weygang, F. *Chem. Ber.* **1971**, *104*, 687–690.
(6) (a) De Jersey, J.; Zerner, B. *Biochemistry* **1969**, *8*, 1967–1974. (b) De Jersey, J. *Biochemistry* **1970**, *9*, 1761–1767. (c) Daffe, V.; Fastrez, J. *J. Am. Chem. Soc.* **1980**, *102*, 3601–3605. (d) Previero, A.; Coletti-Previero, M. A. *C. R. Acad. Sci., Ser. D* **1969**, *268*, 1798–1801.
(7) Gu, R. L.; Lee, I. S.; Sih, C. J. *Tetrahedron Lett.* **1992**, *33*, 1953–1956.

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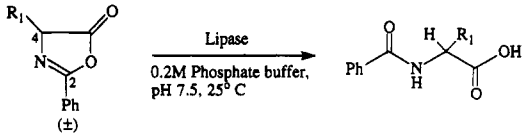
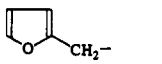
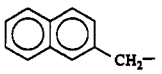
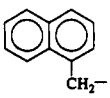
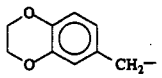
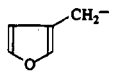
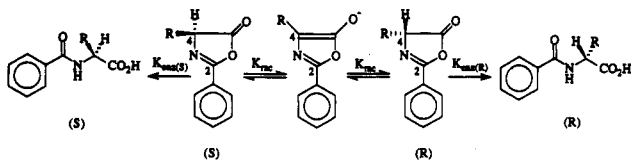
Table I. Lipase-Catalyzed Asymmetric Hydrolysis of 4-Substituted 2-Phenyloxazolin-5-ones



R_1 group	enzyme	time (h)	$[\alpha]_D$ ($CHCl_3$, c = 1)	ee (%)
p-CH_3OPh	PL	46	−10.4	52
	AP	46	+11.0	57
	PL	24	−12.7	60
	AP	24	+17.0	85
	PL	144	−0.61	
	AP	144	+1.8	
	PL	21	−107.5	85
p-CH_3PhCH_2–	PL	24	−13.1	61
	AP	24	+11.9	57
	PL	30	−9.3	55
	AP	30	+2.4	20
	PL	24	−1.8	43
	AP	24	+3.7	57
$PhCH_2CH_2$	PL	48	−5.0	33
	AP	48	−3.6	30

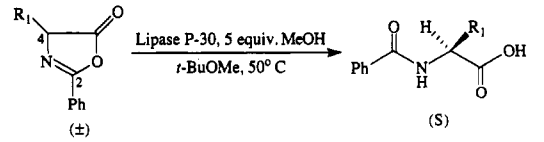
configurationally unstable 5(4*H*)-oxazolone antipodes is sufficiently rapid and the rate of enzyme-catalyzed ring opening (k_{enz}) occurs at a substantially faster rate than the rate of nonenzymatic cleavage (k_{chem}) [$k_{rac} \geq k_{enz} \gg k_{chem}$]. Using these two lipases, we then examined their

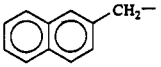


abilities in catalyzing the asymmetric hydrolysis of other 2-phenyloxazolin-5-ones, modified at C-4, with a view to determining the versatility of this methodology. In these experiments, the enzymatic transformations were allowed to proceed to completion, for some residual oxazolone may be hydrolyzed during the workup procedure resulting in the lowering of the optical purity of the *N*-benzoyl-α-amino acids. While both enzymes retained their chiral preference on all the substrates studied, the reaction rate and the degree of enantioselectivity varied markedly depending on the C-4 substituents (Table I). For substrates with moderate size C-4 side chains, the lipase-catalyzed ring-opening reactions proceeded with good to moderate enantioselectivity. However, the reaction rate decreased for substrates having bulky C-4 substituents (e.g., R = isopropyl). Conversely, good reaction rates were attained with substrates possessing small appendages at C-4 (e.g., R = CH_3), but the enantioselectivity of ring cleavage was poor because, in the absence of severe steric interactions, the carbonyl group is more susceptible to nonenzymatic hydrolysis. Unfortunately, the enantioselectivity of the lipase-catalyzed ring cleavages of all the substrates examined was not as high as that for (±)-1.

Enantioselective Methanolysis of 5(4*H*)-Oxazolone Derivatives in Organic Solvents. Because the 5(4*H*)-oxazolones are relatively unstable in aqueous media,

Table II. Lipase P-30 Catalyzed Enantioselective Methanolysis of 4-Substituted 2-Phenyloxazolin-5-ones



R_1	amt of H_2O (equiv)	time (h)	$[\alpha]_D$ ($CHCl_3$, c = 1)	yield (%)	ee (%)	conf
Me_2CH	5	130	+30.9	47	77	*S*
Me_2CHCH_2	5	72	+18.6	85	90	*S*
	0	72	+16.1	82	78	*S*
$MeSCH_2CH_2$	5	48	+26.0	31	82	
	0	114	+24.8	56	71	*S*
	5	91	+40.0	83	49	
	0	86	+61.9	90	75	
p-CH_3PhCH_2	5	84	+59.2	78	63	
	0	156	+72.0	86	66	
Ph	5	84	+71.7	46	75	
$PhCH_2$	5	35	+73.0	80	78	*S*
	0	22	+62.0	93	69	*S*
$PhCH_2CH_2$	5	42	+41.0	61	93	
$PhCH_2CH_2CH_2$	5	72	+33.7	76	95	
	0	72	+24.9	91	84	

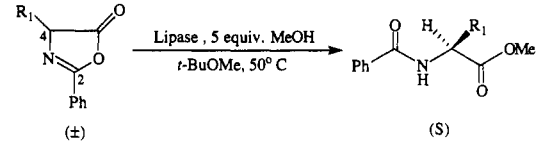
especially when the C-4 substituent is small in size (e.g., R = CH_3), Bevinakatti *et al.*[8] investigated the lipase-catalyzed enantioselective cleavage of 2-phenyl-4-methyloxazolin-5-one in anhydrous diisopropyl ether using *n*-butanol as the nucleophile. The best lipase was that of *Mucor miehei* which catalyzed the solvolysis of 2-phenyl-4-methyloxazolin-5-one into (*S*)-butyl-*N*-benzoylalanine in 45% yield, but unfortunately, the optical purity of the product was only modest (57% ee).

As the *Pseudomonas* lipases (AK, K-10, P-30) have been successfully employed for a variety of enantioselective transformations in aqueous and nonaqueous media,[9] we decided to investigate the possible use of these bacterial lipases in nonaqueous media for the enantioselective cleavage of 5(4H)-oxazolone derivatives. Thus, a variety of 4-substituted 2-phenyl-oxazolin-5-one derivatives were first exposed to the P-30 lipase in *tert*-butyl methyl ether at 50 °C in the presence of 5 equiv of methanol with or without 5 equiv of water. In general, the reaction rate was somewhat faster in the presence of 5 equiv of water, but nonenzymatic hydrolysis also occurred under these conditions resulting in a lowering of the product yield in most cases. The enantioselectivity ranged from 66 to 95% ee and appeared to improve as the C-4 substituent increased in size. The (*S*)-preferred chirality of the P-30 lipase toward the oxazolones was deduced by comparing the optical rotations of several of the products to those of known *N*-benzoyl-L-α-amino acid methyl esters. We next examined the enantioselective properties of the three *Pseudomonas* lipases on several other 2-phenyloxazolin-5-ones, and the results are shown in Table III. It is interesting to note that all three lipases behaved similarly toward the two substrates (entries 3 and 4, Table III), but their actions toward 4-(*p*-hydroxyphenyl)-2-phenyloxazolin-5-one were very different. Only the AK lipase catalyzed the methanolysis of this substrate with good enantioselectivity (75% ee), whereas lipases P-30 and K-10 catalyzed

(8) (a) Bevinakatti, H. S.; Newadkar, R. V.; Banerji, A. A. *J. Chem. Soc., Chem. Commun.* **1990**, 1091–1092. (b) Bevinakatti, H. S.; Banerji, A. A.; Newadkar, R. V.; Mokashi, A. A. *Tetrahedron Asymmetry* **1992**, *3*, 1505–1508.

(9) Xie, Z. F. *Tetrahedron Asymmetry* **1991**, *2*, 733–750.

Table III. Asymmetric Methanolysis of 4-Substituted 2-Phenyloxazolin-5-ones Catalyzed by Bacterial Lipases

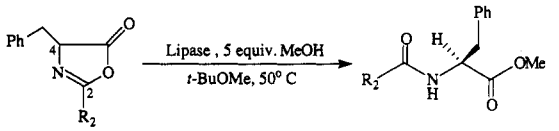


(±) (S)

R_1	lipase	time (h)	yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	conf.
Me	AK	69	76	−0.6		*R*
	AK (5 equiv of H_2O)	15	46	+5.0	18	*S*
	P-30	23	91	−3.6	59	*R*
	P-30 (5 equiv of H_2O)	38	39	+1.8	50	*S*
	K-10	69	80	−2.2	13	*R*
	K-10 (5 equiv of H_2O)	86	50	−3.9	15	*R*
p-OHPh	AK	76	52	+72.6	75[a]	*S*
	P-30	112	32	+17.7	18[a]	*S*
	K-10	88	22	+4.8	5[a]	*S*
p-$OHPhCH_2$	AK	16	39	+52.0	61[a]	*S*
	P-30	66	40	+60.1	71[a]	*S*
	K-10	97	57	+58.5	69[a]	*S*
$PhCH_2SCH_2$	AK	15	69	−6.3	79	*S*
	P-30	23	64	−6.4	80	*S*
	K-10	23	61	−9.6	90	*S*

[a] The ee value is determined as the *p*-methoxyphenyl derivative.

Table IV. Enzymatic Enantioselective Methanolysis of 2-Substituted 4-Benzyloxazolin-5-ones



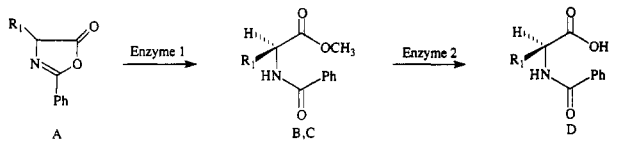
R_2	enzyme	time (h)	$[\alpha]$ ($CHCl_3$, c = 1)	yield (%)	ee (%)
p-ClPh	P-30	23	+74.9	73	75
	K-10	72	+74.8	78	76
CF_3	P-30	73	+30.9	31	39
	AK	88	+28.7	58	30
CH_3	P-30	24	+18.4	52	20

the solvolysis of this substrate slowly with poor enantioselectivity. For the substrate 4-methyl-2-phenyloxazolin-5-one, it is interesting to note that in the presence of 5 equiv of water both lipases P-30 and AK preferentially catalyzed the methanolysis of the (*S*)-enantiomer. However, in the absence of exogenous water, the enzymes changed their preferred chirality to (*R*) suggesting a significant change in enzyme conformation in different microenvironments. On the other hand, the lipase K-10 retained its (*R*)-stereochemical preference in either environment. These results demonstrate the dissimilarity of the stereochemical behavior of these bacterial lipases.

In an attempt to further enhance the enantioselectivity of these enzymes, we decided to change the substituent at the C-2 position. For this purpose, we synthesized three substrates and exposed them to the action of the bacterial lipases (Table IV). Unfortunately, no improvement in enantioselectivity was observed. While the enantioselectivity of the 2-(4-chlorophenyl)oxazolin-5-one is very similar to that of 2-phenyloxazolin-5-one, the enantioselectivity for the methanolysis of 2-methyl- and 2-(trifluoromethyl)oxazolin-5-one were relatively low for the lipases. However, the (*S*)-preferred chirality for all the lipases was retained (Table IV).

Kinetic Resolution of *N*-Benzoylamino Acid Methyl Esters. While the *Pseudomonas* lipases possess broad substrate specificities, their enantioselectivities toward various 5(4*H*)-oxazolone derivatives varied from 66 to 95% ee, depending on the C-4 substituent. In our estimation, it would be difficult to find a single enzyme that could catalyze the cleavage of all 5(4*H*)-oxazolones with a high degree of enantioselectivity (e.g., >95% ee). Consequently, we felt that it would be more convenient to couple the asymmetric solvolysis to a second enzymatic kinetic resolution step to prepare *N*-benzoyl-L-α-amino acids of high optical purity.

With this objective in mind, our next task was to find a suitable enzyme that has high enantioselectivity in cleaving the methyl ester of **BC**, the product of the lipase-

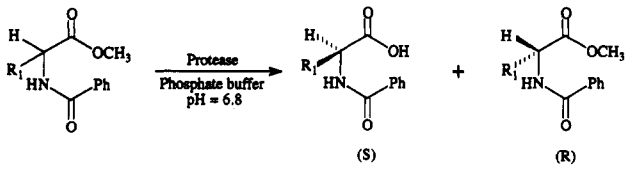


catalyzed asymmetric cleavage of 5(4*H*)-oxazolones (**A**). Although the P-30 lipase is capable of catalyzing the cleavage of the methyl ester of **BC** in aqueous medium, the reaction rate was too low to be of use. Hence, we turned our attention to the use of proteases for this kinetic resolution. The results in Table V clearly show that two proteases were uniquely suited to catalyze this hydrolytic reaction with a high degree of enantioselectivity (E = >100). Both small and larger amino acid side chains (R_1) were accommodated by the commercially available enzymes, prozyme 6 and protease N. This finding allows one to couple the two reactions for the preparation of virtually any amino acid, natural or unnatural, in high enantiomeric excess and in greater than 50% yield.

Enzymatic Asymmetric Cleavage of 5(4*H*)-Thiazolin-5-ones. Coletti-Previero *et al.*[10] first examined the enzymatic cleavage of 5(4*H*)-thiazolin-5-one using the enzyme chymotrypsin. Although these investigators claimed that the hydrolysis was enantioselective, unfortunately, the optical purity of the product was not given. This observation aroused our interest in reexamining the enzymatic asymmetric hydrolysis of 5(4*H*)-thiazolin-5-ones. Our initial experiment was conducted in 0.2 M phosphate buffer, pH 6.8, using several 4-substituted 2-phenylthiazolin-5-ones as model substrates. The results in Table VI show that the reaction rates were very slow, even though a stoichiometric quantity of chymotrypsin was used. After 8 days, the yield of the products for two of the substrates were less than 30% and the products were found to be racemic. While chymotrypsin cleaved 4-benzyl-2-phenylthiazolin-5-one with a moderate degree of enantioselectivity, the reaction rate was very slow. On the other hand, for 4-[(methylthio)ethyl]-2-phenylthiazolin-5-one, the rate of enzymatic hydrolysis was considerably faster, and the optical purity of the product was found to be greater than 90% ee. We then examined a variety of proteases with a view to finding one that is more suitable for catalyzing the asymmetric hydrolysis of 4-substituted 2-phenylthiazolin-5-ones. From our initial experiments, it appears that the most suitable enzymes are the proteases, prozyme 6 and 2A and N. We, therefore, examined the asymmetric hydrolyses of a variety of 4-substituted 2-phenylthiazolin-5-ones using these proteases. The results in Table VII show that both enzymes (prozyme 6 and protease 2A) have an (*S*)-stereochemical preference on all substrates examined. The reaction rate was slow but may be improved by the addition of 10% CH_3CN to the medium; the optical purity of the product

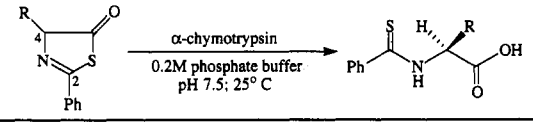
(10) Coletti-Previero, M. A.; Kraicsovits, F.; Previero, A. *FEBS Lett.* **1973**, *37*, 93–96.

Table V. Protease-Catalyzed Kinetic Resolution of *N*-Benzoylamino Acid Methyl Esters



			substrate (rem)		product			
R_1	enzyme	time (h)	yield (%)	ee (%)	yield (%)	ee (%)	convsn (%)	*E*
Me	prozyme 6	6	42	95	43	85	52	45
	protease N	6	43	95	40	95	50	>100
Me_2CH	prozyme 6	6	41	95	43	95	50	>100
	protease N	6	42	95	39	95	50	>100
$PhCH_2$	prozyme 6	30	31	100	44	100	50	>100
	protease N	21	43	100	37	100	50	>100

Table VI. Asymmetric Hydrolysis of 4-Substituted 2-Phenylthiazolin-5-ones Catalyzed by α-Chymotrypsin



R	time (h)	[α] (MeOH, c = 1)	yield (%)	ee (%)	conf.
Me	192		12	racemic	
$MeSCH_2CH_2$	60	+48.2	97	94	*S*
$PhCH_2$	192	+141.4	32	80	*S*
H_2NCOCH_2	48		30	racemic	

was excellent. Since *N*-(benzoylthio)amino acid esters racemize under strong acidic conditions, they may be first converted into the corresponding *N*-benzoyl derivatives without racemization using Boudet's method[11] and then cleaved.[17]

In an attempt to further improve the enzymatic reaction rate, we prepared several 4-substituted 2-methylthiazolin-5-one derivatives to determine whether the smaller substituent at C-2 is beneficial or not. The results in Table VIII show that indeed the reaction rate was markedly improved but the enantioselectivity of ring cleavage was not as high as in the 2-phenyl series. In all cases, the enzymes retained an (*S*)-stereochemical preference.

Discussion

For the past 2 years, our efforts have been devoted to the development of a general enzymatic asymmetric synthesis of α-amino acids. To date, most of the L-α-amino acids are prepared by fermentation or by enzymatic kinetic resolution processes.[12] As far as enzymatic asymmetric syntheses are concerned, most of these are tailored for a particular α-amino acid, the exception being the asymmetric hydrolysis of hydantoins using bacterial enzymes.[13] Although several types of hydantoinases have been discovered, their substrate specificities are only moderately relaxed. Tedious screening is often required to find a suitable hydantoinase for a particular transformation.

The hydantoins are interesting substrates for asymmetric induction studies. Depending on the substituent, the C-4 proton may undergo ready enolization so that in principle 100% of the substrate is convertible into the product. However, in some cases, the rate of racemization of different hydantoins may differ by at least 3 orders of magnitude, and a racemase is often required to bring about this equilibration.[14] Although several types of D- and L-hydantoinases are known, these processes have not attained the versatility suitable for industrial utilization except the processes for the preparation of D-phenylglycine and D-hydroxyphenylglycine.[13]

Initially, we looked into the possibility of utilizing commercially available lipases to asymmetrically cleave several hydantoins (R = propyl, Ph) in aqueous and nonaqueous media. Unfortunately, none of the lipases tested were capable of catalyzing this ring-opening reaction, a finding that is consistent with the general notion that lipases are sluggish toward the cleavage of amide bonds. For this reason, we turned our attention to the use of 5(4*H*)-oxazolones as substrate for lipase-catalyzed asymmetric induction. Our results clearly demonstrate the feasibility of conducting asymmetric hydrolysis of 5(4*H*)-oxazolones in aqueous buffer at pH 7.6, but the enantioselectivity of the enzyme-catalyzed ring opening depends markedly on the size of the C-4 substituent. By far the best substrate was when the C-4 substituent was benzyl for both lipases, AP and PL, to furnish D- and L-*N*-benzoylphenylalanine of high enantiomeric purity, respectively.

The bacterial lipases have very broad substrate specificities, and moreover, the *Pseudomonas* lipases, such as AK and P-30, are remarkably stable in nonpolar organic solvents when the water content is low. However, a layer of core water (2–3%) is needed to maintain the structural integrity and catalytically active conformation(s) of lipases.[15] Since our objective has been the development of a general asymmetric synthesis of natural and unnatural α-amino acids, it seemed fitting to take advantage of the unique properties of the *Pseudomonas* P-30 lipase by conducting the asymmetric cleavage of oxazolones in organic solvents. In such a microenvironment, nonenzymatic hydrolysis proceeds very slowly but the rate of enolization of the C-4 proton is sufficiently rapid[8] so that 100% of the substrate is convertible into product. When water was used as the nucleophile in nonaqueous medium, the rate of ring fission was too slow to be useful. However, the chemoselectivity and reaction rate of the lipase-catalyzed reaction in nonaqueous media can often be

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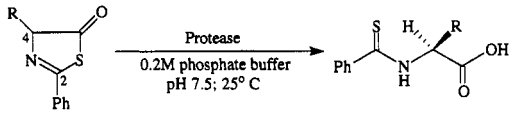
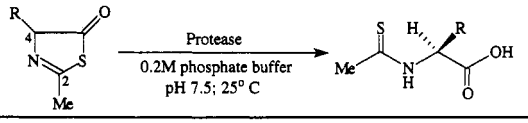
Table VII. Enantioselective Hydrolysis of 4-Substituted 2-Phenylthiazolin-5-ones Catalyzed by Proteases



R	enzyme	cosolvent, CH_3CN (v/v)	time (h)	[α] (MeOH, c = 1)	yield (%)	ee (%)	conf.
Me	prozyme 6	0	7	~0	67	90	
	protease 2A	0	96	−1	25	>95	S
Me_2CH	prozyme 6	10%	120	+21.3	39	94	S
		0	120	+36.7	49	94	S
	protease 2A	10%	156	+13.0	14	81	S
		0	156	+15.7	29	98	S
Me_2CHCH_2	prozyme 6	10%	88	+57.9	78	97	S
		0	120	+58.1	59	96	S
	protease 2A	10%	115	+47.1	61	95	S
		0	192	+41.1	49	96	S
$MeSCH_2CH_2$	prozyme 6	10%	12	+54.3	94	98	S
		0	48	+51.6	91	94	S
	protease 2A	10%	24	+48.4	90	96	S
		0	60	+50.4	91	96	S
$MeCH_2CH_2CH_2$	prozyme 6	10%	41	+34.2	86	99	S
		0	144	+34.6	85	93	S
	protease 2A	10%	110	+38.3	67	98	S
		0	240	+25.3	35	90	S
$PhCH_2$	prozyme 6	0	192	+100	30	78	S
H_2NCOCH_2	prozyme 6	0	17	+60.5	98	57	S

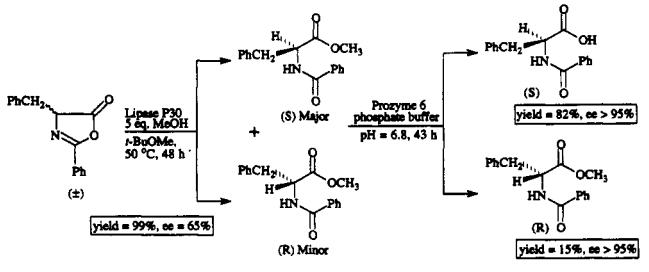
Table VIII. Asymmetric Hydrolysis of 4-Substituted 2-Methylthiazolin-5-ones Catalyzed by Proteases



R	enzyme	cosolvent, CH_3CN (v/v)	time (h)	[α] ($CHCl_3$, c = 1)	yield (%)	ee (%)
Me	prozyme 6	10%	19	−1.5	50	58
	protease N	10%	19	−1.1	37	47
Me_2CH	prozyme 6	10%	19	~0	79	0
	protease 2A	10%	19	+3.2	72	24
Me_2CHCH_2	prozyme 6	10%	4	+0.9	90	82
		0	45	+0.8	92	77
	protease 2A	0	45	+0.8	84	67
		0	45	+0.9	90	83
$PhCH_2$		10%	6	+150.5	84	72
	prozyme 6	10% DMF	6	+133.6	90	66
		0	22	+125.6	98	64
	protease N	0	47	+112.4	87	60
	protease 2A	0	78	+106.8	76	53

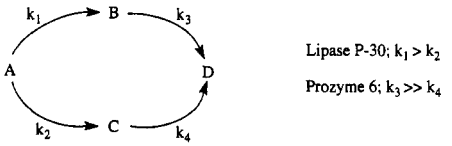
altered by the use of different nucleophiles.[16] We have found that when methanol was used as the nucleophile methanolysis of oxazolones proceeded at a useful rate to furnish methyl esters of *N*-benzoyl-L-α-amino acids. The optical purity of the products ranged from 66 to 98% ee.

As the likelihood of finding a single enzyme to accomplish the enantioselective cleavage of all different types of oxazolones with high degrees of enantiocontrol appeared rather remote, we decided to use an alternative strategy



to overcome this problem. While the substrate specificity of the *Pseudomonas* lipases, such as P-30, is broad, the enantioselectivity in most cases needs to be improved. This may be readily achieved by the use of a second enzyme to effect a kinetic resolution of the enriched product. We have found two proteases that are capable of catalyzing the enantioselective hydrolysis of the methyl ester (**BC**) with *E* values of >100, leading to enantiopure L-*N*-benzoyl-α-amino acids (**D**). In turn, the *N*-benzoyl function may be chemically cleaved without racemization under acidic conditions (6 N HCl).[17]

This type of synergistic coupling of an enantioselective reaction ($k_1 > k_2$) with a subsequent kinetic resolution ($k_3 > k_4$) leads to a marked enhancement in the enantiomeric purity. This concept has been extensively employed in enzymatic and non-enzymatic asymmetric syntheses.[18]



The relationship between the enantiomeric excess of the product of lipase-catalyzed methanolysis, ee_o = (**B** − **C**)/(**B** + **C**), and the enantiomeric purity of the final *N*-benzoyl-L-α-amino acids and the *E* value of the protease (*E* = 100) is governed by the following equation,[19] where *c* is the extent of conversion of protease-catalyzed methyl ester hydrolysis. A plot of this equation gives a useful

$$\left[1 - c\left(\frac{1 + ee'}{1 + ee_o}\right)\right] = \left[1 - c\left(\frac{1 - ee'}{1 - ee_o}\right)\right]^E \qquad (1)$$

theoretical curve (Figure 1), which relates the variables

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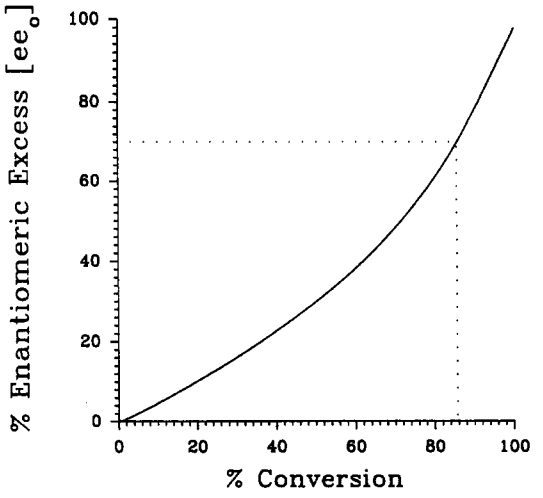

Figure 1. Plot relating the initial percent enantiomeric excess (ee_o), the percent conversion (c), and the final enantiomeric excess (ee'), fixed at 98% for the enzyme with an enantiomeric ratio (E) of 100. The figure was computer generated from eq 1 by equating c and ee_o to a function of x for values $0 \le x \le 1$, with ee' fixed at 0.98; $c = [100(1-x)(1-x^{100})]/(100-99x-x^{100})$; and $ee_o = (98-99x+x^{100})/(100-99x-x^{100})$.

ee_o and c for fixed values of ee' (set at 98%) and $E = 100$. For example, let us assume that the product of lipase-catalyzed solvolysis of oxazolone affords an antipodal mixture with an ee_o of 0.70, which is then subjected to enantioselective hydrolysis by a protease ($E = 100$). To secure the *N*-benzoyl-L-α-amino acid with an ee' of ≥98%, it is necessary to stop the reaction when $c \le 0.856$. Equation 1 is general, and plots may be obtained to relate different values of ee_o, ee', E, and c. This synergistic deployment of two enzymes allows the ready preparation of virtually all L-α-amino acids of high enantiopurity in yields greater than 50%, which is an inherent advantage over conventional resolution procedures.

As far as the asymmetric hydrolysis of thiazolin-5-ones is concerned, the two proteases, prozyme 6 and 2A, are uniquely suited for this transformation. These enzymes are superior to chymotrypsin in terms of reaction rates and enantioselectivity on most 4-substituted 2-phenylthiazolin-5-ones, especially those bearing small C-4 substituents such as 4-methyl-2-phenylthiazolin-5-one (Table VII). Hence, this finding nicely complements the P-30 lipase-catalyzed asymmetric solvolysis of 4-substituted 2-phenyloxazolin-5-ones, which prefers larger C-4 substituents.

Experimental Section

Materials. The following lipases were purchased from the Amano Co.: *Pseudomonas cepacia* (P-30), *Pseudomonas sp.* (AK and K-10), and *A. niger* (AP). Porcine pancreatic lipase (Fermlipase PL) was a product of Genencor. Proteases prozyme 6, N, and 2A were purchased from the Amano Co. α-Chymotrypsin was a product of Sigma Chemical Co. All other chemicals and solvents were of the highest quality grade available and were purchased from Aldrich Chemical Co. or Sigma Chemical Co.

The substrates, 5(4*H*)-oxazolin-5-one[20] and 5(4*H*)-thiazolin-5-one derivatives,[21] were synthesized by standard methods.

Thin-layer chromatography (TLC) was performed on glass plates coated with a 0.25-mm layer of silica gel. Flash column chromatography was performed with silica gel (40 μm). All solvents were glass distilled prior to use. All combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. For the determination of enantiomeric excess (ee), the methyl ester group was analyzed by ^1H NMR ($CDCl_3$) in the presence of the chiral shift reagent, $Eu(hfc)_3$. All products (amino acids) were first converted into their methyl esters and then analyzed as above. The absolute configurations of the *N*-benzoyl-L-amino acid methyl esters and *N*-benzoyl-L-amino acids obtained from the enzymatic reactions were established by comparison of their optical rotations to the corresponding known derivatives prepared from L-amino acids. All yields reported herein are isolated yields.

Enantioselective Hydrolysis of 4-Substituted α-Phenyloxazolin-5-ones in Aqueous Buffer. General Procedure. To a suspension of either lipase (AP) or lipase (PL) (50 mg) in 2 mL of 0.2 M phosphate buffer, pH 7.5, was added 50 mg of a 4-substituted 2-phenyloxazolin-5-one. The reaction mixture was stirred with a magnetic stirrer for the indicated times at 25 °C until the reaction was complete. After acidification of the mixture (2 N HCl), the contents were extracted exhaustively with ethyl acetate three times. The residue was chromatographed over a silica gel column (1 × 10 cm), and the column was eluted with a solvent mixture consisting of $CHCl_3$–methanol (98:2 to 95:5) to yield the product, *N*-benzoylamino acids. The results are tabulated in Table I.

Enantioselective Methanolysis of 5(4*H*)-Oxazolone Derivatives in Organic Solvent. General Procedure. To a solution of 50 mg of racemic substrate in 2 mL of *tert*-butyl methyl ether containing 5 equiv of methanol (in some cases 5 equiv water was present) was added 50 mg of a crude lipase. The mixture was incubated at 50 °C until complete conversion was achieved. The progress of the reaction was monitored by TLC using the solvent system ethyl acetate–hexanes (this solvent ratio varies depending on R_1 and R_2, e.g., R_f value is 0.60 for *N*-benzoylphenylalanine methyl ester using 1:1 ethyl acetate/hexanes as the mobile phase). The crude lipase was separated from the reaction mixture by filtration and then washed with ethyl acetate. The product was purified by silica gel flash chromatography (e.g., 4:1 hexanes/ethyl acetate for *N*-benzoylamino acid methyl ester, this ratio varies with different R_2).

The results of the enantioselective methanolyses are summarized in Tables II–IV.

Kinetic Resolution of *N*-Benzoylamino Acid Methyl Esters. General Procedure. To 50 mg of a (±)-*N*-benzoylamino acid methyl ester, suspended in 4 mL of 0.2 M phosphate buffer (pH 6.8), was added 50 mg of a crude protease (prozyme 6 or protease N), 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). The reaction was terminated by the addition of saturated $NaHCO_3$ and extracted with ethyl acetate (3 × 20 mL). Evaporation of the organic extract to dryness afforded the remaining *N*-benzoyl-D-amino acid methyl ester. The aqueous phase was acidified to pH 2 with 3 N HCl and extracted with ethyl acetate (3 × 20 mL). The organic extract was evaporated to yield the product *N*-benzoyl-L-α-amino acid. The results are tabulated in Table V.

Enantioselective Solvolysis Followed by Kinetic Resolution Procedure. The reaction mixture contained 250 mg of (±)-4-benzyl-2-phenyloxazolin-5-one and 250 mg of crude lipase P-30 in 20 mL of *tert*-butyl methyl ether. The mixture was incubated at 50 °C for 48 h. The lipase P-30 was separated from the reaction mixture by filtration and washed with ethyl acetate. The combined organic layer was concentrated to dryness under reduced pressure to yield 280 mg of *N*-benzoyl-L-phenylalanine methyl ester (99% yield; 65% ee).

A suspension of 280 mg of the above residue and 280 mg of crude prozyme 6 in 20 mL of 0.2 M phosphate buffer (pH 6.8) was stirred vigorously at room temperature for 43 h. The reaction mixture was terminated by the addition of saturated $NaHCO_3$ solution and then extracted with ethyl acetate (3 × 30 mL). Concentration of the combined organic layers gave the remaining substrate, 43 mg (15.3%). The aqueous phase was acidified to

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pH 2 with 3 N HCl and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were concentrated to dryness to give 220 mg of *N*-benzoyl-L-phenylalanine (82%) (ee > 95%), 82.1%, $[\alpha]^{25}_D = -30°$ (*c* 1.0, MeOH).

Enantioselective Hydrolysis of (±)-4-Benzyl-2-phenylthiazolin-5-one in Aqueous Buffer. To 52 mg of racemic (±)-4-benzyl-2-phenylthiazolin-5-one (0.2 mM) suspended in 4 mL of 0.2 M phosphate buffer (pH 6.8) was added 52 mg of a crude enzyme (for α-chymotrypsin, 10 mg was added), and the mixture was stirred vigorously with magnetic stirrer at 25 °C for the time indicated in Table VI. The reaction was terminated by the addition of saturated $NaHCO_3$, and the mixture was then extracted with ethyl acetate (3 × 20 mL) to remove the remaining substrate. The aqueous layer was acidified to pH 2 with 3 N HCl and extracted with ethyl acetate (3 × 20 mL). The residue consisted of (+)-*N*-(thiobenzoyl)-L-phenylalanine. The results are shown in Table VI.

The absolute configuration of the product was established by comparison of their optical rotations to those known for *N*-(thiobenzoyl)-L-α-amino acid derivatives, prepared by the method of Kjaer.[21a]

Enzymatic Asymmetric Cleavage of 5(4*H*)-Thiozolin-5-ones in Aqueous Buffer. General Procedure. To a suspension consisting of 40 mg of substrate in 2 mL of 0.2 M sodium phosphate buffer (pH 6.8) was added 40 mg of a crude protease. The reaction mixture was stirred vigorously at room temperature to complete conversion, monitored by TLC using the solvent system ethyl acetate–hexanes (1:1). When acetonitrile (10%) was used, only 1.8 mL of 0.2 M sodium phosphate buffer and 0.2 mL of acetonitrile were added. The isolation and characterization procedures are the same as described above. The results are shown in Tables VII and VIII.

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