Molecular Basis for the Enantioselective Ring Opening of β-Lactams Catalyzed by *Candida antarctica* Lipase B

Seongsoon Park,[a] Enikö Forró,[a,b] Harjap Grewal,[a] Ferenc Fülöp,[b] Romas J. Kazlauskas[a,*]

[a] Department of Chemistry, McGill University, 801 Sherbrooke St. W., Montréal, Québec, H3A 3K6, Canada
E-mail: romas.kazlauskas@mcgill.ca
[b] Institute of Pharmaceutical Chemistry, University of Szeged, H-6701 Szeged, PO Box 121, Hungary

Received: April 11, 2003; Accepted: May 20, 2003

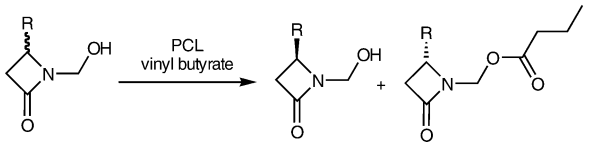
Abstract: Lipase B from *Candida antarctica* (CAL-B) catalyzes the slow, but highly enantioselective ($E > 200$), ring-opening alcoholysis of two bicyclic and two 4-aryl-substituted β-lactams. Surprisingly, the rate of the reaction varies with the nature of the alcohols and was fastest with either enantiomer of 2-octanol. A 0.5-g scale reaction with 2-octanol as the nucleophile in diisopropyl ether at 60 °C yielded the unreacted β-lactam in 39–46% yield (maximum yield is 50%) with ≥96% ee. The product β-amino acid esters reacted further by polymerization (not isolated or characterized) or by hydrolysis due to small amounts of water in the reaction mixture yielding β-amino acids (7–11% yield, ≥96% ee). The favored enantiomer of all four β-lactams had similar 3-D orientation of substituents, as did most previously reported β-lactams and β-lactones in similar ring-opening reactions. Computer modeling of the ring opening of 4-phenylazetidin-2-one suggests that the reaction proceeds *via* an unusual substrate-assisted transition state, where the substrate alcohol bridges between the catalytic histidine and the nitrogen of the β-lactam. Computer modeling also suggested that the molecular basis for the high enantioselectivity is a severe steric clash between Ile189 in CAL-B and the phenyl substituent on the slow-reacting enantiomer of the β-lactam.

Keywords: β-amino acids; *Candida antarctica*; enantioselectivity; enzyme catalysis; β-lactam ring opening; lipase B; molecular modeling

Introduction

β-Lactams, key structures in β-lactam antibiotics, are also important synthetic intermediates for β-amino acids,[1] short peptide segments,[2] natural products,[3] and heterocycles.[4] For this reason, many researchers are searching for good enantioselective routes to β-lactams.[5] Although lipase-catalyzed reactions are often a good enantioselective route to many chiral intermediates,[6] lipases and esterases do not usually catalyze the ring opening of the β-lactams. In contrast, β-lactams and β-lactones inhibit serine hydrolases by forming a stable acyl-enzyme intermediate. The natural protein target of β-lactam antibiotics is the serine in transpeptidase and possibly other penicillin-binding proteins, which catalyze the last step in bacterial cell wall biosynthesis. Similarly, medicinal chemists used β-lactams as mechanism-based inhibitors of serine proteases[7] and β-lactones as mechanism-based inhibitors of lipases, which also have a nucleophilic serine.[8]

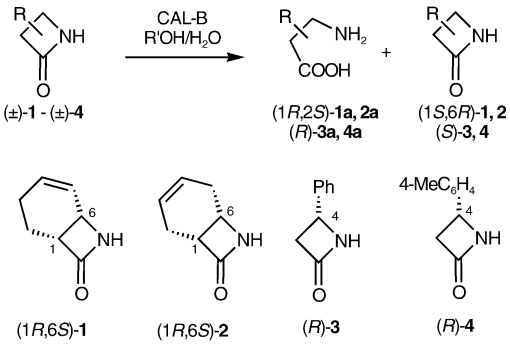
However, researchers can use lipase-catalyzed enantioselective reactions with pendant groups to resolve β-lactams. For example, lipase from *Pseudomonas cepacia* catalyzes the highly enantioselective acylation of the *N*-hydroxymethylated β-lactams even though the reaction site (the hydroxy group) is far from the stereocenter, Eq. (1).[9,10] In a related approach, Achilles et al. resolved β-lactams by chymotrypsin-catalyzed hydrolysis of the ester in a pendant *N*-CH_2COOEt group.[11]



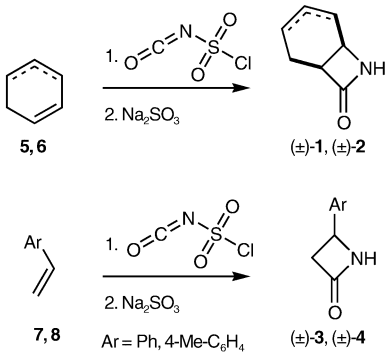
(1)

In spite the ability of β-lactams to inhibit some serine hydrolases, three groups reported lipase- or esterase-catalyzed ring opening of β-lactams. Jones and Page reported the ring opening of the β-lactam in benzylpenicillin catalyzed by pig liver esterase.[12] Sih's group reported the enantioselective ring opening of an *N*-benzoyllactam catalyzed by lipase from *Pseudomonas cepacia*.[13] The *N*-benzoyl group is unusual in that it even further activates the strained β-lactam ring by increasing the leaving group ability of the nitrogen of the

  DOI: 10.1002/adsc.200303069



Scheme 1. Enantioselective ring opening of β-lactams yields a β-amino acid and unreacted β-lactam. The initial product may be the β-amino acid ester, but this ester hydrolyzes to the acid under the reaction conditions. The structures show the fast reacting enantiomers.



Scheme 2. Synthesis of racemic β-lactams.

β-lactam. Adam et al. recently reported the direct ring opening of α-methylene-β-lactams by lipase B from *Candida antarctica.* The reactions were slow, but highly enantioselective (E usually >100).[14] This β-lactam ring is unusual due to the α-methylene group, which increases ring strain, flattens the conformation of the ring and withdraws electrons from the carbonyl group.

Although most esterases and lipases do not cleave β-lactams, β-lactamases do cleave them efficiently using either a zinc hydrolase mechanism (class B β-lactamases) or modified serine hydrolase mechanisms (class A, C, and D β-lactamases). Even though both serine esterases/lipases and serine β-lactamases have a serine as the nucleophile, their protein folds and catalytic machinery differ.[15] Two β-lactamases showed no enantioselectivity in the hydrolysis of an *N*-benzoyl-β-lactam.[18]

In this paper, we report a similar enantioselective ring opening of β-lactams, but for the unstrained normal β-lactams: the bicyclic (±)-**1** and (±)-**2** and 4-aryl-substituted β-lactams (±)-**3** and (±)-**4**, Scheme 1. Ring opening yields the ring-opened β-amino acids **1a**–**4a** and unreacted β-lactam enantiomers **1**–**4**. Change of nucleophiles, alcohols, causes dramatic changes in reaction rate. We used modeling to propose a possible transition state for this unusual reaction and propose a molecular basis for the enantioselectivity.

Results

CAL-B-Catalyzed Ring Opening of β-Lactams

1,2-Dipolar cycloaddition of chlorosulfonyl isocyanate to 1,3- and 1,4-cyclohexadiene, styrene, and 4-methylstyrene yielded the bicyclic β-lactams (±)-**1** and (±)-**2** and the 4-aryl-substituted β-lactams (±)-**3** and (±)-**4**, Scheme 2.[16] Initial screening of commercial lipases, esterases and proteases for their ability to catalyze hydrolysis of (±)-**1** revealed several that catalyzed the hydrolysis, but with low enantioselectivity.[17] The most promising hydrolase was lipase B from *Candida antarctica* (Novozym 435, CAL-B) since it was slightly enantioselective ($E=5$, Table 1, row 1). For four bicyclic β-lactams similar to (±)-**1** and (±)-**2**, changing the solvent from water to diisopropyl ether dramatically increased the enantioselectivity ($E>200$), but the reactions were very slow.[18]

However, upon changing from a hydrolysis reaction to an alcoholysis reaction, we could increase the reaction rate depending on the nature of the alcohol. Although ethanol as the nucleophile showed only 2% conversion under the standard reaction conditions, replacing ethanol with longer chain alcohols (heptanol and dodecanol [rows 3 and 4]), 2,2,2-trichloroethanol (row 5) or *tert*-butyl alcohol (row 6) increased the conversion to 14–31% while maintaining high enantioselectivity ($E>45$). Some secondary alcohols [2-octanol and 1-phenyl-1-ethanol (rows 8 and 9)] showed even higher conversions (20–34%) while maintaining high enantioselectivity ($E>40$). However, reactions with isopropyl alcohol (row 7) showed only 3% conversion under these conditions. Two halogenated secondary alcohols (rows 10 and 11) showed low enantioselectivity ($E\sim1$), while no reaction occurred with phenol (row 12). We concluded that 2-octanol showed the best combination of enantioselectivity and reaction rate (row 8). Later experiments in diisopropyl ether as the solvent showed that either enantiomer 2-octanol gave the same conversion and enantioselectivity as racemic 2-octanol (rows 16–18). Consistent with the important role of the alcohol on the reaction rate, the reaction rate decreased if the reaction mixture contained less alcohol (data not shown.).

Next we optimized the solvent. The CAL-B-catalyzed alcoholysis of (±)-**1** with 2-octanol was very slow when toluene was replaced by acetonitrile, tetrahydrofuran or dichloromethane (1–2% conversion after 24 h; data not shown), but the conversion was marginally higher in diisopropyl ether (row 8 *vs.* 13) and we chose to continue

Table 1. Conversion and enantioselectivity of CAL-B-catalyzed ring opening of (±)-**1**.[a]

Row	CAL-B (mg cm^{-3})	Solvent: R'OH (15:1, v/v)	Time (h)	Conv. (%)	ee$_s$[b] (%)	ee$_p$[c] (%)	*E*
1	20	H_2O	72	36[d]	32	57	5
2	20	Toluene:CH_3CH_2OH	44	2	2	>95	>40
3	20	Toluene:$CH_3(CH_2)_6OH$	44	14	16	>95	>46
4	20	Toluene:$CH_3(CH_2)_{11}OH$	44	31	43	>95	>60
5	20	Toluene:Cl_3CCH_2OH	44	16	18	>95	>47
6	20	Toluene:$(CH_3)_3COH$	46	14	16	>95	>45
7	20	Toluene:$(CH_3)_2CHOH$	48	3	3	>95	>40
8	20	Toluene:$CH_3(CH_2)_5CH(CH_3)OH$	44	34	48	>95	>63
9	20	Toluene:$C_6H_5CH(CH_3)OH$	46	20	24	>95	>49
10	20	Toluene:$(ClCH_2)_2CHOH$	44	5	1	19	~1
11	20	Toluene:$(BrCH_2)(CH_3CH_2)CHOH$	44	36	*Rac*	*Rac*	1
12	20	Toluene:C_6H_5OH	44	No reaction			
13	20	*i*-Pr_2O:$CH_3(CH_2)_5CH(CH_3)OH$	44	36	53	>95	>66
14	30	*i*-Pr_2O:$CH_3(CH_2)_5CH(CH_3)OH + Et_3N$	43	39	71	>95	>73
15	10	*i*-Pr_2O:$CH_3(CH_2)_5CH(CH_3)OH$	43	15	17	>95	>46
16	10	*i*-Pr_2O:$CH_3(CH_2)_5CH(CH_3)OH$	40[e]	28	36	>95	>56
17	10	*i*-Pr_2O:(+)-$CH_3(CH_2)_5CH(CH_3)OH$	40[e]	28	37	>95	>56
18	10	*i*-Pr_2O:(−)-$CH_3(CH_2)_5CH(CH_3)OH$	40[e]	28	37	>95	>56

[a] 0.05 M substrate (approx. 15 mg in 2 cm^3), 60 °C.
[b] ee$_s$ is the enantiomeric excess of the unreacted starting β-lactam. It was measured by gas chromatography on a Chiralsil Dex CB column.
[c] ee$_p$ is the enantiomeric excess of the products. This was not measured, but calculated from the conversion and the enantiomeric purity of the remaining starting material, which were measured by gas chromatography.
[d] Determined by ^{1}H NMR.
[e] At 70 °C.

Table 2. Optimum temperatures for the CAL-B-catalyzed ring opening of (±)-**1** – (±)-**4** by racemic 2-octanol.[a]

Row	Compound	Temp. (°C)	ee$_s$ (%)	ee$_p$ (%)	conv. (%)	*E*
1	(±)-**1**	7–8	*No reaction*			
2	(±)-**1**	35	5	~48	~9	~3
3	(±)-**1**	40	7	~55	~11	~4
4	(±)-**1**	50	18	~72	~20	~7
5	(±)-**1**	55	20	>99	17	>200
6	(±)-**1**	60	40	>99	29	>200
7	(±)-**1**	70	67	>99	40	>200
8	(±)-**1**	75	72	>99	42	>200
9	(±)-**1**	80	88	~70	~56	~16
10	(±)-**2**	60	82	>95	46	>100
11	(±)-**3**	60	96	>95	50	>154
12	(±)-**4**	60	83	>95	47	>102

[a] Conversion (conv) and enantiomeric excess of the starting β-lactam (ee$_s$) were measured by GC after 24 h. The enantiomeric excess of the products (ee$_p$) was calculated from these values. 30 mg cm^{-3} enzyme, substrate concentration: 0.05 M, in 2-octanol : *i*-Pr_2O (1:15, v/v).

our studies in diisopropyl ether. Although Parker et al. reported faster CAL-B-catalyzed ring opening of 4-substituted oxazol-5(4*H*)-ones upon addition of triethylamine,[19] in our reaction adding triethylamine had little effect on conversion (rows 13 and 14).

Finally, we optimized the temperature of the reaction. CAL-B catalyzed alcoholysis of (±)-**1** with racemic 2-octanol at 35–50 °C was slower and much less enantioselective (Table 2, rows 1–4) than at 55–75 °C. The enantioselectivity dropped again at 80 °C and we chose 60 °C as the optimum temperature. Ring opening of the other β-lactams (±)-**2** – (±)-**4** also showed excellent enantioselectivity under these conditions.

Preparative-scale resolution (0.5 gram) of **1–4** yielded the unreacted β-lactams in 39–46% yield (maximum yield is 50% for a resolution) with 96–99% ee, Table 3. We used a higher ratio of lipase to substrate in the preparative-scale resolution (50 mg cm^{-3} *vs.* 10–20 mg

Table 3. Preparative-scale CAL-B-catalyzed ring opening[a] of (±)-**1** – (±)-**4**.

	Time (h)	Conv. (%)	*E*	β-Lactam recovered (**1**–**4**)				β-Amino acid produced (**1a**–**4a**)			
				Yield (%)	Isomer	ee[b] (%)	$[\alpha]_D^{25}$	Yield (%)	Isomer	ee[c] (%)	$[\alpha]_D^{25}$
(±)-**1**	44	50	>200	39	1*S*,6*R*	99	+161[d]	11	1*R*,2*S*	97	+120[e]
(±)-**2**	47	50	>200	42	1*S*,6*R*	99	−29[f]	9	1*R*,2*S*	99	−39[g]
(±)-**3**	20	50	>200	46	*S*	99	−139[h]	11	*R*	96	+6.8[i]
(±)-**4**	48	50	>200	40	*S*	96	−121.9[j]	7	*R*	98	−8[k]

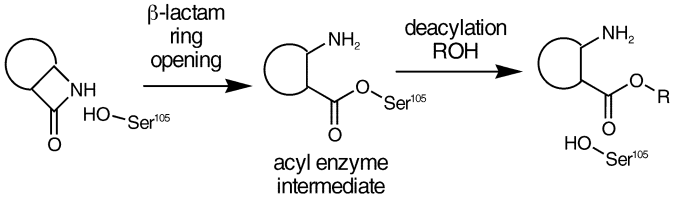
[a] 0.5 g substrate, 4.0 g immobilized CAL-B, 80 cm^3 2-octanol:*i*-Pr_2O (1:15, v/v) at 60 °C.
[b] According to GC.
[c] According to HPLC.
[d] $c = 0.29$; $CHCl_3$.
[e] $c = 0.27$; H_2O.
[f] $c = 0.26$; $CHCl_3$.
[g] $c = 0.5$; H_2O.
[h] $c = 0.19$; EtOH.
[i] $c = 0.45$; H_2O.
[j] $c = 0.5$; EtOH.
[k] $c = 0.1$; H_2O.

cm^{-3} for the small-scale reactions) to increase the conversion to 50% in 20–48 h. We did not isolate any β-amino acid octyl esters because they further polymerized (polymer not characterized) or hydrolyzed to the β-amino acids under the reaction conditions. We isolated the β-amino acids with high enantiomeric purity (96–99% ee), but low yield (7–11%). The small amounts of water needed for the hydrolysis may have come from either the enzyme preparation or the solvent.

The absolute configurations of the fast-reacting enantiomers were (1*R*,6*S*) for **1** and **2** and (*R*) for **3** and **4** as shown in Scheme 1. We established these configurations by comparing the specific rotations of the slow-reacting enantiomers with those reported in the literature, Table 3 and Experimental Section. The fast-reacting enantiomers have a similar shape in all four cases. The substituent at the stereocenter next to the nitrogen (position 6 for **1** and **2**, position 4 for **3** and **4**) points down in Scheme 1 in all four cases, even though the sequence priority rules yield a 6*S* configuration for **1** and **2** and a 4*R* configuration for **3** and **4**.

Molecular Modeling

To rationalize the reactivity and high enantioselectivity of CAL-B toward these β-lactams and the critical role of the alcohol, we used computer modeling of the ring-opening step for β-lactam **3**. The lipase-catalyzed reaction of β-lactams presumably proceeds in two stages, Scheme 3. First, ring opening of the β-lactam forms an acyl enzyme intermediate and second, deacylation of the enzyme yields the product ester. (This ester may undergo subsequent polymerization or hydrolysis by small amounts of water in the reaction medium. Indeed, we isolated the hydrolysis product in low yield



Scheme 3. The CAL-B catalyzed transesterification of β-lactams with alcohols proceeds in two steps: ring opening of the β-lactam followed by deacylation of the acyl enzyme intermediate.

as discussed above.) The first step involves the β-lactams, while the second step involves the more flexible β-amino acyl group. Since the less flexible β-lactams would be more difficult to accommodate in the active site, we hypothesized that enantioselectivity originates in the ring-opening step and focused our computer modeling on this step.

First, we identified a catalytically productive conformation of (*R*)-**3**, the fast reacting enantiomer. We modeled a phosphonate as an analogue of the tetrahedral intermediate that results upon attack of the active site serine at the carbonyl of β-lactam. We define a catalytically productive conformation as one that maintains all the key hydrogen bonds required for catalysis (see below) and avoids severe steric clashes with the enzyme. It was difficult to find a catalytically productive conformation for the fast reacting enantiomer of **3**.

The tetrahedral intermediate for hydrolysis of (*R*)-(+)-**3** (fast enantiomer) is relatively rigid and can adopt only two conformations, which differ in the pucker of the four-membered ring, Scheme 4. (Although the β-lactam ring is flat, upon formation of the tetrahedral intermediate, the ring adopts one of two puckered butterfly conformations.) Neither conformation contained all of

Table 4. Key hydrogen bonds in possible catalytically productive models of the CAL-B-catalyzed ring opening of the fast reacting enantiomer β-lactam (*R*)-(+)-**3**.

	Hydrogen bonds[a]	H-bond distance, Å (angle)[b]	Comments
Conformation 1	a	3.02 (130°)	H-bond 'c' is weak or missing. Shows severe steric strain between Ile285 and phenyl substituent of the β-lactam. Unlikely to be a catalytically productive structure.
	b	3.19 (146°)	
	c	3.36 (148°)	
	d	2.73 (157°)	
	e	2.74 (163°)	
Conformation 2	a	2.81 (127°)	H-bond 'b' is weak or missing.
	b	3.37 (140°)	
	c	2.79 (144°)	
	d	2.80 (168°)	
	e	2.74 (175°)	
Alcohol bridged	a	2.82 (125°)	H-bond 'b' is missing, but may not be needed because 2-octanol forms a bridge of alternate hydrogen bonds, but H-bond 'g' is weak.
	b	3.70 (125°)	
	c	2.82 (147°)	
	d	2.81 (168°)	
	e	2.75 (171°)	
	f	2.96 (125°)	
	g	3.25 (136°)	
Alkoxide-bridged tautomer	a	2.81 (126°)	H-bond 'b' is missing, but may not be needed because 2-octanol forms a bridge of alternate hydrogen bonds. Bridging H-bonds 'f' and 'g' are stronger because 2-octanol is deprotonated and the β-lactam nitrogen is protonated.
	b	3.76 (130°)	
	c	2.77 (139°)	
	d	2.81 (168°)	
	e	2.74 (173°)	
	f	2.86 (125°)	
	g	3.00 (145°)	

[a] Scheme 4 defines the hydrogen bonds.

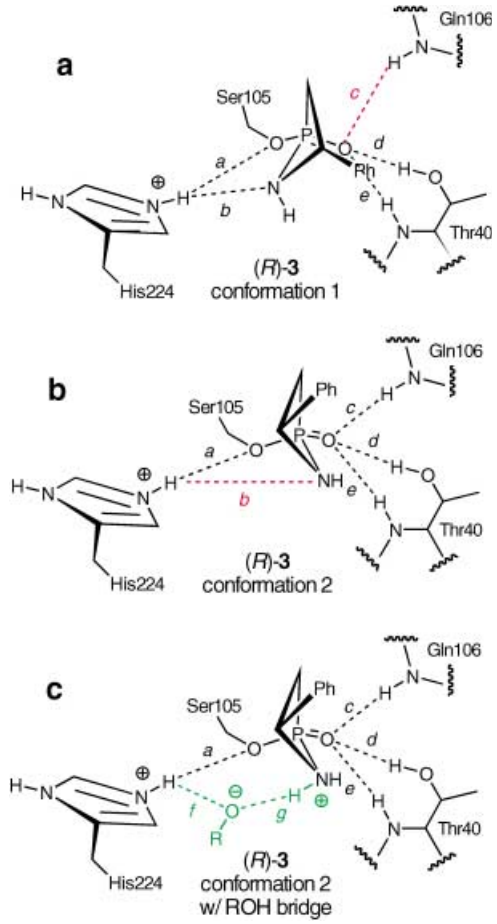
[b] Distance between non-hydrogen atoms (N–N, N–O or O–O). Distances of 2.7 – 3.2 Å are consistent with a hydrogen bond. Angle refers to the N–H–O or similar angle. For an ideal hydrogen bond, this angle is 180°, but angles of $>120°$ are consistent with a hydrogen bond.

the key hydrogen bonds, Table 4. For conformation 1, the hydrogen bond between the N–H of Gln106 and the phosphonyl oxygen was weak or missing. The N–O distance is 3.36 Å, beyond the limit for a hydrogen bond of ~3.2 Å. More importantly, the phenyl substituent encountered severe steric strain with Ile285. Because of the missing hydrogen bond and the severe steric clash, conformation 1 cannot be catalytically productive.

Conformation 2 is also unlikely to be catalytically productive because the hydrogen bond between His 224 Nε – H and the β-lactam nitrogen is weak or missing. The calculated N–N distance is 3.37 Å, also beyond the limit of ~3.2 Å. Without this hydrogen bond, the leaving group would be the highly basic RNH^-, which is chemically very unlikely. However, conformation 2 fits well in the active site of CAL-B and did not encounter any steric clashes.

However, upon adding the co-substrate 2-octanol to the conformation-2 model, we did find a catalytically productive conformation. The 2-octanol formed a hydrogen-bond bridge between the His 224 Nε – H and the β-lactam nitrogen and restored the missing hydrogen bond (not shown). One hydrogen bond of this bridge was weak (3.25 Å), Table 4. This hydrogen bond strengthened (to 3.00 Å), when the proton is transferred from the 2-octanol (leaving the alkoxide) to the β-lactam nitrogen creating a protonated amine, Figure 1c. This transfer of a proton strengthened the hydrogen bond because it created complementary charges at each end of the hydrogen bond. Either the alcohol-bridged structure or the alkoxide-bridged structure could be the catalytically productive conformation of the fast-reacting enantiomer of **3**. This complex is an example of substrate-assisted catalysis.

Rationalizing the observed enantioselectivity was straightforward since the slow-reacting enantiomer, (*S*)-**3**, encountered severe steric clashes in both conformations. In conformation 1, the phenyl substituent of the β-lactam clashed with Ile189 and Leu278 (on the lower left side of the binding pocket in Figure 1), while in conformation 2, it clashes with Ile189 and Val190 (structure shown in Figure 1b). Energy minimization of either structure distorted the phenyl group to a non-planar geometry. On the other hand, the fast-reacting



Scheme 4. Line diagrams of possible catalytically productive conformations for the ring opening of β-lactam (*R*)-(+)-**3** (fast enantiomer) catalyzed by CAL-B showing the phosphonate analogues of the tetrahedral intermediates. **a**) Conformation 1 orients the lactam amide towards His224. Hydrogen bond 'c' (red) between the main chain amide N−H of Gln106 and the phosphonyl oxygen, which mimics the oxyanion of the tetrahedral intermediate is weak or missing in this structure. In addition, the phenyl group encounters severe steric strain with Ile285 (not shown). **b**) Conformation 2 orients the amide away from His224. Hydrogen bond 'b' (red) between His 224 Nε – H and the β-lactam nitrogen is weak or missing. **c**) The substrate alcohol forms a hydrogen-bonded bridge (green) between His 224 Nε – H and the lactam nitrogen of conformation 2. This structure shows the alkoxide-bridged tautomer, but the alcohol-bridged structure is the same except the hydrogen being transferred (green) is on the oxygen of the alcohol. We propose that either the alkoxide-bridged or the alcohol-bridged structures are the catalytically productive conformations. (A sixth key hydrogen bond present in all structures is between His 224 Nδ – H and the carboxylate of Asp264. For clarity, neither this hydrogen bond nor Asp264 are shown in the line diagrams.)

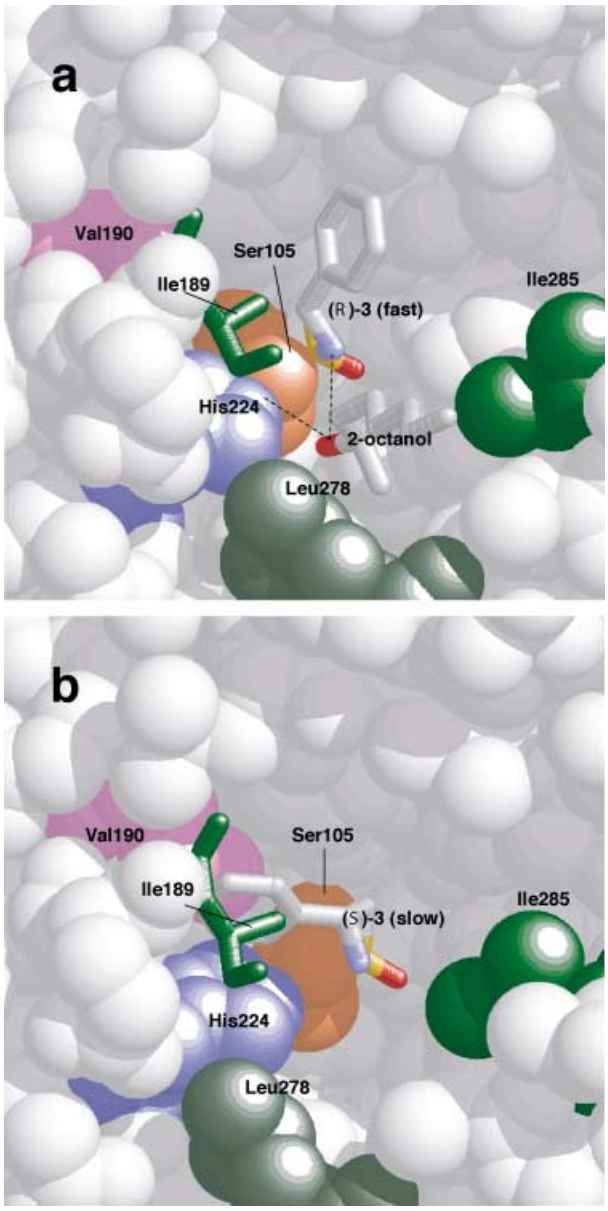


Figure 1. Proposed tetrahedral intermediate analogues of the enantiomers of β-lactam **3**. CAL-B is shown in space-filling representation, except for Ile189, which is shown as stick representations. The tetrahedral intermediate analogue for the β-lactam is shown in stick representation as is the 2-octanol in part **a**. **a**) The proposed productive conformation for the fast-reacting enantiomer, (*R*)-**3**, corresponds to structure **c** in Scheme 4. The phenyl ring points upward and toward the reader and makes good hydrophobic interactions with Ile189, Ala141, Thr138. **b**) We did not identify any catalytically productive structures for the slow-reacting enantiomer, (*S*)-**3**. The picture shows a non-energy minimized structure of the slow enantiomer in an orientation similar to that in part **a**. Upon energy minimization, the phenyl ring distorts to an unrealistic non-planar geometry due to severe steric clash with the side chains of Val190 and Ile189.

enantiomer fits well in conformation 2 as discussed above and in Figure 1a. The phenyl ring makes favorable hydrophobic contacts to Ile189 (3.3 Å between carbon atoms), Ala141 (4.0 Å) and Thr138 (3.4 Å).

Discussion

CAL-B catalyzes the highly enantioselective ($E > 200$) ring opening of unactivated bicyclic and 4-aryl-substituted β-lactams in diisopropyl ether at 60 °C. Adding 2-octanol to this reaction increased both the enantioselectivity and reaction rate. We previously resolved the same β-lactams using *Pseudomonas cepacia* lipase-catalyzed butyrylation of the *N*-hydroxymethyl derivatives.[10] Both preparative methods show high enantioselectivity and favor the same enantiomer. The CAL-B-catalyzed direct ring opening avoids the addition and removal of the *N*-hydroxymethyl group, but requires more enzyme because it is slower. Also a disadvantage was the low yield (7 – 11%) of the ring-opened products, the β-amino acids **1a** – **4a**. We also recently reported CAL-B-catalyzed direct ring opening of similar β-lactams by hydrolysis,[18] but these reactions are five to ten times slower than alcoholysis.

Our experimental results and computer modeling suggest that this ring opening of β-lactams **1** – **4** proceeds through an unusual substrate-assisted transition state, which involves a hydrogen-bond bridge. Other researchers also reported substrate-assisted catalysis in subtilisin and even in the same lipase CAL-B. Carter et al.[20] removed the catalytic histidine (His64) in subtilisin BPN′ by site-directed mutagenesis. The mutant had 10^5-fold lower activity toward normal substrates, but only ten-fold lower activity with a histidine-containing substrate. Apparently the histidine in the substrate restores the missing hydrogen bond. In another example, Magnusson et al.[21] increased the enantioselectivity of CAL-B through substrate-assisted catalysis. The side chain hydroxy of Thr40 makes a key hydrogen bond in the transition state. Mutation from Thr40 to Val decreased the reaction rate dramatically. However, for ethyl 2-hydroxypropanoate, which contained a pendant hydroxy group, the mutant showed increased enantioselectivity ($E = 1.6$ to $E = 22$). Presumably, only one enantiomer can restore the key hydrogen bond.

Since CAL-B also catalyzed the hydrolysis of β-lactams,[18] a water molecule can also serve as a hydrogen-bond bridge. There are several well-characterized examples of proton transfer *via* water bridges in proteins[22] including a suggested bridging water in a class A β-lactamase[23] and in penicillin G acylase.[24] In our case the long-chain alcohols such as octanol and dodecanol were more effective than water or short-chain alcohols like ethanol presumably because the longer chain alcohols bind to the active site more strongly due to their higher hydrophobicity. Kobayashi and coworkers also noted that addition of long-chain alcohols (1-octanol in his case) increased the rate of ring opening polymerization of β-butyrolactone (4-methyl-oxetan-2-one).[25] Their increased rate may also be due to a similar alcohol-assisted catalysis.

Although CAL-B shows high enantioselectivity in the acylation of 2-octanol and in the hydrolysis of esters of 2-octanol, it showed no enantioselectivity toward 2-octanol in the ring opening of β-lactams. Racemic or either enantiomer of 2-octanol gave indistinguishable rates of reaction. Initially, this result was surprising, but the molecular modeling suggests a rationalization. In its hydrogen-bond-bridging role, 2-octanol does not bind in the same location as it does when it undergoes acylation or hydrolysis of its esters. In this new location, both enantiomers fit.

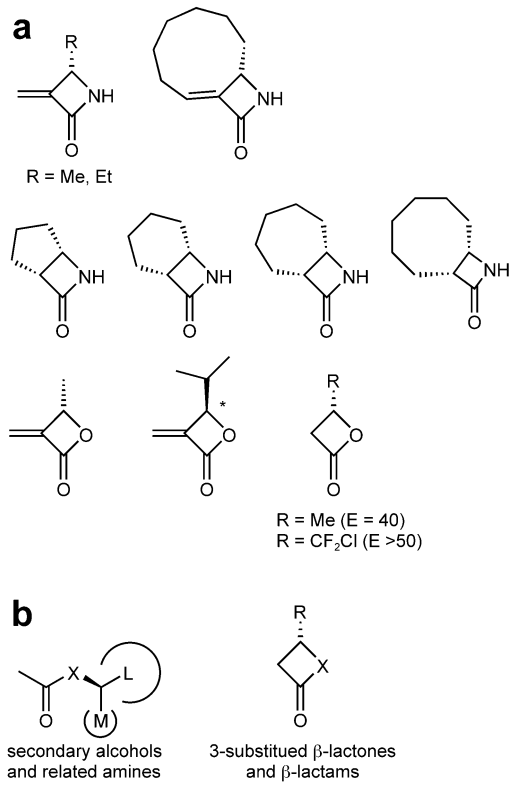
The favored enantiomer in the CAL-B-catalyzed ring opening of other β-lactams and the related β-lactones usually has a similar three-dimensional shape to the four β-lactams in this paper, Scheme 5. As discussed above, steric strain of the substituent with the walls of the active site pocket account for the enantioselectivity. The enantioselectivity is lowest for the methyl-substituted compound presumably because the smaller steric stain caused by small methyl group. The isopropyl-substituted β-lactam is either an exception or a misassignment of the absolute configuration.[26]

Although the alcohol portion of γ- and δ-lactones is a secondary alcohol, the secondary alcohol rule cannot be used here because the stereocenter lies in a different position, Scheme 5. Indeed the molecular basis for enantioselectivity is likely different in the two cases: steric strain in the acyl binding pocket for β-lactones and β-lactams, and fit of the medium substituent in the stereospecificity pocket of the alcohols binding site for secondary alcohols.[27] Esterases and lipases cleave lactones much slower than the corresponding acyclic esters, but a different class of enzymes, lactonases,[28] efficiently cleaves lactones.

Experimental Section

General

Esterase, lipase and protease were from Fluka, Sigma, while chemicals were from Aldrich. Solvents were of the highest analytical grade. CAL-B immobilized on a macroporous poly(acrylic) beads (Novozym 435) was from Novozymes A/S (Denmark). ^{1}H NMR spectra were run at 400 MHz in $CDCl_3$ unless otherwise noted. Chemical shift values, δ, are in ppm. The β-lactams (±)-**1** – (±)-**4** were prepared by 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate to the corresponding cyclohexadiene, styrene and 4-methylstyrene.[10e,16]



Scheme 5. Enantiopreference of CAL-B in the ring opening of β-lactams and β-lactones. **a**) Examples of enantioselective CAL-B-catalyzed ring opening of β-lactams and β-lactones. The structures show the fast reacting enantiomer. All but one example have the substituent pointing back. An asterisk marks the exception. **b**) Generalized structure for the fast reacting enantiomer. The secondary alcohol rule cannot be used for lactones because the stereocenter lies in a different position. Acyclic esters adopt a *syn* conformation along the carbonyl C – alcohol – O-bond. The crystal structure of transition state analogues bound to lipases suggest that this conformation persists in the active site. On the other hand, the lactone ring forces an *anti* conformation along the carbonyl C – alcohol – O-bond, which places the stereocenter in a different part of the enzyme. In particular, the lactone stereocenter appears to lie entirely within the L-pocket of the alcohol-binding crevice. Indeed, many of the lactone examples in this section do not follow the secondary alcohol rule.

Initial Screening

A mixture of enzyme (10 mg) and racemic β-lactam (10 mg) in potassium phosphate buffer (100 mM, pH 7, 1 cm^3) was stirred for 7 days at 25 °C. The conversion was estimated by TLC analysis ($CHCl_3$:MeOH:AcOH:H_2O = 70:20:8:2). Before 50% conversion, unreacted β-lactam was extracted with ethyl acetate (3 cm^3). The ee of the unreacted β-lactam was determined by gas chromatography.

Typical Small-Scale Experiment

A mixture of racemic β-lactam (0.05 M solution) in an organic solvent (2 cm^3), Novozyme 435 (10 – 75 mg cm^{-3}), alcohol (65 μL cm^{-3}) and *n*-decane as an internal standard (1 μL) was stirred magnetically at the selected temperature. The progress of the reaction was followed by gas chromatography on a Chromopak Chiralsil-Dex CB column (25 m × 0.25 mm, Raritan, NJ). The enantiomeric purity of the remaining starting material was measured directly and the conversion was measured by comparison to the internal standard *n*-decane. The enantiomeric purity of the products (ring-opened β-amino acid derivatives) was calculated from the enantiomeric purity of the starting material and the conversion.[29] The enantiomeric purity of the isolated β-amino acids was determined by HPLC on a reversed-phased (C18) column after derivatization of the sample with (1*S*,2*S*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate (DANI).[30]

Preparative-Scale Resolution of 7-Azabicyclo[4.2.0]oct-4-en-8-one (±)-1

Racemic **1** (0.500 g, 4.06 mmol) was dissolved in diisopropyl ether (80 cm^3). CAL-B (4 g, 50 mg cm^{-3}) and 2-octanol (5.3 cm^3) were added and the mixture was shaken in an incubator shaker at 60 °C for 44 h. The reaction was stopped by filtering off the enzyme at 50% conversion (ee **1** = 99%). The solvent was evaporated and the residue was chromatographed on silica eluted with ethyl acetate:hexane (7:3) yielding unreacted (1*S*,6*R*)-**1** {0.19 g, 39%; $[\alpha]_D^{25}$: +161 (*c* 0.29; $CHCl_3$); mp 111 – 113 °C (recrystallized from diisopropyl ether); ee 99%}. ^{1}H NMR: δ = 1.63 – 2.11 (4H, m, $2CH_2$), 3.51 (1H, m, H-1), 4.01 – 4.04 (1H, m, H-6), 5.93 – 6.14 (2H, m, C*HCH*), 5.94 (1H, bs, NH). Anal. calcd. for C_7H_9NO: C 68.27, H 7.37, N 11.37; found: C 67.99, H 7.34, N 11.28.

The filtered enzyme was washed with distilled water (5 × 10 cm^3), and the water was evaporated, yielding the crystalline β-amino acid (1*R*,2*S*)-**1a** {65 mg, 11%; $[\alpha]_D^{25}$: +120 (*c* 0.27; H_2O); mp 236 – 238 °C (recrystallized from water); lit.[10b] mp 220 – 221 °C; ee = 97%}. ^{1}H NMR (D_2O): δ = 1.83 – 2.17 (4H, m, $2CH_2$), 2.72 – 2.76 (1H, m, H-1) 3.99 – 4.01 (1H, m, H-2) 5.72 – 6.13 (2H, m, C*HCH*). Anal. calcd. for $C_7H_{11}NO_2$: C 59.56, H 7.85, N 9.92; found: C 59.44, H 7.80, N 9.79.

Preparative-Scale Resolution of 7-Azabicyclo[4.2.0]oct-3-en-8-one (±)-2

With the procedure described above, the ring opening of racemic **2** (0.500 g, 4.06 mmol) with 2-octanol (5.3 cm^3) in diisopropyl ether (80 cm^3) in the presence of CAL-B (4 g, 50 mg cm^{-3}) at 60 °C for 47 h afforded unreacted (1*S*,6*R*)-**2** {0.2 g, 42%; $[\alpha]_D^{25}$: −29 (*c* 0.26; $CHCl_3$); mp 152 – 153 °C (recrystallized from diisopropyl ether); ee = 99%}. ^{1}H NMR: δ = 1.58 – 2.11 (4H, m, $2CH_2$), 3.51 (1H, m, H-1), 4.01 – 4.04 (1H, m, H-6), 5.93 – 6.15 (2H, m, C*HCH*), 6.01 (1H, bs, NH). Anal. calcd. for C_7H_9NO: C 68.27, H 7.37, N 11.37; found: C 68.12, H 7.33, N 11.37. Amino acid (1*R*,2*S*)-**2a** {55 mg, 9%; $[\alpha]_D^{25}$: −39 (*c* 0.5, H_2O); lit.[10b] $[\alpha]_D^{25}$: −36.2 (*c* 0.5, H_2O); mp 233 – 235 °C (recrystallized from water/acetone); lit.[10b] mp 224 – 225 °C; ee = 99%}. ^{1}H NMR (D_2O): δ = 2.23 – 2.51 (4H, m, $2CH_2$), 2.74 – 2.78 (1H, m, H-1) 3.76 – 3.79 (1H, m, H-2), 5.63 – 5.83 (2H, m, C*HCH*). Anal. calcd. for $C_7H_{11}NO_2$: C 59.56, H 7.85, N 9.92; found: C 59.51, H 7.69, N 9.89.

Preparative-Scale Resolution of 4-Phenyl-2-azetidinone (±)-3

With the procedure described above, the ring opening of racemic **3** (0.500 g, 3.39 mmol) with 2-octanol (4.6 cm^3) in diisopropyl ether (70 cm^3) in the presence of CAL-B (3.5 g, 50 mg cm^{-3}) at 60 °C for 20 h afforded unreacted (*S*)-**3** {0.23 g, 46%; $[\alpha]_D^{25}$: −139 (*c* 0.19; EtOH); mp 114 °C (recrystallized from diisopropyl ether); ee = 99%}; ^{1}H NMR: δ = 2.85–2.89 (1H, dd, *J* = 2, 14.8 Hz, CH_AH) 3.41–3.47 (1H, ddd, *J* = 2.4; 5.2; 7.6 Hz, CH_BH), 4.71–4.73 (1H, dd, *J* = 2.5; 5.3 Hz, CH), 6.27 (1H, bs, NH) 7.26–7.40 (5H, m, Ph). Anal. calcd. for C_9H_9NO: C 73.45, H 6.16, N 9.52; found: C 73.23, H 6.41, N, 9.62.and the amino acid (*R*)-**3a** {59 mg, 11%; $[\alpha]_D^{25}$: +6.8 (*c* 0.45, H_2O); lit.[31] for (*R*)-β-phenyl-β-alanine $[\alpha]_D^{25}$: +6.5 (*c* 0.9; H_2O); mp 233–235 °C (recrystallized from water/acetone); lit.[18] mp for (*R*)-β-phenyl-β-alanine 221–223 °C; ee = 96%}; ^{1}H NMR (D_2O): δ = 2.84–2.91 (2H, m, CH_2), 4.65–4.66 (1H, m, C*H*NH_2), 7.46–7.50 (5H, m, Ph). Anal. calcd. for $C_9H_{11}NO_2$: C 65.44, H 6.71, N 8.48; found: C 65.23, H 6.72, N 8.33.

Preparative-Scale Resolution of 4-(*p*-Tolyl)-2-azetidinone (±)-4

With the procedure described above, the ring opening of racemic **4** (0.500 g, 3.1 mmol) with 2-octanol (4 cm^3) in diisopropyl ether (60 cm^3) in the presence of CAL-B (3 g, 50 mg cm^{-3}) at 60 °C for 48 h afforded unreacted (*S*)-**4** {0.2 g, 40%; $[\alpha]_D^{25}$: −121.9 (*c* 0.5; EtOH); mp 56 °C (recrystallized from diisopropyl ether); ee 96%}; ^{1}H NMR: δ = 2.35 (3H, s, CH_3) 2.83–2.88 (1H, dd, *J* = 1.7; 14.8 Hz, CH_AH), 3.39–3.44 (1H, ddd, *J* = 2.4; 5.2; 7.6 Hz, CH_BH), 4.68–4.69 (1H, dd, *J* = 2.3; 5.2 Hz, CH), 6.12 (1H, bs, NH), 7.17–7.27 (4H, m, Ph). Anal. calcd. for $C_{10}H_{11}NO$: C 74.51, H 6.88, N 8.69; found: C 74.62, H 6.89, N 8.66 and the amino acid (*R*)-**4a** {39 mg, 7%; $[\alpha]_D^{25}$: −8 (*c* 0.1, H_2O); mp 241–243 °C (recrystallized from water/acetone); ee = 98%}; ^{1}H NMR: δ = 2.35 (3H, s, CH_3) 2.82–2.89 (2H, m, CH_2) 4.60–4.64 (1H, m, C*H*NH_2) 7.31–7.37 (4H, m, Ph). Anal. calcd. for $C_{10}H_{13}NO_2$: C 67.02, H 7.31, N 7.82; found: C 66.89, H 7.22, N 7.77.

Computer Modeling of Transition State Analogues in CAL-B

All modeling was done with Discover, version 2.9.7 (Accelrys, San Diego, CA) using the AMBER[32] force field. Results were displayed using Insight II version 95.0 (Accelrys). The starting structure was the X-ray crystal structure of CAL-B containing a covalently linked phosphonate inhibitor (Protein Data Bank[33] file 1lbs). Using the biopolymer module of Insight II, hydrogen atoms were added to correspond to pH 7.0. Histidines were uncharged, aspartates and glutamates were negatively charged and arginines and lysines were positively charged. The catalytic histidine (His224) was protonated. The phosphonate group, which covalently linked to Ser105 in the X-ray structure, was replaced by a phosphonate analogue of β-lactam **3**.

Energy minimization proceeded in four stages. First, 100 iterations of steepest descent algorithm, all protein atoms constrained with a force constant of 10 kcal mol^{-1} $Å^{-2}$; second, 500 iterations of conjugate gradients algorithm with the same constraints; and third, 500 iterations of conjugate gradients algorithm with only the backbone constrained by a 10 kcal mol^{-1} $Å^{-2}$ force constant. For the fourth stage, minimization was continued using conjugate gradients algorithm without any constraints until the rms derivatives reached less than 0.005 kcal mol^{-1} $Å^{-1}$. Crystallographic water molecules were included in all minimizations. Water molecules and the substrate were not constrained through any of the minimization cycles.

Acknowledgements

The authors thank the Natural Sciences and Engineering Research Council of Canada for financial support and Hungarian Government for Eötvös Scholarship to EF. The authors also thank Christopher K. Savile (McGill) for useful discussions about modeling and Karl Hult (Royal Institute of Technology, Stockholm) for critical reading of the manuscript.

References and Notes

[1] a) F. Fülöp, *Chem. Rev.* **2001**, *101*, 2181–2204; F. Fülöp, in *Studies in Natural Product Chemistry*, Vol. 22. (Ed.: Atta-ur-Rahman), Elsevier Science Publishers, **2000**, pp. 273–306; b) P. Sonnet, P. Dallemagne, J. Guillon, C. Enguehard, S. Stiebing, J. Tanguy, R. Bureau, S. Rault, P. Auvray, S. Moslemi, P. Sourdaine, G.-E. Séralini, *Bioorg. Med. Chem.* **2000**, *8*, 945–955; c) C. Palomo, J. M. Aizpurua, I. Ganboa, M. Oiarbide, *Synlett* **2001**, *12*, 1813–1826.

[2] a) C. Palomo, M. Oiarbide, A. Landa, A. Esnal, A. Linden, *J. Org. Chem*. **2001**, *66*, 4180–4186; b) C. Palomo, I. Ganboa, M. Oiarbide, G. T. Sciano, J. L. Miranda, *Arkivoc* **2002**, 8–16; http://www.arkat-usa.org/ark/journal/2002/MManas/MM-334C/MM-334C.pdf.

[3] H. K. Lee, J. S. Chun, C. S. Park, *Tetrahedron Lett.* **2001**, *42*, 3483–3486.

[4] a) L. A. Cabell, J. S. McMurray, *Tetrahedron Lett.* **2002**, *43*, 2491–2493; b) B. Alcaide, P. Almendros, J. M. Alonso, M. F. Aly, *J. Org. Chem*. **2001**, *66*, 1351–1358.

[5] Reviews: P. A. Magriotis, *Angew. Chem. Int. Ed.* **2001**, *40*, 4377–4379; C. Palomo, J. M. Aizpurua, I. Ganboa, M. Oiarbide, *Eur. J. Org. Chem.* **1999**, 3223–3235.

[6] U. T. Bornscheuer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis Regio- and Enantioselective Reactions*, Wiley-VCH, Weinheim, **1999**.

[7] Examples: W. B. Knight, B. G. Green, R. M. Chabin, P. Gale, A. L. Maycock, H. Weston, D. W. Kuo, W. M. Westler, C. P. Dorn, P. E. Finke, W. K. Hagmann, J. J. Hale, J. Liesch, M. MacCoss, M. A. Navia, S. K. Shah, D. Underwood, J. B. Doherty, *Biochemistry* **1992**, *31*, 8160–8170; R. C. Wilmouth, S. Kassamally, N. J. Westwood, R. J. Sheppard, T. D. W. Claridge, R. T. Aplin, P. A. Wright, G. J. Pritchard, C. J. Shofield, *Biochemistry* **1999**, *38*, 7989–7998.

[8] Tetrahydrolipstatin: Q. Lüthi-Peng, H. P. Märki, P. Hadváry, *FEBS Lett.* **1992**, *299*, 111–115; LDL phospholi-

pase A_2: D. G. Tew, H. F. Boyd, S. Ashman, C. Theobald, C. A. Leach, *Biochemistry* **1998**, *37*, 10087 – 10093.
[9] H. Nagai, T. Shiozawa, K. Achiwa, Y. Terao, *Chem. Pharm. Bull.* **1993**, *41*, 1933 – 1938.
[10] a) P. Csomós, L. T. Kanerva, G. Bernáth, F. Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1789 – 1796; b) J. Kámán, E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2000**, *11*, 1593 – 1600; c) F. Fülöp, M. Palkó, J. Kámán, L. Lázár, R. Sillanpää, *Tetrahedron: Asymmetry* **2000**, *11*, 4179 – 4187; d) E. Forró, J. Árva, F. Fülöp, *Tetrahedron: Asymmetry* **2001**, *12*, 643 – 649; e) E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2001**, *12*, 2351 – 2358.
[11] K. Achilles, T. Schirmeister, H.-H. Otto, *Arch. Pharm. Pharm. Med. Chem.* **2000**, *333*, 243 – 253.
[12] M. Jones, M. I. Page, *J. Chem. Soc. Chem. Commun.* **1991**, 316 – 317.
[13] R. Brieva, J. Z. Crich, C. J. Sih, *J. Org. Chem.* **1993**, *58*, 1068 – 1075.
[14] W. Adam, P. Groer, H.-U. Humpf, C. R. Saha-Möller, *J. Org. Chem.* **2000**, *65*, 4919 – 4922; Another ring opening reaction, but for five-membered γ-lactams, is the subtilisin-catalyzed hydrolysis of two *N*-substituted bicyclic γ-lactams: M. Mahmoudian, A. Lowdon, M. Jones, M. Dawson, C. Wallis, *Tetrahedron: Asymmetry* **1999**, *10*, 1201 – 1206.
[15] J. R. Knox, P. C. Moews, J. M. Frère, *Chem. Biol.* **1996**, *3*, 937 – 947; L. Maveyraud, D. Golemi, L. P. Kotra, S. Tranier, S. Vakulenko, S. Mobashery, J.-P. Samama, *Structure* **2000**, *8*, 1289 – 1298.
[16] a) H. Bestian, H. Biener, K. Clauss, H. Heyn, *Justus Liebigs Ann. Chem.* **1968**, *718*, 94 – 100; b) R. Singh, R. D. G. Cooper, *Tetrahedron* **1994**, *50*, 12049 – 12064; c) P. Furet, C.Garcia-Echeverria, B. Gay, J. Scoepfer, M. Zeller, J. Rahuel, *J. Med. Chem.* **1999**, *42*, 2358 – 2363; d) C. Palomo, M. Oiarbide, S. Bindi, *J. Org. Chem*. **1998**, *63*, 2469 – 2474.
[17] Other hydrolases that catalyzed hydrolysis of (±)-**1** include: lipase from wheat germ (type I, Sigma), rennin from *Mucor meihei* (mucorpeptin, Fluka), protease from *Aspergillus oryzae* (Type XIII, Sigma), bacterial proteinase (Fluka), protease subtilisin Carlsberg (bacterial type VIII from *Bacillus licheniformis,* Sigma), cholesterol esterase from bovine pancreas (Genzyme), α-chymotrypsin (Sigma), and subtilisin from *Bacillus licheniformis* (Fluka).
[18] E. Forró, F. Fülöp, *Org. Lett.* **2003**, *5*, 1209 – 1212.
[19] M. C. Parker, S. A. Brown, L. Robertson, N. J. Turner, *Chem. Commun.* **1998**, 2247 – 2248.
[20] P. Carter, L. Abrahmsen, J. A. Wells, *Biochemistry* **1991**, *30*, 6142 – 6148.
[21] A. Magnusson, K. Hult, M. Holmquist, *J. Am. Chem. Soc*. **2001**, *123*, 4354 – 4355.
[22] Examples: carbonic anhydrase: K. M. Jude, S. K. Wright, C. Tu, D. N. Silverman, R. E. Viola, D. W. Christianson, *Biochemistry* **2002**, *41*, 2485 – 2491; gramicidin: R. Pomès, *Isr. J. Chem.* **1999**, *39*, 387 – 395; uracil DNA glycosylase: N. Luo, E. Mehler, R. Osman, *Biochemistry* **1999**, *38*, 9209 – 9220.
[23] J. Lamotte-Brasseur, G. Dive, O. Dideberg, P. Charlier, J. M. Frére, J. M. Ghuysen, *Biochem. J.* **1991**, *279*, 213 – 221.
[24] H. J. Duggleby, S. P. Tolley, C. P. Hill, E. J. Dodson, G. Dodson, P. C. E. Moody, *Nature* **1995**, *373*, 264 – 268.
[25] H. Uyama, S. Suda, H. Kikuchi, S. Kobayashi, *Chem. Lett.* **1997**, 1109 – 1110; H. Kikuchi, H. Uyama, S. Kobayashi, *Macromolecules* **2000**, *33*, 8971 – 8975.
[26] W. Adam, V. O. N. Salgado, B. Wegener, E. Winterfeldt, *Chem. Ber.* **1993**, *126*, 1509 – 1510. The authors assigned the absolute configuration of this lactone on the basis of the ^{1}H NMR spectra of an adduct with an enantiomerically pure cyclopentadiene derivative.
[27] M. Cygler, P. Grochulski, R. J. Kazlauskas, J. D. Schrag, F. Bouthillier, B. Rubin, A. N. Serreqi, A. K. Gupta, *J. Am. Chem. Soc.* **1994**, *116*, 3180 – 3186.
[28] S. Shimizu, M. Kataoka, K. Honda, K. Sakamoto, *J. Biotechnol.* **2001**, *92*, 187 – 194.
[29] Footnote 17 in C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294 – 7299.
[30] M. Péter, A. Péter, F. Fülöp, *J. Chromatogr. A* **2000**, *871*, 115 – 126.
[31] V. A. Soloshonok, N. A. Fokina, A. V. Rybakova, I. P. Shiskina, S. V. Galushko, A. E. Sorochinsky, V. P. Kukhar, M. V. Savchenko, V. K. Svedas, *Tetrahedron: Asymmetry* **1995**, *6*, 1601 – 1610.
[32] S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Ghio, G. Alagona, S. Profeta, P. Weiner, *J. Am. Chem. Soc.* **1984**, *106*, 765 – 784.
[33] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, *Nucleic Acids Res.* **2000**, *28*, 235 – 242; http://www.rcsb.org/pdb/.

