Direct and Indirect Enzymatic Methods for the Preparation of Enantiopure Cyclic β-Amino Acids and Derivatives from β-Lactams

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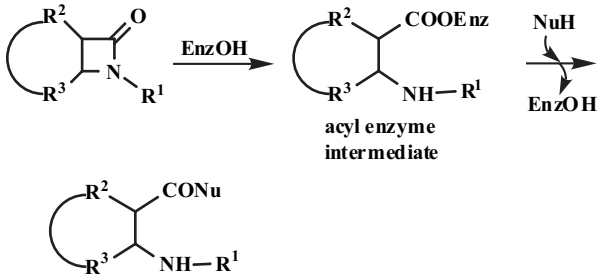
Abstract: Direct enzymatic methods for the preparation of enantiopure cyclic β-amino acids (*e.g*. cispentacin) and β-lactams through the enzyme-catalyzed enantioselective ring opening of β-lactams in water and organic solvents are reviewed. Indirect methods through the lipase-catalyzed asymmetric acylation of *N*-hydroxymethylated β-lactams or the lipase-catalyzed hydrolysis of the corresponding ester derivatives, followed by ring opening, are also surveyed.

Key Words: β-lactam, β-amino acid, cispentacin, ring opening, enzymatic catalysis.

I. INTRODUCTION

The β-amino acids and β-lactams are of biological and chemical importance. Some β-amino acids themselves exhibit antibacterial activity (*e.g*. cispentacin) [1-6]. The use of β-lactams [7-10] and β-amino acids or their derivatives [11-20] for synthetic purposes has been accentuated in the past few years, and it is therefore, not surprising that the development of their synthesis in optically pure form has become a real challenge for organic chemists. A number of new enzymatic and asymmetric syntheses of β-amino acids or their derivatives have been elaborated, and most of them have been reviewed [1-3, 7, 8, 21, 22]. Methods have been established for the selective enzymatic cleavage of β-lactam rings by different nucleophiles, resulting in enantiomerically pure β-lactams and valuable cyclic or acyclic β-amino acids or their derivatives. β-Lactamases (class C) have the ability to catalyze the N_1-C_2 cleavage of the β-lactam ring, leading to β-amino acids (Scheme **1**) [23-26]. The use of *N*-activated (*N*-acyl or *N*-Boc) β-lactams has proved to be of major importance.

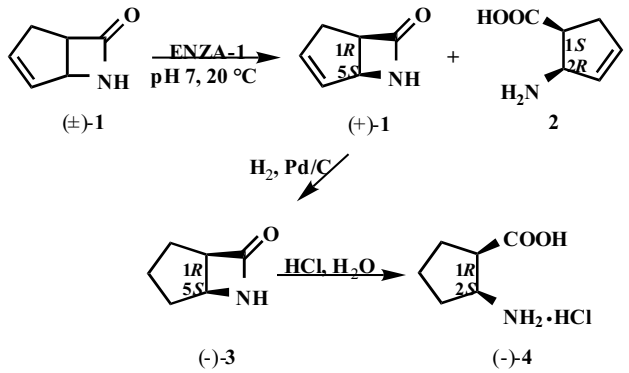


Scheme 1.

In recent years, there has been increasing interest in the use of hydrolytic enzymes to produce enantiopure β-lactams and β-amino acids or their derivatives. In particular, lipases and esterases have been applied successfully for the hydrolysis and alcoholysis of β-lactams with high enantioselectivity: *E* usually > 200. An important indirect enzymatic route to enantiopure β-lactams and β-amino acids or their derivatives proceeds through the lipase-catalyzed asymmetric acylation of the primary hydroxy group of *N*-hydroxymethylated β-lactams, or the lipase-catalyzed hydrolysis of the corresponding ester derivatives, followed by ring opening to the β-amino ester or acid, respectively. This mini-review provides an overview of the direct and indirect enzymatic methods leading to enantiopure cyclic β-amino acids or their derivatives and β-lactams, with synthetic applications. Some acyclic analogs are also described comparatively.

II. DIRECT ENZYMATIC METHODS

Even though a large number of commercially available isolated enzymes achieved little or no hydrolysis of unactivated racemic 6-azabicyclo[3.2.0]hept-3-en-7-one, (±)-**1**, lactamases in a special whole-cell preparation, ENZA-1 (*Rhodococcus equi NCIMB 40213*), catalyzed its enantioselective ring opening in water (Scheme **2**) [27]. The ENZA-1-catalyzed highly enantioselective hydrolysis, performed in two steps at 20 °C in phosphate buffer (pH 7), afforded the β-lactam (+)-**1** and β-amino acid **2** in high enantiomeric excess: ee > 96%. The lactam (+)-**1** was hydrogenated, and then hydrolyzed to the corresponding



Scheme 2.

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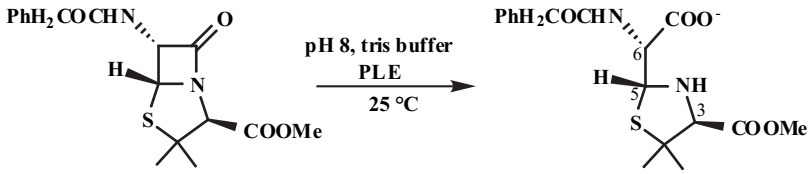
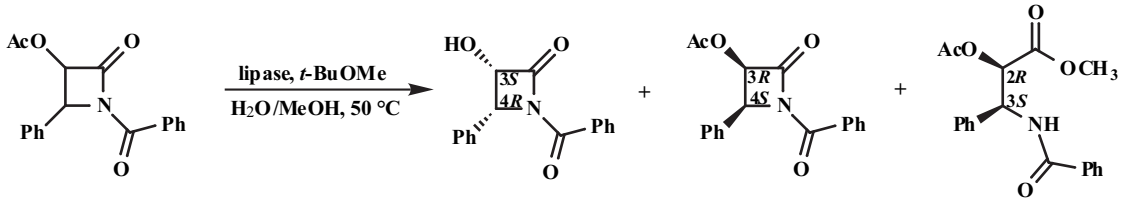


(3*S*,5*R*,6*R*)-**5** (3*S*,5*R*,6*R*)-**6**

Scheme 3.



(±)-**7** (-)-**8** (+)-**9** (-)-**10**

Scheme 4.

enantiomerically pure amino acid (-)-**4**, the well-known cispentacin hydrochloride. It is interesting that ENZA-1 displays poor activity towards the saturated β-lactam (±)-**3**.

The PLE (porcine liver esterase) -catalyzed selective hydrolysis of the methyl (3*S*,5*R*,6*R*)-benzylpenicilloate, **5** was described by Jones and Page [28]. When the reaction was carried out at pH 8.0, in tris buffer at 25 °C, the ester did not undergo hydrolysis, but formation of the ring-opened derivative **6** was observed (Scheme **3**).

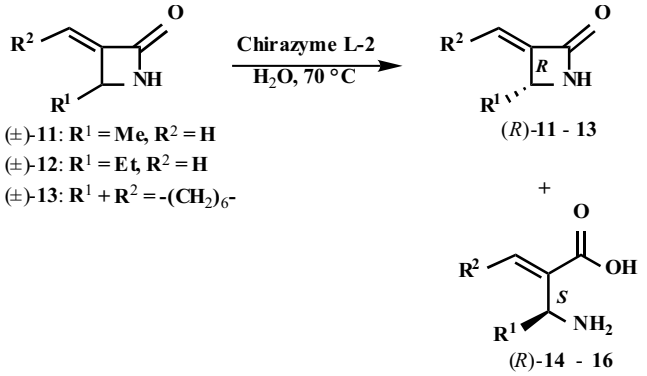
Although the lipases catalyze a large number of enantioselective transformations, few of them have been found capable of enantioselectively cleaving the β-lactam ring to yield a β-amino acid or derivative and unreacted β-lactam enantiomers. The enantioselective ring opening of racemic *cis*-1-benzoyl-3-acetoxy-4-phenylazetidin-2-one, (+)-**7**, catalyzed by lipase P-30 (*Pseudomonas cepacia*) and lipase AK (*Pseudomonas fluorescens*) in organic solvent has been reported (Scheme **4**) [29]. Since the reaction rate in the organic solvent (quantity of water < 0.4%) was considerably lower than that in aqueous phosphate buffer, the reactions were performed at elevated temperatures (up to 50 °C). It was found that the reaction pathway was markedly influenced by the nucleophile: when water was used, only hydrolysis of the acetoxy ester (±)-**7** with the formation of (-)-**8** and (+)-**9** was observed; when methanol was used as nucleophile, the enantioselective cleavage of β-lactam took place, with the formation of β-amino ester (-)-**10** (Table **1**).

Table 1. Conversion and Enantioselectivity of Lipase-Catalyzed Ring Opening of (±)-7[a]

		(-)-8		(+)-9		(-)-10	
enzyme	time (h)	yield (%)	ee (%)	yield (%)	ee (%)	yield (%)	ee (%)
P-30	90	19	95	18	100	19	100
AK	48	4	n.d.[b]	35	100	42	100

[a]Data from ref. [29], 60 mg substrate, 5 equiv. MeOH, 20 mg enzyme in 3 mL *t*-BuOMe, 50 °C. [b]Not determined.

The synthesis of a variety of optically active α-methylene-β-lactams (+)-**11**, (+)-**12** and (-)-**13**, through Chirazyme L-2 (lipase B from *Candida antarctica*) -catalyzed *S*-selective hydrolysis of the lactams (±)-**11**-(±)-**13** was reported recently (Scheme **5**, Table **2**) [30]. Chirazyme L-1 (*Burkhold eria sp*.) and Chirazyme L-6 (*Pseudomonas sp*.) were also tested for the ring opening of (±)-**12**, but no activity was observed, even on prolonged reaction times. At room temperature, the reactions were highly selective, but very slow, whereas at relatively high temperature (70 °C), the reaction rate was enhanced considerably, but the enzyme rapidly lost activity. To ensure preservation of the enzyme activity in time at this high temperature, and ready separation of the enzyme from the product β-amino acid **14-16** (ee ≥ 90%), the carrier-fixed Chirazyme L-2 was applied successfully. For *N*-protected β-lactam derivatives (*p*-methoxyphenyl- and benzyl-protected), no ring opening reactions were observed under the optimized conditions.



(±)-**11**: R^1 = Me, R^2 = H
(±)-**12**: R^1 = Et, R^2 = H
(±)-**13**: $R^1 + R^2 = -(CH_2)_6-$

(*R*)-**11** - **13** + (*R*)-**14** - **16**

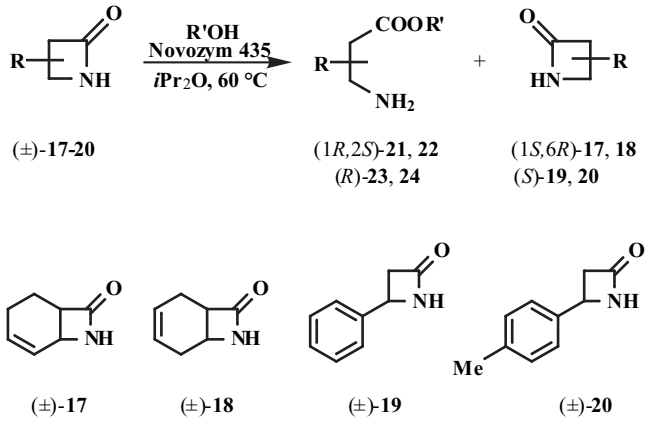
Scheme 5.

Table 2. Conversion and Enantioselectivity of Chirazyme L-2-Catalyzed Ring Opening of (±)-11-13 at 70 °C[a]

substrate	time (h)	conv. (%)	ee_s (%)	ee_p (%)	*E*
(±)-**11**[b]	86	52	96	90	74
(±)-**12**[b]	24	50	99	98	>200
(±)-**13**[c]	68	49	94	97	>200

[a]Data from ref. [30]. [b]Substrate:enzyme 315:250.
[c]Substrate:enzyme 315:500.

Novozym 435 (lipase B from *Candida antarctica*) -catalyzed enantioselective alcoholysis via the ring opening of two alicyclic-fused and two 4-aryl-substituted β-lactams (±)-**17-20** in an organic solvent has been described (Scheme **6**) [31].



Scheme 6.

The ring opening reactions were slow (the fastest one was observed with (±)-2-octanol, in iPr$_2$O), but highly enantioselective (E > 200). The enantioselectivity and reaction rate were influenced strongly by the reaction temperature: the alcoholysis of (±)-**17** with (±)-2-octanol at 35-50 °C was slower and much less enantioselective than at 55-75 °C, and a drop in enantioselectivity was observed at 80 °C (Table **3**). The preparative-scale reactions of (±)-**17-20** with 2-octanol as nucleophile in iPr$_2$O at 60 °C yielded the unreacted β-lactams (1*S*,6*R*)-**17**, (1*S*,6*R*)-**18**, (*S*)-**19** and (*S*)-**20** in high yield (39-46%) with high ee (≥ 96%) (Table **4**). However, instead of the product β-amino esters (which were not isolated, but probably reacted further by hydrolysis), β-amino acids (1*R*,2*S*)-**21**, (1*R*,2*S*)-**22**, (*R*)-**23** and (*R*)-**24** were isolated with high ee (≥ 96%) but in low yield (7-11%) (Table **4**) [31].

Table 3. Effect of Temperature on the Novozym 435-Catalyzed Ring Opening of (±)-17 by (±)-2-Octanol[a]

temp. (°C)	ee_s (%)	ee_p (%)	conv. (%)	E
35	5	~48	~9	~3
40	7	~55	~11	~4
50	18	~72	~20	~7
55	20	>99	17	>200
60	40	>99	29	>200
70	67	>99	40	>200
75	72	>99	42	>200
80	88	~70	~56	~16

[a]Data from ref. [31], 30 mg/mL enzyme, 0.05 M substrate concentration, 2-octanol:iPr$_2$O (1:15 v/v), after 24 h.

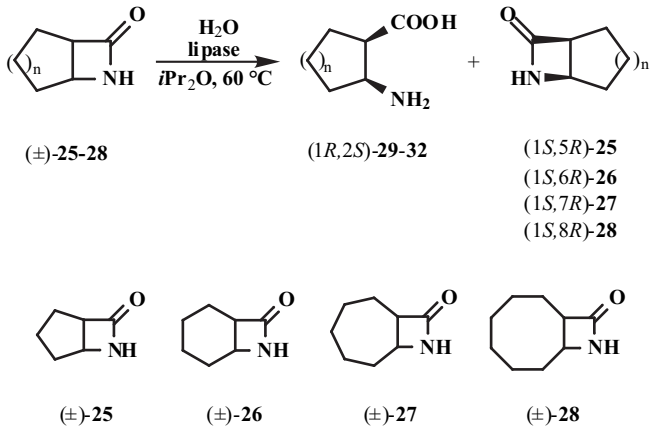
To examine the high enantioselectivity and the critical role of the nucleophile alcohol (2-octanol), computer modeling of the ring opening of β-lactam (±)-**19** was performed. This suggested that the reaction proceeds via an unusual substrate-assisted transition state, where the alcohol forms a bridge between the catalytic histidine and the nitrogen of the β-lactam. The computer modeling also suggested that the molecular basis for the high enantioselectivity is a severe steric clash between Ile 189 and the phenyl substituent on the slow-reacting enantiomer of the β-lactam [31].

Table 4. Preparative-Scale Novozym 435-Catalyzed Ring Opening of (±)-17 - 20[a]

substrate	time (h)	conv. (%)	E	β-lactam		β-amino acid	
				yield (%)	ee (%)	yield (%)	ee (%)
(±)-**17**	44	50	>200	39	99	11	97
(±)-**18**	47	50	>200	42	99	9	99
(±)-**19**	20	50	>200	46	99	11	96
(±)-**20**	48	50	>200	40	96	7	98

[a]Data from ref. [31], 0.5 g substrate, 4.0 g Novozym 435, 80 cm^3 2-octanol:iPr$_2$O (1:15 v/v), 60 °C.

The present authors have developed a very efficient and simple enzymatic method for the enantioselective ring opening of unactivated alicyclic-fused β-lactams (±)-**25-28** in organic media, yielding β-amino acids (1*R*,2*S*)-**29-32** (*e.g.* cispentacin **29**) and unreacted β-lactams (1*S*,5*R*)-**25**, (1*S*,6*R*)-**26**, (1*S*,7*R*)-**27** and (1*S*,8*R*)-**28** (Scheme **7**) [32].



Scheme 7.

Highly enantioselective ring opening was observed with CAL-A (lipase A from *Candida antarctica*), Novozym 435, Chirazyme L-2 and Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) in diisopropyl ether at 60 °C for the model compound (±)-**27** (Table **5**). It was found that the catalytic activity of the tested Lipolase was progressively lowered on increase of the amount of water added (optimal quantity 0-1 equiv. water), through E was apparently not affected (Table **6**) [32].

The resolved products, obtained in good chemical yields (36-45% β-lactams and 43-47% β-amino acids; Table **7**), could be easily separated. The transformations of β-lactams (1*S*,5*R*)-**25**, (1*S*,6*R*)-**26**, (1*S*,7*R*)-**27** and (1*S*,8*R*)-**28** with 18% aqueous HCl resulted in the corresponding enantiomers

of the β-amino acid hydrochlorides (1*S*,2*R*)-**33**-**36** (Scheme **8**), without a drop in enantiomeric excess (ee ≥ 99%) [32].

Table 5. Conversion and Enantioselectivity of Lipase-Catalyzed Ring Opening of (±)-27 after 20 h[a]

enzyme (50 mg/mL)	conv. (%)	ee_s (%)	ee_p (%)	*E*
Chirazyme L-2	~9	5	~48	~3
Lipolase	~11	7	~55	~4
Novozym 435	~20	18	~72	~7
CAL-A	17	20	>99	>200

[a]Data from ref. [32], 0.05 M substrate and 1 equiv. H_2O, in iPr_2O, 60 °C.

Table 6. Conversion and Enantioselectivity of Lipolase-Catalyzed Ring Opening of (±)-27 after 23 h[a]

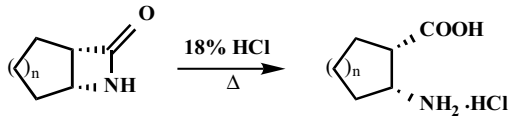
H_2O (equiv.)	conv. (%)	ee_s (%)	ee_p (%)	*E*
1	48	89	>95	>117
-	49	90	>95	>120
2	46	82	>95	>99
3	43	73	>95	>85
4	30	40	>95	>57
10	13	14	>95	>44

[a]Data from ref. [32], 0.05 M substrate in iPr_2O, 60 °C.

Table 7. Preparative-scale Lipolase-Catalyzed Ring Opening of (±)-25 - 28[a]

substrate	time (h)	conv. (%)	*E*	β-lactam		β-amino acid	
				yield (%)	ee (%)	yield (%)	ee (%)
(±)-**25**	249	48	>200	42	93	44	99
(±)-**26**	141	50	>200	45	98	45	98
(±)-**27**	31	50	>200	41	99	47	98
(±)-**28**	170	50	>200	36	99	43	95

[a]Data from ref. [32], 0.5 g substrate, 4.0 g Novozym 435, 80 cm^3 2-octanol:iPr_2O (1:15 v/v), 60 °C.

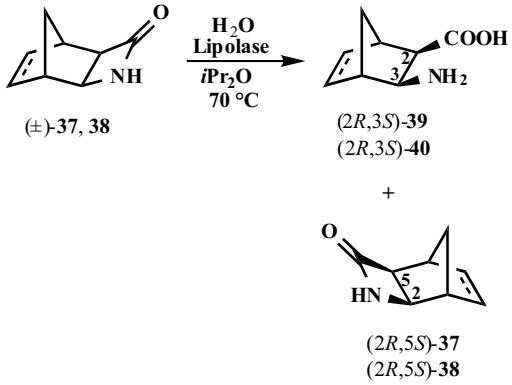


(1*S*,5*R*)-**25**
(1*S*,6*R*)-**26**
(1*S*,7*R*)-**27**
(1*S*,8*R*)-**28**

(1*S*,2*R*)-**33**-**36**

Scheme 8.

1,4-Ethyl- and 1,4-ethylene-bridged cispentacin enantiomers were prepared through the lipase-catalyzed enantioselective ring opening of racemic *exo*-3-azatricyclo[4.2.1.$0^{2,5}$]nonan-4-one, (±)-**37**, and *exo*-3-azatricyclo[4.2.1.$0^{2,5}$]non-7-en-4-one, (±)-**38** (Scheme **9**) [33]. High enantioselectivity (*E* > 200) was observed when the Lipolase-catalyzed reactions were performed with 1 equiv. H_2O in toluene at 70 °C. The resolved β-amino acids (2*R*,3*S*)-**39** (yield 40%) and (2*R*,3*S*)-**40** (yield 39%) and β-lactams (2*R*,5*S*)-**37** (yield 46%) and (2*R*,5*S*)-**38** (yield 46%) could be easily separated. The ring opening of lactam enantiomers with 18% HCl afforded the corresponding β-amino acid hydrochloride enantiomers (ee≥94%).



(±)-**37**, **38**

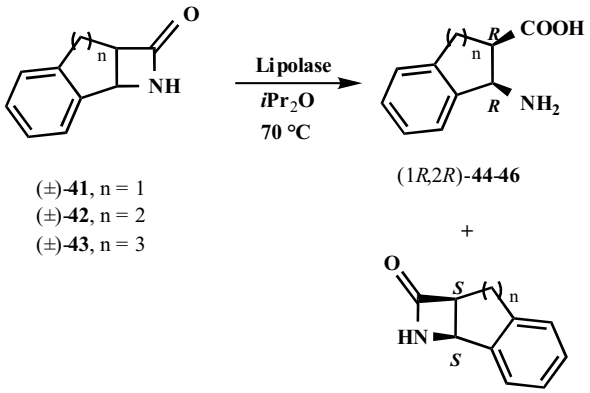
(2*R*,3*S*)-**39**
(2*R*,3*S*)-**40**

+

(2*R*,5*S*)-**37**
(2*R*,5*S*)-**38**

Scheme 9.

Enantiopure 1-aminoindane-2-carboxylic acid, benzocispentacin and its six- and seven-membered homologs were prepared in high yield (43-46%) through the Lipolase-catalyzed enantioselective ring opening of 3,4-benzo-6-azabicyclo[3.2.0]heptan-7-one, (±)-**41**, 4,5-benzo-7-azabicyclo[4.2.0]octan-8-one, (±)-**42**, and 5,6-benzo-8-azabicyclo[5.2.0]nonan-9-one, (±)-**43**, (Scheme **10**) in iPr_2O at 70 °C [34]. In accordance with the earlier observation (Table **6**) [32], the degree of hydrolysis was complete without the addition of any water (the water present in the reaction medium or in the enzyme preparation was sufficient for the lactam ring opening). Besides the β-amino acid hydrochloride enantiomers (18% HCl), the corresponding β-amino ester enantiomers (1*R*,2*R*)-**47**-**49** (HCl/EtOH, indirect method) and (1*S*,2*S*)-**47**-**49** were also prepared. Isomerization of the esters, followed by hydrolysis, resulted in the *trans*-β-amino acid hydrochloride enantiomers (1*S*,2*R*)-**50**-**53** and (1*R*,2*S*)-**50**-**53** (Scheme **11**).



(±)-**41**, n = 1
(±)-**42**, n = 2
(±)-**43**, n = 3

(1*R*,2*R*)-**44**-**46**

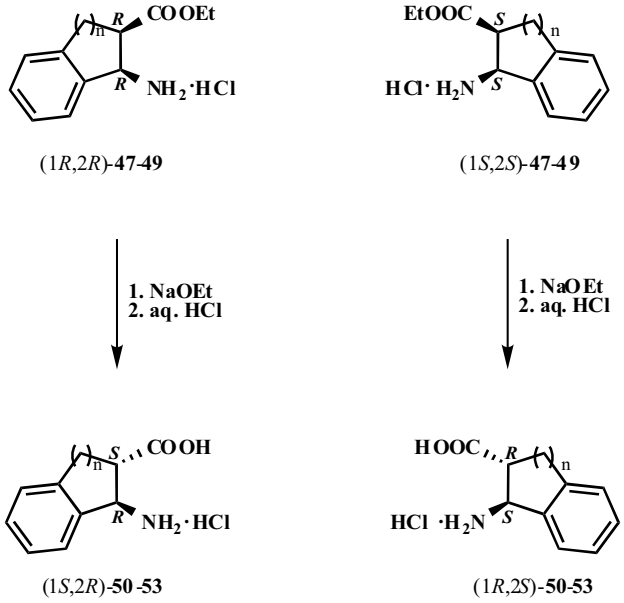
+

(1*S*,5*S*)-**41**
(1*S*,6*S*)-**42**
(1*S*,7*S*)-**43**

Scheme 10.

Data on the lipase-catalyzed enantioselective ring opening of activated and inactivated β-lactams with different nucleophiles, in both water and organic solvents, yielding

the desired β-amino acids or derivatives and unreacted β-lactams, are presented in Table **8**.



Scheme 11.

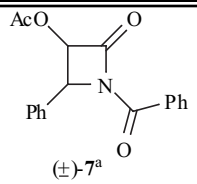
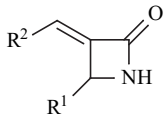
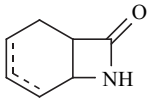
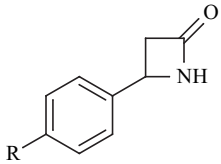
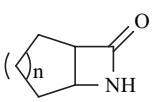
III. INDIRECT ENZYMATIC METHODS

Enantioselective acylation of *N*-hydroxymethylated β-lactams in the presence of lipases affords optically active precursors for the preparation of enantiopure β-amino acids or derivatives. In comparison with the direct enzymatic methods, the indirect ones, take place with the same high enantioselectivity (E usually > 200), but involve the addition and removal of the *N*-hydroxymethyl group, and therefore, result in the products in lower overall yields.

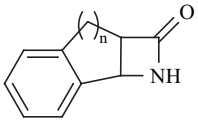
The preparation of a variety of optically active 3,4-substituted β-lactams through lipase-catalyzed kinetic resolution by using the enantioselective hydrolysis of *N*-acyloxymethyl β-lactams or the acylation of *N*-hydroxymethyl β-lactams in an organic solvent was pioneered by Japanese authors [35, 36]. Lipase B and lipase PS proved suitable catalysts for both hydrolysis and transesterification reactions (E usually > 200). Lipase PS generally gave better results for acylation than lipase B, which was favorable in the hydrolysis (Table **9**).

The *N*-hydroxymethylated alicyclic β-lactam (±)-**56** was successfully resolved (E = 90) through lipase AK (from *Pseudomonas fluorescens)* -catalyzed butyrylation at the 5*S* stereogenic center in acetone at 0 °C (Scheme **12**) [37]. The

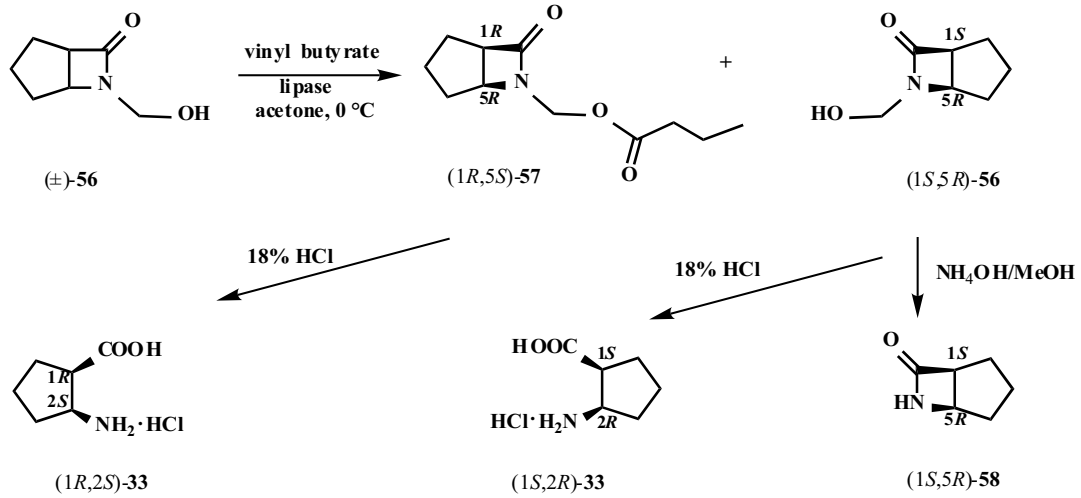
Table 8. Optimal Conditions of Lipase-Catalyzed Enantioselective Ring Opening of β-Lactams

substrate	enzyme	nucleophile	solvent	temp. (°C)	time (h)	conv. (%)	*E*
 (±)-**7**[a]	AK	MeOH	*t*-BuOMe	50	48	n.d.[b]	n.d.[b]
 (±)-**11-13**[c]	Chirazyme L-2	H_2O	H_2O	70	**11**: 86 **12**: 24 **13**: 68	**11**: 52 **12**: 50 **13**: 49	**11**: 74 **12**: >200 **13**: >200
 (±)-**17**[d], (±)-**18**[d]	Novozym 435	(±)-2-octanol	*i*Pr_2O toluene	60	**17**: 44 **18**: 47	**17**: 50 **18**: 50	>200
 R = H, *p*-Me (±)-**19**[d], (±)-**20**[d]	Novozym 435	(±)-2-octanol	*i*Pr_2O toluene	60	**19**: 20 **20**: 48	**19**: 50 **20**: 50	>200
 n = 1, 2, 3, 4 (±)-**25-28**[e]	Lipolase	H_2O	*i*Pr_2O	60	**25**: 249 **26**: 141 **27**: 31 **28**: 170	**25**: 48 **26**: 50 **27**: 50 **28**: 50	>200

(Table 8). contd.....

substrate	enzyme	nucleophile	solvent	temp. (°C)	time (h)	conv. (%)	*E*
 (±)-**37**[f]	Lipolase	H_2O	*i*Pr_2O	70	266	50	>200
 (±)-**38**[f]	Lipolase	H_2O	*i*Pr_2O	70	191	49	>200
 n = 1, 2, 3 (±)-**41**-**43**[g]	Lipolase	-	*i*Pr_2O	60	**41**: 6 **42**: 54 **43**: 51	**41**: 50 **42**: 50 **43**: 50	>200

[a]Data from ref. [29]. [b]Not determined. [c]Data from ref. [30]. [d]Data from ref. [31]. [e]Data from ref. [32]. [f]Data from ref. [33]. [g]Data from ref. [34].

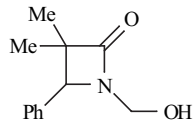


Scheme 12.

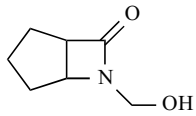
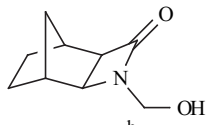
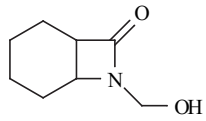
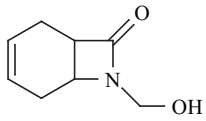
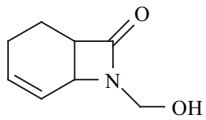
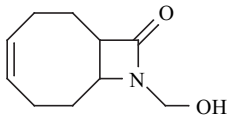
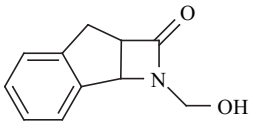
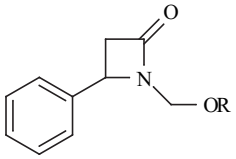
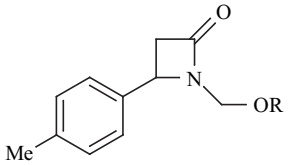
product butyrate (1*R*,5*S*)-**57** and the less reactive *N*-hydroxymethyl derivative (1*S*,5*R*)-**56** were converted to the corresponding amino acid hydrochlorides (1*R*,2*S*)-**33** and (1*S*,2*R*)-**33**. Treatment of (1*S*,5*R*)-**56** with NH_4OH/MeOH afforded the enantiopure β-lactam (1*S*,5*R*)-**58**.

The present authors have also resolved a variety of alicyclic and acyclic β-lactams through lipase-catalyzed asymmetric acylation of the primary hydroxy group of *N*-hydroxymethylated β-lactams or by lipase-catalyzed asymmetric deacylation of 1-acyloxymethyl-2-azetidinones (Table **9**).

Table 9. Optimal Conditions of Lipase-Catalyzed Enantioselective Acylation of *N*-Hydroxymethylated β-Lactams and Lipase-Catalyzed Enantioselective Hydrolysis of 1-Acyloxymethyl-2-azetidinones

substrate	enzyme (mg/mL)	acyl donor or nucleophile	solvent	temp. (°C)	time (h)	conv. (%)	*E*	substrate		product	
								yield (%)	ee (%)	yield (%)	ee (%)
 (±)-**54**[a]: R = H (±)-**55**[a] : R = COMe	lipase PS (10)	VA	CH_2Cl_2		9	50	>200	46	99	49	98
	lipase B (5)	VA	CH_2Cl_2	RT	24	48	143	42	88	47	96
	lipase B (5)	H_2O	*i*Pr_2O		8	50	>200	46	99	48	98

(Table 9). contd.....

substrate	enzyme (mg/mL)	acyl donor or nucleophile	solvent	temp. (°C)	time (h)	conv. (%)	*E*	substrate		product	
								yield (%)	ee (%)	yield (%)	ee (%)
 (±)-**56**[b]	lipase AK (50)	VB	acetone	0	2.7	52	90	41	98	45	90
 (±)-**59**[b]	lipase AK (50)	VB	acetone	0	3.5	53	62	34	98	37	86
 (±)-**60**[c]	lipase PS (50)	VB	acetone	RT	5	50	>200	36	97	40	98
 (±)-**61**[c]	lipase PS (50)	VB	acetone	RT	4	49	>200	25	97	38	99
 (±)-**62**[c]	lipase PS (50)	VB	acetone	RT	6	49	>200	32	94	34	99
 (±)-**63**[d]	lipase PS (30)	VB + Et_3N	iPr_2O	-15	4	51	94	31	96	43	92
 (±)-**64**[e]	lipase AK (50)	VB	THF	RT	2.5	50	>200	41	99	44	99
 (±)-**65**[f]: R = H (±)-**67**[f] : R = COPr	lipase PS (30)	VB EtOH	toluene iPr_2O	25 40	1.5 7	50	>200 57	31 47	98 97	43 46	97 96
 (±)-**66**[f]: R = H (±)-**68**[f] : R = COPr	lipase PS (30)	VB EtOH	toluene iPr_2O	25 40	1.5 6	52	>200 89	28 40	95 97	45 45	88 91

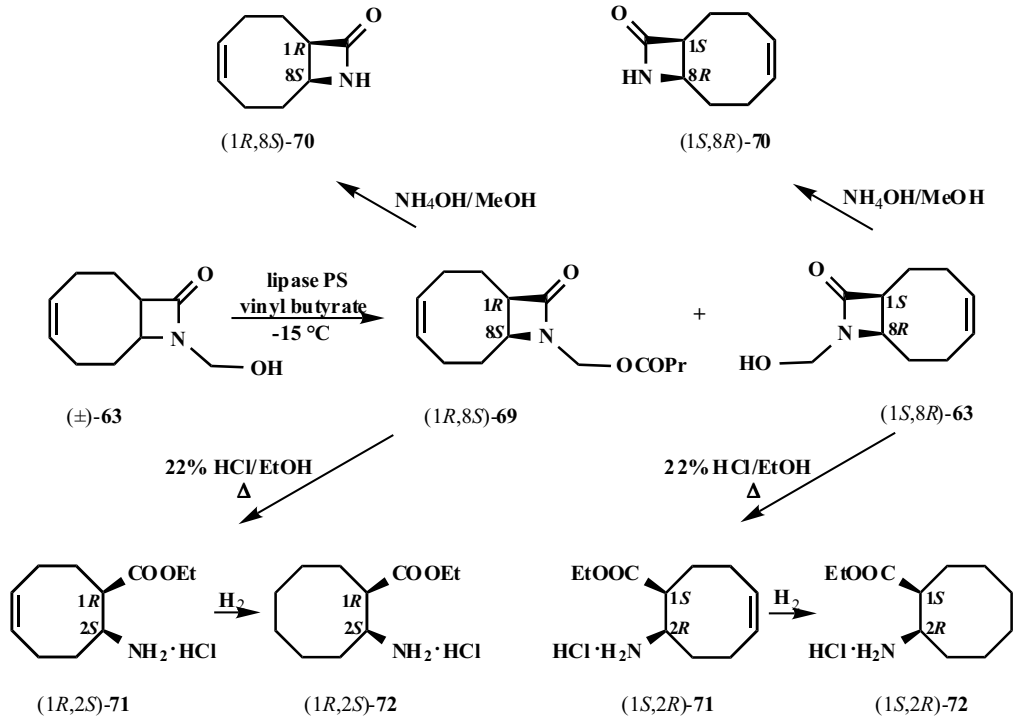
[a]Data from ref. [36]. [b]Data from ref. [37]. [c]Data from ref. [38]. [d]Data from ref. [39]. [e]Data from ref. [40]. [f]Data from ref. [41].

The alicyclic β-lactams (±)-**54**, (±)-**56** and (±)-**59**-**63** were resolved through the lipase PS or AK-catalyzed asymmetric acylation with vinyl acetate or butyrate of the hydroxy group at the (*S*) stereogenic center, while in the case of (±)-**64**, the lipase AK-catalyzed butyrylation occurred at the (*R*) stereogenic center. It should be mentioned that, in this latter case, the same stereochemical demands are fulfilled around the asymmetric center, but only the sequence of CIP priority of the substituents on the substrates differs [40].

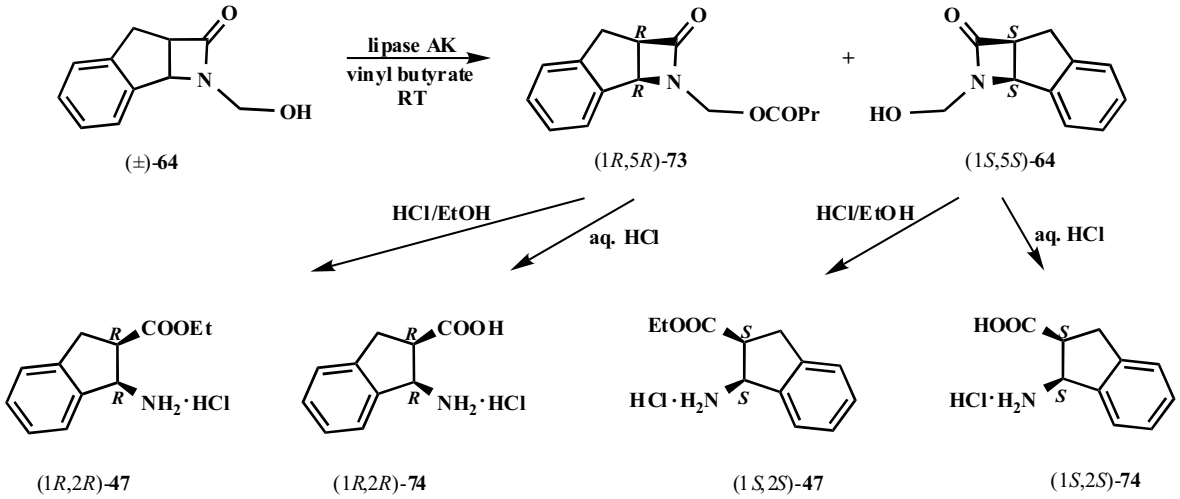
The enzymatic resolution of 9-hydroxymethyl-9-azabicyclo[6.2.0.]dec-4-en-10-one, (±)-**63** resulted in the (1*R*,8*S*)-ester and (1*S*,8*R*)-unreacted β-lactam enantiomers; on treatment with NH_4OH/MeOH, these afforded the corresponding β-lactams (1*R*,8*S*)-**70** and (1*S*,8*R*)-**70**, potential starting compounds in anatoxin-*a* synthesis (Scheme 13) [39]. The ring opening of lactam enantiomers (22% HCl/EtOH) followed by catalytic reduction resulted in the enantiomers of β-amino esters (1*R*,2*S*)-**72** and (1*S*,2*R*)-**72**.

All four enantiomers of 1-aminoindane-2-carboxylic acid, (1*R*,2*R*)-**50**, (1*S*,2*S*)-**50**, (1*R*,2*R*)-**74** and (1*S*,2*S*)-**74**, have been prepared through the lipase AK-catalyzed asymmetric acylation with vinyl butyrate of 3,4-benzo-6-hydroxymethyl-6-azabicyclo[3.2.0]heptan-7-one, (±)-**64** (Scheme **14**) [40]. The hydrolysis and isomerization of the β-amino ester hydrochloride enantiomers (1*R*,2*R*)-**47** and (1*S*,2*S*)-**47** resulted in the *trans*-β-amino acid hydrochlorides.

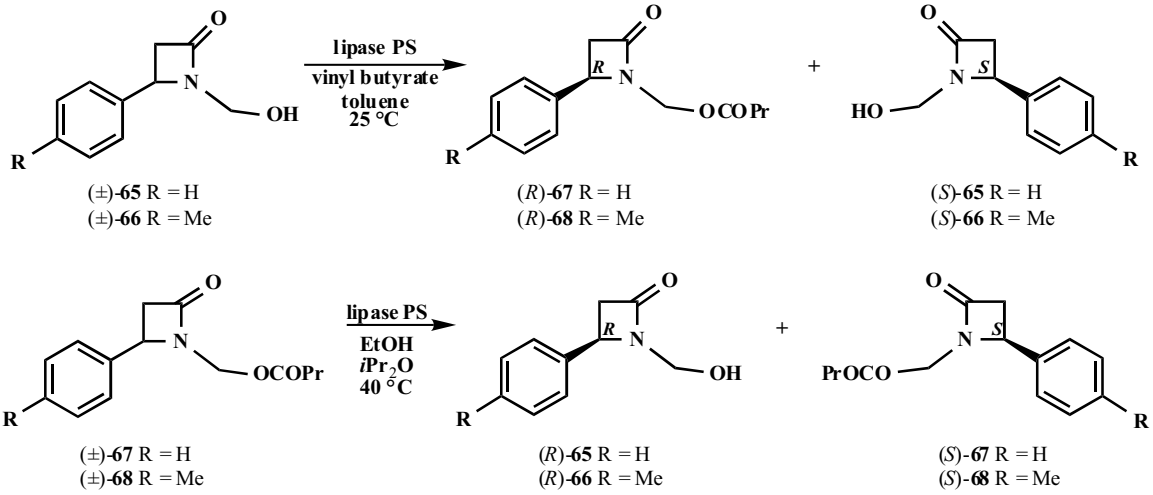
4-Phenyl- and 4-(*p*-tolyl)-2-azetidinone enantiomers were prepared via lipase PS-catalyzed *R*-selective butyrylation and debutyrylation (Scheme **15**). In the cases of the phenyl-substituted β-lactams (±)-**65** and (±)-**67**, both butyrylation



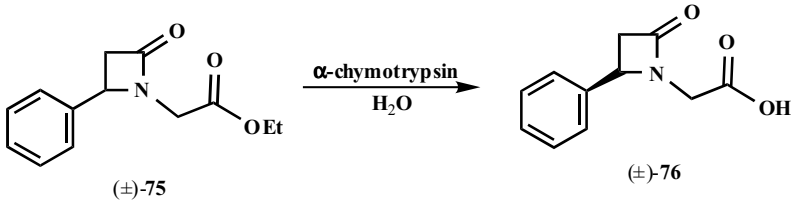
Scheme 13



Scheme 14



Scheme 15.



Scheme 16.

and debutyrylation exhibit higher enantioselectivity than in the case of the 4-(*p*-tolyl) derivatives (±)-**66** and (±)-**68** [41]. With HCl/EtOH, the product lactam enantiomers afforded the corresponding enantiopure β-amino ester hydrochlorides (ee ≥ 92%).

The hydrolysis of ethyl 2-(2-oxo-4-phenylazetidin-1-yl)acetate, (±)-**75**, with α-chymotrypsin under pH-stat conditions has been described (Scheme **16**) [42]. The only product isolated from the enzymatic reaction was (*R*)-2-(2-oxo-4-phenylazetidin-1-yl)acetic acid, (*R*)-**76** (ee = 84%). No data were reported with regard to the enantioselectivity and yield.

IV. CONCLUSIONS AND OUTLOOK

The lipase-catalyzed enantioselective ring opening of activated and inactivated β-lactams in an organic solvent is a very simple and efficient direct method for the preparation of enantiopure cyclic β-amino acids. Although a number of enzymatic and asymmetric syntheses have already been developed for valuable β-amino acids (cispentacin, with strong antibiotic and antifungal activities), this direct method will almost certainly be of interest, as it involves a very simple and inexpensive new synthetic route. The indirect enzymatic method for the preparation of enantiopure β-amino acids or derivatives is a somewhat less efficient and longer procedure, but it ensures the simultaneous preparation of both β-lactam enantiomers.

V. ABBREVIATIONS

Ac	=	Acetyl
CAL-A	=	Lipase A from *Candida antarctica*
Chirazyme L-1	=	*Burkhold eria* sp.
Chirazyme L-2	=	Lipase B from *Candida antarctica*
Chirazyme L-6	=	*Pseudomonas* sp.
conv.	=	Conversion
E	=	Enantioselectivity
ee, ee_S, ee_P	=	Enantiomeric excess, ee of unreacted substrate, ee of product
ENZA-1	=	*Rhodococcus equi NCIMB 40213*
Enz	=	Enzyme
Et	=	Ethyl
His	=	Histidine
iPr_2O	=	Diisopropyl ether
lipase PS	=	Lipase from *Pseudomonas cepacia*
lipase P-30	=	Lipase from *Pseudomonas cepacia*
lipase AK	=	Lipase from *Pseudomonas fluorescens*
lipase B	=	Lipase from *Pseudomonas fragi*
Lipolase	=	Lipase B from *Candida antarctica*
Me	=	Methyl
MeOH	=	Methanol
Novozym 435	=	Lipase B from *Candida antarctica*
Nu	=	Nucleophile
Ph	=	Phenyl
PLE	=	Porcine liver esterase
Pr	=	Propyl

RT	=	Room temperature
t-BuOMe	=	*tert*-Butyl methyl ether
temp.	=	Temperature
THF	=	Tetrahydrofuran
VA	=	Vinyl acetate
VB	=	Vinyl butyrate

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