Direct enzymatic routes to β-substituted β-amino acid enantiomers

PhD Thesis

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Publications

I. E. Forró, T. Paál, **G. Tasnádi**, F. Fülöp
A new route to enantiopure β-aryl-substituted β-amino acids and 4-aryl-substituted β-lactams through lipase-catalyzed enantioselective ring cleavage of β-lactams
Adv. Synth. Catal. **2006**, *348*, 917-923.

II. **G. Tasnádi**, E. Forró, F. Fülöp
Candida antarctica lipase B-catalyzed ring opening of 4-arylalkyl-substituted β-lactams
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III. **G. Tasnádi**, E. Forró, F. Fülöp
An efficient new enzymatic method for the preparation of β-aryl-β-amino acid enantiomers
Tetrahedron: Asymmetry **2008**, *19*, 2072-2077.

IV. **G. Tasnádi**, E. Forró, F. Fülöp
Burkholderia cepacia lipase as an excellent enzyme for the enantioselective hydrolysis of β-heteroaryl-β-amino esters
Tetrahedron: Asymmetry **2009**, *20*, 1771-1777.

V. **G. Tasnádi**, E. Forró, F. Fülöp
Improved enzymatic syntheses of valuable β-arylalkyl-β-amino acid enantiomers
Org. Biomol. Chem. **2010**, *8*, 793-799.

VI. **G. Tasnádi**, E. Forró, F. Fülöp
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Magy. Kém. Foly. **2010**, accepted manuscript.

VII. M. Utczás, E. Székely, **G. Tasnádi**, É. Monek, L. Vida, E. Forró, F. Fülöp, B. Simándi
Kinetic resolution of 4-phenyl-2-azetidinone in supercritical carbon dioxide
J. Supercrit. Fluids **2010**, submitted manuscript.

Lectures

I. Vegyészkonferencia, 28-30 June, 2005, Hajdúszoboszló, Hungary, poster presentation:
Enikő Forró, Tihamér Paál, **Gábor Tasnádi**, Ferenc Fülöp
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IV. Training school in biocatalysis, 28 April - 3 May, 2007, Siena, Italy, oral presentation:
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V. "Szegedi Ifjú Szerves Kémikusok Támogatásáért" Alapítvány 8. tudományos előadó ülése, 16 April, 2008, Szeged, Hungary, oral presentation:
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VI. 20th International Symposium on Chirality, 6-9 July, 2008, Geneva, Switzerland, poster presentation:
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VII. 16th European Symposium on Organic Chemistry, 12-16 July, 2009, Prague, Czech Republic, poster presentation:
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VIII. Foldamers: building blocks, structure and function, 24-26 September, 2009, Szeged, Hungary, poster presentation:
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An improved enzymatic method for the preparation of valuable β-arylalkyl-β-amino acid enantiomers (P-11)

Abbreviations

AC I	aminoacylase I from *Aspergillus melleus*
Alcalase	subtilisin from *Bacillus licheniformis*
Amano lipase PS	lipase from *Burkholderia (Pseudomonas) cepacia*
α-CT	α-chymotrypsin
BCL	*Burkholderia cepacia* lipase
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bz	benzoyl
CAL	*Candida antarctica* lipase
CAL-A	*Candida antarctica* lipase A
CAL-B	*Candida antarctica* lipase B
Cam	carbamoylmethyl
Cbz	benzyloxycarbonyl
CCL	*Candida cyclindracea* lipase
Conv.	conversion
CRL	*Candida rugosa* lipase
CSI	chlorosulfonyl isocyanate
DABCO	1,4-diazabicyclo[2.2.2]octane
DKR	dynamic kinetic resolution
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
E	enantioselectivity
ee	enantiomeric excess
ee_p	enantiomeric excess of product
ee_s	enantiomeric excess of substrate
Gl	glyceryl
lipase PS	*Burkholderia (Pseudomonas) cepacia* lipase
lipase PS-D	*Burkholderia (Pseudomonas) cepacia* lipase
lipase PS IM	*Burkholderia (Pseudomonas) cepacia* lipase
Lipolase	*Candida antarctica* lipase B
(*S*)-NIFE	(*S*)-*N*-(4-nitrophenoxycarbonyl)phenylalanine methoxyethyl ester
PLE	pig liver esterase
PPL	porcine pancreas lipase
protease A	protease from *Aspergillus oryzae*
$scCO_2$	supercritical CO_2
Subtilisin BL	subtilisin from *Bacillus lentus*
Subtilisin BPN’	subtilisin from *Bacillus amyloliquefaciens*
t	reaction time
T	temperature
THF	tetrahydrofuran

1. Introduction and aims

In view of their unique chemical and biological behaviour, β-amino acids are currently a hot topic.[1] They can act in biological systems as single molecules,[2] but their bifunctional structure offers a good possibility to insert them into more complex molecules.[3]

Enantiomerically pure β^3-amino acids, including β-aryl-, β-heteroaryl- and β-arylalkyl-β-amino acids (Figure 1), have numerous pharmaceutical applications, as illustrated below.

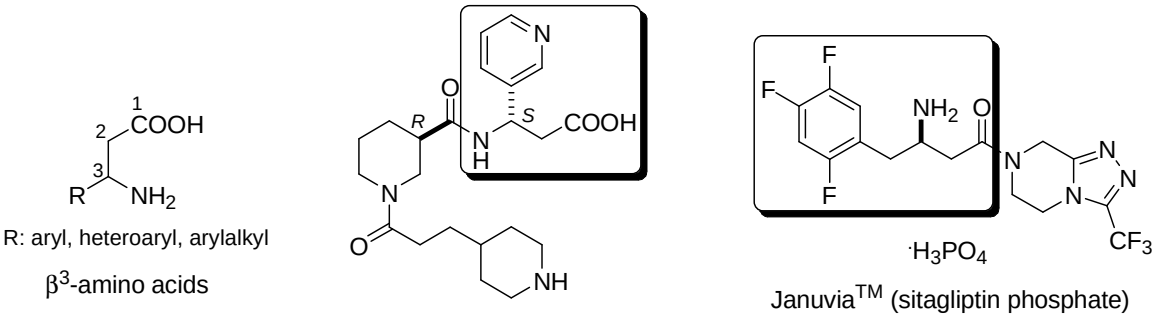


Figure 1

The integrin receptor $\alpha_v\beta_3$ is an interesting therapeutic target in the treatment of osteoporosis, restenosis, cancer growth and metastasis formation. Derivatives of (*R*)-3-amino-3-(3,5-dichlorophenyl)propanoic acid and (*S*)-3-amino-3-(3-pyridyl)propanoic acid have been tested as integrin receptor antagonists.[4,5]

Elarofiban (RWJ-53308) (Figure 1) is a fibrinogen receptor antagonist with an antithrombotic effect.[6-9] It contains an (*S*)-3-amino-3-(3-pyridyl)propanoic acid subunit. Elarofiban has progressed successfully through human phase II clinical trials involving oral or intravenous administration.[6-9]

Derivatives of (*R*)-3-amino-3-phenylpropanoic acid, (*R*)-3-amino-3-(3-pyridyl)propanoic acid and (*R*)-3-amino-5-phenylpentanoic acid have been tested as hepatitis C virus (HCV) NS5B polymerase inhibitors, a valid target for antiviral therapy against HCV.[10,11]

(*R*)-3-Amino-3-phenylpropanoic acid and (*R*)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoic acid have been inserted into anti-inflammatory bradykinin B1 receptor

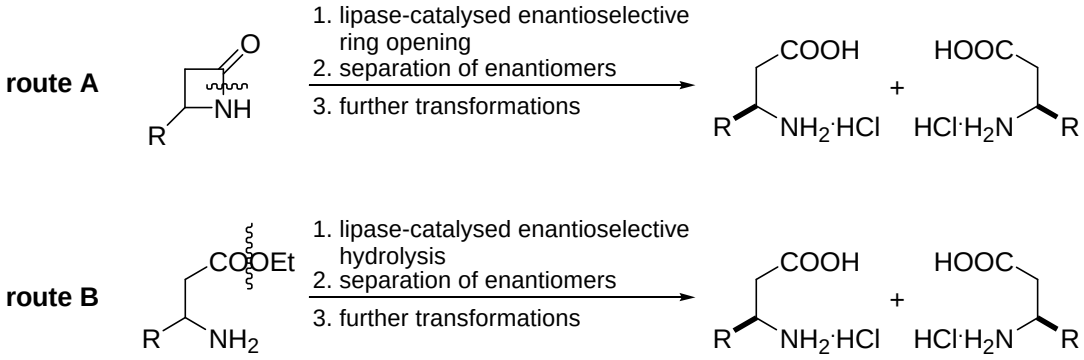
antagonists.[12,13] Another β-amino acid, (*R*)-3-amino-3-(3-fluorophenyl)propanoic acid, has also been used in the synthesis of anti-inflammatory agents, antagonists of chemokine receptors.[14]

Moreover, numerous β-aryl-, β-heteroaryl- and β-arylalkyl-β-amino acid enantiomers have been tested as components of anticancer agents.[15-21]

Dipeptidyl peptidase IV is a new therapeutic target for the treatment of type 2 diabetes. Inhibition of this peptidase results in increased levels of incretins (glucagon-like peptide 1 and gastric inhibitory polypeptide), which control the blood glucose concentration.[22] Sitagliptin phosphate (Januvia^{TM}) (Figure 1) was the first approved drug for the inhibition of dipeptidyl peptidase IV. Sitagliptin contains an (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid subunit.[23] Many further derivatives of sitagliptin have been synthetized and tested as potential antidiabetic drugs.[24-30]

Consequently, there are numerous reasons to prepare β-substituted β-amino acids in enantiomerically pure form. One aim of my thesis was the enzymatic kinetic resolution of 4-aryl- and 4-arylalkyl-substituted β-lactams through opening of the lactam ring (Scheme 1, route A).[I,II] The second aim was to develop the enzymatic hydrolysis of β-aryl-, β-heteroaryl- and β-arylalkyl-substituted β-amino esters (Scheme 1, route B).[III-V]

A number of reaction conditions, *e.g.* the amounts and effects of the enzymes, the nature of the solvent, the temperature and the presence of additives, were investigated in order to achieve the best reaction rate and enantioselectivity. The optimum reaction conditions were then extended to preparative-scale resolutions.



Scheme 1 Reactions planned for the work reported in this thesis

2. Literature

In the literature section of this thesis, the main features of hydrolases (especially lipases, esterases and proteases) and the results on the enzyme-catalysed hydrolyses of α- and β-amino esters in the past decade are surveyed. The publications on which this thesis is based are referred to via Roman numbers as superscripts, while other literature references are given by Arabic numbers as superscripts.

2.1. *Main characteristics of hydrolases*

2.1.1. Lipases and esterases

Hydrolases are enzymes that catalyse the formation of two products from a substrate by hydrolysis with H_2O. In the Enzyme Commission number (EC number) system, the hydrolases form group 3. The main natural role of hydrolases is the digestion of nutrients. For example, proteases break down the amide bonds of proteins, producing smaller peptides or amino acids, while lipases hydrolyse triglycerides to glycerol and fatty acids. Hydrolases can be classified on the basis of the type of the bond hydrolysed. The enzymes which are able to hydrolyse ester bonds belong in group 3.1, and carboxylic ester hydrolases (*e.g.* lipases and esterases) in group 3.1.1.

Hydrolases featuring lipases (EC 3.1.1.3) and esterases (EC 3.1.1.1) have several andvantages which make them useful in organic chemistry: (i) broad substrate specificity, (ii) high stereoselectivity, (iii) catalysis of reactions other than hydrolysis (*e.g.* condensation and alcoholysis), (iv) commercial availability, and (v) stability in organic solvents (especially lipases and esterases).[31]

Lipases and esterases are α/β-hydrolases (Figure 2).[32] All lipases whose structures have been solved exhibit a similar 3D structure. This consists of central, mostly parallel β-sheets, which are surrounded on both sides by α-helices. An esterase from *Pseudomonas fluorescens* displays a similar structure.[33]

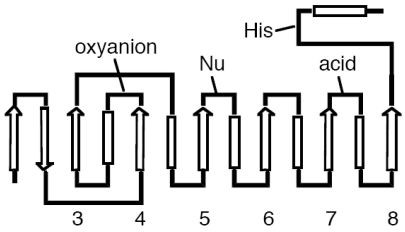


Figure 2 Schematic diagram of the α/β-hydrolase fold. Oxyanion: residues that stabilize the oxyanion, Nu: nucleophilic residue; for lipases, esterases and proteases, this is a serine; α-helices are shown as rectangles, and β-sheets as arrows.[31]

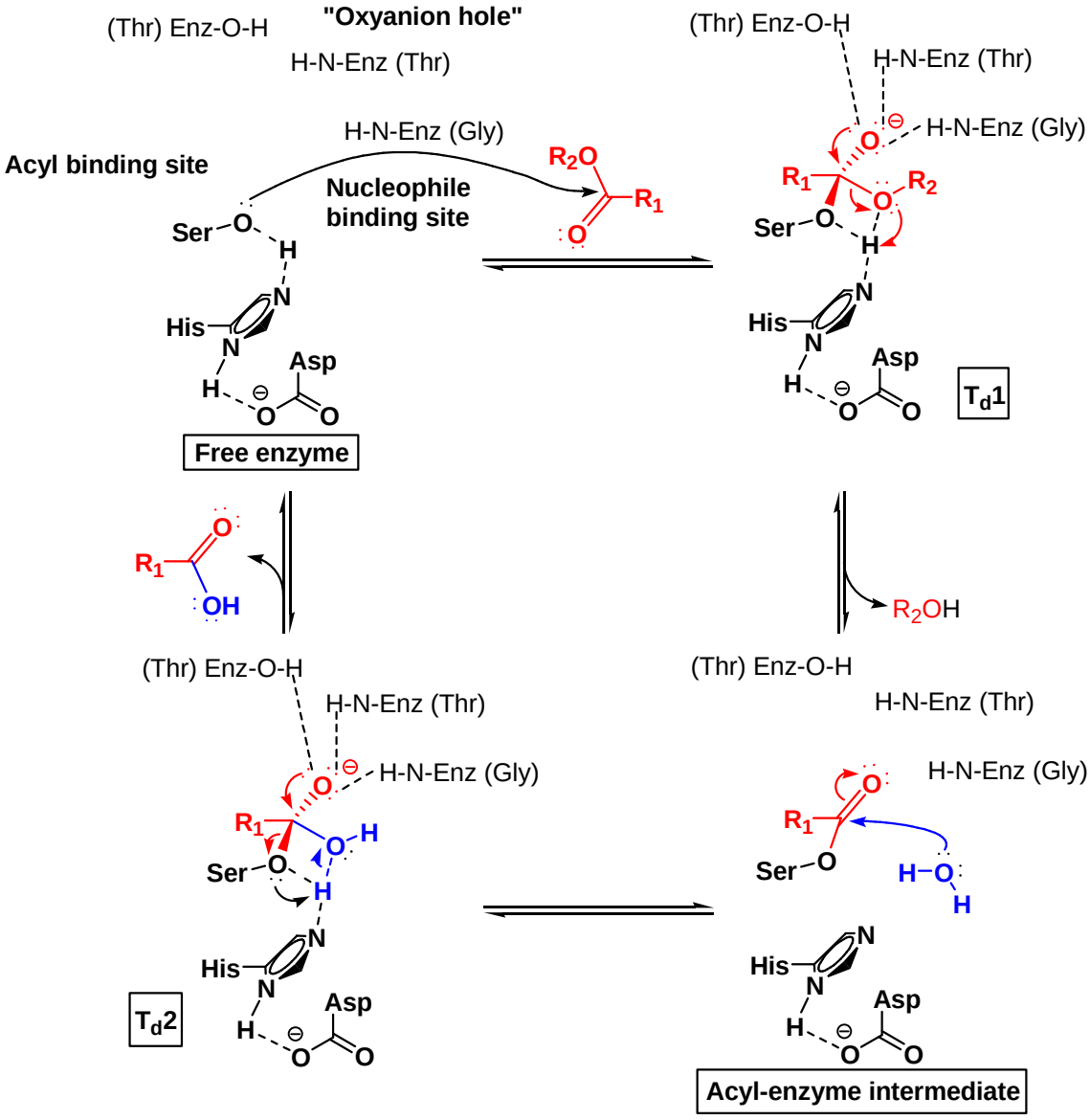


Figure 3 The serine-hydrolase mechanism

Lipases and esterases are serine hydrolases. They have a catalytic Ser, His and Asp triad in the active site, and an "oxyanion hole" to stabilize the transition state. The sequence and the orientation of the catalytic triad are the same in the α,β-hydrolase family.[34] The special arrangement of the catalytic triad causes a decrease in the pK value of the Ser hydroxy group, making it more nucleophilic (Figure 3). Thus, it can attack the carbonyl group of the substrate, forming a tetrahedral intermediate (T_d1), which is stabilized by the amino acid side-chains of the "oxyanion hole". Liberation of the alcohol results in an acyl-enzyme intermediate. In the case of a hydrolytic reaction, H_2O acts as a nucleophile, forming a second tetrahedral intermediate (T_d2). The enzyme is regenerated after release of the product acid. If the reaction is performed in an organic medium, H_2O can be replaced by another nucleophile, leading to transformations other than hydrolysis (Figure 4).[35]

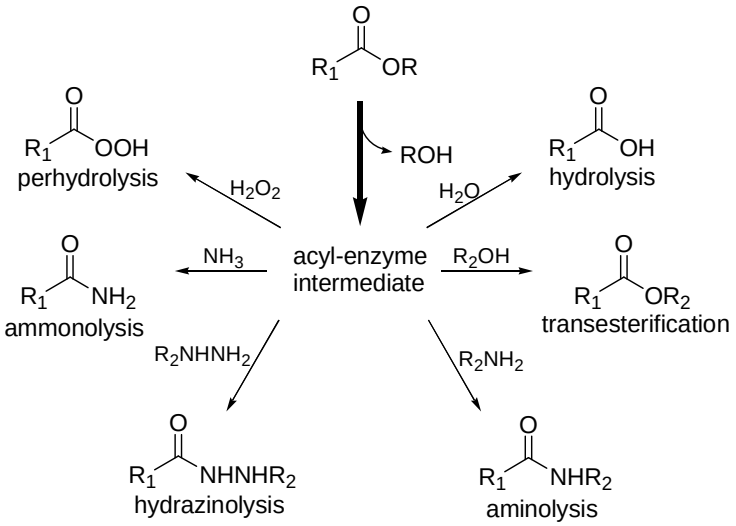


Figure 4 Possible reaction pathways in a hydrolase-catalysed reaction[35]

The difference between lipases and esterases is that lipases prefer to hydrolyse H_2O-insoluble substrates, and demonstrate poor activity towards H_2O-soluble substrates.[36] The activities of lipases are low in true monomeric solutions, but enhanced when an aggregated form (such as an emulsion or a micellar solution) is present.[37] As the hydrolysis by lipases is performed on the H_2O-oil interface, this phenomenon is called "interfacial activation". In the absence of an aqueous-lipid interface, the lipase is inactive; a closed conformation appears where a lid (a helical segment) covers the active site. The

residues from the internal face of the lid and from other chains of the protein form a large hydrophobic surface that surrounds the catalytic site. When the lipase binds to a hydrophobic interface such as a H_2O-insoluble aggregate, the lid opens, as a result of which the lid is folded back, and the catalytic activity increases.[38] This binding and conformational change increases the activity of most, but not all lipases. For example, CAL-B has a small lid, but shows no interfacial activation.[37] Due to this fact, an esterase cannot be defined as a lipase on the basis of interfacial activation. Lipases are rather defined as carboxyl ester hydrolases that act on long-chain acyl glycerols.

Lipases have different binding sites for the nucleophile and the acyl residues. The nucleophile binding site contains a large hydrophobic pocket and a small pocket. This region is responsible for the enantiodiscrimination, although numerous examples are to be found in the literature with excellent chiral recognition in the acyl part of a substrate.[39,40]

The binding site for the acid part of the ester differs among the lipases. For example, in CRL (other name: CCL) the acyl chain binds in a tunnel which is long enough to admit a chain of at least 18 carbons. In contrast, in CAL-B and in BCL the acyl moiety can be accommodated in a funnel-like binding pocket. In all three structures, the α-carbon of the acyl chain binds below the hydrophobic pocket of the alcohol binding site and substituents on the α-carbon can be situated in the hydrophobic pocket as well.[41]

2.1.2. Proteases

Besides lipases and esterases, some proteases are often used for the preparation of enantiomeric compounds. The most important and commercially available proteases are α-CT (EC 3.4.21.1) and different types of subtilisins (EC 3.4.21.62) (*e.g.* Subtilisin BL, Subtilisin BPN' and Alcalase). The latter enzymes are able to tolerate 10-20% organic co-solvent, *e.g.* MeOH, EtOH, *t*-BuOH, DMF, DMSO, MeCN and Me_2CO, to help dissolve the substrate.[42]

α-CT and subtilisins are serine-hydrolases. Due to the fact that the acyl-binding site contains non-polar amino acid units, they prefer aromatic or large non-polar residues. In subtilisins this binding site is rather a shallow groove,[43] while in α-CT it is a well-defined hydrophobic pocket.[44]

2.1.3. Hydrolases in organic solvents

Hydrolases are the enzymes most commonly used both in academic practice[45,46] and in industry.[47,48] The most frequent substrates of hydrolases are prochiral diols or dicarboxylic diesters, and racemic alcohols or carboxylic esters. Hydrolysis, acylation and alcoholysis (transesterification) can be easily performed with hydrolases. However, to carry out transesterification, an almost anhydrous medium is needed in order to avoid hydrolysis of the substrate, and a nucleophile other than H_2O must be used.

Lipases, esterases and proteases are able to function in aqueous solution, in a mixture of H_2O and an organic solvent, or in a neat organic solvent.[49] The use of organic solvents has some advantages, *e.g.* (i) better recovery and yield of the product, (ii) the use of apolar substrates and compounds that are unstable in H_2O, (iii) less side-reactions (*e.g.* hydrolysis), (iv) in many cases the enzyme is thermodynamically more active, (v) the possibility to perform reactions other than hydrolysis, and (vi) denaturation of the enzyme is minimized (excpet in ethanol).[50] However, an appropriate amount of H_2O, which can originate from the enzyme preparation (< 5%) or the solvent (< 0.1%), is required for the sufficient functioning of the enzymes.[51]

Lipases form a special group among hydrolases due to their ability to act in almost anhydrous media.[52] They frequently exhibit higher activity in two-phase systems or in H_2O-saturated organic solvents such as *i*-Pr_2O, *t*-BuOMe, *n*-hexane or cyclohexane than in aqueous buffers. It should be mentioned that the type of the solvent can have a drastic effect on the selectivity of the enzyme, and can even invert it.[53]

As the main topic of this thesis is the hydrolysis of ester and lactam moieties, the main characteristics and some examples of the enzyme-catalysed hydrolyses of esters, α- and β-amino esters and β-lactams will be presented below.

2.2. *Application of hydrolases in organic chemistry*

2.2.1. Hydrolyses of aliphatic and aromatic esters

The enantioselective hydrolyses of (i) prochiral or *meso* diacetates of diols, (ii) dicarboxylic diesters, (iii) racemic carboxylic esters and (iv) esters of racemic alcohols were reviewed in 2002 by Drauz and Waldmann.[45] Since 2002, many further papers have reported the enantioselective kinetic resolution of anti-inflammatory arylpropionic acid esters,[54-58] α- or β-hydroxy-substituted esters,[59-61] carbocyclic[62,63] and heterocyclic[64-69] esters, α-halogenated esters[70] and chiral phosphorus-containing esters.[72] Several dynamic kinetic resolutions[73] and desymmetrizations[73-81] have also been reported. It should be mentioned that the chemo- and regioselectivities of enzymes may offer a mild and efficient route for the removal of protecting groups of carboxylic esters.[82-86]

2.2.2. Hydrolyses of α-amino esters

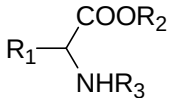
Numerous natural and non-natural α-amino esters have been resolved with various hydrolytic enzymes. The hydrolyses of *N*-protected and *N*-unprotected, aliphatic, aromatic and heterocyclic, and natural and non-natural α-amino esters reported before 2002 have been compiled in review articles.[45,87] These results indicate that subtilisin, α-CT and different lipases are among the most suitable enzymes at ambient temperature in a buffer (pH 6-8) or in a mixture of solvents.

Here, only results reported in the past decade will be mentioned.

Table 1 relates to the *S*-selective (≡ L-selective) resolution of different aromatic and aliphatic α-amino esters [(±)-**1**–(±)-**23**]. Amino esters with a hydrophobic *N*-protecting group [(±)-**1**–(±)-**5**] have been resolved in the presence of an organic co-solvent (5-15%).[88-92] As an exception, however, the racemic methyl ester of *N*-Boc-*tert*-leucine [(±)-**6**] gave better results in a buffer without an organic co-solvent.[91] In several cases, the application of an organic co-solvent can be explained by enzyme inhibition at higher substrate concentrations.[89] Subtilisin has been applied not only in the resolution of amino esters, but also in the syntheses of *N*-protected non-symmetric aspartate and

glutamate diesters, which are important building blocks in peptide chemistry (data not shown).[92]

Table 1 Examples of protease-catalysed hydrolyses of aromatic and aliphatic α-amino esters



Substrate	R_1	R_2	R_3	Enzyme	t	ee_s (%)	ee_p (%)	Conv. (%)	*E*
(±)-**1**[88a]	$(CN)_2CHCH_2$	Me	Boc	α-CT	2 h	95	96	50	183
(±)-**2**[89b]	$NH_2(CO)CH_2$	Me	Cbz	Alcalase	8 h	> 99	> 99	49	> 200
(±)-**3**[89c]	$NH_2(CO)CH_2$	Bn	Bz		17.9 h	> 99	> 99	50	> 200
(±)-**4**[90d]	cyclopropyl	Me	Boc	papain	36 h	99	99	50	> 200
(±)-**5**[91e]	Et	Me	Boc	Alcalase	4 h	98	98	50	> 200
(±)-**6**[91]	*t*-Bu	Me	Boc		18 h	91	99	48	> 200
(±)-**7**[93]	Et	Me	H	AC I	3.2 h	92	82	53	33
(±)-**8**[93]		Et	H		4 h	97	90	52	80
(±)-**9**[93]	Ph	Me	H		5 h	50	54	48	5
(±)-**10**[93]	*p*-OH-Ph	Me	H		5 h	88	69	56	15
(±)-**11**[94]	*n*-Pr	Me	H	protease A	170 min	39	58	40	5
(±)-**12**[94]		Et	H		280 min	48	72	40	10
(±)-**13**[94]		*n*-Pr	H		250 min	50	75	40	11
(±)-**14**[94]		*i*-Pr	H		510 min	55	82	40	17
(±)-**15**[94]		*n*-Bu	H		150 min	59	88	40	28
(±)-**16**[94]		*i*-Bu	H		77 min	62	92	40	45
(±)-**17**[94f]	Et	*i*-Bu	H		22.5 h	63	95	40	75
(±)-**16**[94f]	*n*-Pr	*i*-Bu	H		400 min	65	97	40	129
(±)-**18**[94f]	*n*-Bu	*i*-Bu	H		19.7 h	68	98	41	> 200
(±)-**19**[94]	*i*-Bu	*i*-Bu	H		320 min	63	94	40	62
(±)-**20**[94]	*n*-pentyl	*i*-Bu	H		280 min	44	89	33	26
(±)-**21**[94]	*i*-pentyl	*i*-Bu	H		14.5 h	38	77	33	11
(±)-**22**[94]	*p*-Cl-Bn	Me	H		59 min	35	46	43	4
(±)-**23**[94]	*p*-Cl-Bn	*i*-Bu	H		30 min	83	98	46	> 200

[a] Containing 5% DMSO. [b] Containing 10% THF. [c] Containing 15% Me_2CO. [d] Containing 33% MeCN and 33% DMF. [e] Containing 50% *t*-BuOMe. [f] Performed at 5 °C.

The extraordinary behaviour of AC I (EC 3.5.1.14) in catalysing the hydrolysis of *N*-unprotected non-natural amino esters with moderate selectivity has been demonstrated.[93] However, the unprotected 2-aminobutanoic acid ester derivatives [Table 1, (±)-**7** and (±)-**8**] have been resolved with lower *E* as compared with the *N*-Boc-protected derivative [(±)-**5**] catalysed by Alcalase. In the case of phenylglycine derivatives [(±)-**9** and (±)-**10**], the hydroxy substitution exerted marked effects on the conversion and *E*.

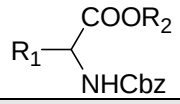
Miyazawa et al. applied protease A (EC 3.4.24.27) to prepare *N*-unprotected aliphatic and aromatic α-amino acid enantiomers through hydrolysis of the ester group.[94] They found that *E* increased greatly on the use of 2-aminopentanoic acid esters with a longer alkyl chain [Table 1, (±)-**11**–(±)-**16**]. Thus, *iso*-butyl aminopentanoate [(±)-**16**] proved to be the most suitable, giving *E* = 45 at room temperature and *E* = 129 at 5 °C. Good to excellent *E* values were obtained with substrates bearing 2-4 carbon atoms in the side-chain [(±)-**16**–(±)-**18**] at 5 °C. However, longer substituents were not suitable substrates of protease A [(±)-**19**–(±)-**21**]. This observation was confirmed by two Japanese groups.[95,96] A similar relation between *E* and the alkyl chain length was observed in the case of substituted phenylalanine derivatives [(±)-**22** and (±)-**23**]. The *iso*-butyl ester derivative [(±)-**22**] gave excellent conversion and *E* relative to those for the methyl ester derivative [(±)-**23**]. Further substrates have been successfully resolved with α-CT[97] and subtilisin[98,99] on a kilogramm scale.

In the cases of *N*-Cbz chloroethyl esters of aliphatic amino acids [Table 2, (±)-**24a**–(±)-**32a**], PPL directed the *S*-selective resolutions of substrates with 3-5 carbon atoms in the side-chain [(±)-**26a**–(±)-**30a**] with higher *E* values than with shorter [(±)-**24a** and (±)-**25a**] or longer chains [(±)-**31a** and (±)-**32a**].[95] More recently, Kagawa et al. confirmed these results.[96] They prepared Gl and Cam esters of *N*-Cbz aliphatic and aromatic amino acids and applied them in hydrolytic reactions catalysed by PPL at room temperature. They used buffer/MeCN (70/30%) as solvent to help dissolve the hydrophobic substrates. Increased *E* values were observed in comparison with the previous results[95] [(±)-**24a**–(±)-**36a**]. The reaction rates of (±)-**26b**, (±)-**27b** and (±)-**29b** were much better than those of (±)-**25b** and (±)-**31b**. These results again indicate that PPL can readily accept amino esters with straight side-chains of 3-5 carbon atoms into its

pocket. When aromatic amino esters were used as substrates [(±)-**33a,b**–(±)-**36a,b**], the Gl esters in aqueous MeCN displayed higher *E* than those for the chloroethyl esters.

Further racemic aliphatic[100], aromatic[101,102] and cyclic[103] amino esters have been resolved with different hydrolases.

Table 2 Examples of PPL-catalysed hydrolyses of aliphatic and aromatic α-amino esters

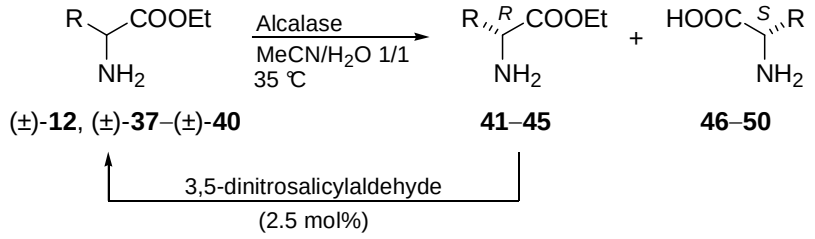


		$R_2 = CH_2ClCH_2$[95] (**24a**–**36a**)[a]					$R_2 = Gl$[96] (**24b**–**36b**)				
	R_1	t (h)	ee_s (%)	ee_p (%)	Conv. (%)	*E*	t (h)	ee_s (%)	ee_p (%)	Conv. (%)	*E*
(±)-**24**	Me	68	16	24	40	2	24	11	75	13	8
(±)-**25**	Et	21	61	88	41	29	12	40	> 99	29	> 200
(±)-**26**	*n*-Pr	23	58	94	38	58	3	81	> 99	45	> 200
(±)-**27**	*n*-Bu	66	70	97	42	138	3	66	> 99	40	> 200
(±)-**28**	*i*-Bu	47	66	> 99	40	> 200	-	-	-	-	-
(±)-**29**	*n*-pentyl	44	67	93	42	56	3	78	> 99	44	> 200
(±)-**30**	*i*-pentyl	42	63	95	40	75	-	-	-	-	-
(±)-**31**	*n*-hexyl	160	25	68	27	7	9	37	> 99	27	> 200
(±)-**32**	*n*-heptyl	18[b]	14	52	21	4	-	-	-	-	-
(±)-**33**	Ph	160	88	69	56	15	9	55	97	36	114
(±)-**34**	Bn	90	63	95	40	75	0.3	> 99	> 99	50	> 200
(±)-**35**	Phenylethyl	30	39	58	40	5	9	44	97	31	101
(±)-**36**	*p*-Cl-Bn	72	48	72	40	10	0.3	> 99	> 99	50	> 200

[a] Except for (±)-**30a**, (±)-**32a** and (±)-**35a**, where $R_2 = CF_3CH_2$. [b] Reaction time is given in days.

DKRs and desymmetrizations allow a maximum theoretical yield of 100%. DKR combines enzyme, chemocatalyst or microbial kinetic resolution with the *in situ* enzyme, metal or base-catalysed racemization.[104-106] The acidic proton of the α-carbon makes the α-amino esters useful substrates for DKRs. Several processes have been developed for the DKR of α-amino esters in the presence of an aldehyde in catalytic amount.[107-109] α-CT-catalysed *S*-selective hydrolyses of Schiff bases of amino esters have been studied in aqueous MeCN.[107] When phenylalanine ethyl ester was applied, racemization occurred in the presence of DABCO.

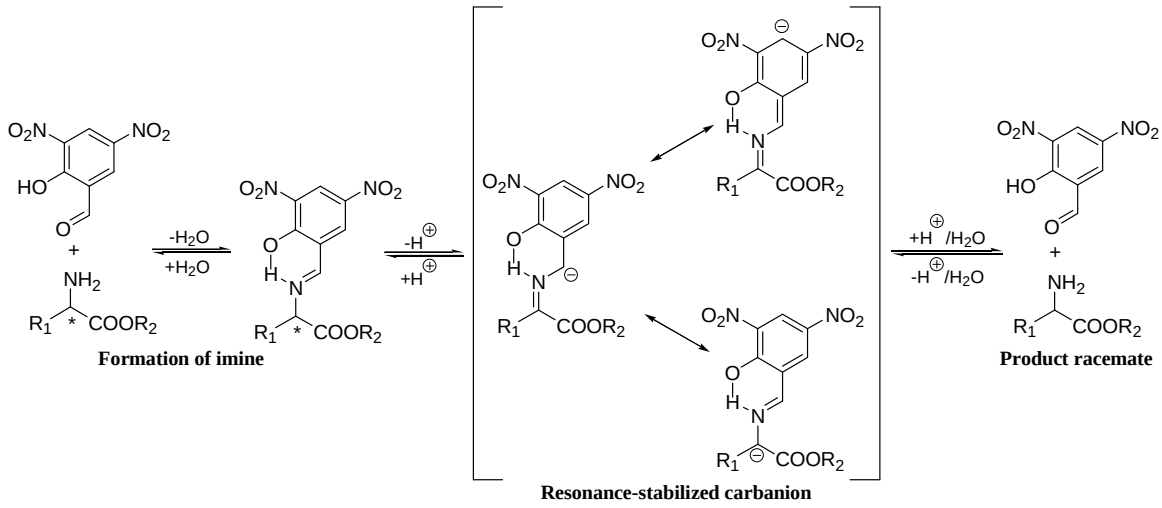
Table 3 Aldehyde-assisted DKR of several α-amino esters[109]



	R	t (d)	Yield (%) (**46**–**50**)	ee_p (%) (**46**–**50**)
(±)-**12**	*n*-Pr	4	98	64
(±)-**37**	Bn	1	95	99
(±)-**38**	*p*-OH-Bn	2	94	95
(±)-**39**	$MeS(CH_2)_2$	2	96	89
(±)-**40**	*i*-Bu	3	99	97

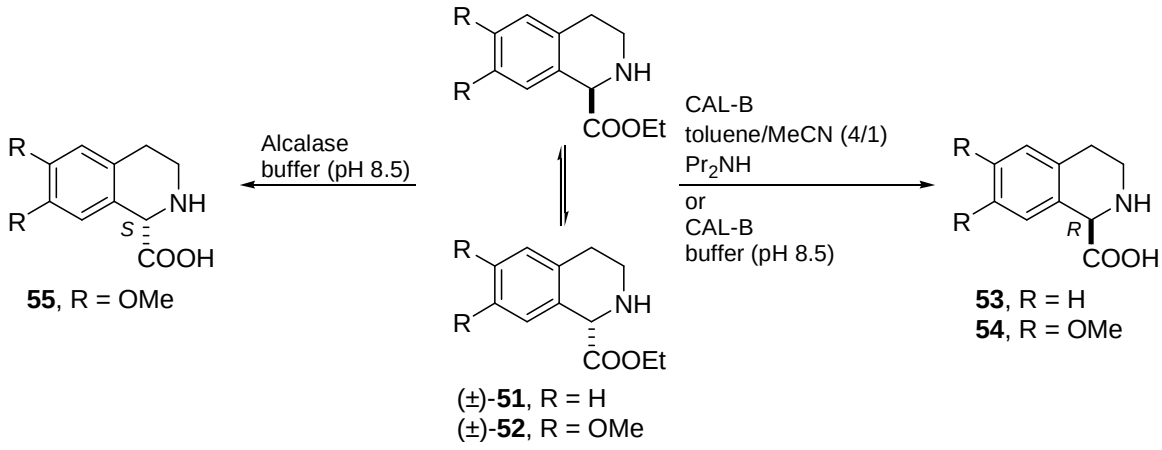
Schichl and co-workers studied the properties of the aldehyde and found that electron-withdrawing groups on positions 3 and 5 of the aromatic ring, combined with a hydroxy group at position 2, is advantageous in the case of α-amino esters.[109] In this way, the use of amines for the racemization can be avoided. These findings have resulted in the efficient DKR of several α-amino esters [(±)-**18**, (±)-**37**–(±)-**40**] being performed with Alcalase in 1:1 MeCN/buffer in the pH range 7-8 at 35 °C without the use of base (Table 3).[109] At the end of the reactions, the product (*S*)-amino acids (**46**–**50**, ee_p = 65-99%) were almost quantitatively (yield = 94-99%) precipitated by the addition of 1,2-dimethoxyethane.

The proposed mechanism of the racemization is presented in Scheme 2. The condensation of the amino ester with the aldehyde enhances the acidity of the chiral proton. A rapid protonation-deprotonation of the α-carbon through a resonance-stabilized carbanion causes racemization. It is important to note that hydrogen-bonding between the imine and the aromatic hydroxy group increases the possibility of protonation-deprotonation.



Scheme 2 Proposed mechanism of aldehyde-assisted racemization of α-amino esters[109]

DKRs of tetrahydroisoquinoline-1-carboxylic acid ethyl esters have been developed without additional aldehyde (Scheme 3).[110,111] Hydrolyses were performed in an organic solvent in the presence of an amine [(±)-**51**] or in an alkaline buffer [(±)-**51** and (±)-**52**]. In the cases of 6,7-diOMe-substituted compounds, the hydrolysis was directed toward the *R*- or the *S*-product by the choice of the enzyme (CAL-B to produce **54** and Alcalase to produce **55**). The amino acid enantiomers (**53**–**55**) were obtained with high *ee* (92-98%) and in good yields (80-92%).



Scheme 3 DKRs of tetrahydroisoquinoline-1-carboxylic ethyl ester derivatives

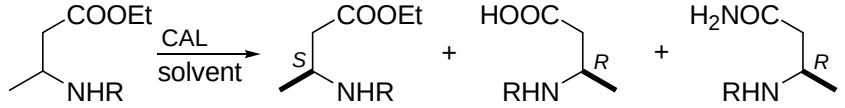
In summary, methods exist to prepare aliphatic or aromatic α-amino acid enantiomers from their ester derivatives with proteases and lipases. Protection of the NH_2

group is not necessary. The chain-lengths of the substituents, the type of the ester group and the use of organic co-solvents can strongly influence the reaction rate and *E*.

2.2.3. Hydrolyses of β-amino esters

In contrast with the α-amino acids, relatively few examples are to be found in the literature for the preparation of enantiopure β-amino acids from their racemic ester derivatives via enzyme-catalysed hydrolysis. Some early results relate to the α-CT- and PLE-catalysed hydrolyses of *N*-protected aspartate,[112] glutarate[113] and β-phenylalanine[114] derivatives. The few results include the hydrolyses of cyclic and acylic *N*-protected and unprotected esters in buffers and in organic solvents, mainly with the use of CAL-B or BCL.

Table 4 Ammonolysis and hydrolysis of ethyl (±)-3-aminobutanoate [(±)-**56**] and ethyl (±)-*N*-Cbz-3-aminobutanoate [(±)-**57**]



(±)-**56**, R = H
(±)-**57**, R = Cbz
58, R = H
59, R = Cbz
60, R = Cbz
61, R = H

Entry	R	Solvent	t (h)	ee_s (%)		ee_p (%)		Conv. (%)	*E*
				58	**59**	**60**	**61**		
1	H	1,4-dioxane[a]	8	-	71	-	36	66	4
2	Cbz	1,4-dioxane[a]	8	87	-	94	84[b]	-	-
3	Cbz	1,4-dioxane[c]	8	21	-	93	-	18	33
4	Cbz	1,4-dioxane[d]	8	67	-	98	-	41	> 200
5	Cbz	Buffer, pH 7	1	77	-	83	-	48	25

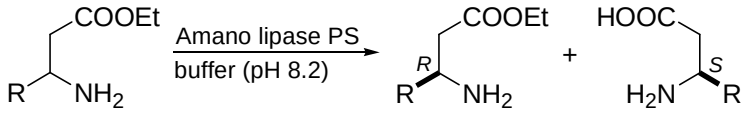
[a] For the ammonolysis process, $NH_{3(g)}$ was bubbled through the solvent at 0 °C for 10 min, under a N_2 atmosphere. [b] Only a 3% yield of amide product. [c] With H_2O (l equiv.). [d] With H_2O (1 equiv.) and Et_3N (l equiv.).

Sánchez et al. examined the possibilities of the resolution of racemic 3-aminobutyric acid derivatives (Table 4).[115] They used CAL (the type of CAL was not given) in 1,4-dioxane and found that ammonolysis of the *N*-unprotected derivative [(±)-**56**] furnished the (*R*)-amide product **61** (T was not given) (entry 1). However, when the starting material was changed to the *N*-Cbz derivative [(±)-**57**], the (*R*)-hydrolytic product **60** was also isolated, and hydrolysis seemed to be the main reaction pathway (entry 2).

When 1 equiv. of H_2O was used instead of NH_3, a lower reaction rate and *E* were observed (entry 3). When, besides the H_2O, 1 equiv. of Et_3N was also added (instead of NH_3 to avoid amide formation), excellent *E* was achieved at high conversion (entry 4). This might be due to the fact that Et_3N removed the product by formation of its ammonium salt. Hydrolysis was attempted in an aqueous buffer as well (entry 5). A higher reaction rate, but lower *E* were observed.

The first aqueous resolution of aromatic *N*-unprotected β-amino esters was reported by Faulconbridge et al. (Table 5).[116] Amano lipase PS proved to be suitable for the hydrolytic reaction in a phosphate buffer (T was not given). The authors noted the important role of the pH on the *ee* of the product amino acid. In the case of (±)-β-phenylalanine ethyl ester [(±)-**62**], the *ee* of amino acid enantiomer **74** was 73% at pH 7, but 99% at pH 8 at the same conversion. Six different β-aryl-substituted β-amino esters [(±)-**62**–(±)-**67**] have been resolved on a preparative scale, giving good to high enantiomeric excesses (74-99%) for the unreacted ester (**68**–**73**) and product (**74**–**79**) enantiomers.

Table 5 Amano lipase PS-catalysed hydrolyses of β-aryl-β-amino esters in a phosphate buffer[a]



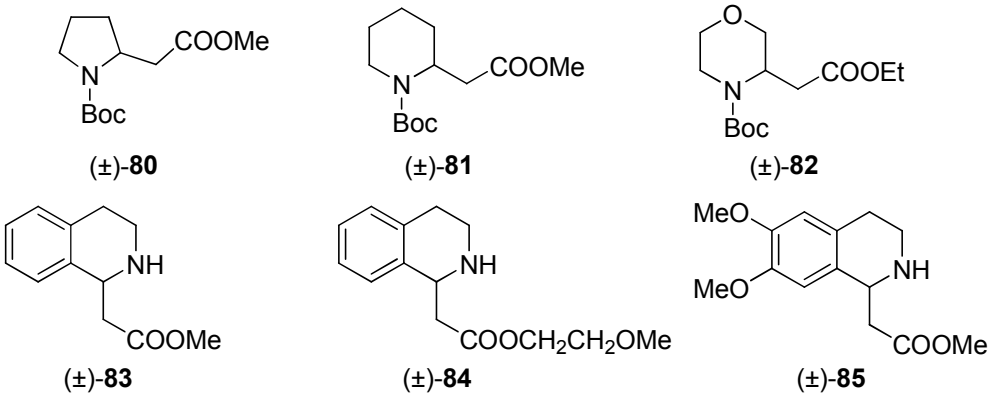
(±)-**62**–(±)-**67** **68**–**73** **74**–**79**

	R	*ee*$_s$ (%) (**68**–**73**)	*ee*$_p$ (%) (**74**–**79**)	Conv. (%)	*E*
(±)-**62**	Ph	98	99	50	> 200
(±)-**63**	2-Br-Ph	96	99	49	> 200
(±)-**64**	3-Br-Ph [b]	74	77	49	17
(±)-**65**	4-Br-Ph	99	99	50	> 200
(±)-**66**	4-F-Ph	90	91	50	65
(±)-**67**	1-Naphthyl	99	98	50	> 200

[a] The reaction time was given only for (±)-**62**: 15 h. [b] Reaction performed on the HCl salt.

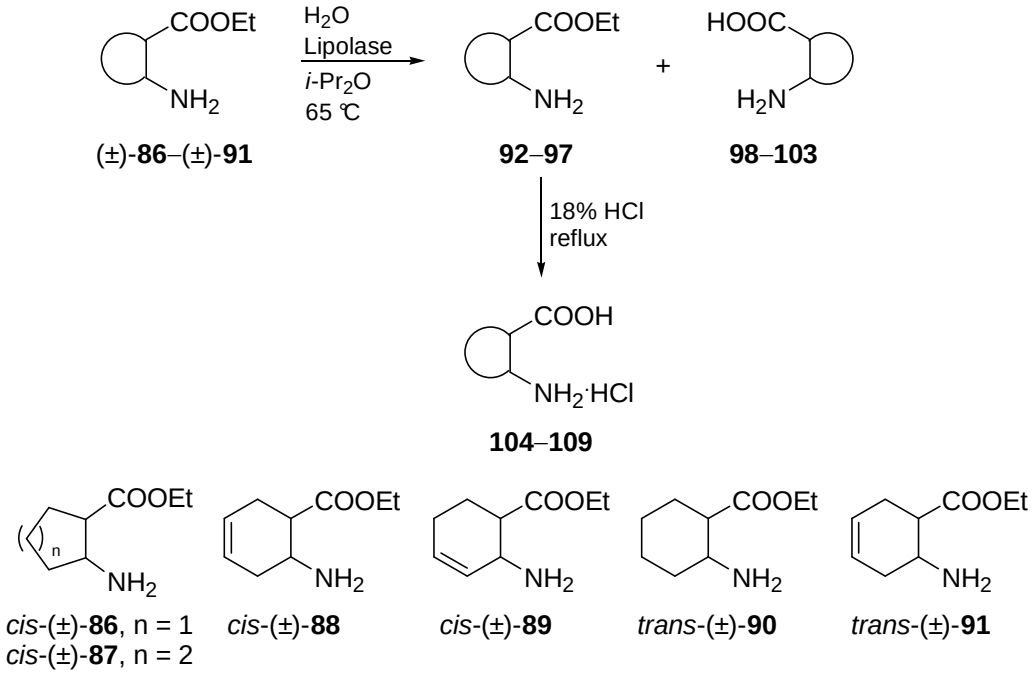
Further methods have been developed for the production of β-phenylalanine enantiomers, including microbial[117] and enzymatic[118] processes, and reactions in a biphasic system.[119]

Heterocyclic β-amino esters[120,121] and alicyclic β-amino esters[122] have also proved to be suitable substrates for lipases. A very efficient process (E > 200) has been developed for the kinetic resolution of *N*-Boc-protected homoproline [(±)-**80**], homopipecolic acid [(±)-**81**] and 3-carboxymethyl-morpholine esters [(±)-**82**], using BCL in a buffer at room temperature (Scheme 4).[120] Later, tetrahydroisoquinoline-1-acetic acid esters [(±)-**83**–(±)-**85**] were subjected to hydrolysis with lipase PS-D.[121] The effects of the solvents were examined and *i*-Pr_2O was found to be the most appropriate in the presence of 2-4 equiv. of H_2O at room temperature (E > 200). Hydrolysis in a buffer was not enantioselective. Among the esters studied, the 2-methoxyethyl derivative [(±)-**84**] exhibited an improved reactivity relative to the ethyl ester.



Scheme 4 Structures of resolved cyclic secondary β-amino esters

The first new direct enzymatic method for the enantioselective (E usually > 100) hydrolyses of *cis* [(±)-**86**–(±)-**89**] and *trans* [(±)-**90** and (±)-**91**] carbocyclic β-amino esters in an organic solvent was reported by Forró and Fülöp (Scheme 5, Table 6).[122] *i*-Pr_2O proved to be the most suitable solvent at 65 °C when Lipolase was used, resulting in the product β-amino acids (**98**–**103**) in high *ee* (96-99%) and in good yields (42-48%). The unreacted ester enantiomers (**92**–**97**) were immediately hydrolysed to the corresponding β-amino acid hydrochlorides (**104**–**109**). It should be mentioned that the selectivity of Lipolase for the hydrolysis of carbocyclic β-amino esters was opposite to that for the Lipolase-catalysed ring opening of carbocyclic β-lactams.[123]



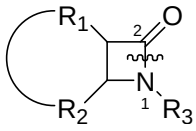
Scheme 5 Resolution of carbocyclic β-amino esters

Table 6 Lipolase-catalysed hydrolyses of carbocyclic β-amino esters in an organic solvent

Substrate	Conv. (%)	*E*	β-Amino acid·HCl (**104**–**109**)			β-Amino acid (**98**–**103**)		
			Yield (%)	Abs. conf.	*ee* (%)	Yield (%)	Abs. conf.	*ee* (%)
cis-(±)-**86**	48	74	42	1*R*,2*S*	94	42	1*S*,2*R*	96
cis-(±)-**87**	49	> 200	46	1*R*,2*S*	99	47	1*S*,2*R*	98
cis-(±)-**88**	50	110	45	1*R*,2*S*	98	46	1*S*,2*R*	98
cis-(±)-**89**	49	133	45	1*R*,2*S*	98	46	1*S*,2*R*	99
trans-(±)-**90**	49	> 200	46	1*S*,2*S*	99	48	1*R*,2*R*	99
trans-(±)-**91**	50	183	44	1*S*,2*S*	99	45	1*R*,2*R*	99

2.2.4. Enantioselective ring opening of β-lactams

β-Lactamases (EC 3.5.2.6) (class C) are bacterial enzymes which are able to catalyse the cleavage of the N_1-C_2 bond of β-lactams, *e.g.* β-lactam antibiotics, causing bacterial resistance (Scheme 6).[124]



Scheme 6 The N_1-C_2 bond cleavage of β-lactams by β-lactamases from class C

Until the past few years, no general method had been available for the ring opening of unactivated β-lactams through the use of commercially available isolated enzymes. Since 2003, numerous papers have been published on the enantioselective ring opening of unactivated carbocyclic β-lactams. The results on the enzyme-catalysed ring opening of β-lactams have been reviewed, and will not be mentioned in detail here.[40,125]

3. Materials and methods

3.1. *Materials*

The enzymes used in this work were commercially available. The CAL-B preparations, *i.e.* Lipolase (Sigma-Aldrich), Chyrazyme L-2 (Roche Diagnostics Corp.), Novozym 435 (Novozyme) and lipase PS IM (Amano), were originally immobilized. PPL, lipases PS and lipase AK (all from Sigma-Aldrich) (5 g) were dissolved in Tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g)[126] before use. The solvents and the chemicals were of the highest analytical grade.

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. The results of elemental analyses (CHNS) corresponded closely (within ± 0.3%) with the calculated ones in all cases.

3.2. *Enzymatic experiments*

Each enzymatic project started with determination of the optimum conditions of the reaction with the use of a model compound on a small scale. As a first attempt, the enzymes mentioned above were screened to identify the best catalyst. To determine the further reaction conditions with the best enzyme, the effects of solvents, temperature, additives and the amount of the enzyme were usually examined. In a small-scale experiment, the enzyme tested (10, 20, 30, 40, 50 or 75 mg mL^{-1}) was added to a racemic compound (0.05 M solution) in an organic solvent (1 mL), followed by additive (0, 0.5, 1 or 5 equiv.). The mixture was shaken at 25, 30, 40, 45, 50 or 60 °C.

After the preliminary experiments, preparative-scale reactions were performed. The unreacted and product enantiomers and the enzyme could be easily separated by filtration and extraction. The resulting enantiomers were characterized by NMR, elemental analysis, melting point and specific rotation. Finally, the absolute configurations were proved by comparing the specific rotations of the enantiomers or their derivatives with the literature data. When specific rotations were not available, the

analysed chromatograms or the comparative specific rotations indicated the enantiopreference of the enzyme.

3.3. *Analytical methods*

To follow the progress of the reactions, an appropriate analytical method had to be developed for all the compounds prepared. The sample from the enzymatic mixture was injected directly or after derivatization into the chiral GC or HPLC column. From the analysed chromatograms, the *ee*, conversion and *E* were calculated via the following equations:

$$ee_s = (A_2 - A_1)/(A_1 + A_2)$$

$$ee_p = (A_3 - A_4)/(A_3 + A_4)$$

$$\text{Conv.} = ee_s/ee_s + ee_p$$

$$E = \{\ln[(1-ee_s)/(1+ee_s/ee_p)]\}/\{\ln[(1+ee_s)/(1+ee_s/ee_p)]\},$$

where A_1, A_2, A_3 and A_4 are the areas of peaks and $A_2 > A_1$, $A_3 > A_4$.

The *ee* values for 4-aryl-substituted β-lactams **121a-g** were determined by GC on a Chrompack Chiralsil-Dex CB column, and the *ee* values for 4-arylalkyl-substituted β-lactams **121h,i** on a Chirasil-L-Val column, the samples being injected directly into the column.

The *ee* values for β-aryl-β-amino esters **68** and **124b-e** and for β-heteroaryl-β-amino esters **124h,j** were determined by GC on a Chirasil-L-Val column after derivatization with an appropriate acid anhydride in the presence of pyridine containing 10% DMAP.

The *ee* values for β-arylalkyl-β-amino esters **124m-o** and for β-heteroaryl-β-amino ester **124k** were determined by HPLC on a Chiralpak IA column. In the cases of **124m-o**, samples were injected directly into the column, while **124k** was pre-column hydrolysed with aq. HCl and then derivatized with CH_2N_2. The *ee* values for β-heteroaryl-β-amino esters **124f,g,i,l** were determined by HPLC on a Chirobiotic TAG column after hydrolysis with aq. HCl to the corresponding β-amino acid hydrochlorides.

The *ee* values for β-aryl-β-amino acids **122a-g**, **74** and **125b-e** and for β-heteroaryl-β-amino acids **125h,j** were determined by GC on a Chirasil-L-Val column

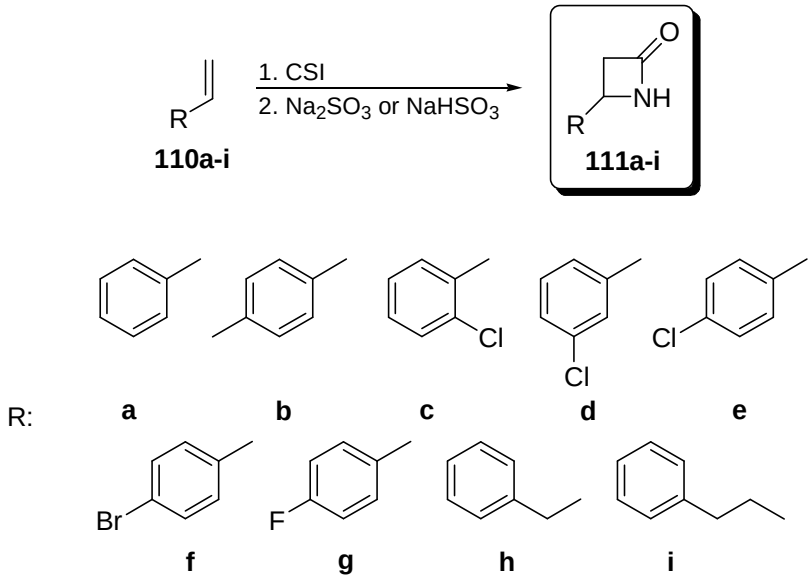
after double derivatization with CH_2N_2 and an appropriate acid anhydride in the presence of pyridine containing 10% DMAP.

The *ee* values for β-heteroaryl-β-amino acids **125f,g,i,l** and for β-arylalkyl-β-amino acid **125m** were determined by HPLC on a Chirobiotic TAG column, the samples being injected directly into the column, while the *ee* values for β-heteroaryl-β-amino acid **125k** and for β-arylalkyl-β-amino acid **125o** were determined on a Chiralpak IA column after derivatization with CH_2N_2. The *ee* values for β-arylalkyl-β-amino acids **122h,i** and **125n** were determined on an APEX Octadecyl column after derivatization with (*S*)-NIFE.

The exact conditions and retention times for the GC and HPLC analyses can be found in the original papers.[I-V]

3.4. *Syntheses of racemic starting materials*

(±)-4-Phenyl-2-azetidinone (**111a**), (±)-4-(*p*-tolyl)-2-azetidinone (**111b**), 4-benzyl-2-azetidinone (**111h**) and 4-phenylethyl-2-azetidinone (**111i**) were prepared from styrene (**110a**),[127] 4-methylstyrene (**110b**),[127] allylbenzene (**110h**)[128] and 4-phenyl-1-butene (**110i**)[129] by the addition of CSI, according to literature methods (Scheme 7).



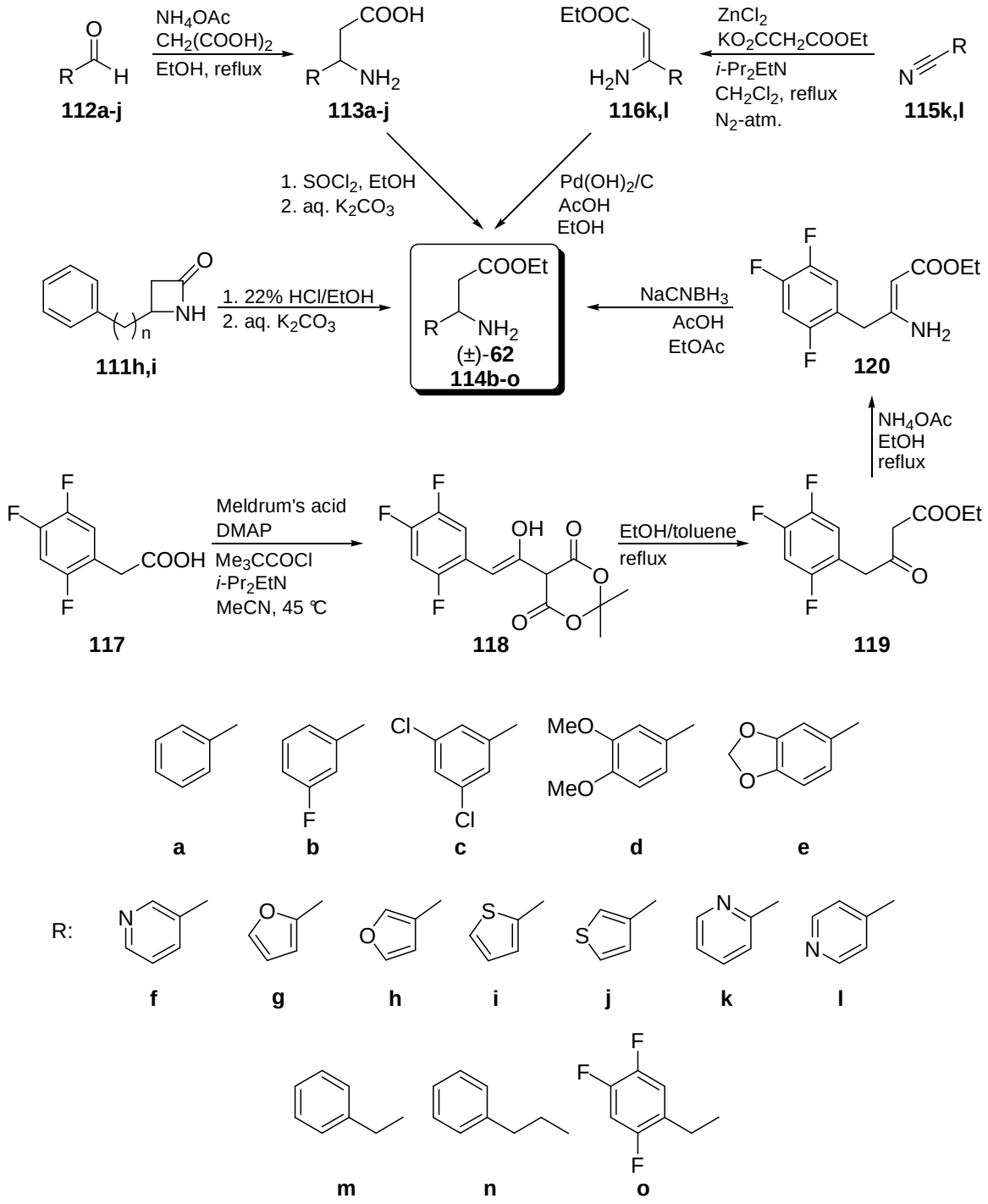
Scheme 7 Syntheses of racemic β-lactams **111a-i**

β-Lactams (±)-4-(2-chlorophenyl)-2-azetidinone (**111c**), (±)-4-(3-chlorophenyl)-2-azetidinone (**111d**), (±)-4-(4-chlorophenyl)-2-azetidinone (**111e**), (±)-4-(4-bromophenyl)-2-azetidinone (**111f**) and (±)-4-(4-fluorophenyl)-2-azetidinone (**111g**) were also prepared by CSI addition to the corresponding alkenes **110c-g**, but in a slightly modified literature procedure:[127] alkenes **110c-g** were added to a solution of CSI in absolute toluene at 0 °C instead of CH_2Cl_2 at room temperature.

(±)-3-Amino-3-phenylpropanoic acid (**113a**), (±)-3-amino-3-(3-fluorophenyl)propanoic acid (**113b**), (±)-3-amino-3-(3,5-dichlorophenyl)propanoic acid (**113c**), (±)-3-amino-3-(3,4-dimethoxyphenyl)propanoic acid (**113d**), (±)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoic acid (**113e**), (±)-3-amino-3-(3-pyridyl)propanoic acid (**113f**), (±)-3-amino-3-(2-furyl)propanoic acid (**113g**), (±)-3-amino-3-(3-furyl)propionic acid (**113h**), (±)-3-amino-3-(2-thienyl)propanoic acid (**113i**) and (±)-3-amino-3-(3-thienyl)propanoic acid (**113j**) were synthetized by a modified Rodionov synthesis from the corresponding aldehydes (**112a-j**) through condensation with malonic acid in the presence of NH_4OAc in EtOH under reflux (Scheme 8).[130]

Racemic β-amino ester hydrochlorides (±)-**62**·HCl and **114b-j**·HCl were prepared by the esterification of β-amino acids **113a-j** in the presence of $SOCl_2$ in EtOH. The free β-amino ester bases (±)-**62** and **114b-j** were liberated by the treatment of (±)-**62**·HCl and **114b-j**·HCl with aqueous K_2CO_3.

Ethyl (±)-3-amino-3-(2-pyridyl)propionate (**114k**) and ethyl (±)-3-amino-3-(4-pyridyl)propionate (**114l**) were prepared by the decarboxylative Blaise reactions[131] of 2-cyanopyridine (**115k**) and 4-cyanopyridine **(115l**), following $Pd(OH)_2$-catalysed reduction of enamines **116k,l** (Scheme 8).



Scheme 8 Syntheses of racemic β-amino esters (±)-**62** and **114b-o**

Ethyl (±)-3-amino-4-phenylbutanoate (**114m**) and ethyl (±)-3-amino-5-phenylpentanoate (**114n**) were prepared by the ring opening of **111h,i** with 22% HCl/EtOH, followed by treatment with aqueous K_2CO_3 (Scheme 8).

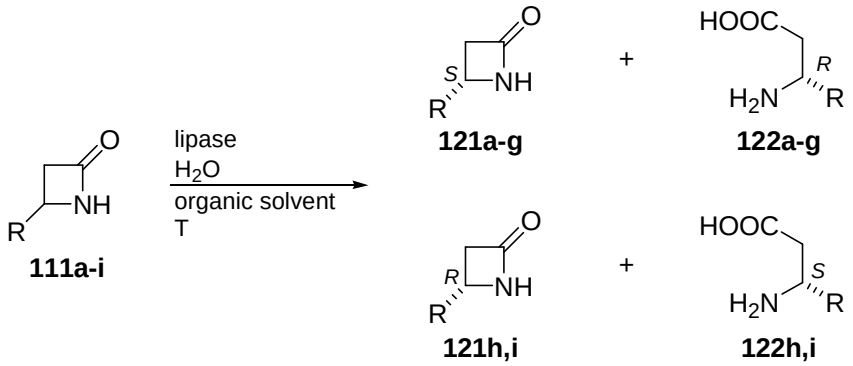
Starting from 2,4,5-trifluorophenylacetic acid (**117**), enamine **120** was prepared through the intermediates **118** and **119** by a slightly modified literature method:[28] during the transformation of **118**, an EtOH/toluene mixture was used instead of a mixture of MeOH and toluene, affording ethyl 3-oxo-4-(2,4,5-trifluorophenyl)butanoic acid (**119**). Ethyl (±)-3-amino-4-(2,4,5-trifluorophenyl)butanoate (**114o**) was obtained by the reduction of enamine **120** with $NaCNBH_3$ in the presence of AcOH (Scheme 8).

4. Results and discussion

4.1. *Enzymatic ring opening of β-lactams*[I,II,VII]

CAL-B-catalysed kinetic resolution has been exploited previously for the alcoholysis of (±)-7-azabicyclo[4.2.0]oct-4-en-8-one, (±)-7-azabicyclo[4.2.0]oct-3-en-8-one, (±)-4-phenyl-2-azetidinone and (±)-4-(*p*-tolyl)-2-azetidinone.[132] The preparative-scale reactions were performed in *i*-Pr_2O at 60 °C with 2-octanol as a nucleophile, yielding the unreacted β-lactams in high enantiomeric excesses (*ee* ≥ 96%) and in high yields (39-46%). However, the product β-amino acid octyl esters were not isolated, because they further polymerized or hydrolysed to the β-amino acids, which were isolated with high optical purity (*ee* ≥ 96%), but in low yields (7-11%). The first direct, highly enantioselective (*E* > 200) lipase-catalysed hydrolytic ring opening of unactivated carbocyclic β-lactams in an organic medium was published in 2003.[123] When CAL-B was used in *i*-Pr_2O at 60 °C with H_2O as nucleophile, the unreacted β-lactams were obtained in high enantiomeric excesses (*ee* ≥ 93%) and good yields (36-45%). The product β-amino acids, cispentacin and its 6-, 7- and 8-membered homologues, were also isolated in high enantiomeric excesses (*ee* ≥ 95%) and good yields (43-47%). An advantage of this method was that it was not necessary to activate the lactam ring, and the unreacted and product enantiomers could be easily separated and obtained in good yields. This method was then extended to the synthesis of ethyl- and ethylene-bridged cispentacins,[133] to the resolution of unsaturated[134] and *trans*[135] carbocyclic β-lactams and to the synthesis of benzocispentacin and its 6- and 7-membered homologues.[136]

After the successful application of the above method to carbocyclic β-lactams, 4-aryl- and 4-arylalkyl-substituted β-lactams **111a-i** were prepared and lipase-catalysed ring opening was attempted (Scheme 9).



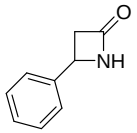
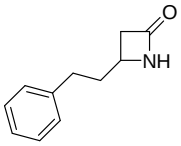
Scheme 9 Lipase-catalysed ring opening of **111a-i** (for the meanings of letters **a-i**, see Scheme 7)

4.1.1. Small-scale resolutions

Our model compounds for the preliminary experiments were 4-phenyl-2-azetidinone (**111a**) and 4-phenylethyl-2-azetidinone (**111i**).

Earlier results[123-136] on the lipase-catalysed hydrolysis of carbocyclic β-lactams suggested preliminary experiments with an enzyme screening using *i*-Pr_2O as solvent and 0.5 or 1 equiv. of H_2O as nucleophile. The temperature of the reactions was 45 °C for CAL-A, lipase AK, lipase AY and PPL, and 60 °C for Lipolase, Novozym 435 and Chyrazyme L-2 (all CAL-B preparations). CAL-A, lipase AK, lipase AY and PPL showed very low activity and selectivity in *i*-Pr_2O with H_2O as nucleophile (conv. ≤ 3%, $E \leq 2$). Lipolase, Novozym 435 and Chyrazyme L-2 directed the ring opening of 4-phenyl-2-azetidinone (**111a**) with excellent E (> 200) at conversions of 24-28% (Table 7, entries 1-3). 4-Phenylethyl-2-azetidinone (**111i**) was not a suitable substrate for CAL-B preparations, as very low E values (~ 11) were observed at conversions of 19-22% (Table 8, entries 4-6). Earlier, low E values were reported when 4-benzyl-2-azetidinone (**111h**) was subjected to CAL-B-catalysed alcoholysis and hydrolysis on a small scale.[128] However, those authors did not attempt to optimize the reaction conditions, and the enantiomers were prepared through the esterification and hydrolysis of racemic *N*-hydroxymethyl- and *N*-alkoxymethyl-4-benzyl-2-azetidinone.

Table 7 Conversions and enantioselectivities of the ring openings of **111a,i**[a]

Entry	Substrate	Enzyme	Enzyme (mg mL^{-1})	t (h)	ee_s[b] (%)	ee_p[c] (%)	Conv. (%)	*E*
1		Lipolase	50	1	31	> 99	24	> 200
2		Novozym 435	50	1	33	> 99	25	> 200
3		Chyrazyme L-2	50	1	38	> 99	28	> 200
4		Lipolase	30	2	19	81	19	11
5		Novozym 435	30	2	22	80	22	11
6		Chyrazyme L-2	30	2	21	80	21	11

[a] 0.05 M substrate, 1 mL *i*-Pr_2O, 1 equiv. H_2O for **111a**; 0.5 equiv. H_2O for **111i**, 60 °C.
[b] According to GC. [c] According to GC in the case of **111a**, and to HPLC in the case of **111i**.

It is known that the temperature can influence the *E* and the reaction rate of a lipase-catalysed reaction.[137-139] To achieve a higher *E* value in the hydrolysis of 4-phenylethyl-2-azetidinone (**111i**), we decreased the temperature to 50, 45, 40 and 30 °C. The reaction rate decreased, while *E* did not change (Table 8). Finally, 45 °C was chosen as the optimum temperature (entry 3).

Table 8 Effect of temperature on the ring opening of **111i**[a]

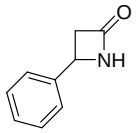
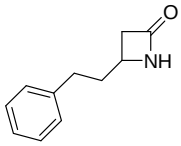
Entry	T (°C)	t	ee_s (%)[b]	ee_p (%)[c]	Conv. (%)	*E*
1	60	7 h	46	67	41	8
2	50	7 h	24	74	24	8
3	45	7 h	18	74	20	8
4	40	7 h	13	71	15	7
5	30	5 d	64	42	60	5

[a] 0.05 M substrate, 1 mL *i*-Pr_2O, 30 mg mL^{-1} Lipolase, 0.5 equiv. H_2O.
[b] According to GC. [c] According to HPLC.

As mentioned above, the nature of the solvent can strongly influence the *E* value.[53] Accordingly, the effects of different solvents on the reaction rate and *E* of the ring opening of 4-phenyl-2-azetidinone (**111a**) and 4-phenylethyl-2-azetidinone (**111i**) were examined (Table 9). Very low or no conversion (1-2%, after 24 h) was observed for both the aryl- and arylalkyl-substituted substrates **111a,i** in Me_2CO, THF and $CHCl_3$ (data not shown). *i*-Pr_2O and toluene proved to be suitable reaction media for the enantioselective ring opening of 4-phenyl-2-azetidinone (**111a**), but the conversion was much lower in toluene than in *i*-Pr_2O (entries 1 and 2). The highest reaction rates in the

Lipolase-catalysed ring opening of 4-phenylethyl-2-azetidinone (**111i**) were observed in *i*-Pr_2O and toluene, but *t*-BuOMe, Et_2O and *n*-hexane also proved to be acceptable reaction media for the ring opening of **111i**. However, low *E* values (5-8) were observed in all the tested solvents (entries 3-7). Further experiments on **111a,i** were performed in *i*-Pr_2O.

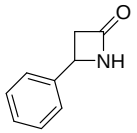
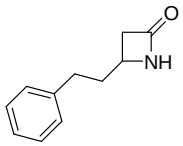
Table 9 Effects of solvents on the ring opening of **111a,i**[a]

Entry	Substrate	Solvent (1 mL)	T (°C)	t (h)	ee_s[b] (%)	ee_p[c] (%)	Conv. (%)	*E*
1		*i*-Pr_2O	60	2	64	> 99	39	> 200
2		toluene	60	2	22	> 99	18	> 200
3		*i*-Pr_2O	45	24	46	67	41	8
4		*t*-BuOMe	45	24	24	74	24	8
5		Et_2O	45	24	18	74	20	8
6		*n*-hexane	45	24	13	71	15	7
7		toluene	45	24	64	42	60	5

[a] 0.05 M substrate, 50 mg mL^{-1} Chyrazyme L-2 and 1 equiv. H_2O for **111a**; 50 mg mL^{-1} Lipolase and 0.5 equiv. H_2O for **111i**. [b] According to GC. [c] According to GC in the case of **111a**, and to HPLC in the case of **111i**.

Certain additives are known to exert beneficial effects on the reaction rate and/or the *E* of lipase-catalysed reactions.[140,141] Thus, 1 equiv. of Et_3N, *i*-Pr_2EtN or 2-octanol was added to the reaction mixture. Unfortunately, the hydrolysis of 4-phenyl- (**111a**) or 4-phenylethyl-substituted (**111i**) β-lactams was not affected by the additives: no enhancement in the reaction rate or *E* was observed (Table 10, entries 2-4 and 6-8). Moreover, the hydrolysis was complete even without added H_2O (entries 1 and 5). This effect was demonstrated in earlier studies on the lipase-catalysed ring opening of β-lactams.[123,136] The H_2O present in the reaction medium (< 0.1%) or on the surface of the enzyme preparation (< 5% w/w H_2O) was sufficient for the lactam ring opening.

Table 10 Effects of additives on the ring opening of **111a,i**[a]

Entry	Substrate	Additives (1 equiv.)	t (h)	ee_s[b] (%)	ee_p[c] (%)	Conv. (%)	*E*
1		-	1	35	> 99	26	> 200
2		Et_3N	1	35	> 99	26	> 200
3		*i*-Pr_2EtN	1	37	> 99	27	> 200
4		2-octanol	1	33	> 99	25	> 200
5		-	24	79	61	56	10
6		Et_3N	24	87	55	61	9
7		*i*-Pr_2EtN	24	81	63	56	11
8		2-octanol	24	74	64	54	10

[a] 0.05 M substrate, 1 mL *i*-Pr_2O, 50 mg mL^{-1} Chyrazyme L-2 and 60 °C for **111a**; 50 mg mL^{-1} Lipolase and 45 °C for **111i**. [b] According to GC. [c] According to GC in the case of **111a**, and to HPLC in the case of **111i**.

The reaction rate of the ring opening of 4-phenyl-2-azetidinone (**111a**) clearly increased as the amount of Lipolase was increased (Table 11). The reaction rate was lowest in the presence of 10 mg mL^{-1} enzyme and it needed 41 h to reach 49% conversion (entry 1). Although the optimum enzyme quantity proved to be 75 mg mL^{-1} (entry 6), preparative-scale reactions were performed with 30 mg mL^{-1} Lipolase for economic reasons (entry 3).

Comparison of Table 7 entry 4 with Table 11 entry 3 reveals that the reaction rate of the ring opening of 4-phenylethyl-2-azetidinone (**111i**) is lower (Conv. = 19% after 2 h) than that of 4-phenyl-2-azetidinone (**111a**) (Conv. = 17% after 1h).

Table 11 Effect of the enzyme amount on the ring opening of **111a**[a]

Entry	Lipolase (mg mL^{-1})	ee_s (%)	ee_p (%)	Conv. (%)	*E*
1	10	9	> 99	8 (49 after 41 h)	> 200
2	20	16	> 99	14	> 200
3	30	20	> 99	17	> 200
4	40	28	> 99	22	> 200
5	50	31	> 99	24	> 200
6	75	47	> 99	32	> 200

[a] 0.05 M substrate, 1 mL *i*-Pr_2O, 1 equiv. H_2O, 60 °C after 1 h.

Since *N*-Boc-protected cyclic β-lactams have been opened selectively,[142] we synthetized *N*-Boc-4-phenylethyl-2-azetidinone (*N*-Boc-**111i**) and attempted Lipolase

(50 mg/mL)-catalysed ring opening at 45 ºC. Unfortunately, we did not achieve a better result (conv. = 94% after 24 h, E = 3).

In cooperation with the Budapest University of Technology and Economics, the Lipolase-catalysed ring opening of 4-phenyl-2-azetidinone (**111a**) was investigated in $scCO_2$. Reaction kinetics, pressure and temperature effects were studied in detail. The optimum pressure and temperature for the β-lactam ring-opening reaction proved to be 14 MPa and 70 °C. The resulting β-amino acid **122a** (*ee* ≥ 98%) and β-lactam **121a** (*ee* > 99%) could be easily separated after 120 h by $scCO_2$ extraction of the (*S*)-β-lactam and subsequent washing of the enzyme with hot H_2O to recover the amino acid. The results are under publication.[VII]

4.1.2. Preparative-scale resolutions

Further 4-aryl-substituted β-lactams (**111b-g**) were also subjected to enzymatic ring opening in *i*-Pr_2O as solvent in the presence of 30 mg mL^{-1} Lipolase with 1 equiv. of H_2O as nucleophile at 60 ºC. Excellent E values (> 200) and reaction rates were observed. Preparative-scale resolutions of β-lactams **111a-g** were performed. After a H_2O/organic solvent extraction, the unreacted β-lactam (**121a-g**) and the product β-amino acid (**122a-g**) enantiomers were isolated in good yields (41-49%) and with high enantiomeric excesses (≥ 95%) (Table 12).

Table 12 Preparative-scale resolutions of β-lactams **111a-g**[a]

				β-Lactam (**121a-g**)				β-Amino acid (**122a-g**)			
	t (h)	Conv. (%)	E	Yield (%)	Abs. conf.	*ee*[b] (%)	$[\alpha]_D^{25}$ (EtOH)	Yield (%)	Abs. conf.	*ee*[b] (%)	$[\alpha]_D^{25}$ (H_2O)
111a	40	50	> 200	46	*S*	> 99	−137 (*c* 0.28)	47	*R*	> 99	+7 (*c* 0.17)
111b	60	49	> 200	45	*S*	95	−113 (*c* 0.26)	42	*R*	99	+8 (*c* 0.20)
111c	47	50	> 200	48	*S*	99	−271 (*c* 0.47)	47	*R*	99	+30.3 (*c* 0.43)
111d	60	50	> 200	46	*S*	99	−118 (*c* 0.46)	46	*R*	99	+5 (*c* 0.51)
111e	42	50	> 200	46	*S*	99	−110 (*c* 0.49)	48	*R*	99	+16.3 (*c* 0.33)
111f	67	49	> 200	41	*S*	96	−73 (*c* 0.16)	47	*R*	98	+4 (*c* 0.10)
111g	24	50	> 200	49	*S*	99	−117 (*c* 0.45)	43	*R*	99	+4.9 (*c* 0.45)

[a] 30 mg mL^{-1} Lipolase in *i*-Pr_2O, 1 equiv. H_2O, 60 °C. [b] According to GC.

The Lipolase-catalysed ring opening of 4-benzyl-2-azetidinone (**111h**) also gave a low *E* value (= 11) in *i*-Pr_2O with 0.5 equiv. of H_2O at 45 ºC. In order to isolate the products in good enantiomeric excesses, the preparative-scale resolutions of 4-benzyl-2-azetidinone (**111h**) and 4-phenylethyl-2-azetidinone (**111i**) were performed in two consecutive steps. This method was earlier attempted in the case of piperidine hydroxy esters.[137] The preparative-scale resolutions were carried out by working up the reactions at about 25% conversion (ee_p ~ 60%), and then continuing the reactions with the unreacted substrates and fresh enzyme until about 85% conversion (ee_s ~ 90%). This method resulted in the unreacted β-lactam (**121h,i**) and the product β-amino acid (**122h,i**) enantiomers in good to excellent enantiomeric excesses (≥ 87%) (Table 13). The disadvantage of this procedure was the fact that the enantiomers were isolated in only moderate yields (27-36%).

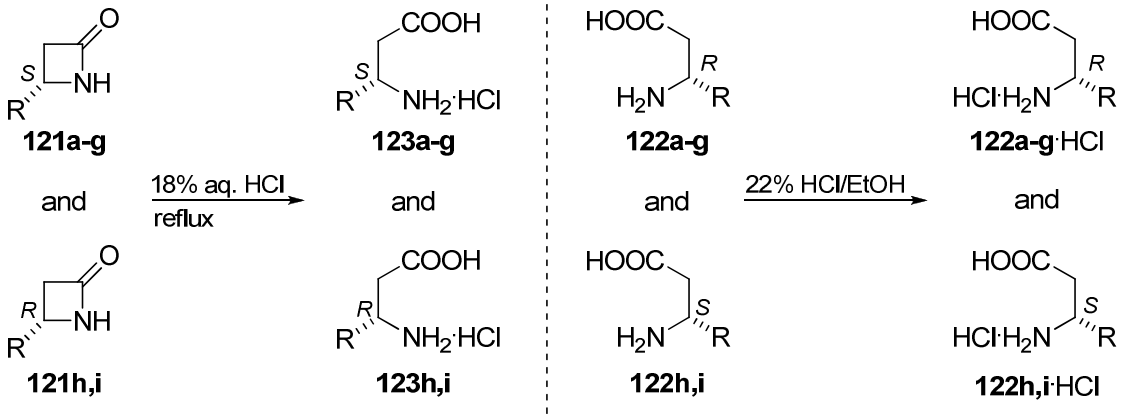
Table 13 Preparative-scale resolutions of β-lactams **111h,i**[a]

			β-Lactam (**121h,i**)				β-Amino acid (**122h,i**)			
	t (h)	Conv. at workup (%)	Yield (%)	Abs. conf.	*ee*[b] (%)	$[\alpha]_D^{25}$ ($CHCl_3$)	Yield (%)	Abs. conf.	*ee*[c] (%)	$[\alpha]_D^{25}$ (H_2O)
111h	13	24					27	*S*	89	+7 (*c* 0.20)
	88	81	36	*R*	> 99	+38.8 (*c* 0.65)				
111i	11	29					31	*S*	87	+24 (*c* 0.28)
	22	89	30	*R*	> 99	+19 (*c* 0.21)				

[a] 30 mg mL^{-1} Lipolase in *i*-Pr_2O, 0.5 equiv. H_2O, 45 °C. [b] According to GC. [c] According to HPLC.

4.1.3. Further transformations

Both the aryl- and arylalkyl-substituted β-lactam enantiomers were transformed into the corresponding β-amino acid hydrochlorides (**123a-i**) (Scheme 10). The ring opening reactions were carried out under reflux in 18% aqueous HCl. Treatment of the β-amino acid enantiomers **122a-i** with 22% HCl/EtOH resulted in the corresponding β-amino acid hydrochlorides **122a-i**·HCl (Scheme 10). These further transformations resulted in antipode enantiomer hydrochloride salts with opposite specific rotations (Table 14).



Scheme 10 Transformations of enantiomers **121a-i** and **122a-i** (for the meanings of letters **a-i**, see Scheme 7)

Table 14 Physical data on the prepared enantiomers **123a-i** and **122a-i**·HCl

	Substrate	β-Amino acid·HCl (**123a-i**)			β-Amino acid·HCl (**122a-i**·HCl)		
		Abs. conf.	*ee*[a] (%)	$[\alpha]_D^{25}$ (H_2O)	Abs. conf.	*ee*[a] (%)	$[\alpha]_D^{25}$ (H_2O)
Aryl	**a**	*S*	> 99	+3 (*c* 0.28)	*R*	> 99	−3 (*c* 0.30)
	b	*S*	95	+4 (*c* 0.28)	*R*	99	−4.5 (*c* 0.36)
	c	*S*	99	−8.6 (*c* 0.46)	*R*	99	+8.4 (*c* 0.47)
	d	*S*	99	+3.3 (*c* 0.35)	*R*	99	−3.2 (*c* 0.43)
	e	*S*	99	+5.3 (*c* 0.46)	*R*	99	−5.2 (*c* 0.46)
	f	*S*	96	+3.8 (*c* 0.45)	*R*	98	−4.1 (*c* 0.45)
	g	*S*	99	+1.5 (*c* 0.47)	*R*	99	−1.2 (*c* 0.37)
Aryl-alkyl	**h**	*R*	> 99	−8 (*c* 0.11)	*S*	89	+6 (*c* 0.21)
	i	*R*	> 99	−15 (*c* 0.21)	*S*	87	+12 (*c* 0.21)

[a] According to GC in the cases of **123a-g** and **122a-g**·HCl, and to HPLC in the cases of **123h,i** and **122h,i**·HCl.

4.1.4. Absolute configurations

Following the ring opening of β-lactams **111a,b,e,g-i**, the absolute configurations of the produced enantiomers were proved by comparing the specific rotations of the enantiomers with literature data, while for **111c,d,f** the analysed chromatograms indicated the same enantiopreference for Lipolase.

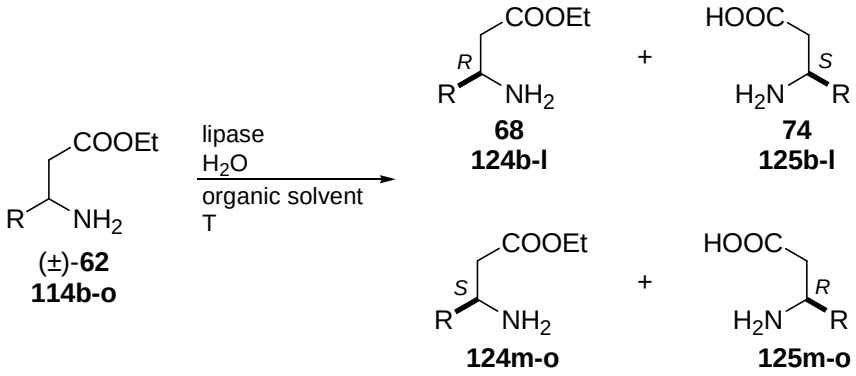
Thus, the absolute configurations indicated *R*-selectivity for the ring opening of β-aryl-β-lactams **111a-g**, and *S*-selectivity for β-arylalkyl-β-lactams **111h,i**. It should be mentioned that Lipolase did not display opposite selectivity for β-arylalkyl-β-lactams

111h,i, but the priority of the substituents varied according to the Cahn–Ingold–Prelog priority rules.

4.2. *Enzymatic hydrolyses of β-amino esters*[III-VI]

As detailed in the Literature section, the first direct lipase-catalysed enantioselective (*E* usually > 100) hydrolyses of carbocyclic β-amino esters were recently reported with Lipolase in *i*-Pr_2O at 65 °C (Scheme 5).[122] This method resulted in a new, simple approach for the preparation of carbocyclic β-amino acid enantiomers in high optical purity (≥ 94%) and good yields (≥ 42%).

In consequence of these findings,[122] racemic β-aryl- [(±)-**62** and **114b-e**], β-heteroaryl- (**114f-l**) and β-arylalkyl- (**114m-o**) substituted β-amino esters were synthetized and subjected to the lipase-catalysed hydrolytic reaction (Scheme 11).



Scheme 11 Lipase-catalysed hydrolyses of (±)-**62** and **114b-o** (for the meanings of letters **b-o**, see Scheme 8)

4.2.1. Small-scale resolutions

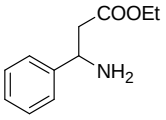
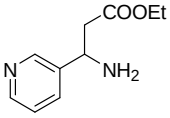
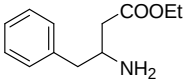
Our model compounds were ethyl 3-amino-3-phenylpropionate [(±)-**62**], ethyl 3-amino-3-(3-pyridyl)propionate (**114f**) and ethyl 3-amino-4-phenylbutanoate (**114m**).

As in the cases of the ring opening of the aryl- and arylalkyl-substituted β-lactams, we started our preliminary experiments with an enzyme test (Table 15). In contrast with the ring openings of β-lactams,[I,II] Lipolase did not exhibit any selectivity towards β-amino esters (±)-**62**, **114f,m** (data not shown). It should be mentioned that

carbocyclic β-lactams[123] and carbocyclic β-amino esters[122] were resolved with Lipolase. PPL (entries 1, 5 and 9) and lipase AK (entries 2, 6 and 10) were insufficient enzymes in the ring opening of aryl- and arylalkyl-substituted β-lactams, but exhibited low to moderate *E* (≤ 35) for β-aryl-, β-heteroaryl- and β-arylalkyl-substituted β-amino esters. Lipase PS preparations proved to be suitable enzymes for the enantioselective hydrolyses of ethyl 3-amino-3-phenylpropionate [(±)-**62**], ethyl 3-amino-3-(3-pyridyl)propionate (**114f**) and ethyl 3-amino-4-phenylbutanoate (**114m**) in *i*-Pr_2O [(±)-**62** and **114f**, entries 3 and 7] or in *t*-BuOMe (**114m**, entry 12) at 45 °C. However, the reaction time required to reach 50% conversion was several times longer for the arylalkyl-substituted model compound (**114m**, 72 h) than for the aryl- and heteroaryl-substituted substrates [(±)-**62**, 5 h, and **114f**, 17 h]. Decreasing the temperature to 25 °C did not influence the hydrolysis of 3-amino-3-phenylpropionate [(±)-**62**] (entry 4), but decreased the reaction rate of the hydrolysis of ethyl 3-amino-4-phenylbutanoate (**114m**) (entry 13). In the case of ethyl 3-amino-3-(3-pyridyl)propionate (**114f**), an enhancement of the *E* value was observed (entry 8). In view of these results, further experiments were performed at 45 °C with the β-aryl- and β-arylalkyl-substituted models [(±)-**62** and **114m**] and at 25 °C with the β-heteroaryl-substituted model (**114f**).

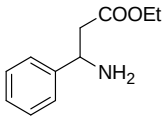
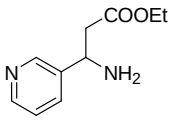
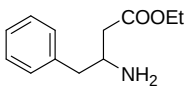
Next, the effects of the solvents on the reaction rate and *E* were investigated. *i*-Pr_2O, *t*-BuOMe, *n*-hexane and toluene proved to be suitable reaction media for the hydrolyses of the β-aryl- and β-heteroaryl-substituted substrates (±)-**62** and **114f** (Table 16, entries 1-4 and 5-8). Other solvents, such as THF, 1,4-dioxane, $CHCl_3$ or Me_2CO, gave low reaction rates or *E* values (data not shown). When β-arylalkyl-substituted substrate **114m** was hydrolysed with lipase PS IM, the highest *E* values were achieved in *i*-Pr_2O and *t*-BuOMe (entries 9 and 10), while in *n*-hexane and toluene the *E* value and/or the reaction rate was low (entries 11 and 12). Our earlier results on the vapour-assisted ring opening of carbocyclic β-lactams[143] prompted us to perform the hydrolysis of ethyl 3-amino-4-phenylbutanoate (**114m**) under solvent-free conditions. A higher conversion, but a lower *E* value was observed (entry 13), demonstrating the low applicability of this method for this substrate. As in the ring opening of 4-aryl- and 4-arylalkyl-substituted β-lactams,[I,II] ether-type solvents furnished high conversions and *E* values in the hydrolyses of β-amino esters, suggesting the use of *i*-Pr_2O and *t*-BuOMe in the further tests.

Table 15 Conversions and enantioselectivities of the hydrolyses of (±)-**62** and **114f,m**[a]

Entry	Substrate	Enzyme	*T* (ºC)	t (h)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1		PPL[c]	45	5	66	88	43	31
2		lipase AK[c]	45	5	67	89	43	35
3		lipase PS[c]	45	5	96	> 99	49	> 200
4		lipase PS[c]	25	6	92	> 99	49	> 200
5		PPL[c]	45	17	43	74	37	10
6		lipase AK[c]	45	17	70	8	90	2
7		lipase PS[c]	45	17	> 99	90	52	100
8		lipase PS[c]	25	17	> 99	98	50	> 200
9		PPL[c]	25	87	2	88	2	16
10		lipase AK[c]	25	87	19	88	18	19
11		lipase PS IM	45	26	37	96	28	70
12		lipase PS IM	45	72	99	96	51	> 200
13		lipase PS IM	25	26	14	96	13	56

[a] 0.05 M substrate, 1 mL *i*-Pr_2O for (±)-**62** and **114f**, 1 mL *t*-BuOMe for **114m**, 50 mg mL^{-1} enzyme for (±)-**62** and **114m**, 30 mg mL^{-1} enzyme for **114f**, 0.5 equiv. H_2O. [b] According to GC in the case of (±)-**62**, and to HPLC in the cases of **114f,m**. [c] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

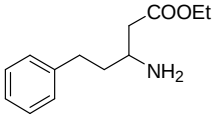
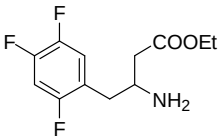
Table 16 Effects of solvents on the hydrolyses of (±)-**62** and **114f,m**[a]

Entry	Substrate	Solvent	t (h)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1		*i*-Pr_2O	5	96	> 99	49	> 200
2		*t*-BuOMe	5	92	> 99	48	> 200
3		*n*-hexane	5	98	> 99	50	> 200
4		toluene	5	60	> 99	38	> 200
5		*i*-Pr_2O	18	> 99	98	48	> 200
6		*t*-BuOMe	18	> 99	98	50	> 200
7		*n*-hexane	18	> 99	92	52	126
8		toluene	18	66	98	40	197
9		*i*-Pr_2O	35	60	96	38	90
10		*t*-BuOMe	35	50	96	34	81
11		*n*-hexane	35	50	89	36	28
12		toluene	35	5	82	6	11
13		solvent-free	35	74	66	53	11

[a] 0.05 M substrate, 1 mL solvent, 50 mg mL^{-1} lipase PS for (±)-**62**, 50 mg mL^{-1} lipase PS IM for **114m**, 30 mg mL^{-1} lipase PS for **114f**, 0.5 equiv. H_2O, 45 ºC for (±)-**62** and **114m**, 25 ºC for **114f**. [b] According to GC in the case of (±)-**62**, and to HPLC in the cases of **114f,m**.

It should be noted that ethyl (±)-3-amino-5-phenylpentanoate (**114n**) and ethyl (±)-3-amino-4-(2,4,5-trifluorophenyl)butanoate (**114o**) were also hydrolysed with 0.5 equiv. of H_2O in the presence of 50 mg mL^{-1} lipase PS IM in *t*-BuOMe or in *i*-Pr_2O at 45 °C. Higher reaction rates and better *E* values were obtained in *i*-Pr_2O than in *t*-BuOMe for both of these β-amino esters (Table 17). This observation confirms that solvents can have a strong influence on the reaction rate and the *E* value.

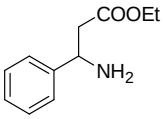
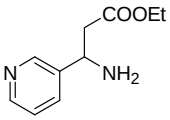
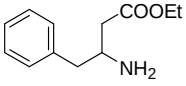
Table 17 Effects of solvents on the hydrolyses of **114n,o**[a]

Entry	Substrate	Solvent (1 mL)	t (h)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1		*i*-Pr_2O	43	79	96	45	119
2		*t*-BuOMe	43	54	91	37	36
3		*i*-Pr_2O	65	85	97	47	179
4		*t*-BuOMe	65	61	97	39	123

[a] 0.05 M substrate, 50 mg mL^{-1} lipase PS IM, 0.5 equiv. H_2O at 45 °C. [b] According to HPLC.

The presence of H_2O in the reaction medium affects the enzymatic activity.[144] Accordingly, the enzyme-catalysed hydrolyses of ethyl 3-amino-3-phenylpropionate [(±)-**62**], ethyl 3-amino-3-(3-pyridyl)propionate (**114f**) and ethyl 3-amino-4-phenylbutanoate (**114m**) were performed with increasing amounts of H_2O (0, 1, and 5 equiv.) (Table 18). In the cases of the β-aryl- and β-heteroaryl-substituted compounds (±)-**62** and **114f**, the reaction rate increased slightly with increasing amount of added H_2O (entries 1-3 and 4-6). However, in the case of ethyl 3-amino-3-(3-pyridyl)propionate (**114f**), the *E* value of the hydrolysis fell sharply, an effect which was not observed with ethyl 3-amino-3-phenylpropionate [(±)-**62**]. The increasing amount of the added H_2O slowed down the hydrolysis of β-arylalkyl-substituted compound **114m**, but did not affect the *E* value (entries 7-9). In this latter case, we persumed that the poorer solubility of **114m** in the presence of more H_2O could cause a decrease in the reraction rate. It should be mentioned that in a small-scale experiment we could perform the hydrolysis without any added H_2O (entries 1, 4 and 7). In good correlation with our results on the ring opening of β-lactams,[I,II] the H_2O present in the reaction medium (< 0.1%) or on the surface of the enzyme preparation (< 5% w/w H_2O) was responsible.

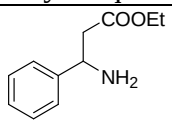
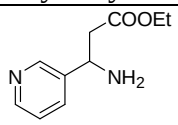
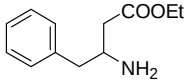
Table 18 Effects of added H_2O on the hydrolyses of (±)-**62** and **114f,m**[a]

Entry	Substrate	H_2O (equiv.)	t (h)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1		0	2	85	> 99	46	> 200
2		1	2	93	> 99	48	> 200
3		5	2	> 99	> 99	50	> 200
4		0	7	80	98	46	> 200
5		1	7	94	83	53	38
6		5	7	> 99	81	55	49
7		0	25	38	96	28	71
8		1	25	31	96	24	66
9		5	25	22	96	19	61

[a] 0.05 M substrate, 1 mL *i*-Pr_2O for (±)-**62** and **114f**, 1 mL *t*-BuOMe for **114m**, 50 mg mL^{-1} enzyme for (±)-**62** and **114m**, 30 mg mL^{-1} enzyme for **114f**, 45 °C for (±)-**62** and **114m**, 25 °C for **114f**. [b] According to GC in the case of (±)-**62**, and to HPLC in the cases of **114f,m**.

We performed the reactions with different concentrations of enzyme. For all of the model compounds (±)-**62**, **114f,m**, the reaction rate clearly increased as the quantity of the lipase was increased, while *E* did not change significantly (Table 19). The highest reaction rates were observed in the presence of 75 mg mL^{-1}, but in the case of ethyl 3-amino-4-phenylbutanoate (**114m**), the reaction rate was much lower relative to the conversions of the β-aryl- and β-heteroaryl-substituted substrates (±)-**62** and **114f** (entry 7). The hydrolyses of the β-aryl- and β-heteroaryl-β-amino esters were complete in 1 day, even in the presence of only 10 mg mL^{-1} enzyme (entry 2). For economic reasons, preparative-scale resolutions were performed with a 30 [for (±)-**62** and **114f**] or 50 (for **114m**) mg mL^{-1} lipase PS preparation. A similar correlation between the enzyme quantity and the conversion was noted in studies on the enzymatic ring opening of β-lactams.[I,II]

Table 19 Effects of the enzyme quantity on the hydrolyses of (±)-**62** and **114f,m**[a]

										
Entry	Enzyme (mg mL^{-1})	t (h)	Conv. (%)	*E*	t (h)	Conv. (%)	*E*	t (h)	Conv. (%)	*E*
1	10	1	17	> 200	3	16	118			
2	10	24	45	> 200	22	49	> 200			
3	20	1	27	> 200	3	24	134			
4	30	1	31	> 200	3	32	158	26	20	62
5	40	1	35	> 200	3	37	179			
6	50	1	38	> 200	3	42	> 200	26	28	70
7	75	1	45	> 200	3	49	> 200	26	33	79

[a] 0.05 M substrate, 1 mL *i*-Pr_2O for (±)-**62** and **114f**, 1 mL *t*-BuOMe for **114m**, lipase PS for (±)-**62** and **114f**, lipase PS IM for **114m**, 0.5 equiv. H_2O, 45 ºC for (±)-**62** and **114m**, 25 ºC for **114f**.

It can be clearly seen from the above results that the enantioselective enzyme-catalysed hydrolysis of ethyl 3-amino-4-phenylbutanoate (**114m**) was much slower than those of ethyl 3-amino-3-phenylpropionate [(±)-**62**] and ethyl 3-amino-3-(3-pyridyl)propionate (**114f**). A similar, but smaller difference in the reaction rates was observed when the enzymatic ring openings of 4-phenyl- and 4-phenylethyl-2-azetidinones (**111a,i**) were studied.[I,II]

Due to this fact, we attempted to improve the reaction rate of the hydrolysis of ethyl 3-amino-4-phenylbutanoate (**114m**) by the addition of 1 equiv. of Et_3N, *i*-Pr_2EtN or 2-octanol (Table 20) to the reaction mixture. Unfortunately, the conversions of the reactions with these additives (entries 2-4) relative to H_2O (entry 1) were not better.

Table 20 Effects of additives on the hydrolysis of ethyl 3-amino-4-phenylbutanoate (**114m**) [a]

Entry	Additive	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	H_2O	44	96	31	76
2	Et_3N	43	96	31	75
3	*i*-Pr_2EtN	41	96	30	73
4	2-octanol	39	96	29	72

[a] 0.05 M substrate, 1 mL *t*-BuOMe, 50 mg mL^{-1} lipase PS IM, 1 equiv. additive, 45 °C after 31 h. [b] According to HPLC.

4.2.2. Preparative-scale resolutions

Further β-aryl- (**114b-e**) and β-heteroaryl- (**114g-l**) substituted β-amino esters were hydrolysed with excellent *E* (> 200) under the optimum conditions, *i.e.* 30 mg mL^{-1} lipase PS with 0.5 equiv. of H_2O in *i*-Pr_2O at 45 °C (**114b-e**) or 25 °C (**114g-l**). The optimum conditions for the β-arylalkyl-substituted compounds **114m-o** were 50 mg mL^{-1} lipase PS IM with 0.5 equiv. of H_2O in *t*-BuOMe (**114m**) or in *i*-Pr_2O (**114n,o**) at 45 °C. Preparative-scale resolutions of all 15 racemic β-amino esters (±)-**62** and **114b-o** were performed. The reaction time to reach 50% conversion varied between 16 and 120 h. The lowest reaction rates were observed in the cases of ethyl 3-amino-4-phenylbutanoate (**124m**) and ethyl 3-amino-4-(2,4,5-trifluorophenyl)butanoate (**124o**) (120 h), and the highest one in the case of ethyl 3-amino-3-benzo[1,3]dioxol-5-ylpropanoate (**114e**). At close to 50% conversion, the reactions were stopped and the product β-aryl-β-amino acid (**74** and **125b-e**), β-heteroaryl-β-amino acid (**125f-l**) and β-arylalkyl-β-amino acid (**125m-o**) enantiomers were isolated with high enantiomeric excesses (≥ 96%) and in good yields (40-47%) (Table 21).

4.2.3. Further transformations

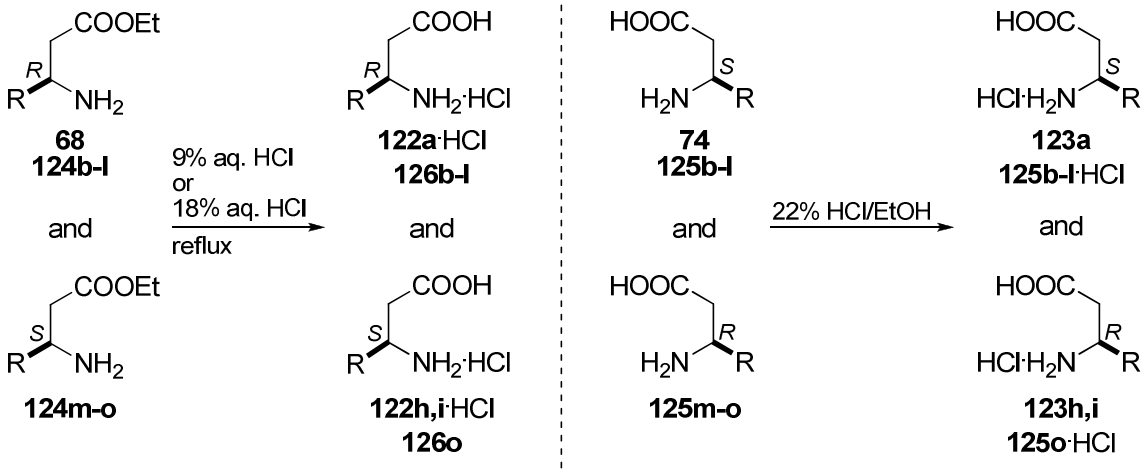
Because of the low stability of the unreacted β-amino ester enantiomers (**74** and **124b-o**) even at low temperature, β-amino acid hydrochlorides (**122a,h,i**·HCl and **126b-l,o**) were readily prepared by hydrolysis of the unreacted ester enantiomers with aqueous HCl, and were characterized with high *ee* (≥ 96%) and in good yields (40-49%) (Scheme 12, Table 21) (note: compounds **122a,h,i**·HCl were defined in Scheme 10). Treatment of β-amino acids **74** and **125b-o** with 22% HCl/EtOH resulted in the corresponding β-amino acid hydrochlorides **123a,h,i** and **125b-l,o**·HCl, antipodes of hydrochlorides **122a,h,i**·HCl and **126b-l,o** (Scheme 12, Table 22) (note: compounds **123a,h,i** were defined in Scheme 10).

Table 21 Preparative-scale resolutions of β-amino esters (±)-**62** and **114b-o**[a]

					β-Amino acid·HCl (**122a,h,i**·HCl and **126b-l,o**)				β-Amino acid (**74** and **125b-o**)			
		t (h)	Conv. (%)	*E*	Yield (%)	Abs. conf.	*ee*[b] (%)	$[\alpha]_D^{25}$ (H_2O)	Yield (%)	Abs. conf.	*ee*[b] (%)	$[\alpha]_D^{25}$ (H_2O)
	(±)-**62**	22	50	> 200	44	*R*	> 99	−4 (*c* 0.3)	44	*S*	> 99	−8 (*c* 0.27)
Aryl	**114b**	23	49	> 200	40	*R*	> 99	−6.5 (*c* 0.31)	40	*S*	> 99	−1.8 (*c* 0.38)
	114c	74	50	> 200	43	*R*	> 99	−5.1 (*c* 0.34)	44	*S*	> 99	−5.5 (*c* 0.38)
	114d	18	50	> 200	41	*R*	> 99	−7.9 (*c* 0.32)	41	*S*	> 99	+1.3 (*c* 0.51)
	114e	16	52	> 200	44	*R*	97	−8.4 (*c* 0.33)	46	*S*	> 99	+4 (*c* 0.3)
Heteroaryl	**114f**	40	50	> 200	40	*R*	> 99	+4.1 (*c* 0.33)	46	*S*	> 99	−5.1 (*c* 0.41)
	114g	60	49	> 200	44	*R*	97	+5.4 (*c* 0.32)	46	*S*	> 99	−5.8 (*c* 0.52)
	114h	47	50	> 200	49	*R*	> 99	+5.3 (*c* 0.42)	44	*S*	> 99	−6.7 (*c* 0.34)
	114i	60	50	> 200	46	*R*	> 99	+4.1 (*c* 0.33)	44	*S*	> 99	−3.1 (*c* 0.33)
	114j	42	50	> 200	46	*R*	> 99	+4.0 (*c* 0.34)	43	*S*	> 99	−3.2 (*c* 0.32)
	114k	67	50	> 200	45	*R*	98	+9.7 (*c* 0.32)	42	*S*	> 99	−18.2 (*c* 0.32)
	114l	24	50	> 200	43	*R*	97	+3.2 (*c* 0.36)	45	*S*	98	−11.7 (*c* 0.36)
Arylalkyl	**114m**	120	50	194	42	*S*	96	+4.6 (*c* 0.36)	45	*R*	96	−4.6 (*c* 0.30)
	114n	72	51	> 200	47	*S*	98	+11.5 (*c* 0.40)	47	*R*	96	−12.1 (*c* 0.34)
	114o	120	50	> 200	44	*S*	96	−9.8 (*c* 0.32)	43	*R*	97	+15.5 (*c* 0.35)

[a] 30 mg mL^{-1} lipase PS for (±)-**62** and **114b-l**, 50 mg mL^{-1} lipase PS IM for **114m-o**, *i*-Pr_2O for (±)-**62** and **114b-l,n,o**, *t*-BuOMe for **114m**, 0.5 equiv. H_2O, 45 °C for (±)-**62**, **114b-e,m-o**, 25 °C for **114f-l**. [b] According to GC in the cases of (±)-**62** and **114b-e,h,j**, and to HPLC in the cases of **114f,g,i,k-o**.

It should be mentioned that this method offers a better choice for the preparation of β-arylalkyl-substituted β-amino acid enantiomers as compared with the ring opening of the corresponding lactams.[II]



Scheme 12 Transformations of enantiomers **68**, **124b-o**, **74** and **125b-o** (for the meanings of letters **b-o**, see Scheme 8) (note: compounds **122a,h,i**·HCl and **123a,h,i** were defined on Scheme 10)

Table 22 Physical data on the prepared enantiomers (**123a,h,i** and **125b-l,o**·HCl)

	β-Amino acid·HCl	Abs. conf.	*ee*[a] (%)	$[\alpha]_D^{25}$ (H_2O)
Aryl	**123a**	*S*	> 99	+4 (*c* 0.30)
	125b·HCl	*S*	> 99	+5.7 (*c* 0.31)
	125c·HCl	*S*	> 99	+5.7 (*c* 0.34)
	125d·HCl	*S*	> 99	+7.2 (*c* 0.32)
	125e·HCl	*S*	> 99	+8.9 (*c* 0.33)
Heteroaryl	**125f**·HCl	*S*	> 99	−3.9 (*c* 0.33)
	125g·HCl	*S*	> 99	−4.9 (*c* 0.32)
	125h·HCl	*S*	> 99	−4.6 (*c* 0.42)
	125i·HCl	*S*	> 99	−3.1 (*c* 0.33)
	125j·HCl	*S*	> 99	−3.6 (*c* 0.34)
	125k·HCl	*S*	> 99	−8.6 (*c* 0.31)
	125l·HCl	*S*	98	−3.6 (*c* 0.35)
Arylalkyl	**123h**	*R*	96	−3.9 (*c* 0.35)
	123i	*R*	96	−10.5 (*c* 0.40)
	125o·HCl	*R*	97	+10.6 (*c* 0.31)

[a] According to GC in the cases of **123a** and **125b-e,h,j**·HCl, and to HPLC in the cases of **123h,i** and **125f,g,i,k,l,o**·HCl.

4.2.4. Absolute configurations

In the cases of the hydrolyses of β-amino esters (±)-**62** and **114d,e,g-j,m,n**, the absolute configurations of the produced enantiomers were proved by comparing the specific rotations of the enantiomers with literature data. For **114b,c**, the analysed chromatograms indicated the same enantiopreference for lipase PS, while for **114k,l**, the comparative specific rotations of the products (**125f-l** negative, **126f-l** positive) pointed to the same enantiopreference for lipase PS.

In the case of **114f**, the unreacted ester enantiomer **124f** was treated with 5% TFA/EtOH and the [α] value of **124f**·TFA was compared with literature data.

To prove the absolute configuration of the produced enantiomers in the hydrolysis of **114o**, *N*-Boc-**125o** was prepared,[145] which was known in the literature.

Thus, the absolute configurations indicated *S*-selectivity for the hydrolyses of β-aryl- and β-heteroaryl-β-amino esters (±)-**62** and **114b-l**, and *R*-selectivity for β-arylalkyl-β-amino esters **114m-o**. It should be mentioned that lipase PS IM did not show opposite selectivity for β-arylalkyl-β-amino esters **114m-o**, but the priority of the substituents varied according to the Cahn–Ingold–Prelog priority rules.

5. Summary

Racemic 4-aryl-substituted and 4-arylalkyl-substituted β-lactams were prepared by the addition of chlorosulphonyl isocyanate to the corresponding alkenes. β-Aryl-β-amino esters, β-heteroaryl-β-amino esters and β-arylalkyl-β-amino esters were synthetized by the modified Rodionov synthesis followed by esterification; or by the reduction of enamines; or by the ring opening of β-lactams with 22% HCl/EtOH under reflux.

We found that Lipolase catalysed the ring opening of 4-phenyl-2-azetidinone (**111a**) with excellent enantioselectivity (*E* > 200) in *i*-Pr_2O at 60 °C with 1 equiv. of H_2O. However, in the case of 4-phenylethyl-2-azetidinone (**111i**), a low *E* value (~ 11) was observed even at 45 °C. *i*-Pr_2O and toluene proved to be suitable solvents for **111a**, while the nature of the solvent did not influence the reaction rate or the *E* value for the ring opening of **111i**. Addition of Et_3N, *i*-Pr_2EtN or 2-octanol to the reaction mixtures did not significantly affect either the enantioselectivity or the reaction rate. The ring opening was complete even without the addition of H_2O. Lipolase-catalysed ring opening of *N*-Boc-4-phenylethyl-2-azetidinone gave a low *E* value (3) at 45 ºC. Lipolase directed the ring opening of 4-phenyl-2-azetidinone (**111a**) in $scCO_2$ at 14 MPa and 70 °C in 120 h with excellent *E* (> 200).

The Lipolase-catalysed preparative-scale resolutions of 4-aryl-substituted β-lactams (**111a-g**) were performed in *i*-Pr_2O with 1 equiv. of H_2O at 60 °C. The unreacted lactam and the product amino acid enantiomers were isolated by H_2O/organic solvent extraction in 41-49% yields and with *ee* ≥ 95%. The preparative-scale resolutions of 4-benzyl-2-azetidinone (**111h**) and 4-phenylethyl-2-azetidinone (**111i**) were performed in two steps, in *i*-Pr_2O with 0.5 equiv. of H_2O using Lipolase at 45 °C. β-Lactam enantiomers were isolated with *ee* > 99%, and β-amino acids with *ee* ≥ 87% in 27-36% yields. β-Lactams were refluxed with 18% aq. HCl and β-amino acids were treated with 22% HCl/EtOH, which afforded antipode β-amino acid hydrochlorides without a decrease in *ee* (≥ 87%).

In the hydrolyses of ethyl 3-amino-3-phenylpropionate [(±)-**62**], ethyl 3-amino-3-(3-pyridyl)propionate (**114f**) and ethyl 3-amino-4-phenylbutanoate (**114m**), lipase PS (from *Burkholderia cepacia*) gave excellent *E* values (> 200) in *i*-Pr_2O or *t*-BuOMe with

0.5 equiv. of H_2O at 45 °C or 25 °C. We found that *i*-Pr_2O, *t*-BuOMe, *n*-hexane and toluene proved to be suitable reaction media for hydrolysis of the β-aryl- and β-heteroaryl-substituted substrates (±)-**62** and **114f**. For the hydrolysis of ethyl 3-amino-4-phenylbutanoate (**114m**), *i*-Pr_2O and *t*-BuOMe were suitable solvents. Increasing amounts of added H_2O slightly increased the reaction rate of the hydrolyses of 3-amino-3-phenylpropionate [(±)-**62**] and ethyl 3-amino-3-(3-pyridyl)propionate (**114f**), but decreased that of β-arylalkyl-substituted compound **114m**. In the case of ethyl 3-amino-3-(3-pyridyl)propionate (**114f**), the *E* value decreased with increasing amount of H_2O. The hydrolysis was also complete without any added H_2O. The reaction rate of the hydrolysis increased as the quantity of lipase PS was increased, while *E* did not change.

The preparative-scale resolutions of β-aryl- [(±)-**62** and **114b-e**], β-heteroaryl- (**114f-l**) and β-arylalkyl- (**114m-o**) β-amino esters were performed in *i*-Pr_2O or *t*-BuOMe in the presence of lipase PS with 0.5 equiv. of H_2O at 45 °C or 25 °C. The β-amino acids produced were isolated with *ee* ≥ 96% and in 40-47% yields at 50% conversions. The unreacted β-amino esters were immediately transformed to the corresponding β-amino acid hydrochlorides (*ee* ≥ 96%, yield = 40-49%) with aq. HCl. Treatment of the β-amino acids produced with 22% HCl/EtOH resulted in antipode β-amino acid hydrochlorides with *ee* ≥ 96%.

The resulting 75 enantiomers (among them 40 new) were characterized by NMR, elemental analysis, melting point and specific rotation. The absolute configurations were proved by comparing the specific rotations of the enantiomers or their derivatives with literature data or, if this was not possible, the analysed chromatograms or the comparative specific rotations helped us to assume the enantiopreference of the enzyme.

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ANNEX

I.

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A New Route to Enantiopure β-Aryl-Substituted β-Amino Acids and 4-Aryl-Substituted β-Lactams through Lipase-Catalyzed Enantioselective Ring Cleavage of β-Lactams

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Abstract: A simple and efficient direct enzymatic method was developed for the synthesis of 4-aryl-substituted β-lactams and the corresponding β-amino acid enantiomers through the CAL-B (lipase B from *Candida antarctica*)-catalyzed enantioselective ($E>200$) ring cleavage of the corresponding racemic β-lactams with 1 equiv. of H_2O in *i*-Pr_2O at 60 °C. The product (*R*)-β-amino acids (ee ≥ 98%, yields ≥ 42%) and unreacted (*S*)-β-lactams (ee ≥ 95%, yields ≥ 41%) could be easily separated. The ring opening of enantiomeric β-lactams with 18% HCl afforded the corresponding enantiopure β-amino acid hydrochlorides (ee ≥ 99%).

Keywords: β-aryl-substituted β-amino acids; 4-aryl-substituted β-lactams; enantioselectivity; enzyme catalysis; lipase; ring cleavage

Introduction

β-Aryl-substituted β-amino acids and the corresponding β-lactams have been intensively investigated, due to their unique biological activity[1] and their utility in synthetic chemistry[2] and drug research.[3] In the free form, they have neurological activity and are known to be receptor antagonists and enzyme inhibitors.[4] They are also components of naturally occurring cyclic peptide astins with antitumor properties.[3] A large number of syntheses for racemic and enantiopure acyclic β-amino acids and β-lactams have already been reported.[5] As an example, 3-amino-3-(4-cyanophenyl)propanoic acid and its heteroaryl-substituted analogues have been successfully prepared in enantiomerically pure form through *Candida antarctica* lipase A-catalyzed *N*-acylation of the corresponding racemic esters.[6] Enantiomers of 4-phenyl- and 4-(*p*-tolyl)-2-azetidinones and β-aryl-substituted β-amino acids or their derivatives have been synthesized *via* lipase PS-catalyzed *R*-selective butyrylation ($E \geq 57$)[7] of the primary hydroxy group of *N*-hydroxymethylated β-lactams, or hydrolysis ($E \geq 89$) of the corresponding ester derivatives, followed by ring opening to the β-amino ester or acid, respectively.[8] This indirect enzymatic method ensures the simultaneous preparation of both β-lactam enantiomers. We have also reported a direct enzymatic method through Novozym 435 (lipase B from *Candida antarctica*)-catalyzed enantioselective alcoholysis *via* the ring opening of β-lactams ($E>200$), leading to enantiopure β-lactams in high yields (39–46%), with high ee (≥ 96%), and β-amino acids with high ee (≥ 96%) but in low yields (7–11%).[9] We recently developed a very simple and efficient new enzymatic hydrolysis method for the enantioselective ($E>200$) ring opening of alicyclic β-lactams (the synthesis of cispentacin, for instance).[10] A great advantage of this method is that the lactam ring does not necessarily need to be activated and the product β-amino acid and β-lactam are obtained in good chemical yields (≥ 36%). These results on the lipase-catalyzed enantioselective hydrolysis of alicyclic β-lactams suggested the possibility of the enantioselective ring cleavage of racemic 4-aryl-substituted β-lactams. Accordingly, in this paper we report the lipase-catalyzed enantioselective ring opening of racemic 4-aryl-substituted β-lactams, in an organic solvent. The aryl substituents were selected with regard to the synthetic applicability of the products, with different electronic characters and in different positions.

Results and Discussion

Syntheses of (±)-1–(±)-7

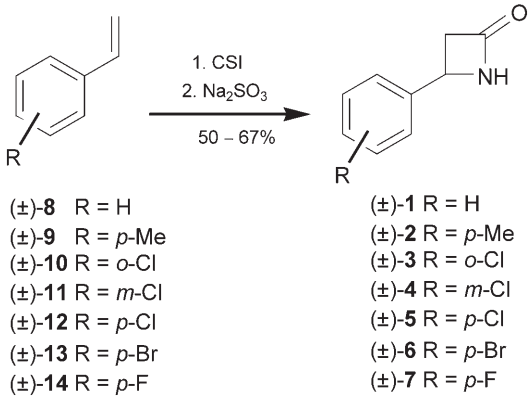
The racemic β-lactams (±)-**1** and (±)-**2** were prepared according to the literature procedure, starting from styrene or 4-methylstyrene by chlorosulfonyl isocyanate (CSI) addition[4] (Scheme 1). β-Lactams (±)-**3**–(±)-**7** were prepared in a similar, but slightly modified way, as described in the Experimental Section.

Lipase-Catalyzed Enantioselective Ring Cleavage of (±)-1–(±)-7

The earlier results[6] on the lipase-catalyzed enantioselective hydrolysis of alicyclic β-lactams suggested the possibility of the enantioselective ring opening of (±)-**1**–(±)-**7** with H_2O in an organic solvent (Scheme 2).

We explored the ring cleavage reactions on (±)-**1** with 1 equiv. of H_2O as nucleophile and Lipolase (lipase B from *Candida antarctica*) as catalyst, in *i*-Pr_2O, at 60 °C (Table 1, entry 7), but also tested the reactions with Chirazyme L-2 (entry 2) and Novozym 435 (entry 8) (both lipase B from *Candida antarctica*). High enantioselectivities ($E > 200$) were observed in all cases, with no significant differences in reactivity. Chirazyme L-5 (lipase A from *Candida antarctica*), lipase AY (*Candida rugosa*), lipase AK (*Pseudomonas fluorescens*) and lipase PS (*Pseudomonas cepacia*) did not exhibit any reactivity (no products detected after 24 h) at 45 °C. When the ring opening reaction of (±)-**1** catalyzed by Chirazyme L-2 was performed at 3 °C, a much slower reaction was observed (the reaction needed 336 h to reach 46% conversion), but with the same high enantioselectivity ($E > 200$).

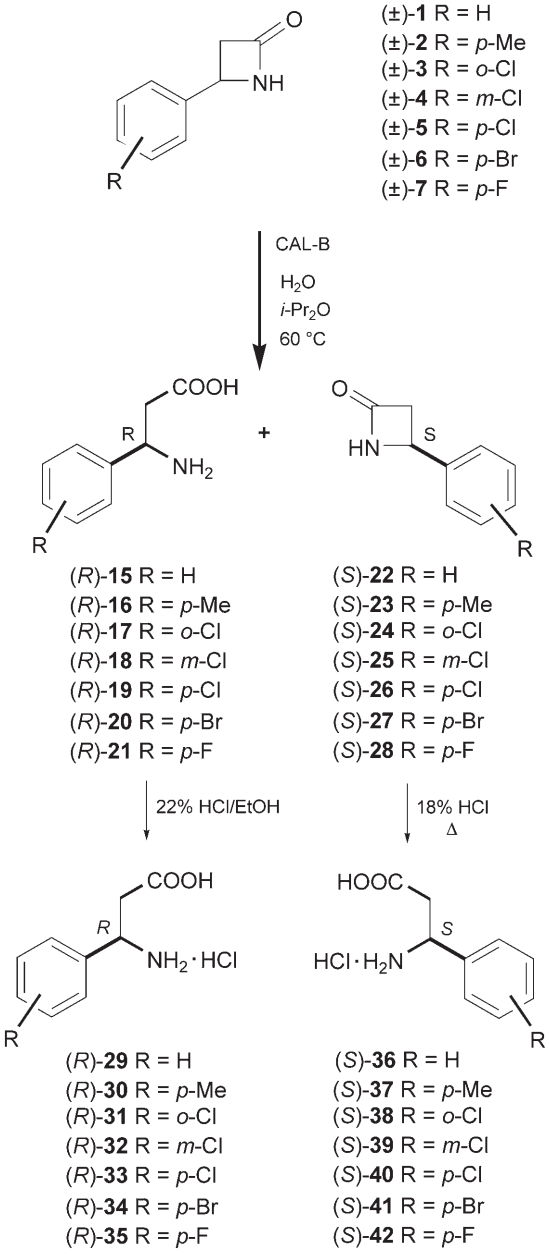
Although the CAL-B-catalyzed ring opening of (±)-**1** with H_2O (1 equiv.) at 60 °C proceeded with excellent enantioselectivity ($E > 200$), in a relatively short time



(±)-**8** R = H
(±)-**9** R = *p*-Me
(±)-**10** R = *o*-Cl
(±)-**11** R = *m*-Cl
(±)-**12** R = *p*-Cl
(±)-**13** R = *p*-Br
(±)-**14** R = *p*-F

(±)-**1** R = H
(±)-**2** R = *p*-Me
(±)-**3** R = *o*-Cl
(±)-**4** R = *m*-Cl
(±)-**5** R = *p*-Cl
(±)-**6** R = *p*-Br
(±)-**7** R = *p*-F

Scheme 1. Syntheses of (±)-**1**–(±)-**7**.



Scheme 2. Lipase-catalyzed enantioselective ring opening of (±)-**1**–(±)-**7**.

(conversion 24–26% after 1 h), several additives (2-OctOH, Et_3N and *i*-Pr_2EtN) were also tested in an attempt to enhance the reaction rate (Table 1, entries 3–5). Since no significant changes in enantioselectivity ($E > 200$) or reaction rate (conversion 25–27% after 1 h) were observed, and as the hydrolysis was complete even without the addition of H_2O (Table 1, entries 1 and 6), we concluded that the H_2O in the reaction medium (< 0.1%) or present in the enzyme preparation (< 5%) was responsible for the lactam ring opening.

We next analyzed the effect of the solvent on the enantiodiscrimination and the reaction rate. The CAL-B-catalyzed ring opening of (±)-**1** with H_2O (1 equiv.) at

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

Entry	Enzyme [50 mg mL^{-1}]	Additive [equivs.]	Conv. [%]	ee$_s$[b] [%]	ee$_p$[c] [%]	E
1	Chirazyme L-2	–	26	35	>99	>200
2	Chirazyme L-2	1 equiv. H_2O	28	38	>99	>200
3	Chirazyme L-2	1 equiv. Et_3N	26	35	>99	>200
4	Chirazyme L-2	1 equiv. *i*-Pr_2EtN	27	37	>99	>200
5	Chirazyme L-2	1 equiv. 2-OctOH	25	33	>99	>200
6	Lipolase	–	24	31	>99	>200
7	Lipolase	1 equiv. H_2O	24	31	>99	>200
8	Novozym 435	1 equiv. H_2O	25	33	>99	>200

[a] 0.05 M substrate in *i*-Pr_2O, at 60 °C, after 1 h.
[b] According to GC.
[c] According to GC after double derivatization.

60 °C was very slow when *i*-Pr_2O was replaced by acetone, acetonitrile, 1,4-dioxan, *tert*-amyl alcohol, tetrahydrofuran or chloroform (1–2% conversion after 24 h; data not shown), but the conversion was acceptable in toluene (22% conversion after 2 h), with excellent enantioselectivity ($E>200$).

The reactivity for the hydrolysis of (±)-**1** clearly increased as the quantity of enzyme was increased (Table 2). In the presence of 10 mg mL^{-1} enzyme, the reaction was relatively slow (49% conversion after 41 h; entry 1). In spite of the fact that the optimal enzyme quantity (resulting in the shortest reaction time needed to reach 50% conversion) proved to be 75 mg mL^{-1} (entry 6), for reasons of economy 30 mg mL^{-1} (entry 3) Lipolase was chosen for the preparative-scale resolutions of (±)-**1**–(±)-**7**.

On the basis of the preliminary results, the gram-scale resolutions of (±)-**1**–(±)-**7** were performed with 1 equiv. of H_2O in the presence of Lipolase (30 mg mL^{-1}) in *i*-Pr_2O at 60 °C. The products were characterized by an excellent enantiomeric excess at 49–50% conversion. The results are reported in Table 3 and in the Experimental Section.

Transformations of the Enantiomers

The transformations involving the ring opening of β-lactams **23**–**28** with 18% HCl resulted in the enantiomers of the β-amino acid hydrochlorides **36**–**42** (Scheme 2). Treatment of amino acids **15**–**21** with 22% EtOH/HCl resulted in enantiopure hydrochlorides **29**–**35**. The physical data on the enantiomers prepared are reported in the Experimental Section.

The absolute configurations in the cases of **21**, **22**, **23**, **33** and **35** were assigned by comparing the [α] values with the literature data[9,11] (see Table 3 and Experimental Section), while for **17**, **18** and **20** the analyzed chromatograms indicated the same enantiopreference for Lipolase.

Conclusions

In conclusion, an efficient direct enzymatic method was developed for the synthesis of optically pure β-aryl-substituted β-amino acids and β-lactams *via* the enantioselective ring cleavage of the corresponding β-lactams in an organic medium. The Lipolase-catalyzed highly enantioselective reactions ($E>200$) when H_2O (1 equiv.) was used as a nucleophile in *i*-Pr_2O at 60 °C led to β-amino acid and β-lactam enantiomers (ee ≥

Table 2. Effect of the quantity of Lipolase on the ring opening of (±)-**1**.[a]

Entry	Lipolase [mg mL^{-1}]	Conv. [%]	ee$_s$[b] [%]	ee$_p$[c] [%]	E
1	10	8 (49 after 41 h)	9	>99	>200
2	20	14	16	>99	>200
3	30	17	20	>99	>200
4	40	22	28	>99	>200
5	50	24	31	>99	>200
6	75	32	47	>99	>200

[a] 0.05 M substrate in *i*-Pr_2O, with 1 equiv. H_2O, at 60 °C, after 1 h.
[b] According to GC.
[c] According to GC after double derivatization.

Table 3. Lipolase-catalyzed ring opening of (±)-**1**–(±)-**7**.[a]

	Time [h]	Conv. [%]	*E*	β-Amino acid (**15**–**21**)				β-Lactam (**22**–**28**)			
				Yield [%]	Isomer	ee[b] [%]	$[\alpha]_D^{25}$	Yield [%]	Isomer	ee[c] [%]	$[\alpha]_D^{25}$
(±)-**1**	24	50	>200	47	*R*	>99	+7[d]	46	*S*	>99	−137[e]
(±)-**2**	30	49	>200	42	*R*	99	+8[f]	45	*S*	95	−113[g]
(±)-**3**	14	50	>200	47	*R*	99	+30.3[h]	48	*S*	99	−271[i]
(±)-**4**	11	50	>200	46	*R*	99	+5[j]	46	*S*	99	−118[k]
(±)-**5**	15	50	>200	48	*R*	99	+16.3[l]	46	*S*	99	−110[m]
(±)-**6**	14	49	>200	47	*R*	98	+4[n]	41	*S*	96	−73[o]
(±)-**7**	13	50	>200	43	*R*	99	+4.9[p]	49	*S*	99	−117[q]

[a] 3 mg mL^{-1} enzyme in *i*-Pr_2O, 1 equiv. H_2O, 60 °C.
[b] Determined by GC [after double derivatization (i) diazomethane; (ii) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine (Experimental Section).
[c] According to GC (Experimental Section).
[d] $c=0.17$; H_2O.
[e] $c=0.28$; EtOH, lit.[9] $[\alpha]_D^{25}$: −139 ($c=0.19$; EtOH).
[f] $c=0.20$; H_2O.
[g] $c=0.26$; EtOH, lit.[9] $[\alpha]_D^{25}$: −121.9 ($c=0.5$; EtOH).
[h] $c=0.43$; H_2O.
[i] $c=0.47$; EtOH.
[j] $c=0.51$; H_2O.
[k] $c=0.46$; EtOH.
[l] $c=0.33$; H_2O.
[m] $c=0.49$; EtOH.
[n] $c=0.10$; H_2O.
[o] $c=0.16$; EtOH.
[p] $c=0.45$; H_2O, lit.[11] $[\alpha]_D^{25}$: +3.9 ($c=0.4$; H_2O).
[q] $c=0.45$; EtOH.

95%) in good chemical yields (41–49%). The products could be easily separated. Transformations through the ring opening of β-lactams with 18% HCl resulted in the corresponding β-amino acid hydrochlorides (ee ≥ 99%). It is important to note that no significant correlation was found between the steric and electronic nature of the substituent on the aryl ring and the activation of the ring cleavage. The present method proved to be a very simple, inexpensive route, and could be easily scaled up. The synthetized enantiopure β-amino acids and β-lactams are promising building blocks for the synthesis of peptides, peptidomimetics and potential pharmacons.

Experimental Section

Materials and Methods

Lipolase (lipase B from *Candida antarctica*), produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin, was from Sigma-Aldrich. Novozym 435, as an immobilized lipase (lipase B from *Candida antarctica*) on a macroporous acrylic resin, was from Novo Nordisk. Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*) was purchased from Roche Diagnostics Corporation. Chlorosulfonyl isocyanate, styrene and substituted styrenes were from Aldrich. The solvents were of the highest analytical grade.

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus.

Synthesis of Racemic β-Lactams, (±)-1 and (±)-2

The racemic β-lactams (±)-**1** and (±)-**2** were prepared according to the literature,[7] starting from styrene **8** (2 g, 19.20 mmol) or 4-methylstyrene **9** (2 g, 16.92 mmol).

(±)-4-Phenyl-2-azetidinone [(±)-1]: yield: 2.22 g (79%); mp 104–105 °C (lit.[8] mp 108–109 °C).

(±)-4-(*p*-Tolyl)-2-azetidinone [(±)-2]: yield: 1.73 g (63%); mp 87–88 °C (lit.[8] mp 85–86 °C).

Synthesis of Racemic β-Lactams (±)-3–(±)-7

β-Lactams (±)-**3**–(±)-**7** were prepared with a slightly modified synthetic procedure: a solution of substituted styrene **10** (2 g, 14.43 mmol) or **11** (2 g, 14.43 mmol) or **12** (2 g, 14.43 mmol) or **13** (2 g, 10.92 mmol) or **14** (2 g, 12.10 mmol) in absolute toluene (10 mL) was added dropwise to a stirred solution of CSI (1 equiv.) in absolute toluene (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 8 h and then left to stand overnight. The solution was added dropwise to a vigorously stirred solution of Na_2SO_3 (0.8 g) and

Na_2CO_3 (3.6 g) in H_2O (30 mL). The organic layer was separated and the aqueous phase was extracted with toluene. The combined organic layers were dried (Na_2SO_4) and, after filtration, concentrated. The resulting crude β-lactams were recrystallized from *i*-Pr_2O.

(±)-4-(2-Chlorophenyl)-2-azetidinone [(±)-3]: yield: 1.30 g (50%); mp 123–125 °C (lit.[12] mp 121–122 °C).

(±)-4-(3-Chlorophenyl)-2-azetidinone [(±)-4]: yield: 1.75 g (67%); mp 93–95 °C.

(±)-4-(4-Chlorophenyl)-2-azetidinone [(±)-5]: yield: 1.52 g (58%); mp 97–99 °C (lit.[13] mp 98–99 °C).

(±)-4-(4-Bromophenyl)-2-azetidinone [(±)-6]: yield: 1.55 g (50%); mp 104–108 °C (lit.[14] mp 105 °C).

(±)-4-(4-Fluorophenyl)-2-azetidinone [(±)-7]: yield: 1.49 g (55%); mp 58–62 °C.

Typical Small-Scale Experiment

Racemic β-lactam (0.05 M solution) in an organic solvent (2 mL) was added to the lipase tested (10, 20, 30, 40, 50 or 75 mg mL^{-1}). H_2O or additive (0 or 1 equiv.) was added. The mixture was shaken at 60 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analyzing them by gas chromatography. The ee values for the unreacted β-lactam enantiomers were determined by gas chromatography on a Chromopack Chiralsil-Dex CB column (25 m) [160 °C for 4 min → 190 °C (temperature rise 20 °C min^{-1}), 140 kPa; retention times (min); **22**: 18.28 (antipode: 16.85); **23**: 20.48 (antipode: 19.55); 190 °C isothermal, 140 kPa; **24**: 41.77 (antipode: 40.10); **25**: 50.75 (antipode: 47.81); **26**: 53.77 (antipode: 52.01); **27**: 50.76 (antipode: 47.88); **28**: 23.15 (antipode: 12.28)], while the ee values for the β-amino acids produced were determined by using a gas chromatograph equipped with a Chirasil-L-Val column (20 m) after double derivatization with (i) diazomethane [**Caution!** the derivatization with diazomethane should be performed under a well-working hood]; (ii) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine [120 °C for 2 min → 180 °C (temperature rise 20 °C min^{-1}), 140 kPa; retention times (min); **15**: 11.74 (antipode: 11.95); **16**: 16.27 (antipode: 16.67); 150 °C for 30 min → 180 °C (temperature rise 20 °Cmin^{-1}), 100 kPa: **17**: 35.91 (antipode: 36.27); 120 °C for 10 min → 190 °C (temperature rise 10 °C min^{-1}), 140 kPa; **18**: 27.34 (antipode: 27.75); **21**: 19.61 (antipode: 19.78); 150 °C for 15 min → 180 °C (temperature rise 5 °C min^{-1}), 120 kPa: **19**: 36.62 (antipode: 37.47); 180 °C isothermal, 140 kPa; **20**: 28.68 (antipode: 29.76)].

Gram-Scale Resolution of (±)-1

Racemic β-lactam **1** (0.5 g, 3.39 mmol) was dissolved in *i*-Pr_2O (70 mL). Lipolase (2.1 g, 30 mg mL^{-1}) and H_2O (61 μL, 3.39 mmol) were added, and the mixture was shaken in an incubator shaker at 60 °C for 24 h. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residue (*S*)-**22** crystallized; yield: 230 mg (46%); recrystallized from *i*-Pr_2O {$[\alpha]_D^{25}$: −137 (*c* 0.28; EtOH) lit.[9] $[\alpha]_D^{25}$: −139 (*c* 0.19; EtOH); mp 116–118 °C, lit.[9] mp 114 °C; ee > 99%}.

The filtered-off enzyme was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off, affording the crystalline β-amino acid (*R*)-**15** [yield: 263 mg (47%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +7 (*c* 0.27; H_2O); mp 242–246 °C; ee > 99%].

When **15** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**29** was obtained [yield: 98 mg (81%); $[\alpha]_D^{25}$: −3 (*c* 0.30; H_2O); mp 195–198 °C, ee > 99%].

Gram-Scale Resolution of (±)-2

Via the procedure described above, the reaction of racemic **2** (0.5 g, 3.10 mmol) and H_2O (56 μL, 3.10 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**23** {yield: 225 mg (45%); $[\alpha]_D^{25}$: −113 (*c* 0.26; EtOH), lit.[9] $[\alpha]_D^{25}$: −121.9 (*c* 0.5; EtOH); mp 60–61 °C (recrystallized from *i*-Pr_2O), lit.[9] mp 56 °C; ee = 95%} and β-amino acid (*R*)-**16** {yield: 233 mg, 42%; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +8 (*c* 0.20; H_2O), (lit.[9] $[\alpha]_D^{25}$: given by mistake with the opposite sign); mp 244–248 °C, lit.[9] mp 241–243 °C; ee = 99%} in 30 h.

When **16** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**30** {yield: 97 mg (81%); $[\alpha]_D^{25}$: −4.5 (*c* 0.36; H_2O); mp 197–202 °C (recrystallized from EtOH and Et_2O), ee > 99%} was formed.

Gram-Scale Resolution of (±)-3

Via the procedure described above, the reaction of racemic **3** (0.5 g, 2.75 mmol) and H_2O (50 μL, 3.10 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**24** {yield: 240 mg (48%); $[\alpha]_D^{25}$: = −271 (*c* 0.47; EtOH); mp 108–112 °C (recrystallized from *i*-Pr_2O); ee = 99%} and β-amino acid (*R*)-**17** {yield: 258 mg (47%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +30.3 (*c* 0.43; H_2O); mp 235–239 °C (with sublimation), ee = 99%} in 14 h.

When **17** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**31** {yield: 104 mg (88%); $[\alpha]_D^{25}$: +8.4 (*c* 0.47; H_2O); mp 175–178 °C (recrystallized from EtOH and Et_2O), ee > 99%} was formed.

Gram-Scale Resolution of (±)-4

Via the procedure described above, the reaction of racemic **4** (0.5 g, 2.75 mmol) and H_2O (50 μL, 3.10 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**25** {yield: 230 mg (46%); $[\alpha]_D^{25}$: −118 (*c* 0.47; EtOH); mp 95–98 °C, (recrystallized from *i*-Pr_2O); ee = 99%} and β-amino acid (*R*)-**18** {252 mg (46%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +5 (*c* 0.43; H_2O); mp 230–233 °C, ee 99%} in 11 h.

When **18** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**32** {yield: 106 mg (90%); $[\alpha]_D^{25}$: −3.2 (*c* 0.43, H_2O); mp 200–204 °C (recrystallized from EtOH and Et_2O), ee = 99%} was formed.

Gram-Scale Resolution of (±)-5

Via the procedure described above, the reaction of racemic **5** (0.5 g, 2.75 mmol) and H_2O (50 μL, 3.10 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at

60 °C afforded the unreacted (*S*)-**26** {yield: 232 mg (46%); $[\alpha]_D^{25}$: −110 (*c* 0.49; EtOH); mp 123–127 °C, (recrystallized from *i*-Pr_2O); ee = 99%} and β-amino acid (*R*)-**19** {yield: 263 mg (48%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +16.3 (*c* 0.33; H_2O); mp 241–244 °C with sublimation, lit.[10] mp 223–225 °C; ee = 99%} in 15 h.

When **19** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**33** {yield: 106 mg, 90%; $[\alpha]_D^{25}$: −5.2 (*c* 0.46, H_2O), lit.[10] $[\alpha]_D^{25}$: −3.33 (1.6 N HCl); mp 184–188 °C (recrystallized from EtOH and Et_2O), ee = 99%} was formed.

Gram-Scale Resolution of (±)-6

Via the procedure described above, the reaction of racemic **6** (0.5 g, 2.21 mmol) and H_2O (40 μL, 2.21 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**27** {220 mg (41%); $[\alpha]_D^{25}$: −73 (*c* 0.16; EtOH); mp 151–154 °C, (recrystallized from *i*-Pr_2O); ee = 96%} and β-amino acid (*R*)-**20** {yield: 234 mg (47%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +4 (*c* 0.45; H_2O); mp 258–260 °C, ee = 98%} in 14 h.

When **20** (50 mg) was treated with 18% HCl (3 mL), (*R*)-**34** {yield: 52 mg (90%); $[\alpha]_D^{25}$: −4.1 (*c* 0.45, H_2O); mp 191–193 °C with sublimation (recrystallized from EtOH and Et_2O), ee = 99%} was formed.

Gram-Scale Resolution of (±)-7

Via the procedure described above, the reaction of racemic **7** (0.5 g, 3.03 mmol) and H_2O (55 μL, 3.03 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**28** {yield: 219 mg (44%); $[\alpha]_D^{25}$: = −117 (*c* 0.45; EtOH); mp 97–101 °C (recrystallized from *i*-Pr_2O); ee = 99%} and β-amino acid (*R*)-**21** {yield: 268 mg (48%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +4.9 (*c* 0.45; H_2O), lit.[10] $[\alpha]_D^{25}$: +3.9 (*c* 0.4; H_2O); mp 245–247 °C, ee = 99%} in 13 h.

When **21** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**35** {110 mg (91%); $[\alpha]_D^{25}$: −1.2 (*c* 0.37, H_2O), lit.[10] $[\alpha]_D^{25}$: −1.9 (1.9 N HCl); mp 166–169 °C (recrystallized from EtOH and Et_2O), ee = 99%} was formed.

Ring Opening of (*S*)-22–(*S*)-28 with 18% HCl

The β-lactam enantiomer (*S*)-**22** (50 mg, 0.34 mmol) or (*S*)-**23** (50 mg, 0.31 mmol) or (*S*)-**24** (100 mg, 0.55 mmol) or (*S*)-**25** (100 mg, 0.55 mmol) or (*S*)-**26** (100 mg, 0.55 mmol) or (*S*)-**27** (50 mg, 0.22 mmol) or (*S*)-**28** (100 mg, 0.60 mmol) was dissolved in 18% HCl (12 mL) and the solution was refluxed for 2 h. The solvent was then evaporated off, and the product was recrystallized from EtOH and Et_2O, which afforded white crystals of the β-amino acid hydrochlorides.

(*S*)-**36**: {yield: 49 mg (72%); $[\alpha]_D^{25}$: +3.0 (*c* 0.28; H_2O); mp 197–201 °C}.

(*S*)-**37**: {yield: 52 mg (78%); $[\alpha]_D^{25}$: +4.0 (*c* 0.28; H_2O); mp 196–201 °C}.

(*S*)-**38**: {yield: 104 mg (80%); $[\alpha]_D^{25}$: −8.6 (*c* 0.46; H_2O); mp 176–179 °C}.

(*S*)-**39**: {yield: 111 mg (86%); $[\alpha]_D^{25}$: +3.3 (*c* 0.35; H_2O); mp 201–204 °C}.

(*S*)-**40**: {yield: 109 mg (84%); $[\alpha]_D^{25}$: +5.3 (*c* 0.46; H_2O); mp 185–188 °C}.

(*S*)-**41**: {yield: 57 mg (91%); $[\alpha]_D^{25}$: +3.8 (*c* 0.45; H_2O); mp 191–193 °C with sublimation}.

(*S*)-**42**: {yield: 108 mg (81%); $[\alpha]_D^{25}$: +1.5 (*c* 0.47; H_2O); mp 164–168 °C}.

Supporting Information

The spectroscopic and analytical data for racemates **1**–**7** and enantiomers **15**–**42** are presented in the Supporting Information.

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Candida antarctica lipase B-catalyzed ring opening of 4-arylalkyl-substituted β-lactams

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Abstract—The Lipolase-catalyzed ring opening of racemic 4-benzyl- **3** and 4-phenylethyl-2-azetidinone **4** was performed with 0.5 equiv of H_2O in diisopropyl ether at 45 °C. The resulting (*S*)-β-amino acid **5** or **6** (ee ⩾ 87%) and (*R*)-β-lactam **7** or **8** (ee >99%) enantiomers could easily be separated. The ring opening of enantiomeric β-lactams with 18% aqueous HCl afforded the corresponding enantiopure β-amino acid hydrochlorides **9** and **10** (ee >99%).

1. Introduction

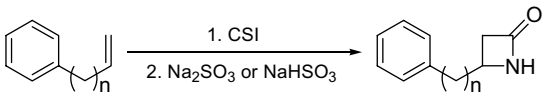
In recent years, β-amino acids have aroused considerable interest as potentially biologically active compounds.[1] (*S*)-Homo-β-phenylalanine increases the μ-type opioid receptor affinity[2] and is a valuable building block for two tripeptidomimics exhibiting angiotensin-converting enzyme inhibitor activity[3] and an optically active poly(β-peptide).[4] It has been tested as a catalyst in intra- and intermolecular aldol reactions.[5] (*R*)-Homo-β-phenylalanine derivatized with a thiazolidine is a potent inhibitor of dipeptidyl peptidase IV, which allows a novel therapeutic approach to the treatment of diabetes type 2.[6] A photochemical application is also known.[7] β-Phenylethyl-β-alanine has been applied as a model compound in analytical studies[8] and its antiseizure activity has also been studied.[9] Valuable antiplatelet[10] and antiviral agents[11] have been synthesized from the title β-lactams.

Since their widespread investigation, over the last few years a number of new enzymatic and asymmetric syntheses of β-amino acids and β-lactams have been published most of which have been reviewed.[12] An important indirect enzymatic method for the preparation of β-amino acid enantiomers is the lipase-catalyzed asymmetric acylation of the primary hydroxy group of the N-hydroxymethylated β-lactams, or the lipase-catalyzed hydrolysis of the corresponding ester derivatives, followed by ring opening to give the desired β-amino acids.[13] We recently discovered a simple and efficient direct enzymatic method for the enantioselective (E >200) ring cleavage of β-lactams.[14] Later, the enantioselective (E >200) ring opening of several 4-aryl-substituted β-lactams was reported.[15]

Herein, we report the lipase-catalyzed ring cleavage of 4-benzyl- and 4-phenylethyl-2-azetidinones (±)-**3** and (±)-**4**.

2. Results and discussion

The starting racemic β-lactams **3** and **4** were prepared by the addition of chlorosulfonyl isocyanate to allylbenzene **1** or 4-phenyl-1-butene **2**, according to a literature method[10,13d] (Scheme 1).



1, n = 1 (±)-**3**, n = 1
2, n = 2 (±)-**4**, n = 2

Scheme 1.

The earlier results on the lipase-catalyzed enantioselective hydrolysis of 4-aryl-substituted β-lactams[15] suggested the possibility of the enantioselective ring opening of (±)-**3** and (±)-**4**. Relatively low enantioselectivity (E = 12) was

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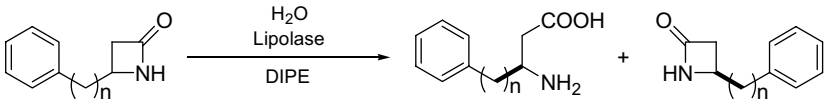


(±)-**3**, n = 1 (±)-**4**, n = 2 **5**, n = 1 **6**, n = 2 **7**, n = 1 **8**, n = 2

Scheme 2.

observed when the ring in (±)-**4** was opened with H_2O in diisopropyl ether (DIPE), with Lipolase (30 mg/mL) as a catalyst at 60 °C (Scheme 2).

To increase the enantioselectivity, several further enzymes were tested. In addition to Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin), Chyrazyme L-2 and Novozym 435 (both lipase B from *C. antarctica*) also proved to be promising catalysts, directing the hydrolysis of (±)-**4** with similar enantioselectivities ($E \sim 12$). Lipase A from *C. antarctica*, lipase AY from *Candida rugosa* and Lecitase did not show any reactivity at 45 °C (no conversion after 24 h), while PPL (porcine pancreas lipase) and lipase AK from *Pseudomonas fluorescens* catalyzed the reaction at 45 °C, although the reaction rates and the enantioselectivities were low (after 24 h, conv. ~ 3%, $E \sim 2$). Therefore Lipolase was chosen as the enzyme for further studies.

Next, we tested the ring-cleavage reactions of (±)-**4** at different temperatures: 60, 50, 45, 40 and 30 °C. Decreasing the reaction temperature also caused the reaction rate to decrease as well, but without an increase in enantioselectivity (after 7 h, conv. = 41% at 60 °C; 24% at 50 °C; 20% at 45 °C; and 15% at 40 °C; after 5 days, conv. = 61% at 30 °C). Thus, 45 °C was chosen as the optimal temperature.

Several solvents were also tested. No reaction was observed after 24 h when the Lipolase (50 mg/mL)-catalyzed ring cleavage of (±)-**4** was performed in chloroform, tetrahydrofuran or acetone. The reaction proceeded more slowly in toluene (conv. = 19% after 24 h) and much more slowly in 1,4-dioxane (conv. = 4% after 24 h) than in DIPE (conv. = 58% after 24 h), *tert*-butyl methyl ether (conv. = 60% after 24 h), diethyl ether (conv. = 49% after 24 h) or *n*-hexane (conv. = 69% after 24 h). So we chose to continue our studies in DIPE.

Certain additives can have a beneficial influence by increasing the enantioselectivity and/or the reaction rate.[16] As an attempt, 1 equiv of triethylamine, 2-octanol and *N*,*N*-diisopropylethylamine were added to the reaction mixture. However, no significant changes in the reaction rate or enantioselectivity were observed (conv. ~ 57% after 24 h, $E \sim 10$).

Since the enantioselective ring cleavage of some *N*-Boc-protected cyclic β-lactams has been described,[17] we synthesized *N*-Boc-protected-(±)-**4**. Unfortunately, the Lipolase (50 mg/mL)-catalyzed ring opening of *N*-Boc-(±)-**4** at 45 °C did not give a better result (conv. = 94% after 24 h, $E = 3$).

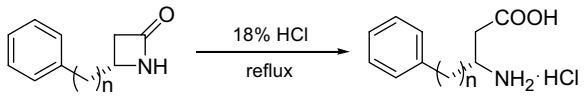
On the basis of the preliminary results, we decided to perform the gram-scale resolutions of (±)-**3** and (±)-**4** in DIPE with Lipolase as catalyst and H_2O (0.5 equiv) as the nucleophile at 45 °C. We planned to stop the reactions at about 25% conversion [ee(β-amino acids) ~60%], and then perform the reactions until about 85% conversion [ee(β-lactams) ~90%], following recrystallization of the crude β-amino acid and β-lactam enantiomers. The results are shown in Table 1 and in Section 4.

Table 1. Lipolase-catalyzed ring opening of (±)-**3** and (±)-**4**

	Time (h)	Conv. at workup (%)	Enantiomer	Yield (%)	Isomer	ee[a] (%)	$[\alpha]_D^{25}$
(±)-**3**	13	24	β-Amino acid **5**	27	(*S*)	89[c]	+7[b,e]
	88	81	β-Lactam **7**	36	(*R*)	>99[d]	+38.8[b,f]
(±)-**4**	11	29	β-Amino acid **6**	31	(*S*)	87[c]	+24[b,g]
	22	89	β-Lactam **8**	30	(*R*)	>99[d]	+19[b,h]

[a] After recrystallization.
[b] Specific rotations were measured with a Perkin–Elmer 341 polarimeter.
[c] According to HPLC [APEX Octadecyl 5 μ column (0.04 cm × 25 cm); precolumn derivatization with (*S*)-NIFE according to the literature;[18] the mobile phases were H_2O (A) and MeCN (B), both of which contained 0.1% TFA; the gradient slopes were 95% A + 5% B at 0 min, increased to 25% A + 75% B within 60 min.; flow rate: 0.8 mL/min; room temperature; detection at 205 nm; retention times (min): **5**, 38.69 (antipode: 39.67); **6**, 40.00 (antipode: 40.90)].
[d] According to GC [Chrompack Chirasil-L-Val column (25 m × 0.25 mm); 90 °C for 20 min (**3**) and 10 min (**4**) → 140 °C; temperature rise 5 °C/min; 140 kPa; retention times (min): **7**, 35.77 (antipode: 36.66); **8**, 38.52 (antipode: 39.27)].
[e] *c* 0.2; H_2O lit.[19] $[\alpha]_D^{25} = -8.5$ (*c* 0.2, H_2O) for (*R*)-**5**, ee = 95%.
[f] *c* 0.65; $CHCl_3$ lit.[13d] $[\alpha]_D^{25} = +30.1$ (*c* 0.65, $CHCl_3$), ee = 98%.
[g] *c* 0.28 lit.[20] $[\alpha]_D^{25} = -28.4$ (*c* 0.56, H_2O) for (*R*)-**6**, ee >99%; H_2O.
[h] *c* 0.21; $CHCl_3$.

The transformations involving the ring opening of β-lactams **7** and **8** with 18% aqueous HCl afforded β-amino acid hydrochlorides **9** and **10** (Scheme 3), while treatment of amino acids **5** and **6** with 18% aqueous HCl resulted in the corresponding β-amino acid hydrochlorides **5**·HCl and **6**·HCl.



7, n = 1
8, n = 2

9, n = 1
10, n = 2

Scheme 3.

The absolute configurations were proven by comparing the specific rotations with the literature data[13d,19,20] (Table 1). Thus, the absolute configuration for **5** and **6** is (*S*), and for **7** and **8** it is (*R*).

3. Conclusion

In conclusion, 4-benzyl- and 4-phenylethyl-2-azetidinones (±)-**3** and (±)-**4** were resolved via opening of the β-lactam ring in an organic medium. The Lipolase-catalyzed reactions when H_2O (0.5 equiv) was used as nucleophile in DIPE at 45 °C led to (*S*)-β-amino acids **5** and **6** (ee ⩾ 87%) and (*R*)-β-lactams **7** and **8** (ee >99%). The products could be separated with ease. Transformations of β-lactams **7** and **8** by ring opening with 18% aqueous HCl gave the corresponding enantiomers of the β-amino acid hydrochlorides **9** and **10** (ee >99%).

4. Experimental

4.1. Small-scale resolutions

In a small-scale experiment, (±)-**4** (0.05 M solution) in DIPE (1 mL) was added to Lipolase (30 or 50 mg/mL). H_2O (0.5 equiv) was added. The mixture was shaken at 30, 40, 45, 50 or 60 °C. The progress of the reaction was followed by taking samples from the mixture at intervals and analyzing them by gas chromatography and HPLC (Table 1).

4.2. Gram-scale resolution of racemic 4-benzyl-2-azetidinone (±)-3

Racemic **3** (1.2 g, 7.44 mmol) was dissolved in DIPE (40 mL). Lipolase (1.2 g, 30 mg/mL) and H_2O (67 μL, 3.72 mmol) were added. The mixture was stirred at 45 °C for 13 h. The reaction was stopped by filtering off the enzyme at 24% conversion. The solvent was evaporated off, affording the unreacted β-lactam **7** (0.85 g, 5.27 mmol, ee = 18%). The filtered enzyme was washed with distilled H_2O (3 × 20 mL), and the H_2O was evaporated off, yielding the crystalline (*S*)-β-amino acid **5** {0.36 g, 27%; $[\alpha]_D^{25} = +7$ (*c* 0.2; H_2O); mp = 207–210 °C (recrystallized from H_2O/acetone); ee = 89%; lit.[19] $[\alpha]_D^{25} = -8.5$ (*c* 0.2, H_2O) for (*R*)-**5**; mp = 222–225 °C; ee = 95%}.

To obtain β-lactam **7** with high ee, the above unreacted lactam (0.85 g) was dissolved in DIPE (30 mL). Lipolase (0.8 g, 27 mg/mL) and H_2O (47 μL, 2.64 mmol) were added. The mixture was stirred at 45 °C for 88 h. The reaction was stopped by filtering off the enzyme at 81% conversion. The solvent was evaporated off, affording (*R*)-β-lactam **7** {0.43 g, 36%; $[\alpha]_D^{25} = +38.8$ (*c* 0.65; $CHCl_3$); mp = 67–69 °C (recrystallized from DIPE); ee >99%; lit.[13d] $[\alpha]_D^{25} = +30.1$ (*c* 0.65, $CHCl_3$); ee = 98%}. When **5** (36 mg) was treated with 18% HCl (5 mL), **5**·HCl was obtained {38 mg, 88%; $[\alpha]_D^{25} = +6$ (*c* 0.21; H_2O); mp = 172–175 °C; ee = 89%; lit.[13d] mp = 176–178 °C}.

^{1}H NMR (D_2O, 400 MHz) δ (ppm) for **5**: 2.40–2.46 (dd, *J* = 16.8, 8.2 Hz, 1H, C*H*$_2$CO$_2$H), 2.52–2.57 (dd, *J* = 16.8, 4.9 Hz, 1H, C*H*$_2$CO$_2$H), 2.91–3.04 (m, 2H, C*H*$_2$Ph), 3.73–3.76 (m, 1H, CH), 7.30–7.43 (m, 5H, Ph).

^{1}H NMR (D_2O, 400 MHz) δ (ppm) for **5**·HCl: 2.66–2.82 (m, 2H, CH_2CO), 3.03–3.05 (m, 2H, CH_2Ph), 3.90–3.93 (m, 1H, CH), 7.32–7.45 (m, 5H, Ph).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) for **7**: 2.68–2.72 (d, *J* = 14.8 Hz, 1H), 2.82–2.87 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.95–3.00 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.05–3.10 (m, 1H), 3.83–3.86 (m, 1H), 5.83 (br s, 1H), 7.17–7.35 (m, 5H).

4.3. Gram-scale resolution of racemic 4-phenylethyl-2-azetidinone (±)-4

With the procedure described above, the ring cleavage of racemic **4** (0.8 g, 4.57 mmol) in DIPE (30 mL) in the presence of Lipolase (0.8 g, 27 mg/mL) and H_2O (41 μL, 2.29 mmol) afforded (*S*)-β-amino acid **6** [0.27 g, 31%; $[\alpha]_D^{25} = +24$ (*c* 0.28; H_2O); mp = 215–219 °C (recrystallized from H_2O/acetone); ee = 87%; lit.[20] $[\alpha]_D^{25} = -28.4$ (*c* 0.56, H_2O) for (*R*)-**6**; mp = 215–217 °C; ee >99%] in 11 h and (*R*)-β-lactam **8** [0.24 g, 30%; $[\alpha]_D^{25} = +19$ (*c* 0.21; $CHCl_3$); mp = 46–48 °C (recrystallized from DIPE); ee >99%] in 22 h. When **6** (30 mg) was treated with 18% HCl (5 mL), **6**·HCl was obtained [33 mg, 92%; $[\alpha]_D^{25} = +12$ (*c* 0.21; H_2O); mp = 150–152 °C; ee = 87%].

^{1}H NMR (D_2O, 400 MHz) δ (ppm) for **6**: 1.96–2.02 (m, 2H), 2.45–2.52 (dd, *J* = 16.8, 8.4 Hz, 1H), 2.61–2.67 (dd, *J* = 16.8, 4.6 Hz, 1H), 2.72–2.79 (m, 2H), 3.48–3.53 (m, 1H), 7.29–7.42 (m, 5H).

^{1}H NMR (D_2O, 400 MHz) δ (ppm) for **6**·HCl: 2.00–2.05 (m, 2H), 2.69–2.86 (m, 4H), 3.62 (m, 1H), 7.28–7.41 (m, 5H).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) for **8**: 1.94–2.00 (dd, *J* = 14.5, 7.5 Hz, 2H, CH_2Ph), 2.55–2.59 (d, *J* = 14.8 Hz, 1H, C*H*H), 2.64–2.73 (m, 2H, CH_2), 3.02–3.08 (m, 1H, CH*H*), 3.61–3.65 (m, 1H, CH), 5.67 (br s, 1H, NH), 7.16–7.32 (m, 5H, Ph).

4.4. Ring opening of β-lactam enantiomers 7 and 8

Compounds **7** (50 mg) or **8** (25 mg) were refluxed in 18% HCl (10 mL) for 3 h. The solvent was evaporated off to afford **9** [60 mg, 90%; $[\alpha]_D^{25} = -8$ (*c* 0.11; H_2O); mp = 182–185 °C; ee >99%] or **10** {26 mg, 79%; $[\alpha]_D^{25} = -15$ (*c* 0.21; H_2O); mp = 146–148 °C; ee >99%}. The ^1H NMR (H_2O, 400 MHz) δ (ppm) data for **9** and **10** are similar to those for **5**·HCl and **6**·HCl.

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An efficient new enzymatic method for the preparation of β-aryl-β-amino acid enantiomers

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ABSTRACT

An efficient synthesis of β-aryl-β-amino acid enantiomers has been developed via the lipase-catalysed enantioselective hydrolysis of the corresponding racemic ethyl esters in an organic solvent. High enantioselectivities (E >100) were observed when the lipase PS-catalysed reactions were performed with H_2O (0.5 equiv) in diisopropyl ether at 45 °C. The products could be easily separated and were obtained in good yields of ⩾40%.

1. Introduction

β-Amino acids, including β-aryl-substituted β-amino acids, are widely used in drug research, and their pharmacological significance has been described.[1] There are several methods for the synthesis of enantiopure β-aryl-β-amino acids, such as (i) the enantioselective reduction of a β-enamino ester;[2] (ii) the addition of a chiral sulfoxide to a substituted *N*-sulfonylimine;[3] (iii) an asymmetric catalytic Mannich reaction;[4] (iv) the conjugate addition of a chiral lithium amide to a β-aryl α,β-unsaturated ester;[5] (v) the reaction of a Reformatsky reagent with an imine;[6] or (vi) the preparation of a diastereomeric salt.[7] In addition to asymmetric synthetic routes,[2–7] enzyme-catalysed processes have been developed through (i) the transesterification of an N-protected ester in isobutanol;[8] (ii) stereoselective degradation of a racemic amino acid;[9] (iii) hydrolysis of a racemic β-amino ester;[10] (iv) hydrolysis of a racemic N-acetylated β-amino acid;[11] or (v) an aminomutase-catalysed α,β-rearrangement[12] in aqueous medium.

We recently reported some direct and indirect enzymatic methods for the preparation of enantiomers of β-phenylalanine or its derivatives from racemic β-lactams.[13] Previously, we devised the first direct enzymatic highly enantioselective (E >100) hydrolysis of carbocyclic β-amino esters in *i*-Pr_2O with Lipolase at 60 °C.[14]

Herein, our goal was to extend this method to β-aryl-substituted β-amino esters, hydrolysing them to enantiopure β-aryl-β-amino acids. β-Phenylalanine was chosen as the model compound. There are complex structures which include an (*R*)- or (*S*)-β-phenylalanine **6a** or **7a** moiety. For example, **6a** has been inserted into NS5B polymerase inhibitors against the hepatitis C virus,[15] anti-inflammatory bradykinin B1 receptor antagonists,[16] anticancer matrix metalloproteinase (MMP-12) inhibitors[17] or analgesic endomorphin-1 analogue tetrapeptides.[18] (*S*)-β-Phenylalanine **7a** has been applied in the synthesis of novel antibiotics.[19] In addition to β-phenylalanine, we set out to prepare β-amino acid enantiomers with pharmaceutical potential. (*S*)-3-Amino-3-(3-fluorophenyl)propanoic acid **7b** was used to obtain chemokine receptor (CCR5) antagonists with anti-inflammatory and anti-HIV effects.[20] The (*R*)-isomer of 3-amino-3-(3,5-dichlorophenyl)propanoic acid **6c** was investigated as part of an integrin receptor $\alpha_v\beta_3$ antagonist.[21] This receptor is an interesting therapeutic target in the treatment of osteoporosis, restenosis, cancer growth and metastasis formation. Several VLA-4 integrin receptor antagonists have been synthetized, including the (*S*)-3-amino-3-(3,4-dimethoxyphenyl)propanoic acid **7d** unit, and used against inflammatory and autoimmune diseases.[22] One non-peptide bradykinin B1 receptor antagonist with anti-inflammatory and analgesic effects contains an (*R*)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoic acid **6e** moiety.[23]

2. Results and discussion

2.1. Syntheses of ethyl 3-amino-3-arylpropanoates 3a–e

The racemic compounds **2a–e** were first synthesized by a modified Rodionov synthesis from the corresponding **1a–e** through condensation with malonic acid and in the presence of NH_4OAc, the mixture was heated at reflux in EtOH (Scheme 1).[11c] Compounds **3a–e**·HCl were prepared by the esterification of **2a–e** in the presence of $SOCl_2$. The free bases **3a–e** were then liberated by treatment of **3a–e**·HCl with aqueous KOH.

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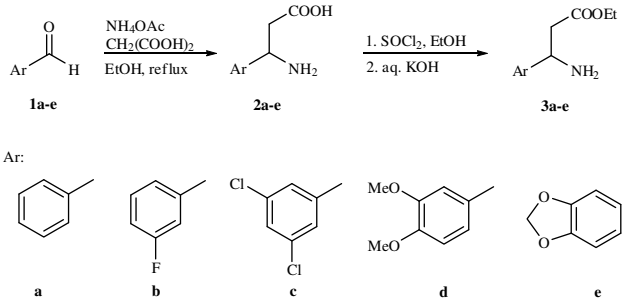


Scheme 1. Synthesis of **3a–e**.

2.2. Lipase-catalysed enantioselective ring cleavage of 3a–e

Previous results[14] on the lipase-catalysed enantioselective hydrolysis of carbocyclic β-amino esters suggested the possibility of the enantioselective hydrolysis of **3a–e** with 0.5 equiv of H_2O in the presence of Lipolase (CAL-B) in *i*-Pr_2O at 60 °C. Since Lipolase did not exhibit any selectivity towards **3a**, further enzymes were screened, including lipase PS (*Pseudomonas cepacia*), PPL (porcine pancreatic lipase), lipase AK (*Pseudomonas fluorescens*), lipase AY (*Candida rugosa*) and Chirazyme L-5 (lipase A from *Candida antarctica*) (Table 1).

Table 1
Conversion and enantioselectivity of the hydrolysis of **3a**[a]

Entry	Enzyme (50 mg mL^{-1})	H_2O (equiv)	T (°C)	t (h)	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	PPL	0.5	45	5	43	66	88	31
2	lipase AK[d]	0.5	45	5	43	67	89	35
3	Lipase PS[d]	0.5	25	6	49	92	>99	>200
4	Lipase PS[d]	0.5	45	5	49	96	>99	>200
5	Lipase PS[d]	—	45	2	46	85	>99	>200
6	Lipase PS[d]	1	45	2	48	93	>99	>200
7	Lipase PS[d]	2	45	2	50	99	>99	>200
8	Lipase PS[d]	5	45	2	50	>99	>99	>200

[a] 0.05 M substrate, 1 mL *i*-Pr_2O.
[b] According to GC after derivatization (Section 4).
[c] According to GC after double derivatization (Section 4).
[d] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

A key issue was to develop a simple analytical method allowing the progress of the reactions to be followed. The unreacted amino ester and the product amino acid were detected in the same run by GC on a chiral column, where the hydrolysed amino acid was derivatized (Section 4).

All enzymes were active in the presence of 0.5 equiv of H_2O in *i*-Pr_2O at 45 °C, but lipase AY and Chirazyme L-5 did not exhibit any selectivity towards **3a**, while PPL and lipase AK catalysed the reaction with moderate enantioselectivities (entries 1 and 2). Lipase PS directed the hydrolysis with excellent enantioselectivity (E >200) (entry 4). When the lipase PS-catalysed hydrolysis of **3a** was performed at 25 °C, a slight decrease in the reaction rate was observed (entry 3).

Next, we analysed the effects of the amount of added H_2O on the enantioselectivity and the reaction rate. The added H_2O amount (1–5 equiv) apparently did not affect the enantioselectivity (E >200) or the reaction rate (Table 1, entries 6, 7 and 8), but when an *i*-Pr_2O/H_2O 1/1 (v/v) mixture was used, a dramatic decrease in the enantioselectivity was observed (E = 30). In a small-scale experiment, the hydrolysis of **3a** was complete, even without the addition of H_2O (entry 5). In this case, the H_2O originated from the reaction medium (<0.1%) or from the enzyme preparation (<5%).

Next, we analysed the effect of the solvent (Table 2). All the solvents tested proved to be suitable for the enantioselective (E >200) hydrolysis of **3a**, with significant differences in the reaction rate. The highest reaction rates were observed in *i*-Pr_2O, *t*-BuOMe and *n*-hexane (entries 1, 2 and 4), while the hydrolysis was slowest in $CHCl_3$ (entry 7). We chose to continue our experiments with *i*-Pr_2O.

Table 2
Effect of solvents on the hydrolysis of **3a**[a]

Entry	Solvent (1 mL)	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	*i*-Pr2O	49	96	>99	>200
2	*t*-BuOMe	48	92	>99	>200
3	Et_2O	39	63	>99	>200
4	*n*-Hexane	50	98	>99	>200
5	Toluene	38	60	>99	>200
6	1,4-Dioxane	26	34	>99	>200
7	$CHCl_3$	4	4	>99	>200
8	THF	14	16	>99	>200
9	Me_2CO	13	15	>99	>200

[a] 0.05 M substrate, 0.5 equiv. of H_2O, 50 mg mL^{-1} lipase PS[d], after 5 h.
[b] According to GC after derivatization.
[c] According to GC after double derivatization.
[d] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

The reaction rate for the hydrolysis of **3a** clearly increased as the amount of enzyme was increased (Table 3). The highest reaction rate was observed in the presence of 75 mg mL^{-1} lipase PS (entry 6). However, for economic reasons, 30 mg mL^{-1} lipase PS was chosen for the preparative-scale resolutions of **3a–e**.

Table 3
Effect of the quantity of lipase PS on the hydrolysis of **3a**[a]

Entry	Lipase PS[d] (mg mL^{-1})	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	10	17 (45 after 24 h)	20	>99	>200
2	20	27	36	>99	>200
3	30	31	45	>99	>200
4	40	35	54	>99	>200
5	50	38	60	>99	>200
6	75	45	82	>99	>200

[a] 0.05 M substrate, 0.5 equiv of H_2O, 1 mL *i*-Pr_2O, after 1 h.
[b] According to GC after derivatization.
[c] According to GC after double derivatization.
[d] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

In view of the results of the preliminary experiments, the preparative-scale resolutions of **3a–e** were performed with 0.5 equiv of H_2O in the presence of lipase PS in *i*-Pr_2O at 45 °C. The products were characterized by a good enantiomeric excess at close to 50% conversion. The results are reported in Table 4 and in Section 4.

2.3. Transformations of the enantiomers

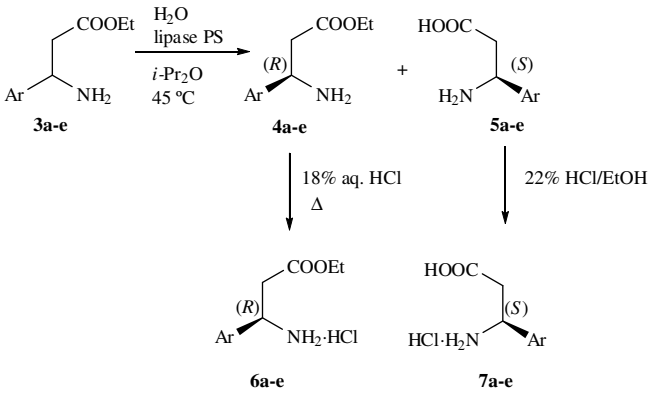
The transformations involving the hydrolysis of **4a–e** with aqueous HCl afforded **6a–e**·HCl (ee ⩾ 97%) (Scheme 2). Treatment of **5a–e** with 22% HCl/EtOH resulted in the corresponding enantiopure **7a–e**·HCl (ee >99%).

The absolute configurations in the cases of **5a**, **5d** and **5c** were proved by comparing the [α] values with literature data (Section 4), while for **5b** and **5c** the chromatograms analysed indicated the same enantiopreference for all the enzymes applied. Thus, the absolute configurations indicated the (*S*)-selective hydrolysis of **3a–e**. Although the enantiomers **5b**[10c] and the ethyl ester of **5c**[24] have been prepared, physical data for them (specific rotations and melting points) have not been described. It should be

Table 4
Lipase PS-catalysed hydrolysis of **3a–e**[a]

	Time (h)	Conv. (%)	*E*	β-Amino acid·HCl (**6a–e**)				β-Amino acid (**5a–e**)			
				Yield (%)	Isomer	ee[b] (%)	$[\alpha]_D^{25}$ (H_2O)	Yield (%)	Isomer	ee[c] (%)	$[\alpha]_D^{25}$ (H_2O)
3a	22	50	>200	44	(*R*)	>99	−4[d]	44	(*S*)	>99	−8[e]
3b	23	49	>200	40	(*R*)	>99	−6.5[f]	40	(*S*)	>99	−1.8[g]
3c	74	50	139	43	(*R*)	>99	−5.1[h]	44	(*S*)	>99	−5.5[g]
3d	18	50	>200	41	(*R*)	>99	−7.9[i]	41	(*S*)	>99	+1.3[j]
3e	16	52	110	44	(*R*)	97	−8.4[k]	46	(*S*)	>99	+4[d]

[a] 30 mg mL^{-1} enzyme in *i*-Pr_2O, 0.5 equiv H_2O, 45 °C.
[b] According to GC after derivatization.
[c] According to GC after double derivatization.
[d] *c* = 0.3.
[e] *c* = 0.27.
[f] *c* = 0.31.
[g] *c* = 0.38.
[h] *c* = 0.34.
[i] *c* = 0.32.
[j] *c* = 0.51.
[k] *c* = 0.33.



Scheme 2. Lipase PS-catalysed enantioselective hydrolysis of **3a–e** (for the meanings of **a–e**, see Scheme 1).

mentioned that lipase PS demonstrated the opposite selectivity in the hydrolysis of **3a–e** as compared with the CAL-B-catalysed enantioselective ring-opening of 4-aryl-substituted β-lactams, which was *R*-selective.[13c]

3. Conclusions

A simple and efficient direct enzymatic method has been developed for the synthesis of pharmacologically valuable, optically active β-aryl-substituted β-amino acids via the enantioselective hydrolysis of the corresponding racemic β-amino esters in an organic medium. The lipase PS-catalysed (*S*)-selective hydrolysis of **3a–e** with H_2O (0.5 equiv) as a nucleophile in *i*-Pr_2O at 45 °C led to enantiomers of **4a–e** (ee ⩾ 97%) and **5a–e** (ee >99%) with high enantioselectivities (*E* >100) in good chemical yields (40–46%). The products could be easily separated. Transformations of **4a–e** with 18% aqueous HCl resulted in the enantiomers of **6a–e**·HCl (ee ⩾ 97%).

4. Experimental

4.1. Materials and methods

Lipase PS and lipase AK were from Amano Pharmaceuticals, Lipolase (lipase B from *C. antarctica* was produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) and PPL (type II) were from Sigma, and Chirazyme L-5 (lipase B from *C. antarctica*) was from Novo Nordisk. Before use, lipase PS, lipase AK and CAL-A (5 g) were dissolved in Tris–HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g) (Sigma). Substituted benzaldehydes were from Aldrich, except for 3,5-dichlorobenzaldehyde, which was from Fluorochem. The solvents were of the highest analytical grade.

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. Elemental analyses (CHN) corresponded closely (within ±3%) with the calculated ones in all cases.

The ee values for the unreacted β-amino ester and the β-amino acid enantiomers produced were determined by GC on a Chirasil-L-Val column (20 m) after double derivatization with (i) CH_2N_2 [Caution! derivatization with CH_2N_2 should be performed under a well-working hood]; (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine [100 °C for 10 min→150 °C (temperature rise 10 °C min^{-1}), 140 kPa; retention times (min); **4a**: 23.16 (antipode: 23.65); **5a**: 21.11 (antipode: 20.80); 120 °C for 10 min→190 °C (temperature rise 10 °C min^{-1}), 140 kPa; **4b**: 16.32 (antipode: 16.45); **5b**: 15.81 (antipode: 15.69); 130 °C for 10 min→160 °C (temperature rise 10 °C min^{-1}), 140 kPa; **4c**: 40.59 (antipode: 42.29); **5c**: 37.60 (antipode: 36.06); **4d**: 56.58 (antipode: 58.20); **5d**: 48.42 (antipode: 47.39); **4e**: 43.33 (antipode: 45.00); **5e**: 38.17 (antipode: 37.06)].

4.2. General procedure for the syntheses of racemic β-amino acids 2a–e

Compounds **2a–e** were prepared by a slightly modified literature method[11c] based on the modified Rodionov synthesis from the corresponding **1a–e** (5 mmol) through condensation with malonic acid (2 equiv) in the presence of NH_4Ac (2 equiv) in EtOH at reflux for 6 h. The resulting precipitated crystals were filtered off, washed with Me_2CO and recrystallized from H_2O and Me_2CO.

4.2.1. (±)-3-Amino-3-phenylpropanoic acid 2a

Yield: 0.46 g (56%); mp 235–235 °C (lit.[25] mp 220–222 °C). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[2]

4.2.2. (±)-3-Amino-3-(3-fluorophenyl)propanoic acid 2b

Yield: 0.52 g (57%); mp 237–239 °C. The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[10c]

4.2.3. (±)-3-Amino-3-(3,5-dichlorophenyl)propanoic acid 2c

Yield: 0.51 g (44%); mp 225–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.74–2.88 (2H, m, CH_2), 4.59–4.63 (1H, m, CH), 7.41 (2H, m, Ar), 7.53 (1H, m, Ar). ^{13}C NMR (100.62 MHz, DMSO-d_6) δ (ppm): 40.2, 51.7, 125.6, 129.0, 134.9, 139.5, 176.7.

4.2.4. (±)-3-Amino-3-(3,4-dimethoxyphenyl)propanoic acid 2d

Yield: 0.52 g (46%); mp 238–240 °C (lit.[26] mp 248–249 °C). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[27]

4.2.5. (±)-3-Amino-3-benzo[1,3]dioxol-5-ylpropanoic acid 2e

Yield: 0.54 g (52%); mp 230–233 °C. The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[5b]

4.3. General procedure for the syntheses of racemic β-amino esters 3a–e

To 40 mL of EtOH was added dropwise 0.55 mL of $SOCl_2$, with the temperature being kept under −10 °C with saline ice. To this solution, **2a–e** (5 mmol) were added. The mixture was stirred at 0 °C for 30 min, and then at room temperature for 3 h, and finally heated at reflux for 1 h. The solvent was evaporated off and the resulting **3a–e**·HCl were recrystallized from EtOH and Et_2O. Treatment of **3a–e**·HCl with aqueous KOH resulted in the free **3a–e** as pale-yellow oils.

4.3.1. Hydrochloride salt of ethyl (±)-3-amino-3-phenylpropanoate 3a·HCl

Yield: 1.05 g (91%); mp 141–144 °C (lit.[25] mp 138–141 °C). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[25]

4.3.2. Hydrochloride salt of ethyl (±)-3-amino-3-(3-fluorophenyl)propanoate hydrochloride 3b·HCl

Yield: 1.14 g (92%); mp 148–151 °C. The ^{1}H NMR data are in accordance with those reported in the literature.[28] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.6, 38.4, 51.5, 62.9, 114.5, 114.7, 116.9, 117.1, 131.8, 131.8, 137.2, 161.5, 164.0, 171.3.

4.3.3. Hydrochloride salt of ethyl (±)-3-amino-3-(3,5-dichlorophenyl)propanoate hydrochloride 3c·HCl

Yield: 1.22 g (82%); mp 180–185 °C. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 1.13–1.17 (3H, t, J = 7.16 Hz, CH_3), 3.06–3.21 (2H, m, CH_2CO), 4.08–4.15 (2H, m, CH_2CH_3), 4.77–4.81 (1H, t, J = 7.27 Hz, CH), 7.44–7.45 (2H, m, Ar), 7.54 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.6, 38.2, 51.0, 62.9, 126.4, 130.1, 135.8, 138.7, 171.5.

4.3.4. Hydrochloride salt of ethyl (±)-3-amino-3-(3,4-dimethoxyphenyl)propanoate hydrochloride 3d·HCl

Yield: 1.27 g (88%); mp 218–221 °C (lit.[29] mp 206–208 °C). ^{1}H NMR (400 MHz, D_2O) δ (ppm): 1.10–1.14 (3H, t, J = 7.15 Hz, CH_3), 3.05–3.21 (2H, m, CH_2CO), 3.84 (1H, s, OCH_3), 3.86 (1H, s, OCH_3), 4.07–4.12 (2H, m, CH_2CH_3), 4.73–4.76 (1H, t, J = 7.44 Hz, CH), 7.04–7.09 (3H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.1, 37.9, 51.1, 55.8, 62.2, 110.5, 112.2, 120.5, 128.1, 148.6, 171.4.

4.3.5. Hydrochloride salt of ethyl (±)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoate hydrochloride 3e·HCl

Yield: 1.26 g (92%); mp 189–191 °C. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 1.10–1.13 (3H, t, J = 7.15 Hz, CH_3), 3.00–3.15 (2H, m, CH_2CO), 4.05–4.11 (2H, m, CH_2CH_3), 4.68–4.70 (1H, t, J = 7.48 Hz, CH), 5.96 (2H, s, OCH_2O), 6.88–6.94 (3H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.2, 37.9, 51.5, 62.5, 101.6, 107.3, 121.5, 128.6, 148.2, 171.6.

4.4. Typical small-scale enzyme tests

Racemic **3a** (0.05 M solution) in an organic solvent or H_2O (1 mL) was added to the enzyme tested (10, 20, 30, 40, 50 or 75 mg mL^{-1}), followed by H_2O (0, 0.5, 1, 2 or 15 equiv). The mixture was shaken at either 3, 25, 45 or 60 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analysing them by GC.

4.5. General procedure for the preparative-scale resolutions of 3a-e

Racemic compounds **3a–e** (3 mmol) were dissolved in *i*-Pr_2O (25 mL). Lipase PS (0.75 g, 30 mg mL^{-1}) and H_2O (27 μL, 1.5 mmol) were added and the mixture was shaken in an incubator shaker at 45 °C for 16–74 h (Table 4). The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residues (*R*)-**4a–e** were immediately hydrolysed by refluxing (5 h) with 18% aqueous HCl solution (6 mL) to give (*R*)-**6a–e**·HCl (in the case of **3e**, the unreacted **4e** was hydrolysed by shaking at room temperature for 12 h with 3.6% aqueous HCl solution). The filtered off enzyme was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off, yielding the crystalline (*S*)-**5a–e**.

When (*S*)-**5a–e** (50 mg) were treated with 22% HCl/EtOH (5 mL), (*S*)-**7a–e**·HCl were obtained.

4.5.1. Hydrochloride salt of (*R*)-3-amino-3-phenylpropanoic acid 6a

Yield: 266 mg (44%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -4$ (*c* 0.3, H_2O) {lit.[13c] $[\alpha]_D^{25} = -3$ (*c* 0.3, H_2O)}; mp 195–198 °C (lit.[13c] mp 195–198 °C); ee >99%. The ^{1}H NMR data for **6a** are in accordance with those reported in the literature.[13c] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.3, 52.0, 127.5, 129.8, 130.1, 135.6, 174.1.

4.5.2. (*S*)-3-Amino-3-phenylpropanoic acid 5a

Yield: 218 mg (44%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -8$ (*c* 0.27; H_2O) {lit.[13c] $[\alpha]_D^{25} = +7$ (*c* 0.27, H_2O) for (*R*)-**5a**}; mp 251–253 °C (lit.[13c] mp 242–246 °C); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5a** are similar to those for **2a**.

4.5.3. Hydrochloride salt of (*S*)-3-amino-3-phenylpropanoic acid 7a

Quantitative yield; $[\alpha]_D^{25} = +4$ (*c* 0.3, H_2O) {lit.[13c] $[\alpha]_D^{25} = +3$ (*c* 0.28; H_2O)}; mp 197–200 °C (lit.[13c] mp 197–201 °C); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7a** are similar to those for **6a**.

4.5.4. Hydrochloride salt of (*R*)-3-amino-3-(3-fluorophenyl)propanoic acid 6b

Yield: 264 mg (40%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -6.5$ (*c* 0.31, H_2O); mp 181–190 °C (slow melting); ee >99%. The ^{1}H NMR data for **6b** are in accordance with those reported in the literature.[30] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.1, 51.5, 114.4, 114.7, 116.8, 117.1, 123.4, 131.7, 131.8, 137.9, 164.3, 173.9.

4.5.5. (*S*)-3-Amino-3-(3-fluorophenyl)propanoic acid 5b

Yield: 220 mg (40%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -1.8$ (*c* 0.38, H_2O); mp 226–229 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5b** are similar to those for **2b**.

4.5.6. Hydrochloride salt of (*S*)-3-amino-3-(3-fluorophenyl)propanoic acid 7b

Quantitative yield; $[\alpha]_D^{25} = +5.7$ (*c* 0.31, H_2O); mp 185–194 °C (slow melting); ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7b** are similar to those for **6b**.

4.5.7. Hydrochloride salt of (*R*)-3-amino-3-(3,5-dichlorophenyl)propanoic acid 6c

Yield: 349 mg (43%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -5.1$ (*c* 0.34, H_2O); mp 208–210 °C; ee = 97%. ^1H NMR (400 MHz, D_2O) δ (ppm): 3.07–3.13 (2H, m, CH_2), 4.74–4.78 (1H, t, *J* = 7.04 Hz, CH), 7.45 (2H, m, Ar), 7.56 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 37.7, 50.8, 125.8, 129.7, 135.2, 138.5, 173.4.

4.5.8. (*S*)-3-Amino-3-(3,5-dichlorophenyl)propanoic acid 5c

Yield: 309 mg (44%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -5.5$ (*c* 0.38, H_2O); mp 219–221 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5c** are similar to those for **2c**.

4.5.9. Hydrochloride salt of (*S*)-3-amino-3-(3,5-dichlorophenyl)propanoic acid 7c

Quantitative yield; $[\alpha]_D^{25} = +5.7$ (*c* 0.34, H_2O); mp 212–214 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7c** are similar to those for **6c**.

4.5.10. Hydrochloride salt of (*R*)-3-amino-3-(3,4-dimethoxyphenyl)propanoic acid 6d

Yield: 322 mg (41%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -7.9$ (*c* 0.32, H_2O); mp 178–181 °C; ee >99%. ^1H NMR (400 MHz, D_2O) δ (ppm): 2.98–3.14 (2H, m, CH_2), 3.79–3.80 (3H, d, *J* = 3.3 Hz, OCH_3), 3.81–3.82 (3H, d, *J* = 2.1 Hz, OCH_3), 4.68–4.70 (1H, m, CH), 7.02–7.04 (3H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.3, 51.8, 56.2, 56.3, 111.0, 112.6, 120.7, 128.6, 148.9, 149.4, 174.1.

4.5.11. (*S*)-3-Amino-3-(3,4-dimethoxyphenyl)propanoic acid 5d

Yield: 277 mg (41%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = +1.3$ (*c* 0.51, H_2O) {lit.[5b] $[\alpha]_D^{25} = +0.9$ (*c* 0.7, H_2O)}; mp 224–229 °C (lit.[5b] mp 216–219 °C); ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5d** are similar to those for **2d**.

4.5.12. Hydrochloride salt of (*S*)-3-amino-3-(3,4-dimethoxyphenyl)propanoic acid 7d

Quantitative yield; $[\alpha]_D^{25} = +7.2$ (*c* 0.315, H_2O); mp 177–181 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7d** are similar to those for **6d**.

4.5.13. Hydrochloride salt of (*R*)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoic acid 6e

Yield: 324 mg (44%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -8.4$ (*c* 0.33, H_2O); mp 203–207 °C; ee = 97%. The ^1H NMR data for **6e** are in accordance with those reported in the literature.[31] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.0, 51.5, 101.9, 107.5, 109.2, 121.7, 129.4, 148.4, 173.8.

4.5.14. (*S*)-3-Amino-3-benzo[1,3]dioxol-5-ylpropanoic acid 5e

Yield: 289 mg (46%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = +4$ (*c* 0.3, H_2O) {lit.[5b] $[\alpha]_D^{25} = +42.4$ (*c* 0.3, H_2O)}; mp 231–234 °C (lit.[5b] mp 219–220 °C); ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5e** are similar to those for **2e**.

4.5.15. Hydrochloride salt of (*S*)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoic acid 7e

Quantitative yield; $[\alpha]_D^{25} = +8.9$ (*c* 0.33, H_2O); mp 204–208 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7e** are similar to those for **6e**.

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IV.

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Burkholderia cepacia lipase is an excellent enzyme for the enantioselective hydrolysis of β-heteroaryl-β-amino esters

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ABSTRACT

The enantioselective (E >200) lipase PS-catalysed hydrolysis of β-heteroaryl-β-amino esters is described. The reactions were performed with H_2O (0.5 equiv) in either diisopropyl ether or *tert*-butyl methyl ether at 25 °C. The resulting β-heteroaryl-substituted β-amino acid enantiomers were formed in high enantiomeric excess (ee ⩾ 97%) and in good yield (⩾40%).

1. Introduction

In view of their successful applications in peptidomimetics and as valuable building blocks, β-amino acids are currently a focus of pharmaceutical research.[1] β-Heteroaryl-substituted β-amino acids have wide-ranging potential applications as indicated by the following examples. A substituted (*R*)-3-amino-3-(2-pyridyl)propionic acid **6f** moiety has been identified as the β-amino acid component of kedarcidin, a potent antitumour antibiotic.[2] Additionally, the synthetic L-azatyrosine analogue methyl (*R*)-3-amino-3-(5-hydroxy-2-pyridyl)propanoate is an important compound in anticancer research.[3] Several promising antithrombotic fibrinogen receptor antagonists contain a β-heteroaryl-β-amino acid unit. A valuable member of this family is elarofiban (RWJ-53308), which contains (*S*)-3-amino-3-(3-pyridyl)propionic acid **7a**.[4] Elarofiban has progressed successfully through human phase II clinical trials involving oral or intravenous administration.[4d] Compound **7a** has also been used in the synthesis of a peptidomimetic $\alpha_v\beta_3$-receptor antagonist which could be a valuable agent in the treatment of osteoporosis.[5] (*R*)-3-Amino-3-(3-pyridyl)propionic acid **6a** has been tested as a component of an inhibitor of hepatitis C virus (HCV) NS5B polymerase, a valid target for antiviral therapy against HCV.[6] Acylated (*R*)-3-amino-3-(4-methoxy-3-pyridyl)propionic acid has been described as a potent, specific and orally bioavailable antagonist of VLA-4.[7] Therapeutic targets for this receptor include asthma, rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease. Moreover, several heteroaromatic taxanes have been prepared and display good to excellent activity in a microtubule assembly assay in comparison with paclitaxel.[8]

The development of new enantioselective approaches for the preparation of heteroaryl-substituted β-amino acid enantiomers is of high priority, even if numerous asymmetric strategies have been described, for example, (i) asymmetric Mannich reactions;[9] (ii) stereoselective reductions of an enamine;[10] (iii) preparation of a diastereomeric salt with (1*R*,2*S*)-ephedrine or quinine;[11] (iv) enolate additions to a chiral imine;[12] (v) addition of a Reformatsky reagent to a chiral imine;[13] (vi) Michael addition to an α,β-unsaturated ester;[14] or (vii) ozonolysis of a chiral N-acylated allylamine.[15]

In addition to the asymmetric methods,[9–15] a number of enzymatic studies have been reported for the preparation of β-aryl- and β-heteroaryl-β-amino acid enantiomers. As an example, we initially performed the indirect enantioselective resolution of acyclic β-lactams through the acylation of *N*-hydroxymethyl-β-lactams and hydrolysis of the corresponding *N*-hydroxymethyl esters,[16] followed by ring opening of the enantiomeric lactams with aqueous HCl. Next, we devised a direct enzymatic method for the enantioselective ring cleavage of β-lactams.[17a] Later, we extended the method to 4-aryl- and 4-arylalkyl-substituted β-lactams.[17b,c] Recently, on the basis of a newly patented direct enzymatic method,[18] we reported the synthesis of carbocyclic *cis*- and *trans*-β-amino acid enantiomers through enantioselective hydrolysis of β-amino esters in organic media, enantioselective hydrolysis of β-aryl-substituted β-amino esters resulting in biologically valuable enantiomers has also been achieved.[19] Some other methods relate to (i) hydrolysis of N-acylated β-amino esters,[4a,20] (ii) N-acylation of β-amino esters,[21] (iii) hydrolysis of β-amino esters in an aqueous medium[22] or (iv) β-aminotransferase-catalysed amination of β-keto esters.[23]

Herein, we turned our attention to β-heteroaryl-substituted β-amino esters and planned to carry out the lipase-catalysed hydrolysis of the pharmaceutically important substrates **3a**–**g**.

2. Results and discussion

2.1. Syntheses of ethyl 3-amino-3-heteroaryl-propanoates 3a–g

Racemic compounds **2a**–**e** were synthesized by a modified Rodionov synthesis[19] through the reactions of **1a**–**e** with malonic

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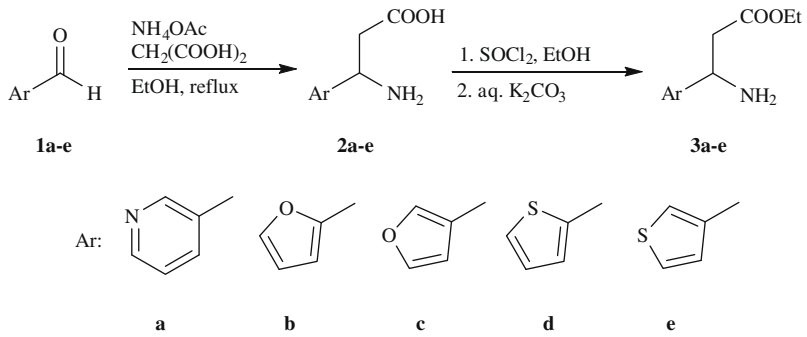


Scheme 1. Synthesis of **3a–e**.

acid in the presence of NH_4OAc in EtOH at reflux (Scheme 1). Compounds **3a–e**·HCl were prepared by the esterification of **2a–e** in the presence of $SOCl_2$ in EtOH. The free bases **3a–e** were liberated by treatment of **3a–e**·HCl with aqueous K_2CO_3 (Scheme 1).

Racemic **3f** and **3g** were prepared by the decarboxylative Blaise reaction[24] of **1f** and **1g** following $Pd(OH)_2$-catalysed reduction of enamines **2f** and **2g** (Scheme 2).

2.2. Lipase-catalysed enantioselective hydrolysis of 3a–g

The preliminary experiments were started with enzyme screening, using **3a** as the model compound (Scheme 3). Among the lipases tested (Table 1), Chyrazyme L-5 (lipase A from *Candida antarctica*) and Lipolase (lipase B from *C. antarctica*) did not exhibit any selectivity towards **3a** at 45 °C in *i*-Pr_2O with 0.5 equiv of H_2O, while PPL (porcine pancreas lipase) and lipase AK (*Pseudomonas fluorescens*) catalysed the reaction with moderate enantioselectivity ($E \leqslant 6$) (entries 1 and 2). As lipase PS (*Burkholderia cepacia*) afforded high enantioselectivity ($E = 99$) (entry 3), we chose this lipase for further investigation.

When we decreased the temperature, the enantioselectivity increased to an excellent value ($E > 200$) (Table 1, entries 4 and 5). Moreover, as the reaction did not slow down at 25 °C (entry 4) when compared with 45 °C (entry 3), we continued further experiments at 25 °C.

Next, we analysed the effects of solvents (Table 2). The reaction rates were highest in *i*-Pr_2O, *t*-BuOMe and *n*-hexane (entries 1, 2 and 3) and lowest in Me_2CO (entry 9). The enantioselectivities were high ($E > 100$) in all cases except in *tert*-amyl alcohol (entry 5). On the basis of our previous results,[25] we developed a fully green method for the resolution of **3a** in a solvent-free system: we achieved high enantioselectivity ($E = 116$), but a low reaction rate (entry 10), when the reaction was performed with 0.5 equiv of H_2O using 30 mg mL^{-1} of lipase PS at 25 °C. Further experiments were carried out in *i*-Pr_2O.

Table 1
Conversion and enantioselectivity of the hydrolysis of **3a**[a]

Entry	Enzyme[c]	*T* (°C)	*t* (h)	Conv. (%)	ee_s[b] (%)	ee_p[b] (%)	*E*
1	PPL	45	17	37	43	74	10
2	Lipase AK	45	17	90	70	8	2
3	Lipase PS	45	17	52	>99	90	99
4	Lipase PS	25	17	50	>99	98	>200
5	Lipase PS	3	72	50	>99	98	>200

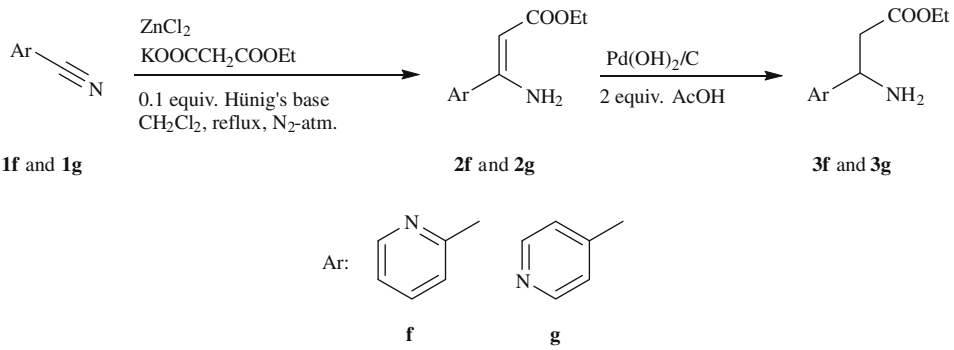
[a] 0.05 M substrate, 1 mL *i*-Pr_2O, 30 mg mL^{-1} enzyme, 0.5 equiv of H_2O.
[b] According to HPLC (Section 4).
[c] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

When we increased the amount of added water (1–5 equiv), the enantioselectivities decreased, while the reaction rates slightly increased (Table 3). We observed that the enantioselectivities were low ($E \leqslant 3$) in a 1/1 (v/v) mixture of H_2O and *i*-Pr_2O, and also in neat H_2O. In a small-scale experiment, we could perform the

Table 2
Effects of solvents on the hydrolysis of **3a**[a]

Entry	Solvent (1 mL)	Conv. (%)	ee_s[b] (%)	ee_p[b] (%)	*E*
1	*i*-Pr_2O	48	>99	98	>200
2	*t*-BuOMe	50	>99	98	>200
3	*n*-hexane	52	>99	92	126
4	Toluene	40	66	98	197
5	*t*-amyl alcohol	31	38	83	16
6	THF	17	20	98	120
7	1,4-dioxane	13	14	98	114
8	$CHCl_3$	6	6	98	105
9	Me_2CO	2	2	98	101
10	—[c]	14	16	98	116

[a] 0.05 M substrate, 30 mg mL^{-1} lipase PS, 0.5 equiv of H_2O at 25 °C after 18 h.
[b] According to HPLC (Section 4).
[c] Without solvent.



Scheme 2. Syntheses of **3f** and **3g**.

Table 3
Effect of added water on the hydrolysis of **3a**[a]

Entry	H_2O (equiv)	Conv. (%)	ee_s[b] (%)	ee_p[b] (%)	*E*
1	0	46	80	98	>200
2	0.5	48	90	98	>200
3	1	53	94	83	38
4	5	55	>99	81	49

[a] 0.05 M substrate, 1 mL *i*-Pr_2O, 30 mg mL^{-1} lipase PS at 25 °C after 7 h.
[b] According to HPLC (Section 4).

hydrolysis without the addition of any added H_2O with excellent enantioselectivity (entry 1). As a conclusion, H_2O in the reaction medium (<0.1%) or at the surface of the enzyme preparation (<5% w/w H_2O) was responsible for the hydrolysis of **3a**.

When more enzyme was added to the reaction mixture, higher reaction rates were observed (Table 4). 75 mg mL^{-1} of lipase PS resulted in the highest rates with excellent enantioselectivity (entry 6). For economic reasons, preparative-scale resolutions were performed with 30 mg mL^{-1} of enzyme.

Table 4
Effect of the quantity of lipase PS on the hydrolysis of **3a**[a]

Entry	Lipase PS[c] (mg mL^{-1})	Conv. (%)	ee_s[b] (%)	ee_p[b] (%)	*E*
1	10	16 (49% after 22 h)	18	98	118
2	20	24	31	98	134
3	30	32	47	98	158
4	40	37	58	98	179
5	50	42	72	98	>200
6	75	49	93	98	>200

[a] 0.05 M substrate, 1 mL *i*-Pr_2O, 0.5 equiv of H_2O at 25 °C after 3 h.
[b] According to HPLC (Section 4).
[c] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

Racemic compounds **3b–g** were also hydrolysed with excellent enantioselectivities (*E* >200) under the optimum conditions, that is, with 0.5 equiv of H_2O in the presence of 30 mg mL^{-1} lipase PS in *i*-Pr_2O at 25 °C. The preparative-scale resolutions of **3a–g** were performed. The products were characterized by good enantiomeric excess (ee ⩾ 97%) at close to 50% conversion. The results are reported in Table 5 and in Section 4.

2.3. Transformations of the enantiomers

The transformations involving the hydrolysis of **4a–g** with aqueous HCl afforded **6a–g** (ee ⩾ 97%) (Scheme 4). Treatment of **5a–g** with 22% HCl/EtOH resulted in the corresponding enantiopure **7a–g** (ee ⩾ 98%).

2.4. Absolute configurations

The absolute configurations and selectivities were proven by comparing the specific rotation values with the literature data (Section 4). The absolute configurations of **3f** and **3g** were given on the basis of comparative specific rotations (**5a–g** negative, **6a–g** positive), assuming the same selectivity of lipase PS towards **3a–g**. Thus, the absolute configurations indicated the (*S*)-selective hydrolysis of **3a–g**.

3. Conclusions

An efficient, direct enzymatic hydrolysis of the desired pharmacologically valuable β-heteroaryl-substituted β-amino acid enantiomers has been devised. The lipase PS-catalysed (*S*)-selective hydrolysis of **3a–g** with H_2O (0.5 equiv) as a nucleophile in *i*-Pr_2O or in *t*-BuOMe at 25 °C (*E* >200) resulted in the enantiomers of **4a–g** (ee ⩾ 97%) and **5a–g** (ee ⩾ 98%) in good yields (⩾40%). The products could be easily separated. Ester enantiomers **4a–g** were readily hydrolysed with 18% aqueous HCl, resulting in acids **6a–g** (ee ⩾ 97%).

4. Experimental

4.1. Materials and methods

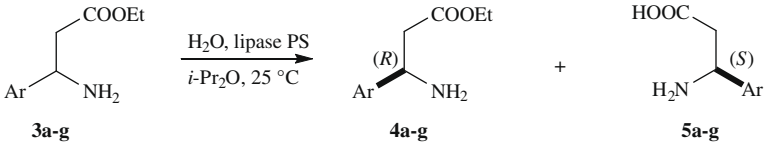
Lipase PS and lipase AK were from Amano Pharmaceuticals, Lipolase (lipase B from *C. antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) and PPL (type II) were from Sigma, and Chyrazyme L-5 was from Novo Nordisk. Before use, lipase PS, lipase AK, CAL-A and PPL (5 g) were dissolved in Tris–HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g) (Sigma). Heteroaromatic aldehydes were from Aldrich. 2-Cyanopyridine and 4-cyanopyridine were from Fluka. Diethylamine (DEA), triethylamine (TEA) and glacial acetic acid (AcOH) were from Aldrich. Triethylammonium acetate buffer (TEAA) was prepared by adding AcOH to a 0.1% aqueous solution of TEA to give pH 4.1. The solvents were of the highest analytical grade.

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on a

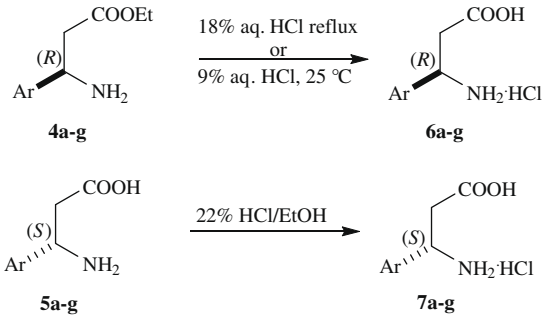
Table 5
Lipase PS-catalysed hydrolysis of **3a-g**[a]

	Time (h)	Conv. (%)	*E*	β-Amino acid·HCl (**6a–g**)				β-Amino acid (**5a–g**)			
				Yield (%)	Isomer	ee (%)	$[\alpha]_D^{25}$ (H_2O)	Yield (%)	Isomer	ee (%)	$[\alpha]_D^{25}$ (H_2O)
3a	40	50	>200	40	(*R*)	>99[b]	+4.1[d]	46	(*S*)	>99[b]	−5.1[e]
3b	60	49	>200	44	(*R*)	97[b]	+5.4[f]	46	(*S*)	>99[b]	−5.8[g]
3c	47	50	>200	49	(*R*)	>99[c]	+5.3[h]	44	(*S*)	>99[c]	−6.7[i]
3d	60	50	>200	46	(*R*)	>99[b]	+4.1[d]	44	(*S*)	>99[b]	−3.1[d]
3e	42	50	>200	46	(*R*)	>99[c]	+4.0[i]	43	(*S*)	>99[c]	−3.2[f]
3f	67	50	>200	45	(*R*)	98[b]	+9.7[f]	42	(*S*)	>99[b]	−18.2[f]
3g	24	50	>200	43	(*R*)	97[b]	+3.2[j]	45	(*S*)	98[b]	−11.7[j]

[a] 30 mg mL^{-1} lipase PS in *i*-Pr_2O, 0.5 equiv of H_2O at 25 °C.
[b] According to HPLC.
[c] According to GC.
[d] *c* 0.33.
[e] *c* 0.41.
[f] *c* 0.32.
[g] *c* 0.52.
[h] *c* 0.42.
[i] *c* 0.34.
[j] *c* 0.36.



Scheme 3. Lipase PS-catalysed enantioselective hydrolysis of **3a–g** (for the meanings of letters **a–g**, see Schemes 1 and 2).



Scheme 4. Transformation of **4a–g** to **6a–g** and **5a–g** to **7a–g** (for the meanings of letters **a–g**, see Schemes 1 and 2).

Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. Elemental analyses (CHNS) corresponded closely (within ±3%) with the calculated ones in all cases.

In a typical small-scale enzyme test, racemic **3a** (0.05 M solution) in an organic solvent or in a 1/1 (v/v) mixture of *i*-Pr_2O and H_2O or in neat H_2O (1 mL) was added to the enzyme tested (10, 20, 30, 40, 50 or 75 mg mL^{-1}), followed by H_2O (0, 0.5, 1 or 5 equiv). The mixture was shaken at 3, 25 or 45 °C.

The ee values for the unreacted β-amino ester and the β-amino acid enantiomers produced were determined by HPLC or GC as follows:

Compounds **4a**, **4b**, **4d** and **5a**, **5b**, **5d**: HPLC [**4a**, **4b**, **4d** were pre-column hydrolysed with aq HCl to **6a**, **6b**, **6d**]; Chirobiotic TAG column (4.6 mm × 250 mm); eluent: MeOH/AcOH/TEA (100/0.1/0.1); flow rate: 0.8 mL min^{-1} for **5a**, **5b**, 0.3 mL min^{-1} for **5d**; detection at 205 nm; retention times (min) for **5a**: 33.70, **6a**: 26.95; **5b**: 12.98, **6b**: 14.25; **5d**: 39.62, **6d**: 36.86.

Compounds **4c**, **4e** and **5c**, **5e**: GC [**5c**, **5e** were pre-column derivatized[26] with (i) CH_2N_2 (**Caution!** derivatization with CH_2N_2 should be performed under a well-working hood) and (ii) **5c** with $(PrCO)_2O$, **5e** with $(EtCO)_2O$ in the presence of 4-dimethylaminopyridine; **4c** with $(PrCO)_2O$; and **4e** with $(EtCO)_2O$]; Chirasil-L-Val column (20 m), 130 °C for **4c** and **5c**, 140 °C for **4e** and **5e**; column flow: 0.7 mL min^{-1}; retention times (min) for **4c**: 43.78 (antipode: 44.58); **5c**: 33.98 (antipode: 32.09); **4e**: 43.13 (antipode: 44.55); **5e**: 34.44 (antipode: 33.20).

Compounds **4f** and **5f**: HPLC, [**5f** was pre-column derivatized[26] with CH_2N_2, and **4f** was pre-column hydrolysed with aq HCl to **6f**, then derivatized with CH_2N_2]; Chiralpak IA column (4.6 mm × 250 mm); eluent: *n*-hexane (0.1% DEA)/EtOH (80/20); flow rate: 0.5 mL min^{-1}; detection at 250 nm; retention times (min) for **5f**: 30.11, **6f**: 25.88.

Compounds **4g** and **5g**: HPLC [**4g** was pre-column hydrolysed with aq HCl to **6g**]; Chirobiotic TAG column (4.6 mm × 250 mm); eluent: TEAA/MeOH (20/80); flow rate: 0.15 mL min^{-1}; detection at 250 nm; retention times (min) for **5g**: 67.80, **6g**: 70.85.

4.2. General procedure for the synthesis of racemic β-amino acids 2a–e

The synthesis was based on a modified Rodionov synthesis.[19] To a solution of the corresponding **1a–e** (5 mmol) in EtOH (40 mL) were added malonic acid (1 equiv) and NH_4OAc (2 equiv). The mixture was refluxed for 6 h. The resulting precipitated crystals were filtered off, washed with Me_2CO and recrystallized.

4.2.1. (±)-3-Amino-3-(3-pyridyl)propanoic acid 2a

Yield: 0.39 g (44%), white crystals; mp 225–228 °C (recrystallized from H_2O/Me_2CO) lit.[27] mp 224–228 °C. The ^1H NMR data are in accordance with those reported in the literature.[27] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 40.5, 51.2, 125.7, 133.5, 137.4, 148.0, 149.9, 177.3.

4.2.2. (±)-3-Amino-3-(2-furyl)propanoic acid 2b

Yield: 0.34 g (44%), brown crystals; mp 222–225 °C (decomp.) (recrystallized from H_2O/Me_2CO) lit.[17] mp 219–221 °C). The ^1H NMR data are in accordance with those reported in the literature.[17] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.1, 46.6, 109.7, 111.3, 144.6, 149.3, 177.4.

4.2.3. (±)-3-Amino-3-(3-furyl)propanoic acid 2c

Yield: 0.18 g (23%), dark-brown crystals; mp 232–235 °C (decomp.) (recrystallized from H_2O/Me_2CO) lit.[21] mp >250 °C. The ^1H NMR data are in accordance with those reported in the literature.[21] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 39.5, 45.0, 108.2, 121.4, 141.2, 144.7, 177.5.

4.2.4. (±)-3-Amino-3-(2-thienyl)propanoic acid 2d

Yield: 0.36 g (42%), white crystals; mp 225–228 °C (recrystallized from H_2O/Me_2CO) lit.[21] mp 225–228 °C. The ^1H NMR data are in accordance with those reported in the literature.[21] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 41.6, 48.9, 108.2, 127.9, 128.3, 128.4, 177.6.

4.2.5. (±)-3-Amino-3-(3-thienyl)propanoic acid 2e

Yield: 0.35 g (41%), pale-brown crystals; mp 237–239 °C (recrystallized from H_2O/Me_2CO) lit.[21] mp 239–242 °C. The ^1H NMR data are in accordance with those reported in the literature.[21] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 40.6, 48.8, 124.7, 126.3, 128.2, 136.9, 177.3.

4.3. General procedure for the syntheses of racemic β-amino esters 3a–e

To 40 mL of EtOH were added dropwise 0.47 mL (1.3 equiv) of $SOCl_2$, with the temperature being kept under −10 °C with saline ice. To this solution, **2a–e** (5 mmol) was added. The mixture was stirred at 0 °C for 30 min, and then at room temperature for 3 h, and then finally heated at reflux for 1 h. The solvent was evaporated off and the resulting **3a–f**·HCl were recrystallized from EtOH and Et_2O. Treatment of **3a–e**·HCl with aqueous K_2CO_3 resulted in the **3a–e** as oils.

4.3.1. Ethyl (±)-3-amino-3-(3-pyridyl)propanoate hydrochloride 3a·HCl

Yield: 0.83 g (82%), white crystals; mp 168–171 °C (recrystallized from EtOH). The ^1H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[28]

4.3.2. Ethyl (±)-3-amino-3-(2-furyl)propanoate hydrochloride 3b·HCl

Yield: 1.01 g (92%), brown crystals; mp 95–97 °C. The ^{1}H NMR data are in accordance with those reported in the literature.[21] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.7, 36.2, 45.5, 63.1, 110.4, 111.4, 145.0, 148.2, 171.9.

4.3.3. Ethyl (±)-3-amino-3-(3-furyl)propanoate hydrochloride 3c·HCl

Yield: 0.93 g (85%), brown crystals; mp 118–120 °C. The ^{1}H NMR data are in accordance with those reported in the literature.[21] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.7, 38.0, 44.2, 63.0, 108.8, 120.4, 141.4, 144.8, 171.8.

4.3.4. Ethyl (±)-3-amino-3-(2-thienyl)propanoate hydrochloride 3d·HCl

Yield: 1.07 g (91%), off-white crystals; mp 109–111 °C. The ^{1}H NMR data are in accordance with those reported in the literature.[21] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 14.0, 39.5, 47.7, 63.4, 128.4, 128.5, 128.8, 137.8, 172.1.

4.3.5. Ethyl (±)-3-amino-3-(3-thienyl)propanoate hydrochloride 3e·HCl

Yield: 1.04 g (88%), brown crystals; mp 88–90 °C. The ^{1}H NMR data are in accordance with those reported in the literature.[21] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.7, 38.5, 47.5, 63.0, 125.4, 126.2, 128.6, 136.2, 172.2.

4.4. Synthesis of β-enamino esters 2f and 2g

Compounds **2f** and **2g** were synthesized by a decarboxylative Blaise reaction according to a literature method.[24] To the solution of the corresponding **1f** and **1g** (5 mmol) in CH_2Cl_2 (5 mL) were added $KOOCCH_2COOEt$ (1.5 equiv), dry $ZnCl_2$ (0.5 equiv) and Hünig's base (0.1 equiv). After refluxing for 7 h under a nitrogen atmosphere, the mixture was cooled to room temperature and saturated NH_4Cl solution (5 mL) was added. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo, resulting in the crude solid.

4.4.1. Ethyl 3-amino-3-(2-pyridyl)propenoate 2f

Yield: 0.82 g (85%), pale-yellow crystals; mp 58–59 °C (recrystallized from *n*-hexane). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[24]

4.4.2. Ethyl 3-amino-3-(4-pyridyl)propenoate 2g

Yield: 0.80 g (83%), yellow crystals; mp 109–111 °C (recrystallized from *n*-hexane/EtOAc 2/1). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.19–1.22 (3H, t, J = 7.10 Hz, CH_2CH_3), 4.05–4.10 (2H, m, CH_2CH_3), 4.90 (1H, s, CH), 7.57–7.59 (2H, m, Ar), 8.65–8.66 (2H, m, Ar). ^{13}C NMR (100.62 MHz, DMSO-d_6) δ (ppm): 14.9, 59.2, 83.4, 121.7, 143.4, 151.0, 158.5, 169.7 (the signals of C2,C6 and C3,C5 are overlapping).

4.5. Synthesis of racemic β-amino esters 3f and 3g

To a solution of **2f** or **2g** (5 mmol) in EtOH (30 mL) were added 0.96 g (10 wt %) of $Pd(OH)_2$ (20% on carbon) and AcOH (2 equiv). The mixture was hydrogenated at atmospheric pressure at room temperature for 24 h. The reaction was stopped by filtering the catalyst off. The solvent was evaporated off, resulting in the acetate salt of **3f** or **3g** as a brown oil. Treatment of **3f**·AcOH and **3g**·AcOH with aqueous K_2CO_3 resulted in the formation of free **3f** and **3g** as yellow oils.

4.5.1. Ethyl (±)-3-amino-3-(2-pyridyl)propanoate 3f

Yield: 0.80 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.23–1.27 (3H, t, J = 7.14 Hz, CH_2CH_3), 2.69–2.91 (2H, m, CH_2CO), 4.13–4.18 (2H, m, CH_2CH_3), 4.44–4.48 (1H, m, CH), 7.18–7.19 (1H, m, Ar), 7.36–7.38 (1H, m, Ar), 7.67–7.68 (1H, m Ar), 8.57–8.58 (1H, m, Ar). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ (ppm): 14.6, 43.4, 54.2, 60.9, 121.4, 122.6, 137.1, 149.7, 172.3.

4.5.2. Ethyl (±)-3-amino-3-(4-pyridyl)propanoate 3g

Yield: 0.72 g (74%); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.19–1.22 (3H, t, J = 7.15 Hz, CH_2CH_3), 2.63–2.71 (2H, m, CH_2CO), 4.13–4.19 (2H, m, CH_2CH_3), 4.41–4.44 (1H, m, CH), 7.30–7.33 (2H, m, Ar), 8.57–8.59 (2H, m, Ar). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ (ppm): 14.6, 44.0, 52.1, 61.2, 121.8, 150.5, 153.7, 171.7.

4.6. General procedure for the preparative-scale resolutions of 3a–g

Racemic compounds **3a**–**g** (3 mmol) were dissolved in *i*-Pr_2O (25 mL). Lipase PS (0.75 g, 30 mg mL^{-1}) and H_2O (27 μL, 1.5 mmol) were added and the mixture was shaken in an incubator shaker at 25 °C for 24–67 h (Table 4). The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residues (*R*)-**4a**–**g** were immediately hydrolysed either by refluxing with 6 mL of 18% aqueous HCl solution for 5 h (**4a**, **4f** and **4g**) or by shaking with 6 mL of 9% aqueous HCl at 25 °C (**4b**–**e**) for 12 h to give (*R*)-**6a**–**g**·HCl. The filtered-off enzyme was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off, yielding the crystalline (*S*)-**5a**–**g**. When (*S*)-**5a**–**g** (50 mg) were treated with 22% HCl/EtOH (5 mL), (*S*)-**7a**–**g** were obtained.

4.6.1. Hydrochloride salt of (*R*)-3-amino-3-(3-pyridyl)propanoic acid 6a

Yield: 243 mg (40%), white crystals; recrystallized from MeOH and Et_2O; $[\alpha]_D^{25} = +4.1$ (*c* 0.33, H_2O); mp 220–223 °C (with decomp.); ee >99%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.26–3.41 (2H, m, CH_2), 5.18–5.22 (1H, t, J = 6.98 Hz, CH), 8.24–8.28 (1H, m, Ar), 8.83–8.85 (1H, m, Ar), 8.95–8.97 (1H, m, Ar), 9.07 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 37.6, 49.1, 128.6, 136.2, 141.8, 143.2, 146.0, 172.6. When **4a** was treated with 5% TFA/EtOH (5 mL), **4a**·TFA was obtained {yellow oil, $[\alpha]_D^{25} = -2.3$ (*c* 4, DMF) lit.[14a] $[\alpha]_D^{25} = +3.3$ (*c* 10, DMF) for the (*S*) enantiomer}.

4.6.2. (*S*)-3-Amino-3-(3-pyridyl)propanoic acid 5a

Yield: 230 mg (46%), white crystals; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -5.1$ (*c* 0.41, H_2O); mp 212–215 °C (with decomp.); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5a** are similar to those for **2a**.

4.6.3. Hydrochloride salt of (*S*)-3-amino-3-(3-pyridyl)propanoic acid 7a

Quantitative yield, white crystals; $[\alpha]_D^{25} = -3.9$ (*c* 0.33; H_2O); mp 218–220 °C (with decomp.); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7a** are similar to those for **6a**.

4.6.4. Hydrochloride salt of (*R*)-3-amino-3-(2-furyl)propanoic acid 6b

Yield: 253 mg (44%), off-white crystals; recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = +5.4$ (*c* 0.32; H_2O); mp 170–173 °C; ee = 97%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.17–3.20 (2H, m, CH_2), 4.94–4.98 (1H, m, CH), 6.56–6.58 (1H, m, Ar), 6.64–6.65 (1H, m, Ar), 7.65–7.66 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 35.9, 45.5, 110.2, 111.4, 144.9, 148.3, 173.9. When **4b** (20 mg) was treated with 22% HCl/EtOH (5 mL), **4b**·HCl was

obtained {brown oil, $[\alpha]_D^{25} = +9.3$ (*c* 0.97, MeOH) lit.[21] $[\alpha]_D^{25} = +9.5$ (*c* 1.00, MeOH)}.

4.6.5. (*S*)-3-Amino-3-(2-furyl)propanoic acid 5b

Yield: 213 mg (46%), white crystals; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -5.8$ (*c* 0.52, H_2O) lit.[11b] $[\alpha]_D^{25} = +12.9$ (*c* 1.00, H_2O) for (+)-3-amino-3-(2-furyl)propanoic acid, which could be assigned to the L series,[11b] but the absolute configuration was not given; mp 208–211 °C (with decomp.) lit.[11b] mp 205–207 °C (with decomp.); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5b** are similar to those for **2b**.

4.6.6. Hydrochloride salt of (*S*)-3-amino-3-(2-furyl)propanoic acid 7b

Quantitative yield, off-white crystals; $[\alpha]_D^{25} = -4.9$ (*c* 0.32, H_2O); mp 197–200 °C; ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7b** are similar to those for **6b**.

4.6.7. Hydrochloride salt of (*R*)-3-amino-3-(3-furyl)propanoic acid 6c

Yield: 282 mg (49%), off-white crystals; recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = +5.3$ (*c* 0.42, H_2O); mp >145 °C (with decomp.); ee >99%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.11–3.24 (2H, m, CH_2), 4.87–4.91 (1H, m, CH), 6.68 (1H, m, Ar), 7.66 (1H, m, Ar), 7.78 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 37.6, 44.3, 108.8, 121.0, 142.0, 145.3, 174.0. When **4c** (20 mg) was treated with 22% HCl/EtOH (5 mL), **4c**·HCl was obtained {brown oil, $[\alpha]_D^{25} = +6.4$ (*c* 1.30, MeOH) lit.[21] $[\alpha]_D^{25} = +7.25$ (*c* 1.00, MeOH)}.

4.6.8. (*S*)-3-Amino-3-(3-furyl)propanoic acid 5c

Yield: 203 mg (44%), white crystals; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -6.7$ (*c* 0.34, H_2O); mp >219 °C (with decomp.); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5c** are similar to those for **2c**.

4.6.9. Hydrochloride salt of (*S*)-3-amino-3-(3-furyl)propanoic acid 7c

Quantitative yield, off-white crystals; $[\alpha]_D^{25} = -4.6$ (*c* 0.42, H_2O); mp >142 °C (with decomp.); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7c** are similar to those for **6c**.

4.6.10. Hydrochloride salt of (*R*)-3-amino-3-(2-thienyl)propanoic acid 6d

Yield: 287 mg (46%), off-white crystals; recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = +4.1$ (*c* 0.33, H_2O); mp 183–186 °C; ee >99%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.20–3.25 (2H, m, CH_2), 5.15–5.18 (1H, t, *J* = 7.00 Hz, CH), 7.18–7.21 (1H, m, Ar), 7.35–7.36 (1H, m, Ar), 7.61–7.62 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.9, 47.4, 128.1, 128.2, 128.4, 137.6, 173.2. When **4d** (20 mg) was treated with 22% HCl/EtOH (5 mL), **4d**·HCl was obtained {yellow oil, $[\alpha]_D^{25} = +6.8$ (*c* 1.07, MeOH) lit.[21] $[\alpha]_D^{25} = +4.6$ (*c* 1.00, MeOH)}.

4.6.11. (*S*)-3-Amino-3-(2-thienyl)propanoic acid 5d

Yield: 228 mg (44%), white crystals; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -9.9$ (*c* 0.41, H_2O) lit.[11b] $[\alpha]_D^{25} = +15.3$ (*c* 1.00, H_2O) for (+)-3-amino-3-(2-thienyl)propanoic acid, which could be assigned to the L series,[11b] but the absolute configuration was not given; mp 212–215 °C (with decomp.) lit.[11b] mp 206–208 °C (with decomp.); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5d** are similar to those for **2d**.

4.6.12. Hydrochloride salt of (*S*)-3-amino-3-(2-thienyl)propanoic acid 7d

Quantitative yield, off-white crystals; $[\alpha]_D^{25} = -3.1$ (*c* 0.33, H_2O); mp 184–187 °C; ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7d** are similar to those for **6d**.

4.6.13. Hydrochloride salt of (*R*)-3-amino-3-(3-thienyl)propanoic acid 6e

Yield: 274 mg (44%), brown crystals; recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = +4.0$ (*c* 0.34, H_2O); mp >150 °C (with decomp.); ee >99%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.20–3.25 (2H, m, CH_2), 5.15–5.18 (1H, t, J = 7.00 Hz, CH), 7.18–7.21 (1H, m, Ar), 7.35–7.36 (1H, m, Ar), 7.61–7.62 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.9, 47.4, 128.1, 128.2, 128.4, 145.3, 174.0. When **4e** (20 mg) was treated with 22% HCl/EtOH (5 mL), **4e**·HCl was obtained {brown oil, $[\alpha]_D^{25} = +2.3$ (*c* 1.00, MeOH) lit.[21] $[\alpha]_D^{25} = +1.00$ (*c* 1.00, MeOH)}.

4.6.14. (S)-3-Amino-3-(3-thienyl)propanoic acid 5e

Yield: 221 mg (43%), white crystals; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -3.2$ (*c* 0.32, H_2O); mp 220–222 °C (with decomp.); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5e** are similar to those for **2e**.

4.6.15. Hydrochloride salt of (*S*)-3-amino-3-(3-thienyl)propanoic acid 7e

Quantitative yield, pale-brown crystals; $[\alpha]_D^{25} = -3.6$ (*c* 0.34, H_2O); mp >140 °C (with decomp.); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7e** are similar to those for **6e**.

4.6.16. Hydrochloride salt of (*R*)-3-amino-3-(2-pyridyl)propanoic acid 6f

Yield: 262 mg (45%), white crystals; recrystallized from MeOH and Et_2O; $[\alpha]_D^{25} = +9.7$ (*c* 0.32, H_2O); mp 155–158 °C; ee = 98%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.24–3.30 (2H, m, CH_2), 5.00–5.05 (1H, m, CH), 7.61–7.67 (2H, m, Ar), 8.07–8.12 (1H, m, Ar), 8.69–8.72 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 37.1, 51.1, 125.2, 127.4, 145.5, 145.7, 172.8.

4.6.17. (*S*)-3-Amino-3-(2-pyridyl)propanoic acid 5f

Yield: 209 mg (42%), white crystals; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -18.2$ (*c* 0.32; H_2O); mp 208–210 °C; ee >99%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.25–3.28 (2H, m, CH_2), 5.02–5.05 (1H, t, *J* = 6.82 Hz, CH), 7.65–7.77 (2H, m, Ar), 8.13–8.19 (1H, m, Ar), 8.70–8.71 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 40.2, 53.3, 122.7, 124.7, 138.9, 148.2, 153.7, 176.8.

4.6.18. Hydrochloride salt of (*S*)-3-amino-3-(2-pyridyl)propanoic acid 7f

Quantitative yield, white crystals; $[\alpha]_D^{25} = -8.6$ (*c* 0.31; H_2O); mp 159–162 °C; ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7f** are similar to those for **6f**.

4.6.19. Hydrochloride salt of (*R*)-3-amino-3-(4-pyridyl)propanoic acid 6g

Yield: 251 mg (43%), white crystals; recrystallized from MeOH and Et_2O; $[\alpha]_D^{25} = +3.2$ (*c* 0.36, H_2O); mp >195 °C (with decomp.); ee = 97%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.29–3.39 (2H, m, CH_2), 5.20–5.24 (1H, m, CH), 8.25–8.28 (2H, m, Ar), 8.95–8.98 (2H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 37.5, 50.8, 126.5, 142.8, 155.9, 173.1.

4.6.20. (*S*)-3-Amino-3-(4-pyridyl)propanoic acid 5g

Yield: 224 mg (45%), white crystals; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -11.7$ (*c* 0.36, H_2O); mp 220–223 °C (with decomp.); ee = 98%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 2.92–2.95 (2H, m, CH_2), 4.77–4.78 (1H, t, *J* = 7.09 Hz, CH), 7.56–7.58 (2H, m, Ar), 8.66–8.67 (2H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 40.5, 55.0, 122.8, 146.1, 150.0, 176.4.

4.6.21. Hydrochloride salt of (*S*)-3-amino-3-(4-pyridyl)propanoic acid 7g

Quantitative yield, white crystals; $[\alpha]_D^{25} = -3.6$ (*c* 0.35, H_2O); mp >191 °C (with decomp.); ee = 98%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7g** are similar to those for **6g**.

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V.

Improved enzymatic syntheses of valuable β-arylalkyl-β-amino acid enantiomers†

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The enantioselective ($E \sim 200$) *Burkholderia cepacia*-catalysed hydrolyses of β-amino esters with H_2O (0.5 equiv.) in *t*-BuOMe or in *i*-Pr_2O at 45 °C are described. The enantiomers of biologically relevant β-arylalkyl-substituted β-amino acids, and especially (*R*)-3-amino-3-(2,4,5-trifluorophenyl)butanoic acid, the intermediate of the new antidiabetic drug sitagliptine, were prepared with high enantiomeric excesses (*ee* ≥96%) and in good yields (≥42%).

Introduction

In recent years, extensive investigations have been carried out on the chemistry of β-amino acids, in particular because of their importance in pharmaceutical research.[1] Optically pure β-arylalkyl-substituted β-amino acids have wide-ranging applications, *e.g.* β-peptides containing an (*S*)-homo-β-phenylalanine unit (**5c**), such as a matrix metalloproteinase-2 inhibitor β-tetrapeptide, utilized for the diagnosis of cancer and atherosclerosis,[2] or β-dipeptides, used for studying the conformational behaviour of foldamers.[3] Moreover, various heterocyclic compounds have been tested as modulators of protein kinase B, a potential therapeutic target for diseases associated with abnormal cell growth, cancer, inflammation or metabolic disorders.[4] (*S*)-β-Phenylethyl-β-alanine (**6c**) has been built into a heat shock protein 70 (Hsp70) modulator.[5] Hsp70 probably contributes to a number of diseases, including cancer and neurodegeneration. **6c** has also been applied in an α-helical peptide mimetic compound,[6] while a derivative of the (*R*) enantiomer (**6d**) has been tested as a hepatitis C virus inhibitor.[7] Type 2 diabetes is a major health problem in the 21st century. Unfortunately, the current modes of therapy are associated with undesirable side-effects, such as hypoglycaemia or cardiovascular abnormalities. Dipeptidyl peptidase IV is a new therapeutic target for the treatment of this form of diabetes. Inhibition of this peptidase results in increased levels of incretins (glucagon-like peptide 1 and gastric inhibitory polypeptide), which control the blood glucose concentration.[8] Januvia™ (sitagliptin phosphate) (Fig. 1), the first approved drug for the inhibition of dipeptidyl peptidase IV, contains a β-amino acid subunit, (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid (**11b**).[9] Besides Januvia™, numerous derivatives of **11b** have been synthesized and tested as potential antidiabetic drugs.[10]

Since β-arylalkyl-β-amino acids are of considerable importance, many asymmetric synthetic routes have been developed for their preparation, *e.g.* (i) enantioselective hydrogenation of an enamine,[10e,11] (ii) cross-coupling of an enantiomeric organozinc reagent with aryl iodides,[12] (iii) homologation of an optically pure α-amino acid by the Arndt–Eistert method,[10a,13] (iv) homologation of an optically pure α-amino acid by the formation of a β-amino alcohol following substitution of the OH group with a CN group,[2,14] (v) conversion of L- or D-aspartic acid to a lactone, followed by reduction and substitution,[15] (vi) conjugate addition of an amine to an α,β-unsaturated carbonyl compound,[16] (vii) S_N2 ring opening of an enantiopure β-lactone with a nitrogen-based nucleophile,[17] or (viii) ring opening of an enantiopure β-lactam.[18]

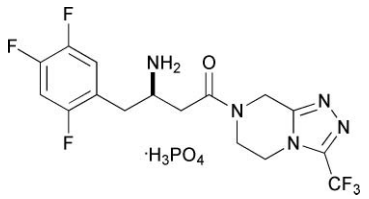

Fig. 1 Januvia™ (sitagliptin phosphate).

Enzymatic methods have also been used for the preparation of enantiopure β-amino acids. Indirect methods are based on acylation of the corresponding *N*-hydroxymethyl-β-lactam or hydrolysis of the *N*-acyloxymethyl-β-lactam.[19] However, indirect methods have some disadvantages, such as the addition and elimination of the hydroxymethyl group or the separation of the product enantiomers by column chromatography.[20] These additional steps can cause relatively low yields. We recently developed an efficient direct enzymatic method for the synthesis of carbocyclic and aryl-substituted β-amino acid enantiomers through the selective ($E > 200$) ring opening of racemic β-lactams.[21] On extension of this method to 4-arylalkyl-substituted β-lactams, surprisingly, low *E* values (≤12) were observed.[22] Preparative-scale resolutions were carried out in two steps, which led to relatively low yields (≤36%). Later, we devised a highly selective direct enzymatic method (*E* usually >100) through the lipase-catalysed hydrolysis of carbocyclic-,[23a] aryl-[23b] and heteroaryl[23c]-substituted β-amino esters.

Our aim was to develop a direct enzymatic method for the resolution of racemic β-arylalkyl-substituted β-amino esters under non-aqueous conditions, resulting in valuable β-amino acid

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enantiomers, *e.g.* (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid (**11b**), an intermediate of sitagliptin.

Results and discussion

Syntheses of (±)-5, (±)-6 and (±)-11

β-Lactams (±)-**3** and (±)-**4** were prepared from alkenes **1** and **2** by the addition of chlorosulfonyl isocyanate (CSI), according to known literature methods.[19b,24] β-Amino esters (±)-**5** and (±)-**6** were synthesized by the ring opening of (±)-**3** and (±)-**4** with 22% HCl/EtOH, followed by treatment with aqueous K_2CO_3 (Scheme 1).

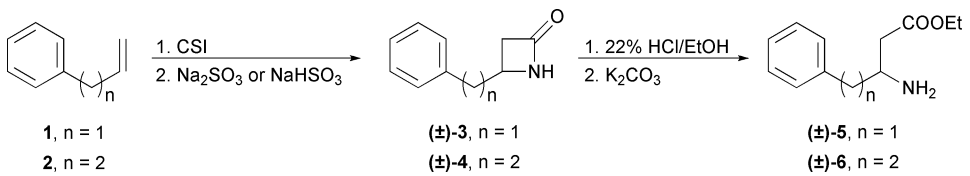
Starting from acid **7**, enamine **10** was prepared by a slightly modified literature method.[10e] β-Amino ester (±)-**11** was obtained by the reduction of enamine **10** with $NaCNBH_3$ in the presence of AcOH (Scheme 2).

Enzymatic hydrolysis of (±)-5, (±)-6 and (±)-11

Preliminary experiments. Preliminary experiments were performed on the hydrolysis of model compound (±)-**5** (Scheme 3). First, a number of lipases were tested in *t*-BuOMe at 25 °C with 0.5 equiv. of H_2O (the quantity of the racemic compound is considered as 1 molar equivalent). The use of organic solvents in enzymatic reactions has numerous advantages, and lipases are able to hydrolyse ester and amide bonds in organic solvents with high enantioselectivity.[25] CAL-A (*Candida antarctica* lipase A) and Lipolase (*Candida antarctica* lipase B) catalysed the reaction with low *E* values (Table 1, entries 1 and 2), while PPL (porcine pancreas lipase) and lipase AK (*Pseudomonas fluorescens*) displayed moderate *E* values (entries 3 and 4). Lipase PS IM (*Burkholderia cepacia*) proved to be the best enzyme for the hydrolysis of (±)-**5** (entry 5), therefore it was chosen for further experiments and for the preparative-scale resolutions.

When the temperature was increased from 25 to 45 °C, the conversion increased considerably (Table 1, entries 5 and 6), but a further increase of the reaction temperature did not exert any additional beneficial effect on the reaction rate (entry 10), though *E* remained high (≥56). The enantioselective ($E > 200$) hydrolysis of (±)-**5** was complete in 72 h, even at 45 °C (entry 7); accordingly, subsequent experiments were planned at 45 °C.

When the amount of enzyme was increased from 30 to 50 and then 75 mg mL^{-1}, the reaction rates clearly increased, while the *E*

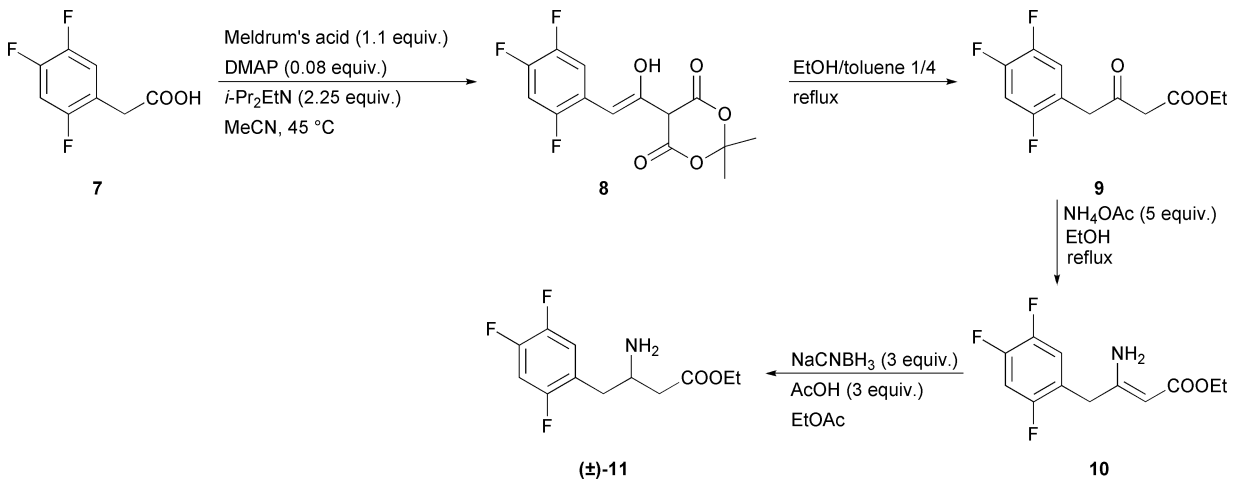


1, n = 1
2, n = 2

(±)-3, n = 1
(±)-4, n = 2

(±)-5, n = 1
(±)-6, n = 2

Scheme 1 Syntheses of (±)-**5** and (±)-**6**.



7

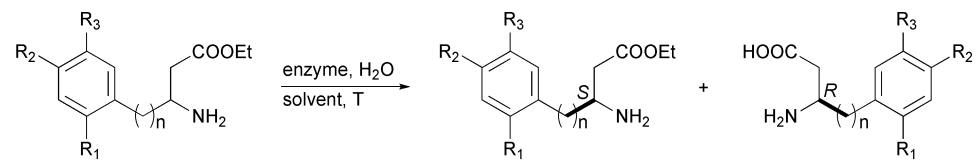
8

9

10

(±)-11

Scheme 2 Synthesis of (±)-**11**.



(±)-5, n = 1, $R_1 = R_2 = R_3$ = H
(±)-6, n = 2, $R_1 = R_2 = R_3$ = H
(±)-11, n = 1, $R_1 = R_2 = R_3$ = F

5a
6a
11a

5b
6b
11b

Scheme 3 Enzyme-catalysed hydrolyses of (±)-**5**, (±)-**6** and (±)-**11**.

Table 1 Conversion, enantiomeric excesses (*ee*) and enantioselectivities (*E*) of the hydrolysis of (±)-**5**[a]

Entry	Enzyme	Enzyme/mg mL^{-1}	*t*/h	*T*/°C	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	CAL-A[c]	50	87	25	7	37	16	2
2	Lipolase	50	87	25	88	15	85	3
3	PPL[c]	50	87	25	2	88	2	16
4	Lipase AK[c]	50	87	25	19	88	18	19
5	Lipase PS IM	50	26	25	14	96	13	56
6	Lipase PS IM	50	26	45	37	96	28	70
7	Lipase PS IM	50	72	45	99	96	51	>200
8	Lipase PS IM	30	26	45	24	96	20	62
9	Lipase PS IM	75	26	45	48	96	33	79
10	Lipase PS IM	50	26	60	39	96	29	72

[a] 0.05 M substrate, 1 mL of *t*-BuOMe, 0.5 equiv. of H_2O. [b] According to HPLC (Experimental section). [c] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

values were apparently not affected (Table 1, entries 6, 8 and 9). For economic reasons, preparative-scale resolutions were carried out with 50 mg mL^{-1} enzyme.

We next analysed the effects of solvents on the reaction rate and *E* (Table 2). The highest *E* values and conversions were observed in *i*-Pr_2O and in *t*-BuOMe (entries 1 and 2). None of the other solvents tested were suitable for the hydrolysis of (±)-**5** (entries 3–5). In view of our earlier results on the vapour-assisted ring opening of carbocyclic *cis*-β-lactams,[26] the hydrolysis of (±)-**5** was attempted under solvent-free conditions (entry 6): the reaction rate increased considerably, while *E* decreased.

Certain additives can influence the enantioselectivity or the reaction rate of lipase-catalysed reactions (Table 3).[27] However, no enhancement relative to H_2O was achieved by the addition of *i*-Pr_2EtN, Et_3N or 2-octanol.

We then attempted to increase the reaction rate by increasing the amount of H_2O (1–10 equiv.) (Table 4). In contrast with our previous experience,[23b,c] the reaction rate decreased on increasing the amount of H_2O; moreover, the degree of hydrolysis was complete and the reaction was fastest without the addition of any H_2O (entry 1). We presume that the poorer solubility of (±)-**5** in the presence of more H_2O can cause a decrease in the reaction rate. In good correlation with our earlier observation,[21] the H_2O present in the reaction medium (<0.1%) or at the surface of the enzyme preparation (<5% w/w H_2O) was responsible for the hydrolysis of (±)-**5**.

Table 2 Effects of solvents on the hydrolysis of (±)-**5**[a]

Entry	Solvent (1 mL)	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	*i*-Pr_2O	60	96	38	90
2	*t*-BuOMe	50	96	34	81
3	Et_2O	8	74	10	7
4	*n*-Hexane	50	89	36	28
5	Toluene	5	82	6	11
6	Solvent-free	74	66	53	11

[a] 0.05 M substrate, 50 mg mL^{-1} lipase PS IM, 0.5 equiv. of H_2O at 45 °C after 35 h. [b] According to HPLC (Experimental section).

Table 3 Effects of additives on the hydrolysis of (±)-**5**[a]

Entry	Additive (1 equiv.)	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	H_2O	44	96	31	76
2	*i*-Pr_2EtN	41	96	30	73
3	Et_3N	43	96	31	75
4	2-Octanol	39	96	29	72

[a] 0.05 M substrate, 1 mL of *t*-BuOMe, 50 mg mL^{-1} lipase PS IM at 45 °C after 31 h. [b] According to HPLC (Experimental section).

Table 4 Effects of added H_2O on the hydrolysis of (±)-**5**[a]

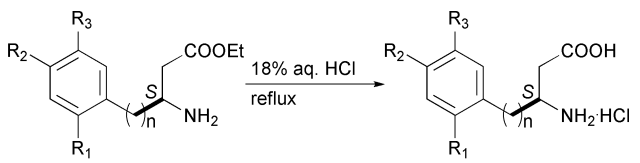
Entry	H_2O (equiv.)	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	0	38	96	28	71
2	0.5	35	96	27	69
3	1	31	96	24	66
4	5	22	96	19	61
5	10	8	96	8	53

[a] 0.05 M substrate, 1 mL of *t*-BuOMe, 50 mg mL^{-1} lipase PS IM at 45 °C after 25 h. [b] According to HPLC (Experimental section).

Racemic **6** and **11** were hydrolysed under the optimized conditions for (±)-**5**: with 0.5 equiv. of H_2O in the presence of 50 mg mL^{-1} lipase PS IM in *t*-BuOMe or in *i*-Pr_2O at 45 °C. When the reaction was performed in *i*-Pr_2O instead of *t*-BuOMe, higher reaction rates and better *E* values were observed (Table 5).

With regard to the results of the preliminary experiments, preparative-scale resolutions were performed with lipase PS IM in *t*-BuOMe or in *i*-Pr_2O with 0.5 equiv. of H_2O at 45 °C. The reactions were stopped at close to 50% conversion, and the products were obtained in good yields (≥42%) and with good *ee* values (≥96%).

Enantiomeric **5a**, **6a** and **11a** were hydrolysed with aqueous HCl, affording **5c**, **6c** and **11c** (*ee* ≥96%) (Scheme 4). Treatment of **5b**, **6b** and **11b** with 22% HCl/EtOH resulted in the corresponding enantiopure **5d**, **6d** and **11d** (*ee* ≥96%) (Scheme 5).



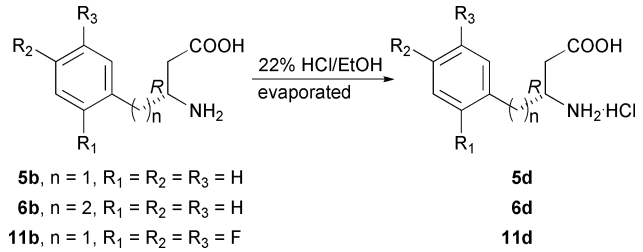
5a, n = 1, $R_1 = R_2 = R_3 = H$ — **5c**
6a, n = 2, $R_1 = R_2 = R_3 = H$ — **6c**
11a, n = 1, $R_1 = R_2 = R_3 = F$ — **11c**

Scheme 4 Syntheses of **5c**, **6c** and **11c**.

Table 5 Effects of solvents on the hydrolyses of (±)-**6** and (±)-**11**[a]

Entry	Substrate	*t*/h	Solvent (1 mL)	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	(±)-**6**	43	*i*-Pr_2O	79	96	45	119
2	(±)-**6**	43	*t*-BuOMe	54	91	37	36
3	(±)-**11**	65	*i*-Pr_2O	85	97	47	179
4	(±)-**11**	65	*t*-BuOMe	61	97	39	123

[a] 0.05 M substrate, 50 mg mL^{-1} lipase PS IM, 0.5 equiv. of H_2O at 45 °C. [b] According to HPLC (Experimental section).



Scheme 5 Syntheses of **5d**, **6d** and **11d**.

The absolute configurations and *E* values were proved by comparing the [α] values with literature data (Experimental section).

Conclusions

A simple and efficient direct enzymatic method has been developed for the synthesis of pharmacologically valuable optically active β-arylalkyl-substituted β-amino acids *via* enantioselective hydrolysis of the corresponding racemic β-amino esters in an organic medium. The *R*-selective hydrolyses of (±)-**5**, (±)-**6** and (±)-**11** were performed with H_2O (0.5 equiv.) as a nucleophile, using *Burkholderia cepacia* lipase (lipase PS IM) as an enzyme in *t*-BuOMe or in *i*-Pr_2O at 45 °C (*E* ~ 200). The enantiomers of **5a**, **6a** and **11a** (*ee* ≥96%), and **5b**, **6b** and **11b** (*ee* ≥96%) were isolated in good yields (≥42%) and could be easily separated. Ester enantiomers **5a**, **6a** and **11a** were readily hydrolysed with 18% aqueous HCl, resulting in acids **5c**, **6c** and **11c** (*ee* ≥96%).

This method offers a better choice for the preparation of β-arylalkyl-substituted β-amino acid enantiomers as compared with the ring opening of the corresponding lactam.[22] It should be mentioned that, although derivatives of **11b** have been prepared by asymmetric routes (see ref. 10*a*, 11 and 18), to the best of our knowledge they have not yet been obtained by enzymatic procedures.

Experimental section

Materials and methods

Lipase PS IM (*Burkholderia cepacia*, immobilized on diatomaceous earth) was a gift of Amano Enzyme Europe Ltd. Lipase AK (*Pseudomonas fluorescens*) was from Amano Pharmaceuticals, Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) and PPL (porcine pancreas lipase type II) were from Sigma, and Chyrazyme L-5 (lipase A from *Candida antarctica*) was from Novo Nordisk. Before use, lipase AK, CAL-A and PPL (5 g) were dissolved in Tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g) (Sigma). Allylbenzene, 4-phenylbut-1-ene and Meldrum's acid were from Aldrich. (2,4,5-Trifluorophenyl)acetic acid was from Matrix Scientific. The solvents were of the highest analytical grade.

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. Elemental analyses (CHNS) corresponded closely (within ±0.3%) with the calculated ones in all cases.

In a typical small-scale enzyme test, (±)-**5**, (±)-**6** or (±)-**11** (0.05 M solution) in an organic solvent (1 mL) was added to the enzyme tested (30, 50 or 75 mg mL^{-1}), followed by H_2O, *i*-Pr_2EtN, Et_3N or 2-octanol (0, 0.5, 1, 5 or 10 equiv.). The mixture was shaken at 25, 45 or 60 °C.

The *ee* values for the unreacted β-amino ester and the β-amino acid enantiomers produced were determined by HPLC as follows:

5a, **6a**, **11a**, **11b**: [**11b** was pre-column derivatized with CH_2N_2[28] (**Caution!** derivatization with CH_2N_2 should be performed under a well-working hood)]: a Chiralpak IA column (4.6 mm × 250 mm); eluent: *n*-hexane (0.1% DEA)–*i*-PrOH (95 5); flow rate: 0.3 mL min^{-1}; detection at 276 nm; retention times (min) for **5a**: 35.2 (antipode: 33.9); for **6a**: 37.5 (antipode: 36.6); for **11a**: 48.5 (antipode: 39.6); for **11b**: 44.4 (antipode: 50.0).

5b: a Chirobiotic TAG column (4.6 mm × 250 mm); eluent: MeOH–AcOH–TEA (100 : 0.1 : 0.1); flow rate: 0.8 mL min^{-1}; detection at 205 nm; retention times (min): 17.2 (antipode: 19.2).

6b: an APEX Octadecyl 5 μ column (0.04 cm × 25 cm); pre-column derivatization with (*S*)-NIFE according to the literature;[29] the mobile phases were H_2O (A) and MeCN (B), both of which contained 0.1% TFA; the gradient slopes were: 95% A + 5% B at 0 min, increased to 25% A + 75% B within 60 min; flow rate: 0.8 mL min^{-1}; room temperature; detection at 205 nm; retention times (min): 42.7 (antipode: 41.9).

Syntheses of β-amino esters (±)-5 and (±)-6

Racemic β-lactams (±)-**3** and (±)-**4** were prepared by the addition of chlorosulfonyl isocyanate to allylbenzene (**1**) or 4-phenylbut-1-ene (**2**), respectively, according to known literature methods.[19b,24] (±)-**3** and (±)-**4** (6.0 mmol) were then refluxed with 22% HCl/EtOH (15 mL) for 8 h, after which the solvent was evaporated off, resulting in the corresponding β-amino ester hydrochlorides (±)-**5**·HCl and (±)-**6**·HCl, which were immediately treated with aqueous K_2CO_3 to afford (±)-**5** and (±)-**6**, as oils.

Ethyl (±)-3-amino-4-phenylbutanoate [(±)-5]. Yield: 1.07 g (86%), a pale-yellow oil; δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.27-1.31

(3 H, t, *J* 7.1, CH_2CH_3), 2.32-2.38 (1 H, dd, *J* 8.7 and 15.9, CH_2COOH), 2.49-2.54 (1 H, dd, *J* 4.2 and 15.9, CH_2COOH), 2.62-2.68 (1 H, dd, *J* 8.1 and 13.4, CH_2Ar), 2.78-2.82 (1 H, dd, *J* 5.6 and 13.4, CH_2Ar), 3.49-3.52 (1 H, m, C*H*), 4.15-4.20 (2 H, q, *J* 7.1 and 14.3, CH_2CH_3), 7.22-7.36 (5 H, m, Ar); δ_C(100.62 MHz; $CDCl_3$; Me_4Si) 14.6, 42.4, 44.4, 50.1, 60.8, 126.9, 129.0, 129.7, 139.0, 172.8.

Ethyl (±)-3-amino-5-phenylpentanoate [(±)-6]. Yield: 1.10 g (83%), a pale-yellow oil. The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[30]

Syntheses of β-amino ester (±)-11

To a mixture of 2,4,5-trifluorophenylacetic acid **7** (2.28 g, 12.0 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (1.90 g, 13.2 mmol), *N*,*N*-dimethylaminopyridine (0.12 g, 0.96 mmol) and *N*,*N*-diisopropylethylamine (4.7 mL, 27.0 mmol) in MeCN (8 mL), trimethylacetylchloride (1.6 mL, 13.2 mmol) was added at 40 °C. The reaction mixture was stirred at 45 °C for 3 h, cooled to 0 °C, and 1 M HCl (20 mL) was then slowly added to the reaction mixture to form a solid. The resulting solid was washed with 20% CH_3CN–H_2O (50 mL) to give 5-(1-hydroxy-2-(2,4,5-trifluorophenyl)ethylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (**8**): 3.37 g (85%), white crystals; mp 99-101 °C (from MeCN) [lit.,[31] 117 °C (decomp.)]. The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[31]

In the next step, **8** (3.30 g, 10.0 mmol) was refluxed in EtOH–toluene 1 : 4 (80 mL) for 3 h. The reaction mixture was subsequently diluted with EtOAc (35 mL), and washed with brine (2 × 50 mL). The organic layer was dried with Na_2SO_4 and evaporated to give ethyl 3-oxo-4-(2,4,5-trifluorophenyl)butanoate (**9**): 2.45 g (94%), white crystals; mp 29-30 °C (from *n*-hexane). δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.30-1.34 (3 H, t, *J* 6.9, CH_2CH_3), 3.55 (2 H, s, $CH_2COOCH_2CH_3$), 3.88 (2 H, s, CH_2Ar), 4.22-4.27 (2 H, q, *J* 7.1 and 14.3, CH_2CH_3), 6.94-7.01 (1 H, m, Ar), 7.04-7.11 (1 H, m, Ar); δ_C(100.62 MHz; $CDCl_3$; Me_4Si) 14.6, 42.4, 49.1, 62.1, 106.1, 119.9, 145.8, 147.8, 155.1, 157.1, 167.1, 198.5.

A mixture of **9** (2.60 g, 10.0 mmol) and NH_4OAc (3.85 g, 50.0 mmol) in EtOH (40 mL) was refluxed for 7 h. The reaction mixture was evaporated, diluted with EtOAc (50 mL) and washed with H_2O (2 × 50 mL). The organic layer was dried with Na_2SO_4 and evaporated to give ethyl 3-amino-4-(2,4,5-trifluorophenyl)but-2-enoate (**10**): 2.31 g (89%), white crystals; mp 115-117 °C (from *n*-hexane and EtOAc). δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.27-1.31 (3 H, t, *J* 7.2, CH_2CH_3), 3.44 (2 H, s, CH_2Ar), 4.12-4.17 (2 H, q, *J* 7.1 and 14.2, CH_2CH_3), 4.59 (1 H, s, CH), 6.94-7.01 (1 H, m, Ar), 7.09-7.15 (1 H, m, Ar); δ_C(100.62 MHz; $CDCl_3$; Me_4Si) 14.9, 35.1, 59.3, 85.8, 105.6, 119.2, 145.7, 147.9, 154.4, 158.8, 169.8.

10 (1.56 g, 6.0 mmol) was dissolved in EtOAc (15 mL), the mixture was cooled to 0 °C, and $NaCNBH_3$ (1.13 g, 18.0 mmol) and glacial AcOH (1.0 mL, 18.0 mmol) were added. After 6 h, the mixture was extracted with 10% Na_2CO_3 (3 × 15 mL), and the organic phase was dried with Na_2SO_4 and evaporated, resulting in crude (±)-**11**. This was then dissolved in 22% HCl/EtOH (10 mL), and the solution was evaporated, resulting in (±)-**11**·HCl, which was immediately treated with aqueous K_2CO_3 to afford (±)-**11**: 0.85 g (54%), a pale-yellow oil; δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.28-1.32 (3 H, t, *J* 7.1, CH_2CH_3), 2.32-2.39 (1 H, dd, *J* 8.5 and 15.9, CH_2COOH), 2.48-2.54 (1 H, dd, *J* 4.2 and 15.9, CH_2COOH), 2.65-2.71 (1 H, dd, *J* 7.7 and 13.7, CH_2Ar), 2.76-2.81 (1 H, dd, *J* 5.8 and 13.7, CH_2Ar), 3.46-3.52 (1 H, m, C*H*), 4.16.4.21 (2 H, q, *J* 7.1 and 14.2, CH_2CH_3), 6.93-6.96 (1 H, m, Ar), 7.08-7.11 (1 H, m, Ar); δ_C(100.62 MHz; $CDCl_3$; Me_4Si) 14.6, 36.8, 42.1, 49.1, 61.0, 105.6, 120.3, 145.3, 147.7, 155.0, 157.0, 172.0.

General procedure for the preparative-scale resolutions of (±)-5, (±)-6 and (±)-11

Racemic (±)-**5**, (±)-**6** and (±)-**11** (3 mmol) were dissolved in *t*-BuOMe [(±)-**5**] or in *i*-Pr_2O [(±)-**6** and (±)-**11**] (15 mL). Lipase PS IM (0.75 g, 50 mg mL^{-1}) and H_2O (27 μL, 1.5 mmol) were added and the mixture was shaken in an incubator shaker at 45 °C for 3–5 d (Table 6). The reaction was stopped by filtering off the enzyme at close to 50% conversion. The solvent was evaporated and the residues (*S*)-**5a**, (*S*)-**6a** and (*S*)-**11a** were immediately hydrolysed by refluxing with 6 mL of 18% aqueous HCl solution for 7 h to give (*S*)-**5c**, (*S*)-**6c** and (*S*)-**11c**. The filtered-off enzyme was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off, yielding crystalline (*R*)-**5b**, (*R*)-**6b** and (*R*)-**11b**. When (*R*)-**5b**, (*R*)-**6b** or (*R*)-**11b** (50 mg) was treated with 22% HCl/EtOH (5 mL), then evaporated, (*R*)-**5d**, (*R*)-**6d** or (*R*)-**11d** was obtained.

***(R)*-3-Amino-4-phenylbutanoic acid (5b).** Yield: 242 mg (45%), white crystals; *ee* = 96%; $[\alpha]_D^{25}$ –4.6 (*c* 0.30 in H_2O) [lit.,[22] +7 (*c* 0.20 in H_2O) for the (*S*) enantiomer]; mp 209-211 °C (from H_2O and Me_2CO) (lit.,[22] 207-210 °C) The ^{1}H NMR data are in accordance with those reported in the literature.[22] δ_C(100.62 MHz; D_2O; Me_4Si) 38.5, 38.6, 51.2, 128.1, 129.6, 130.0, 136.2, 178.3.

Hydrochloride salt of (*S*)-3-amino-4-phenylbutanoic acid (5c). Yield: 272 mg (42%), off-white crystals; *ee* = 96%; $[\alpha]_D^{25}$ +4.6 (*c* 0.36 in H_2O) [lit.,[22] +6 (*c* 0.21 in H_2O)]; mp 174-176 °C (from EtOH and Et_2O) (lit.,[22] 172-175 °C). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[19b]

Table 6 Lipase PS IM-catalysed hydrolyses of (±)-**5**, (±)-**6** and (±)-**11**[a]

				β-Amino acid·HCl (**5c**, **6c**, **11c**)				β-Amino acid (**5b**, **6b**, **11b**)			
	t/d	Conv. (%)	*E*	Yield (%)	Isomer	*ee* (%)[b]	$[\alpha]_D^{25}$	Yield (%)	Isomer	*ee* (%)[b]	$[\alpha]_D^{25}$
(±)-**5**	5	50	194	42	*S*	96	+4.6[c]	45	*R*	96	–4.6[d]
(±)-**6**	3	51	>200	47	*S*	98	+11.5[e]	47	*R*	96	–12.1[f]
(±)-**11**	5	50	>200	44	*S*	96	–9.8[g]	43	*R*	97	+15.5[h]

[a] 50 mg mL^{-1} lipase PS IM in *i*-Pr_2O, 0.5 equiv. of H_2O at 45 °C. [b] According to HPLC (Experimental section). [c] *c* 0.36. [d] *c* 0.30. [e] *c* 0.40. [f] *c* 0.34. [g] *c* 0.32. [h] *c* 0.35.

Hydrochloride salt of (*R*)-3-amino-4-phenylbutanoic acid (5d). Quantitative yield; off-white crystals; *ee* = 96%; $[\alpha]_D^{25}$ −3.9 (*c* 0.35 in H_2O) [lit.,[22] −8 (*c* 0.11 in H_2O)]; mp 169-172 °C (lit.,[22] 182-185 °C). The ^{1}H NMR and ^{13}C NMR data for **5d** are similar to those for **5c**.

(*R*)-3-Amino-5-phenylpentanoic acid (6b). Yield: 272 mg (47%), white crystals; *ee* = 96%; $[\alpha]_D^{25}$ −12.1 (*c* 0.34 in H_2O) [lit.,[22] +24 (*c* 0.28 in H_2O) for the (*S*) enantiomer]; mp 214-216 °C (from H_2O and Me_2CO) (lit.,[22] 215-219 °C). The ^{1}H NMR data are in accordance with those reported in the literature.[22] δ_C(100.62 MHz; D_2O; Me_4Si) 31.3, 34.4, 38.7, 49.6, 127.0, 129.0, 129.4, 141.4, 178.6.

Hydrochloride salt of (*S*)-3-amino-5-phenylpentanoic acid (6c). Yield: 324 mg (47%), off-white crystals; *ee* = 98%; $[\alpha]_D^{25}$ +11.5 (*c* 0.40 in H_2O) [lit.,[22] +12 (*c* 0.21 in H_2O)]; mp 144-146 °C (from EtOH and Et_2O) (lit.,[22] 150-152 °C). The ^{1}H NMR data are in accordance with those reported in the literature.[22] δ_C(100.62 MHz; D_2O; Me_4Si) 31.1, 34.1, 36.3, 48.5, 127.1, 129.0, 129.4, 141.0, 174.8.

Hydrochloride salt of (*R*)-3-amino-5-phenylpentanoic acid (6d). Quantitative yield; off-white crystals; *ee* = 96%; $[\alpha]_D^{25}$ −10.5 (*c* 0.40 in H_2O) [lit.,[22] −15 (*c* 0.21 in H_2O)]; mp 147-149 °C (lit.,[22] 146-148 °C). The ^{1}H NMR and ^{13}C NMR data for **6d** are similar to those for **6c**.

(*R*)-3-Amino-4-phenyl(2,4,5-trifluorophenyl)butanoic acid (11b). Yield: 301 mg (43%), white crystals; *ee* = 97%; $[\alpha]_D^{25}$ +15.5 (*c* 0.35 in H_2O); mp 217-219 °C (from H_2O and Me_2CO). δ_H(400 MHz; D_2O; Me_4Si) 2.48-2.54 (1 H, dd, *J* 7.9 and 16.5, CH_2COOH), 2.59-2.65 (1 H, dd, *J* 5.0 and 16.9, CH_2COOH), 3.08-3.09 (2 H, d, *J* 6.9, CH_2Ar), 3.81-3.88 (1 H, m, C*H*), 7.22-7.27 (1 H, m, Ar), 7.31-7.37 (1 H, m, Ar); δ_C(100.62 MHz; D_2O; Me_4Si) 31.1, 38.0, 49.1, 105.8, 118.9, 145.6, 148.0, 155.1, 157.5, 177.3. To prove the absolute configuration, *N*-Boc-**11b** was prepared by a literature method[11*b*] {white crystals; $[\alpha]_D^{25}$ +24.6 (*c* 0.46 in $CHCl_3$) [lit.,[11*b*] +32.3 (*c* 1.0 in $CHCl_3$)]; mp 112-114 °C (from *n*-hexane) (lit.,[11*b*] 124-125 °C)}.

Hydrochloride salt of (*S*)-3-amino-4-(2,4,5-trifluorophenyl)-butanoic acid (11c). Yield: 356 mg (44%), off-white crystals; *ee* = 96%; $[\alpha]_D^{25}$ −9.8 (*c* 0.32 in H_2O); mp 177-179 °C (from EtOH and Et_2O). δ_H(400 MHz; D_2O; Me_4Si) 2.75-2.89 (2 H, m, CH_2COOH), 3.08-3.19 (2 H, m, CH_2Ar), 3.96-4.05 (1 H, m, C*H*), 7.21-7.28 (1 H, m, Ar), 7.32-7.38 (1 H, m, Ar); δ_C(100.62 MHz; D_2O; Me_4Si) 31.5, 36.1, 48.8, 106.5, 119.4, 146.0, 148.1, 155.4, 156.6, 174.0.

Hydrochloride salt of (*R*)-3-amino-4-(2,4,5-trifluorophenyl)-butanoic acid (11d). Quantitative yield; off-white crystals; *ee* = 97%; $[\alpha]_D^{25}$ +10.6 (*c* 0.31 in H_2O); mp 179-181 °C. The ^{1}H NMR and ^{13}C NMR data for **11d** are similar to those for **11c**.

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