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Biocatalysis as a profound tool in the preparation of highly enantiopure β-amino acids

Arto Liljeblad* and Liisa T. Kanerva

Laboratory of Synthetic Drug Chemistry, University of Turku, Lemminkäisenkatu 5 C, FIN-20520 Turku, Finland

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Abbreviations: Ac, acetyl; Asp, aspartic acid; Bn, benzyl; Bz, benzoyl; Bu, butyl; CAL-A, lipase A from *Candida antarctica*; CAL-B, lipase B from *Candida antarctica*; CRL, lipase from *Candida rugosa*; de, diastereomeric excess; DIPE, diisopropyl ether; *E*, enantiomer ratio; ee, enantiomeric excess; ee_s, enantiomeric excess of the substrate; ee_p, enantiomeric excess of the product; Et, ethyl; Gln, glutamine; His, histidine; Ile, isoleucine; LG, leaving group; MOPS, 4-morpholinepropanesulfonic acid; PDF, peptide deformylase; Ph, phenyl; PG, protective group; PGA, penicillin G amidase; PLE, pig liver esterase; PPL, porcine pancreatic lipase; Pr, propyl; Ser, serine; TBME, *tert*-butyl methyl ether; Thr, threonine; TMS, trimethylsilyl; Trp, tryptophan; Ts, *p*-toluenesulfonyl; Z, benzyloxycarbonyl.
* Corresponding author. Tel.: +358 2 333 7956; fax: +358 2 333 7955; e-mail: artlilje@utu.fi

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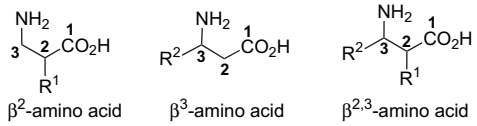
1. Introduction

β-Amino acids have unique pharmacological properties and their utility as building blocks of β-peptides, pharmaceutically important compounds and natural products is of growing interest.[1–6] This arises, for instance, from the ability of β-peptides to fold to secondary structures in predictable ways. Furthermore, the enzymes in the body do not act on β-peptide bonds. Several review articles on the asymmetric synthesis of β-amino acids are available.[7–14] This review covers the enzymatic methodology developed up to the present day for the preparation of various highly enantiopure β^2-, β^3- and $\beta^{2,3}$-amino acids (Scheme 1). Most of the reported methods exploit hydrolytic enzymes in kinetic resolutions of racemic mixtures.

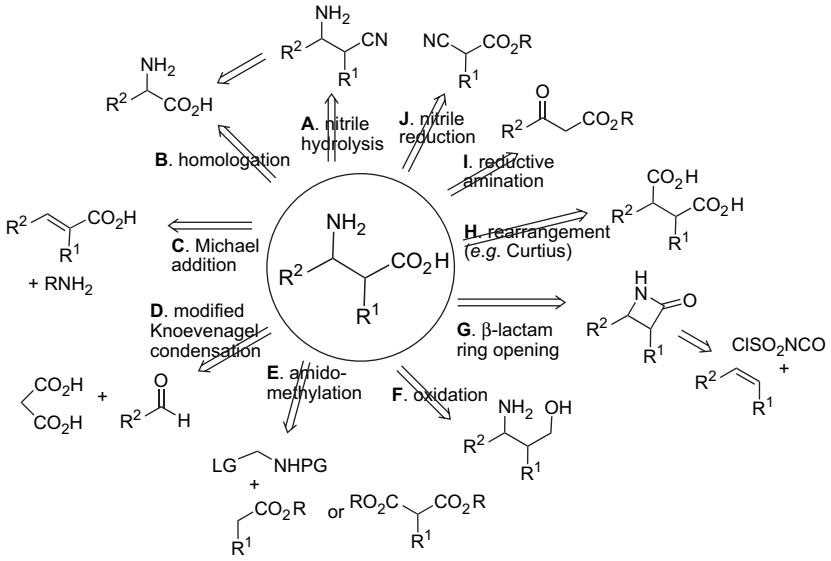


Scheme 1. General structures of β-amino acids.

A plethora of methods have been used for the preparation of racemic β-amino acids. Some useful alternatives are outlined retrosynthetically in Scheme 2. Thus, β-amino acids have been prepared by hydrolysis of β-amino nitriles (A),[15–21] homologation of α-amino acids (B),[22] Michael-type additions to double bonds (C),[15,23–25] Knoevenagel-type condensations of an aldehyde and malonic acid in the presence of ammonium acetate (D),[26–28] amidomethylation of aryl acetic or malonic esters (E),[29–32] oxidation of amino alcohols (F), ring opening of β-lactams (G),[33] transformation of a carboxylic functionality of a dicarboxylic acid into an amine (like the Curtius or Hofmann rearrangements),[34] reductive amination via an enamine (I)[35] and reduction of α-cyano carboxylic esters (J).[36,37] In particular, the Michael-type addition (C) and β-lactam ring opening (G) after formation of the ring from an alkene and chlorosulfonyl isocyanate have been widely employed in the preparation of racemic β^3- and $\beta^{2,3}$-amino acids. Homologation of α-amino acids (B) is usually performed by the Arndt–Eistert homologation, which requires the use of diazomethane. For this reason, it is not suitable for large-scale use. α-Amino acids can also serve as synthons for β-amino nitriles, which can be hydrolysed into β-amino acids (A). Method F, the preparation of β-amino acids by oxidation of 1,3-amino alcohols, is seldom applied due to the limited availability of appropriate 1,3-amino alcohols. In fact, 1,3-amino alcohols are often prepared by reducing β-amino acids.[38–40] In addition to 1,3-amino alcohols, 1,2-amino alcohols serve as synthons for β-amino nitriles in method A. Amino alcohols and dicarboxylic acids (H) form a field of their own, and their preparation in an enantiopure form is excluded from this review.

The main chemo-enzymatic paths for obtaining highly enantiopure β-amino acids are based on the kinetic resolution of racemic β-amino acids or some of their derivatives and intermediates. In this review, the efficiency of the kinetic resolution is described by the enantioselectivity ratio, *E*.[41] Besides kinetic resolutions, biocatalytic studies towards enantiopure β-amino acids encompass homologation of α-amino acids (B) by aminomutases, the Michael addition (method C) and the reductive amination of ketones by β-aminotransferase (I). Reaction steps in methods D, E, F, H and J have not been biocatalytically performed, to the best of our knowledge.

This review is divided into kinetic resolutions and other biotransformations. Each utilized enzyme is discussed separately in a specific section. The resolutions of β^2-amino acids have not yet been studied to the same extent as their β^3- and $\beta^{2,3}$-counterparts. This is possibly due to the more laborious synthesis of β^2-amino acids, which is often based on the use of acrylic, cyanoacetic or malonic ester derivatives,[14] whereas the synthesis of the β^3- and $\beta^{2,3}$-counterparts is relatively straightforward by many methods in Scheme 2.



Scheme 2. Retrosynthetic analysis for the synthesis of β-amino acids.

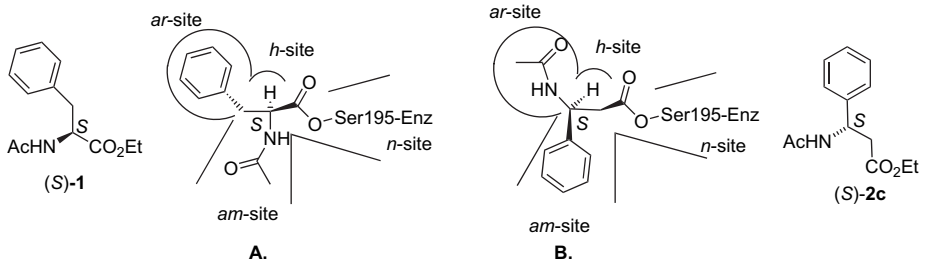
2. Kinetic resolutions

2.1. Enzymes acting on C–N bonds

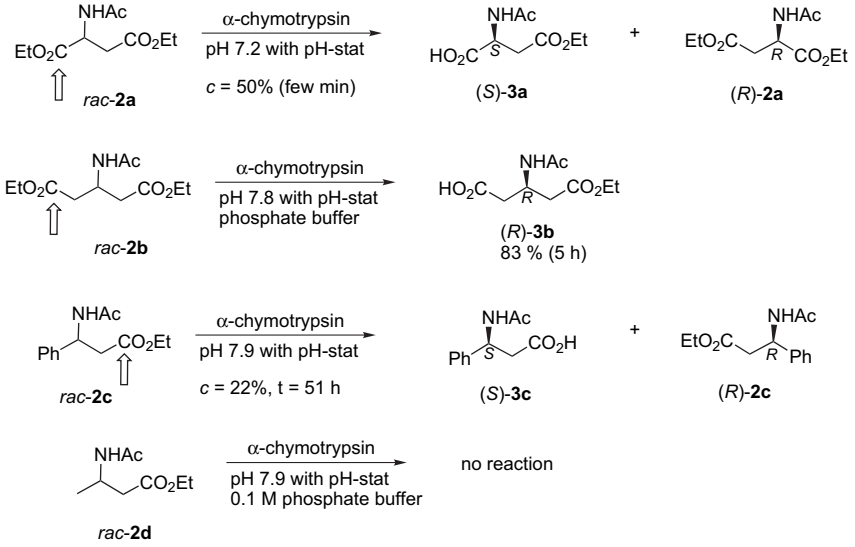
2.1.1. α-Chymotrypsin. A proteolytic enzyme, α-chymotrypsin [EC 3.4.21.1], was applied to one of the earliest resolutions of β^3-amino acids in the 1960s.[42–45] The structure and characteristics of the enzyme are well known, and a good deal of molecular modelling has been carried out for explaining the substrate–chymotrypsin interactions.[46–51] α-Chymotrypsin is a serine protease catalysing the hydrolysis of peptide bonds of protein foods in the gut via an acyl-enzyme intermediate in the same way as lipases (see Scheme 18). The enzyme acts specifically on non-terminal amide bonds adjacent to aromatic α-L-tryptophan, -tyrosine or -phenylalanine. It has a pH optimum of 7.8, digesting itself at neutral pH. Thus, the enzyme can best be used for hydrolysing ester groups at pH 5, where it is more stable.

α-Chymotrypsin exhibits a high enantioselectivity, especially towards *N*-acetyl-α-amino esters, giving highly enantiopure (*S*)-acids as the reaction products. This was first rationalised by Cohen's active site model, which is still useful for rough predictions.[48] According to this model, at the asymmetric centre the four substituents of *N*-acetyl-(*S*)-phenylalanine derivative [(*S*)-**1**], as an example, bind into four different pockets (Scheme 3, A). The ester group with the Ser195-hydroxyl of the catalytic triad locates at the *n*-site. The closed hydrophobic pocket (*ar*-site) accommodates the aromatic residue of the substrate. The site is large enough to accommodate e.g., an indole ring. The third pocket (*am*-site) contains Ser214, which is able to hydrogen bond to the carbonyl oxygen of the substrate. Both the *n*- and the *am*-sites are open to the solvent and are able to accommodate long or large groups. The small *h*-site accepts H, Cl or HO, but not, for instance, CH_3.

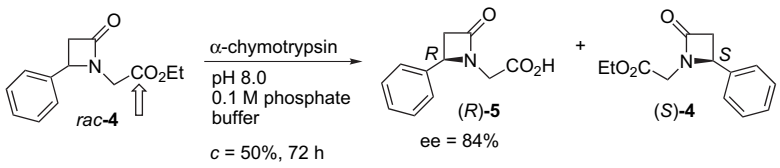
The reported kinetic resolutions of β-amino acid derivatives with α-chymotrypsin have been collected in Scheme 4. The reported high enantioselectivities are based on the measured $[\alpha]_D$ values of the substrate and the product. Cohen's model for α-amino acids also holds for diethyl *N*-acetyl aspartate **2a** (both α- and β-amino acids) and diethyl 3-acetamidoglutarate **2b**.[42,44] Thus, the more hindered α-ester group of diethyl *N*-acetyl aspartate **2a** is hydrolysed in a few minutes with high (*S*)-enantioselectivity into (*S*)-**3a**. The hydrolysis of *meso*-diethyl β-acetamidoglutarate **2b** was shown to proceed to 83% conversion in 5 h, yielding the highly enantiopure (*R*)-monoacid **3b**. On the other hand, the α-chymotrypsin-catalysed highly enantioselective ester hydrolysis of ethyl 3-acetamido-3-phenylpropanoate **2c** contradicts the model, as the resolution yields the corresponding (*R*)-**2c** and the hydrolysed (*S*)-**3c** (Scheme 3, B).[43,45] Even though many subtle behavioural forms of α-chymotrypsin have been solved by molecular modelling, an exhaustive explanation for this phenomenon has not been presented.



Scheme 3. Cohen's active site model of α-chymotrypsin.[48]



Scheme 4. Kinetic resolution of β^3-amino acid derivatives by α-chymotrypsin.[42–45]



Scheme 5. Kinetic resolution of *rac*-**4** by α-chymotrypsin.[52]

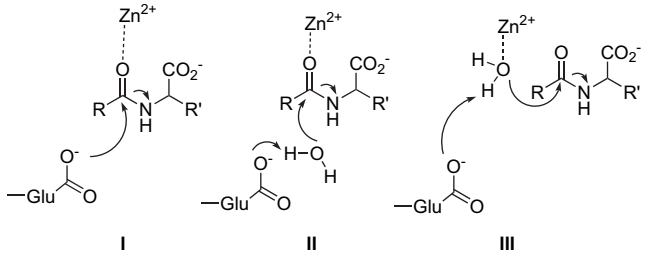
Despite the high enantioselectivity, the hydrolysis of **2c** is slow (c=22%, t=51 h). Moreover, **2c** was reported to have a low solubility in water (pH 7.8), limiting the preparative utility of the reaction. Ethyl 3-acetamidobutanoate **2d** is not hydrolysed.

As an alternative method, Achilles et al. applied α-chymotrypsin for the resolution of a β-lactam derivative **4** (Scheme 5).[52] The reaction yields the (*R*)-product (*R*)-**5** with 84% ee at an estimated 50% conversion. Thus, α-chymotrypsin produces the enantiomer, which corresponds to the less reactive enantiomer for the hydrolysis of the hydrolysed ester **2c**.

The above results indicate that α-chymotrypsin may have some potential for the enantioselective hydrolysis of β^3-amino esters with aromatic or polar substituents. The reactions are considerably slower when compared to the hydrolysis of the corresponding α-amino esters.

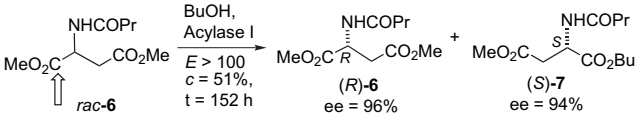
2.1.2. Acylase I. Acylases are cytosolic enzymes, the concentrations of which are high, especially in the kidney.[53,54] Various acylase I enzymes [EC 3.5.1.14] have been isolated and purified from different sources. Acylases from porcine kidney and different *Aspergillus* species are commercially available. Acylase I has been applied to industrial-scale resolutions for the production of enantiopure α-L-amino acids using the fungal *Aspergillus* enzyme, which has a higher stability, compared to the renal enzyme.[55] Other reported acylase I enzymes have been isolated, e.g., from human or rat kidney, bovine liver, *Thermococcus litoralis*, *Pyrococcus horikoshii* and *Bacillus stearothermophilus*.[56–61] In addition, acylases hydrolysing D-amino acids have been isolated from a variety of microorganisms and used in the industrial production of D-amino acids.[62–67]

Even though a considerable amount of structural information on different acylase I enzymes has been unravelled,[56–61,68–70] only the partial crystal structure of the Thr347Gly mutant of human acylase I has been reported in the Protein Data Bank (PDB).[71,72] A preliminary X-ray diffraction analysis of acylase I from *T. litoralis* has also been carried out.[73] The mechanism is generally assumed to be similar to that of carboxypeptidase A, a Zn(II)-exopeptidase (Scheme 6).[74,75]



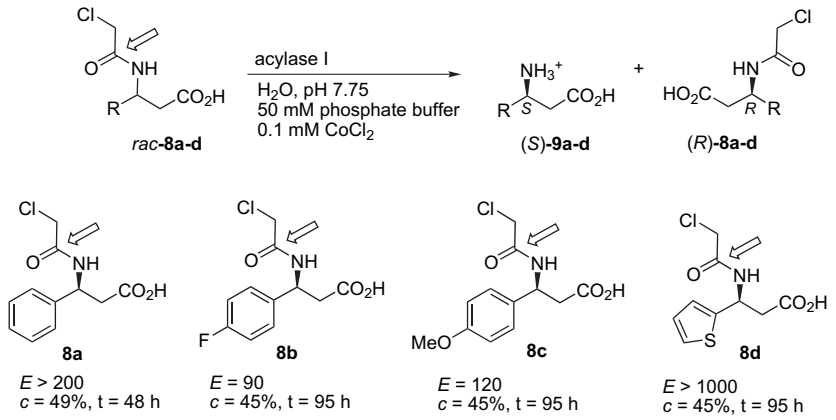
Scheme 6. Proposed mechanisms of carboxypeptidase A as generally used models for acylase I.[74,75]

Acylase I, typically, catalyses the hydrolysis of *N*-acyl groups of *N*-acyl-α-L-amino acids other than *N*-acyl-L-aspartic acid.[76] Moreover, the hydrolysis and alcoholysis of *N*-acyl-α-L-amino esters with exquisite chemo- and regioselectivity towards the ester bond have been reported.[77–79] In such cases, even dimethyl *N*-butanoyl aspartate *rac*-**6** is a substrate (Scheme 7).[77] It is crucial that only the α-ester group of the aspartate reacts, giving (*S*)-**7** and, under the alcoholysis conditions, no *N*-acyl transfer is observed. Based on these experimental findings, it can be proposed that an amino acid substrate of acylase I needs structural elements, which make the C–N bond sufficiently weak (Scheme 6). A carboxylate group at the α-position to the cleavable amide bond, together with the coordination of the carbonyl oxygen to Zn^{2+}, is sufficient for hydrolysis, but not for alcoholysis. It is therefore not surprising that the acylase I-catalysed hydrolyses of *N*-chloroacetyl-3-aminobutanoic and *N*-acetyl-3-amino-2-methylpropanoic acid have failed.[76] Acylase I from porcine kidney was, however, recently reported to hydrolyse *N*-chloroacetyl-3-aryl-β-amino acids *rac*-**8a–d** with high enantioselectivity, giving (*S*)-**9a–d** (Scheme 8).[80] Evidently, the electron-withdrawing chlorine at the *N*-acetyl group and the aromatic ring at the adjacent carbon to the cleavable bond make the hydrolysis possible. The predictability of other β-amino acid substrates for acylase I or the synthetic utility of the enzyme cannot, however, be determined on the basis of the present knowledge.



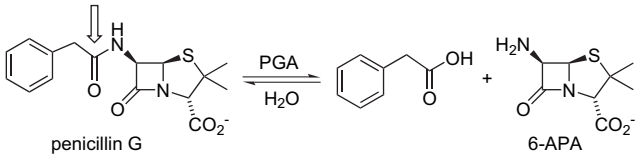
Scheme 7. Kinetic resolution of *rac*-**6** by acylase I from *Aspergillus melleus*.[77]

2.1.3. Penicillin G amidase from *Escherichia coli*. Among the penicillin amidases [EC 3.5.1.11], penicillin G amidase (PGA, also called penicillin acylase, main source *E. coli*) was discovered in the 1950s.[81–86] PGA catalyses the hydrolysis of the phenylacetyl group of **10** (penicillin G), giving 6-aminopenicillanic acid **11** (6-APA, Scheme 9). The enzyme has been immobilised on several supports in order to optimise its performance.[87] Penicillin amidases have been applied to the industrial production of β-lactam antibiotics.[88,89] The physiological role of PGA is obscure, even though it has been suggested to play a role as a scavenger enzyme with an ability to detach the phenylacetyl group for use as a carbon source.[90] The key catalytic amino acid



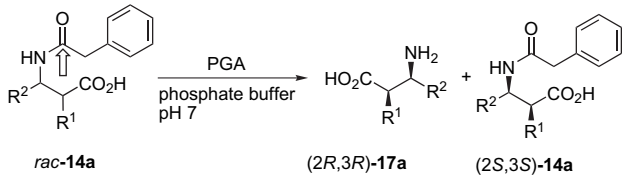
Scheme 8. Kinetic resolution of *rac*-**8** by acylase I from porcine kidney.[80] *E* values have been calculated according to the reported ee_p and *c* values. More reactive enantiomers have been presented.

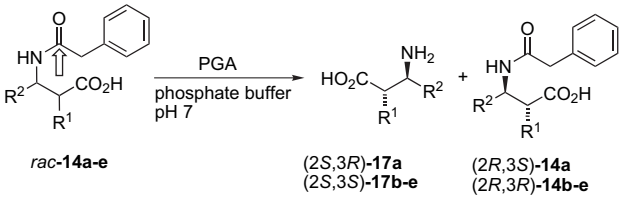
residue is SerB1, which has been proposed to act as an attacking nucleophile for **10**, leading to the formation of an anionic tetrahedral intermediate.[91,92] Asn241 of the B-subunit stabilises the intermediate. The main mechanistic difference to the serine hydrolases (see Scheme 18) is the lack of general acid–base catalysis by adjacent histidine in the formation of the intermediate. In addition, pH affects the catalytic properties of PGA. Hydrolysis takes place over the pH range 7–8.5, whereas the equilibrium favours acylation at pH 5–6.[81,82] To some extent, PGA accepts groups other than phenylacetyl, e.g., 4-pyridylacetyl and phenoxyacetyl, in the substrate, whereas a wide variety of structurally different leaving groups (the amine part of amides) are tolerated, which makes the enzyme highly useful.[93]



Scheme 9. Natural reaction of penicillin G amidase.

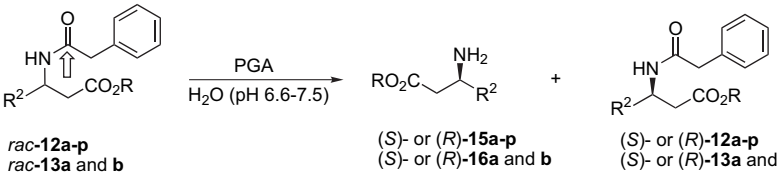
PGA has proved to be one of the most powerful tools for the preparation of highly enantiopure β^3- and $\beta^{2,3}$-amino acids **12a**–**p**, **13a**,**b** and **14a**–**e** (Schemes 10 and 11).[94–101] Over 95% ee values of the unreacted substrate enantiomer and the produced product enantiomers **15a**–**p**, **16a**,**b** and **17a**–**e** are typically obtained at 50% conversion within a few hours. The catalyst is readily available and functions at catalytic amounts in aqueous buffers. Substrate concentrations of up to 0.22 M have been used with *rac*-**12i**–**n**.[96]





(a) R^1 = Me, R^2 = CF_3
(b) R^1 = Et, R^2 = Me
(c) R^1 = $CH_2CH{=}CH_2$, R^2 = Me
(d) R^1 = CH_2Ph, R^2 = Me
(e) R^1 = Me, R^2 = Me

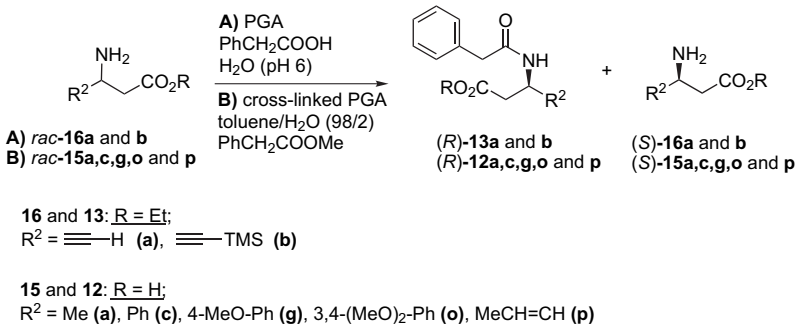
Scheme 11. Kinetic resolution of N-phenylacetylated $\beta^{2,3}$-amino acids by PGA.[100,101] The starting material consists of one enantiomeric pair.



12 and **15**: R = H;
R^2 = Me **(a)**, Et **(b)**, Ph **(c)**, 2-F-Ph **(d)**, 4-F-Ph **(e)**, 4-Cl-Ph **(f)**, 4-MeO-Ph **(g)**, 3,4,5-$(MeO)_3$-Ph **(h)**, CF_3 **(i)**, C_2F_5 **(j)**, C_3F_7 **(k)**, CHF_2 **(l)**, $H(CF_2)_2$ **(m)**, $H(CF_2)_4$ **(n)**, 3,4-$(MeO)_2$Ph **(o)**, MeCH=CH **(p)**

13 and **16**: R = Et;
R^2 = ≡—H **(a)**, ≡—TMS **(b)**

Scheme 10. Kinetic resolution of N-phenylacetylated β^3-amino acids by PGA.[94–99]



Scheme 12. Kinetic resolution of *rac*-**15** and *rac*-**16** by PGA.[98,102]

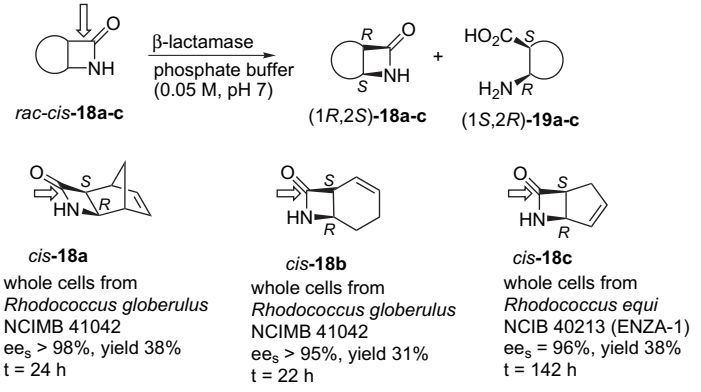
Kinetic resolutions based on enzymatic N-acylation of β^3-amino acid derivatives are scarce, compared to the hydrolysis reactions (Scheme 12).[98,102] For substrates *rac*-**16a** and **b**, the equilibrium for amide hydrolysis is diminished, because the resolution product crystallises from the solution (Scheme 12, method A).[98] Under the acylation conditions for *rac*-**15a**,**c**,**g**,**o** and **p**, the activity of cross-linked PGA strongly increases by adding 2% of water in toluene as a solvent. The *E* values are, typically, over 100 for compounds with aromatic substituents (**15c**, **g** and **o**), whereas, with *rac*-**15a** and **p**, the *E* values are only around 10 (Scheme 12, method B).

PGA has been shown to work excellently for the kinetic resolution of β^3- and $\beta^{2,3}$-amino acids when the phenylacetyl group is transferred to or from the amino group. On the other hand, the PGA-catalysed hydrolysis of N-phenylacetylated α-methyl-β-alanine takes place with low enantioselectivity, suggesting that β^2-amino acid derivatives are less suitable substrates for the enzyme.[94]

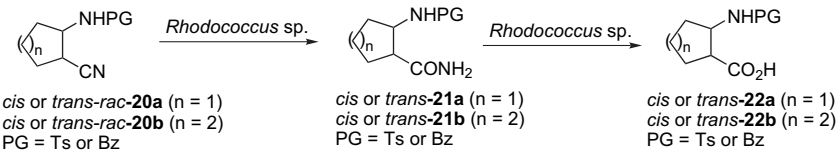
2.1.4. β-Lactamases. β-Lactamases [EC 3.5.2.6] are bacterial enzymes, naturally cleaving the β-lactam ring, and are therefore responsible for challenging β-lactam antibiotics. β-Lactamases have been divided into classes A, B, C and D, those in classes A, C and D are serine hydrolases, whereas the active site of class B β-lactamases utilises a metal ion, which is usually zinc.[103–105]

Even though β-lactamases impact negatively on therapies with β-lactam antibiotics, they have been successfully used in organic synthesis. Promising whole-cell systems from *Rhodococcus* sp. have been reported for *rac-cis*-**18a–c** (Scheme 13).[106,107] Although the enantiomeric excess of the product (1*S*,2*R*)-**19a–c** was not determined, the relatively high substrate recoveries with high ee values point to highly enantioselective reactions. In gram-scale resolutions of *cis*-**18a** and *cis*-**18b**, substrate concentrations of 0.38 and 0.30 M were used, respectively. Enzyme screenings revealed that, among a number of other commercially available β-lactamases, the enzymes from *Bacillus cereus*, *Enterobacter cloacae*, *E. coli* and *Staphylococcus aureus* do not catalyse the ring opening of *cis*-**18c**.[106]

2.1.5. Nitrilases and nitrile hydratases. In recent years, nitrile-hydrolysing enzymes have been introduced as an attractive alternative in the search for mild reaction conditions for nitrile hydrolysis. Nitrilases (EC 3.5.5.1) transform nitriles directly into acids, whereas nitrile hydratases (EC 4.2.1.84) transform them into amides. The ability of various strains of *Rhodococcus* sp. to hydrolyse β-amino nitriles into the β-amino amides and acids has been investigated (Scheme 14).[108–110] Because there is also amidase activity in the whole-cell system, sequential kinetic resolution results. The authors found five- and six-membered alicyclic racemic nitriles (*cis*- and *trans*-**20a** and **b**) to be suitable substrates for *Rhodococci* (Table 1). Racemic substrates *cis*-**20a** and **b** (PG=Ts) yield exclusively the amide *cis*-**21a** and **b** (entries 10–12 and 16–18). The enantioselectivities of the reactions, however, remain low. With the trans-compounds, both products amide **21a** and **b** and acid **22a** and **b** are formed and, typically, only one of these displays a relatively



Scheme 13. Kinetic resolution of *rac*-**18a–c** by β-lactamases.[106,107] More reactive enantiomers have been presented.



Scheme 14. Nitrile hydrolysis by whole cells from *Rhodococcus* sp.[110]

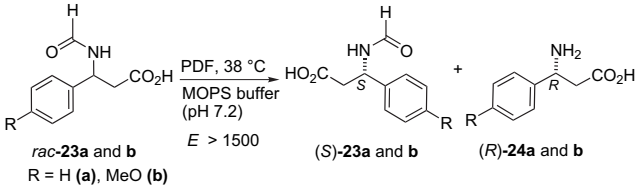
Table 1. Isolated yields and ee values for nitrile hydrolysis by whole cells from *Rhodococcus* sp. (after a reaction time of 24 h)[110]

Entry	Substrate	Nitrile % (ee %)	Amide % (ee %)	Acid % (ee %)	Microorganism
1	*trans*-**20a** (PG=Bz)	0	40 (94)	55 (75)	*R. equi* A4
2		0	30 (>99)	63 (48)	*R. erythropolis* NCIMB 11540
3		0	7 (>99)	87 (15)	*R.* sp. R 312
4	*trans*-**20b** (PG=Bz)	38 (99)	22 (56)	36 (>95)	*R. equi* A4
5		59 (44)	16 (67)	15 (>95)	*R. erythropolis* NCIMB 11540
6		61 (82)	14 (38)	7 (>95)	*R.* sp. R 312
7	*trans*-**20a** (PG=Ts)	40 (47)	14 (>99)	44 (2)	*R. equi* A4
8		0	13 (>99)	86 (5)	*R. erythropolis* NCIMB 11540
9		46 (30)	10 (>99)	34 (14)	*R.* sp. R 312
10	*cis*-**20a** (PG=Ts)	71 (5)	14 (51)	0	*R. equi* A4
11		50 (16)	49 (15)	0	*R. erythropolis* NCIMB 11540
12		11 (51)	75(7)	0	*R.* sp. R 312
13	*trans*-**20b** (PG=Ts)	26 (78)	54 (65)	13 (>99)	*R. equi* A4
14		24 (98)	56 (59)	15 (97)	*R. erythropolis* NCIMB 11540
15		33 (47)	42 (77)	16 (87)	*R.* sp. R 312
16	*cis*-**20b** (PG=Ts)	47 (8)	48 (6)	0	*R. equi* A4
17		50 (10)	41 (8)	0	*R. erythropolis* NCIMB 11540
18		44 (10)	43 (4)	0	*R.* sp. R 312

high enantiopurity. Thus, the sequential resolution of the trans-five-membered **20a** (PG=Ts or Bz) yields the amides **21a** (PG=Ts or Bz) with high enantiopurity (entries 1–3 and 7–9), whereas the trans-six-membered **20b** (PG=Ts or Bz) yields the acids **22b** (PG=Ts or Bz) with high enantiopurity (entries 4–6 and 13–15). The tosyl protective group is favoured over its benzoyl counterpart, e.g., for benzoylated *cis*-**20a** and **b** are not accepted as substrates for the enzymes. The absolute configurations of the resolved products were not determined.

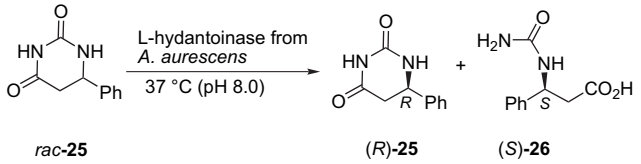
Nitrile-hydrolysing enzymes provide a mild method to hydrolyse nitriles into amides or carboxylic acids. The real value of nitrile-hydrolysing enzymes for the kinetic resolution of β-amino acids and amides has, however, not yet been discovered.

2.1.6. Peptide deformylases. Peptide deformylases (PDF [EC 3.5.1.31]) catalyse the hydrolysis of the *N*-terminal formyl group from nascent polypeptides. The recent work with PDF from *E. coli* describes good activity and high enantioselectivity with certain N-formylated α-amino acids, α-amino acid amides and α-amino nitriles.[111] In the same study, the hydrolysis of *N*-formyl-3-amino-3-arylpropanoic acid **23a** and **b** (10 mM) was observed to take place with excellent enantioselectivity (E>1500), giving highly enantiopure (*R*)-**24a** and **b** as reaction products (Scheme 15). The exact reaction time was not reported, but it was stated to be approximately the same as that for the α-amino acid derivatives (ca. 1 h). Studies of PDF with other β-amino acids need to be carried out before it is possible to say more about the synthetic potency of the method.



Scheme 15. Kinetic resolution of *rac*-**23** by PDF.[111]

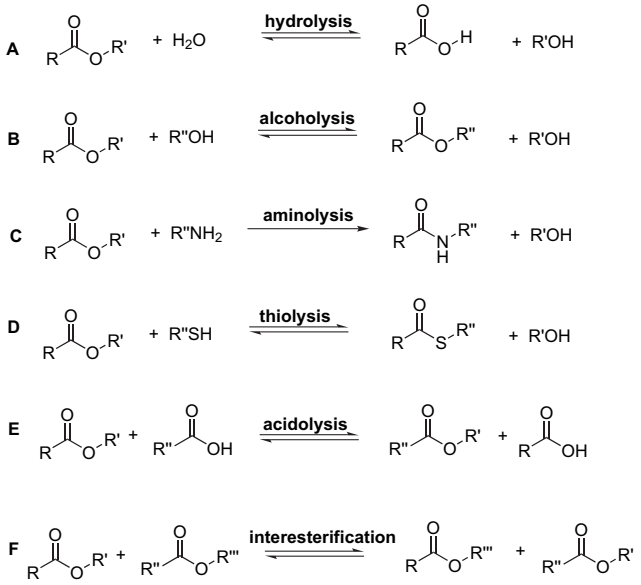
2.1.7. Hydantoinases. Hydantoinases [EC 3.5.2.x] hydrolyse cyclic amides. The process is well known in the industrial production of enantiopure α-amino acids.[112] In the Biotrans 2005 meeting (Delft, The Netherlands), a new L-hydantoinase from *Arthrobacter aurescens* was reported to catalyse the hydrolysis of 6-substituted aromatic dihydrouracils *rac*-**25** as β-amino acid-derived substrates into *N*-carbamoyl derivative (*S*)-**26** with good enantioselectivity (Scheme 16).[113,114] The use of this novel enzyme could therefore open up a new kinetic resolution route to enantiopure β-amino acids. On the other hand, commercially available hydantoinases from *E. coli* and *V. angularis* catalyse the same reactions with low enantioselectivity.



Scheme 16. Kinetic resolution of *rac*-**25** by L-hydantoinase from *Arthrobacter aurescens*.[113,114]

2.2. Enzymes acting on C–O-bonds

2.2.1. Lipases: reaction types and mechanism. Lipases are serine hydrolases [EC 3.1.1.3], the natural reaction of which is the hydrolysis of triglycerides. Lipases accept a wide range of compounds as their substrates. Besides ester hydrolysis (A), in organic solvents lipases catalyse alcoholysis (B), aminolysis (C), thiolysis (D), acidolysis (E) and interesterification (F) when water is not present (Scheme 17). In a broad sense, the reactions can be considered as acylations of a nucleophile (Nu^2H) and as deacylations of an acyl donor ($RCONu^1$) for the reaction $RCONu^1+Nu^2H=RCONu^2+Nu^1H$ (Scheme 18). It is worth mentioning that the terms inter- and transesterification are often used as synonyms.[115] We felt that it is necessary to reserve the term interesterification to describe reactions (F) where two esters change their alkyl parts. Examples of interesterifications are scarce.[116–120]



Scheme 17. Types of lipase-catalysed reactions.

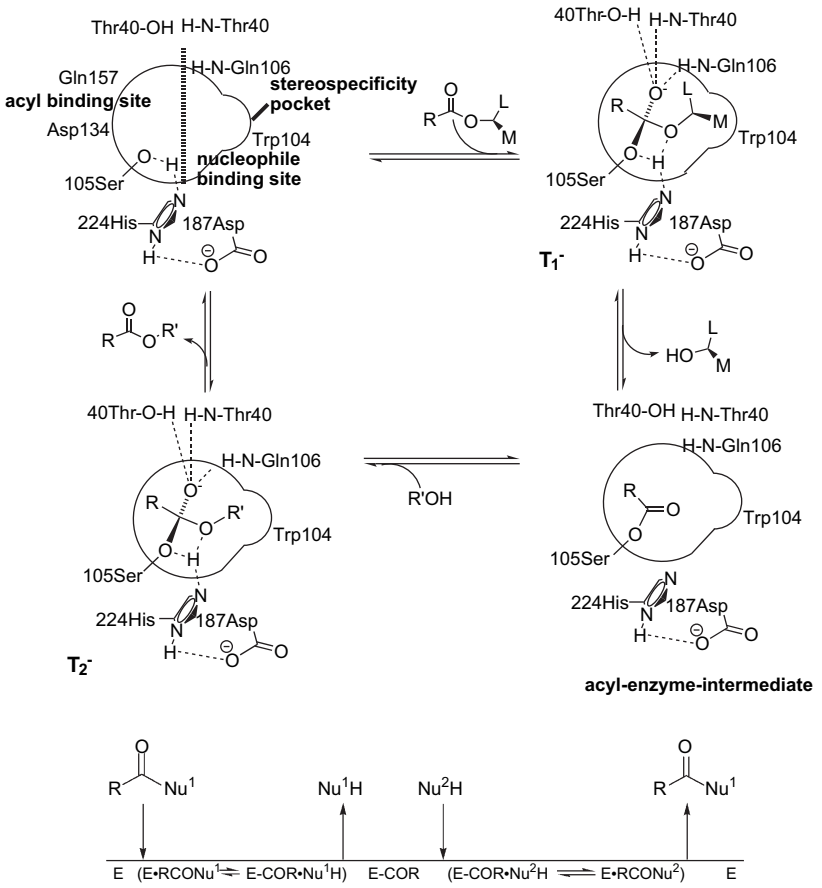
The ping-pong bi–bi mechanism for the lipase-catalysed reaction of an ester enantiomer (RCO_2CHLM, an acyl donor $RCONu^1$) with R′OH (a nucleophile Nu^2H) and CAL-B is presented in Scheme 18 as a model reaction (L and M denote large- and medium-sized groups, respectively). The mechanism is known as a serine hydrolase mechanism. At the beginning, the ester substrate enters the active site where the serine residue (Ser105) is activated by a hydrogen bond to histidine (His224). Nucleophilic attack of the serine hydroxyl at the carbonyl carbon of the substrate leads to the formation of the anionic tetrahedral intermediate (T_1^-), which is stabilised by three hydrogen bonds at the oxyanion hole, one of which is formed with Gln106 and two with Thr40. The intermediate breaks down with the formation of an acyl-enzyme intermediate liberating the LMOH (Nu^1H). The attack of R′OH (an added nucleophile Nu^2H) leads to the formation of a second anionic tetrahedral intermediate (T_2^-), which finally leads to the second product (RCO_2R' or $RCONu^2$). The enzyme (E) is released and the cycle recurs.

2.2.2. Lipase B from *Candida antarctica*.

2.2.2.1. Characteristics. Two entirely different lipases, CAL-A and CAL-B, have been isolated from *C. antarctica* yeast.[121,122] CAL-B is, perhaps, the most common enzyme used by synthetic chemists. CAL-B could better be classified as an esterase, or as an intermediate form of a lipase and an esterase. This is because of the lack of typical interfacial activation characteristics for lipases.[123] Moreover, CAL-B hydrolyses long-chain triglycerides slowly.[124] The most common form of CAL-B in organic synthesis is Novozym 435 from Novo Nordisk. The enzyme was also available earlier with the trade name Chirazyme L2 (carrier-fixed C2, lyophilised) from Roche. In these preparations, CAL-B is immobilised on a polyacrylic resin. CAL-B is stable in aqueous solutions over the pH range 3.5–9.5 and the denaturation temperature of the free enzyme varies between 50 and 60 °C, depending on the pH and buffer. CAL-B accepts a wide range of substrates and solvents from polar to apolar, which makes it a highly useful catalyst for synthetic work.[125] A lot of effort has been focused on tailoring and extending the catalytic properties of CAL-B.[126]

The three-dimensional structure of CAL-B has been elucidated.[127,128] The funnel-shaped active site is located in the core of the enzyme. CAL-B-catalysed reactions are assisted by a catalytic triad Ser105-His224-Asp187, where Ser105 is situated at the bottom of the active site in rather polar surroundings (Scheme 18). In addition to His224 being projected into the active site, Gln157 is hydrogen bonded to Asp134 and Thr40. Otherwise, the active site is coated with hydrophobic amino acids, Trp104 being the only aromatic residue. The acyl and nucleophile-binding sites run almost parallel from the bottom to the surface and are barely separated by the hydrophobic side chains of Ile189 and Ile285. The nucleophile-binding site contains the stereospecificity pocket where substituents smaller than propyl can fit.[129] The pocket is delineated by the side chain of Trp104. The structure of the acyl binding site has not been thoroughly investigated, but it is known to be more spacious than the nucleophile site. Accordingly, chiral recognition by CAL-B is different for reactions at the amino and carboxylate functionalities of the amino acid-based substrates of Scheme 1. Generally, higher enantioselectivities are expected for chiral recognition at the nucleophile-binding site.[128] The results reviewed below, however, indicate excellent chiral recognitions of the acyl part with proper substrate structures. The most prominent feature of CAL-B catalysis is the low chemoselectivity observed when alicyclic β-amino esters react with an achiral ester.[119,120] For this reason, we want to distinguish the terms interesterification and transesterification.

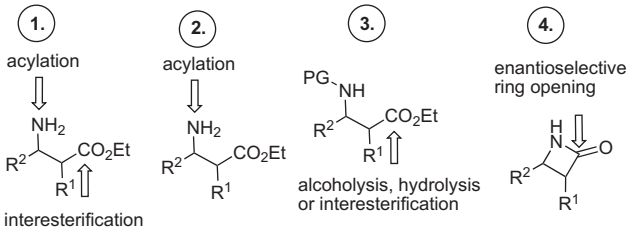
The mechanism of interesterification seems to be more complex than is assumed on the basis of the ping-pong bi–bi mechanism (Scheme 18). This is most clearly seen when 2,2,2-trifluoroethyl butanoate $PrCO_2R$ ($R=CF_3CH_2$) acts as an acyl donor for the reaction with **27a**, leading to the formation of an interesterification product **28a** (Scheme 19). As expected, alcoholysis of **27a** with a weak nucleophile, 2,2,2-trifluoroethanol, in DIPE does not take place.[119] This may indicate that 2,2,2-trifluoroethanol is not able to enter the active site, but rather needs to be liberated from its ester by the enzyme and to stay in the polar surroundings of the



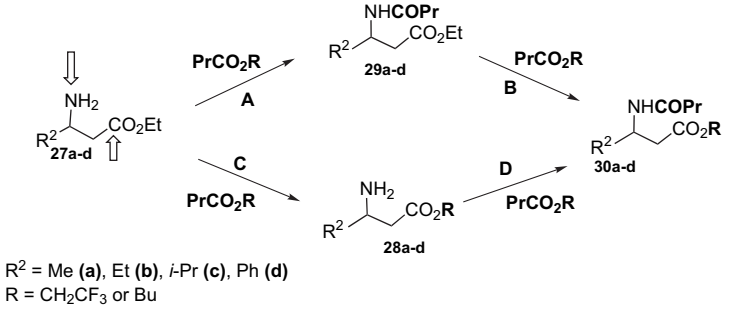
Scheme 18. Top view for the ping-pong bi–bi mechanism of CAL-B.

active site close to the catalytic triad. Possible hydrogen bonds from the active site amino acid residues to fluorine atoms can increase the nucleophilic character of 2,2,2-trifluoroethanol. Moreover, hydrogen bonds from His224 can further activate the hydroxyl group of the alcohol, as previously predicted by computer modelling.[130] When the released alcohol remains in the active site, the effect on the enantioselectivity is evident. Thus, acylation of a chiral alcohol with 2,2,2-trifluoroethyl and other alkyl butanoates has been observed to proceed with differing enantioselectivities, although the acyl-enzyme intermediate is the same butanoate ester in each case.[131,132] This effect has also been observed with PPL and CAL-A.[77,133,134]

The most successful approaches to the enantiomers of β-amino acid derivatives by CAL-B are shown in Scheme 20 in general forms. The first method exploits the low enzymatic chemoselectivity, and the second method is based on highly chemoselective N-acylation. In the third method, the reaction is directed to the ester group of an *N*-protected β-amino ester and, in the fourth method, a β-lactam ring is enzymatically cleaved.

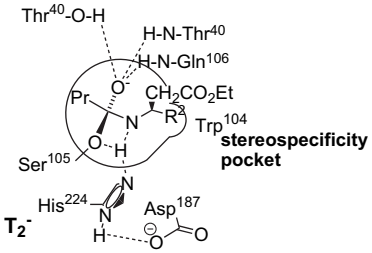


Scheme 20. Strategies for the kinetic resolution of β-amino acid derivatives by CAL-B.



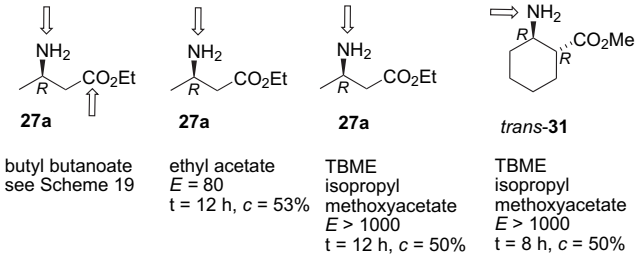
Scheme 19. Chemoselective reaction possibilities of **27a–d** with $PrCO_2R$ by CAL-B.[119,120]

2.2.2.2. N-acylation and/or interesterification. The reaction of an acyclic β-amino ester with an achiral acyl donor leads to competitive N-acylation (route A) and interesterification reactions (route B) in the presence of CAL-B (Scheme 19).[119,120] As a consequence, when one of the bifunctional substrates **27a–d** is subjected to the CAL-B-catalysed reaction with butanoate esters ($PrCO_2R$, R=Bu or CF_3CH_2), the reaction gives **30a–d** as the most advanced products through routes A+B and C+D. The chemoselectivity depends on the nature of $PrCO_2R$ and R^2. With **27a** in neat butyl butanoate, the route C+D prevails and, at 80% total conversion, the initially racemic mixture is transformed into a mixture of enantiopure products (*S*)-**27a** (20%), (*S*)-**28a** (R=Bu, the amount 24%) and (*R*)-**30a** (R=Bu, the amount 54%) with all ees over 95%. The amount of (*R*)-**29a** stays always minimal. Accordingly, the enantiomers were effectively obtained by a sequential resolution path on a preparative scale in 2 h. For the reaction of **27a** with 2,2,2-trifluoroethyl butanoate in DIPE, the route A+B clearly prevails over C+D. Steps C+D, however, proceed smoothly with low enantioselectivity, finally ruining the attempt for successful kinetic resolution. The increased size of R^2 favours interesterification (C+D), due to the stereospecificity pocket of CAL-B becoming too crowded with increasing size of R^2, preventing the substrate binding as a nucleophile (Scheme 21). Accordingly, when R^2=^{i}Pr in the case of **27c**, interesterification with low enantioselectivity (*E*=15 for butyl butanoate and *E*=4 for trifluoroethyl butanoate in DIPE) through route C is the only reaction observed. With phenyl-substituted **27d**, the reaction proceeds sluggishly with low enantioselectivity, leading to a product distribution (**29d**:**28d**:**30d**) of 20:100:7 after 55 h.



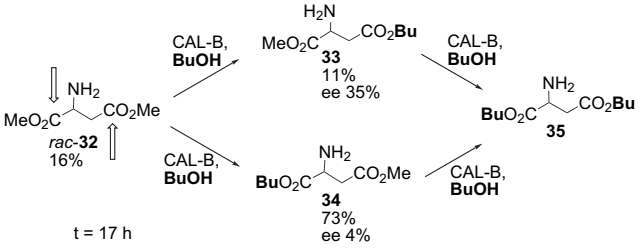
Scheme 21. Top view for T_2^- for N-acylation of β-amino ethyl esters **27**.

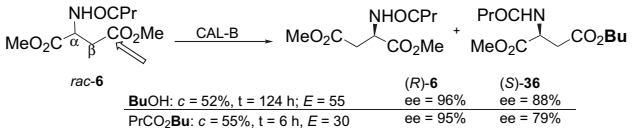
Interesterification is, of course, seen only when two esters with different alkyl parts react. The resolution of **27a** in ethyl acetate was successfully performed with *E*=80 (Scheme 22).[135] On the other hand, interesterification was suppressed by using isopropyl methoxyacetate as an acyl donor for the kinetic resolution of **27a** (1.7 M) in TBME.[136] The total isolated yield of 86% for the unreacted (*S*)-**27a** and the formed product were reported after a reaction time of 12 h and distillation. The same method results in excellent enantioselectivities also for the kinetic resolution of methyl *trans*-2-aminocyclohexanecarboxylate **31** (1.6 M, *t*=8 h) as an alicyclic β-amino acid, suggesting further versatility of the method. N-Acylations of ethyl *cis*- and *trans*-2-aminocyclohexane-1-carboxylates as $\beta^{2,3}$-amino acid derivatives with trifluoroethyl acetate in diethyl ether were, however, reported to proceed with low enantioselectivity.[137]



Scheme 22. Kinetic resolution of *rac*-**27a** and *rac*-**31** by CAL-B. More reactive enantiomers have been presented.[119,135,136]

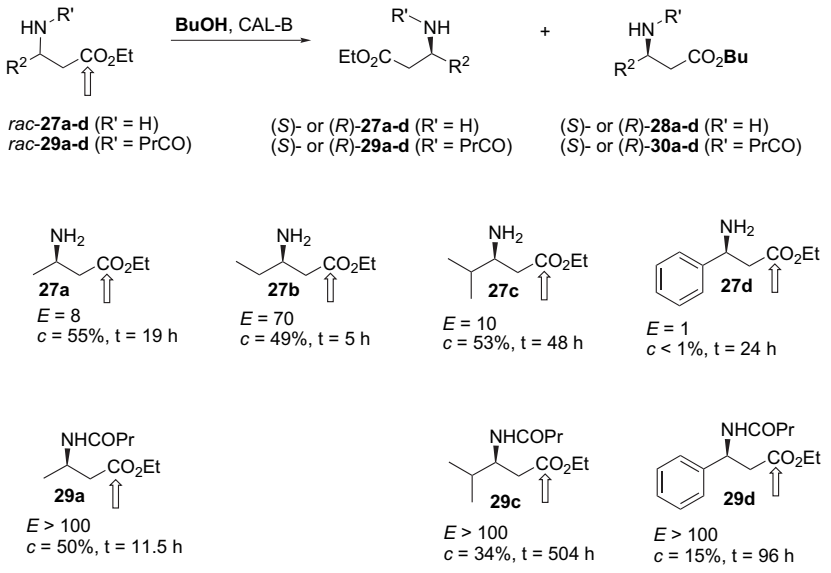
2.2.2.3. Alcoholysis. Two ester groups of acidic amino esters entail the possibility for enzymatic regioselective recognition through alcoholysis and interesterification. This is shown for the butanolysis of racemic dimethyl aspartate **32** and dimethyl *N*-butanoyl aspartate **6** in Scheme 23.[77,120] The reaction of *rac*-**32** yields a 1:7 mixture of β- and α-butyl methyl esters **33** and **34** at 84% conversion in 17 h with negligible enantioselectivity. At this stage, the dibutyl ester **35** is not yet formed. After *N*-protection (formation of *rac*-**6**), the reaction in neat butanol (*E*=55) and in neat butyl butanoate (*E*=30) turns regiospecific, i.e., CAL-B recognises the amino ester as a β-amino ester, leading to the enantioselective formation of (*S*)-**36**. Butanolysis has been successfully used for the kinetic resolution of *rac*-**6** at 52% conversion, yielding (*R*)-**6** (ee=96%) and (*S*)-**36** (ee=88%), and for the enantiomeric purification of (*S*)-**6** (ee=65%) to an enantiopure stage (ee>99%). Competitive inhibition of CAL-B by alcohols and/or solvent effects may explain the slow reaction in butanol (ca. 55% conversion in 124 h), compared to interesterification (ca. 55% conversion in 6 h) in butyl butanoate.[123,138]





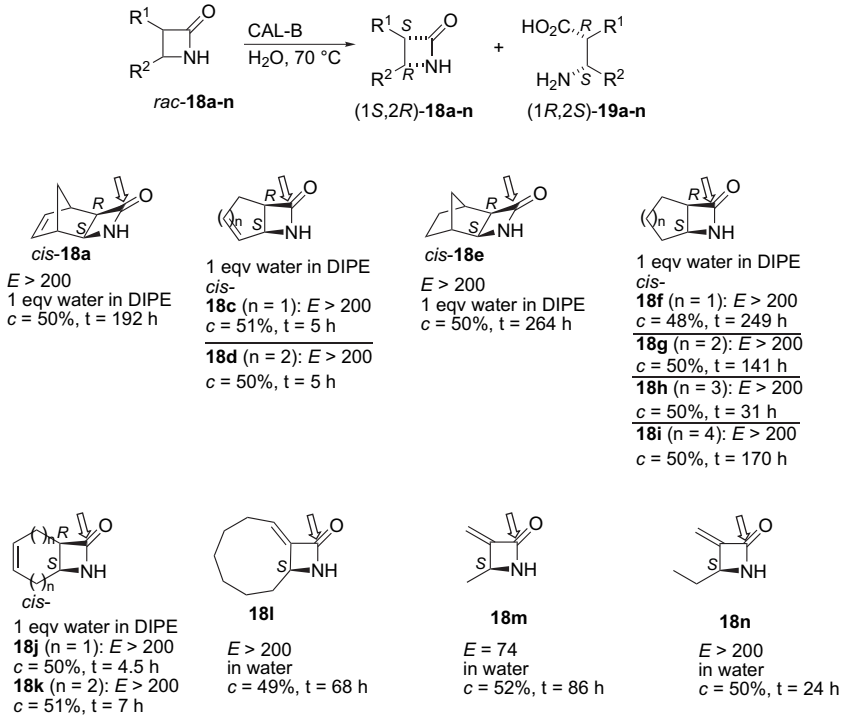
Scheme 23. Regioselective alcoholysis of *rac*-**32** and kinetic resolution of *rac*-**6** by CAL-B.[77,120]

Acyclic β-amino esters *rac*-**27a–d** and *rac*-**29a–d** have been subjected to alcoholysis in neat butanol in the presence of CAL-B (Scheme 24).[120] As with the aspartate above, *rac*-**29** with *N*-protection gives high enantioselectivities (*E*>100). Due to long reaction times, the method is of practical value only for the kinetic resolution of *rac*-**29a**, where (*S*)-**29a** (ee 99%) and (*R*)-**30a** (ee 99%) were separated after 11.5 h.



Scheme 24. Kinetic resolution of *rac*-**27a–d** and *rac*-**29a–d** in neat butanol.[120] More reactive enantiomers are presented.

2.2.2.4. Hydrolysis of β-lactams. CAL-B-catalysed ring opening of β-lactams shows a high potential for the preparation of enantiopure β^3- and $\beta^{2,3}$-amino acids (Scheme 25).[130,139–142] Even though lipases are generally unable to act on stable amide bonds, CAL-B has been found to catalyse the hydrolysis and alcoholysis of β-lactam rings, due to diminished resonance stabilisation at the amide bond in a strained four-membered ring. This method allows high enantioselectivities and easy purification of the products (1*S*,2*R*)-**18a–n** and (1*R*,2*S*)-**19a–n**. Water activity under low water conditions is of importance for enzymatic reactivity. Accordingly, high water contents clearly decrease both enantioselectivity and conversion as shown by an increase in *E* from 44 (conversion 13%) to 117 (conversion 48%) when 10 equiv of water are replaced by 1 equiv for the ring opening of *cis*-**18h**.[140] The reactions tend to proceed sluggishly, especially with saturated *cis*-**18e**,**f**,**g** and **i**, even at elevated temperature, taking 141–264 h before 50% conversion is reached.[140,142] This may be partly due to the consumption of water by the reaction under low water contents. With unsaturated *cis*-**18c**,**d**,**j** and **k**, 50% conversion is reached in a few hours (4.5–7 h) at 70 °C. The resolutions of *rac*-**18l–n** have been carried out as 0.03 M solutions in water at 70 °C, and 50% conversion is reached in 86, 86 and 24 h, respectively. The method can also be readily applied to β^3-lactams.[130] The highest substrate concentrations



Scheme 25. Hydrolysis of β-lactams. More reactive enantiomers have been presented.[130,139–142]

used in the gram-scale resolutions have been 0.2 M with *cis*-**18a**,**c**,**j** and **k**. It has been crucial for high enantioselectivity that the reactions are performed at elevated temperatures, usually at 60–70 °C. It is also worth noting that the enantiopreference is different than in the β-lactamase-catalysed reactions (Scheme 13).

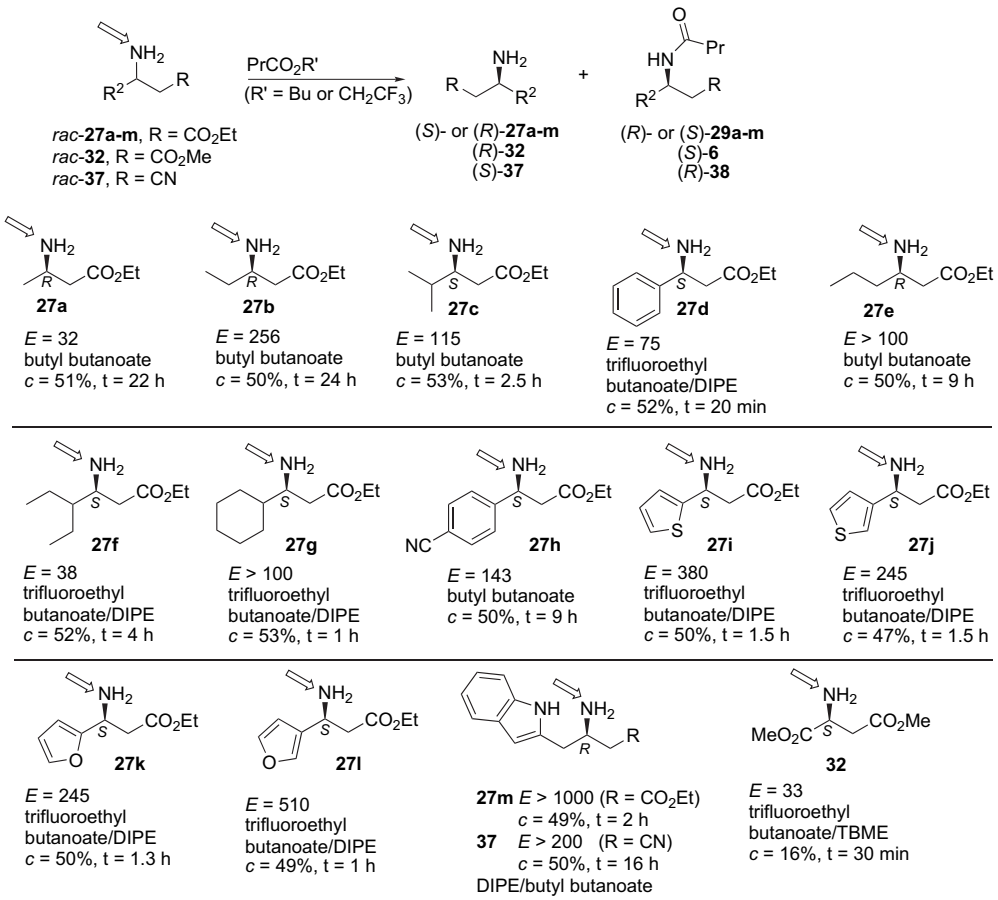
2.2.2.5. Summary. The above results show that, although CAL-B is generally of high practical value for kinetic resolution of racemates, its use is relatively limited for the kinetic resolution of β-amino esters. This is mainly due to the low chemoselectivity of the enzyme, which allows both functional groups of an amino ester to react with achiral acyl donors, except when an isopropyl ester is used. On the other hand, CAL-B-catalysed hydrolysis of β-lactams is a highly valuable method for the preparation of many alicyclic β-amino acid enantiomers by kinetic resolution.

2.2.3. Lipase A from *C. antarctica*. The crystal structure of CAL-A has not been elucidated, but its primary structure is available.[143] The enzyme consists of 431 amino acids, and it has a unique structure deviating from other known protein structures. The ability of CAL-A to catalyse the acylation of bulky substrates is noteworthy. Even tertiary alcohols have been esterified with good enantioselectivity.[144,145] Henke et al. have used molecular modelling for explaining the recognition of bulky substrates.[146] This was shown to be based on a GGGX-motif at the active site coating, whereas other lipases consist of a GX-motif.

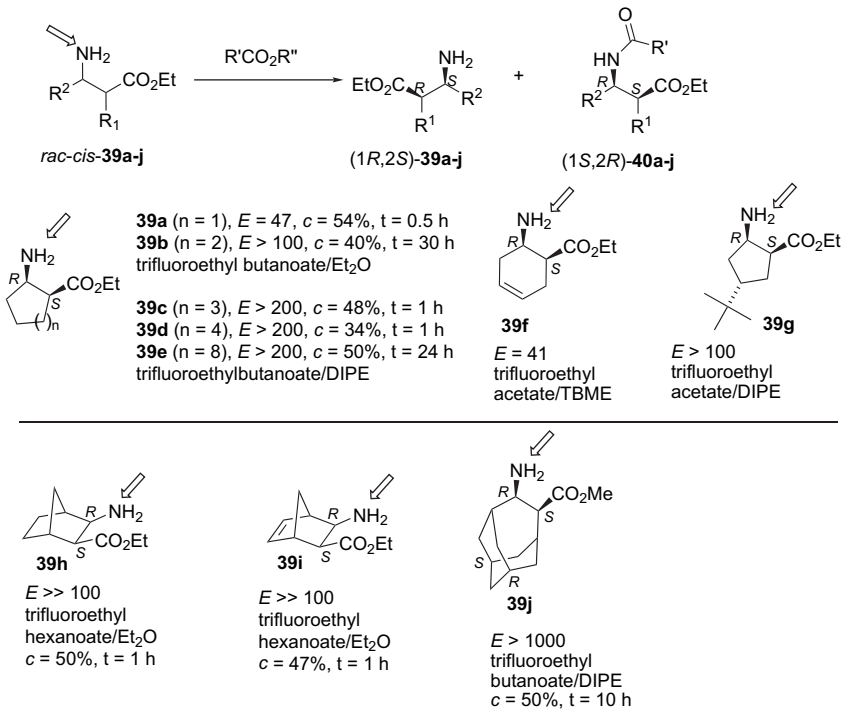
Several promising properties are making CAL-A increasingly interesting today. Even though *C. antarctica* is not a thermophilic organism, lipase A is one of the most thermostable enzymes known to date. Its denaturation temperature is 93 °C at pH 7 and 96 °C at pH 4.5.[147] Because of its thermostability, it is used in the pulp and textile industries to hydrolyse high-melting fatty acid glycerides and resin esters.[148] Earlier trade names of CAL-A encompass lyophilised SP 526 and Novozym 868 (both from Novo Nordisk). The latter product was an aqueous solution and seems to be mentioned only once in the literature.[149] Later, CAL-A has been produced with the trade name Chirazyme L5 (from Roche) and Novozym 735 (from Novo Nordisk). Novozym 735 is an aqueous solution containing 50% glycerol. Chirazyme L5 distributed by Roche corresponds to Novozym 735, except that L5 is lyophilised.

The crucial action for enabling the synthetic use of CAL-A in organic solvents is immobilisation. The common method is based on adsorption of CAL-A (10–20%) on Celite in the presence of sucrose (12%).[150] Polypropylene powder, Accurel EP-100, has also proved to be an excellent carrier.[144]

We belong to pioneers working with CAL-A as a highly enantioselective catalyst. In this laboratory, CAL-A has been especially useful for the highly enantioselective N-acylation of several β^3- and $\beta^{2,3}$-amino esters (Schemes 26 and 27).[77,137,151–157] Kinetic resolution of all compounds

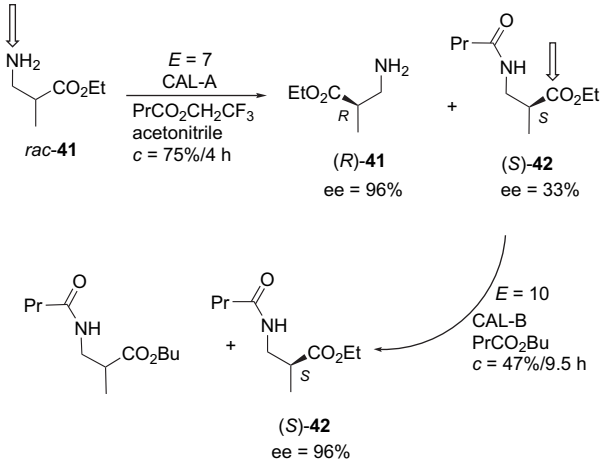


Scheme 26. N-Acylation of β^3-amino esters by CAL-A. More reactive enantiomers have been presented.[77,151,153,156,157]



Scheme 27. N-Acylation of racemic $\beta^{2,3}$-amino esters *cis*-**39a–j** by CAL-A. More reactive enantiomers have been presented.[137,154,155]

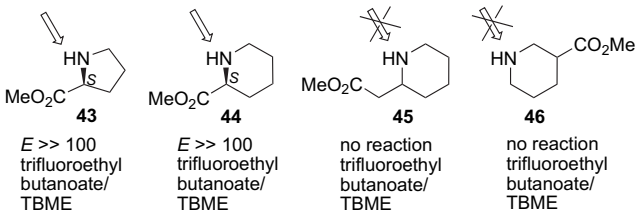
27a–m, **32**, **37** and **39a–j** has been performed on a preparative scale, and the enantiomers of the starting material and the product **29a–m**, **6**, **38** and **40a–j** have been separated and characterised. The reactions have been typically performed at room temperature (22–24 °C), the N-acylation of **39e** (47 °C) being an exception. Unlike CAL-B, CAL-A does not catalyse interesterification. The enantioselectivities are generally high, except for the N-acylation of **41** (E=7) as a β^2-amino acid (Scheme 28).[156] This is not surprising, because the amino group is not attached directly to the asymmetric centre, and the enzyme prefers sterically hindered substrates. CAL-A has also performed successfully in the kinetic resolution of a β-amino nitrile **37** (R=CN). Even though the enantioselectivity of the reaction is slightly lower (E>200) than the corresponding β-amino ester **27m** (E>1000), the reaction is roughly 8-fold faster under the same conditions. Thus, 50% conversion into (*R*)-**38** and (*R*)-**29m** is reached in 2 and 16 h, respectively. Another possibly useful method for CAL-A might be the enantioselective opening of a β-lactam ring. Thus, the slow hydrolysis of *cis*-**18h** takes place (E=47) in the presence of CAL-A.[140] This reaction cannot, however, compete with that in the presence of CAL-B (Scheme 25).



Scheme 28. Kinetic resolution of *rac*-**41** by CAL-A and CAL-B.[152]

To our knowledge, only two enzymatic kinetic resolutions of β^2-amino acids have been reported. Penicillin G amidase-catalysed resolution of *N*-phenylacetyl-α-methyl-β-alanine has already been mentioned to take place with low enantioselectivity.[94] CAL-A-catalysed N-acylation of *rac*-**41** with 2,2,2-trifluoroethyl butanoate also proceeds at negligible enantioselectivity (Scheme 28).[152] Surprisingly, the best enantioselectivity (E=7) was observed with acetonitrile as a solvent. The chemoselectivity of CAL-A for N-acylation and the possibility of CAL-B for interesterification were, however, exploited in two successive kinetic resolutions. Moreover, the opposite enantioselectivities of the lipases in these reactions were utilised. In this method, CAL-A produces the *R*-enantiomer at 75% conversion as an unreacted isomer, whereas CAL-B-catalysed interesterification in butyl butanoate purifies the enantiomerically enriched (*S*)-**42**, now as an unreacted counterpart.

Among the α-amino esters, acylation of a secondary ring nitrogen of *N*-heterocyclic methyl esters of proline **43** and pipecolic acid **44** proceeds with high enantioselectivity (E>100) (Scheme 29).[158,159] This is an intriguing result, because the enzymatic acylation of the secondary nitrogen atom with high enantioselectivity has proved to be a difficult task.[160–166] The method was tried for extension to the homologous β-amino esters **45** and **46**, but these were found not to be substrates for CAL-A.[167]

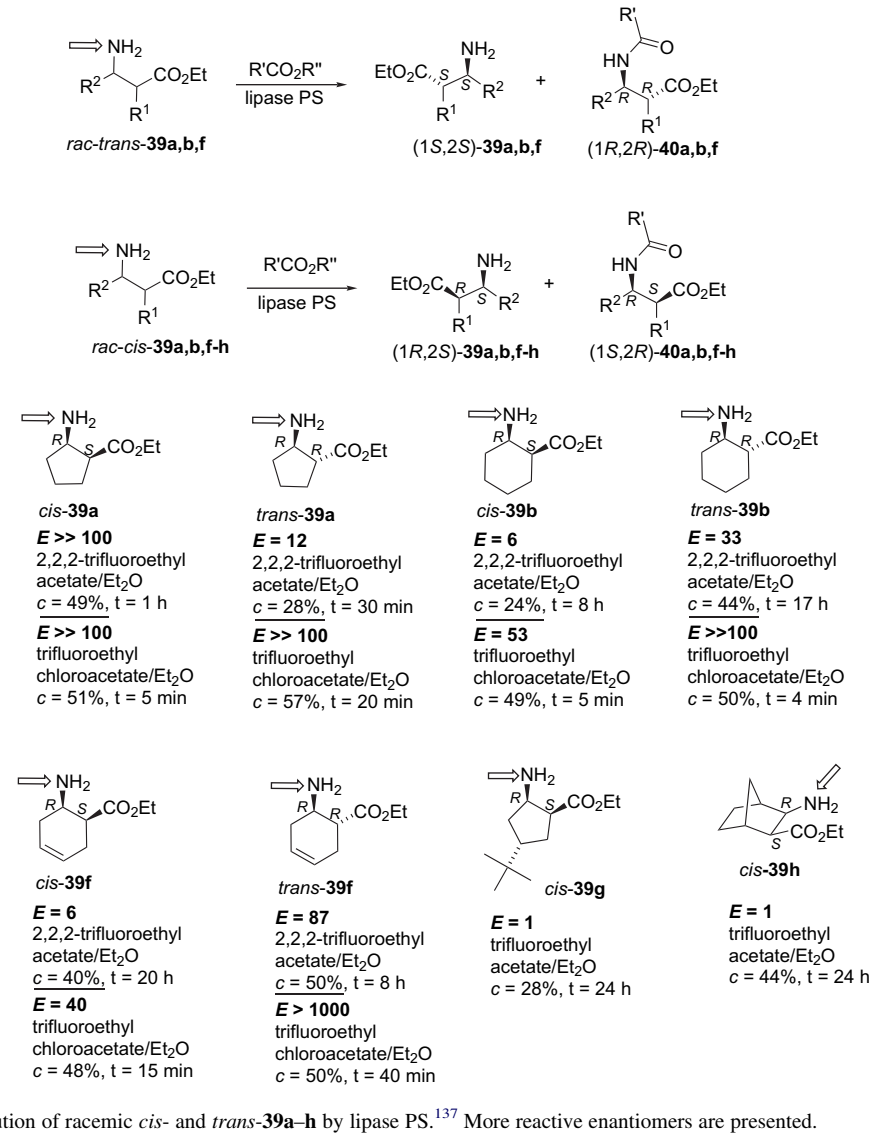


Scheme 29. N-Acylation of α- and β-amino esters **43**–**46**. More reactive enantiomers have been presented.[158,159,167]

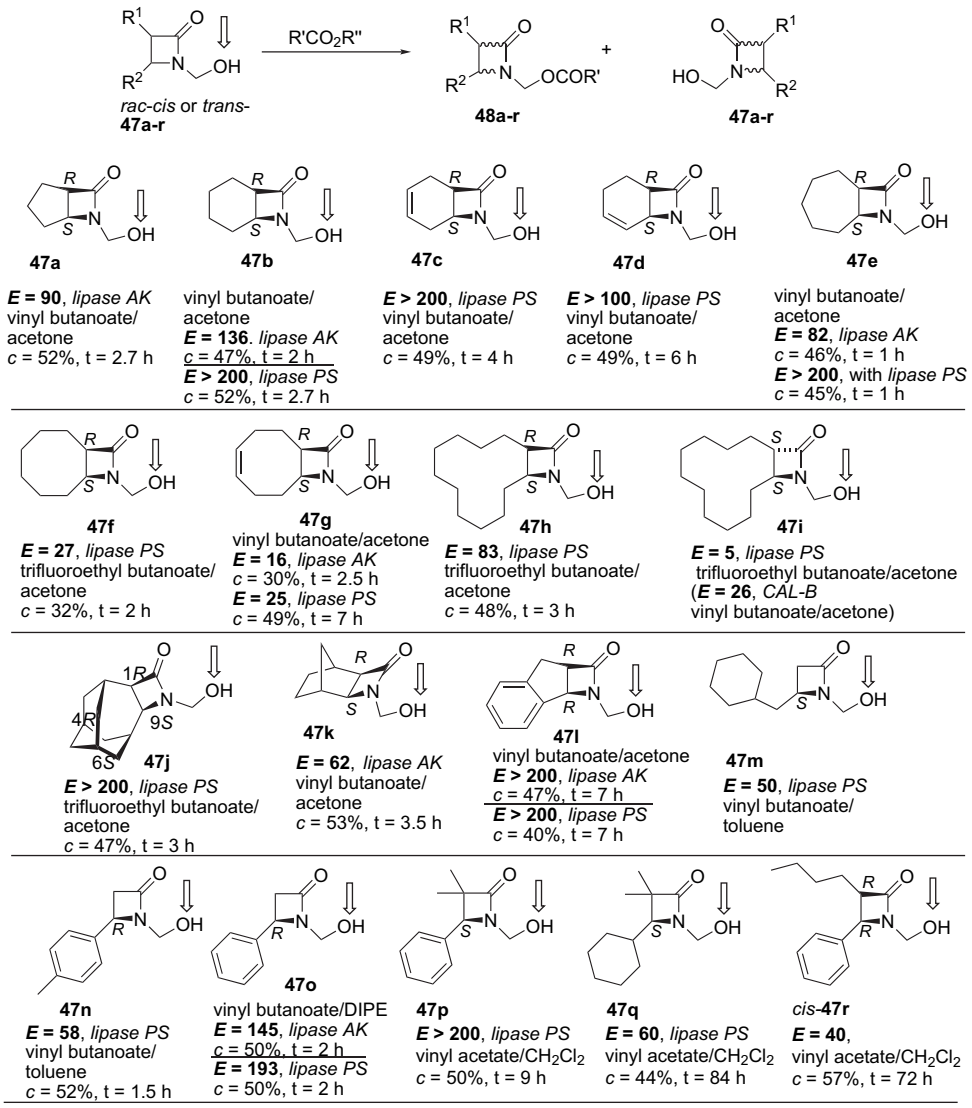
The reported results suggest CAL-A to be the most useful enzyme for the preparation of various highly enantiopure β^3- and $\beta^{2,3}$-amino acids by N-acylation. Even though further scale up of the substrate concentration to over 0.1 M has not been studied, the method conceals a very high synthetic potential. Combined with CAL-B catalysis, CAL-A is also important for the preparation of the enantiomers of *rac*-**41** as the only β^2-amino ester resolved enzymatically so far.

2.2.4. Lipases from *Pseudomonas* species and *Burkholderia cepacia*. Concerning the enzymatic kinetic resolution of β-amino acids and their precursors, three lipases have been reported to catalyse these reactions in a rather similar manner. The most common are lipase PS (from *B. cepacia*, formerly *Pseudomonas cepacia*) and lipase AK (from *Pseudomonas fluorescens*) from Amano, whilst lipase Amano P-30 from *P. cepacia* has been less frequently used. Concerning lipase PS, neither microorganism nor the lipase has been changed by the change in the name. The pH optimum for lipase PS is 7.0 and is 8.0 for lipase AK. The structure of lipase PS is well known,[168–175] with Ser87, His86 and Asp264 forming a catalytic triad. Three binding pockets constitute the active site, and two hydrophobic pockets are responsible for the enantiodiscrimination. Molecular modelling has not, however, been applied to β-amino acids.

Low enantioselectivity in the presence of lipase PS and AK has been reported for the reaction of acyclic *rac*-**27a** with 2,2,2-trifluoroethyl butanoate in DIPE.[119] On the other hand, the enzyme has been highly useful for the acylation of various hydroxymethylated β-lactams and $\beta^{2,3}$-amino esters (Schemes 30 and 31).[137,154,155,176–183] Thus, with racemic $\beta^{2,3}$-amino esters **39a**–**h** lipase PS displays excellent enantioselectivity towards the N-acylation of trans-compounds (Scheme 30) in particular, whereas CAL-A favours



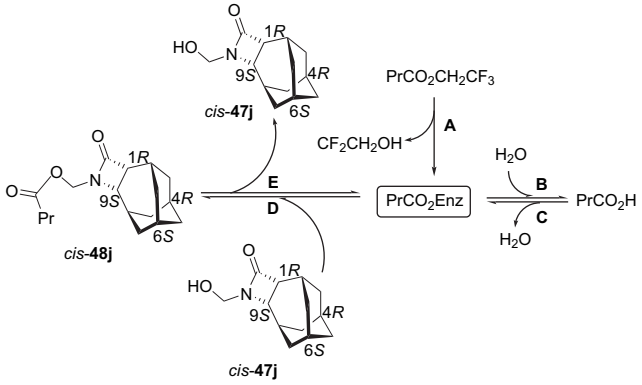
Scheme 30. Kinetic resolution of racemic *cis*- and *trans*-**39a**–**h** by lipase PS.[137] More reactive enantiomers are presented.



Scheme 31. Kinetic resolution of N-hydroxymethylated β-lactams by lipase PS and AK in organic solvents.[154,155,176–183] More reactive enantiomers are presented.

the acylation of cis-compounds (Scheme 27), the two lipases thus showing a nice complementary behaviour.[137] It has been observed that, for lipase PS catalysis, enantioselectivity and reactivity decrease when the acyl part of the achiral acyl donor becomes more hydrophobic, i.e., from Ac to PrCO. Accordingly, 2,2,2-trifluoroethyl chloroacetate has been, surprisingly, the best acyl donor for these N-acylations.

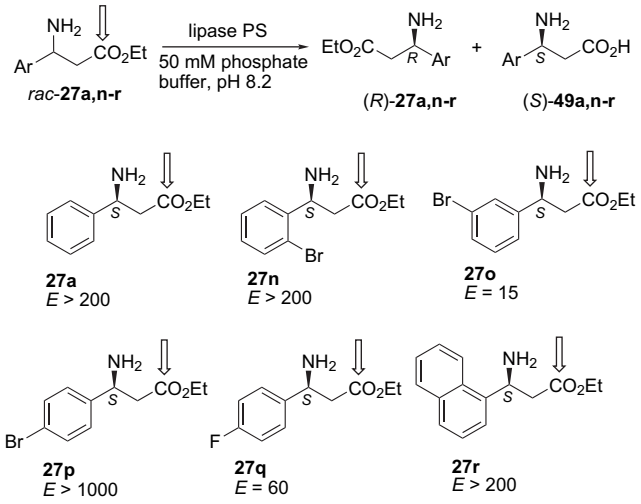
As shown by the data in Scheme 31, the lipase-catalysed acylation of N-hydroxymethylated β-lactams by lipase AK and PS is a highly valuable kinetic resolution method. The produced resolution products **47a–r** and **48a–r** can be easily transformed into the corresponding β-lactams and β-amino acids or β-amino esters.[183,184] As a drawback of this method, the drop in ee of the unreacted substrate enantiomer through enzymatic hydrolysis seems sometimes to hinder the total applicability of the method. This is suggested to be due to a complicated equilibrium in the system where the ester product e.g., **48j** produced through steps A+D and an achiral acyl donor are hydrolysed by the water in the seemingly dry enzyme preparation (steps A+B and E+B, Scheme 32).[155] On the other hand, the esterification of the acid (step C) acts as a continuous source of new water. It was also shown that, under strongly acidic conditions, the hydrolysis of an *N*-hydroxymethyl β-lactam and its ester into the corresponding β-amino acid results in the formation of a dimer.[155] For this reason, the hydrolysis through the corresponding β-lactam (cleavage of the hydroxymethyl group by



Scheme 32. Equilibria in the mixture of *cis*-**47j**, 2,2,2-trifluoroethyl butanoate, butanoic acid and water in the presence of lipase PS in TBME.[155]

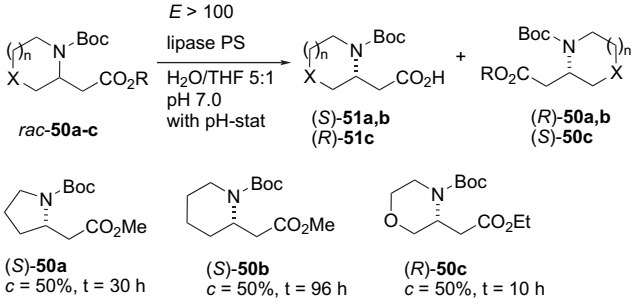
NH_4OH followed by hydrolysis of the β-lactam ring with HCl) can be recommended.[183]

As another strategy, lipase PS-catalysed hydrolysis at pH 8.2 has proved to be an excellent method for the resolution of 3-aryl-3-aminopropanoic acid esters **27a**,**n**–**r**, giving (*S*)-**49a**,**n**–**r** as resolution products (Scheme 33).[185] Thus, excellent enantioselectivities are observed with various aryl substituents. The reaction time (15 h) was mentioned only for **27a**. The method was reported to be scaleable to 200 g/dm^3 (1 M). A patent describing a closely similar system, however, reports solubility problems with **27a** (0.37 M) at pH 8.2 (no buffer).[186] This was overcome by using *tert*-butyl methyl ether as a cosolvent (1:1; pH 8.2 with a pH stat), allowing the use of 1.2 M of **27a**. Isolation of (*S*)-**49a** with 99.6% ee and a yield of 42% (t=15 h) were reported. The extendability of the method to other β-amino esters has not been studied.



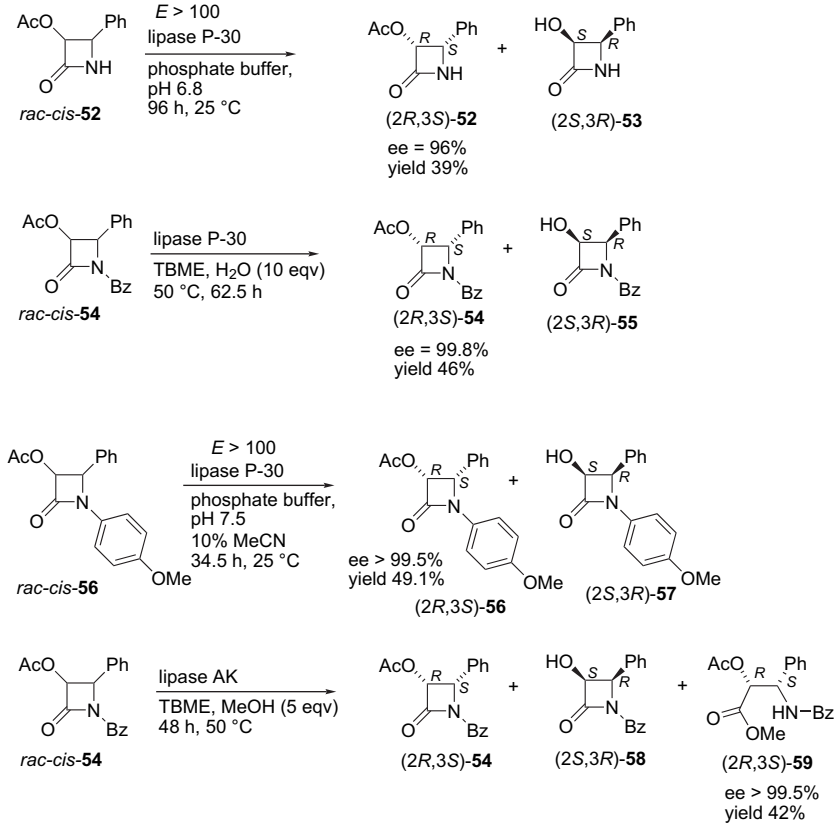
Scheme 33. Kinetic resolution of *rac*-**27a**,**n**–**r** by lipase PS.[185] More reactive enantiomers are presented.

Lipase PS has been successfully used in the kinetic resolution of *N*-Boc-protected heterocyclic β-amino esters **50a**–**c**, yielding highly enantiopure acids **51a**–**c** (Scheme 34).[187] Here, the β-amino functionality is a secondary nitrogen as part of the ring structure. Enantioselectivities of the resolutions of all tested compounds are excellent (E>100).



Scheme 34. Kinetic resolution of heterocyclic β-amino esters *rac*-**50a**–**c** by lipase PS.[187] More reactive enantiomers are presented.

Pseudomonas lipases have been efficiently used in the kinetic resolution of *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine, the Taxol C-13 side chain (Scheme 35).[188] Thus, lipases



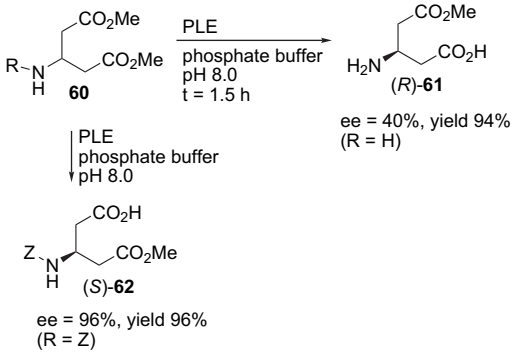
Scheme 35. Kinetic resolution of β-lactams by *Pseudomonas* lipases for the synthesis of Taxol C-13 side chain.[188]

P-30 and AK exhibit high enantioselectivity for the hydrolysis of the acetyl group of racemic *cis*-**52**, **54** and **56**, giving (2*S*,3*R*)-**53**, -**55** and -**57** as the hydrolysed product, respectively. In addition to this hydrolysis, β-lactam ring opening of *rac*-**54** with methanol is observed. This is possibly the first report on a lipase-catalysed β-lactam ring opening. The ring opening preferentially takes place with the (2*R*,3*S*)-enantiomer in the formation of (2*R*,3*S*)-**59**, simultaneously with the hydrolysis of the 3-acetoxy group of the (2*S*,3*R*)-enantiomer in the formation of (2*S*,3*R*)-**58**. When methanol is replaced by water, only hydrolysis of the C-3 acetoxy group is observed. Experiments on β-lactam ring opening with racemic *cis*-**52** and **56** were not reported.

For the resolution of the Taxol C-13 side chain, phenyl glycidic esters and 3-azido-2-hydroxy-3-phenylpropanoates have also been used as starting materials.[189,190] As another strategy, Baker's yeast and various microorganisms have also been used for the enantioselective reduction of the keto group of 3-amino-2-oxo-3-phenylpropanoic esters.[191,192]

2.2.5. Pig liver esterase. Pig liver esterase (PLE) [EC 3.1.1.1.] has been successfully used for the resolution of various prochiral dicarboxylic esters and acetylated prochiral diols.[50] With dicarboxylic esters, the reaction usually stops after the hydrolysis of the first ester group, which has been attributed to the charged carboxylate group. PLE is a serine esterase, which has been isolated with three different isoenzymes.[193–196] Even though the catalytic properties of the isoenzymes differ from each other, PLE has been commonly used as a crude mixture. The crystal structure of PLE has not been elucidated, but an empirical active site model has been developed, based on the hydrolysis of prochiral methyl esters.[197–199]

With prochiral **60** (R=H), enzymatic hydrolysis into (*R*)-**61** proceeds at low enantioselectivity (Scheme 36).[200] This is due to considerable chemical hydrolysis parallel to the enzymatic reaction. It was suggested that the amino group aids the reaction by hydrogen bonding to the carbonyl oxygen. Accordingly, *N*-benzyloxycarbonyl (Z) protection prevents hydrogen bonding, and (*S*)-**62** is produced with a high ee and yield (both 96%) with reversed enantiotopic selectivity. In order to find an enzyme of non-mammalian origin, the authors performed an extensive enzyme screening and reported *Flavobacterium lutescens* IFO 3084 as catalysing a highly regioselective hydrolysis of the pro-*R* ester group.[201] PLE



Scheme 36. PLE-catalysed hydrolysis of the prochiral **60**.[200]

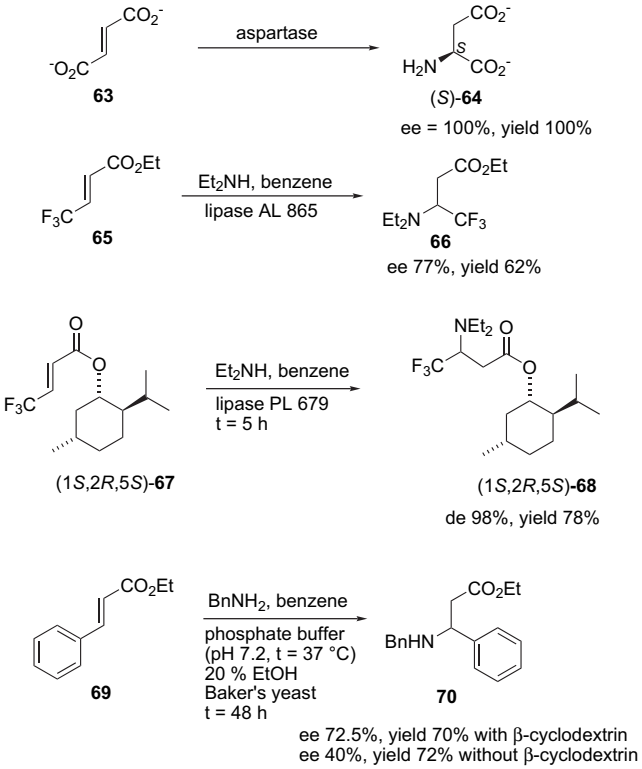
has also been observed to catalyse the ring opening of the methyl ester of penicillin G (**10**).[202]

PLE has limited use in the resolutions of β-amino acids. This can be seen in the hydrolysis of methyl *N*-benzoyl-3-aminobutanoic acid, which proceeds with low enantioselectivity.[203]

3. Other biotransformations

3.1. Michael-type additions

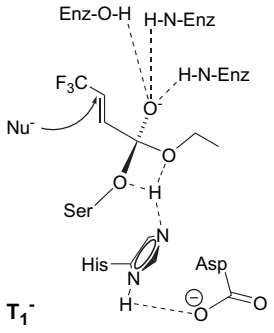
Lyases [EC 4.b.c.d] catalyse the addition of small molecules to C=C, C=N or C=O bonds, or the reverse reaction. Lyases may possess a high synthetic potential, but poor commercial availability hinders their use. As one of the most successful examples, the aspartase-catalysed [EC 4.3.1.1] addition of ammonia to fumaric acid **63** has been operated commercially for the production of (*S*)-aspartic acid, (*S*)-**64**, on a multithousand-ton scale by diverse producers (Scheme 37).[204,205] Even though the method utilising aspartase looks appealing, the enzyme has proved to be very substrate specific.



Scheme 37. Towards β^3-amino acids by Michael addition.[206–208]

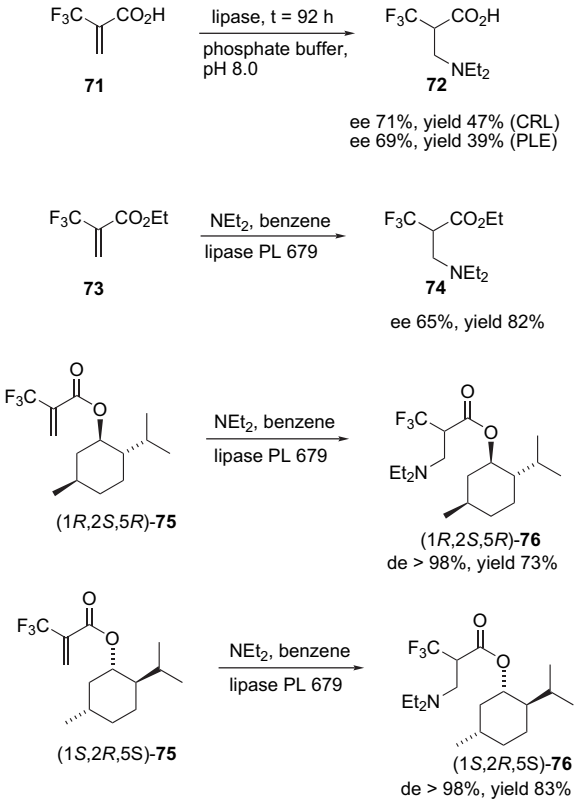
Another strategy is the use of lipase catalysis in the Michael-type additions. The method suffers from relatively low enantiopurities of the products, but is of high value in the future, in the expectation that more suitable lipases are found or developed. The Michael addition is not the natural reaction of lipases, but may take place if the first anionic tetrahedral intermediate serves as a template for the addition of a nucleophile (Scheme 38). Thus, the formation of **66** by the addition of diethylamine to ethyl (*E*)-4,4,4-trifluoro-2-butenoate **65** with various lipases has been tested (Scheme 37).[206,207] Lipase AL 865 from *Achromobacter* is one of

the best enzymes, with 77% product ee and 62% yield. The reaction has not been reported with ethyl (*E*)-2-butenoate. Thus, the necessity of fluorines as double-bond activators remains obscure. A further improvement has been achieved by using the (1*S*,2*R*,5*S*)-menthol ester **67** as a Michael acceptor (78% yield and 98% de of (1*S*,2*R*,5*S*)-**68**). Baker's yeast-catalysed Michael addition of various amines to ethyl *trans*-cinnamate **69** has also been reported.[208] The presence of β-cyclodextrin has been observed to increase the ee of the product **70** from 40 to 72.5%, the yields being around 70%. The absolute configurations were not determined, and the possible side products as a result of ester hydrolysis were not mentioned.



Scheme 38. Mechanism of Michael addition with lipases.

β²-Amino acids have also been obtained by the lipase-catalysed Michael addition (Scheme 39).[206,207,209] Lipases from *Candida rugosa* (CRL) and pig liver esterase (PLE) are able to catalyse the reaction of **71** with diethylamine, even though the enantiopurites and yields of the product **72** remain low.

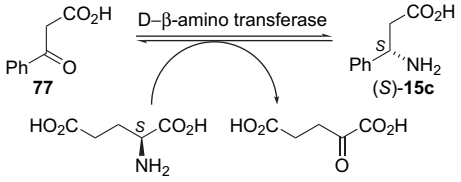


Scheme 39. Towards β²-amino acids by Michael addition.[206,207,209]

The use of lipase PL 679 and the esterified substrate **73** allows an improved yield of the product **74**. For the addition of diethylamine to 2-trifluoromethyl-2-propenoic acid menthol esters **75** catalysed by lipase PL 679, excellent diasteroselectivities (over 98% de) of the product **76** have been obtained with both enantiomers of menthol.

3.2. Transferases

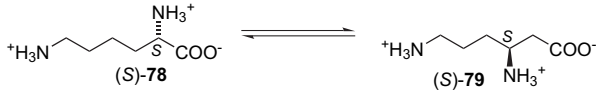
Transferases [EC 2.b.c.d] have not yet found widespread use in organic synthesis. Most aminotransferases (EC 2.6.1) require a cofactor that is often pyridoxal-5-phosphate. The use of β-aminotransferases has recently been introduced especially for the preparation of 3-amino-3-phenylpropanoic acid (Scheme 40).[210] D-β-Amino transferase from *Variovorax paradoxus* and L-β-amino transferase from *Alcaligenes eutrophus* catalyse the reductive amination of **77** into (*S*)- and (*R*)-β-amino acids **15c**, respectively. In this system, the inexpensive cofactors, (*S*)-glutamic acid and (*S*)-alanine, respectively, are oxidised into the corresponding keto acids. The reactions yield exclusively one enantiomer. For the reverse reaction, various racemic β-amino acids were subjected to enzymatic reaction conditions, and consumption of the other enantiomer was observed to take place typically within 24 h. Even though the exact conversion was not determined, the results suggest a wide substrate specificity. Gram-scale reactions were not performed.



Scheme 40. D-β-Amino transferase-catalysed reaction.[210]

3.3. Isomerases

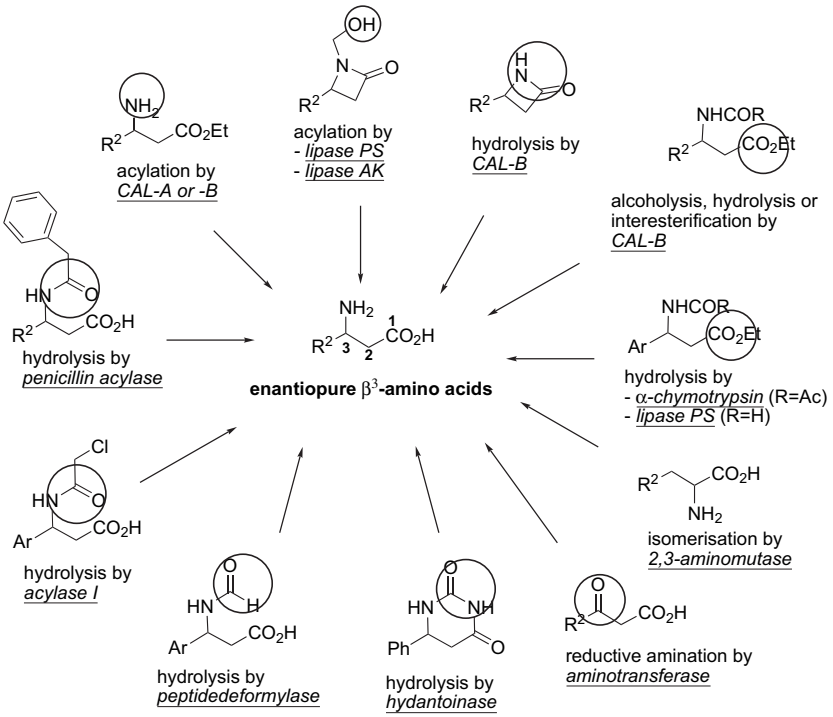
Enzymes in the class number five consist of isomerases. Their subclass [EC 5.4.3] encompasses enzymes transferring amino groups. Among these, enzymes capable of isomerisation of L-amino acids into L-β-amino acids may find synthetic use in the future, e.g., lysine 2,3-aminomutase catalyses the interconversion of L-lysine (*S*)-**78** and L-β-lysine (*S*)-**79** (Scheme 41).[211] This is the first step in the metabolism of lysine into acetate. Lysine 2,3-aminomutase has been claimed to accept also other substrates such as L-alanine, L-aspartate and L-glutamate.[212] At present, there is too little information available on aminomutases to assess their full synthetic potential.



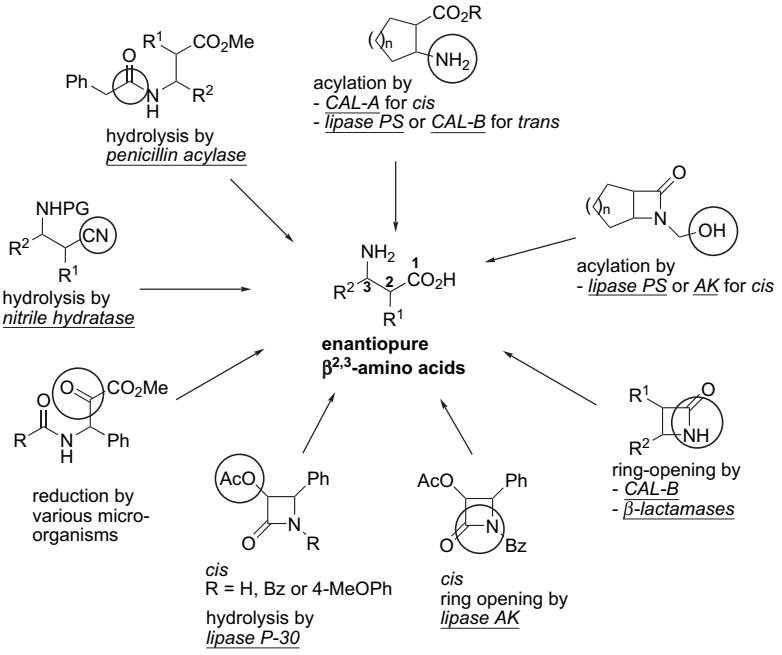
Scheme 41. Natural reaction of lysine 2,3-aminomutase.[212]

4. Conclusions

The enzymatic methods for the preparation of β³- and β²,³-amino acids have been summarised in Schemes 42 and 43,



Scheme 42. Synthesis of enantiopure β^3-amino acid derivatives by enzymatic resolution. The reactive functional group is circled.



Scheme 43. Preparation of enantiopure $\beta^{2,3}$-amino acid derivatives by enzymatic kinetic resolution. The reactive functional group is circled.

respectively. Thus far, the most frequently used methods are based on kinetic resolution with hydrolytic enzymes. The presence of amino and ester functionalities in the substrates enables the development of the various resolution methods. Thus, the highly enantioselective hydrolysis of a phenylacetyl group by penicillin acylase was the first successful approach that is applicable to the preparation of a wide range of enantiopure β^3- and $\beta^{2,3}$-amino acids. Other hydrolytic enzymes with relatively strictly targeted reaction possibilities are acylase I from porcine kidney and α-chymotrypsin. As recently introduced methods, the potency of peptide deformylase, hydantoinases, aminotransferases, 2,3-aminomutases and nitrilases/nitrile hydratases cannot be fully assessed at the present time. β-Lactamase-catalysed $\beta^{2,3}$-lactam ring opening proceeds with high enantioselectivity. Extendability of the method to β^3-amino acids is not known.

Lipases are the most utilised and also the most exploited enzymes for the preparation of β-amino acids and their analogues. Lipase PS from *B. cepacia* (lipase PS) and lipases A

and B from *C. antarctica* have proved to be the most prominent catalysts, indicating acceptance for a wide variety of substrates (β-amino acids, β-amino esters, β-amino nitriles, β-lactams and N-hydroxymethylated β-lactams). Generally, CAL-A catalysis allows the kinetic resolution of acyclic β^3-amino esters and alicyclic *cis*-$\beta^{2,3}$-amino esters of various types, whereas lipase PS is excellent for the kinetic resolution of alicyclic *trans*-$\beta^{2,3}$-amino esters. Noteworthy is the CAL-B-catalysed β-lactam ring opening, even though the problems of long reaction times and the control of water activity still need to be solved. CAL-B-catalysed N-acylation is, however, less useful than CAL-A- and lipase PS-catalysed N-acylation of amino esters. The reason is the low chemoselectivity of CAL-B, i.e., simultaneous interesterification and N-acylation with achiral acyl donors. The use of isopropyl methoxyacetate as an acyl donor has allowed N-acylation without interesterification also with CAL-B. Lipases PS and AK have been especially useful for the acylation of N-hydroxymethylated β-lactams, even though the method is sometimes hampered by reverse hydrolysis.

Lipases P-30 and AK exhibit high enantioselectivity in the resolution of *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine, the Taxol C-13 side chain. The resolutions are based on hydrolysis of acetyl groups or lipase AK-catalysed β-lactam ring opening. Enantioselective reduction of keto groups has also been used for obtaining the product.

The applicability of the above-mentioned methods to the preparation of enantiopure β^2-amino acids has not been extensively studied, even though they are also valuable synthons. Thus far, α-methyl-β-alanine is the only β^2-amino acid the enantiomers of which have been prepared by kinetic resolution. CAL-A and -B display low enantioselectivities in the kinetic resolution. The high chemoselectivity of CAL-A (allowing N-acylation) and low chemoselectivity of CAL-B (allowing interesterification) with carboxylic acid esters have been exploited in two successive kinetic resolutions on a gram scale.

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Biographical sketch

Arto Liljeblad was born in 1974 in Pori, Finland. He graduated with an M.Sc. in organic chemistry from the University of Turku in 2000. He received his PhD under the supervision of Professor Liisa T. Kanerva in February 2005 in the same University. His research focuses on the enzymatic kinetic and dynamic kinetic resolution of non-natural α- and β-amino acids.

Professor Liisa T. Kanerva was born in Salo, Finland in 1947. She received her M.Sc. in organic chemistry from the University of Turku in 1973 and her PhD in 1983. In 1987, she was appointed as a docent in physical organic chemistry in the University of Turku. In 1988–89 she spent a period in M.I.T. with Professor Alexander M. Klibanov. Since returning to the University of Turku, her group has been active in research where enzymes have been used for the preparation of enantiopure compounds, lipase-catalysed kinetic resolution of amino esters being one of the main fields. In 1992, she was appointed as a Professor of Medical Chemistry and, in 2000, Professor of Synthetic Drug Chemistry.