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# Enantioselective acylation of chiral amines catalysed by serine hydrolases

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**Contents**



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## 1. Introduction

Enantiopure amines are used in the fine-chemicals industry as resolving agents, chiral auxiliaries and chiral synthetic building blocks for pharmaceuticals and agrochemicals. Among the methodologies that have been developed for the industrial production of enantiopure amines, lipase mediated enantioselective acylation[1] is increasing in significance compared with competing procedures, such as the crystallisation of a diastereomeric salt, enantioselective reductive amination using a chiral auxiliairy and enantioselective reductive amination mediated by a transaminase.

Lipases, the lipid hydrolysing catalysts of Nature, are highly stable enzymes with a very simple catalytic machinery (see Fig. 1). These characteristics have made them outstanding catalysts for synthetic biotransformations that involve the carboxyl group, such as esterification, transesterification, perhydrolysis and aminolysis in which the natural nucleophile—water—is replaced by an alcohol, hydroperoxide or amine.[2,3] The principle of lipase-catalysed aminolysis of carboxylic esters was first described in 1984 by Inada et al.[4] in a paper that also contributed to the revival of the use of lipases in organic media, following the largely disregarded example by Sym in the 1930's.[5] A publication by Zaks and Klibanov followed closely[6] and a multitude of publications describing the application of lipase-catalysed aminolysis has been published since.[7]

Although Nature has not designed lipases for enantioselectivity, it was recognised already 100 years ago that they are chiral and, hence, capable of enantiodiscrimination.[8] The first—incompletely documented—examples of enantioselective lipase-mediated amine acylation date from the late 1980's.[9] In the early 1990's, after highly stable lipase preparations had become freely available, papers and patent applications describing the resolution of chiral amines via lipase-catalysed enantioselective acylation (kinetic resolution) started to appear in large numbers. Less than 10 years later, the procedure is applied by BASF at a scale of over 1000 t/a[1] and an extension of the capacity has been announced.

Two other serine hydrolases, the protease subtilisin and penicillin acylase, have received much less attention, although their capability for enantioselective amine acylation was demonstrated in the late 1980's and early 1990's, respectively. In the present paper we will critically review the accomplishments in the field of the kinetic resolution of amines in the presence of serine hydrolases from three classes: lipases, subtilisin and penicillin acylase and compare their capabilities.

## 2. Lipase mediated aminolysis

### 2.1. Mechanism

Lipases, the lipid splitting catalysts of Nature (EC 3.1.1.3), operate by essentially the same mechanism as the serine proteases.[10] The active site (see Fig. 1) contains a serine residue that is activated by histidine and aspartate residues; these together form the catalytic triad. The reactant ester forms a tetrahedral acyl-enzyme intermediate by reaction with the OH group of the catalytic serine residue; the resulting excess of negative charge that develops on the carbonyl oxygen atom is stabilised by the oxyanion hole. Next, the tetrahedral intermediate collapses to the serinate

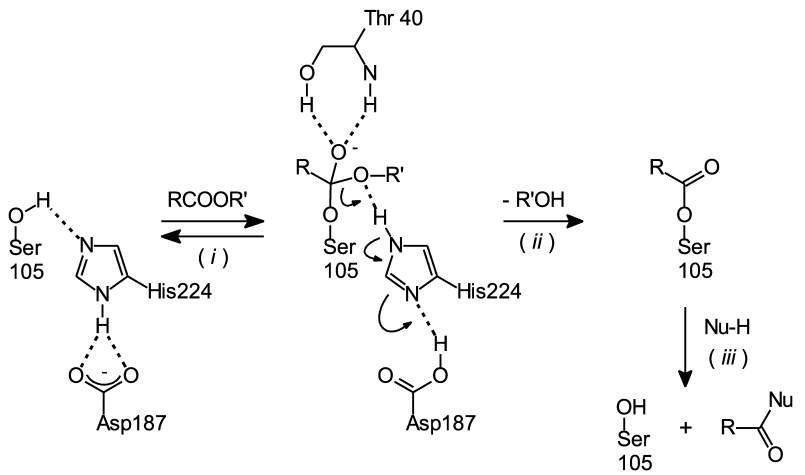



**Figure 1.** Reaction mechanism of lipase catalysis (numbering is for *Candida antarctica* lipase B); - - - denotes a hydrogen bond. Step (iii) is the microscopic reversal of steps (i) and (ii).

ester with elimination of the alcohol. Subsequent reaction of the acyl-enzyme intermediate with a nucleophile—the acyl acceptor—affords the product. In the case of hydrolysis, which is the natural reaction of lipases, the nucleophile is water. The reaction of the acyl-enzyme with an amine affords the corresponding carboxylic amide.[2]

Ester aminolysis can be regarded as irreversible, because the transformation is strongly exothermic under normal reaction conditions.[11] Amide hydrolysis, which is not readily mediated by lipases, can also be excluded due to the almost universally employed anhydrous reaction conditions (see below).

### 2.2. Practical considerations

Water, which is the natural reaction medium of enzymes, is not a good solvent for aminolysis, because hydrolysis of the donor is bound to predominate. In consequence, organic solvents are used almost universally. Even trace quantities of water in the reaction mixture will lead to undesirable hydrolysis of the acyl donor;[11] apart from the resulting loss in yield, the liberated acid may effect deactivation of the lipase. Hence, aminolysis is preferably carried out in a strictly anhydrous medium; in some cases activated zeolites have been added to the reaction mixture to ensure anhydrous reaction conditions. Lipases, in particular the ones from microbial sources that are commonly used nowadays, are exceptionally rugged enzymes and they tolerate anhydrous organic solvents rather well. In particular *Candida antarctica* lipase B (CaLB),[12,13] which actually seems to prefer anhydrous conditions,[14] has been widely used in consequence.

The efficient use of enzymes in non-aqueous media necessitates their immobilisation on a suitable carrier material,[15,16] presumably because the use of 'free' protein dispersions renders the major part of the enzyme molecules inaccessible to the reaction medium. Accordingly, in ammoniolysis lipases adsorbed on macroporous polypropylene (Accurel EP100) are more efficient than a free suspension of an equal amount of lyophilisate.[16] CaLB can be obtained from commercial sources as adsorbate on acrylic resin (Novozym 435), on Accurel EP100 (SP611 from Novozymes) and as a cross-linked enzyme crystal (Altus ChiroCLEC™-CAB). The use of sol–gel encapsulated CaLB in enantioselective aminolysis has quite recently been demonstrated.[17] We have reported the immobilisation of lipases, including CaLB, as cross-linked enzyme aggregates[18] but their use in amine resolution has not yet been published.

*C. antarctica* lipase A[19] (CaLA) has seen little application until now, but recently it has shown particular promise in the selective *N*-acylation of α- and β-amino acid esters (see later). CaLA was immobilised on Celite[15] in the presence of sucrose for optimum activity in aminolysis.

### 2.3. Lipase-catalysed synthesis of carboxylic amides

Because the subject has recently been reviewed[7] a brief introduction will suffice. Lipase-catalysed aminolysis seems to be a fairly general reaction that has been reported for a wide range of lipases and amines, including, besides the primary alkylamines, ammonia, hydroxylamine and hydrazine. Aniline derivatives, which are—similar to phenols—weak nucleophiles, hardly react. Examples of enzymatic acylation of secondary amines are rare (see later).

Lipase-catalysed esterification is well documented but the corresponding reaction of carboxylic acids and amines was thought to be not feasible due to the tendency of the reactants to form unreactive salts, in spite of ample indications to the contrary.[7] It has recently been shown, however, that the lipase catalysed condensation of carboxylic acids and ammonia is quite feasible.[20–22]

### 2.4. Kinetic resolution of amines

The kinetic resolution of chiral amines via enantioselective acylation by an ester is depicted in Figure 2.

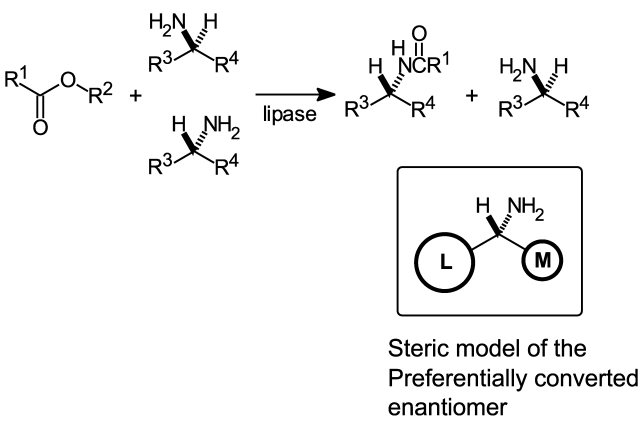



**Figure 2.** Lipase mediated enantioselective acylation of chiral amines. The steric model shows the preferentially acylated enantiomer.[27,28]

It is common practice to discuss a kinetic resolution in terms of its enantiomeric ratio $E$[23,24] which is equal to the ratio of the pseudo second order rate constants of the enantiomers according to Eq. (1). We note that formally this practice is not correct for lipase catalysed resolutions, because lipases do not obey minimal Michaelis–Menten kinetics and, hence, slight deviations of the predicted behaviour are to be expected.[25] It has often been attempted to predict enzymatic kinetic resolutions by measuring the specificity constants $k_{cat}/K_m$ of the enantiomers separately. It has recently been shown, however, that $E$ can only be determined reliably from experiments with the racemic nucleophile.[26]

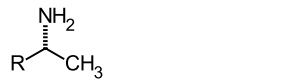
$$E = \frac{\left(\frac{k_{cat}}{K_m}\right)_R}{\left(\frac{k_{cat}}{K_m}\right)_S} \quad (1)$$

The enantiopreference of lipases in the acylation of chiral alkyl and arylalkyl amines corresponds with a steric model (see Fig. 2) that originally has been developed to predict the preferentially acylated enantiomer of secondary alcohols.[27] Interestingly, subtilisin mediates the acylation of chiral alcohols and amines with the opposite enantiopreference.[28]

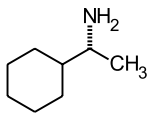
**Table 1**. Non-enzymatic acylation of **6** by 2,2,2-trifluoroethyl butyrate[a]

| Solvent | Conversion (%) | $K$ ($M^{-1}h^{-1}\times10^{3}$)[b] |
|---|---|---|
| Hexane | 47 | 74 |
| DIPE | 25 | 31 |
| Toluene | 15 | 17 |
| Acetonitrile | 10 | 11 |
| Acetone | 10 | 11 |
| 3-Methyl-3-pentanol | 3 | 3 |
| *tert*-Amyl alcohol | 2 | 2 |

[a] Compound **6** (0.32 M) and 2,2,2-trifluoroethyl butyrate (0.70 M) in the chosen solvent, 14 h at 40 °C. Data have been taken from Ref. 29.
[b] Calculated from the conversion by standard second-order kinetics.




(*R*)-**1a-e**




(*R*)-**1i**

a: R = $C_2H_5$ e: R = $C_5H_{11}$
b: R = $C_3H_7$ f: R = $C_6H_{13}$
c: R = $C_4H_9$ g: R = $C_9H_{19}$
d: R = *t*-$C_4H_9$ h: R = $CH_3OCH_2$

**Figure 3.** Lipase mediated enantioselective acylation of the alkylamines **1a**–**i**; the preferentially acylated enantiomers are shown.

## 3. Lipase-mediated enantioselective acylation of amines

The number of chiral amines that has been resolved via lipase mediated enantioselective acylation has grown explosively over the last few years; most of these have involved *C. antarctica* lipase B. A wide range of acyl donors and reaction conditions has been employed but systematic investigations are few.

It should always be kept in mind that amines, in contrast with alcohols, react spontaneously with esters[29–31] and that such an uncatalysed background reaction will erode the enantiomeric excess of the product. Hence, the acyl donor should not be too reactive[30] and the reaction conditions should be chosen with some consideration for this issue, which will be discussed more fully later.

*Effects of the medium on the non-enzymatic reaction*. Gutman et al. have found that the non-enzymatic reaction of 1-aminoindane and the activated ester 2,2,2-trifluoroethyl butyrate (see Table 1) is severely influenced by the solvent.[29] The rate decreased with increasing solvent polarity as the reaction was 30 times faster in hexane than in *tert*-amyl alcohol or 3-methyl-3-pentanol. One could conclude from these results that tertiary alcohols are attractive resolution media. Alcohols are lipase inhibitors,[32] however. Moreover, it is the ratio of the rates of the enzymatic and the non-enzymatic reactions, rather than either parameter in isolation, that should be optimised.

### 3.1. Alkylamines

2-Aminobutane (**1a**, see Fig. 3) was one of the first amines to be resolved via enantioselective acylation.[33,34] The $E$ values that we have estimated from the—often incomplete—literature data generally are rather low, reflecting, it would seem, the difficulty of discriminating a methyl and an ethyl group. Acetic acid esters were inefficient resolution reagents,[35,36] presumably because of the small size of the acetyl group (see Table 2). Methyl methacrylate seems to give the best results ($E$>55), but the reaction was very slow and the conversion was low even after 11 days reaction time.[33] BASF, who has developed a robust process for the resolution of a wide range of alkyl- and arylalkylamines, has claimed a modest $E$ of 8 for **1a**.[1]

**Table 2**. Resolution of chiral alkylamines **1**

| Amine | Lipase | Donor | Solvent | $T$ (°C) | $E$ | Reference |
|---|---|---|---|---|---|---|
| **1a** | CaLB | Ethyl acetate | – | 21 | >4.5 | 36 |
| | BpL | Methoxyacetic ester[a] | – | | 8 | 1 |
| | CaLB | Methyl methacrylate | THF | 30 | 55 | 33 |
| | CaLB | Dimethyl succinate | Dioxane | 30 | 35 | 34 |
| | CaLB | Dimethyl succinate | Hexane | 30 | 6 | 34 |
| | CalB | 1-Phenylethyl acetate | Dioxane | 30 | 4 | 35 |
| **1b** | CaLB | Ethyl acetate | – | | >200 | 37 |
| | PaL | Ethyl acetate | Diethyl ether | rt | >100 | 38 |
| | BpL | Methoxyacetic ester[a] | – | | 50 | 1 |
| | CaLB | Dibenzyl carbonate | Hexane | rt | 4 | 42 |
| **1c** | CaLB | Ethyl acetate | – | 21 | >31 | 36 |
| **1d** | CaLB | Ethyl acetate | DME | rt | 29 | 41 |
| | CaLB | Isopropyl acetate | DME | rt | >700 | 41 |
| | BpL | Methoxyacetic ester[a] | – | | >1000 | 1 |
| **1e** | CaLB | Ethyl acetate | – | 21 | >55 | 36 |
| | CaLB | Dimethyl succinate | Dioxane | 30 | 35 | 34 |
| | CaLB | Dimethyl succinate | Hexane | 30 | 68 | 34 |
| | CaLB | 1-Phenylethyl acetate | Dioxane | | >200 | 35 |
| **1f** | CaLB | Ethyl acetate | – | 21 | >110 | 36 |
| | CaLB | Ethyl octanoate | – | 39 | >100 | 40 |
| | CaLB | Dibenzyl carbonate | Hexane | rt | 5 | 42 |
| **1g** | CaLB | Ethyl acetate | – | 21 | >40 | 36 |
| **1h** | CaLB | Isopropyl methoxyacetate | – | rt | 58 | 39 |
| **1i** | CaLB | Dibenzyl carbonate | Toluene | rt | 17 | 30 |

[a] The alkyl group was not specified.

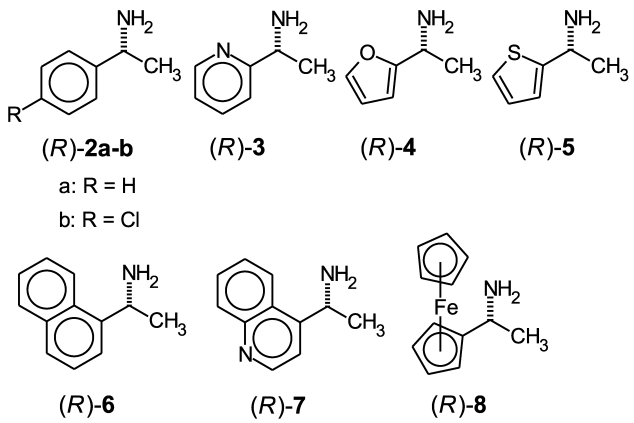



**Figure 4.** Lipase mediated enantioselective acylation of the 1-arylethylamines **2**–**8**; the preferentially acylated enantiomers are shown.

In contrast, the methyl and propyl groups in 2-aminopentane (**1b**) were very efficiently discriminated upon acetylation of the latter amine in the presence of CaLB[37] as well as *Pseudomonas aeruginosa* lipase (PaL).[38] Higher 2-aminoalkanes (**1c**–**1h**) have been resolved using donors such as dimethyl succinate,[34] isopropyl methoxyacetate[39] and ethyl octanoate[40] in the presence of CaLB (see Table 1 for further details). Amine **1d**, in which the **L**-group is *tert*-butyl, even was acylated with absolute enantioselectivity.[1,41] Surprisingly, the *E* ratio improved by a factor of 20 when the acyl donor was isopropyl acetate rather than ethyl acetate.[41]

1-Cyclohexylethylamine (**1i**) was acylated with only a modest *E* of 17.[30] This latter result could, perhaps, be ascribed to the acyl donor—dibenzyl carbonate—which also gave inferior results with **1b** and **1f**.[42] We note, however, that the sterically very similar **2a** (see Fig. 4) was acylated with very high enantioselectivity ($E>300$) under the conditions that failed to resolve **1i**.[30]

### 3.2. Arylalkylamines

**3.2.1. 1-Arylethylamines.** 1-Phenylethylamine (**2a**) seems to have served as a test substrate as it has been subjected to a wide variety of acylation conditions. Most of these have resulted in excellent enantiorecognition. Donor reagents (see Table 3) that have been used include ethyl acetate,[37] esters of methoxyacetic acid[43,44] and dibenzyl carbonate.[30,42] This latter reagent is particularly attractive as it affords the enantiopure Z-protected amine in a single step.

1-(4-Chlorophenyl)ethylamine (**2b**) has similarly been acylated with excellent enantiodiscrimination by a considerable range of esters in the presence of CaLB.[45,46] This work is conspicuous by the use of ethyl chloroacetate, dimethyl malonate and ethyl phenylacetate as acyl donors.[46] The enantioselectivity with ethyl chloroacetate in DME medium was very high ($E$=954) but high-selectivity resolutions could also be accomplished with methyl methoxyacetate, either neat ($E$=232) or in *tert*-amyl methyl ethyl (TAME) medium ($E$=458). Surprisingly, the reaction was significantly more enantioselective in TAME medium than in TBME.[45]

The resolution of the heteroaryl-substituted ethylamine derivatives **3**–**5** in the presence of CaLB illustrates the

**Table 3**. Resolution of the chiral 1-arylethylamines **2**–**8**

| Amine | Lipase | Donor | Solvent | *T* (°C) | *E* | Reference |
|---|---|---|---|---|---|---|
| **2a** | CaLB | Ethyl acetate | – | | 110 | 37 |
| | BpL | Ethyl methyoxyacetate | TBME | | 114 | 43 |
| | CaLB | Isopropyl methyoxyacetate | TBME | | >1000 | 44 |
| | CaLB | Dimethyl succinate | Dioxane | 30 | 95 | 34 |
| | CaLB | Dimethyl succinate | Hexane | 30 | 6 | 34 |
| | CaLB | 1-Phenylethyl acetate | Dioxane | | >200 | 35 |
| | CaLB | Dibenzyl carbonate | Toluene | rt | >300 | 30 |
| | CaLB | Dibenzyl carbonate | Hexane | rt | 21 | 30 |
| | CaLB | Dibenzyl carbonate | Hexane | rt | 50 | 42 |
| **2b** | CaLB | Methyl methoxyacetate | – | rt | 232 | 45 |
| | CaLB | Methyl methoxyacetate | TBME | 40 | 198 | 45 |
| | CaLB | Methyl methoxyacetate | TAME | 40 | >458 | 45 |
| | CaLB | Ethyl chloroacetate | DME | rt | 954 | 46 |
| | CaLB | Ethyl chloroacetate | TBME | rt | 131 | 46 |
| | CaLB | Ethyl butyrate | TAME | 45 | 449 | 46 |
| | CaLB | Ethyl phenylacetate | TBME | 45 | 309 | 46 |
| **3** | CaLB | Ethyl acetate | – | 30 | 66 | 47 |
| | CaLB | Ethyl acetate | – | 60 | 41 | 48 |
| | CaLB | Ethyl acetate | Dioxane | 30 | 72 | 47 |
| **4** | CaLB | Ethyl acetate | – | 30 | >100 | 47 |
| | CaLB | Ethyl acetate | Dioxane | 30 | 31 | 47 |
| **5** | CaLB | Ethyl acetate | – | 30 | 32 | 47 |
| | CaLB | Ethyl acetate | Dioxane | 30 | >100 | 47 |
| **6** | CaLB | Ethyl acetate | – | | 24 | 37 |
| | CaLB | Ethyl acetate | – | 60 | 5 | 48 |
| | CaLB | Isopropyl acetate | DME | rt | 650 | 41 |
| | BcL | Isopropyl acetate | – | rt | 18 | 41 |
| **7** | CaLB | Ethyl acetate | DIPE | 60 | 120 | 48 |
| **8** | CaLB | Ethyl formate | Dioxane | 28 | 30 | 49 |
| | CaLB | Ethyl acetate | – | 28 | >200 | 49 |

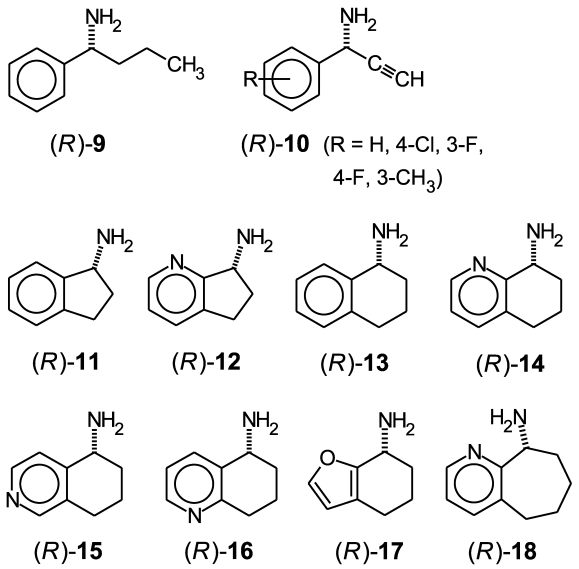



**Figure 5.** Lipase mediated enantioselective acylation of the 1-arylalkylamines **9**–**18**; the preferentially acylated enantiomers are shown.

capricious nature of enantioselective amine acylation. The resolution of the pyridine derivative **3** was less selective than that of its carbocyclic equivalent **2a** under comparable conditions, regardless of whether the reaction was carried out in neat ethyl acetate or dioxane medium.[47,48] 1-(2-Furyl)ethylamine (**4**) was efficiently resolved ($E>100$) by neat ethyl acetate in the presence of CaLB.[47] This latter result contrasts with the somewhat more modest enantiorecognition in the resolution of **1h**,[1] which also has an ether oxygen atom β to the amine function. The effect of the medium on the acylation of **4** is also noteworthy: in dioxane medium the enantioselectivity was much less than in neat ethyl acetate. The thiophene equivalent **5**, in contrast, was very well resolved in dioxane, but in neat ethyl acetate the enantioselectivity was mediocre.[47]

Attempts at resolving 1-(1-naphthyl)ethylamine (**6**) in the presence of CaLB produced conflicting results. Ethyl acetate was found to be an inefficient resolving agent by several authors,[37,41,48] even though one of these had resolved **2a** very efficiently under identical conditions.[37] Isopropyl acetate[41] and 1-phenylethyl acetate,[35] in contrast, gave excellent enantioselectivity. A possible effect by a heteroatom became apparent with the corresponding quinoline system (**7**), which was excellently resolved under conditions that had failed with **6**.[48]

Finally, 1-ferrocenylethylamine (**8**) was acylated with near-quantitative enantioselectivity by neat ethyl acetate in the presence of CaLB, but, in contrast, an attempt at enantioselective formylation resulted in a mediocre enantiodiscrimination.[49]

**3.2.2. 1-Arylalkylamines.** In all of the above examples the **M** group is methyl, but the acylation of 1-phenylbutylamine (**9**, see Fig. 5 and Table 4) demonstrated the ability of CaLB to discriminate larger **M** groups, although with a lower enantioselectivity ($E=60$), than observed with **2a** by the same authors.[35] Hence, the discrimination of a phenyl and a butyl group is less clear-cut than that between a phenyl and a methyl group but, in contrast, the CaLB mediated resolution of a range of 1-phenyl-2-propynylamines (**10**) was accomplished with high enantioselectivity ($E\gg100$).[50] Apparently, the acetylene moiety is readily accommodated in the subsite that binds the **M** group in the resolution of **2**.

The **M** subsite in CaLB is also able to accommodate an alicyclic ring, as shown by the efficient resolution, via acylation with dibut-3-enyl carbonate in toluene, of the bicyclic amine 1-aminoindane (**11**).[30] The closely related compound 1-aminotetraline (**13**), in contrast, reacted with only modest enantioselectivity ($E\sim18$) with either dibenzyl carbonate[42] or ethyl acetate[41,48] in the presence of CaLB. The effect of the leaving group that had already been noted in the resolutions of **1d** and **6** also became manifest with **13** as in the acylation of the latter with neat isopropyl acetate the reaction was highly enantioselective.[41]

Surprisingly, a series of analogs of **13** that contained a heteroaromatic nitrogen, such as **14**–**16**, were acylated with excellent enantioselectivity by ethyl acetate, but, in contrast, the $E$ ratio of the furan equivalent **17** was only mediocre. It is also noteworthy that the expansion of the alicyclic ring in **14** by one methylene group (**18**) caused a complete loss of enantiorecognition.[48] The obvious conclusion is that the discrimination of a phenyl or pyridyl group and an alicyclic ring becomes more difficult when the latter is increased in size. The low $E$ ratio of **17** likewise could be attributed to the

**Table 4**. Resolution of the chiral 1-arylalkylamines **9**–**18**

| Amine | Lipase | Donor | Solvent | $T$ (°C) | $E$ | Reference |
|---|---|---|---|---|---|---|
| **9** | CaLB | 1-Phenylethyl acetate | Dioxane | 30 | 60 | 35 |
| **10** | CaLB | Ethyl acetate | – | | >100 | 50 |
| **11** | CaLB | Dibut-3-enyl carbonate | Toluene | rt | >200 | 30 |
| | CaLB | Dibenzyl carbonate | Hexane | rt | 72 | 42 |
| **12** | CaLB | Ethyl acetate | – | 60 | 49 | 48 |
| **13** | CaLB | Ethyl acetate | DIPE | 60 | 17 | 48 |
| | CaLB | Isopropyl acetate | – | rt | 458 | 41 |
| | CaLB | Dibenzyl carbonate | Hexane | rt | 19 | 42 |
| | BcL | Isopropyl acetate | – | rt | 28 | 41 |
| **14** | CaLB | Ethyl acetate | DIPE | 60 | >500 | 48 |
| **15** | CaLB | Ethyl acetate | DIPE | 60 | 210 | 48 |
| **16** | CaLB | Ethyl acetate | DIPE | 60 | >500 | 48 |
| **17** | CaLB | Ethyl acetate | DIPE | 60 | 27 | 48 |
| **18** | CaLB | Ethyl acetate | DIPE | 60 | 5 | 48 |

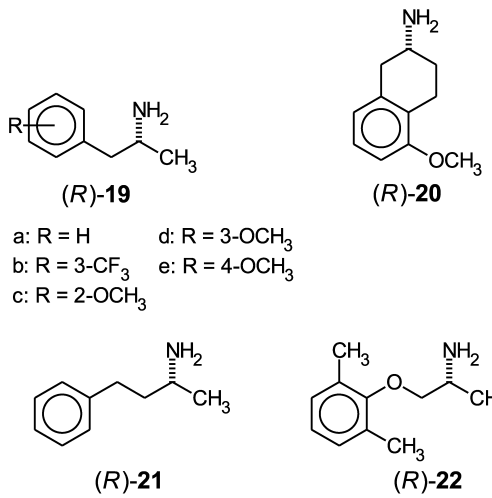



**Figure 6.** Lipase mediated enantioselective acylation of the arylalkylamines **19**–**22**; the preferentially acylated enantiomers are shown.

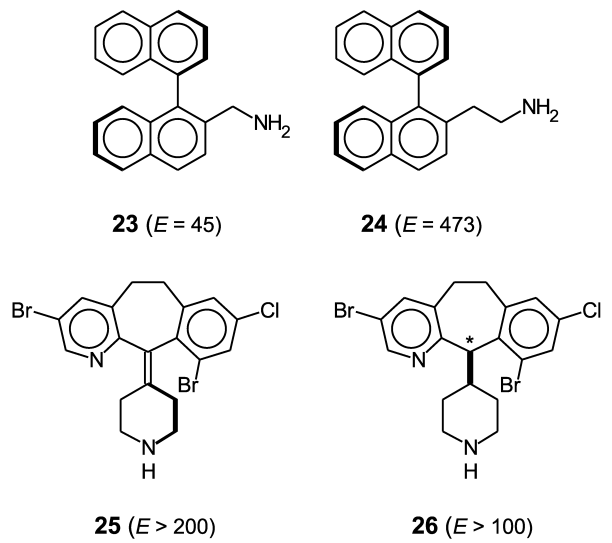



**Figure 7.** Lipase-mediated resolution of highly sterically demanding amines; the preferentially acylated enantiomers are shown.

somewhat smaller size of a furyl group compared with phenyl or pyridyl.

Summarising, the effect of a heteroaromatic nitrogen atom in the **L** group on the enantiorecognition is ambiguous. A positive effect on the enantiorecognition is apparent in the acylation of **7** compared with that of **6** and also with **14**–**16** in comparison with **13**. In contrast, the acylations of **3** and **12** were not more enantioselective than those of **2a** and **11**, respectively.

**3.2.3. Various arylalkylamines.** When an extra methylene group was inserted between the phenyl group and the amine group (**19a**, see Fig. 6), the extent of enantiodiscrimination seems to become less,[51] although the 3-trifluoromethyl derivative **19b** was resolved with near-absolute enantioselectivity (Table 5).[35]

The enantiomer discrimination by *Burkholderia cepacia* lipase (BcL) in the acylation of **20** seems to be rather modest[52]—the experimental data are incomplete—which fails to surprise as BcL did not perform very well in other amine resolutions either. The successful resolutions of **21** and **22** show that efficient enantiodiscrimination with CaLB is also possible when three atoms separate the aromatic moiety and the amine function.[45,51]

In the resolution of the very bulky binaphthyl-substituted amines **23** and **24** (see Fig. 7) PaL performed better than CaLB or any other common lipase.[53] 2,2,2-Trifluoroethyl butyrate gave the best result ($E$=45) in the enantioselective acylation of **23**, whereas the optimum donor for **24**, which reacted more than 25 times as fast, was ethyl butyrate. This latter resolution was accomplished with absolute enantioselectivity ($E$=473).[53]

PaL was also the optimum biocatalyst in the enantioselective acylation of **25** and **26** ($E$>200), with 2,2,2-trifluoroethyl isobutyrate in TBME.[54] The latter resolution was quite sensitive to the solvent. In THF, for example, the enantiomeric ratio only was 27. These resolutions are rare examples of the enantioselective enzymatic acylation of secondary amines.

### 3.3. Alkyl- and arylalkylamines in perspective

Summarising, an impressive number of alkyl- and arylalkylamines has been acylated and nearly every amine can be adequately resolved by fine-tuning the reaction conditions. In every case the enantiomeric bias was in agreement with Kazlauskas' rule. A comparison of the examples in Tables 2 and 3 seems to hint that an aromatic **L** group increases the enantiomeric bias, indicating that non-steric interactions also contribute to the enantiorecognition. The unpredictable effect of heteroatoms in the aromatic system points to the same conclusion.

Tables 2–4 contain numerous examples where the acyl

**Table 5**. Resolution of the chiral arylalkylamines **19**–**22**

| Amine | Lipase | Donor | Solvent | $T$ (°C) | $E$ | Reference |
|---|---|---|---|---|---|---|
| **19a** | CaLB | Ethyl acetate | – | 28 | 37 | 51 |
| **19b** | CaLB | 1-Phenylethyl acetate | Dioxane | 30 | 168 | 35 |
| **19c** | CaLB | Ethyl acetate | – | 28 | 79 | 51 |
| **19d** | CaLB | Ethyl acetate | – | 28 | 70 | 51 |
| **19e** | CaLB | Ethyl acetate | – | 28 | 52 | 51 |
| **20** | BcL | Ethyl acetate | Toluene | | >16 | 52 |
| **21** | CaLB | Methyl methoxyacetate | TBME | 35 | 99 | 45 |
| | CaLB | Ethyl acetate | – | 28 | 41 | 51 |
| **22** | CaLB | Ethyl acetate | – | 28 | 123 | 51 |

donor and the reaction medium exert an appreciable, and sometimes even a vital, effect on the outcome of the resolution. Such effects raise the issue of a competing uncatalysed background reaction. The rate of the latter depends on the activity of the donor[30,31] and the reaction medium.[29]

**3.3.1. Leaving group effects.** In the kinetic resolution of **6**, mediated by CaLB or BcL, in neat alkyl acetate, reported by Reeve[41] (see Table 6), the leaving group exerts a remarkable effect on the enantiorecognition that seems difficult to explain. Taken together with the beneficial effect of dilution on *E*, however, the suspicion arises that the leaving group predominantly influences the background reaction. It is to be expected that the uncatalysed aminolysis of an acetic acid ester by **6** will be slower with the isopropyl ester than with the methyl one, due to the electropositive effect of the isopropyl group. It is also noteworthy that the enantioselectivity was lower in neat isoamyl acetate ($E$=28) than in neat isobutyl acetate ($E$=37). Apparently, the retarding effect of the increase in electropositive character of the leaving group on the uncatalysed reaction is less, in this case, than the accelerating effect of the decrease in medium polarity. Very similar effects were noted in the acylations of **1d** and **13** in the presence of two different CaLB preparations as well as BcL.[41]

**Table 6**. Effect of the leaving group and the medium on the enantiodiscrimination of 1(-1-naphthyl)ethylamine (**6**)

| Donor | Lipase | *E* (neat) | *E* (20% in DME) |
|---|---|---|---|
| Methyl acetate | CaLB | 1 | 10 |
| Ethyl acetate | CaLB | 15 | 33 |
| Propyl acetate | CaLB | 16 | 35 |
| Butyl acetate | CaLB | 18 | 43 |
| Isopropyl acetate | CaLB | 100 | 650 |
| Isobutyl acetate | CaLB | 37 | 95 |
| Isoamyl acetate | CaLB | 28 | 61 |
| Ethyl acetate | BcL | 3 | n.d. |
| Isopropyl acetate | BcL | 18 | n.d. |

In seeming contradiction, the high enantiomeric ratios that have been observed by other authors upon acylation by methyl and ethyl esters strongly indicate that the above problems are by no means universal. In our view the phenomenon is of sufficient importance to warrant a systematic investigation, which has not yet been performed, however. A somewhat speculative hint that we could offer is the possibility of an additive in the enzyme preparations catalysing a non-chiral reaction. An obvious candidate would be Tris buffer, which is known to act as a transesterification catalyst.

**3.3.2. Acyl donors.** Although acetic acid esters have performed adequately in many cases, the reaction is often undesirably slow. It has been shown that methoxyacetic acid esters are >100 times more active[43] and also have other characteristics that are desirable in an industrial resolution process.[1] Thus, BASF has developed a robust process for the resolution of a wide range of alkyl- and arylalkylamines in the presence of, presumably, a proprietary BpL, using an undisclosed ester of 2-methoxyacetic acid.[1]

**3.3.3. Reaction media.** The numerous examples of successful resolutions in neat esters of acetic and methoxyacetic acid demonstrate that a solvent is not really required, unless there is a background reaction to cope with. Such solventless procedures are particularly attractive in an industrial context as they obviate the need for a solvent recycle.

Many authors have used diisopropyl ether (DIPE), in spite of its propensity to form peroxides, which renders its large-scale use unattractive. In such applications it could easily be replaced by DME, TBME or TAME.

When different solvent systems can be compared, hexane generally gives the worst outcome. Thus, the CaLB-mediated acylations of **1a**, **1e** and **2a** with dimethyl succinate proceeded 4–15 times slower in hexane than in dioxane,[34] whereas a relatively fast non-enzymatic reaction should be expected in hexane medium.[29] These effects probably account for the mediocre enantioselectivity in hexane ($E$=6 and 5 for **1a** and **2a**, respectively), whereas in dioxane these values were 35 and 95.[34] Similarly, attempts at resolving **1b** and **1f** with dibenzylcarbonate in hexane met with scant success[42] and the acylations of **2a** and **11**, using the same donor, were much less enantioselective in hexane[30,42] than in toluene.[30] Only the resolution of **1e** with dimethyl succinate, which occurred with comparable enantioselectivity in hexane and dioxane, is possibly an exception.[34†] Summarising, hexane seems not a good solvent for enantioselective amine acylation, although it is an excellent medium for many other lipase-catalysed reactions.

Because of the non-enzymatic background reaction, the issue of how the enantiorecognition of amines by lipases is influenced by the medium remains obscure, as no systematic study has been performed. Extrapolating from alcohol resolutions, such an effect should be expected, however.

**3.3.4. Lipases compared.** CaLB is emerging as nearly everybody's first choice as amine resolution catalyst. It should be noted, however, that BASF has claimed an equally robust resolution, using its proprietary BpL biocatalyst[1] and that encouraging results have been reported with PaL.[38,53,54] The enantiorecognition of (*R*)-**2a** by BpL and CaLB seems of the same order under slightly varying reaction conditions. BcL, in contrast, which has resolved alcohols with excellent results, did not perform very well in amine resolution. Thus BcL did not discriminate the enantiomers of **6** and **13** under conditions that worked very well with CaLB.[41] The resolution of **20** is possibly an exception, but the incomplete data preclude a firm conclusion.[52]

### 3.4. Hydroxyalkylamines and diamines

Kazlauskas' rule correctly predicted the CaLB-mediated (*R*)-selective acylation of the *trans* aminoalcohols **27** and **28** (Fig. 8) by dimethyl glutarate.[55] The resolution of **28** was subject to a considerable solvent effect: the enantiomeric

† As the ee of the acylation products of **1a** and **1e** was calculated from the optical rotation, a considerable error is to be expected, in view of the very low specific rotation of these compounds.

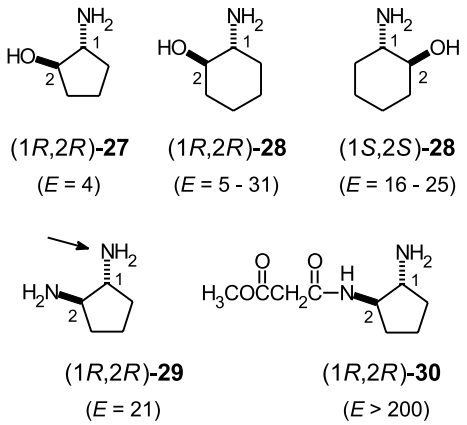



**Figure 8.** Lipase mediated enantioselective acylation of the hydroxyalkylamines and diamines **27**–**30**; the preferentially acylated enantiomers are shown.

ratio was 31 when the reaction was conducted in dioxane medium, whereas in toluene *E* was only 5. Surprisingly, **28** was acylated with opposite enantiopreference by dibenzyl carbonate in dioxane or toluene,[30,55] indicating that non-steric forces also play a role in determining the enantiomer discrimination.

Kazlauskas-type enantioselectivity was also found in the acylation of *trans*-1,2-diaminocyclopentane (**29**) with dimethyl malonate in the presence of CaLB ($E$=20); the subsequent acylation of the resulting monomalonic amide (**30**) occurred with a higher enantiomeric ratio ($E$=200).[56] Steric forces could account for this effect, but alternative explanations are also possible.

### 3.5. N-Acylation of α-amino acid derivatives

1-Amino-1-phenylacetonitrile (**31**, see Fig. 9) reacted with 2,2,2-trifluoroethyl acetate in diisopropyl ether with moderate (*S*)-selectivity ($E$=12–15) in the presence of PaL or *Chromobacterium viscosum* lipase (CvL), but BcL displayed a slight pro-(*R*) bias.[57] Acylation of **31** with ethyl acetate in the presence of CaLB resulted in an unexpected turnover-related racemisation of the reactant.[58] With phenylacetic acid ethyl ester, in contrast, no racemisation was observed and the reaction was highly (*S*)-selective.[58] This latter result may be explained as a beneficial effect of steric congestion in the active site.

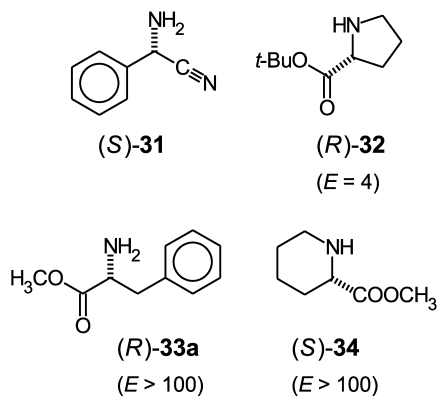



**Figure 9.** Lipase mediated enantioselective acylation of α-amino acid derivatives; the preferentially acylated enantiomers are shown.

A few α-amino acid esters have been acylated at the amino function. The reaction of proline *tert*-butyl ester (**32**) with dibenzyl carbonate in the presence of CaLB was slightly ($E$=4) (*R*)-selective.[30] It is not surprising that the *tert*-butyloxycarbonyl group in **32** is recognised as the **L** group by CaLB but in the acylation of the methyl ester of phenylalanine (**33a**) with ethyl methoxyacetate the preference of CaLB for the (*R*)-enantiomer was nearly quantitative ($E$>100).[59] Hence, the methoxycarbonyl group, rather than the benzyl group, is accommodated in the **L** subsite, which strongly indicates that non-steric interactions determine the enantiomeric bias in this case. It is also noteworthy that the ester group in **33a** did not react, presumably because the ethyl methoxyacetate donor is a highly active one.

The Kanerva group, which has been exploring the lipase-catalysed reactions of α and β-aminoacid esters for some years, has found that CaLB mainly catalysed interesterification reactions of the carboxyl group, whereas CaLA showed a strong preference for amine acylation. These authors surprisingly found that methyl pipecolinate (**34**) could be selectively acylated at the *N*-atom, by the highly active 2,2,2-trifluoroethyl esters, although this is a secondary amine function.[60] 2,2,2-Trifluoroethyl butanoate was the preferred acyl donor as it was nearly eight times as reactive as the acetate; $E$>100 was observed with both donors. Remarkably, the enantiorecognition was not significantly affected by the reaction medium as $E$>100 was observed in a wide range of solvents, from acetonitrile to ethers and even hexane.[60] If it is assumed that the active site geometry of CaLA is similar to that of the common lipases, the latter result indicates that the methoxycarbonyl group binds in the **M** subsite, in contrast with the acylations of **32** and **33a**.

### 3.6. N-Acylation of β-amino esters

A range of β-amino acid ethyl esters (**35a**–**e**, see Fig. 10) likewise reacted regioselectively at the amino function in the presence of CaLA.[61] Two acyl donors were used: 2,2,2-trifluoroethyl butyrate and butyl butyrate (see Table 5). Ethyl 3-aminobutyrate (**35a**) reacted with moderate

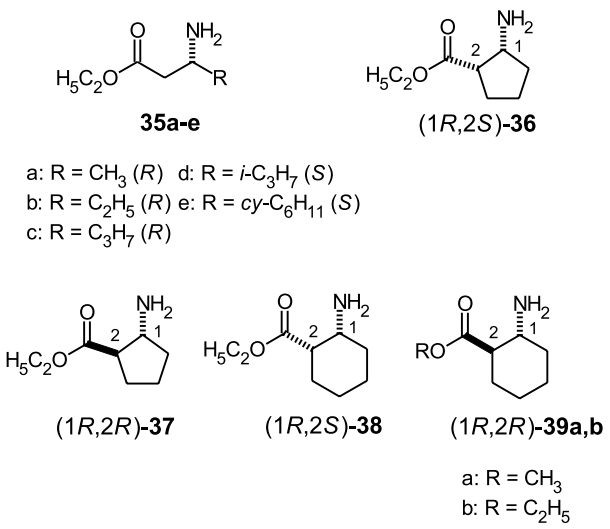



**Figure 10.** Lipase mediated enantioselective acylation of the β-aminoesters **35**–**39**; the preferentially acylated enantiomers are shown.

enantioselectivity ($E$=32) upon acylation with butyl butyrate. Acylation with the 2,2,2-trifluoroethyl ester, under otherwise similar conditions, was uncharacteristically slow and hardly enantioselective.[61] We note that **35a** was also resolved very efficiently in a CaLB-mediated acylation with isopropyl methoxyacetate.[59] The enantioselectivity of the latter procedure was nearly quantitative ($E$=200),[59] in favour of the same enantiomer as preferentially acylated by CaLA.[61] The acylation of **35b**, also using 2,2,2-trifluoroethyl and butyl butyrate as donors, was highly enantioselective with both and the reaction with the fluoroethyl ester was nearly 20 times faster, as would be expected.[61] This pattern was maintained with **35c** and **35d**; the latter nucleophile reacted almost 100 times faster with the trifluoroethyl ester than with the butyl ester. The acylation of the cyclohexyl derivative **35e** with 2,2,2-trifluoroethyl butyrate was highly enantioselective ($E$=100), but with the butyl ester the enantiomeric bias was very much reduced ($E$=9).[61] These results show, the confusing effects of the leaving group notwithstanding, that CaLA is able to discriminate the enantiomers of **35** efficiently under the right conditions. A systematic background reaction is not involved, as is demonstrated by the excellent enantiodiscrimination of **35d** with butyl butyrate, which required 130 h reaction time.[61] We can only guess that some batches of β-amino ester and/or acyl donor may have been contaminated with small amounts of acid chlorides, which are known to be powerful lipase inhibitors.

**3.6.1. Monocyclic β-amino esters.** The acylation of a range of cyclic β-amino esters (**36**–**39**) is conspicuous because some of these were rather well resolved by BcL (see Table 7).[62] The enantiomeric ratio of the latter enzyme in the acylation of **36** was high (~100) in a wide range of solvents but, in contrast, the effect of the solvent on the enantiodiscrimination of **39b** was dramatic (see Table 5). CaLA discriminated the enantiomers of the *cis*-isomers **36** and **38** rather well, but **37** and **39b**, in which the substituents are *trans*, not at all. CaLB acted with high enantioselectivity ($E \gg 100$) in the acylation of the methyl ester **39a** with isopropyl methoxyacetate.[59] In the acylation of **39a** and **39b**, CaLB and CaLA again showed a preference for the same enantiomer.

From the results obtained with **33a**, **35a** and **39a**[59] we tentatively conclude that amino esters can selectively react at the amino group rather than at the ester, in the presence of CaLB, if the acyl donor is a highly active one. It also becomes clear that CaLA and CaLB preferentially convert the same enantiomer of this type of reactants, which could indicate that the active sites have a similar structure. We note that, in the CaLA mediated resolutions of **35a**–**d**, a bulky alkyl group seems to exert a favourable effect on the enantiorecognition, which could indicate that its **M** subsite can accommodate more bulky groups than that of CaLB and that polarity, rather than steric interactions, dominates the enantiorecognition of amino esters by CaLA.

**3.6.2. Bicyclic β-amino esters.** CaLA also mediated the acylation of the bicyclic β-amino esters **40**–**42** with enantiomeric ratios (under optimum conditions) of approx. 30, but BcL, in contrast, was not enantioselective in these acylations.[62] Dramatic effects of the solvent on $E$, even when comparing different ethers, were manifest in the resolutions of **40** and **41**, in which the substituents are *exo*. Thus, the enantiomeric ratio of the acylation of **40** was 26 when the reaction was performed in TBME, which contrasts with $E$ ratios of 10 in diethyl ether, 7 in DIPE and 1 in toluene. In the acylation of **41** the differences between the ethers were less (see Table 7), but, in contrast with **40**, the enantioselectivity was relatively high ($E$=41) when the reaction was performed in 3-methyl-3-pentanol (Fig. 11).[62]

**3.6.3. Aryl-substituted β-amino esters.** Finally a number

**Table 7**. Enantioselective *N*-acylation of the β-amino acid esters **35**–**42**

| Amine | Lipase | Donor | Solvent | $T$ (°C) | $E$ | Reference |
|---|---|---|---|---|---|---|
| **35a** | CaLB | Isopropyl methoxyacetate | – | | 200 | 59 |
| | CaLA | 2,2,2-Trifuoroethyl butyrate | DIPE | rt | 6 | 61 |
| | CaLA | Butyl butyrate | DIPE | rt | 32 | 61 |
| **35b** | CaLA | 2,2,2-Trifuoroethyl butyrate | DIPE | rt | 168 | 61 |
| | CaLA | Butyl butyrate | DIPE | rt | 256 | 61 |
| **35c** | CaLA | 2,2,2-Trifuoroethyl butyrate | DIPE | rt | 72 | 61 |
| | CaLA | Butyl butyrate | DIPE | rt | >100 | 61 |
| **35d** | CaLA | 2,2,2-Trifuoroethyl butyrate | DIPE | rt | 106 | 61 |
| | CaLA | Butyl butyrate | DIPE | rt | 115 | 61 |
| **35e** | CaLA | 2,2,2-Trifuoroethyl butyrate | DIPE | rt | >100 | 61 |
| | CaLA | Butyl butyrate | DIPE | rt | 9 | 61 |
| **36** | CaLA | 2,2,2-Trifuoroethyl acetate | DIPE | rt | 46 | 61 |
| | BcL | 2,2,2-Trifuoroethyl acetate | DIPE | rt | >100 | 61 |
| **37** | BcL | 2,2,2-Trifuoroethyl acetate | Diethyl ether | rt | 12 | 62 |
| **38** | CaLA | 2,2,2-Trifuoroethyl acetate | DIPE | rt | 61 | 62 |
| **39a** | CaLB | Isopropyl methoxyacetate | TBME | rt | ≫ 100 | 59 |
| **39b** | BcL | 2,2,2-Trifuoroethyl acetate | DIPE | rt | 13 | 62 |
| | BcL | 2,2,2-Trifuoroethyl acetate | Diethyl ether | rt | 33 | 62 |
| | BcL | 2,2,2-Trifuoroethyl acetate | Dibutyl ether | rt | 4 | 62 |
| **40** | CaLA | 2,2,2-Trifuoroethyl acetate | Diethyl ether | rt | 10 | 62 |
| | CaLA | 2,2,2-Trifuoroethyl acetate | TBME | rt | 26 | 62 |
| | CaLA | 2,2,2-Trifuoroethyl acetate | DIPE | rt | 7 | 62 |
| | CaLA | 2,2,2-Trifuoroethyl acetate | Toluene | rt | 1 | 62 |
| **41** | CaLA | 2,2,2-Trifuoroethyl acetate | $Et_2MeCOH$ | rt | 41 | 62 |
| | CaLA | 2,2,2-Trifuoroethyl acetate | TBME | rt | 46 | 62 |
| **42** | CaLA | 2,2,2-Trifuoroethyl acetate | Diethyl ether | rt | 30 | 62 |

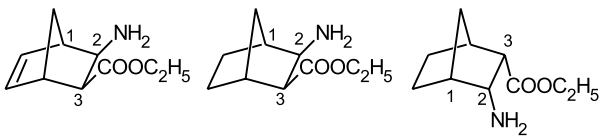



**Figure 11.** Lipase mediated enantioselective acylation of the bicyclic β-aminoesters **40**–**42**; the preferentially acylated enantiomers are shown.

of 3-amino-3-arylpropionic acid ethyl esters (**43**–**47**, see Fig. 12) were resolved via acylation with various butyric acid esters, in the presence of CaLA. The acylation of **43a** by 2,2,2-trifluoroethyl butyrate in DIPE was highly enantioselective ($E>100$, see Table 8); with butyl butyrate, in contrast, the reaction was approx. 200 times slower and the enantiomeric ratio was only a modest 29.[61]

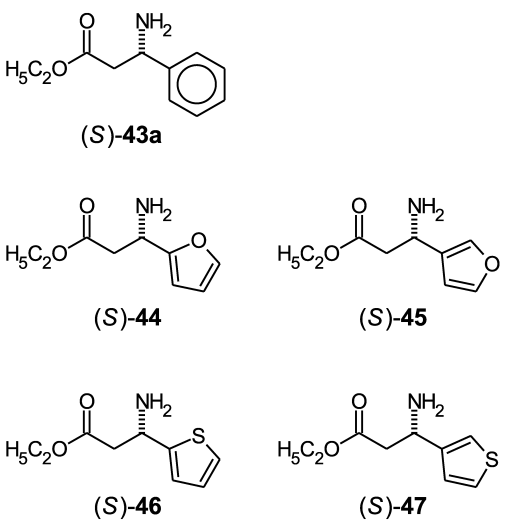



**Figure 12.** Lipase mediated enantioselective acylation of the ethyl 3-amine-3-arylpropionates **43**–**47**; the preferentially acylated enantiomers are shown.

The furyl-substituted β-amino esters **44** and, in particular, **45** were very well resolved via acylation with neat ethyl or butyl butyrate.[63] Surprisingly, the acylation of **44** with neat ethyl acetate was six times slower and also less enantioselective than with ethyl butyrate. The acylation of **44** with 2,2,2-trifluoroethyl butyrate in DIPE was also highly enantioselective, but much less so in ACN or TBME medium.[63] A very similar pattern was observed with the thienyl-substituted amines **46** and **47**, which were also efficiently resolved via acylation with butyl and ethyl butyrate, respectively.[63]

### 3.6.4. Enantiorecognition of β-amino esters by CaLA.

The enantiomer selectivity of CaLA in the *N*-acylation of β-amino esters is uniform in all of the studied examples. In terms of Kazlauskas' lipase steric model, the ester group preferentially binds in the **L** subsite and the alkyl or aryl group in the **M** subsite. The enantiomeric bias of CaLA is the same as those of CaLB and PcL, although not always to the same extent, whenever a comparison can be made. Hence, the conclusion seems justified that the **L** subsite preferentially binds a polar group, such as an ester, whereas a non-polar substituent is preferentially bound in the **M** subsite. From the available data, it seems that a bulky or aromatic substituent increases its affinity for the latter subsite in CaLA.

## 3.7. Lipases: enantiorecognition revisited

It is generally accepted that the enantiodiscrimination of lipase can be rationalised in terms of **L** and **M** substituents that bind in their respective subsites, and which exchange their positions when the 'wrong' enantiomer reacts (see the preceding discussion). It quite recently became clear, however, that this attractive mechanistic picture, which logically emanates from Kazlauskas' rule, is an over simplification. An X-ray analysis of *C. rugosa* lipase inhibited by (*R*)- and (*S*)-menthyl phosphonates showed that the oxygen and hydrogen atoms at C-1 in menthol exchange their positions in the active site and not the **L** and **M** groups.[64] A similar, quite recent, study of the resolution of **2a** by CaLB indicated that the hydrogen atom and the methyl group at C-1 exchange their positions.[65] It would seem that the mechanistic explanation of lipase enantioselectivity is a good deal more complex and interesting than expected.

## 3.8. Lipases: concluding remarks

A very wide range of chiral amines has been successfully resolved via lipase-catalysed acylation and the limit seems

**Table 8**. Enantioselective *N*-acylation of the 3-amino-3-arylpropionic acid esters **43a**–**47**

| Amine | Lipase | Donor | Solvent | *T* (°C) | *E* | Reference |
|---|---|---|---|---|---|---|
| **43a** | CaLA | 2,2,2-Trifuoroethyl butyrate | DIPE | rt | 75 | 61 |
| | CaLA | Butyl butyrate | DIPE | rt | 29 | 61 |
| **44** | CaLA | Ethyl acetate | – | rt | 100 | 63 |
| | CaLA | 2,2,2-Trifuoroethyl butyrate | ACN | rt | 90 | 63 |
| | CaLA | 2,2,2-Trifuoroethyl butyrate | TBME | rt | 60 | 63 |
| | CaLA | 2,2,2-Trifuoroethyl butyrate | DIPE | rt | 210 | 63 |
| | CaLA | Ethyl butyrate | – | rt | 150 | 63 |
| | CaLA | Butyl butyrate | – | rt | 220 | 63 |
| **45** | CaLA | Ethyl butyrate | – | rt | 460 | 63 |
| **46** | CaLA | Ethyl acetate | – | rt | 60 | 63 |
| | CaLA | Ethyl butyrate | – | rt | 305 | 63 |
| | CaLA | 2,2,2-Trifuoroethyl butyrate | ACN | rt | 130 | 63 |
| | CaLA | 2,2,2-Trifuoroethyl butyrate | TBME | rt | 380 | 63 |
| | CaLA | Butyl butyrate | – | rt | 580 | 63 |
| **47** | CaLA | Ethyl butyrate | – | rt | 220 | 63 |

nowhere in sight. CaLB is a highly enantioselective and stable biocatalyst, which tends to eclipse many other potentially useful lipases. The, hereto, little-used CaLA has shown unexpected strength in the enantioselective acylation of α- and β-amino esters. The enantioselectivity often, sometimes vitally, depends on the proper selection of the acylating agent and the reaction medium. The role of the non-enzymatic background reaction in these phenomena is still obscure, but the conclusion that it is a major actor seems inescapable.

## 4. Subtilisin as an amine resolution catalyst

### 4.1. Subtilisin

The subtilisin-type endoproteases (EC 3.4.21.14) are structurally very similar to serine proteases. The Ser-His-Asp triad in the active site is sterically arranged as the mirror image of the lipase active site. The subtilisins also act as esterases on *N*-acyl-L-amino acid esters and short-chain fatty acid esters, such as butyrates.

Subtilisin is, similar to the lipases, a very stable enzyme that maintains its activity in anhydrous organic media. For optimum catalytic efficiency subtilisin should be immobilised, for example via adsorption on glass beads[29] or Accurel EP100.[66] Immobilised subtilisin is commercially available as a cross-linked enzyme crystal (Altus ChiroCLEC™-BL).

### 4.2. Subtilisin-catalysed enantioselective amine acylation

**4.2.1. Alkyl- and arylalkylamines.** The potential of subtilisin as an amine resolution catalyst was already demonstrated in an early stage in the development of non-natural enzymology. With few exceptions, the acylations were conducted with 2,2,2-trifuoroethyl butyrate in an anhydrous organic solvent. The solvent exerted a very considerable effect on the enantiomeric ratio, as measured in separate experiments with pure enantiomers,‡ of the acylation of **2a** and **6** (see Fig. 13).[67] *E* ranged from 1.0 in octane to 22 in 3-methyl-3-pentanol but, in hindsight, it would seem that the uncatalysed background reaction[29] (cf. Table 1) accounts for at least some of these solvent effects.

Subsequently, 3-methyl-3-pentanol was adopted as the solvent for the resolution of a range of alkyl- and arylalkylamines (see Fig. 13). Most of these were resolved with $E>20$, as calculated from the experimental data.[29,67,68] It would seem that a subtilisin CLEC[68] afforded a somewhat better enantiorecognition than a suspension of lyophilisate, although a reduction of the non-enzymatic reaction, perhaps due to a more active biocatalyst, could also account for the improvement.

The enantioselective acylation of **11** was scaled up to 300 g scale and that of **6** to 1.6 kg scale, using a tube reactor containing subtilisin immobilised on glass beads, with a reactant-to-enzyme ratio of 90 and 280 (g/g), respectively.[29] Thus, racemic **6** was resolved into unconverted (*R*)-**6**

‡ This is now known not to be the most reliable approach.[26]

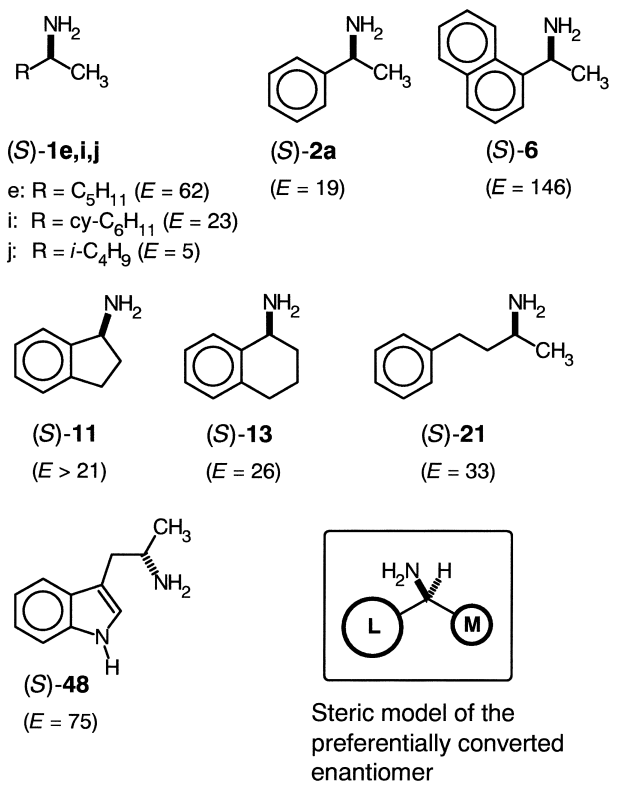



**Figure 13.** Subtilisin mediated enantioselective acylation of alkyl- and arylalkylamines; the preferentially acylated enantiomers and the seric model[28] are shown. The *E* ratios have been calculated from the literature data: **1e**,**i**,**j**, **2**, **13**, **21**;[67] **6**, **48**;[68] **11**.[29]

(ee>95%) and (*S*)-amide, which afforded (*S*)-**6** with ee>90% upon hydrolysis.

The enantiomeric bias of subtilisin in the acylation of the alkyl- and arylalkylamines corresponds with a steric model[28] that is the mirror image of the one that predicts the enantiomeric preference of the lipases (see Fig. 13). These authors have also correlated the steric model with the three-dimensional structure of subtilisin.

**4.2.2. Functionalised amines.** A few amines bearing various functional groups have also been resolved in the presence of subtilisin. An attempt at the acylation of **28** (Fig. 14) with neat diallyl carbonate was hampered by a significant background reaction. It was surprisingly found, however, that the acylations of **28** and **49** could be carried out, without any background reaction, in aqueous buffer at pH 8.[69] Only a modest excess of donor was required; hence,

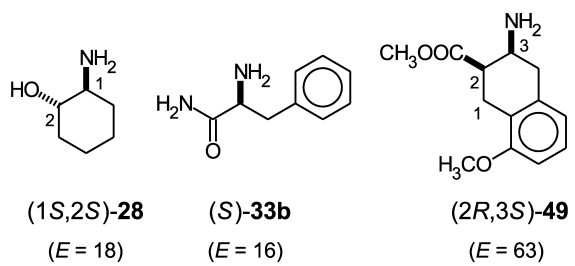



**Figure 14.** Subtilisin mediated enantioselective acylation of the α- and β-amino acid derivatives **28**[69], **33b**[67], **49**[69]; the preferentially acylated enantiomers are shown.

these amines seem to compete efficiently with water in the final enzymatic reaction step (step iii in Fig. 1). Subtilisin favoured the (*S*)-L-enantiomer of phenylalanine amide (**33b**) upon acylation with 2,2,2-trifluoroethyl butyrate,[67] as would be expected for a protease.

The correlation in enantiomeric bias of CaLB and subtilisin with these functionalised amines is striking, although the proteins have completely different structures. With both enzyme classes the hydroxyl group in **28** as well as the ester and amide groups in **33a** (Fig. 8), **33b** and **49** bind in the **L**-subsite.

### 4.3. Subtilisin: concluding remarks

Summarising, subtilisin is a promising amine resolution catalyst with an enantiomeric bias complementary to that of the lipases. The *E* values of subtilisin in these resolutions may seem quite modest in comparison with those of the lipases, but as subtilisin somehow was neglected early on there could be considerable room for improvement. Resolutions that employ subtilisin, and perhaps other proteases as well, could prove particularly useful in cases where the lipases convert the wrong enantiomer.

## 5. Penicillin acylase as amine resolution catalyst

### 5.1. Penicillin acylase

Penicillin acylase (EC 3.5.1.11) is a serine hydrolase; its mechanism is very similar to that of the lipases and serine proteases. Structurally, penicillin acylase is completely different as it belongs to the class of the N-terminal nucleophile hydrolases, which have no catalytic triad but an N-terminal serine that is activated by a bridging water molecule[70] (see Fig. 15).

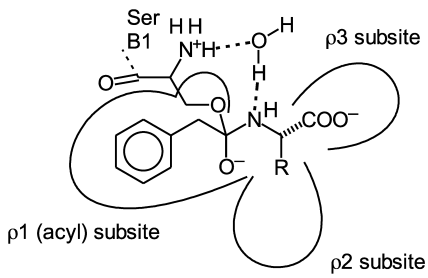



**Figure 15.** A schematic depiction of the active site of penicillin acylase; - - - denotes a hydrogen bond.

Penicillin acylase has a somewhat complex substrate specificity. The acyl binding (ρ1) subsite is highly specific for phenylacetic acid. Only small groups, such as hydroxyl or amino, are allowed at the 2-position and the enantiomer specificity is low. The ρ2 subsite mainly interacts with the reactant through hydrophobic and steric forces. The ρ3 subsite, in contrast, specifically binds negatively charged groups, which explains the high specificity of penicillin acylase for L-amino acid residues, but it also recognises ester groups.[71] The ρ2 and ρ3 sites together form the penicillin-recognising part of the active site.

Penicillin acylase is an industrial catalyst that is mainly used in the manufacture of the β-lactam building block 6-aminopenicillanic acid from penicillin G. The commonly used penicillin acylase from *E. coli* is commercially available in various carrier-bound formulations, such as PGA 400 from Roche, as well as a cross-linked enzyme crystal (Altus ChiroCLEC™-EC). The relatively unknown penicillin acylase from *Alcaligenes faecalis*[72,73] has recently attracted attention because it suffers less from parasitic hydrolysis in amine acylation and is more stable than the *E. coli* enzyme. Penicillin acylases are, compared with CaLB or subtilisin, fragile enzymes that lose their catalytic activity upon dehydration. Cross-linked enzyme aggregates of penicillin acylase maintained their activity in high concentrations of organic solvents,[74] but a few percent of water was an absolute requirement for activity.[75]

### 5.2. Use of penicillin acylase

In the preceding examples, our discussion of the kinetic resolution of amines has remained restricted, with few exceptions, to anhydrous reaction conditions. Hence, the issue of donor hydrolysis could be disregarded. With an enzyme that becomes inactive when dehydrated, such as penicillin acylase, some water must be present. In hydrophobic media, penicillin acylase requires a water activity of approx. 0.5.[76] Alternatively, the resolution can be carried out in a fully aqueous medium. Three kinetic control mechanisms contribute to the outcome of such procedures (see Fig. 16).[77] First, water and the amine compete for the acylated enzyme, causing some donor hydrolysis. Next, the amine enantiomers compete for the acylated enzyme and, hopefully, one is preferentially acylated. Finally, the enantiomerically enriched amide competes with the donor for the enzyme's active site, causing product hydrolysis. This latter process is particularly deleterious, because the enantiomer that is preferentially formed will also react the fastest in the backwards reaction, causing a rapid erosion of

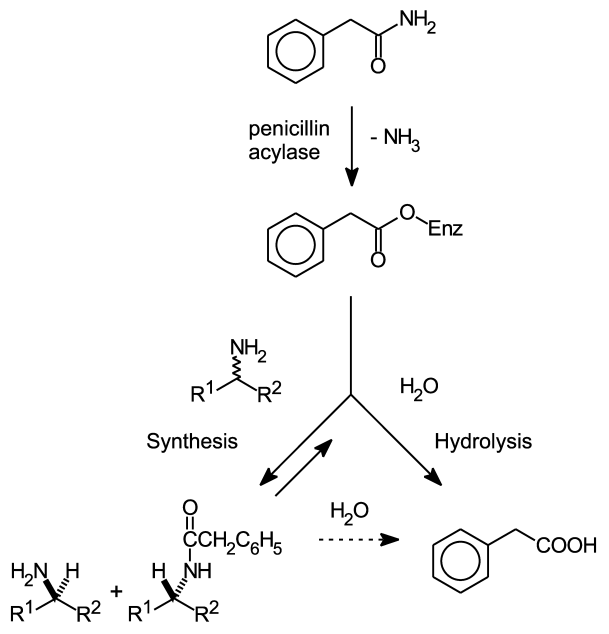



**Figure 16.** In a (partially) hydrous medium enantioselective aminolysis and hydrolysis compete.

the product ee. To be successful, a kinetic resolution in hydrated or aqueous medium absolutely requires an excess of a highly activated donor that monopolises the active site. To restrict the donor losses a good synthesis/hydrolysis ratio is desirable. Both methodologies: reaction in an aqueous buffer[78] as well as in partly hydrated ethyl acetate[79] or toluene[79,80] have been used in amine resolution.

The choice of acyl donor is restricted by the substrate tolerance of penicillin acylase. Simple esters of phenylacetic acid or phenylacetamide are obvious choices; the latter has the advantage of not suffering from spontaneous hydrolysis or aminolysis. Esters and amides of mandelic acid,[81] 4-hydroxyphenylacetic acid[80] or 2-phenylglycine[82] have the advantage of a much better solubility in water.

### 5.3. Amine resolution mediated by penicillin acylase

**5.3.1. Alkyl- and arylalkylamines.** Attempts to resolve alkylamines, such as **1**, **2** and **11** with phenylacetamide in the presence of *E. coli* penicillin acylase met with scant success due to a low enantioselectivity and prevailing donor hydrolysis.[83a] The penicillin acylase from *A. faecalis*, in contrast, required only a modest excess of acyl donor and it was also much more enantioselective.[83] The reaction was performed at pH 11 to deprotonate the amine and increase its competitiveness vs. water. Thus, a number of alkyl- and arylalkylamines (see Fig. 17) were acylated with, in general, the same enantiomeric bias as observed with the lipases. The enantiorecognition of the alkylamines **1b** and **1e** was quite modest however, and **1e** was even acylated with a slight preference for the (*S*)-enantiomer.[83a] The resolution of **1b**, in particular, could be improved by performing the reaction in 10–25% aqueous acetonitrile.[83a] A much better enantiomeric ratio was observed with 1-arylethylamines, which may indicate a specific interaction with the ρ2 binding site in *A. faecalis* penicillin acylase. Interestingly, such an effect was not observed with the *E. coli* enzyme. 1-Aminoindane (**11**) remained an exception as it was not very well resolved by either penicillin acylase, perhaps because the planar bicyclic systems prevents the proper orientation of the aromatic ring in the ρ2 subsite.

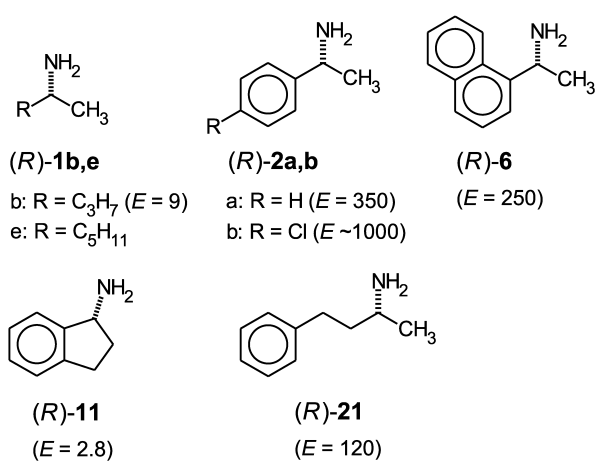



**Figure 17.** The enantioselective acylation of alkyl- and arylalkylamines in the presence of *A. faecalis* penicillin acylase (acyl donor: fenylacetamide); the preferentially acylated enantiomers are shown.[83]

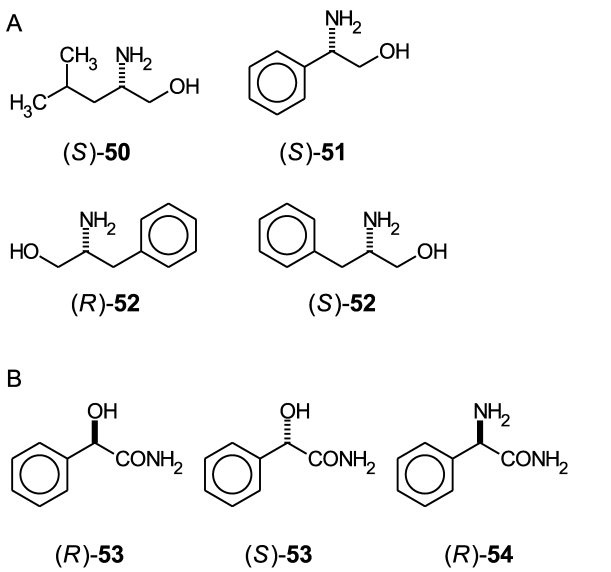



**Figure 18.** (A) The enantioselective acylation of the hydroxyalkylamines **50**–**52** in the presence of penicillin acylase; the preferentially acylated enantiomers are shown. (B) The acyl donors **53**–**54**. For further details see Table 9.

**5.3.2. Aminoalcohols.** The acylation of 2-amino-4-methylpentanol (leucinol, **50**, see Fig. 18 and Table 9) with phenylacetamide in the presence of *A. faecalis* penicillin acylase was much more enantioselective than that of the alkylamines **1b** and **1e**.[82] When comparing the enantioselectivities of 2-amino-2-phenylethanol (**51**) and **2a**, the opposite effect is observed.[83b] The enantiomeric bias shows that the primary alcohol groups in **50** and **51** selectively bind in the ρ3 subsite, similar to the methyl groups in **1b** and **2**. Surprisingly, the enantioselectivity was lost and a slight pro-(*R*) bias developed when the phenyl group in **2a** was replaced by benzyl (**52**).[82] 4-Phenyl-2-aminobutane (**21**), in which two C atoms separate the amino and phenyl groups (Fig. 17), in contrast, was acylated with a high selectivity for the (*R*)-enantiomer, similar to **2a**.

**5.3.3. Donor effects in (hydroxy)alkylamine resolution.** Švedas and co-workers surprisingly found that subtle changes in the nature of the acyl moiety of the donor exerted a dramatic effect on the enantiorecognition of alkylamines and aminoalcohols by *A. faecalis* penicillin acylase.[82] These concerned, besides phenylacetamide, (*R*)-mandelic amide ((*R*)-**53**), (*S*)-mandelic amide ((*S*)-**53**) and (*R*)-phenylglycinamide ((*R*)-**54**). The enantiomeric ratio increased, on average, by a factor of eight when phenylacetamide was replaced by (*R*)-**53**; the effect of (*S*)-**53** was somewhat less (see Table 9). The acylation of

**Table 9**. Effect of the acyl donor on amine resolution in the presence of *A. faecalis* penicillin acylase[a]

| | Amine (*E*) | | | | |
|---|---|---|---|---|---|
| Donor | **2a** | **21** | **50** | **51** | **52** |
| Phenylacetamide | 350 (*R*) | 120 (*R*) | 40 (*S*) | >150 (*S*) | 2.3 (*R*) |
| (*R*)-**53** | 950 (*R*) | 800 (*R*) | 350 (*S*) | >1000 (*S*) | 50 (*S*) |
| (*S*)-**53** | 800 (*R*) | 180 (*R*) | 120 (*S*) | >1000 (*S*) | |
| (*R*)-**54** | 1200 (*R*) | 500 (*R*) | | | |

[a] Data taken from Ref. 82.

2-amino-3-phenylpropanol (phenylalaninol, **52**) presents a special case as a slight preference for the (*R*)-enantiomer upon acylation with phenylacetamide changed into an (*S*)-selectivity (*E*=50) when the donor was (*R*)-**53**.

**5.3.4. Amino acid derivatives.** The nucleophile subsite of pencillin acylase is known to be highly specific for (*S*)-L-α-amino acid derivatives from hydrolysis studies.[71] The same enantiomeric bias was observed in the synthetic direction, upon the N-acylation of alaninamide (**55**, see Fig. 19) with (*S*)-**53** mediated by *E. coli* penicillin acylase in aqueous medium at pH 9.[81] The conversion was far from complete, however, because no excess of (*S*)-**53** was used and parasitic hydrolysis was prominent. The amino group in **56** likewise was acylated by methyl 4-hydroxyphenylacetate with a high selectivity in favour of the (*S*)-enantiomer.[80] The reaction was performed in partially hydrated ($a_W$ 0.7–0.8) toluene and dichloromethane, in the presence of immobilised *E. coli* penicillin acylase.

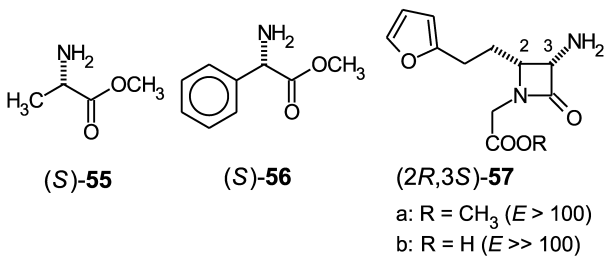



**Figure 19.** The enantioselective acylation of the α-amino acid derivatives **55**–**57** in the presence of the penicillin acylase from *E. coli*; the preferentially acylated enantiomers are shown.

The enantioselective acylation of the amino-β-lactam derivative **57a** with methyl phenylacetate was carried out in the presence of immobilised *E. coli* penicillin acylase in water at pH 6.[78] The enantiodiscrimination was excellent and no product hydrolysis was observed, presumably because the product precipitated. The corresponding product derived from the free acid **57b** remained in solution and some product hydrolysis indeed occurred. A fast and highly enantioselective reaction was, surprisingly, observed with methyl phenoxyacetate as the donor. Product hydrolysis did not occur, as should be expected, because phenoxyacetamides are known not to be substrates for penicillin G acylase. In seeming contradiction, phenoxyacetic acid ester can act as donor nevertheless.

*N*-acyl-β-amino acid derivatives are known to be hydrolysed with the same enantiomeric preference as their α-amino counterparts.[71] Hence, the carboxymethyl moiety is accommodated in the ρ3 subsite. The same enantiopreference was observed in an acylation study, using methyl phenylacetate and ChiroCLEC™-EC biocatalyst in partially hydrated ethyl acetate or toluene.[79] The enantiomer discrimination with the alkyl and alkenyl-substituted compounds **35a** and **35f** (see Fig. 20) was modest. In contrast, **43b** and **43c** were resolved with high enantioselectivity, presumably because of a favourable interaction with the aromatic substituent. A lower *E* was found with the more heavily substituted **43d**, however.

There are few examples of amine resolution using a

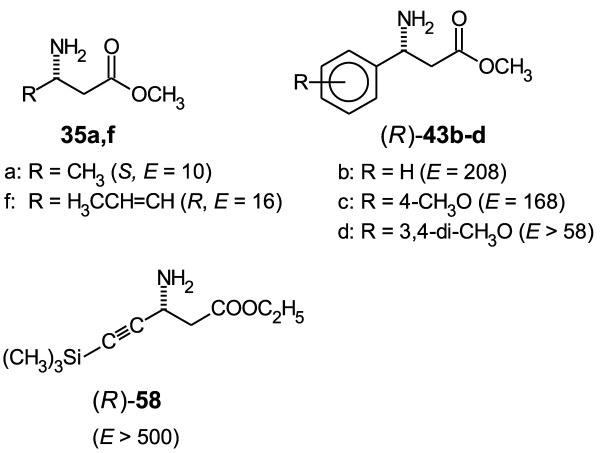



**Figure 20.** The enantioselective acylation of the β-amino acid esters **35**, **43** and **58** in the presence of the penicillin acylase from *E. coli*; the preferentially acylated enantiomers are shown.

carboxylic acid as the acyl donor. The reason is that product hydrolysis, which comes into the play when equilibrium is approached, would erode the ee. It has surprisingly been demonstrated, however, that **58** could be acylated with near-quantitative enantioselectivity via condensation with phenylacetic acid in the presence of immobilised *E. coli* penicillin acylase.[84] The procedure was scaled up to 7 kg and the desired, unconverted (*S*)-**58** was obtained in 43% yield (based on the racemate) and 99% ee.

### 5.4. Penicillin acylase: concluding remarks

Penicillin acylases have shown a surprising capability for the enantioselective acylation of chiral amines in aqueous media. The relatively unknown penicillin acylase from *A. faecalis* was, in contrast with the *E. coli* enzyme, an efficient resolution catalyst with alkyl- and, particularly, arylalkylamines with, in general, the same enantiomeric preference as the lipases. *E. coli* penicillin acylase is an efficient and highly enantioselective catalyst for the resolution of α- and β-amino acid derivatives. Its enantiomeric preference with these latter compounds is the opposite of that observed with the lipases.

## 6. Racemisation and dynamic kinetic resolution

Only one of the pure enantiomers that result from a kinetic resolution will, in general, be required, which renders the racemisation of the unwanted enantiomer a necessity to improve the process economy. BASF has disclosed a procedure that involves racemisation via a Schiff's base derivative.[1] It has also been shown that **9**, as well as its *N*-acetyl derivative, could be racemised by heating at 150°C for 30 min.[48] The racemisation of a range of 1-arylethylamines has been accomplished using a binuclear ruthenium catalyst at 110°C.[85] The formation of side-products was suppressed by the addition of a hydrogen donor. The racemisation conditions were not compatible with lipase-catalysed resolution, however, which means that racemisation has to be performed ex situ.

It would be desirable, however, to integrate racemisation

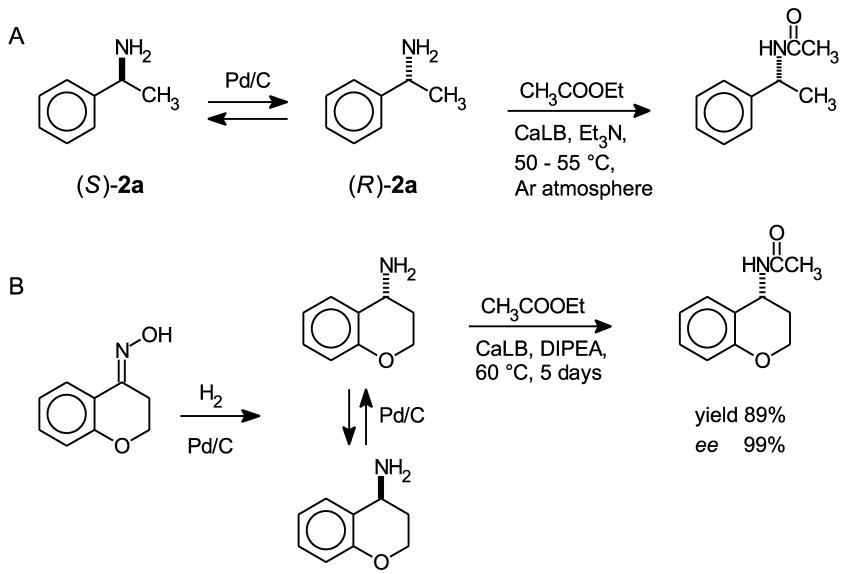



**Figure 21.** Dynamic kinetic resolution of chiral amines combined with in situ racemisation of the slow-reacting enantiomer.

and kinetic resolution, provided, of course, that the preferentially converted enantiomer is the desired one. Such a dynamic kinetic resolution (DKR) is particularly attractive because it requires a much lower enantiomeric ratio for the same product ee than a normal kinetic resolution. The reason is the 50:50 ratio of the reactant enantiomers that is maintained throughout the reaction.

Dynamic kinetic resolution of secondary alcohols is already well developed[86] but, in contrast, that of amines is still in its infancy. The DKR of **2a** has been accomplished by combining its CaLB mediated acylation with palladium catalysed racemisation of the unreacted amine in one pot (Fig. 21(A)).[87] The long reaction time required by the latter procedure could be remedied by starting from the corresponding oxime rather than the racemic amine (Fig. 21(B)).[88]

## 7. Conclusion

In the last decade much progress has been made in the enzymatic resolution of chiral amines, both from the viewpoint of practical utility and of understanding the influence of various parameters on the enantioselectivity. There are two fundamental differences between alcohol and amine resolution. With amines there is a blank non-enzymatic acylation, which has to be taken into account. This seems to have been forgotten by some authors. Secondly, hydrolysis of the acylated product is much more difficult in the case of amides compared to esters. Most of the studies have involved the use of lipases but subtilisin and penicillin acylase are also promising enzymes for amine resolution. Finally, the ultimate goal is an effective method for the dynamic kinetic resolution of amines. This necessitates the development of effective racemisation catalysts that are compatible with the enzymatic resolution step. We are confident that such systems will be developed in the future.

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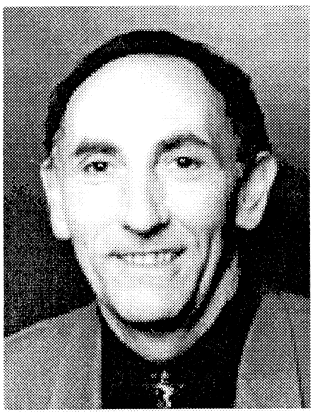
**Biographical sketch**

**Fred van Rantwijk** (1943) studied organic chemistry at the Delft University of Technology where he remainded as a staff-member. He received his PhD in 1980 under the direction of Professor H. van Bekkum. Since the late eighties he works on the application of enzymes in organic synthesis. His particular research interests are the use of enzymes in non-natural reactions, enzyme immobilisation and transformations using multi-enzyme systems.



**Roger Sheldon** was born in Nottingham (UK) in 1942. He has a PhD in organic chemistry (1967) from the University of Leicester (UK), for work on organophosphorus chemistry under the joint supervision of S. Trippett and R. S. Davidson. Following post-doctoral studies with Jay Kochi in the US on reactions of metal ions with free radicals, he joined Shell Research in Amsterdam in 1969 where he carried out research on various catalytic processes, particularly liquid phase oxidations. From 1980 to 1990 he was R & D Director of Andeno (a subsidiary of DSM) in Venlo (Netherlands). In 1991 he moved to his present position as Professor of organic chemistry and catalysis at the Delft University of Technology (Netherlands). His primary research interests are in the application of catalytic methodologies—homogeneous, heterogeneous and enzymatic—in organic synthesis, particularly in relation to fine chemicals production. He is well known for his devevelopment of the of E factor for assessing the environmental impact of chemical processes.