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Invited Review

Lipase-Catalyzed Synthesis of Carboxylic Amides: Nitrogen Nucleophiles as Acyl Acceptor

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Summary. The lipase-catalyzed aminolysis of carboxylic esters is a fairly general reaction that has been performed with a wide range of esters and amines, generally in anhydrous organic media to avoid undesirable hydrolysis of the ester. Alternatively, carboxylic amides can be synthesized by lipase mediated condensation of carboxylic acids and amines if an excess of either reactant is avoided.

Chiral carboxylic esters have been resolved by lipase-catalyzed aminolysis. In the majority of these resolutions, *Candida antarctica* lipase B has been employed as the catalyst. A range of chiral amines has been resolved by lipase mediated acylation, using mainly the lipases from *C. antarctica* (B type) and *Pseudomonas* species. The enantiorecognition was frequently found to depend critically on the acylating agent and the reaction medium.

Keywords. Lipase; *Candida antarctica* lipase; Amine; Carboxylic amide; Enantioselectivity.

Introduction

There is currently a marked trend in the fine chemicals industry towards the reduction, or preferably elimination, of waste by widespread substitution of classical syntheses employing stoichiometric reagents with cleaner catalytic alternatives [1].

Enzymes occupy a special position in the catalysis field because they operate at moderate temperature and mild reaction conditions, which is advantageous with sensitive reactants and products. Moreover, enzymes are inherently enantioselective. In consequence, enzyme catalysis is destined to play a major role in the transition from stoichiometric to catalytic transformations, particularly if it can be made to transcend the limitations of natural reactions in aqueous medium by replacement of the natural reactant by a non-natural one. Lipases, the lipid hydrolyzing catalysts of nature, are eminently suited for such an approach because of their stability and the simplicity of their catalytic machinery (Fig. 1). These characteristics have made them outstanding catalysts for synthetic biotransformations that involve the carboxyl group, such as esterification, transesterification,

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perhydrolysis, and aminolysis in which the natural nucleophile (water) is replaced by alcohols, hydroperoxides, or amines [2, 3]. The principle of lipase-catalyzed aminolysis, resulting in the synthesis of carboxylic amides, has been demonstrated for the first time in 1984/85 [4, 5], and a multitude of publications describing its application has been published since.

This review describes the use of nitrogen nucleophiles in the acyl accepting role as a synthetic route to carboxylic amides as well as the resolution of chiral carboxylic acids, esters, and amides by lipase catalyzed aminolysis.

Mechanistic Considerations

Lipases, the lipid splitting catalysts of nature (EC 3.1.1.3), operate by essentially the same mechanism as that of the serine proteases [6]. 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 stabilized by the oxyanion hole. Next, the tetrahedral intermediate collapses to the serinate ester with elimination of the alcohol. Subsequent reaction of the acylenzyme intermediate with the nucleophile – the acyl acceptor – affords the product. In the case of hydrolysis, which is the natural reaction of lipases, the nucleophile is water. However, almost any nucleophile can, in principle, react with the acyl-enzyme intermediate [2].

Ester aminolysis can be regarded as irreversible, because the transformation is strongly exothermic under normal reaction conditions [7]. Amide hydrolysis, which is, *inter alia*, not readily mediated by lipases, can also be excluded due to the almost universally employed anhydrous reaction conditions (see below). Lipase mediated aminolysis of a carboxylic amide, *i.e.* transamidation, has been reported only once and involved a highly activated N-2,2,2-trifluoroethyl amide as donor [8].

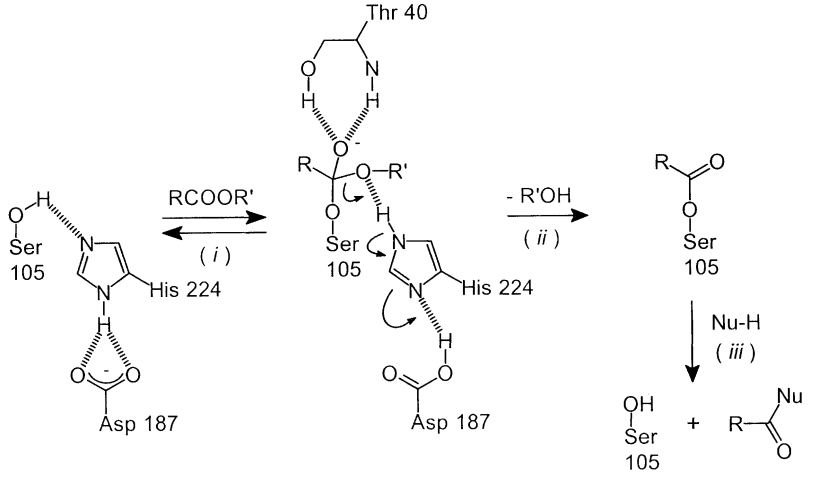


Fig. 1. Reaction mechanism of lipase catalysis; the numbering is for *Candida antarctica* lipase, - - - - - denotes a hydrogen bond; step (*iii*) is the microscopic reversal of steps (*i*) and (*ii*)

Although lipases have not been designed for enantioselectivity, they are chiral and, hence, inherently enantioselective towards all components of the reaction: the acyl group, the leaving group, and the nucleophile. The resolution of chiral amines is one of the major practical applications of lipase-catalyzed aminolysis.

The Use of Lipases in Aminolysis

Lipases[1] are ubiquitous in Nature. Initially the very impure [9] porcine pancreas lipase (PPL) – easily obtained from slaughter waste – and *Candida rugosa*[c2] lipase (CrL), a rather unstable enzyme actually consisting of a mixture of isozymes, were widely used. The recombinant microbial lipases that are now widely available generally do not contain contaminating activity and are much more stable. Some of these have been developed for application in synthetic biotransformations, but most of them were originally intended to be used in laundry formulations or the upgrading of food triglycerides.

Water, which is the natural reaction medium of enzymes, is not a good solvent for aminolysis, because hydrolysis 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; 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 those from microbial sources that are commonly used nowadays, are exceptionally rugged enzymes and tolerate anhydrous organic solvents rather well. In particular, *Candida antarctica* lipase B (CaLB) [10, 11], which actually seems to prefer anhydrous conditions [12], has been widely used in consequence. We note that some lipases, such as that from *Thermomyces lanuginosus*[3] (TIL), that require some water to catalyse esterification, still act as an ammoniolysis catalyst in dry medium [13]. Presumably, ammonia acts as a water replacement.

We have found that ammoniolysis catalyzed by Novozym 435 (CaLB on Lewatit E) is sometimes accompanied by unexpected hydrolysis of the reactant in spite of rigorous exclusion of moisture. Apparently, the acrylic carrier strongly adsorbs traces of water that are flushed out by ammonia. This could be easily remedied by using the hydrophobic Accurel EP 100 (a macroporous polypropylene) as lipase carrier [7, 14].

The efficient use of enzymes in non-aqueous media necessitates their immobilization on a suitable carrier material [15], presumably because the use of 'free' protein dispersions renders the major part of the enzyme molecules inaccessible to the reaction medium. Nevertheless, suspensions of lyophilisate are still often used. Adsorption at the non-polar surface of organic polymers has evolved into a dominant technique for the immobilization of lipases. Lipases generally adsorb readily at a non-polar surface, because in their natural function

[1] A review of the structure, mechanism, and application of lipases is given in Ref. [3]

[2] *Candida rugosa* was previously known as *Candida cylindracea*

[3] Previously classified as *Humicola lanuginosa*

they adsorb at the water-lipid interface; a number of lipases are even interfacially activated. It should be kept in mind, however, that adsorption is a reversible process in aqueous medium [15]. Moreover, a major part of the activity is lost [15], which is probably caused by the partial disruption of the native structure by the hydrophobic interaction of lipase and carrier [16]. We have shown, nevertheless, that in ammoniolysis lipases adsorbed on macroporous polypropylene (Accurel EP100) are more efficient than a free suspension of an equal amount of lyophilisate [15].

Alternatively, lipases can be covalently bound to a solid carrier such as Eupergit C. The activated carrier is expensive, however, and its protein binding capacity is low. The encapsulation of lipases in hydrophobic sol-gel materials which has recently been developed [17] shows considerable potential. The very stable lipase cross-linked crystals [18] (CLCs) such as ChiroCLEC-CR and -PC from CrL and *Burkholderia cepacia*[4] lipase (BcL), respectively, have the advantage that the catalyst material is 100% protein. It has also become clear recently that CLCs of CrL maintain their activity under (aminolysis) conditions that deactivate the native lipase [19].

A number of lipases are commercially available as adsorbed, covalently bound, encapsulated, or cross-linked immobilisates. Moreover, adsorption of lipases on Accurel EP 100 is simple and straightforward [20, 21]. Finally, the modification of lipases to render them soluble in organic media deserves to be mentioned. Although a solubilized lipase has been employed in the first published example of lipase-catalyzed aminolysis [4], this methodology has not found wide application.

Synthesis of Carboxylic Amides

The lipase-catalyzed aminolysis of carboxylic esters has first been described in 1984 by *Inada et al*. [4] in a paper that also constituted the first use of a lipase in organic solvent, closely followed by a publication by *Zaks* and *Klibanov* [5].

Amines

Aminolysis seems to be a fairly general reaction that has been reported for a wide range of alkylamines, including allyl- and benzylamine [22]. Aniline derivatives are weak bases and – similar to phenols – weak nucleophiles. Only a few examples of lipase-catalyzed anilide synthesis, which required massive amounts of CrL, have been published [23,24]. Hydroxyalkylamines are preferentially acylated at the nitrogen atom [25,26], as would be expected on the basis of nucleophilicity as well as bond strength. The competition between the acylation of a secondary amine and a primary alcohol function was less one-sided [27, 28], and the actual outcome of the acylation of N-methylglucamine (Fig. 2) depended on the ionization state and, hence, the nucleophilicity of the nitrogen atom [28].

Although ammonia is the simplest amine, its use as acyl acceptor – ammoniolysis – was reported only fairly recently by us [29] as well as others [22]. Many lipases catalyzed this reaction, although some do not maintain their full

[4] *Burkholderia cepacia* was previously classified as *Pseudomonas capacia*

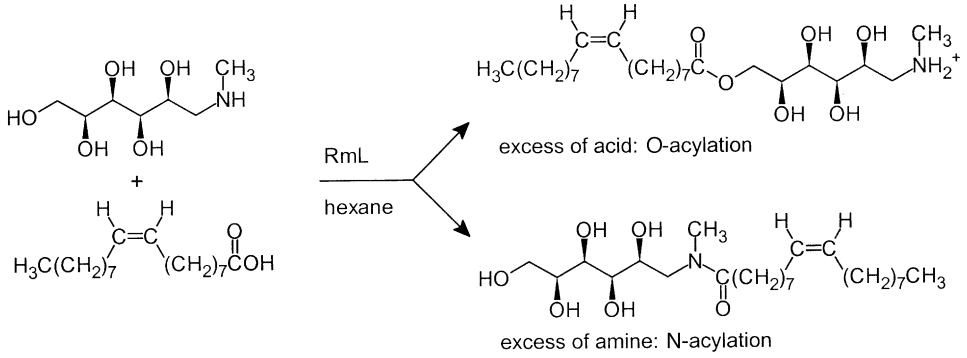


Fig. 2. O-*vs*. N-acylation of N-methylglucamine mediated by *Rhizomucor miehei* lipase (RmL)

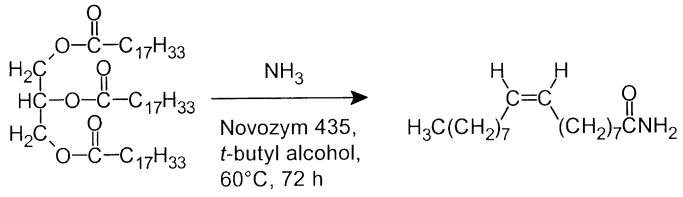


Fig. 3. Ammoniolysis of olive oil

activity at high ammonia concentrations [30]. CaLB (Novozym 435) is particularly stable and efficient in ammoniolysis as well as in aminolysis is general. It has been used, for example, in the transformation of triolein (olive oil) into oleamide (see Fig. 3) [31]. A potential advantage of the enzymatic procedure is that it affords a product of higher purity which obviates the need for purification *via* distillation.

Hydroxylamine and hydrazine, which are also weak bases but, due to the α-effect, strong nucleophiles, readily act as acyl acceptor. Accordingly, the hydroxylaminolysis of fatty acid esters, catalyzed by *Rhizomucor miehei* lipase (RmL), afforded the corresponding hydroxamic acids (N-hydroxyamides) [32]. Hydrazinolysis was, remarkably, first reported for a number of – deactivated – acylhydrazines [33] and only later on also for hydrazine proper [34], using massive amounts of Amano P lipase in both cases.

The hydroxylaminolyis and hydrazinolysis of ethyl octanoate have been extensively investigated in our group [20]. Both nucleophiles require the presence of at least one equivalent of water to prevent irreversible deactivation of the biocatalyst. Hence, hydroxylaminolysis is accompanied by partial hydrolysis of the acyl donor, whereas in hydrazinolysis the hydrolytic side reaction was almost absent. Because the acid resulting from hydrolysis also was converted [32a], although at a lower rate (see below), a nearly quantitative conversion into the hydroxamic acid was eventually achieved.

Acyl donors: carboxylic esters

A wide range of esters, including α,β-alkenic [35, 36] and β-keto esters [22] have been used as acyl donor. Ethyl propiolate, which gave *Michael* adducts with

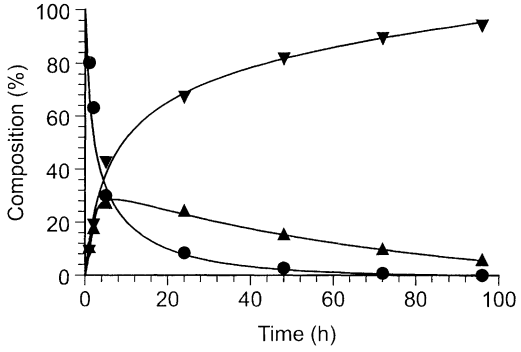


Fig. 4. Time course of the hydroxylaminolysis and competing hydrolysis of ethyl octanoate catalyzed by TIL [20]; ●: ethyl octanoate, ▲: octanoic acid, ▼: octanohydroxamic acid; reaction conditions: 688 mg (4 mmol) ethyl octanoate, 0.5 *M* NH_2OH, 1.75 *M* H_2O, 10 mg immobilized TIL on Accurel EP 100 in 10 cm^3 *tert*-butyl alcohol, 40°C

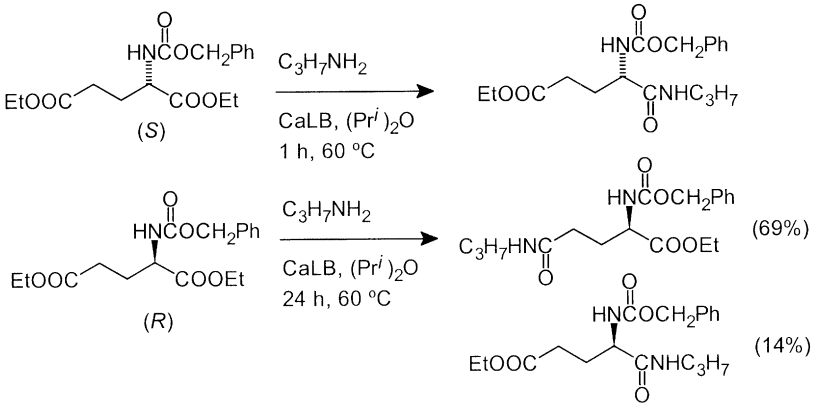


Fig. 5. Aminolysis of diethyl (*S*)- and (*R*)-N-benzyloxycarbonylglutamate

alkylamines [23], reacted with aniline derivatives and ammonia [35] to give the corresponding amides in high yield. The selective transformation of diesters to the monoamide has been observed in hydrophilic solvents [36–39], whereas formation of the succinimide was observed upon aminolysis of diethyl succinate in hexane [38]. An interesting effect of the absolute configuration of the reactant on the regioselectivity is presented by diethyl N-benzyloxycarbonylglutamate (Fig. 5) [37].

Lactams were readily formed by the intramolecular aminolysis of 4- and 5-amino-alkanoic esters, but 6-aminoalkanoic esters reacted only sluggishly to give the ε-lactams [40].

Dialkyl carbonates, the close structural relatives of carboxylic esters, have not often been subjected to lipase-catalyzed transformations. We have found that only CaLB converted carbonates from dibutyl carbonate upwards at a useful rate [41]. Reaction with alkyl amines or ammonia afforded the corresponding carbamate without any further reaction to the ureum derivative (Fig. 6).

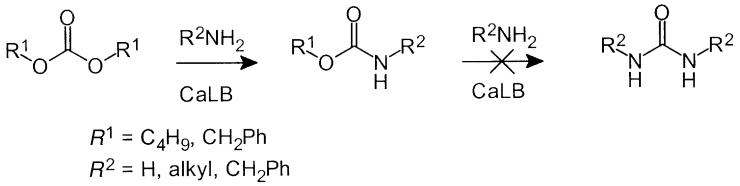


Fig. 6. Aminolysis of dialkyl carbonates

Peptide synthesis

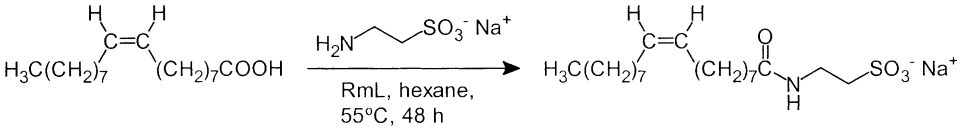
Lipase-catalyzed peptide synthesis offers a number of potential advantages compared with competing methodologies. In contrast to chemical synthesis, circuitous protection/deprotection strategies are not required. Compared with proteases, which are restricted to *L*-amino acids, lipases offer a much more relaxed substrate specificity; moreover, product hydrolysis is virtually absent. These advantages were recognized quite early, and a number of lipase-mediated dipeptide syntheses have been published from 1987 onwards [42–45]. In order to impart the desired selectivity, the amino function in the acyl donor was protected with an acyl group. Out of a large number of lipases tested, only PPL mediated the synthesis at a useful rate [45]. PPL was specific for donors with *L*-configuration [44], whereas the enantiomeric preference towards the acceptor was generally slight [42, 45] (Table 1). These results should be interpreted with some caution, because crude PPL is known to contain protease and carboxylesterase contaminants which, in principle, could be the origin of the observed activity [43]. CrL, which was much less active than PPL, preferentially converted donors with *D*-configuration [44].

Table 1. PPL mediated dipeptide synthesis

Donor	Acceptor	Solvent	Product	Time (h)	Yield (%)	Ref.
Ac-N-*L*-Phe-OEtCl	*L*-Leu-NH_2	Toluene	N-*Ac*-*L*-Phe-*L*-Leu-NH_2	72	83	[42]
Ac-N-*L*-Phe-OEtCl	*D*-Leu-NH_2	Toluene	N-*Ac*-*L*-Phe-*D*-Leu-NH_2	>72	76	[42]
Z-*L*-Phe-OMe	*L*-Ala-OBu^s	Et_2O	*Z*-*L*-Phe-*L*-Ala-OBu^s	16	85	[43]
Z-*L*-Phe-OMe	*D*-Ala-OBu^s	Et_2O	*Z*-*L*-Phe-*D*-Ala-OBu^s	16	60	[43]
Bz-N-*L*-Tyr-OEt	*L*-Thr-OMe	3-Me-pentanol	*Bz*-N-*L*-Tyr-*L*-Thr-OMe	7	92	[45]
Bz-N-*L*-Tyr-OEt	*D*-Thr-OMe	3-Me-pentanol	*Bz*-N-*L*-Tyr-*D*-Thr-OMe	7	73	[45]

Acyl donors: carboxylic acids

Because carboxylic acids and amines tend to form unreactive salts, it was generally assumed that their condensation to the corresponding amides would not be feasible. This opinion has persisted until recently in spite of a number of contrary results that have appeared in the literature. *Tuccio et al*. found that a number of lipases catalyzed the condensation of alkylamines and fatty acids – ranging from acetic to palmitic acid – in anhydrous hexane [46]. The condensation of taurine and oleic acid (Fig. 7) was mediated by Rml as well as CaLB [47], and it has been shown



Oleic acid/taurine (m*M*/m*M*)	Acid conversion (%)
120/30	0
120/60	6
120/120	25
120/240	18
120/480	0

Fig. 7. Condensation of taurine and oleic acid

that for optimum yield the acid and amine reactants should be present in equal amounts to maximize the concentration of both reactants in solution. The synthesis of N-oleyl-N-methylglucamine from oleic acid and N-methylglucamine has been investigated by the same group [28]. Quite recently, *Litjens et al.* showed that the lipase-catalyzed reaction of butyric acid and ammonia is quite feasible [48]; these authors have also established the parameters of the equilibria involved [49].

The lipase-catalyzed condensation of carboxylic acids with hydroxylamine and hydrazine [20, 32a, 50] as well as their derivatives [20] presents a special case because of the stability of the resulting amide bond. These reactions proceed readily to essentially quantitative conversions (Table 2); the condensation of octanoic acid and hydroxylamine has even be performed in water [20]. The advantage of starting from the acid instead of the ester is twofold: it may save a reaction step, and there is virtually no uncatalyzed background reaction, which is important in kinetic resolutions (see later).

Even more surprisingly, many lipases also mediated the condensation of a carboxylic acid with an acyl hydrazide to the N,N′-diacylhydrazine (Fig. 8) [51].

Table 2. Acylation of hydroxylamine and hydrazine derivatives

Donor	Ethyl octanoate	Octanoic acid	
Nucleophile	Initial rate ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Initial rate ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Yield[a] (%)
H_2NOH	910	160	99
H_2NOPh	n.d.	< 5	66
H_2NOCH_2Ph	200	87	98
$HN(CH_2Ph)OH$	n.d.	< 5	65
H_2NNH_2	540	70	91
H_2NNHPh	52	70	95
H_2NNHCH_2Ph	200	100	96

[a] Reaction conditions: 57.6 mg (0.4 mmol) octanoic acid, 1.0 mmol nucleophile, and 50 mg Novozym 435 in 1.0 cm^3 *tert*-butyl alcohol, 20 h, 40°C

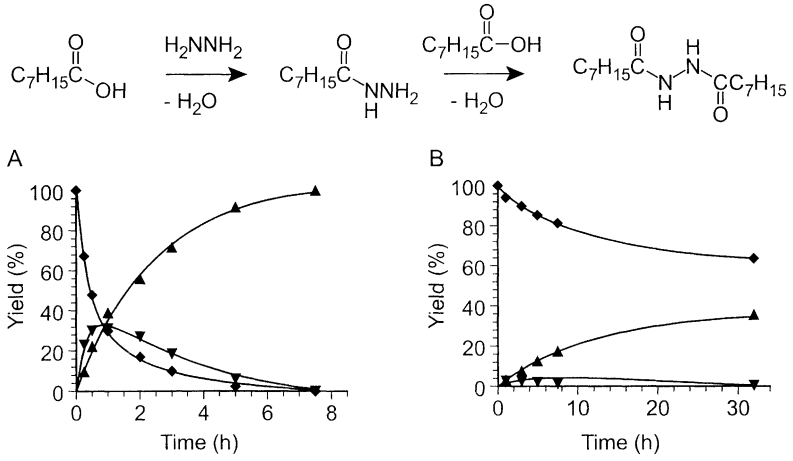


Fig. 8. Time course of the reaction of hydrazine and octanoic acid in isooctane [51]; lipase: A: TIL, B: PaL; ◆: octanoic acid, ▼: octanohydrazine, ▲: N,N′-dioctanoylhydrazine; reaction conditions: 1 mmol hydrazine hydrate, 2 mmol octanoic acid, 50 mg immobilized lipase in 5 cm^3 isooctane, 40°C; 100% yield of N,N′-dioctanoylhydrazine equals 1 mmol

TIL was conspicious for its high activity in this reaction. Some lipases, such as *Pseudomonas* lipoprotein lipase, *P. alcaligenes* lipase (PaL), and *C. antarctica* lipase A acylated octanohydrazide faster than hydrazine itself when both nucleophiles were present in the reaction mixture.

Enantioselective Aminolysis

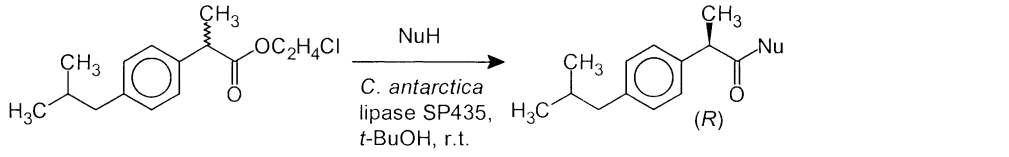
Although lipases have not been designed for enantioselectivity, it was recognized quite early [52] that they are chiral and, hence, inherently enantioselective. This feature has been exploited in aminolysis with the purpose of resolving chiral acyl donors as well as amines. An inherent advantage of aminolysis as a resolution strategy is that, due to the stability of the amide bond, the formation of a carboxylic amide is virtually irreversible [7], which is a prerequisite for an efficient resolution [53].

It is common practice to discuss a kinetic resolution in terms of its enantiomeric ratio E [54] which is equal to the ratio of the *pseudo*-first-order rate constants of the enantiomers according to Eq. 1. We note that formally this practice is not correct for lipase-catalyzed resolutions, because lipases do not obey minimal *Michaelis-Menten* kinetics and, hence, slight deviations of the predicted behaviour are to be expected [55].

$$E = (k_{cat}/K_m)_R/(K_{cat}/K_m)_S \quad (1)$$

Resolution of the acyl donor

Because of the ping-pong mechanism of lipases the conversion of the acyl donor involves a number of steps (see Fig. 1), each of which is subject to – potentially conflicting – enantiomer discriminating effects. The resulting enantiomeric



NuH	Conv. (%)	ee_{ester} (%)	E
H_2O	63	5.8	4
BuOH	56	86	16
NH_3	56	96	28

Fig. 9. Effect of the acceptor on the CaLB-catalyzed resolution of ibuprofen

preference of the transformation is the sum of all these enantiodiscriminatory processes. Hence, the outcome may depend on the reaction conditions and, in particular, on the nature of the acyl acceptor. The CrL mediated aminolysis of methyl 2-chlorobutanoate, for example, was moderately (*S*)-selective ($E = 9$)[5] [8, 56, 57]. In contrast, the esterification of 2-chloropropionic acid, mediated by the same catalyst, was highly (*R*)-specific [58, 59], whereas the hydrolysis of methyl 2-chlorobutanoate was only slightly biased in favour of the (*R*)-enantiomer ($E = 2$) [57, 60]. The N-alkyl-azetidine-2-carboxylates (**1**, Fig. 12) present a comparable case: their resolution could not be accomplished by enzymatic hydrolysis [61], but ammoniolysis catalyzed by CaLB gave the (*R*)-ester and the (*S*)-amide with high selectivity [62]. A final example is the CaLB mediated ammoniolysis of the chloroethyl ester of ibuprofen, which was much more enantioselective than its hydrolysis or alcoholysis (Fig. 9) [29, 30].

In contrast with CaL, CrL is known to convert the physiologically active (*S*)-ibuprofen preferentially [63]. This feature was exploited in the synthesis of (*S*)-ibuproxam, a prodrug, by condensation of racemic ibuprofen with hydroxylamine in aqueous medium (Fig. 10). Crosslinked crystals of CrL (CLEC-CR) were used as the catalyst, because the native enzyme was deactivated under the reaction conditions [19].

Acyl donor resolution would become much more interesting if it could be combined with racemization of the slow-reacting enantiomer to achieve a 100%

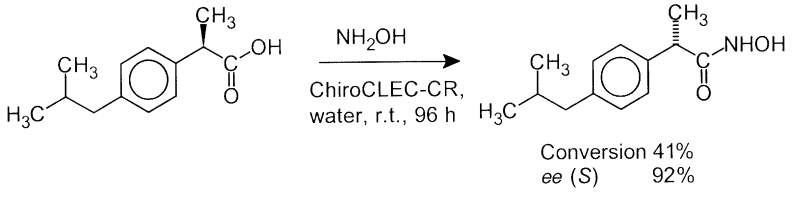


Fig. 10. CLEC-CR mediated synthesis of (*S*)-ibuproxam

[5] *E*-factors, when not provided by the authors, were calculated from the conversion (c) and the enantiomeric purity of the product (ee_p) using the equation derived by *Chen et al.* [54]: $E = \ln(1 - c \cdot (1 + ee_p))/\ln(1 - c \cdot (1 - ee_p))$

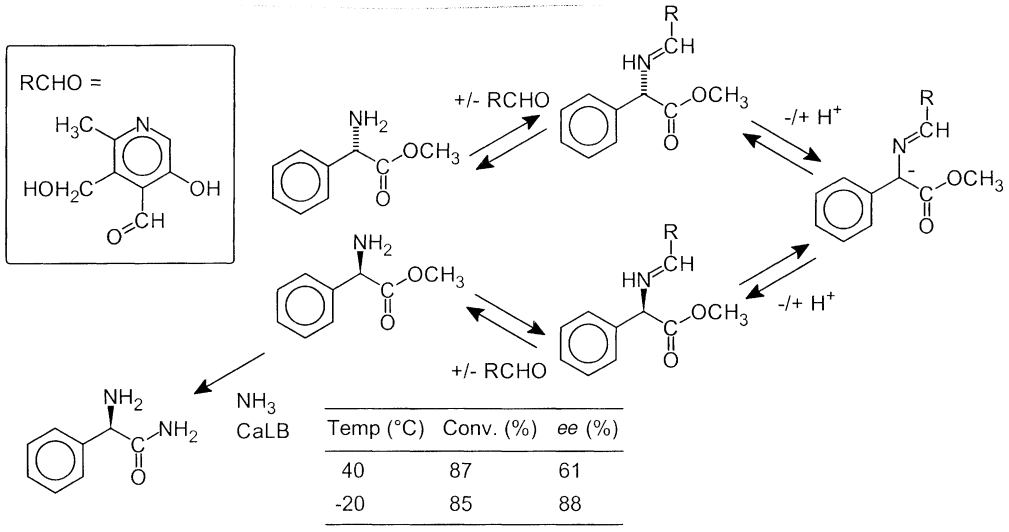


Fig. 11. Ammoniolytic synthesis of (*R*)-phenylglycine amide by dynamic kinetic resolution

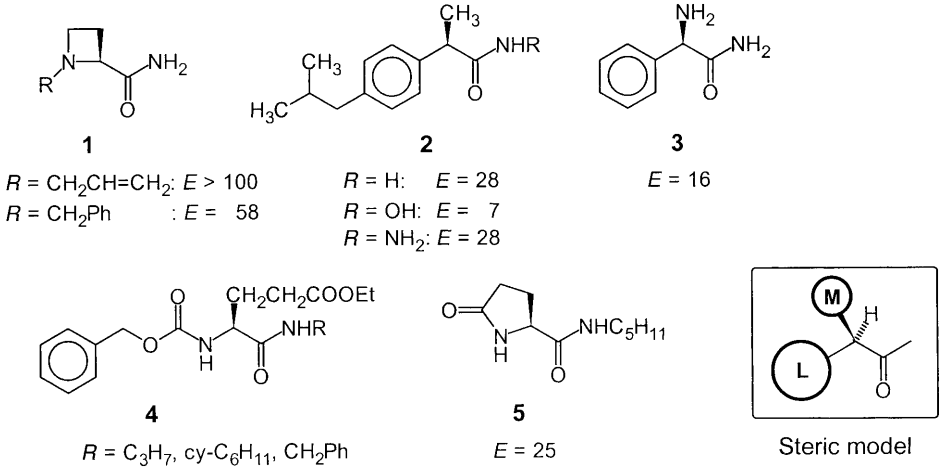


Fig. 12. Enantiorecognition of chiral acyl groups by CaLB

yield of enantiomerically pure product. This has been demonstrated for the CaLB mediated synthesis of (*R*)-phenylglycine amide from its racemic ester [64]. By combining the ammoniolysis with *in situ*-racemization of the slow-reacting (*S*)-ester *via* a *Schiff* base, a dynamic kinetic resolution was developed [65]. Initially the *ee* of the amide was rather low, but it could be improved significantly by lowering the reaction temperature (Fig. 11) [14].

The enantiomeric preference of CaLB in the aminolysis of α-substituted acyl donors is summarized in Fig. 12. The preferentially formed enantiomers of **1** [62], ibuprofen amides (**2**) [19, 29, 30], phenylglycine amide (**3**) [64], and derivatives of glutamic acid (**4**) [37] seem to correspond with a common steric model with the substituents arranged as indicated in Fig. 12. Only the selective ($E = 25$) formation of (*S*)-N-pentylpyroglutamic amide (**5**) [66] does not easily fit into the pattern.

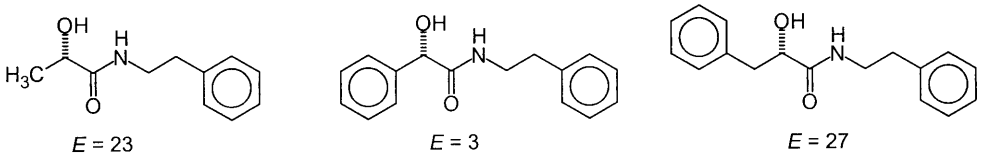


Fig. 13. BcL mediated resolution of benzyl α-hydroxycarboxylates

A number of α-hydroxycarboxylic acid benzyl esters were transformed into their benzylamides in the presence of BcL [67]. The (*S*)-amides were preferentially formed (Fig. 13), whereas the CaLB mediated aminolysis of α-aminocarboxylic acid esters was (*R*)-specific (Fig. 12).

Chiral 3-substituted acyl groups are hardly less efficiently discriminated by CaLB than 2-subsituted ones, in spite of the increasing distance from the reactive centre. The ethyl ester of 3-aminobutyric acid [68] was converted into benzamide **6** with moderate enantioselectivity, but the corresponding 3-hydroxy compound (**7**) was formed with almost absolute enantioselectivity [69, 70] (Fig. 14); the ammoniolysis of ethyl 4-chloro-3-hydroxybutyrate likewise was highly selective [71]. In contrast, BcL acted with negligible enantioselectivity in the benzylaminolysis of ethyl 3-hydroxybutyrate [69].

Prochiral diesters, such as dimethyl 3-hydroxyglutarate, are interesting reactants because their monoaminolysis could, in principle, afford a 100% yield of enantiopure product. Indeed it was found [72] that the *pro-R* ester group in dimethyl 3-hydroxyglutarate was preferentially converted to the monoamides **9** with complete selectivity by CaLB mediated aminolysis (Fig. 14). The enantioselective aminolysis of chiral vinyl carbonates to the corresponding carbamates (**10**), which is not really an acyl resolution, was also quite efficient [73].

It has been explained above that the enantiorecognition of a chiral acyl group involves many steps. Hence, the structure of the amine would be expected to contribute to the overall effect, and intuitively one would expect enantiorecognition to become more efficient with sterically demanding amines. Systematic studies in this field have been few; the available data, which involve β-amino- and β-hydroxyesters, are compiled in Table 3. Based on this very restricted data set, benzylamine would seem to be the resolving agent of choice.

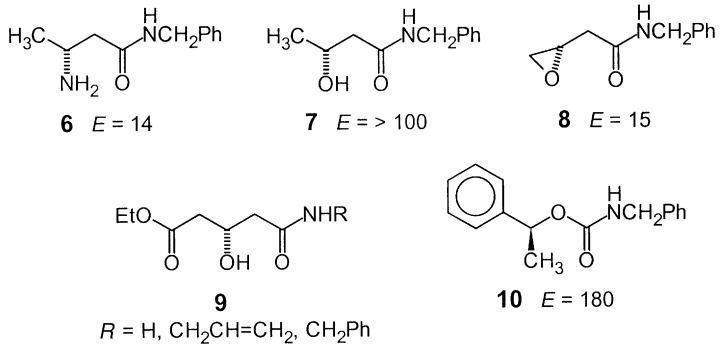
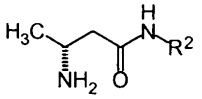
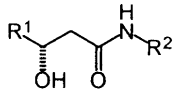
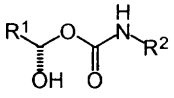


Fig. 14. Enantiomeric preferences of CaLB in the aminolysis of β-substituted acyl donors

Table 3. Effect of the amine on the enantiorecognition of CaLB[a]

Product							
R^1		CH_3	CH_2CH_3	CH_2CH_2Cl	CH_2CH_3	n-C_6H_{13}	C_6H_5
$R^2 = H$	4		44[b]				
$R^2 =$ n-Bu		17	27	43	90	24	63
$R^2 =$ Benzyl	14	>100	52	>100	700	200	183
$R^2 =$ n-Octyl		30	>100	20	160	10	22
Reference	68	70	70	70	73	73	73

[a] Enantiomeric ratios as taken from the references or calculated from conversion and *ee*; [b]data taken from Ref. [71]

Solvents often exert dramatic effects on enzymatic resolutions [74], and an inversion of the enantiopreference has even been observed in certain cases. The effect of the solvent on the CaLB mediated condensation of ibuprofen and hydroxylamine to (*R*)-ibuproxam (**2**, $R = OH$) was only modest, however [19]. *E* ranged from a value of 4 in water and some moderately hydrophilic solvents *via* 8 in isooctane or *tert*-butyl alcohol to 13 in dioxane. The enantiomer discrimination in the CaLB-catalyzed ammoniolysis of phenylglycine methyl ester (Fig. 11) was likewise little affected by the nature of the solvent [14].

Resolution of chiral amines

Acylation studies of secondary alcohols have shown that the nucleophile subsites of all lipases have the same steric preference. A model that has originally been constructed for CrL and BcL [75] was later found to be valid for many other lipases – although not always to the same extent [76] – including CaLB [77]. An overview of the aliphatic and arylaliphatic amines that have been resolved by lipase-catalyzed acylation is given in Fig. 15. The configuration of the preferentially converted enantiomer corresponds with the steric model mentioned above.

The magnitude of the enantiomeric preference, *i.e.* *E*, depends, often critically, on the nature of the acyl reagent and the solvent. Systematic studies in this field are almost non-existent, but we have compiled the available data, which mainly involve CaLB, in Table 4. From these it becomes clear that almost any secondary alkylamine can be resolved once the right reaction conditions have been found. In general, a bulky acyl group, which restricts the conformational freedom of the nucleophile, would be expected to assist the enantiodiscrimination. Indeed 2-aminobutane (**11a**) was much more efficiently resolved by methyl methacrylate than by methyl acrylate [23b]. However, dimethyl succinate also worked rather well in dioxane; in hexane the enantioselectivity was poor [38]. This latter pattern, with the best results being obtained in mildly hydrophilic media, will often be repeated although it is by no means universal.

2-Aminopentane (**11b**) has been resolved, also using CaLB, under completely different conditions: by ethyl acetate in a solvent-free medium [78]. A comparable

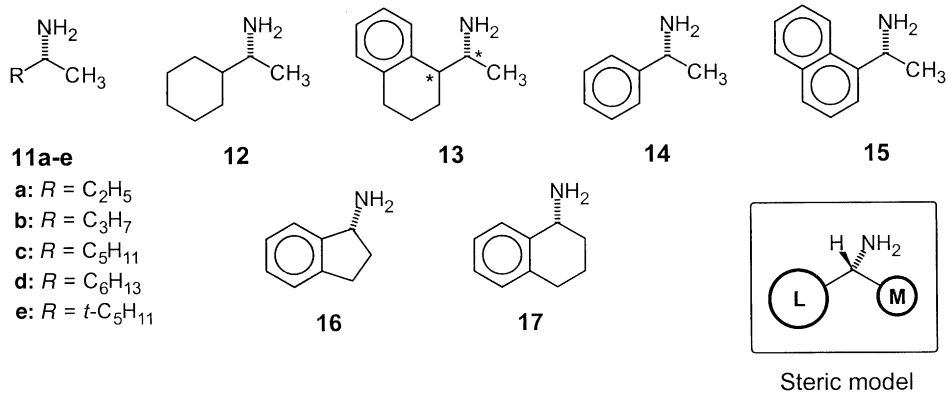


Fig. 15. Preferentially acylated enantiomers of aliphatic and arylaliphatic amines[6]

result was obtained with *Pseudomonas aeruginosa* lipase (PaL) in diethyl ether [79]. The bulky dibenzyl carbonate was an unexpectedly inefficient resolving agent [41], perhaps because hexane is not the optimum solvent considering the solvent effect in the acylation of **11a** [38].

In the resolution of 2-aminoheptane (**11c**) *via* acylation by dimethyl succinate the solvent effect was the opposite of that observed earlier: a good enantiomeric ratio ($E = 68$) was obtained in hexane, whereas reaction in dioxane resulted in only half that value [38]. Another attempt to resolve **11c** in dioxane using the activated reagent octyl vinyl carbonate also failed [80]; **11a** was not resolved by this system either. Finally, ethyl octanoate, an acyl donor that has not been mentioned so far, resolved 2-aminooctane (**11d**) almost quantitatively [81] in a solventless and, hence, hydrophobic reaction medium. However, dibenzyl carbonate in hexane again was quite inefficient [41].

The branched and, therefore, more sterically restricted 2-amino-3,3-dimethylbutane (**11e**) was acylated with low enantioselectivity by neat ethyl acetate [82], whereas this reaction system had given excellent results with **11b** [78]. Even more surprisingly, the *E* factor of **11e** improved by more than an order of magnitude when the acyl donor was isopropyl acetate. Reaction in 1,2-dimethoxyethane (*DME*), which is a more hydrophilic medium, resulted in a further improvement by a factor of seven[6].

1-Cyclohexylethylamine (**12**) was acylated with a moderate *E* of 17 by dibenzyl carbonate in toluene [83]. The more hindered and less flexible amine **13** behaved similarly to **11e** upon acylation by acetic acid esters in the presence of CaLB: the enantiodiscrimination was modest when the ethyl ester was used as donor but, quite remarkably, absolute with the isopropyl ester [82]; BcL was much less selective, although the same trend became apparent.[6] It should be noted that **13** has two chiral centres; the acylation yields [82] indicate that only one (presumable that attached to the nitrogen atom) was resolved.

[6] The absolute configurations of the acylation products of **11e** and **13** were not stated by the authors [82]; by analogy with comparable resolutions, the (*R*)-enantiomer should be expected to be the fast reacting one

Table 4. Resolution of chiral amines **11–17**

Amine	Lipase	Donor	Solvent	*T*/°C	*E*	Ref.
11a	CaLB	Me acrylate	*THF*	30	8	[23b]
	CaLB	Me methacrylate	*THF*	30	55	[23b]
	CaLB	Dimethyl succinate	Dioxane	30	35	[38]
	CaLB	Dimethyl succinate	Hexane	30	6	[38]
	CaLB	Octyl vinyl carbonate	Dioxane		7	[80]
11b	CaLB	Ethyl acetate	–		>200	[78]
	CaLB	Dibenzyl carbonate	Hexane	r.t.	4	[41]
	PaL	Ethyl acetate	Diethyl ether	r.t.	>100	[79]
11c	CaLB	Dimethyl succinate	Dioxane	30	37	[38]
	CaLB	Dimethyl succinate	Hexane	30	68	[38]
	CaLB	Octyl vinyl carbonate	Dioxane	30	3	[80]
11d	CaLB	Ethyl octanoate	–	39	>100	[81]
		Dibenzyl carbonate	Hexane	r.t.	5	[41]
11e	CaLB	Ethyl acetate	–	28	8	[82]
	CaLB	Ethyl acetate	*DME*	r.t.	29	[82]
	CaLB	*i*-Propyl acetate	–	28	109	[82]
	CaLB	*i*-Propyl acetate	*DME*	r.t.	>700	[82]
12	CaLB	Dibenzyl carbonate	Toluene	r.t.	17	[83]
13	CaLB	Ethyl acetate	–	28	14	[82]
	CaLB	*i*-Propyl acetate	–	28	450	[82]
	BcL	Ethyl acetate	–	r.t.	2	[82]
	BcL	*i*-Propyl acetate	–	r.t.	28	[82]
14	CaLB	Ethyl acetate	–		110	[78]
	CaLB	Ethyl octanoate	–	39	>200	[81]
	CaLB	Dimethyl succinate	Dioxane	30	95	[38]
	CaLB	Dimethyl succinate	Hexane	30	6	[38]
	CaLB	Octyl vinyl carbonate	Dioxane		10	[80]
	CaLB	Octyl vinyl carbonate	Di-*i*-propyl ether		200	[80]
	CaLB	Octyl vinyl carbonate	Hexane		200	[80]
	CaLB	Dibenzyl carbonate	Toluence	r.t.	>300	[83]
	CaLB	Dibenzyl carbonate	Hexane	r.t.	20	[83]
	CaLB	Dibenzyl carbonate	Hexane	r.t.	50	[41]
	BpL	Ethyl methoxyacetate	*MTBE*		114	[84]
15	CaLB	Ethyl acetate	–		24	[78]
	CaLB	Ethyl acetate	–	28	15	[82]
	CaLB	*i*-Propyl acetate	–	28	100	[82]
	CaLB	*i*-Propyl acetate	*DME*	r.t.	650	[82]
	BcL	Ethyl acetate	–	r.t.	3	[82]
	BcL	*i*-Propyl acetate	–	r.t.	18	[82]
16	CaLB	Dibut-3-enyl carbonate	Toluene	r.t.	>200	[83]
	CaLB	Dibenzyl carbonate	Hexane	r.t.	72	[41]
17	CaLB	Dibenzyl carbonate	Hexane	r.t.	19	[41]

1-Phenylethylamine (**14**), which has attracted a major share of the efforts in this field, has successfully been resolved under a wide range of reaction conditions. Neat ethyl acetate [78] or octanoate [81] gave a high selectivity; a comparable result was achieved with dimethyl succinate in dioxane, but in hexane the enantioselectivity

was low [38] as observed with **11a**. Octyl vinyl carbonate showed the opposite solvent effect: **14** was resolved with absolute selectivity in diisopropyl ether or hexane, but not in dioxane [80]. Dibenzyl carbonate acted with excellent selectivity in toluene [83], but in hexane [41, 83] the result was much more modest, seemingly confirming that hexane is not the optimum medium for this acyl donor.

Finally, the acyl donor methoxyacetic acid ethyl ester deserves to be mentioned. This compound was developed by BASF as a mildly activated and easily recyclable reagent for the enzymatic resolution of chiral alcohols and amines. **14** was efficiently resolved by *Bukholderia plantarii* lipase (BpL) mediated methoxyacetylation in *tert*-butyl methyl ether (*TBME*) [84].

The enantiodiscrimination of 1-(1-naphthyl)ethylamine (**15**) in the CaLB mediated acylation by neat ethyl acetate was only modest [78, 82], but E could be improved dramatically to over 600 by using isopropyl acetate in *DME* [82] similar to **11e** and **13**.

Finally, the bicyclic amine 1-aminoindane (**16**) was efficiently resolved [41, 83], but the closely related 1-aminotetraline (**17**) reacted with a moderate E of 19 [41]. A dramatic effect of the leaving group (ethyl *vs*. isopropyl) has manifested itself in the acylation of **11e**, **13**, and **15** [82]. The obvious conclusion is that the liberated alcohol influences the enantiorecognition of the amine, presumably because it binds in the active site of the lipase. It is pertinent to note that the interaction with the inihibition of lipases by hydroxylic reaction media is well documented [7, 85].

It has already been noted that *in situ*-racemization of the slowly reacting enantiomer has the potential advantage that the fast reacting enantiomer can be isolated in 100% yield. This has been accomplished with **14** by combining its CaLB mediated acylation with palladium-catalyzed reacemization of the unreacted amine in one pot (Fig. 16) [86].

A few resolutions of more bulky α-phenyl substituted amines have been carried out. The CaLB mediated resolution of a range of 1-phenyl-2-propynylamines (**18**) has been accomplished with absolute enantioselectivity in neat ethyl acetate [87]. Apparently the acetylene moiety is readily accommodated in the part of the nucleophile subsite where the *M*-group binds in the resolutions of **11**–**15**. 1-Amino-1-phenylacetonitrile (**19**), the nitrile analogue of **18**, 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 converted (*R*)-**19** slightly faster [88]. The CaLB mediated reaction of **19** and phenylacetic

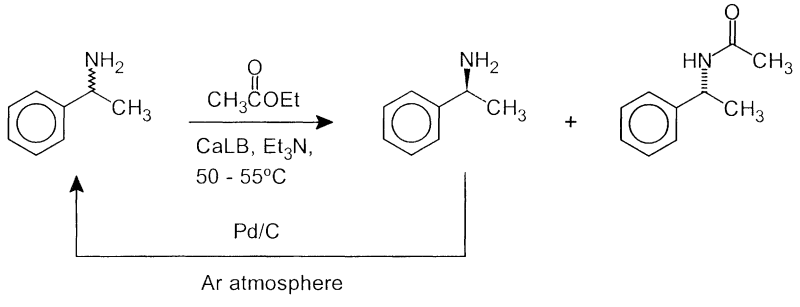


Fig. 16. Dynamic kinetic resolution of 1-phenylethylamine (**14**)

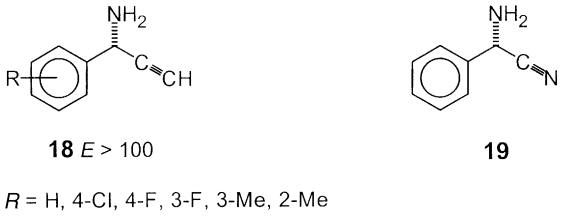


Fig. 17. Enantiopreference of CaLB in the acylation of **18** and **19**

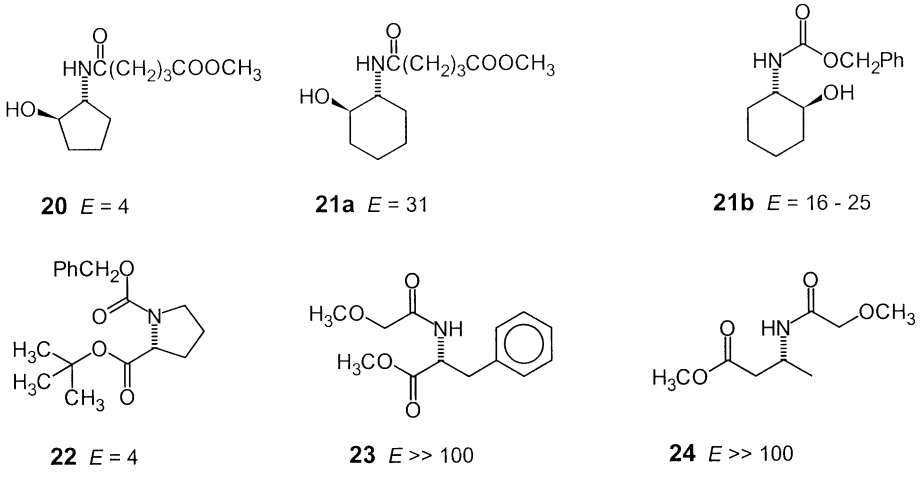


Fig. 18. CaLB mediated enantioselective acylation of functionalized amines **20**–**24**

acid methyl ester was highly (*S*)-selective [89], which may be explained as a beneficial effect of steric congestion in the active site.

A few functionalized amines, such as β-hydroxyamines and amino acid esters have been resolved by enantioselective acylation (Fig. 18), and the question arises whether or not the same steric model applies. The CaLB mediated acylation of *trans*-1-amino-2-hydroxycyclopentane with dimethyl glutarate to **20** was only modestly enantioselective [90]. The enantiomers of its C_6-homologue were more readily discriminated (**21a**) when the reaction was carried out in dioxane, but no resolution was possible in toluene. This enantiopreference would seem to be consistent with the steric model, with the hydroxyl group binding as the *L* substituent. Surprisingly, the enantiopreference was inverted with dibenzyl carbonate as the acyl donor [83, 90]; **21b** was formed with $E = 16$ in either dioxane or toluene. The acylation of amino acid esters was (*R*)-selective, as would be expected on the basis of the peptide synthesis experiments. Proline *tert*-butyl ester (**22**) was inefficiently resolved using dibenzyl carbonate [83] but, surprisingly, the acylation of the methyl esters of phenylalanine (**23**) and β-aminobutyric acid (**24**) with methoxyacetic acid ethyl ester resulted in a nearly quantitative ($E = 200$) resolution [91]. The reaction of β-aminobutyric acid ethyl ester with ethyl acetate was somewhat less enantioselective [68].

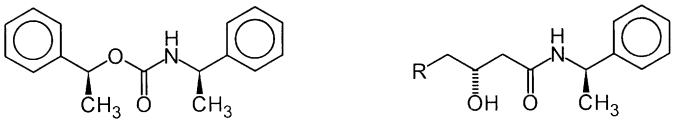


Fig. 19. Combined resolutions

Combined resolutions

Considering that lipases can resolve the acyl donor as well as the acyl acceptor, it might be attractive to accomplish both resolutions in one reaction. Such a one-step resolution of a chiral vinyl carbonate and chiral amines, mediated by CaLB, was only moderately successful, because the products, such as *e.g.* **25** (see Fig. 19), were formed in a moderate diasteromeric and/or enantiomeric excess [92]. In contrast, **26** and some related amides were obtained as the almost pure (3*S*,1′*R*) stereoisomers [93].

Conclusion

The lipase-catalyzed aminolysis of carboxylic esters is a fairly general methodology for the synthesis of carboxylic amides under mild conditions. The condensation of carboxylic acids and the amines provides an alternative route to carboxylic amides. Lipase-catalyzed aminolysis is a useful method to resolve chiral carboxylic esters as well as chiral amines. The enantiorecognition of chiral amines often depends critically on the acylating agent and the reaction medium.

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