Enzymatic kinetic resolution of β-amino acid derivatives

PhD Thesis

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CONTENTS

Publications

Papers related to the thesis

I. **Fitz, M.**; Lundell, K.; Lindross, M.; Fülöp, F.; Kanerva L. T.
An effective approach to the enantiomers of alicyclic β-amino nitriles by using lipase catalysis
Tetrahedron: Asymmetry **2005,** *16*, 3690-3697.

II. **Fitz, M.**; Lundell, K.; Fülöp, F.; Kanerva, L. T.
Lipase-catalysed kinetic resolution of 2-aminocyclopentane- and 2-aminocyclohexanecarboxamides
Tetrahedron: Asymmetry **2006,** *17*, 1129-1134.

III. **Fitz, M.**; Forró, E.; Vigóczki, E.; Lázár, L.; Fülöp, F.
Lipase-catalysed *N*-acylation of β^2-amino esters
Tetrahedron: Asymmetry **2008,** *19*, 1114-1119.

Conference lectures related to the thesis

IV. **Fitz, M**.
A *cisz-* és *transz*-2-amino-ciklopentán- és -ciklohexánkarboxamidok enzim-katalizált kinetikus rezolválása
VII. Clauder Ottó Emlékverseny, Visegrád, 2004.

V. **Fitz, M**.
A *cisz-* és *transz*-2-amino-ciklopentán- és -ciklohexánkarboxamidok enzim-katalizált kinetikus rezolválása
„A Szegedi Ifjú Szerves Kémikusok Támogatásáért” Alapítvány tudományos előadóülése, 2005.

VI. **Fitz, M.**; Lundell, K.; Kanerva, L. T.; Fülöp, F.
Enzyme catalysed kinetic resolution of cyclic β-amino amides and β-amino nitriles
7th International Symposium on Biocatalysis and Biotransformations, Delft, The Netherlands, 2005.

VII. **Fitz M.**; Lundell, K.; Kanerva, L. T.; Fülöp F.
Ciklusos β-amino nitrilek és β-aminosavamidok enzim-katalizált kinetikus rezolválása
Vegyészkonferencia, Hajdúszoboszló, 2005.

Abbreviations

β-Ala	β-alanine
Asp	aspartic acid
Boc	*tert*-butoxycarbonyl
c	concentration
CAL-A	*Candida antarctica* lipase A
CAL-B	*Candida antarctica* lipase B
Cbz	benzyloxycarbonyl
CCL	*Candida cylindracea* lipase
Chirazyme L5	*Candida antarctica* lipase A
Chirazyme L2	immobilized *Candida antarctica* lipase B
chiroCLEC-CR	cross-linked enzyme crystal of *Candida rugosa* lipase
conv.	conversion
CRL	*Candida rugosa* lipase
DIPE	diisopropyl ether
DMAP	4-*N*,*N*-dimethylaminopyridine
DME	dimethoxyethane
DMF	dimethylformamide
E	enantioselectivity, enantiomeric ratio
ee	enantiomeric excess
ee$_p$	enantiomeric excess of product
ee$_s$	enantiomeric excess of substrate
EtOH	ethanol
Et$_2$O	diethyl ether
Fmoc	(9-fluorenyl)methoxycarbonyl
GC	gas chromatography
LAH	lithium aluminium hydride
LIP-300	immobilized preparation of *Pseudomonas aeruginosa*
Lipase AK	*Pseudomonas fluorescens* lipase
Lipase AY	*Candida rugosa* lipase
Lipase PS	*Burkholderia cepacia* lipase
Lipase PS-C II	*Burkholderia cepacia* lipase
Lipolase	*Candida antarctica* lipase B immobilized on acrylic resin
MeCN	acetonitrile
MeOH	methanol
NMR	nuclear magnetic resonance
Novozym 435	immobilized preparation of *Candida antarctica* lipase B
PAL	lipase from *Pseudomonas aeruginosa*
PPL	porcine pancreatic lipase
rt	room temperature
t	reaction time
T	temperature
TAA	*tert*-amyl alcohol
*t*amylOMe	*tert*-amyl methyl ether
TBME	*tert*-butyl methyl ether
TEA	triethylamine
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TMSCN	trimethylsilyl cyanide
Ts	tosyl
VA	vinyl acetate
VB	vinyl butyrate

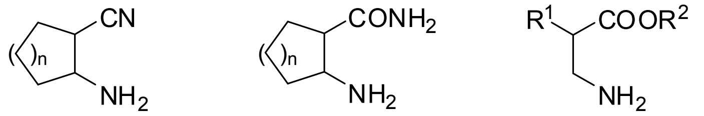
1. Introduction and aims

In a consequence of their unique chemical and biological properties, aliphatic and alicyclic β-amino acids and their derivatives are important subjects of research.[1,2] The most obvious examples are the naturally occurring cispentacin [FR 109615; (1*R*,2*S*)-2-aminocyclopentane-1-carboxylic acid][3,4] and its synthetic derivatives,[5,6] which are potent antifungal antibiotics. β-Amino acids are important intermediates of β-lactams and heterocycles,[1] and are constituents of many biologically active compounds, as antitumour taxoids,[7,8] which contain an α-hydroxy-β-amino acid side-chain. Similarly to α-amino acids, β analogues can be used in the preparation of peptides.[1] The incorporation of β-amino acids in α-peptides may modify the structure and the biological effect, and make the peptide resistant against proteolytic degradation.[9] The β-amino acid-containing peptidomimetics are noteworthy lead compounds in the development of protease inhibitors.[9-12] The secondary structure and folding properties of β-peptides have been studied deeply.[1,10,13-15]

Alicyclic β-amino nitriles can be easily transformed by acid- or base-induced hydrolysis to the corresponding amino acid amides and amino acids. The metal-hydride-catalysed reduction leads to diamines. Amino carboxamides are also intermediates of amino acids. *N*-Substituted 2-aminocyclopentanecarboxamides have been found to be effective modulators of sodium and/or calcium channels; and are potential therapeutics in a wide range of pathologies, including neurological, psychiatric, cardiovascular, metabolic and gastrointestinal diseases.[16] Enantiomeric *N*-cycloalkylcarboxamide derivatives of *N*-benzyl benzenesulfonamides have been prepared, which are useful in the inhibition of the aberrant accumulation of β-amyloid, as in Alzheimer's disease.[17] 2-Aminocyclopentane- and -cyclohexanecarboxamides in their racemic and enantiomeric forms can serve as building blocks of some potential pharmacons, *e.g.* chemokine receptor antagonists,[18] antibacterials[19] and inhibitors of matrix metalloproteinases[20] and tyrosine kinases.[21] These compounds can be useful in the treatment of a wide variety of disorders, such as acute and chronic inflammatory, malignant and degenerative diseases (psoriasis, rheumatoid arthritis, osteoporosis and atherosclerosis), bacterial infections and cancer. Caspases play a role in the activation of interleukins, their increased activity resulting in various neurodegenerative and autoimmune diseases. Certain dipeptides with Asp at the C-terminus can act as caspase inhibitors.[22] Different natural and unnatural amino acids may be present at the *N*-terminus, *e.g.* 2-isopropyl β-Ala.

Numerous methods have been described for the preparation of enantiomerically pure β-amino acids.[1,2,23-25] The preparation of optically active β-amino acids via enzyme-catalysed resolution, in contrast with other approaches, can be performed under mild conditions, using inexpensive reagents and facile procedures.[26]

The basic aim of this PhD work was to perform the enzymatic kinetic resolution of alicyclic *cis* and *trans*-β-aminocyclopentane- and -cyclohexanecarbonitriles (**1**-**4**) and the corresponding amino carboxamides (**5-8**; Figure 1). Besides the development of a suitable method for the preparation of enantiomerically pure β-amino acid derivatives, we planned to follow up the stereochemical preference of lipases in organic solvents. A further aim was to compare our results with those to be found in the literature for the enzymatic resolution of corresponding β-amino esters. It was also planned to develop an enzymatic method for the resolution of ethyl 3-amino-2-ethylpropanoate **9** and methyl 3-amino-2-isopropylpropanoate **10** through lipase-catalysed *N*-acylation (Figure 1).



1: n = 1, *cis*
2: n = 2, *cis*
3: n = 1, *trans*
4: n = 2, *trans*

5: n = 1, *cis*
6: n = 2, *cis*
7: n = 1, *trans*
8: n = 2, *trans*

9: $R^1 = R^2 = Et$
10: $R^1 = i$Pr, $R^2 = Me$

Figure 1

We set out to study the effects of the used enzyme, acyl donor and solvent on the reaction rate and enantioselectivity (*E*) in the frame of preliminary experiments, to summarize these results, and then to perform the gram-scale resolutions of the model compounds under the optimized conditions.

In the literature survey in this thesis, the methods of lipase-catalysed resolution of amines through *N*-acylation, in non-aqueous or solvent-free medium has been overviewed on the basis of the findings of the last 10-12 years.

All the compounds, racemates and enantiomers presented in this thesis are described by numbers (**1**-**148**). The publications on which this thesis is based are given in square brackets in superscript, while other literature references are given as superscripts.

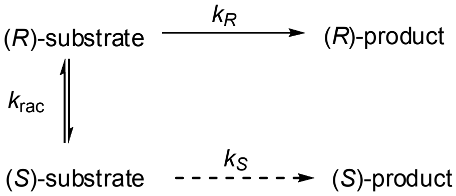
2. Literature

Enantiopure amines are widely used as synthetic building blocks, chiral auxiliaries, catalysts and resolving agents in the pharmaceutical, agrochemical and fine chemical industries.[27] Additionally, numerous drugs contain an amino group at the chiral centre, so there is a great demand for methods for the synthesis of optically active amines.

2.1. *Lipases in the resolution of amines*

The two theoretically possible resolution methods are enantioselective acylation of an amino group, and the hydrolysis of *N*-acylated derivatives. Performance of the enzymatic resolution of amines involves two problems. First of all, amines are more nucleophilic than alcohols and may react non-enzymatically with the acyl donors used in the resolution of alcohols.[28] To overcome this problem, less reactive acylating agents have been applied in the resolution of amines.[29] Since the amide form is energetically more favourable than the amine, it is difficult to hydrolyse it. Consequently, enzyme-catalysed enantioselective *N*-acylation is the appropriate method for the resolution of amines. In recent decades, lipases (EC 3.1.1.3) have acquired great significance in *N*-acylations.[30-35] Substrates containing other functional groups can be resolved through lipase-catalysed *O*-acylation (amino alcohols)[36,37] or ester hydrolysis (amino esters)[38] too.

The enzyme-catalysed kinetic resolution of racemic mixtures is the most common way to prepare enantiomerically pure compounds.[27] The major disadvantage of this technique is that a product yield of at most only 50% can be obtained. Kinetic resolution combined with *in situ* racemization is called dynamic kinetic resolution,[39] which allows a 100% yield of the desired enantiomer (Scheme 1). Significant advances have been made in this field during the last 10 years.[39-43]



Scheme 1

Certain requirements have to be fulfilled in order to achieve succesful dynamic kinetic resolution.[44] The racemization should be faster than the resolution step ($k_{rac} > k_R$). The racemization catalyst must not racemize the product of the reaction. The conditions of kinetic resolution and the racemization must be compatible.

In the last two decades, lipases have mainly been used in organic solvents due to the advantages of these media.[45-50] There has recently been increasing interest in ionic liquids, and in a few cases lipases have been used in this type of media. Sporadically, non-solvent enzymatic reactions have been reported.

To describe enzyme-catalysed reactions, the conversion (conv.) and *E* values are given.[51] Enantiomerically enriched products can be characterized by the ee (enantiomeric excess). Another important characteristic of an enzymatic process is the enantiopreference. The Kazlauskas rule,[52] originally developed to predict the preferentially acylated enantiomer of secondary alcohols, also refers to chiral amines (Figure 2). This empirical rule is based on the structure of the active site of the lipase, which contains two pockets, a medium (M) and a large (L) one, where the two substituents of the substrate fit. M usually binds apolar groups, while L prefers polar groups, leading to disagreement with the Kazlauskas rule in some cases.

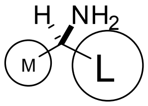


Figure 2

2.2. *Kinetic resolution of amines*

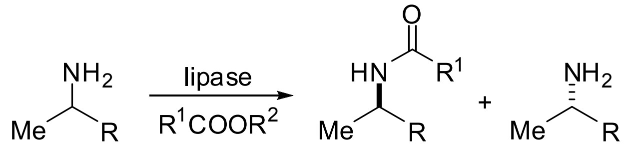
2.2.1. Enantioselective *N*-acylation of alkylamines ($R^1R^2CHNH_2$)

2-Aminobutane **11**, one of the most frequently studied amines, is resolved through lipase-catalysed *N*-acylation (Table 1).[53-55] The low selectivities observed could be explained by using the above-mentioned model: the similarity in size of the methyl and ethyl groups makes the discrimination difficult. The size of the acyl group also influences the selectivity of the reaction. Ethyl decanoate (entry 3)[53] has proved to be an efficient acyl donor, in contrast with others (entries 1 and 2).[54,55]

Compounds containing an R group larger than ethyl can be selectively acylated.[54-61] Possible effects of the leaving group of the acyl donor on the reaction rate were investigated in a study with **13**.[56] The use of isopropenyl acetate instead of ethyl acetate under otherwise the same conditions increased the *E* considerably (entries 7 and 8).

In order to resolve and protect chiral amines in one step, dibenzyl carbonate may be applied as acyl donor.[29,57] In the case of 1-cyclohexylethylamine, modest enantioselectivity (*E* = 17) was detected,[29] but the enantioselective protection of other alkylamines failed (entries 6 and 11).[57]

Table 1. Lipase-catalysed resolution of alkylamines



(±)-**11**-**15** (*R*)-**16**-**20** (*S*)-**11**-**15**

Entry	Substrate	Lipase	Acyl donor	Solvent	*T* (°C)	*t* (h)	Conv. (%)	*E*
1[54]	**11** R = Et	Novozym 435	(phenyl)ethyl acetate	dioxane	30	8	36	4
2[55]			(*R*)/(*S*)-PGE	DIPE	40	3/24	50/90	2/2
3[53]			ethyl decanoate	TBME	rt	24	65	20
4[61]	**12** R = *n*Pr	Novozym 435	MeCOOEt	Et_2O	n.d.	n.d.	44	>200
5[55]			(*R*)/(*S*)-PGE	DIPE	40	3/24	53/58	5/5
6[57]			dibenzyl carbonate	*n*Hex.	rt	288	21	4
7[56]	**13** R = *t*Bu	Chirazyme L2	MeCOOEt	DME	28	336	29	29
8[56]			MeCOO*i*Pr	DME	28	336	36	>>200
9[54]	**14** R = *n*Pent	Novozym 435	(phenyl)ethyl acetate	dioxane	30	17	49	>200
10[55]			(*R*)/(*S*)-PGE	DIPE	40	5.5/24	52/50	11/21
11[57]	**15** R = *n*Hex	Novozym 435	dibenzyl carbonate	*n*Hex.	rt	288	22	5
12[59]			ethyl octanoate	–	39	100	52	>100

Examples of doubly enantioselective reactions[58] catalysed by lipases have appeared in the literature.[54,58] In this process, chiral esters and chiral amines have been reacted in the presence of the enzyme, allowing parallel resolution. In the first example,[54] (±)-(phenyl)ethyl acetate was used as acylating agent, the enzymatic reaction resulting in four enantiomeric products: unreacted amine and ester, free alcohol and amide. The resolution of **11** failed (entry 1), but in the case of **14** this proved to be an excellent resolution method (entry 9) for both acyl donor and nucleophile.[54] In another case, ethyl (±)-4-chloro-3-hydroxybutanoate was chosen as acylating reagent for **14**: after 24 h, at 35% conversion, the amide product contained 92% of the (3*S*,1'*R*) stereoisomer.[58]

Ismail *et al.* recently made an attempt to resolve alkylamines and arylethylamines with enantiomeric α-amino acid esters as acyl donors.[55] Phenylglycine ester enantiomers [(*R*)-phenylglycine propyl ester - (*R*)-PGE, (*S*)-phenylglycine methyl ester - (*S*)-PGE] served as acyl donors and exhibited dramatically different reaction rates, the *E* values being unexpectedly low in each case (entries 2, 5 and 10).

The first example of the solvent-free lipase-catalysed resolution of a racemic amine was described a decade ago (entry 12).[59] Amide enantiomers have subsequently been prepared by the amidation of 2-ethylhexylamine with aliphatic acids by CAL-B catalysis at 90 °C.[60]

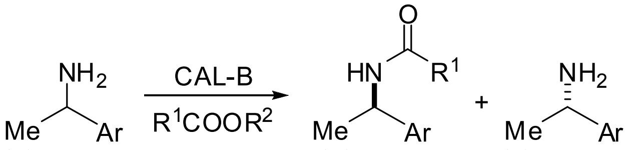
2.2.2. *N*-Acylation of arylalkylamines

Arylethylamines (Table 2) are the most widely studied group of arylalkylamines, and they are therefore discussed separately. Among them, 1-phenylethylamine **21** served as test substrate.[29,54,55,57,61-63] The literature data show that ethyl acetate is suitable for the resolution (entry 1).[61] Change of the acyl or the alcohol part or both resulted in appropriate acyl donors and effective resolution (entries 2-5).[54,55,62,63] The use of dibenzyl carbonate affords the enantiopure Cbz-protected amine in a single step (entries 6 and 7).[29,57] The effect of 3'-bromo substitution was studied by Gill *et al.* (entries 8-10) in an extended screen: diethyl oxalate and ethyl 2-methoxyacetate were found to be optimal acyl donors for the resolution of **22** (entries 9 and 10).[64] 4-Chloro substitution permits excellent enantioselectivity (entries 11-13), even when accomplished in neat methyl methoxyacetate.[65,66] The same substrate was examined by Wagegg *et al*,. who observed worse results (E = 20 and 25) on using ethyl butyrate and ethyl methoxyacetate.[67] Good results were found for the enzymatic acylation of 1-α-naphthylethylamine (entries 14-16), except when ethyl acetate was used as acyl donor.[56,61,62]

In view of the extended utility of ferrocenes, 1-ferrocenylethylamine was prepared in enantiopure form[68] in neat ethyl acetate (entry 17), but with ethyl formate (entry 18) low selectivity was observed, though the reaction proceeded 4 times faster. The resolution of heteroaryl-substituted ethylamines indicated moderate to good enantioselectivities when ethyl acetate was used as acyl donor (entries 19-24).[69] In the case of **26**, higher selectivity was observed in neat ethyl acetate than in dioxane (entries 19 and 20). The opposite tendency appeared with **27** and **28** as substrates (entries 21-24). A recent paper[70] demonstrates the effects of structural modification on the resolution properties of pyridil derivatives. 4-Chloro-2-(1-aminoethyl)pyridine was successfully resolved with ethyl acetate in TBME by CAL-B catalysis (conv. = 50% after 4 h; E > 200). 4-Chloro-3-(1-aminoethyl)pyridine gave similar results in a slower reaction (conv. = 48% after 87.5 h; E > 200).

The above-mentioned doubly enantioselective aminolysis reaction[58] was found to be an appropriate method to resolve **21**, **26** and ethyl 4-chloro-3-hydroxybutanoate, resulting in 97% and 96% (3*S*,1'*S*)-amide at 49% and 48% conversion, respectively.

Table 2. CAL-B-catalysed resolution of aryl- and heteroaryl ethylamines



(±)-21-28 **(*R*)-29-36** **(*S*)-21-28**

Entry	Substrate	Acyl donor	Solvent	*T* (°C)	*t* (h)	Conv. (%)	*E*
1[61]	**21** Ar = phenyl	MeCOOEt	Et_2O	n.d.	n.d.	43	106
2[54]		(phenyl)ethyl acetate	1,4-dioxane	30	17	27	>200
3[62]		ethyl hexanoate	DIPE	30	120	50	>>200
4[63]		isopropenyl methoxyacetate	TBME	35	n.d.	50	>>200
5[55]		(*R*)/(*S*)-PGE	DIPE	40	24	51/55	97/100
6[57]		dibenzyl carbonate	*n*Hex	40	168	27	50
7[29]		dibenzyl carbonate	toluene	rt	n.d.	33	>200
8[54]	**22** Ar = 3-bromo-phenyl	BuCOOEt	TBME	40	20	24	3.5
9[64]		$(COO)_2Et_2$	TBME	40	20	48	>>200
10[64]		ethyl methoxyacetate	TBME	40	20	49	>>200
11[65]	**23** Ar = 4-chloro-phenyl	methyl methoxyacetate		rt	2.5	49	>200
12[66]		ethyl chloroacetate	DME	rt	3.25	50	>>200
13[66]		PrCOOEt	*t*amylOMe	45	6	43	>200
14[61]	**24** Ar = 1-naphthyl	MeCOOEt	Et_2O	n.d.	n.d.	20	23
15[62]		ethyl hexanoate	DIPE	30	120	43	>200
16[56]		MeCOO*i*Pr	DME	28	168	44	>200
17[68]	**25** Ar = 1-ferrocenyl	MeCOOEt	MeCOOEt	28	12	47	>200
18[68]		HCOOEt[a]	1,4-dioxane[a]	28	3	42	29
19[69]	**26** Ar = 2-furyl	MeCOOEt	MeCOOEt	30	6	40	>100
20[69]		MeCOOEt[b]	1,4-dioxane[b]	30	40	42	31
21[69]	**27** Ar = 2-thienyl	MeCOOEt	MeCOOEt	30	14	37	32
22[69]		MeCOOEt[b]	1,4-dioxane[b]	30	40	36	>100
23[69]	**28** Ar = 2-pyridyl	MeCOOEt	MeCOOEt	30	14	50	66
24[69]		MeCOOEt[b]	1,4-dioxane[b]	30	40	47	72

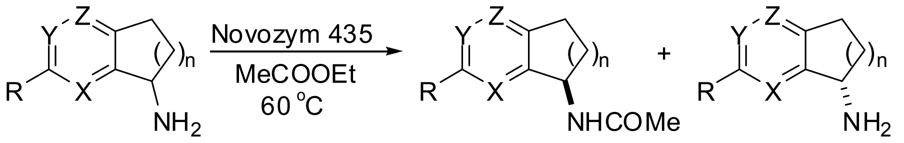
[a]Ethyl formate:1,4-dioxane 1:20.
[b]Ethyl acetate:1,4-dioxane 1:10.
n.d.: not determined

When the pharmacologically valuable *cis* and *trans*-2-phenylcyclopentan- and -cyclohexanamines were subjected to aminolysis catalysed by CAL-B,[71-72] the *trans* compounds could be resolved under very simple conditions: in ethyl acetate at room temperature. In contrast, for the resolution of the *cis* diastereomers, (±)-1-phenylethyl- and (±)-*cis*-2-phenylcyclopentyl methoxyacetates were used as acyl donors. To rationalize these differences caused by the stereochemistry, molecular modelling was applied.[73]

During the past decade, ionic liquids have become accepted as "green" alternatives of organic solvents,[74-75] though they are only sporadically used in amine resolutions.[76-77] The acylation of **21** by CAL-B catalysis with carboxylic acids in ionic liquids and solvent-free systems has been studied. The results clearly show that, without solvent, the reaction rate is lower than in [BMIM][PF_6] (1-butyl-3-methylimidazolium hexafluorophosphate) with 4-pentenoic acid (conv. = 0.6% and 8% after 24 h; *E* >> 200).[78] Different ionic liquids have been investigated in the CAL-B-catalysed acylation of **21** with 4-pentenoic acid, an excellent *E* being observed in each case. For the resolution, [BDMIM][TFMS] (1-butyl-2,3-methylimidazolium trifluoromethane sulfonate) was chosen according to the reaction rate (conv. = 21.2% after 24 h).[79] 2-Phenyl-1-propanamine was the other model compound which exhibited significantly lower selectivity (*E* = 2-6), due to the structural differences. A recent study illustrates the activity of cross-linked CAL-B in ionic liquids.[80] The acylation of **21** with methyl 2-methoxyacetate both in TBME and in [BMIM][NO_3] (1-butyl-3-methylimidazolium nitrate) attained 50% conversion within 24 h, and the selectivities were also comparable: 140 and 95, respectively.

The example of 1-phenylbutylamine demonstrates the ability of CAL-B to catalyse the enantioselective acylation of 1-arylalkylamines (18% conv. after 150 h; *E* = 60).[54] 1-Phenyl-2-propynylamines were resolved by using the same enzyme, which effectively discriminated between phenyl (L) and larger alkyl (M) groups.[81] Different substituents (halogen and methyl) on the phenyl group did not affect the excellent selectivity (*E* > 200), the only exception being 2-substitution, when the reaction was much less selective (*E* = 5) because of the steric hindrance. A number of bicyclic 1-heteroarylamine enantiomers have been prepared by means of CAL-B catalysis (Table 3).[82] The presence of *N* in the aryl ring (**38**-**41**) makes the *E* much higher compared to that for tetrahydronaphthaleneamine (**37**) under the same conditions. The results obtained with substrates **38**, **42** and **43** suggest that the 6,6-fused bicyclic ring system is optimum for the chiral recognition of CAL-B. 2-Methyl substitution (**44**) significantly lowered the selectivity. The resulting acetamides [(*R*)-**45**-**52**] were hydrolysed to the corresponding amines by heating with aqueous HCl without loss of optical purity.

Table 3. Resolution of bicyclic 1-heteroarylamines using Novozym 435[82]



37-44 (*R*)-**45-52** (*S*)-**37-44**

Compound	n	X	Y	Z	R	*t* (h)	Conv. (%)	*E*
37[a]	2	CH	CH	CH	H	24	64	17
38[a]	2	N	CH	CH	H	4	50	>>200
39[a]	2	CH	N	CH	H	23	51	>200

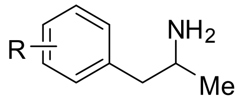
Table 3. (continued) Resolution of bicyclic 1-heteroarylamines using Novozym 435[82]

Compound	n	X	Y	Z	R	*t* (h)	Conv. (%)	*E*
40[a]	2	CH	CH	N	H	6	50	>>200
41[b]	2	N	CH	N	H	2	50	>>200
42[b]	1	N	CH	CH	H	7	55	49
43[a]	3	N	CH	CH	H	24	58	5
44[a]	2	N	CH	CH	Me	9	51	47

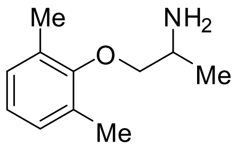
[a] In *i*Pr_2O with 4-5 equivalents ethyl acetate.
[b] In ethyl acetate (the substrate is not soluble in ether solvents).

Other, mostly 2-arylalkylamines were subjected to CAL-B-catalysed reactions in neat ethyl acetate (Table 4).[83] The selectivity for amphetamine **53** was mediocre (*E* = 37, entry 1), and the insertion of an extra methylene (**58**) did not have any effect on *E* (entry 8). Reaction rates and selectivities for the isomeric methoxyamphetamines **53-56** are very similar (entries 1-4), but the *ortho* position (**54**) seems to be more favourable (entry 2). Mexiletine **57** was also successfully resolved (entry 7). By recrystallization of the crude products, highly enantiopure amides (ee > 98%) were obtained. Compound **56** was resolved (entries 5 and 6)[84] under the same conditions and the benefit of added TEA on the reaction rate and selectivity was observed (entry 6). After hydrolysis of the amide product, (*R*)-**56** was applied in the synthesis of the most effective stereoisomer of formoterol.[84]

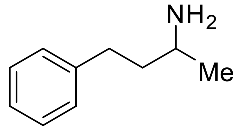
Table 4. Effects of the substituents on the CAL-B-catalysed *R*-selective acylation of 1-phenyl- and 1-phenoxypropan-2-amines and a butan-2-amine derivative in neat ethyl acetate at 30 °C



53: R = H
54: R = 2-OMe
55: R = 3-OMe
56: R = 4-OMe



57

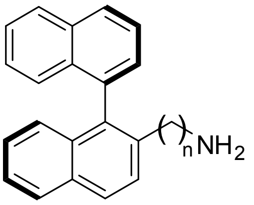


58

Entry	Substrate	*t* (h)	ee_s (%)	ee_p (%)	Conv. (%)	*E*
1[83]	**53**	7	73	89	45	37
2[83]	**54**	7	87	93	48	79
3[83]	**55**	7	87	92	49	68
4[83]	**56**	7	84	91	48	59
5[84]	**56**	15	68	84[b]	45	24
6[84]	**56**[a]	4	56	94[b]	42	66
7[83]	**57**	7	74	96	44	109
8[83]	**58**	7	98	78	56	36

[a] With 0.15 equivalent of TEA.
[b] ee of chiral amine after hydrolysis of the initially formed amide.

58 was applied as a model compound in a recent study[60] that focused on a comparison of carboxylic acids and their ethyl esters as acyl donors. The *R*-selective acylations were carried out by CAL-B catalysis in heptane at 80 °C. The selectivity was mediocre (*E* = 69) with ethyl acetate and high (*E* >> 200) in all the other cases. The reactions with carboxylic acids proceeded faster than those with the corresponding esters (50% conv. after 6 h and 3 h with ethyl laurate and lauric acid, respectively). Alkylamines and 1-arylalkylamines were acylated selectively (*E* >> 200) with lauric acid.[85]



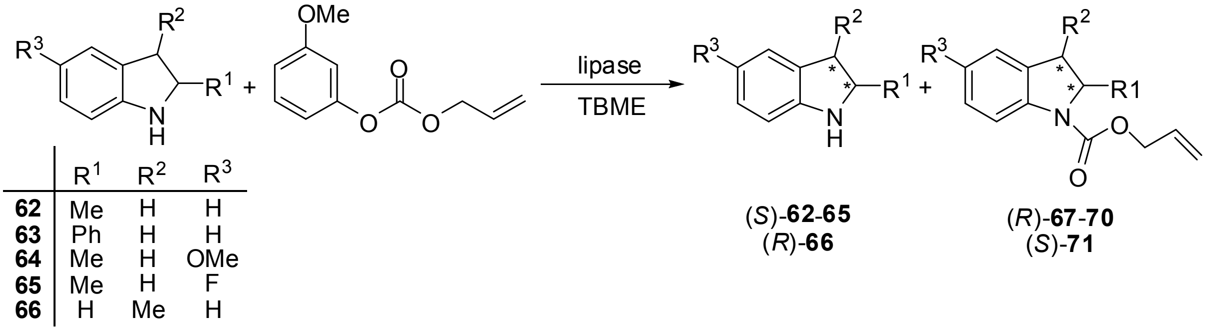
(±)-**59**: n = 0

(±)-**60**: n = 1

(±)-**61**: n = 2

Figure 3

1,1'-Binaphthylamine derivatives (**59-61,** Figure 3) are useful chiral ligands in asymmetric reactions. The acylation of **59** could not be performed under the conditions applied (1 equivalent of acyl donor in DIPE with 20 mg/mL enzyme at 30 °C), due to its bulkiness.[86] Compounds **60** and **61** were successfully resolved via PAL catalysis.[86] The enantioselective acylation of the sterically more hindered **60** requires 2,2,2-trifluoroethyl butanoate as acyl donor (*E* = 45), but for **61** ethyl butyrate seemed to be appropriate (*E* > 200).[86]

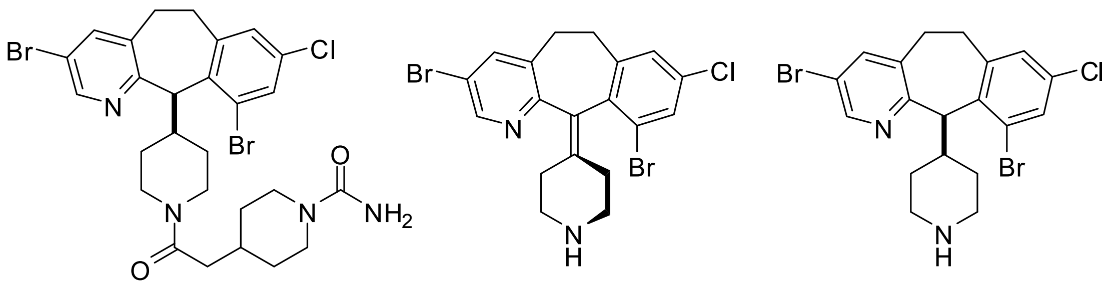


Scheme 2

The selective enzymatic acylation of cyclic secondary amines has been reported less frequently, even though pyrrolidines and piperidines are building blocks of many pharmaceuticals. The chiroCLEC-CR-catalysed acylation of 1-methyltetrahydroisoquinoline with diallyl carbonate in DIPE has been reported, but the allyl alcohol product inhibited further acylation.[87] The use of 3-methoxyphenyl allyl carbonate allowed the separation of the enantiomers (ee ≥ 98%).[87] The same acyl donor was used to resolve indolines (Scheme 2).[88] CAL-A was the appropriate biocatalyst for **62-65** (*E* > 200), but gave poor results with **66** (*E*

= 6), where the amine group is not attached directly to the asymmetric centre. In this case, a similarly high *S* selectivity (*E* > 200) was achieved with diallyl carbonate when CAL-A was replaced with CAL-B and the temperature was decreased from 45 °C to 30 °C.

In order to prepare SCH66336 (**72**), a farnesyl protease transferase inhibitor, enantiomerically pure intermediates are needed (Figure 4).[89,90] The existence of the enantiomers of **73** and **74** is due to the steric hindrance of the 10-bromo substituent. Extensive studies have been performed to find the optimum enzyme, solvent and acyl donor for the resolution of **73**. The best results (42% conv. after 23.5 h; *E* > 200) have been observed with LIP-300 with trifluoroethyl isobutyrate in TBME. The optimal resolution conditions for **74** were similar.



72

(+)-**73**

(*R*)-(+)-**74**

Figure 4

2.2.3. Possibilities of enantioselective amidation of amino alcohols and diamines by lipases

Amino alcohols are bifunctional compounds and both functional groups can behave as nucleophiles during lipase-catalysed acylation. The chemoenzymatic monoacylation of ω-amino-1-alkanols [$HO(CH_2)_nNH_2$, n = 3-6] has been studied.[91,92] The results clearly showed, that the product composition is determined by the nature of the acylating agent, the lipase and the rate of *O*→*N* acyl migration, which takes place in parallel with the preferred enzymatic *O*-acylation. Spontaneous intramolecular *O*→*N* acyl migration is common between 1,2- and 1,3-amino alcohols[93] and lipases can contribute in this process.[94,95] The chemo- and enantioselective acylation of amino alcohols is therefore a challenge. Only a few examples are to be found in the literature of the lipase-catalysed *N*-acylations of amino alcohols.[96,97] The chemoselectivity is due to the steric hindrance in propranolol resolved by CCL,[96] and the fast *O*→*N* acyl migration in the CAL-B-catalysed resolution of *trans*-2-aminocyclohexanol.[97] The highest *E* values achieved were 17 with isopropenyl acetate in DIPE[96] and 27 with dimethyl glutarate in 1,4-dioxane.[97] respectively. *O*-Protected 3-amino-3-phenylpropan-1-ol was resolved through CAL-A-catalysed *N*-acylation with ethyl methoxyacetate (E = 85),[98] yielding the chiral intermediate for the synthesis of (*S*)-dapoxetine.

The derivatives of enantiomeric *trans* alicyclic 1,2-diamines play important roles as intermediates of pharmaceuticals and can function as molecular receptors. Racemic *trans*-cyclohexane-1,2-diamine was resolved by CAL-B-catalysed aminolysis of dimethyl malonate,[99] affording the (*R*,*R*)-bis-amido ester (ee > 99), which was transformed to macrocyclic polyamines.[100] The corresponding cyclopentyl derivative can also undergo sequential resolution by the same catalytic system showing high apparent selectivity (E >200), and each step could be characterized by E (E_1 = 21, E_2 > 200).[101] The resulting bis-amido ester enantiomer was transformed to polyamine without any loss of enantiopurity.[101] *N*,*N*-Disubstituted *trans*-cyclopentane- (E = 20-86) and -cyclohexane-1,2-diamines (E > 200) have been resolved in neat ethyl acetate in the presence of CAL-B.[102,103] One of the resulting enantiomers is an intermediate of a κ-opioid analgesic.[103]

2.2.4. Lipase-catalysed enantioselective *N*-acylation of α-amino acid derivatives

α-Amino acids and their derivatives are of elemental importance in nature, and as building blocks of many pharmaceuticals, and they are also widely used in organic chemistry.[27] 1-Amino-1-phenylacetonitrile (**75**) was subjected to CAL-B (Novozym 435) -catalysed acylation with ethyl acetate in DIPE, when unexpected turnover-related racemization of the product took place (ee$_s$ = 50%, ee$_p$ = 10% at 50% conversion).[104] The racemization is presumably caused by alkaline sites in the zeolite in the enzyme preparation. The acylations of **76** and **77** with ethyl acetate displayed the same characteristics: after 56% and 76% conv., the products were practically racemic, while ee$_s$ was 46% in each case. During the acylation of **75** with ethyl phenylacetate, the starting material was racemized: ee$_s$ = 26%, ee$_p$ > 99% at 47% conv. This process could involve dynamic resolution; it was strongly *S* selective, but the production of benzaldehyde inhibited the enzyme.

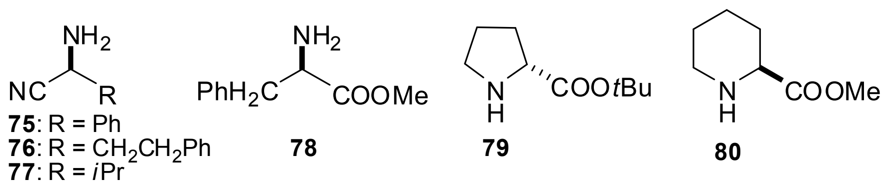
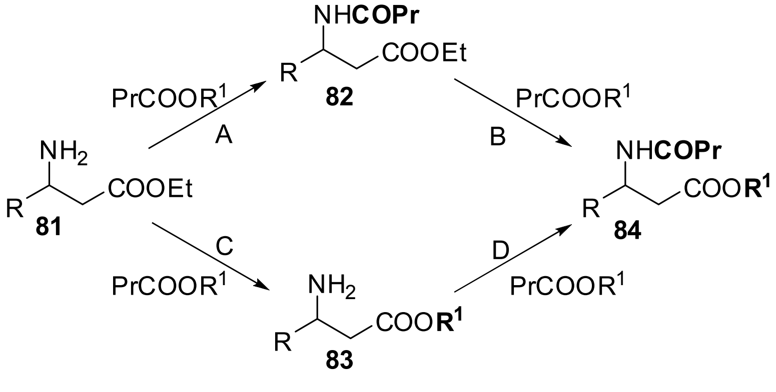


Figure 5. α-Amino acid derivatives resolved; the preferentially acylated enantiomers are shown.

For the resolution of α-amino acid esters, both chemo- and stereoselectivity are required.[105] In the acylation of **78** with ethyl methoxyacetate, strong *R* selectivity ($E > 200$) of CAL-B is observed.[106] In this case, the polar methoxycarbonyl group of the substrate is accommodated on an L subsite instead of the clearly bulkier benzyl group. A slightly enantioselective ($E = 4$) CAL-B-catalysed *N*-acylation of proline ester **79** with dibenzyl carbonate has been described.[29] These examples are exceptions to the observation that CAL-B particularly catalyses interesterification of the carbonyl group, whereas CAL-A preferentially acts on amino group.[107] CAL-A was utilized only rarely until the Kanerva group applied it in the enantioselective *N*-acylation of α- and β-amino acids and esters. The example of methyl pipecolinate **80** clearly illustrates the difference between the chemical preferences of these lipases.[108] *N*-Acylation with 2,2,2-trifluoroethyl butanoate in the presence of CAL-A leads to enantiopure products with strong *S* selectivity ($E > 100$). The transesterification by CAL-B exhibits opposite and low ($E = 2$-15) enantioselectivity.

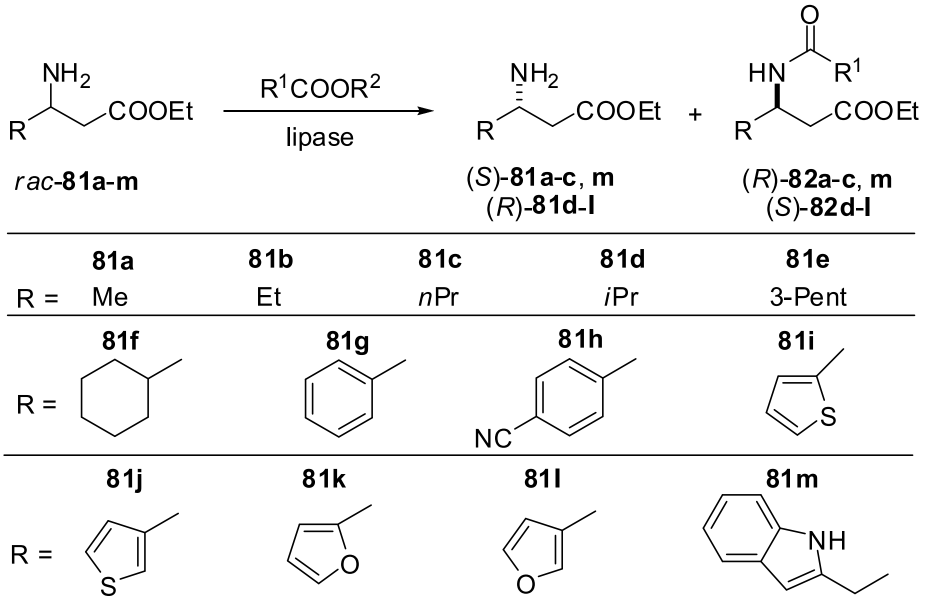
2.2.5. Chemo- and enantioselectivity of lipases towards β-amino esters

The great importance of β-amino acids and their derivatives has been discussed in the introduction of this thesis. The preparation of β-amino acid and ester enantiomers is of growing interest.[109] For example, the resolution of ethyl 3-aminobutyrate **81a** (R = Me) as a model compound has been studied in some detail (Scheme 3).[106,110,111]



Scheme 3

The preference of CAL-B as concerns competitive *N*-acylation and interesterification reactions depends on the nature of the acyl donor and R.[110] When ethyl butanoate was used as acyl donor, interesterification of **81a** was preferred (route C+D),[110] whereas when activated esters such as isopropyl methoxyacetate[106] and 2,2,2-trifluoroethyl butanoate[110] were applied, *N*-acylation (route A+B) was favoured. The sequential resolution of **81a** (R = Me) was successfully carried out in neat butyl butanoate, affording **81a**, **83a** and **84a** with high ee (> 95%).[110] The increasing size of R favours interesterification (C+D), and makes *N*-acylation impossible; other β-amino esters were therefore resolved through *N*-acylation by CAL-A, and lipase PS catalysis.[111] In this way, a large number of β^3-amino esters have been resolved (Scheme 4, Table 6).



Scheme 4. *N*-Acylation of β^3-amino esters.

In a systematic study with **81a-g**, 2,2,2-trifluoroethyl butanoate (in DIPE) and neat butyl butanoate were tested with CAL-A.[112] The reaction rate was higher with the activated ester. In accordance with the enantioselectivities, **81d-g**[112] were resolved with trifluoroethyl butanoate in DIPE, and **81b-c**[112] and 4-cyano derivative **81h**[113] in neat butyl butanoate. The resulting enantiomers of **81h** were transformed to their *N*-protected (Boc or Fmoc) derivatives.[113] Heteroaromatic substrates **81i-l** were resolved with high enantioselectivities in neat ethyl butanoate.[114] β-Tryptophan ethyl ester **81m** and its nitrile derivative **81n** were acylated with butyl butanoate.[115]

Table 6. CAL-A-catalysed *N*-acylation of aliphatic and aromatic β^3-amino esters.

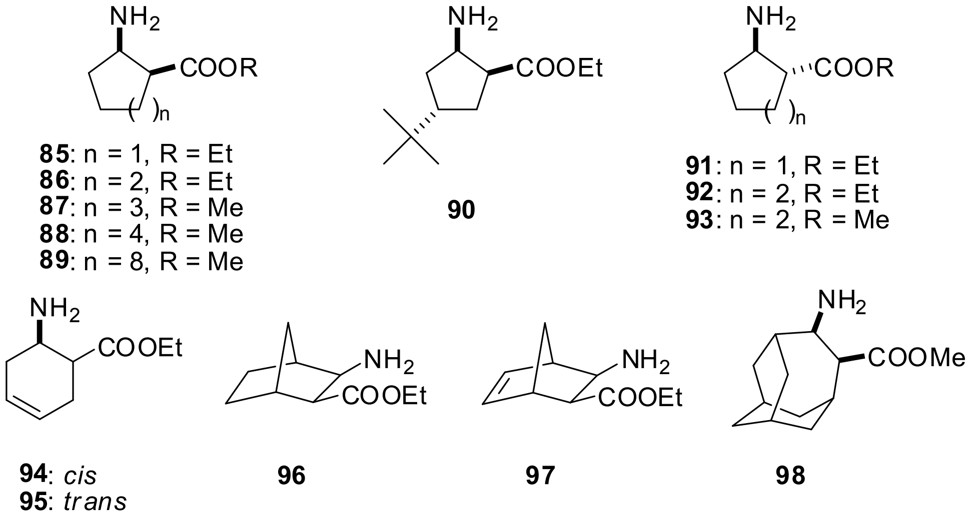
Ref.	Substrate	Acyl donor	Solvent	*t* (h)	Conv. (%)	*E*
112	**81a**	$PrCOOCH_2CF_3$	DIPE	25	50	6
112	**81a**	PrCOOBu	PrCOOBu	22	51	32
112	**81b**	$PrCOOCH_2CF_3$	DIPE	1.33	52	168
112	**81b**	PrCOOBu	PrCOOBu	24	50	>200
112	**81c**	$PrCOOCH_2CF_3$	DIPE	1.5	55	72
112	**81c**	PrCOOBu	PrCOOBu	9	50	>100
112	**81d**	$PrCOOCH_2CF_3$	DIPE	1.5	49	106
112	**81d**	PrCOOBu	PrCOOBu	130	50	115
112	**81e**	$PrCOOCH_2CF_3$	DIPE	4	52	38

Table 6. (Continued) CAL-A-catalysed *N*-acylation of aliphatic and aromatic β^3-amino esters.

Ref.	Substrate	Acyl donor	Solvent	*t* (h)	Conv. (%)	*E*
112	**81e**	PrCOOBu	PrCOOBu	16 days	33	2
112	**81f**	$PrCOOCH_2CF_3$	DIPE	1	53	>100
112	**81f**	PrCOOBu	PrCOOBu	48	23	9
112	**81g**	$PrCOOCH_2CF_3$	DIPE	0.33	52	75
112	**81g**	PrCOOBu	PrCOOBu	70	52	29
113	**81h**	PrCOOBu	PrCOOBu	9	50	143
114	**81i**	PrCOOEt	PrCOOEt	3	50	305
114	**81j**	PrCOOEt	PrCOOEt	4	48	220
114	**81k**	PrCOOEt	PrCOOEt	3	48	150
114	**81l**	PrCOOEt	PrCOOEt	2	47	460
115	**81m**	PrCOOBu[b]	DIPE[b]	16	50	>200
115	**81n**[a]	PrCOOBu[b]	DIPE[b]	2	49	>200

[a] Nitrile derivative of **81m**

A comprehensive study has been carried out on the resolution of a set of $\beta^{2,3}$-amino esters (**85,86**, **90-92**, **94-97,** Figure 6, Table 7) with different 2,2,2-trifluoroethyl esters by CAL-A and lipase PS catalysis.[116] In general, lipase PS displays excellent *E* towards *trans* compounds, while CAL-A preferentially catalyses the *N*-acylation of *cis* compounds, **85** being the only exception. Most gram-scale resolutions have been performed with 2,2,2-trifluoroethyl chloroacetate with lipase PS catalysis. The exceptions **90** and **96** were resolved with 2,2,2-trifluoroethyl hexanoate by CAL-A catalysis. With further increase of the ring size in *cis* alicyclic 1,2-amino esters, slower reactions and high enantioselectivities were observed.[117] Amino ester **93** is the only example of CAL-B-catalysed *N*-acylation among this kind of substrates.[106] CAL-A displayed strong *S* selectivity towards the bulky **98** in an *N*-acylation reaction,[118] illustrating the role of the polarity in enantiorecognition.



85: n = 1, R = Et
86: n = 2, R = Et
87: n = 3, R = Me
88: n = 4, R = Me
89: n = 8, R = Me

90

91: n = 1, R = Et
92: n = 2, R = Et
93: n = 2, R = Me

94: *cis*
95: *trans*

96

97

98

Figure 6. Cyclic amino esters resolved; the preferentially acylated enantiomers are shown.

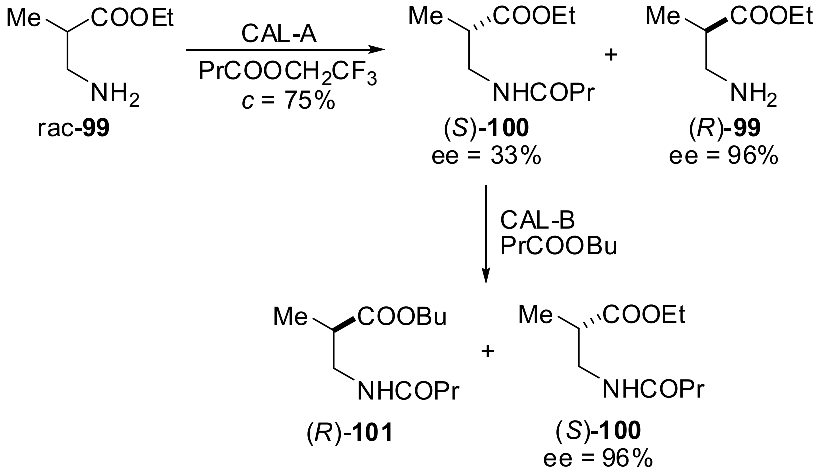
The practical usefulness of lipase-catalysed *N*-acylation was proved by the two-step synthesis of a number of β-dipeptides.[119] Trifluoroethyl *N*-acetyl β-amino ester enantiomers were prepared by CAL-B-catalysed interesterification and were used as acyl donors in the presence of CAL-A in the enantioselective *N*-acylation of other β-amino esters.

Table 7. Lipase-catalysed *N*-acylation of $\beta^{2,3}$-amino esters.

Ref.	Substrate	Lipase	Acyl donor	Solvent	*t* (h)	Conv.(%)	*E*
114	**85**	CAL-A	$PrCOOCH_2CF_3$	Et_2O	0.5	54	47
114	**85**	lipase PS	$ClCH_2COOCH_2CF_3$	Et_2O	5 min	51	>>200
114	**86**	CAL-A	$PrCOOCH_2CF_3$	Et_2O	30	40	>100
114	**86**	lipase PS	$ClCH_2COOCH_2CF_3$	Et_2O	5 min	49	53
115	**87**	CAL-A	$PrCOOCH_2CF_3$	DIPE	1	48	>200
115	**88**	CAL-A	$PrCOOCH_2CF_3$	DIPE	1	33	170
115	**89**[a]	CAL-A	$PrCOOCH_2CF_3$	DIPE	24	50	>200
114	**90**	CAL-A	$PentCOOCH_2CF_3$	Et_2O	3.3	50	>>200
114	**91**	lipase PS	$ClCH_2COOCH_2CF_3$	Et_2O	20 min	57	>>200
114	**92**	lipase PS	$ClCH_2COOCH_2CF_3$	Et_2O	4 min	50	>>200
103	**93**	CAL-B	isopropyl methoxyacetate	TBME	8	50	>>200
114	**94**	CAL-A	$MeCOOCH_2CF_3$	TBME	n.d.	n.d.	41
114	**94**	lipase PS	$ClCH_2COOCH_2CF_3$	Et_2O	15 min	48	40
114	**95**	lipase PS	$ClCH_2COOCH_2CF_3$	Et_2O	40 min	50	>>200
114	**96**	CAL-A	$PentCOOCH_2CF_3$	Et_2O	1	50	>>200
114	**97**	lipase PS	$ClCH_2COOCH_2CF_3$	Et_2O	30 min	51	79
116	**98**	CAL-A	$PrCOOCH_2CF_3$	DIPE	10	50	>>200

[a] T = 47 °C.

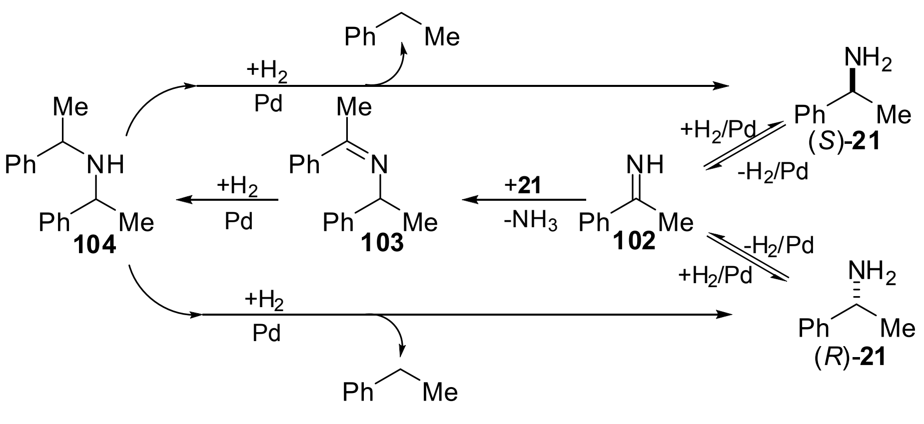
Prior to our work, α-methyl-β-alanine ethyl ester (**99**) was the only β^2-amino ester, that was resolved by *N*-acylation in a two-step protocol (Scheme 5).[120] The CAL-A-catalysed *N*-acylation and the subsequent CAL-B-catalysed transesterification reactions were investigated. The low and opposite enantioselectivities for the above-mentioned two reaction steps allowed gram-scale resolution. The unreacted (*R*)-**99** was separated as the Boc-protected derivative. The enantiomerically enriched **100** was subjected to the second step, leading to (*S*)-**100** (ee = 96%).



Scheme 5

2.3. *Dynamic kinetic resolution of amines, possibilities of amine racemization*

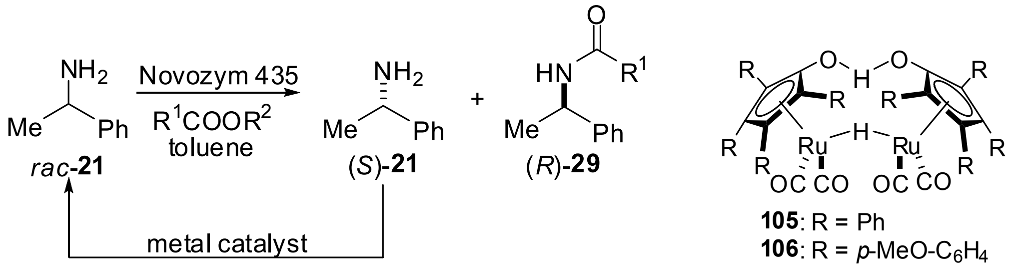
Enzymatic dynamic kinetic resolution methodology has rarely been applied to amines. The non-catalysed racemization of amines usually requires harsh conditions which can denature enzymes, and on the other hand the formation of side-products is possible.[121] Of the known racemization methods,[121] reversible hydrogen transfer and Schiff base-mediated procedures are compatible with lipases. The redox procedures are catalysed by transition metals or organocatalysts.[39,40,44,122,123] The formation of Schiff bases requires the presence of an aldehyde or ketone and an acidic proton at α position of the substrate.[43] Since higher temperature assists the racemization, immobilized lipases (mostly Novozym 435) are used as catalysts in dynamic kinetic resolution to avoid denaturation. The most widely studied substrate is **21**; the dynamic kinetic resolution methods elaborated have been successfully extended to other substrates.



Scheme 6

In the majority of the cases, the reversible hydrogen transfer is catalysed by transition metals or their complexes. Scheme 6 shows the mechanism of the dynamic kinetic resolution on the example of a Pd catalyst, including the formation of intermediates/side-products. Reetz and Schimossek[124] first used Pd-catalysed racemization in the dynamic kinetic resolution of **21**. The reaction was performed in TEA at 50-55 °C, resulting in 64% (isolated yield) *R*-amide (ee = 99%) after 8 days, while 10% Pd/C catalysed the resolution and Novozym 435 (CAL-B) the enantioselective *N*-acylation with ethyl acetate. Some other results with **21** are also listed in Table 8. The De Vos group studied the effects of alkaline earth supports ($BaSO_4$, $BaCO_3$, $SrCO_3$ and $CaCO_3$)[125] on the activity of Pd, Pd/$BaSO_4$ exhibiting the best results (entry 1), even compared with other transition metals (Rh, Ru, Pt and Ir).[126] With Pd on an AlO(OH) support, high yields and enantioselectivities were obtained (entry 2).[127] To improve the performance of the Pd catalyst, microwave irradiation was used as heating source.[128] The Pd/$CaCO_3$-catalysed racemization was faster and less by-product was achieved when the reaction was carried out under microwave conditions (entry 3 vs. 4). Pámies *et al.* investigated different Ru catalysts in the racemization of primary and secondary amines. The addition of ammonia and a hydrogen donor inhibited the formation of side-products during the racemization process with the Shvo's catalyst (**105**),[129] which was performed after the kinetic resolution step. After the second kinetic resolution, moderate yields were obtained (entry 5). **106** catalysed the racemization in a one-pot dynamic kinetic resolution with isopropyl acetate (entry 6).[130] This system was improved by adding 2,4-dimethyl-3-pentanol (a hydrogen donor) with the use of isopropyl methoxyacetate (entry 7).[131] Dibenzyl carbonate can also be used as an acylating agent in dynamic kinetic resolution (entry 8).[132] The widely used hydrogenation catalysts Raney Ni and Co proved to be effective racemization catalysts in one-pot dynamic kinetic resolution (entries 9-10).[133] Pentamethylcyclopentadienyl-iridium(III) iodine dimer, $[IrCp^*I_2]_2$ successfully catalysed the racemization of 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline.[134,135] The substrate was resolved through CRL-catalysed acylation with 3-methoxyphenylpropyl carbonate in toluene. The carbamate formed was achieved with high enantiopurity (ee = 96%) and isolated yield (82%). 1-Methyl-1,2,3,4-tetrahydroisoquinoline was previously kinetically resolved with 3-methoxyphenyl allylcarbonate under similar conditions.[87]

Table 8. Dynamic kinetic resolution of 1-phenylethylamine (**21**) based on reversible hydrogen transfer catalysed by transition metals.



Entry	Metal catalyst	Acyl donor	*T* (°C)	*t* (h)	Yield (%)	ee (%)
1	5% Pd/$BaSO_4$	isopropyl acetate	70	24	86	>99
2	5% Pd/AlO(OH)	ethyl methoxyacetate	70	72	98	>99
3	5% Pd/$CaCO_3$ (microwave)	ethyl methoxyacetate	100	50 min	87	97
4	5% Pd/$CaCO_3$	ethyl methoxyacetate	100	50 min	59	99
5	**105**	MeCOOEt	40/110[a]	n.d.	69	98
6	**106**	isopropyl acetate	90	72	90	98
7	**106**	isopropyl methoxyacetate	100	26	68	98
8	**106**	dibenzyl carbonate	90	72	90	93
9	Raney Co	isopropyl acetate	70	72	63	>99
10	Raney Ni	methyl decanoate	80	120	61	94

[a] T of resolution/racemization step.

Thyol additives can also mediate the racemization of amines via reversible hydrogen transfer. The dynamic kinetic resolution of **58** was carried out in heptane with ethyl laurate by CAL-B catalysis.[136] *N*,*N*-Diethyl-2-mercaptopropanamide proved to be the most effective racemizing agent for arylalkylamines (isolated yield: 70%; ee = 99%).

The aldehyde-promoted racemization of proline methyl ester has recently been reported.[137] The dynamic kinetic resolution method was based on the highly enantioselective CAL-A-catalysed kinetic resolution with VB. The acetaldehyde formed mediated the racemization via the Schiff base. Of the additives tested, TEA used in the gram-scale dynamic kinetic resolution caused an appreciable enhancement in the yield of the product *S*-amide (86% instead of 75%; ee = 97%). The dynamic kinetic resolution was performed under mild conditions: in TBME at room temperature.

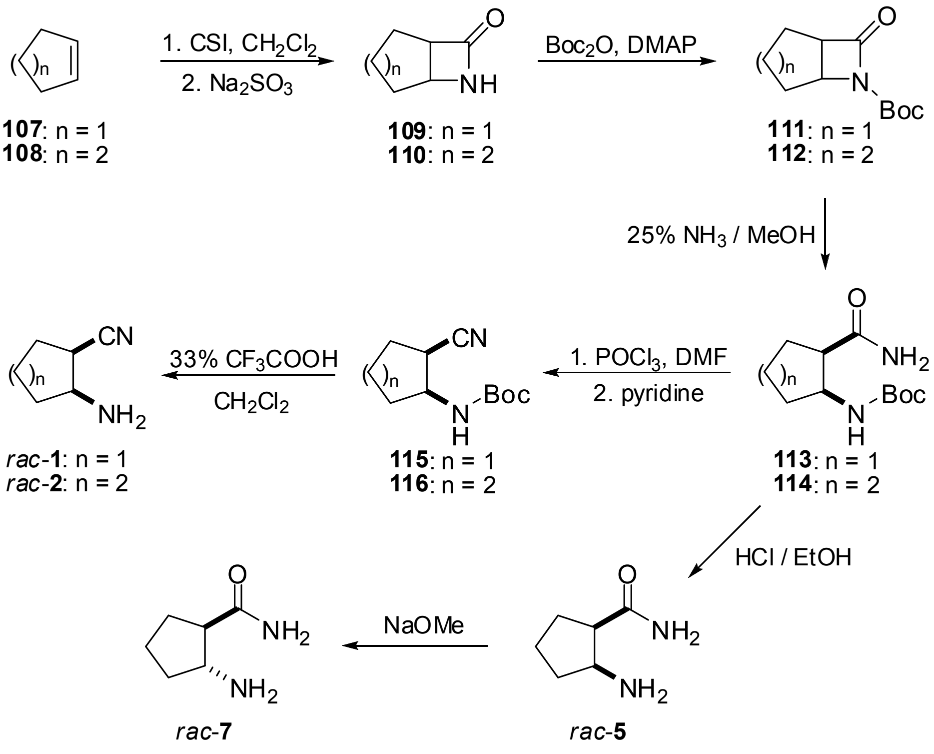
Finally, a special example of enzymatic dynamic kinetic resolution should be mentioned; that of **38** was succesfully performed with ethyl acetate by CAL-B catalysis in toluene.[138] The spontaneously generated product ketone and the less reactive *S*-amine form an enamine, and the subsequent hydrolysis of this dimer provides a great racemization pathway. The enantiomerically enriched *R*-acetamide (ee > 98%) was achieved in good yield (78%) after 48 h at 50 °C.

3. Materials and methods

3.1. *Synthesis of racemic model compounds*

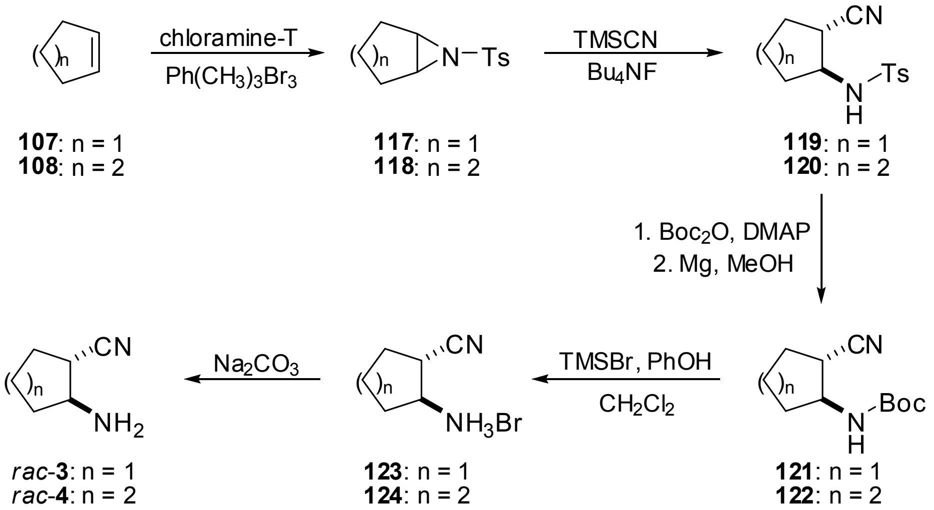
3.1.1. Preparation of racemic *cis* and *trans* amino nitriles *rac*-1-*rac*-4

The cycloaddition of chlorosulfonyl isocyanate to cycloalkenes **107** and **108** afforded alicyclic β-lactams (**109** and **110**).[139-141] The β-lactam ring could be easily opened after the preliminary *N*-Boc protection through ammonolysis, as described for the preparation of **113**.[142] Dehydration of the resulting Boc-protected amide (**113** and **114**) with phosphorus oxychloride[143] and subsequent deprotection with 2,2,2-trifluoroacetic acid, furnished amino nitriles *rac*-**1** and *rac*-**2** (Scheme 7).



Scheme 7

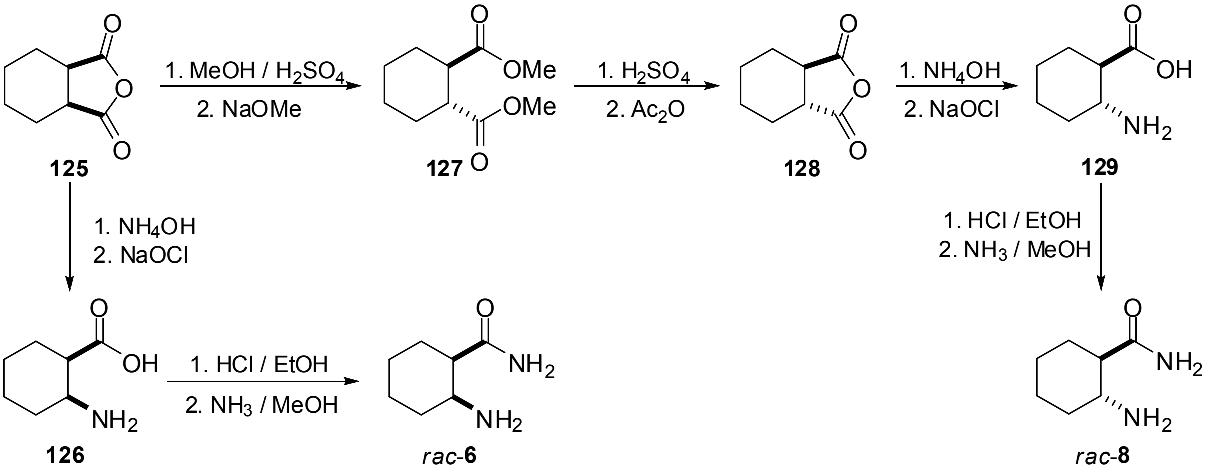
The syntheses of substrates *rac*-**3** and *rac*-**4** are depicted in Scheme 8. Catalytic aziridation of **107** and **108** with chloramine-T, followed by ring opening of the obtained *N*-Ts-aziridines **117** and **118** with TMSCN under tetrabutylammonium fluoride catalysis by a known method, resulted in *N*-Ts-amino nitriles **119** and **120**.[144,145] Use of the Ts protecting group instead of Boc was necessary, since the ring opening of the corresponding *N*-Bocaziridines was incomplete.[145] The Ts group was then changed to Boc within two steps. Removal of the Ts group by the known method was possible with good yields.[146] The Boc rotection was removed from **121** and **122** with bromotrimethylsilane and phenol, and *rac*-**3** and *rac*-**4** were liberated from hydrobromides **123** and **124**.



Scheme 8

3.1.2. Synthesis of racemic β-amino carboxamides *rac*-5-*rac*-8

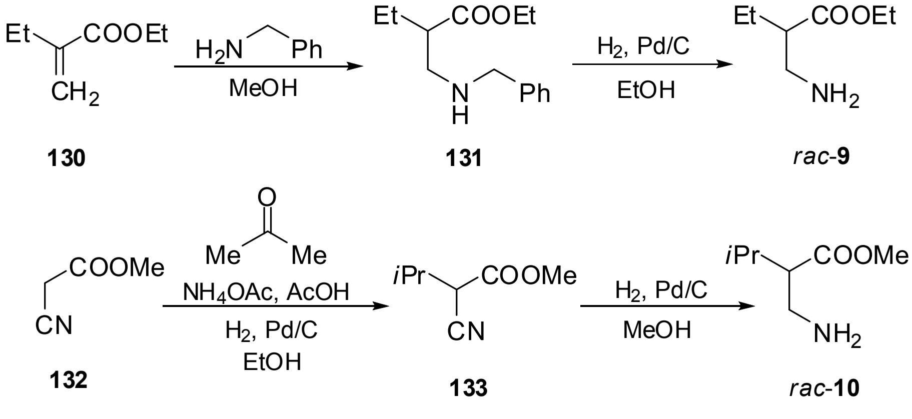
Compounds *rac*-**5**-*rac*-**8** were prepared as reported earlier.[142,147,148] The removal of the Boc protecting group from **113** afforded *rac*-**5**.[142] The isomerization of *rac*-**5** with NaOMe under heating resulted in a *rac*-**7**/*rac*-**5** (9/1) mixture.[142] The hydrochloride of this mixture was prepared; **7**·HCl was separated by recrystallization, and the base was liberated[142] (Scheme 7). The amidation of *cis*-1,2-cyclohexanedicarboxylic anhydride **125**, followed by Hoffman degradation, gave *cis*-2-aminocyclohexanecarboxylic acid **126**.[147] This was converted to its ethyl ester, and the addition of methanolic ammonia afforded *rac*-**6**.[148] *cis*-1,2-Cyclohexanedicarboxylic anhydride **125** was transformed into its *trans* isomer **128** in a small number of steps: esterification, isomerization and subsequent ring closure. *trans*-1,2-Cyclohexanedicarboxylic anhydride **128** was transformed to *rac*-**8** by the procedure described above for *rac*-**6**[148] (Scheme 9).



Scheme 9

3.1.3. Preparation of racemic β-amino acid esters *rac*-**9** and *rac*-**10**

Racemic model compounds *rac*-**9** and *rac*-**10** were prepared by applying literature procedures explored for the synthesis of α-substituted β-amino esters (Scheme 10).[114,149,150] The addition of benzylamine and ethyl ethacrylate **130**[151] afforded the *N*-benzylamino ester **131**, which was deprotected by catalytic hydrogenolysis to give ethyl 3-amino-2-ethylpropanoate **9**. Methyl cyanoacetate **132** and acetone were condensed under reductive conditions to yield the α-substituted malonitrile **133**.[152] This intermediate was reduced by catalytic hydrogenation, affording methyl 3-amino-2-isopropylpropanoate *rac*-**10**.



Scheme 10

3.2. *Small-scale experiments*

In a typical small-scale experiment, a racemic amino nitrile, amino carboxamide or amino ester was dissolved in an appropriate solvent at a concentration of 0.05 M (1 mL). The appropriate amount of enzyme was added. When the acylating agent was different from the solvent, the reaction was initiated by the addition of 0.55, 1 or 2 equivalents of the appropriate ester. The reaction mixtures were shaken at the selected temperature in well-sealed vials.

The progress of the reactions and the ee values of both the substrates and products were monitored by taking samples (0.05 mL) at intervals, filtering off the enzyme and analysing them by GC on a Chrompack CP-Chirasil-DEX CB column (25 m). In the case of ionic liquids, the products were extracted into TBME before analysis. For good baseline separation, the unreacted amino group was acylated with an appropriate acid anhydride in the presence of pyridine containing 1% DMAP. Since the enantiomers of *rac*-**9** could be separated only as the trifluoroacetamide, but not as the *N*-acetyl, *N*-chloroacetyl, *N*-propionyl or *N*-butyryl derivatives, octadecane was used as internal standard to follow the *N*-acylation of this substrate. The determination of the enantioselectivity was based on the equation $E = \ln[(1-ee_s)/(1+ee_s/ee_p]/\ln[(1+ee_s)/(1+ee_s/ee_p)]$ with *conv.* = $ee_s/(ee_s+ee_p)$, as derived from the original equations of Chen *et al.*, where ee_s (enantiomeric excess of the substrate) and ee_p (enantiomeric excess of the product) were determined by GC with a chiral column.[51]

3.3. *Biocatalysts, reagents and solvents*

CAL-A (*Candida antarctica* lipase A, Chirazyme L5) was purchased from Roche, and the immobilized CAL-B (*Candida antarctica* lipase B, Chirazyme L2) preparations, Novozym 435 and lipolase from Novozym and Sigma Aldrich, respectively. Lipases PS (from *Burkholderia cepacia*, formerly *Pseudomonas cepacia*) and AK (*Pseudomonas fluorescens*) were products of Aldrich, while lipase PS-C II (an immobilized preparation of lipase PS) was from Amano Europe, England. Porcine pancreatic lipase (PPL) and *Candida rugosa* lipase (CRL) were purchased from Sigma, and lipase AY (*Candida rugosa*) from Amano pharmaceuticals. Before use, CAL-A and lipases PS, AK and AY were adsorbed on Celite in the presence of sucrose.[153] 2,2,2-Trifluoroethyl butanoate and chloroacetate were prepared from 2,2,2-trifluoroethanol with butanoyl chloride and chloroacetyl chloride, respectively. Ethyl and butyl butanoates were from Sigma-Aldrich, and VA and VB were from Fluka. The organic solvents used were of the highest analytical grade. [BMPy][BF_4] (1-butyl-4-methylpyridinium tetrafluoroborate) and [EMIM][TfO] (1-ethyl-3-methylimidazolium trifluromethane sulfonate) were products of Fluka, while the other ionic liquids were prepared by literature methods.[154-156]

4. Results and discussion

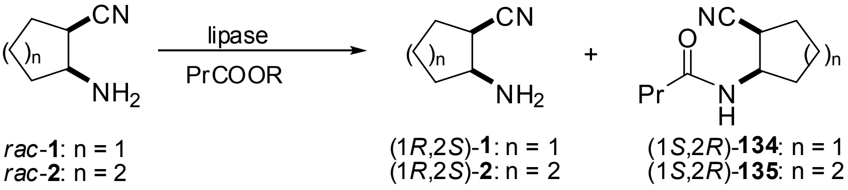
4.1. *Resolution of alicyclic β-amino nitriles and β-amino carboxamides*[I],[II]

Alicyclic β-amino acids were successfully resolved in their ester form,[116] while their intermediates, the corresponding β-amino nitriles and amino carboxamides, have been less extensively examined.

4.1.1. Small-scale experiments with amino nitriles *rac*-1-*rac*-4[I]

Selected enzymes were screened for the acylation of *rac*-**1**-*rac*-**4** with 2,2,2-trifluorethyl butanoate. In previous work, CAL-A and lipase PS were the most enantioselective enzymes towards the corresponding alicyclic *cis*- and *trans*-β-amino esters (**85**, **86** and **91**, **92**), respectively.[116] CAL-B was also selected for testing, although it is rarely used for the resolution of amino esters through *N*-acylation, due to its ability to act on both functional groups, but it proved to be an effective catalyst in the acylation of various amines.

Prior to the enzymatic reaction, the rate of the chemical reaction was investigated: 0.05 M *rac*-**1** was dissolved in TBME, 2 equivalents of 2,2,2-trifluorethyl butanoate was added, and the reaction mixture was shaken at room temperature. After 24 h of shaking, the analysis showed the presence of 0.8% of *N*-acylated product. The enzymatic reactions of substrates *rac*-**1** and *rac*-**2** were started under the above-mentioned conditions in the presence of lipase PS, CAL-A and CAL-B preparations, and for *rac*-**2** the reactions in neat ethyl butanoate were also studied (Scheme 11, Tables 9 and 10).



Scheme 11

Table 9. Lipase screening for the enantioselective acylation of *rac*-**1** (0.05 M) in TBME with 2 equivalents of 2,2,2-trifluorethyl butanoate in the presence of different enzyme preparations (50 mg/mL) at rt (25 °C)

Entry	Lipase	*t* (h)	Conv. (%)	*E*
1	lipase PS	0.5	50	>>200
2	lipase PS-C II	2	51	>200
3	CAL-A	0.5	83	2
4	CAL-B	1	50	>200

With lipase-PS preparations (immobilized on Celite, and PS-C II on ceramic particles), excellent enantioselectivities were observed (Table 9, entries 1 and 2; Table 10, entries 2 and 3). CAL-A resulted in a fast reaction without useful enantioselectivity (Table 9, entry 3; Table 10, entry 4), and it could not be improved by change of the solvent. CAL-B demonstrated good (Table 10, entry 5) to excellent (Table 9, entry 4) enantioselectivities. When the acylation reactions were performed in neat ethyl butanoate, the enzymes lost their enantioselectivity and the reactions proceeded slowly (Table 10). On the basis of the results obtained, the lipase PS-C II-catalysed acylation of *rac*-**2** with 2,2,2-trifluoroethyl butanoate was chosen for further optimization.

Table 10. Lipase screening for the enantioselective acylation of *rac*-**2** with PrCOOR in TBME at rt (25 °C)

Entry	Lipase	R = Et[a]			R = CH_2CF_3[b]		
		t (h)	Conv. (%)	*E*	*t* (h)	Conv. (%)	*E*
1	lipase AK	40	33	1.3	6	12	30
2	lipase PS	40	49	3.2	4	43	>200
3	lipase PS-C II	–	–	–	4	48	>200
4	CAL-A	–	–	–	0.5	83	2
5	CAL-B	40	62	9	6	48	79
6	PPL	40	29	1.1	–	–	–
7	CRL	40	39	1.2	–	–	–

[a] Reaction of *rac*-**2** (0.1 M) in neat ethyl butanoate in the presence of the enzyme preparation (100 mg/mL).

[b] Reaction of *rac*-**2** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) in TBME in the presence of the enzyme preparation (50 mg/mL).

Significant differences were observed for the enantioselectivities of these two enzymes towards *cis* amino nitriles[I] and the corresponding amino esters (Table 11).[116]

Table 11. Enantioselective *N*-acylations of ethyl *cis*-2-aminocyclopentane- (**85**) and -cyclohexane (**86**) carboxylates and their nitrile derivatives *rac*-**1** and *rac*-**2** with $RCOOCH_2CF_3$ by lipase catalysis

Entry	Ref.	Substrate	Acyl donor R	Solvent	*E* lipase PS	*E* CAL-A
1	116	**85**	Me	Et_2O	>>100[a]	31
2	116	**85**	Me	TBME	>100[a]	37
3	I	**1**	Pr	TBME	>200[a]/>200[b]	2
4	116	**86**	Me	Et_2O	6[a]	51
5	116	**86**	Pr	Et_2O	2[a]	>>100
6	116	**86**	Me	TBME	7[a]	57
7	I	**2**	Pr	TBME	>200[a]/>200[b]	2

[a] Lipase PS immobilized on Celite.
[b] Lipase PS-C II.

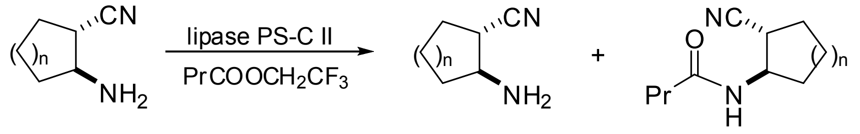
Different enzyme amounts were studied because with 50 mg/mL lipase the otherwise fast reaction tended to slow down at close to 50% conversion (Table 12). A higher reaction rate (about 2 times) was observed with excellent enantioselectivity when the enzyme amount was raised from 25 mg/mL to 75 mg/mL (Table 12, entries 2 and 6).

Table 12. Effect of the amount of lipase PS-C II on the enantioselective acylation of *rac*-**2** (0.05 M) with 2,2,2-trifluoroethyl butanoate (2 equiv) in TBME at rt (25 °C)

Entry	Enzyme (mg/mL)	*t* (h)	Conv. (%)	ee_s (%)[a]	ee_p (%)[b]	*E*
1	25	2	42	70	99	>200
2	25	8	50	96	97	>200
3	50	2	46	83	98	>200
4	50	6	49	92	97	>200
5	75	2	49	93	98	>200
6	75	4	50	97	96	>200

[a] According to GC after derivatization with acetic anhydride in the presence of DMAP and pyridine.
[b] Determined by GC.

Small-scale experiments with *rac*-**3** and *rac*-**4** were started with 2 equivalents of 2,2,2-trifluoroethyl butanoate in the presence of 50 mg/mL lipase PS-C II at room temperature (Scheme 12, Table 13). The only difference was that *rac*-**3** is not soluble in TBME and for this reason a co-solvent was added to the TBME. Excellent enantioselectivities were observed for lipase PS-C II-catalysis in all cases (Table 13, entries 1-5) as expected on the basis of the results with the corresponding β-amino esters.[116] The added TAA lowered the reaction rate in accordance with the known lipase inhibitor effect of the alcohols (Table 13; entry 3 vs entry 4).



rac-**3**: n = 1
rac-**4**: n = 2

(1*S*,2*S*)-**3**: n = 1
(1*S*,2*S*)-**4**: n = 2

(1*R*,2*R*)-**136**: n = 1
(1*R*,2*R*)-**137**: n = 2

Scheme 12

Table 13. Lipase PS-C II (50 mg/mL)-catalysed acylation of *rac*-**3** (0.05 M) and *rac*-**4** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) in TBME after 24 h

Entry	Substrate	Solvent	*T* (°C)	ee_s (%)[a]	ee_p (%)[b]	Conv. (%)	*E*
1	*rac*-**3**	TBME/TAA (8/2)	23	94	>99	49	>>200
2	*rac*-**3**	TBME/TAA (9/1)	23	>99	>99	50	>>200
3	*rac*-**4**	TBME/TAA (9/1)	23	20	>99	17	>>200
4	*rac*-**4**	TBME	23	43	>99	30	>>200
5	*rac*-**4**	TBME	65	47	>99	32	>>200

[a] According to GC after derivatization with acetic anhydride in the presence of DMAP and pyridine.
[b] Determined by GC.

Ionic liquids can serve as alternatives to conventional organic solvents. We decided to study the CAL-B (Novozym 435)-catalysed acylation of *rac*-**2** in such solvents and compare the results with those for TBME (Table 14).

Novozym 435 catalysed this reaction with good enantioselectivity in TBME (Table 10, entry 5). Different enzyme amounts were applied, and it was found that 30 mg/mL or more enzyme content had no effect on the reactivity (Table 14, entries 1-5). The ionic liquids investigated (Figure 7) were selected according to previous studies.[157] The results obtained (Table 14, entries 6-12) did not fulfil our expectations. The reactions were much slower than in TBME, and on the other hand the selectivities were lower; only in EMIM·TfO was a similar value reached as in TBME (Table 14, entry 11), and with the addition of TBME we could not achieve a useful resolution procedure (Table 14, entry 12).

[EMIM][BF$_4$]: R = Et, X = BF$_4$
[BMIM][PF$_6$]: R = Bu, X = PF$_6$
[EMIM][NTf$_2$]: R = Et, X = (CF$_3$SO$_2$)$_2$N
[EMIM][TfO]: R = Et, X = CF$_3$SO$_3$

[BMPyr][BF$_6$]: R = Bu, X = BF$_4$

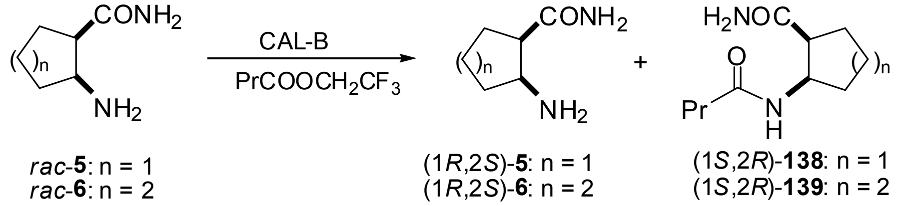
Figure 7

Table 14. Comparison of ionic liquids with TBME in the Novozym 435-catalysed acylation of *rac*-**2** with 2,2,2-trifluoroethyl butanoate at rt (25 °C)

Entry	Solvent	CAL-B (mg/mL)	*t* (h)	Conv. (%)	*E*
1	TBME	10	6	35	57
2	TBME	20	6	45	63
3	TBME	30	6	48	74
4	TBME	50	6	48	79
5	TBME	70	6	48	73
6	BMIM·PF_6	50	96	11	14
7	BMPyr·BF_4	50	96	32	24
8	EMIM·BF_4	50	96	9	12
9	EMIM·NTf_2	50	96	46	22
10	EMIM·TfO	50	96	23	51
11	EMIM·TfO	75	96	26	71
12	EMIM·TfO/TBME	75	96	46	89

4.1.2. Small-scale experiments with amino carboxamides *rac*-5-*rac*-8[III]

For the resolution of alicyclic β-amino carboxamides (*rac*-**5**-**8,** Schemes 13 and 14), the lipases successfully used for the corresponding β-amino carboxylates (**85**, **86**, **91** and **92**)[116] and for alicyclic β-amino nitriles (*rac*-**1**-**4**)[I] in our previous work were tested. In all these examples, the enzymes displayed *R* selectivity.



Scheme 13

The CAL-B-catalysed *N*-acylation of *rac*-**5** proceeded with good to excellent (*E* = 108; *E* > 200; Table 15, entries 1-4) *R* enantioselectivities, whereas in the case of *rac*-**6** the same *R*, but lower (*E* = 8-61; Table 16, entries 1-6) selectivities were observed. In contrast with our expectations, lipase PS preparations and CAL-A (except for lipase PS in the case of *rac*-**5**) yielded low *S* enantioselectivities (Table 15, entry 7; Table 16, entries 7-9). Accordingly, further preliminary experiments were continued with CAL-B.

Table 15. *N*-Acylation of *rac*-**5** (0.05 M) with 2,2,2-trifluoroethyl butanoate (2 equiv) and lipase preparations (50 mg/mL) at 48 °C after 1 h

Entry	Enzyme	Solvent	Conv. (%)	ee_s (%)[a]	ee_p (%)[b]	*E*
1	CAL-B	TBME/TAA (1/1)	46	83	98	>200
2	CAL-B	TBME/TAA (3/1)	50	96	98	>200
3	CAL-B	TBME/TAA (4/1)	50	97	97	>200
4	CAL-B	TBME/MeCN (1/1)	47	85	95	108
5	lipase PS	TBME/TAA (1/1)	28	30	76	11
6	lipase PS-C II	TBME/TAA (1/1)	34	35	67	7
7	CAL-A[c]	TBME/TAA (1/1)	58	69	50	4

[a]According to GC after derivatization with acetic anhydride in the presence of DMAP and pyridine.
[b]Determined by GC.
[c]The reaction proceeded with *S* selectivity, in contrast with the other lipases.

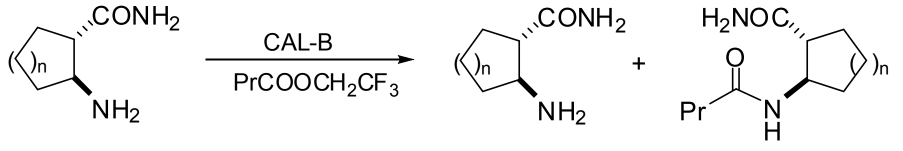
The dissolution of the highly polar *rac*-**5** and *rac*-**6** required a polar solvent, at least as co-solvent, and elevated temperature. Primary and secondary alcohols could not be used for this purpose as they are potential substrates of lipases, but the tertiary alcohol TAA, MeCN or THF could be applied. Since lipases react faster and more selectively in ether solutions (TBME and DIPE),[116] the small-scale resolutions of *rac*-**5** and *rac*-**6** were probed in solvent mixtures of TBME with TAA, MeCN or THF. The addition of the latter two solvents lowered the selectivity in comparison with TAA (Table 15, entry 1 vs. 4; Table 16, entry 3 vs. entries 5-6). The improvement of the amount of TAA in the solvent mixture clearly increased *E*, while the reaction became slower (Tables 15 and 16, entries 1-3). Performing the reactions at 48 °C proved to be more favourable, due to the significantly higher reaction rate (Table 16, entries 3 and 4).

Table 16. *N*-Acylation of *rac*-**6** (0.05 M) with 2,2,2-trifluoroethyl butanoate (2 equiv) by lipase preparations (50 mg/mL) at 48 °C

Entry	Enzyme	Solvent	*t* (h)	Conv. (%)	ee_s (%)[a]	ee_p (%)[b]	*E*
1	CAL-B	TAA	24	30	41	95	61
2	CAL-B	TBME/TAA (3/1)	6	31	42	91	37
3	CAL-B	TBME/TAA (4/1)	6	42	66	91	40
4	CAL-B[c]	TBME/TAA (4/1)	6	9	84	82	20
5	CAL-B	TBME/THF (4/1)	6	59	79	56	8
6	CAL-B	TBME/MeCN (4/1)	24	51	81	79	21
7	CAL-A[d,e]	TBME/MeCN (4/1)	4	99	40	1	1
8	lipase PS-C II[e]	TBME/MeCN (4/1)	24	50	19	19	2
9	lipase PS[e]	TBME/TAA (4/1)	6	16	6	32	2

[a]According to GC after derivatization with acetic anhydride in the presence of DMAP and pyridine.
[b]Determined by GC.
[c]Reaction at room temperature (23 °C).
[d]An unknown side-reaction consumed the product enantiomer obtained.
[e]Reactions proceeded with *S* selectivity, in contrast with CAL-B.

Since the solubilities of the *trans*-amino carboxamides *rac*-**7** and *rac*-**8** in TBME were even worse than those of the corresponding *cis* isomers, a mixture of TBME/TAA (1/1) was used as solvent in the enzyme screening (Table 17). All the tested enzymes were *R*-selective, while CAL-B preparations catalysed the resolutions in a highly enantioselective manner at 48 °C (Scheme 14; Table 17, entries 1, 2 and 5).



rac-**7**: n = 1
rac-**8**: n = 2

(1*S*,2*S*)-**7**: n = 1
(1*S*,2*S*)-**8**: n = 2

(1*R*,2*R*)-**140**: n = 1
(1*R*,2*R*)-**141**: n = 2

Scheme 14

Table 17. *N*-Acylation of *rac*-**7** (0.05 M) and *rac*-**8** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) by lipase catalysis (50 mg/mL) in TBME/TAA (1/1) at 48 °C

Entry	Substrate	Enzyme	*t* (h)	Conv. (%)	ee_s (%)[b]	ee_p (%)[c]	*E*
1	*rac*-**7**	CAL-B	5	50	99	>99	>>200
2	*rac*-**7**	CAL-B[a]	5	43	76	>99	>>200
3	*rac*-**7**	lipase PS	2	38	50	81	16
4	*rac*-**7**	lipase PS-C II	2	49	83	85	25
5	*rac*-**8**	CAL-B	2	47	88	>99	>>200
6	*rac*-**8**	lipase PS	2	56	5	4	1
7	*rac*-**8**	lipase PS-C II	2	3	2	73	10
8	*rac*-**8**	CAL-A	2	1	–	66	6

[a] Lipolase from Sigma.
[b] According to GC after derivatization with acetic anhydride in the presence of DMAP and pyridine.
[c] Determined by GC.

4.1.3. Gram-scale resolutions of *rac*-1-*rac*-8

Finally, *rac*-**1** and *rac*-**2** were resolved on a gram scale in TBME with 2 equivalents of 2,2,2-trifluoroethyl butanoate by lipase PS-C II catalysis at room temperature. For the purpose of the preparation of the enantiomers of *rac*-**2** in reasonable time, the gram-scale resolution was performed in the presence of a 70 mg/mL lipase PS-C II preparation, while in the case of *rac*-**1** 50 mg/mL enzyme effectively catalysed the resolution. Since the *trans* compounds were less soluble in TBME at room temperature, the gram-scale resolution of *rac*-**3** was performed in TBME/TAA (9/1), whereas for *rac*-**4**, due to its somewhat better solubility, neat TBME was used as a reaction medium at 45 °C, with 50 mg/mL lipase PS-C II.

On the basis of the preliminary experiments the gram-scale resolutions of *rac*-**5**, *rac*-**7** and *rac*-**8** were performed in TBME/TAA (1/1) by means of CAL-B catalysis, which allowed the isolation of both enantiomers by stopping the reaction at 50% conversion. The kinetic resolution of *rac*-**6** was carried out in TBME/TAA (4/1) in two stages, due to the low selectivity. The reaction was stopped first at 30% conversion to separate the enantiopure (1*S*,2*R*)-**140**, and the enantiomerically enriched substrate was then subjected to the enzymatic reaction under the same conditions, affording (1*R*,2*S*)-**6**. All these gram-scale resolutions were performed at 45 °C.

The reactions were stopped by filtering off the enzyme. The solvent was then evaporated off and the residue was purified by column chromatography, using CH_2Cl_2-MeOH in different ratios (from 19:1 to 1:1) to separate the resolution products (Table 18).

Table 18.[I],[II] Results of the preparative-scale resolutions of *rac*-**1**-*rac*-**8** (in 0.05 M solutions with 2 equivalents of 2,2,2-trifluoroethyl butanoate), and the physical data on the enantiomers prepared

Substrate	Conditions[a]	*t* (h)	Conv. (%)	Abs. config.	ee[b] (%)	Abs. config.	ee[c] (%)
rac-**1**	A	5	50	1*R*,2*S*-**1**	98	1*S*,2*R*-**134**	98
rac-**2**	B	2.25	50	1*R*,2*S*-**2**	>99	1*S*,2*R*-**135**	96
rac-**3**	C	9.5	50	1*S*,2*S*-**3**	98	1*R*,2*R*-**136**	>99
rac-**4**	D	206	50	1*S*,2*S*-**4**	98	1*R*,2*R*-**137**	>99
rac-**5**	E	1.25	50	1*R*,2*S*-**5**	97	1*S*,2*R*-**138**	99
rac-**6**[d]	F	5.75/150	n.d.	1*R*,2*S*-**6**	99	1*S*,2*R*-**139**	95
rac-**7**	E	10	50	1*S*,2*S*-**7**	98	1*R*,2*R*-**140**	>99
rac-**8**	E	21	50	1*S*,2*S*-**8**	98	1*R*,2*R*-**141**	>99

[a] A: in TBME at rt in the presence of 50 mg/mL lipase PS-C II; B: in TBME at rt in the presence of 70 mg/mL lipase PS-C II; C: in TBME/TAA (9/1) at rt in the presence of 50 mg/mL lipase PS-C II; D: in TBME at 45 °C in the presence of 50 mg/mL lipase PS-C II; E: in TBME/TAA (1/1) at 45 °C in the presence of 50 mg/mL CAL-B;F: in TBME/TAA (4/1) at 45 °C in the presence of 50 mg/mL CAL-B.

[b] According to GC after derivatization with acetic anhydride in the presence of DMAP and pyridine.

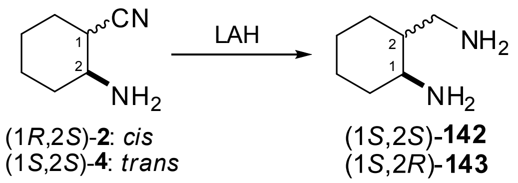
[c] Determined by GC.

[d] Resolution in two steps.

4.1.4. Absolute configurations

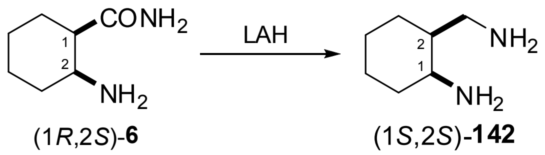
The separated unreacted **2** was reduced with LAH, yielding *cis*-1-amino-2-aminomethylcyclohexane **142** (Scheme 15). The optical rotation of **142**, $[\alpha]_D^{20}$ = +20.5 (*c* 1.0, EtOH), corresponds to the literature value, $[\alpha]_D^{20}$ = +21.8 (*c* 2.18, EtOH),[158] for (1*S*,2*S*)-**142**, which indicates the (1*R*,2*S*) absolute configuration for unreacted **2**. The absolute configuration of unreacted **4** was proven by the same procedure (Scheme 15). The value of $[\alpha]_D^{20}$ = +21.1 (*c* 0.5, EtOH) obtained for the diamine product (**143**) corresponds to the literature value of $[\alpha]_D^{20}$

= +22.8 (*c* 0.724, EtOH)[148] given for (1*S*,2*R*)-**143**. The less reactive enantiomer of **4** has the (1*S*,2*S*) absolute configuration.



Scheme 15

The absolute configuration of the less reactive enantiomer of **6** was confirmed by LAH reduction (Scheme 16), through comparison of the optical rotation of the product diamine (**142**), $[\alpha]_D^{20}$ = +16.8 (*c* 0.5, EtOH), with the literature value for (1*S*,2*S*)-**142**, $[\alpha]_D^{20}$ = +21.8 (*c* 2.18, EtOH)[158]. The optical rotation of the less reactive enantiomer of **8**, $[\alpha]_D^{20}$ = +47.2 (*c* 0.1, EtOH), was in accordance with the literature value given for (1*S*,2*S*)-**8**, $[\alpha]_D^{20}$ = +70.1 (*c* 0.107, EtOH).[148] These observations indicated the same enantiopreference of lipase PS-C II and CAL-B towards alicyclic β-amino nitriles and β-amino carboxamides, respectively.

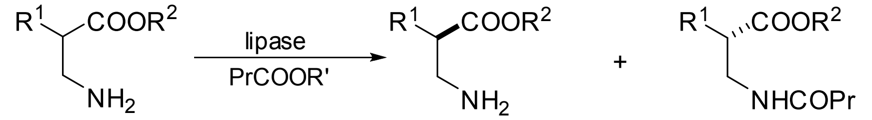


Scheme 16

4.2. *Resolution of α-substituted β-amino esters*[III]

The enzymatic kinetic resolution of β-substituted β-amino esters[108-113] has been extensively investigated, but far fewer results are known in the literature for α-substituted compounds.[114,159,160] The sequential resolution of α-methyl-β-alanine ethyl ester *rac*-**99**[114] is the only example involving *N*-acylation (with subsequent transesterification). We have described the first direct method for the resolution of α-substituted β-amino esters (Scheme 17).

4.2.1. Small-scale experiments with ethyl 3-amino-2-ethylpropanoate *rac*-9 and methyl 3-amino-2-isopropylpropanoate *rac*-10[III]



rac-**9** $R^1 = R^2 = Et$ (*R*)-**9** $R^1 = R^2 = Et$ (*S*)-**144** $R^1 = R^2 = Et$
rac-**10** $R^1 = i\text{Pr}, R^2 = Me$ (*R*)-**10** $R^1 = i\text{Pr}, R^2 = Me$ (*S*)-**145** $R^1 = i\text{Pr}, R^2 = Me$

Scheme 17

Enzyme screening was first performed on *rac*-**9**. Ethyl 3-amino-2-ethylpropanoate (0.05 M) was subjected to lipase (50 mg/mL)-catalysed acylation with 2 equivalents of VA in DIPE at 25 °C. CAL-A, CAL-B and lipases AY, AK, PS and PPL were tested (data not shown). The CAL-B catalysis led to a fast reaction with low chemo- and enantioselectivity. The enantioselectivities were < 2 for all of the enzymes except CAL-A, which displayed a slightly higher selectivity ($E = 4$). Accordingly, the CAL-A (50 mg/mL)-catalysed process was optimized further by testing different acylating agents (ethyl butanoate, butyl butanoate, 2,2,2-trifluoroethyl butanoate, VA, VB and 2,2,2-trifluoroethyl chloroacetate) in reduced amount (1 and 0.55 equivalents) under otherwise constant conditions (0.05 M *rac*-**9** in DIPE at 25 °C; data not shown). The selectivities were still low (up to 6), and the reactions were therefore repeated at 4 °C, using a reduced amount of enzyme (25 mg/mL) with 0.55 equivalents of butyrates, which previously proved to be most promising acyl donors (data not shown). Finally, ethyl butanoate was chosen for further study, since no benefit of using 2,2,2-trifluoroethyl butanoate over the more economical ethyl butanoate could be seen. The results of the solvent screening clearly demonstrated the solvent effect on the reactivity and enantioselectivity (Table 19). The reactions were highly enantioselective, but extremely slow in polar solvents (Table 19, entries 1 and 2), while the other solvents were useless.

Table 19. Solvent screening for the CAL-A (25 mg/mL)-catalysed *N*-acylation of *rac*-**9** (0.05 M) with ethyl butanoate (0.55 equivalent) at 4 ºC

Entry	Solvent	*t* (day)	ee_s[a] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	MeCN	25	45	>99	26	>200
2	TAA	25	68	>99	40	>200
3	CH_2Cl_2	25	4	40	9	2
4	THF	25	8.7	45	16	2.9
5	Et_2O	3	14	24	37	2
6	DIPE	25	64	56	53	6.7

[a] According to GC after derivatization with TFAA in the presence of DMAP and pyridine.
[b] Calculated by using an internal standard (octadecane).

In order to shorten the reaction time (the time needed to reach 50% conversion) without notable loss of selectivity, a higher reaction temperature and an increased enzyme amount were applied. The enzymatic reactions performed at room temperature proceeded faster than expected, but the selectivities were disappointingly low in each solvent and proved to be useless for gram-scale resolution (Table 20, entries 4-5). Elevation of the enzyme content resulted in a faster reaction and a somewhat lower selectivity (Table 20, entries 2 and 3).

Table 20. Effects of temperature and enzyme amount on CAL-A-catalysed *N*-acylation of *rac*-**9** (0.05 M) with ethyl butanoate (0.55 equiv)

Entry	*T* (°C)	Solvent	Enyzme amount (mg/mL)	*t* (day)	ee_s[a] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	4	MeCN	25	25	45	>99	26	>200
2		TAA	25	25	68	>99	40	>200
3			50	14	80	93	46	63
4	rt	MeCN	25	21	30	67	32	17
5		TAA	25	3	31	70	30	8
6			50	2	70	56	55	7

[a] According to GC after derivatization with TFAA in the presence of DMAP and pyridine.
[b] Calculated by using an internal standard (octadecane).

On the other hand, the enantioselectivity can be enhanced by the use of certain additives.[161-163] Most of them have been used previously with lipase PS. In the case of *rac*-**9**, selected additives ($CuCl_2$, TEA and 15-Crown-5) were tested with lipase PS; LiCl, additionally to the previously-mentioned additives, was investigated with CAL-A (Table 21). The additives with lipase PS had no significant effect on the reaction rate (except TEA), nor on the poor selectivity (Table 21, entries 1-4). The same additives used with CAL-A did not fulfil our expectations (Table 21, entries 6-9); moreover LiCl inactivated the lipase.

Table 21. *N*-Acylation of *rac*-**9** (0.05 M) with ethyl butanoate (0.55 equiv) in TAA at 4 ºC by lipase PS and CAL-A catalysis in the presence of additives

Entry	Enzyme	Additive (amount)	*t* (day)	ee$_s$[a] (%)	ee$_p$[b] (%)	Conv. (%)	*E*
1	lipase PS (25 mg/mL)	no additive	20	43	41	51	4
2		$CuCl_2$ (0.1 equiv.)	28	15	34	31	2
3		TEA(0.1 equiv.)	10	39	50	44	4
4		15-Crown-5 (0.33 equiv.)	20	48	30	61	3
5	CAL-A (25 mg/mL)	no additive	25	68	>99	40	>200
6		LiCl (0.1 equiv.)		no reaction detected			
7		$CuCl_2$ (0.1 equiv.)	35	55	84	40	20
8		TEA(0.1 equiv.)	35	74	94	44	74
9		15-Crown-5 (0.33 equiv.)	35	51	88	36	27

[a]According to GC after derivatization with TFAA in the presence of DMAP and pyridine.
[b]Calculated by using an internal standard (octadecane).

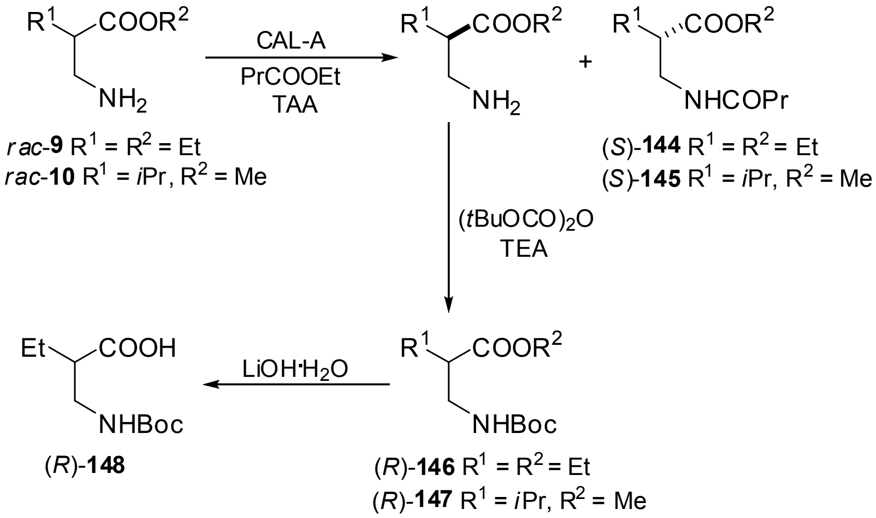
Next, the optimal conditions for *rac*-**9** (0.05 M substrate in TAA, 0.55 equivalent of ethyl butanoate, 50 mg/mL CAL-A at 4 ºC) were applied to *rac*-**10** (data not shown). The reaction rate was similar, but the selectivity was unexpectedly low (42% conversion after 14 days; *E* = 9). In order to increase the selectivity, some other solvents were also tested with a reduced enzyme amount (25 mg/mL) (Table 22), but the use of DIPE or MeCN instead of TAA lowered the selectivity (DIPE; Table 22, entry 2) or the reaction rate (MeCN; Table 22, entry 3). The use of additives ($CuCl_2$ and TEA) did not improve the results either (data not shown).

Table 22. Solvent testing for the *N*-acylation of *rac*-**10** (0.05 M) with ethyl butanoate (0.55 equiv.) in the presence of 25 mg/mL CAL-A at 4 ºC after 35 days

Entry	Solvent	Conv. (%)	*E*
1	TAA	49	9
2	DIPE	48	4
3	MeCN	38	8

4.2.2. Gram-scale resolution of *rac*-9 and *rac*-10

Following the preliminary results, the gram-scale resolutions of *rac*-**9** (0.05 M) and *rac*-**10** (0.05 M) were carried out with an elevated enzyme amount (50 mg/mL) and ethyl butanoate (0.1 M) in TAA at 4 ºC. The enzymatic reactions were stopped by filtering off the enzyme and evaporating off the solvent. Since the substrates could be recovered after column chromatography only with a considerable loss of yield, the unreacted **9** and **10** were transformed to their Boc derivatives (**146** and **147**) and were then separated from the butyramide products (**144** and **145**) by column chromatography with acetone/hexane (1/19) as eluent. The procedure of the gram-scale resolutions and the Boc protection of the unreacted substrates are shown in Scheme 18, while the physical data on the enantiomers prepared presented in Table 23.



Scheme 18

In a consequence of the low selectivity of CAL-A towards *rac*-**10**, the gram-scale resolution was performed in two stages. After the first stage (at 40% conversion), the reaction was stopped as described above, and the residue was dissolved in dilute HCl and extracted with ethyl acetate to obtain **145** with mediocre ee. The aqueous phase was then basified with weak KOH solution and then the unreacted **10** was extracted with ethyl acetate. The recovered substrate was subjected again to enzymatic transformation under the same conditions, allowing the separation of **147** with moderate enantiopurity (Table 23) after Boc protection.

Table 23.[III] Physical data on the isolated enantiomers

Compound	Yield (%)[a]	ee (%)	$[\alpha]_D^{25}$
(*S*)-**144**	36	95[b]	+26.0 (*c* 1.0, MeOH)
(*R*)-**146**	25	85[c]	-20.6 (*c* 0.5, MeOH)
(*S*)-**145**	38	78[c]	+26.4 (*c* 1.0, MeOH)
(*R*)-**147**	18	76[c]	-13.4 (*c* 1.0, MeOH)

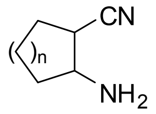
[a] Maximum yield 50% at 50% conversion.
[b] From a comparison of the optical rotation with that of (*R*)-**144**, synthetized from unreacted (*R*)-**9** with ee = 88%.
[c] Determined by GC.

4.2.3. Absolute configurations

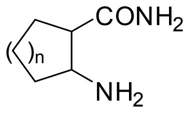
To determine the selectivity of the enzyme, *N*-Boc-protected amino ester **146** was hydrolysed with $LiOH{\cdot}H_2O$,[164] resulting in 3-*tert*-butoxycarbonylamino-2-ethylpropanoic acid **148** as depicted in Scheme 18. The specific rotation for **148** was measured, $[\alpha]_D^{25} = -13.2$ (*c* 1.0, CH_2Cl_2), and comparison with the literature values {$[\alpha]_D^{26} = -17.3$ (*c* 1.3, MeOH)[164] and $[\alpha]_D^{20} = -18.2$ (*c* 1.0, CH_2Cl_2)[165] for (*R*)-**148**} confirmed the *S* selectivity.

5. Summary

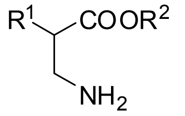
During my PhD work, the effects of the enzyme, enzyme concentration, acyl donor, solvent and temperature on the resolution of β-amino acid derivatives **1-10** were examined, and these compounds were successfully resolved by the methods elaborated.



1: n = 1, *cis*
2: n = 2, *cis*
3: n = 1, *trans*
4: n = 2, *trans*



5: n = 1, *cis*
6: n = 2, *cis*
7: n = 1, *trans*
8: n = 2, *trans*



9: $R^1 = R^2 = Et$
10: $R^1 = i\mathrm{Pr}$, $R^2 = Me$

For the enantioselective *N*-acylation of alicyclic β-amino nitriles **1-4**, lipase PS, CAL-A and CAL-B preparations were tested: the lipase PS preparations allowed the resolution of **1-4** in TBME with 2 equivalents of 2,2,2-trifluoroethyl butanoate (*E* > 200). We monitored the benefit of the activated ester (2,2,2-trifluoroethyl butanoate) in contrast with other acylating agents (*e.g.* ethyl butanoate) on the lipase PS-catalysed *N*-acylation of **2**. We decreased the rate of retardation of the enzymatic acylation to close to 50% conversion by using an increased amount of lipase PS, which permitted the resolution of **2** in a reasonable time. The CAL-B-catalysed *N*-acylation of **2** in TBME and ionic liquids was not successful. We improved the solubility of *trans* compounds in TBME by the addition of TAA as co-solvent (for **3**) or by performing the reactions at elevated temperature (for **4**).

The gram-scale resolutions of **1-4** under the optimized conditions afforded the *N*-acylated (1*S*,2*R*)-**134**, (1*S*,2*R*)-**135**, (1*R*,2*R*)-**136** and (1*R*,2*R*)-**137** and the unreacted enantiomers with high enantiopurity (ee ≥ 98%), which were separated by column chromatography. We demonstrated that, through reduction by LAH, (1*R*,2*S*)-**2** and (1*S*,2*S*)-**4** can be transformed to the corresponding diamines (1*S*,2*S*)-**142** and (1*S*,2*R*)-**143** with only a slight drop in ee, and these results proved the *R* selectivity of the enzymatic acylation.

The high polarity of alicyclic β-amino carboxamides **5-8** required the addition of co-solvents to TBME and elevated temperature (48 °C). CAL-B (50 mg/mL) proved to be a powerful catalyst for the enantioselective acylation of these compounds with 2 equivalents of 2,2,2-trifluoroethyl butanoate. The effects of co-solvents were analysed, and it was found that TAA ensured the best results. Our experiments established that the reaction rate decreased with the elevation of the ratio of TAA. The *N*-acylations of **5**, **7** and **8** were highly selective (*E*

> 200) in TBME-TAA (1:1); whereas for **6** a mixture of TBME-TAA (4:1) proved to be the optimum reaction medium (*E* = 40) and the resolution was performed in two stages.

The unreacted amino carboxamides (1*R*,2*S*)-**5**, (1*R*,2*S*)-**6**, (1*S*,2*S*)-**7** and (1*S*,2*S*)-**8** and the opposite enantiomers as butyramides were obtained with ee ≥ 95%, after separation on silica. The reduction of (1*R*,2*S*)-**6** by LAH resulted in (1*S*,2*S*)-**142**, verifying the *R* selectivity of CAL-B.

On the basis of the results obtained for the CAL-A (50 mg/mL)-catalysed *N*-acylation of **9** with 2 equivalents of VA in DIPE at 25 °C (*E* = 4), further optimizations were developed; the use of lower temperature (4 °C), a reduced amount of CAL-A (25 mg/mL) and ethyl butanoate (0.55 equiv) as acyl donor led to a slightly better result (*E* = 6.7). Solvent screening revealed the influence of the solvent on the enzymatic acylation: in polar solvents (MeCN and TAA), the reactions were slow (26 and 40% conversion after 25 days, respectively) and highly enantioselective (*E* > 200). In order to resolve **9** in a reasonable time, the effects of temperature and the enzyme concentration were examined, and it was found that an elevated amount of CAL-A (50 mg/mL) in TAA at 4 °C offers a faster reaction with good selectivity (46% conversion after 15 days; *E* = 63). When the optimal resolution conditions for **9** with a reduced enzyme amount (25 mg/mL) were applied to **10**, a low reaction rate (49% conversion after 35 days) and low selectivity (*E* = 9) were obtained; the enantiomers of **10** were therefore prepared in two consecutive steps.

After the gram-scale resolution reactions were stopped, the unreacted (*R*)-**9** and (*R*)-**10** were transformed to their Boc-protected forms (*R*)-**146** (ee = 95%) and (*R*)-**147** (ee = 78%), and separated from the butyramides (*S*)-**144** (ee = 85%) and (*S*)-**145** (ee = 76%) by column chromatography. The *S*-enantiopreference of the enzyme was proved by the hydrolysis of (*R*)-**146** with $LiOH{\cdot}H_2O$ to the *N*-Boc-protected amino acid *R*-**148**.

The 20 enantiomers prepared (19 among them new) were characterized by ee values, optical rotations, melting points and ^{1}H-NMR. ^{13}C-NMR and elemental analysis results were also established for β-amino nitrile and β-amino carboxamide enantiomers.

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8. Annex

I.

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An effective approach to the enantiomers of alicyclic β-aminonitriles by using lipase catalysis

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Abstract—Lipase-catalyzed N-acylations of racemic *cis*- and *trans*-2-aminocyclopentane- (and cyclohexane-) carbonitriles with 2,2,2-trifluoethyl butanoate in *tert*-butyl methyl ether (TBME) and in room-temperature ionic liquids (RTILs) were studied. The racemates were effectively resolved ($E > 200$) on a preparative scale by lipase PS-C II (lipase from *Burkholderia cepacia*) in TBME, resulting in two enantiomers in their enantiopure forms at 50% conversion. The reactions in RTILs with Novozym 435 (*Candida antarctica* lipase B) were slow and proceeded with low enantioselectivity.

1. Introduction

Alicyclic $\beta^{2,3}$-amino acids are structural units of oligopeptides, intermediates in the preparation of pharmaceutically important 1,3-heterocycles, and building blocks of potential pharmacons.[1] They include, for instance, cispentacin [FR 109615, (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid] and its derivatives, which exhibit antifungal activity against *Candida* strains.[2,3]

Nitriles are known to be highly valuable intermediates in synthetic chemistry. Both acid- and base-induced hydrolysis of a nitrile gives a carboxylic acid, while metal hydrides reduce nitriles to give primary amines. Accordingly, β-aminonitriles are valuable intermediates in the preparation of β-amino acids and the corresponding diamines. Besides chemical transformations, nitrile-converting enzymes are used to hydrolyze nitriles in the formation of carboxylic acids and amides.[4] Thus, enantioselective hydrolysis of the nitrile group by nitrilases has provided an attractive approach for the production of alicyclic $\beta^{2,3}$-amino acids from the corresponding racemic β-aminonitriles.[5–7] Interestingly, the enantiomers of the same alicyclic $\beta^{2,3}$-amino acids have previously been resolved as amino esters for lipase-catalyzed asymmetric N-acylation, as N-hydroxymethylated β-lactam intermediates for lipase-catalyzed asymmetric O-acylation, and as amino acids for direct lipase-catalyzed hydrolysis of the β-lactam ring.[8–11] These examples indicate a wide area of economical application of the readily availability lipases compared to enzymes with more targeted substrate specificities.

Herein, we report the application of the lipase-induced asymmetric acylation of β-aminonitriles *rac*-**1**–**4** in *tert*-butyl methyl ether (TBME) and in room-temperature ionic liquids (RTILs) for the preparation of enantiopure counterparts (Schemes 1 and 2). To the best of our knowledge, the asymmetric N-acylation of 3-amino-4-(1*H*-3-indolyl)butanenitrile is the only reported lipase-catalyzed kinetic resolution of β-aminonitriles so far.[12] On the other hand, the enzymatic acylation of α-aminonitriles in the presence of lipases has already been described.[13] Although nitriles are not carbonyl compounds, we found it interesting to compare the enzymatic enantioselectivities for the acylation of alicyclic β-aminonitriles herein with those observed earlier for the corresponding β-amino esters. We expected that the absolute configurations of the lipase-catalyzed resolution products **1**–**8** would be the same as in the case of the corresponding β-amino esters.[8] In order to verify this expectation, (1*R*,2*S*)-**2** was reduced with $LiAlH_4$ to the (1*S*,2*S*)-diamine, and (1*S*,2*S*)-**4** to the (1*S*,2*R*)-diamine, with known specific rotations (Scheme 3).[14,15]

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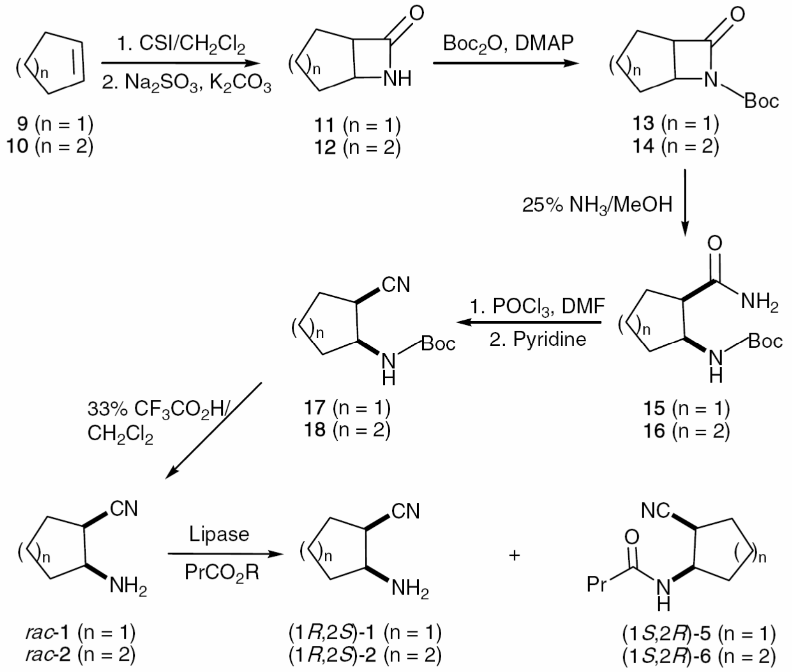
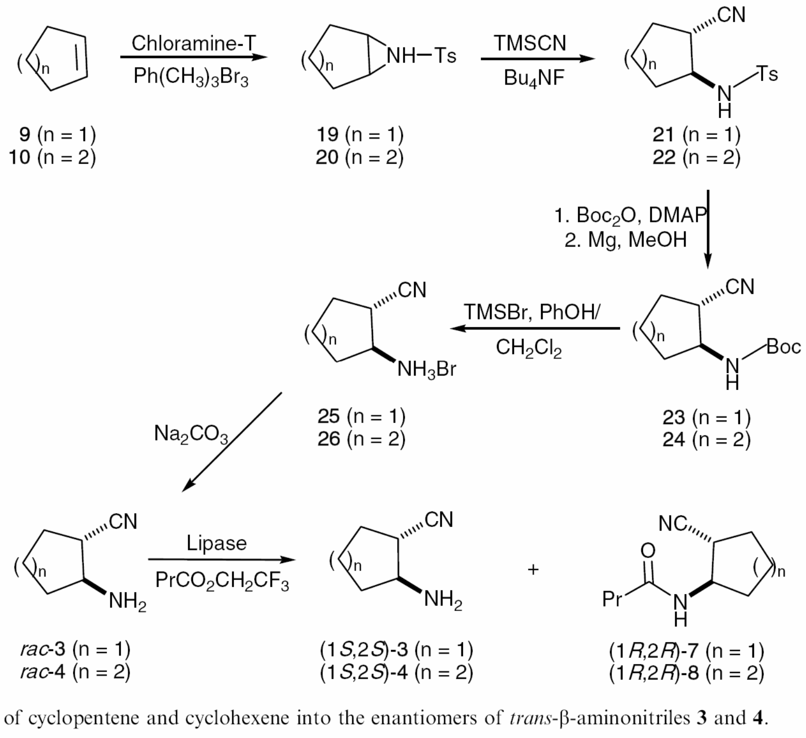


Scheme 1. Transformation of cyclopentene and cyclohexene into the enantiomers of *cis*-β-aminonitriles **1** and **2**.

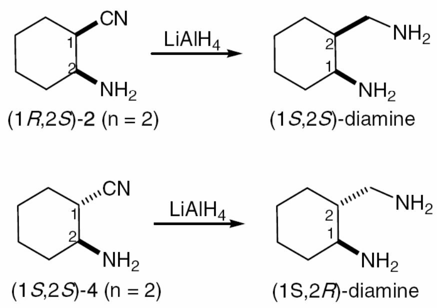


Scheme 2. Transformation of cyclopentene and cyclohexene into the enantiomers of *trans*-β-aminonitriles **3** and **4**.

2. Results and discussion

2.1. Preparation of racemic substrates *rac*-1–4

Substrates *rac*-**1** and *rac*-**2** (the racemic *cis* isomers) were prepared by following the protocol shown in Scheme 1. In this protocol, the alicyclic β-lactams **11** and **12** were readily available by the cycloaddition of chlorosulfonyl isocyanate to cycloalkenes.[9–11] *N*-Boc protection of the β-lactam, followed by ring opening through ammonolysis, gave the Boc-protected amides **15** and **16**, as previously described.[16] Dehydration of the amide with phosphorus oxychloride,[17] followed by *N*-Boc deprotection with 2,2,2-trifluoroacetic acid, furnished the racemic substrates. A three-step synthesis of *rac*-**1** and *rac*-**2** has previously been reported, starting from adiponitrile and heptanedinitrile, respectively, where the *cis* product had to be separated from the *trans* by-product.[7]



Scheme 3. Reduction of β-aminonitrile enantiomers to the diamine enantiomers.

Substrates *rac*-**3** and *rac*-**4** (the racemic *trans*-isomers) were prepared by following the protocol shown in Scheme 2. Catalytic aziridination of the cycloalkenes with chloramine-T gave the *N*-Ts-aziridines **19** and **20,** and subsequent opening of the aziridine ring with trimethylsilyl cyanide in the presence of tetrabutylammonium fluoride proceeded by a known method.[5,6] In the next step, the tosyl group in **21** and **22** was changed to Boc in two steps. The use of the protective groups Ts and Boc was due to the previous observation that the ring opening of *N*-Boc-aziridine was incomplete.[6] Moreover, the removal of Ts with the present procedure was clean.[18] Deprotection of **23** and **24** with bromotrimethylsilane and phenol, and neutralization of the resulting hydrobromides **25** and **26** with sodium carbonate, furnished the racemic substrates as described in the Experimental.

2.2. Kinetic resolution of *rac*-1–4 in TBME

In our earlier studies on the enantioselective N-acylation of alicyclic β-amino esters in ether (Et_2O, $^{i}Pr_2O$ and TBME) solutions, lipase PS from *Burkholderia cepacia* (earlier lipase from *Pseudomonas cepacia*) was the most enantioselective lipase, in the case of the racemic trans-isomers, as was *Candida antarctica* lipase A (CAL-A) in the case of the racemic *cis*-isomers.[8] As an exception to this rule, lipase PS also exhibited excellent enantioselectivity for the acylation of cispentacin ethyl ester. Herein, these lipases, together with some others, were screened for the acylation of *rac*-**1** and *rac*-**2** with 2,2,2-trifluoroethyl butanoate in TBME. The enantioselectivities observed in terms of the enantiomeric ratios *E* and conversions after a certain time are reported in Tables 1 and 2. The lipase PS preparations (PS on Celite and PS-C II on ceramic particles) gave excellent enantioselectivities for both *cis*-β-aminonitriles (entries 1 and 2, Table 1; entries 2 and 3, Table 2), while CAL-A resulted in a fast reaction without enantioselectivity. It was impossible to increase the low enantioselectivity of CAL-A by changing the solvent from TBME to either toluene, acetonitrile, *tert*-amyl alcohol or ethyl butanoate, as indicated by the *E* values constantly being between 2 and 4. Accordingly, there is a clear difference in the enantioselective behavior of the two lipases toward nitrile substrates as compared with the previous ester substrates with a *cis*-configuration (entries 3 and 7 as compared with entries 1, 2, and 4–6, Table 3). Good (entry 5, Table 2) to excellent (entry 4, Table 1) enantioselectivities were observed in the case of *C. antarctica* lipase B (CAL-B) as catalyst. For acylation in neat ethyl butanoate, the lipases totally lost their enantioselectivity (Table 2).

Table 1. Lipase screening for the enantioselective acylation of *rac*-**1** (0.05 M) with $PrCO_2CH_2CF_3$ (0.1 M) in TBME in the presence of the enzyme preparation (50 mg/mL) at room temperature

Entry	Lipase	Time (h)	Conversion (%)	*E*
1	Lipase PS	0.5	50	≫200
2	Lipase PS-C II	2	51	>200
3	CAL-A	0.5	83	2 ± 1
4	CAL-B	1	50	>200

On the basis of the lipase screening, the lipase PS-C II-catalyzed acylation of *rac*-**2** with 2,2,2-trifluoroethyl butanoate in TBME was further optimized with respect to the amount of the enzyme. The reason for this was the observation that, although aminonitrile **1** reacted rapidly to 50% conversion in the presence of 50 mg/mL of the enzyme, the reaction in the case of *rac*-**2** tended to slow down at close to 50% conversion (Table 4). Thus, its took 6 h to reach 49% conversion, whereas the first 46% was reached within only 2 h (entries 3 and 4). In order to obtain both enantiomers in highly enantiopure form in a reasonable time, the gram-scale resolution of *rac*-**2** was performed at 70 mg/mL of lipase PS-C II (entries 5 and 6). Chromatographic purification finally furnished (1*R*,2*S*)-**1** (ee = 98%) and (1*S*,2*R*)-**5** (ee = 98%) from *rac*-**1**, and (1*R*,2*S*)-**2** (ee >99%) and (1*S*,2*R*)-**6** (ee = 96%) from *rac*-**2**, as shown in Section 4.

Table 2. Lipase screening for the enantioselective acylation of *rac*-**2** with $PrCO_2R$ in TBME at room temperature

Entry	Lipase	R = Et[a]			R = CH_2CF_3[b]		
		Time (h)	Conversion (%)	*E*	Time (h)	Conversion (%)	*E*
1	Lipase AK	40	33	1.3 ± 0.1	6	12	30 ± 0.3
2	Lipase PS	40	49	3.2 ± 0.2	4	43	>200
3	Lipase PS-C II	—	—	—	4	48	>200
4	CAL-A	—	—	—	0.5	83	2 ± 1
5	CAL-B	40	62	9 ± 1	6	48	79 ± 5
6	PPL	40	29	1.1 ± 0.01	—	—	—
7	CRL	40	39	1.2 ± 0.1	—	—	—

[a] Reaction of *rac*-**2** (0.1 M) in neat ethyl butanoate in the presence of the enzyme preparation (100 mg/mL).

[b] Reaction of *rac*-**2** (0.05 M) with the acyl donor (0.1 M) in TBME in the presence of the enzyme preparation (50 mg/mL).

Table 3. Enantioselectivity for the lipase-catalyzed N-acylation of ethyl *cis*-2-aminocyclopentyl- **1a** and *cis*-2-aminocyclohexyl- **2a** 1-carboxylates and that of *rac*-**1** and *rac*-**2**

Entry	Ref.	Substrate	Acyl donor	Solvent	Lipase PS *E*	CAL-A *E*
1	8	**1a** (CO_2Et)	$AcOCH_2CF_3$	Et_2O	$\gg100^a$	31
2	8	**1a** (CO_2Et)	$AcOCH_2CF_3$	TBME	$>100^a$	37
3	—	**1** (CN)	$PrCO_2CH_2CF_3$	TBME	$>200^a/>200^b$	2
4	8	**2a** (CO_2Et)	$AcOCH_2CF_3$	Et_2O	6	51
5	8	**2a** (CO_2Et)	$PrCO_2CH_2CF_3$	Et_2O	2	$\gg100$
6	8	**2a** (CO_2Et)	$AcOCH_2CF_3$	TBME	7	57
7	—	**2** (CN)	$PrCO_2CH_2CF_3$	TBME	$>200^a/>200^b$	2

In **1a** and **2a** CO_2Et is in the place of CN of compounds **1** and **2**.

[a] Lipase PS preparation on Celite.

[b] Lipase PS-C II.

Table 4. Effect of the amount of the lipase PS-C II on the enantioselective acylation of *rac*-**2** (0.05 M) with $PrCO_2CH_2CF_3$ (0.1 M) in TBME at room temperature

Entry	Enzyme (mg/mL)	Time (h)	Conversion (%)	$ee^{(1R,2S)\text{-}\mathbf{2}}$ (%)	$ee^{(1S,2R)\text{-}\mathbf{6}}$ (%)	*E*
1	25	2	42	70	99	>200
2	25	8	50	96	97	>200
3	50	2	46	83	98	>200
4	50	6	49	92	97	>200
5	70	2	49	93	98	>200
6	70	4	50	97	96	>200

Excellent enantioselectivities ($E \gg 200$) for the acylations of *rac*-**3** and *rac*-**4** (racemic *trans* isomers) with 2,2,2-trifluoroethyl butanoate were observed in the presence of lipase PS-C II (Table 5). Accordingly, the *trans*-aminonitriles behaved as expected on the basis of the previous results for the corresponding β-aminocarboxylates as substrates.[8] Due to the low solubility of *rac*-**3** in particular, *tert*-amyl alcohol was added to the TBME. However, the presence of *tert*-amyl alcohol somewhat lowered the reactivity as compared with the reaction in neat TBME, as shown for *rac*-**4** (entries 3 and 4). For this reason, and because of the somewhat better solubility of *rac*-**4**, its gram-scale kinetic resolution was performed in TBME at 45 °C, as described in Section 4, which allowed the preparations of (1*S*,2*S*)-**4** (ee = 99%) and (1*R*,2*R*)-**8** (ee >99%) at 50% conversion. *rac*-**3** was resolved in TBME/*tert*-amyl alcohol (9:1) at room temperature, yielding (1*S*,2*S*)-**3** (ee = 98%) and (1*R*,2*R*)-**7** (ee >99%).

2.3. Asymmetric acylation of *rac*-2 with CAL-B in TBME and in RTILs

RTILs serve as non-toxic, non-volatile, and non-flammable alternatives to classical organic solvents in various applications, including their use as biocatalytic reaction media. The results for lipase-catalyzed reactions in RTILs have been promising with respect to enhanced enzymatic activities and stabilities and to enzymatic regio- and enantioselectivities. CAL-B (Novozyme 435) is perhaps the most commonly used enzyme in RTILs. Most of these studies relate to the transformations of secondary alcohols.[19,20] Amines have served as substrates less commonly.[21]

The acylation of *rac*-**2** with 2,2,2-trifluoroethyl butanoate in TBME in the presence of CAL-B gave good (entry 5, Table 2), but not excellent enantioselectivity, as observed in the case of *rac*-**1** (entry 4, Table 1). It was also clear that an enzyme content of 30 mg/mL or higher had no effect on the reactivity of *rac*-**2** (entries 3–5, Table 6), with identical progression curves being obtained (not shown here). As RTILs have been described, which have several attractive properties, with enantioselectivity enhancement for biocatalysis in particular, we decided to study the CAL-B-catalyzed acylation of *rac*-**2** in RTILs. The RTILs were selected according to our previous work and are described in Scheme 4.[22] The results in Table 6 did not meet fully with our expectations (entries 6–12). The reactions in all cases proceeded slowly as compared with the reaction in TBME. The reactivity was best in hydrophobic EMIM·NTf_2 (entry 9), while

Table 5. Acylation of *rac*-**3** (0.05 M) and *rac*-**4** (0.05 M) with $PrCO_2CH_2CF_3$ (0.1 M) in TBME in the presence of lipase PS-C II (50 mg/mL)

Entry	Substrate	Solvent	Temperature (°C)	Conversion (%)	*E*
1	*rac*-**3**	TBME/*tert*-amyl alcohol (8/2)	23	49	$\gg200$
2	*rac*-**3**	TBME/*tert*-amyl alcohol (9/1)	23	50	$\gg200$
3	*rac*-**4**	TBME/*tert*-amyl alcohol (9/1)	23	17	$\gg200$
4	*rac*-**4**	TBME	23	30	$\gg200$
5	*rac*-**4**	TBME	65	32	$\gg200$

Reaction time 24 h.

Table 6. Solvent screening for the CAL-B-catalyzed acylation of *rac*-**2** (0.05 M) with $PrCO_2CH_2CF_3$ (0.1 M) at room temperature

Entry	Solvent	CAL-B (mg/mL)	Time (h)	Conversion (%)	*E*
1	TBME	10	6	35	57 ± 2
2	TBME	20	6	45	63 ± 2
3	TBME	30	6	48	74 ± 10
4	TBME	50	6	48	79 ± 5
5	TBME	70	6	48	73 ± 4
6	$BMIM{\cdot}PF_6$	50	96	11	14 ± 3
7	$BMPyr{\cdot}BF_4$	50	96	32	24 ± 3
8	$EMIM{\cdot}BF_4$	50	96	9	12 ± 1
9	$EMIM{\cdot}NTf_2$	50	96	46	22 ± 1
10	EMIM·TfO	50	96	23	51 ± 6
11	EMIM·TfO	75	96	26	71 ± 4
12	EMIM·TfO/TBME[a]	75	96	46	89 ± 4

[a] A mixture of 1:1; two-phase medium.

[EMIM][BF_4]: R^1 = Et, X = BF_4
[BMIM][PF_6]: R^1 = Bu, X = PF_6
[EMIM][NTf_2]: R^1 = Et, X = $(CF_3SO_2)_2N$
[EMIM][TfO]: R^1 = Et, X = CF_3SO_3

[BMPyr][BF_4]: R^1 = Bu, X = BF_4

Scheme 4. Imidazolium- and pyridinium-based ionic liquids.

the enantioselectivity reached the same level as in TBME only in the case of hydrophilic EMIM·TfO (entry 11). In accordance with what we have seen before,[23] the higher reactivity and enantioselectivity of *rac*-**2** are evident in a solvent mixture of EMIM·TfO and TBME (entry 12).

3. Conclusions

We have shown that alicyclic *cis*- and *trans*-aminonitriles *rac*-**1**–**4** can be effectively resolved on a preparative scale by lipase PS-C II, on acylation with 2,2,2-trifluoroethyl butanoate in TBME. Thus, both enantiomers are simultaneously present at 50% conversion, one as the unreacted aminonitrile **1**–**4** and the other as the produced N-butanoylated counterpart **5**–**8** (Schemes 1 and 2). For both *cis*- and *trans*-isomers, the enantiomer in which the amino group is at the (*R*)-stereocenter reacts. This is in accordance with the previously observed enantiopreference in the N-acylation of the corresponding *cis*- and *trans*-amino esters.[8] In disagreement with the previous results, however, the highly enantioselective (*E* >200) N-acylation of *rac*-**2** proceeded quickly in TBME. The reactions in RTILs with CAL-B catalysis were less successful.

We have also demonstrated that reduction by $LiAlH_4$ can produce *cis*-(1*S*,2*S*)-1-amino-2-aminomethylcyclohexane from (1*R*,2*S*)-**2**, and *trans*-(1*S*,2*R*)-1-amino-2-aminomethylcyclohexane from (1*S*,2*S*)-**4** (Scheme 3). In this process, however, the enantiomeric excess tended to drop somewhat. These reductions were used for the determination of absolute configurations, and need more optimization in order to be used for preparative purposes.

4. Experimental

4.1. Materials and methods

2,2,2-Trifluoroethyl butanoate was prepared from 2,2,2-trifluoroethanol and butanoyl chloride. Organic solvents were of the highest analytical grade. $BMPyr{\cdot}BF_4$ and EMIM·TfO were products of Fluka. The other RTILs were prepared by slightly modifying methods described in the literature.[19,23,24] *C. antarctica* lipase A (lipase SP 526) and B (Novozym 435) were purchased from Roche and Novozyme, respectively. Lipases PS and PS-C II from *B. cepacia* (former *P. cepacia*) and lipase AK from *Pseudomonas fluorescens* were product of Amano Europe, England. Before use, CAL-A and lipases PS and AK were adsorbed on Celite (4.0 g) by dissolving the enzyme and sucrose (0.24 g) in Tris–HCl buffer (pH 7.9), and thereafter left to dry at room temperature, with the final lipase content in the enzyme preparation being 20% (w/w).[25] Porcine pancreatic lipase (PPL) and *Candida rugosa* lipase (CRL) were purchased from Sigma.

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ and D_2O at ambient temperature on a Bruker DRX400 spectrometer. Chemical shifts are given in δ (parts per million) relative to TMS as the internal standard; multiplicities were recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), m (multiplet), or om (overlapping multiplet). Optical rotations were measured with a Perkin Elmer 341 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

In a typical small-scale experiment, one or more of racemates **1**–**4** (0.05 M) was dissolved in TBME (or some other organic solvent) or in one of the RTILs (1 mL), followed by addition of the enzyme preparation (10–75 mg/mL). The addition of 2,2,2-trifluoroethyl butanoate (0.1 M) initiated the reaction. For the reactions in ethyl butanoate, the addition of the enzyme initiated the reaction. The reaction mixture was shaken at room temperature (23 °C) if not otherwise stated. The progress of the reactions and the ee values were calculated by taking samples (0.05 mL) at intervals, filtering off the enzyme, and analyzing them by gas chromatography on a Chrompack CP-Chirasil-DEX CB column (25 m). In the case of RTILs, the products were extracted into TBME before the GC analysis. For good baseline separation, the unreacted amino group in the sample was acylated with acetic anhydride in the presence of pyridine containing 1% 4,4-dimethylaminopyridine. The determination of E was based on the equation $E = \ln[(1 - ee_s)/(1 + ee_s/ee_p)]/\ln[(1 + ee_s)/(1 + ee_s/ee_p)]$, where $c = ee_s/(ee_s + ee_p)$, with the use of linear regression, E being the slope of the line $\ln[(1 - c)(1 - ee_s)]$ versus $\ln[(1 - c)(1 + ee_s)]$ the subscripts referring to the less reactive substrate (s) and to the formed product (p).[26]

4.2. Preparation of *cis*-2-aminocyclopentanecarbonitrile, *rac*-1

The preparation of **15** has already been described (Scheme 1).[16] Compound **15** (3.60 g; 16 mmol) was added to a solution of the freshly formed Vilsmeier reagent (*N*,*N*-dimethylformamide and $POCl_3$; 1.47 mL (19 mmol) and 1.60 mL (18 mmol), respectively) in acetonitrile (20 mL) under an argon atmosphere at 0 °C, followed by the addition of pyridine in order to cleave the intermediate.[17] Compound **17** was obtained as white crystals (2.05 g; 60%), mp: 86–88 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (9H, s, *t*-Bu), 1.55–2.10 (6H, om, 3 × CH_2), 3.21 (1H, m, H-1), 4.12 (1H, m, J = 7.28 Hz, H-2), 4.84 (1H, s, N*H*) ppm; Anal. Calcd for $C_{11}H_{18}N_2O_2$: C, 62.83; H, 8.63; N, 13.32. Found: C, 62.40; H, 8.52; N, 13.01.

Boc-deprotection of **17** involved standard procedures. Compound **17** (1.26 g, 6 mmol) was dissolved in dichloromethane (60 mL), which contained 33% trifluoroacetic acid. The reaction mixture was stirred at room temperature for 49 h before evaporation, leading to the trifluoroacetic salt as an oily residue (4.9 g). The residue (1.0 g) was dissolved in 0.5 M aqueous sodium carbonate (70 mL) and this mixture extracted three times with dichloromethane. After drying of the organic phases and evaporation, the free aminonitrile was purified by column chromatography, using dichloromethane/methanol (19:1) as eluent resulting in *rac*-**1** (0.089 g, 60%) as white crystals, mp: 59–61 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90–2.35 (6H, om, 3 × CH_2), 1.63 (2H, br s, NH_2), 2.92 (1H, m, H-1), 3.59 (1H, m, H-2) ppm, ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.8 (C4), 29.2 (C5), 33.6 (C3), 37.1 (C2), 54.9 (C1), 121.0 (CN) ppm. Anal. Calcd for $C_6H_{10}N_2$: C, 65.42; H, 9.15; N, 25.43. Found: C, 65.23; H, 9.05; N, 25.12.

4.3. Preparation of *cis*-2-aminocyclohexanecarbonitrile, *rac*-2

The preparation of *rac*-**2** was performed in the same way as that of *rac*-**1**. Compound **12** in the synthetic path is well characterized.[10] The characterization of other intermediates is given as below.

4.3.1. 7-*tert*-Butoxycarbonyl-7-azabicyclo[4,2,0]hexan-8-one, 14. White crystals (yield 65%), mp: 69–72 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.52 (9H, m, *t*-Bu), 1.56–2.06 (8H, om, 4 × CH_2), 3.24 (1H, m, J = 3.52 Hz, H-1), 4.10 (1H, m, J = 2.94 Hz, H-2) ppm. Anal. Calcd for $C_{12}H_{19}NO_3$: C, 63.98; H, 8.50; N, 6.22. Found: C, 63.54; H, 8.42; N, 6.14.

4.3.2. *cis*-2-*tert*-Butoxycarbonylaminocyclohexanecarboxamide, 16. White crystals (yield 70%), mp: 163–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (9H, s, *t*-Bu), 1.56–2.04 (8H, om, 4 × CH_2), 2.92 (1H, m, J = 7.23 Hz, H-1), 4.14 (1H, m, J = 7.76 Hz, H-2), 5.02 (1H, d, J = 6.72 Hz, NH), 5.32 (1H, s, NH_2), 5.70 (1H, s, NH_2) ppm. Anal. Calcd for $C_{12}H_{22}N_2O_3$: C, 59.48; H, 9.15; N, 11.56. Found: C, 59.15; H, 9.02; N, 11.37.

4.3.3. *cis*-2-*tert*-Butoxycarbonylaminocyclohexanecarbonitrile, 18. White crystals (yield 56%), mp: 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (9H, s, *t*-Bu), 1.36–2.03 (8H, m, 4 × CH_2), 3.33 (1H, br s, H-1), 3.58–3.63 (1H, m, J = 4.00 Hz, H-2), 4.75 (1H, d, J = 5.47 Hz, N*H*) ppm. Anal. Calcd for $C_{12}H_{20}N_2O_2$: C, 64.26; H, 8.99; N, 12.49. Found: C, 63.91; H, 8.72; N, 12.28.

4.3.4. *cis*-2-Aminocyclohexanecarbonitrile, *rac*-2. White crystals (yield 62%), mp: 74–75.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–2.05 (8H, om, 4 × CH_2), 1.61 (2H, br s, NH_2), 2.86 (1H, m, H-1), 2.97 (1H, m, H-2), ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.3 (C4), 24.6 (C6), 28.1 (C5), 33.0 (C3), 38.0 (C2), 51.0 (C1), 120.9 (CN) ppm. Anal. Calcd for $C_7H_{12}N_2$: C, 67.70; H, 9.74; N, 22.56. Found: C, 67.45; H, 9.65; N, 22.25.

4.4. Preparation of *trans*-2-aminocyclopentanecarbonitrile, *rac*-3

The preparation of *rac*-**3** was performed in the same way as that of *rac*-**4** below, the synthetic path to intermediate **23** following the known procedure (Scheme 2).[5,6]

4.4.1. *trans*-2-Aminocyclopentanecarbonitrile hydrobromide, 25. Light-yellowish crystals (yield 67%), mp: 212–214 °C; ^{1}H NMR (400 MHz, D_2O): δ 1.30–2.25 (6H, om, 3 × CH_2), 1.45 (2H, br s, NH_2), 2.92 (1H, m, H-1), 3.55 (1H, m, H-2) ppm. Anal. Calcd for $C_6H_{10}N_2 \cdot HBr$: C, 37.72; H, 5.80; N, 14.66. Found: C, 37.49; H, 5.76; N, 14.41.

4.4.2. *trans*-2-Aminocyclopentanecarbonitrile, *rac*-3. White crystals (yield 63%), mp: 72–74 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30–2.30 (6H, om, 3 × CH_2), 1.70 (2H, br s, NH_2), 2.42 (1H, m, H-1), 3.54 (1H, m, H-2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.0 (C4), 29.8 (C5), 35.1 (C3), 39.2 (C2), 59.2 (C1), 122.8 (CN) ppm. Anal. Calcd for $C_6H_{10}N_2$: C, 65.42; H, 9.15; N, 25.43. Found: C, 65.06; H, 9.07; N, 25.12.

4.5. Preparation of *trans*-2-aminocyclohexanecarbonitrile, *rac*-4

The preparation of intermediate **24** has been described before (Scheme 2).[5,6] Boc-deprotection in **24** was performed by adding **24** (0.4 g, 1.8 mmol) in dry dichloromethane (4 mL) to a solution of bromotrimethylsilane (0.41 g, 2.7 mmol) and phenol (0.01 g, 0.12 mmol) in dry dichloromethane (5 mL) under an argon atmosphere at room temperature. After stirring for 2.5 h the white crystals produced were filtered off and washed with diethyl ether, resulting in **26** (0.23 g, 66%), mp: 248–250 °C; ^{1}H NMR (400 MHz, D_2O): δ 1.20–2.30 (8H, om, 4 × CH_2), 1.45 (2H, br s, NH_2), 2.89 (1H, m, H-1), 3.48 (1H, m, H-2) ppm. Anal. Calcd for $C_7H_{12}N_2 \cdot HBr$: C, 40.99; H, 6.39; N, 13.66. Found: C, 40.62; H, 6.29; N, 13.32.

Hydrobromide **26** (0.17 g, 0.83 mmol) was neutralized with sodium carbonate (0.5 M, 5 mL) and the aqueous phase extracted with 3 × 50 mL dichloromethane. The combined organic phase was dried and evaporated. The residue was purified by column chromatography, using dichloromethane/methanol (19:1) as eluent yielding *rac*-**4** (0.26 g, 83%) as a yellowish oil; ^{1}H NMR (400 MHz, CDCl3): δ 1.10–2.20 (8H, om, 4 × CH_2), 1,65 (2H, br s, NH_2), 2.46 (1H, m, H-1), 3.47 (1H, m, H-2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.0 (C4), 24.9 (C6), 29.4 (C5), 34.4 (C3), 38.8 (C2), 52.9 (C1), 122.0 (CN) ppm. Anal. Calcd for $C_7H_{12}N_2 \cdot HBr$: C, 67.70; H, 9.74; N, 22.56. Found: C, 67.43; H, 9.62; N, 22.26.

4.6. Preparative-scale resolution of *rac*-1

rac-**1** (0.5 g, 4.54 mmol) was dissolved in TBME (80 mL) and lipase PS-C II (4.50 g, 50 mg/mL) was added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (1.38 mL, 9.14 mmol). The reaction mixture was shaken at room temperature. The reaction was stopped after 5 h at 50% conversion ($ee^{(1R,2S)\text{-}\mathbf{1}} = 98\%$, $ee^{(1S,2R)\text{-}\mathbf{5}} > 99\%$) by filtering off the enzyme. After evaporation, the resolution products were separated by column chromatography, using CH_2Cl_2/MeOH (19:1), yielding (1*R*,2*S*)-**1** {0.182 g, 1.65 mmol; white crystals, mp: 59–61 °C, ee = 98%, $[\alpha]_D^{20} = -5.9$ (*c* 1.0, MeOH); the ^{1}H and ^{13}C NMR and elemental analysis data were identical with those for *rac*-**1**} and (1*S*,2*R*)-**5** {0.280 g, 1.55 mmol; light-yellow oil, ee = 98%, $[\alpha]_D^{20} = +127.5$ (*c* 0.53, MeOH)}. Spectral data for (1*S*,2*R*)-**5**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (3H, t, $J = 7.39$ Hz, CH_3), 1.55–2.25 (6H, om, 3 × CH_2), 1.69 (2H, m, $CH_3CH_2CH_2$), 2.21 (2H, t, $J = 7.13$ Hz, $CH_2CH_2CH_3$), 3.29 (1H, m, H-1), 4.39 (1H, m, H-2), 5.98 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 19.7 ($CH_2CH_2CH_3$), 22.2 (C4), 29.4 (C5), 30.8 (C3), 35.0 ($CH_2CH_2CH_3$), 47.1 (C2), 52.3 (C1), 121.0 (CN), 174.1 (NHCO) ppm. Anal. Calcd for $C_{10}H_{16}N_2O$: C, 66.64; H, 8.95; N, 15.54. Found: C, 66.36; H, 9.21; N, 15.10.

4.7. Preparative-scale resolution of *rac*-2

rac-**2** (0.4 g, 3.23 mmol) was dissolved in TBME (65 mL) and lipase PS-C II (4.52 g, 70 mg/mL) added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (0.97 mL, 6.43 mmol). The reaction mixture was shaken at room temperature. The reaction was then stopped after 2.25 h at 50% conversion [$ee^{(1R,2S)\text{-}\mathbf{2}} = 95\%$, $ee^{(1S,2R)\text{-}\mathbf{6}} = 95\%$], with the following work-up as above, yielding (1*R*,2*S*)-**2** {0.136 g, 1.1 mmol; white crystals, mp: 74–76 °C, ee >99%, $[\alpha]_D^{20} = +13.2$ (*c* 0.5, MeOH); the ^{1}H and ^{13}C NMR and elemental analysis data were identical to those of the racemic *rac*-**2**} and (1*S*,2*R*)-**6** {0.126 g, 0.65 mmol; white crystals, mp: 87–89 °C, ee = 96%, $[\alpha]_D^{20} = +98.7$ (*c* 0.5, MeOH)}. Spectral data for (1*S*,2*R*)-**6**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, $J = 7.38$ Hz, CH_3), 1.30–2.10 (8H, om, 4 × CH_2), 1.66 (2H, m, $CH_3CH_2CH_2$), 2.18 (2H, t, $J = 7.46$ Hz, $CH_2CH_2CH_3$), 2.74 (1H, m, H-1), 4.24 (1H, m, H-2), 7.46 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 19.8 ($CH_2CH_2CH_3$), 21.9 (C4), 25.2 (C6), 28.0 (C5), 29.2 (C3), 34.5 ($CH_2CH_2CH_3$), 39.1 (C2), 48.8 (C1), 120.7 (CN), 172.0 (NHCO) ppm. Anal. Calcd for $C_{11}H_{18}N_2O$: C, 68.01; H, 9.34; N, 14.42. Found: C, 67.64; H, 9.87; N, 14.37.

4.7.1. Absolute configuration of (1*R*,2*S*)-2. The separated unreacted **2** (50 mg, 0.403 mmol) was reduced with lithium–aluminum hydride in the formation of 1-amino-2-aminomethylcyclohexane (30 mg, 0.234 mmol; a yellow oil, ee = 92%) with the following spectral data: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20–2.00 (9H, om, H-2-6), 1.58 (2H, s, CH_2NH_2), 2.80 (2H, br s, CH_2NH_2), 3.40 (1H, m, H-1), 5.10 (2H, br s, $CHNH_2$) ppm. Anal. Calcd for $C_7H_{16}N_2$: C, 65.57; H, 12.58; N, 21.85. Found: C, 65.64; H, 12.87; N, 22.07.

The obtained $[\alpha]_D^{20} = +20.5$ (*c* 1.0, EtOH) corresponds to the literature value[15] $[\alpha]_D^{20} = +21.8$ (*c* 2.18, EtOH) for (1*S*,2*S*)-*cis*-(+)-1-amino-2-aminomethylcyclohexane. This indicates a (1*R*,2*S*)-absolute configuration for the unreacted **2** (Scheme 3).

4.8. Preparative-scale resolution of *rac*-3

rac-**3** (0.255 g, 2.32 mmol) was dissolved in a mixture of TBME and *tert*-amyl alcohol (9:1), after which lipase PS-C II (2.26 g, 50 mg/mL) was added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (0.68 mL, 4.50 mmol). The mixture was shaken at room temperature. The reaction was stopped after 9.5 h at 50% conversion ($ee^{(1S,2S)\text{-}\mathbf{3}} = 98\%$, $ee^{(1R,2R)\text{-}\mathbf{7}}$ >99%), with the following work-up as above, yielding (1*S*,2*S*)-**3** {0.085 g, 0.77 mmol; colorless crystals, mp: 72–74 °C, ee = 98%, $[\alpha]_D^{20} = +54.1$ (*c* 0.5, MeOH); the

^{1}H and ^{13}C NMR and elemental analysis data were identical with those for racemic **3**} and (1*R*,2*R*)-**7** {0.105 g, 0.58 mmol; white crystals, mp: 73–74 °C, ee >99%, $[\alpha]_{D}^{20} = -44.2$ (*c* 0.5, MeOH)}. Spectral data for (1*R*,2*R*)-**7**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, t, $J = 7.36$ Hz, CH_3), 1.55–2.25 (6H, om, $3 \times CH_2$), 1.69 (2H, m, $CH_3CH_2CH_2$), 2.16 (2H, t, $J = 7.30$ Hz, $CH_2CH_2CH_3$), 2.87 (1H, m, H-1), 4.35 (1H, m, H-2), 5.94 (1H, br s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 19.7 ($CH_2CH_2CH_3$), 23.5 (C4), 29.9 (C5), 32.1 (C3), 35.3 ($CH_2CH_2CH_3$), 39.1 (C2), 56.2 (C1), 122.1 (CN), 173.8 (NHCO) ppm. Anal. Calcd for $C_{10}H_{16}N_2O$: C, 66.64; H, 8.95; N, 15.54. Found: C, 66.39; H, 9.30; N, 15.53.

4.9. Preparative-scale resolution of *rac*-4

rac-**4** (0.22 g, 1.77 mmol) was dissolved in TBME (39 mL) and lipase PS-C II (1.95 g, 50 mg/mL) was added. The reaction was started by the addition of 2,2,2-trifluoroethyl butanoate (0.59 mL, 3.91 mmol). The mixture was shaken at 45 °C. The reaction was stopped after 206 h at 50% conversion [$ee^{(1S,2S)\text{-}\mathbf{4}} = 99\%$, $ee^{(1R,2R)\text{-}\mathbf{8}} > 99\%$], with the following work-up as above, yielding (1*S*,2*S*)-**4** {0.101 g, 0.81 mmol; a slowly crystallizing yellowish oil, ee = 98% $[\alpha]_{D}^{20} = +34.6$ (*c* 0.5, MeOH); the ^{1}H and ^{13}C NMR and elemental analysis data were identical with those for racemic **4**}; and (1*R*,2*R*)-**8** (0.110 g, 0.57 mmol), white crystals, mp: 76–78 °C, ee >99% $[\alpha]_{D}^{20} = -6.7$ (*c* 0.5, MeOH). Spectral data for (1*R*,2*R*)-**8**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (3H, t, $J = 7.38$ Hz, CH_3), 1.20–2.25 (8H, om, $4 \times CH_2$), 1.69 (2H, m, $CH_3CH_2CH_2$), 2.19 (2H, t, $J = 7.42$ Hz, $CH_2CH_2CH_3$), 2.66 (1H, m, H-1), 3.99 (1H, m, H-2), 5.84 (1H, s, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3 (CH_3), 19.9 ($CH_2CH_2CH_3$), 24.5 (C4), 29.4 (C6), 32.3 (C5), 35.0 (C3), 39.4 ($CH_2CH_2CH_3$), 49.2 (C2), 50.2 (C1), 121.2 (CN), 173.4 (NHCO) ppm. Anal. Calcd for $C_{11}H_{18}N_2O$: C, 68.01; H, 9.34; N, 14.42. Found: C, 67.83; H, 9.14; N, 13.98.

4.9.1. Absolute configuration of (1*S*,2*S*)-4.

The separated unreacted **4** (30 mg, 0.242 mmol) was reduced with lithium–aluminum hydride in the formation of 1-amino-2-aminomethylcyclohexane (19 mg, 0.148 mmol) as a light-yellowish oil, ee = 90%, $[\alpha]_{D}^{20} = +21.1$ (*c* 0.5, EtOH) with the following spectral data: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05–2.20 (9H, om, H-2-6), 1.50 (2H, s, CH_2NH_2), 2.45 (1H, m, H-1), 2.90 (2H, m, CH_2NH_2), 5.04 (2H, br s, $CHNH_2$) ppm. Anal. Calcd for $C_7H_{16}N_2$: C, 65.57; H, 12.58; N, 21.85. Found: C, 65.72; H, 12.27; N, 21.97.

The obtained $[\alpha]_{D}^{20} = +21.1$ (*c* 0.5, EtOH) corresponds to the literature value[14] $[\alpha]_{D}^{20} = +22.8$ (*c* 0.724, EtOH) for (1*S*,2*R*)-*trans*-(+)-1-amino-2-aminomethylcyclohexane. This indicates a (1*S*,2*S*)-absolute configuration for the unreacted **4** (Scheme 3).

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Tetrahedron: *Asymmetry*

Lipase-catalyzed kinetic resolution of 2-aminocyclopentane- and 2-aminocyclohexanecarboxamides

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Abstract—*Candida antarctica* lipase B (CAL-B)-catalyzed *N*-acylation with 2,2,2-trifluoroethyl butanoate in solvent mixtures of *tert*-butyl methyl ether and *tert*-amyl alcohol was used to prepare all the enantiomers of *cis*- and *trans*-2-aminocyclopentane- and -cyclohexanecarboxamides. An unexpected change in enantiopreference, accompanied by low enantioselectivity, was observed when *Pseudomonas cepacia* lipase (*cis*-cyclohexane substrate) or *C. antarctica* lipase A (*cis*-cyclopentane and -cyclohexane substrates) replaced CAL-B.
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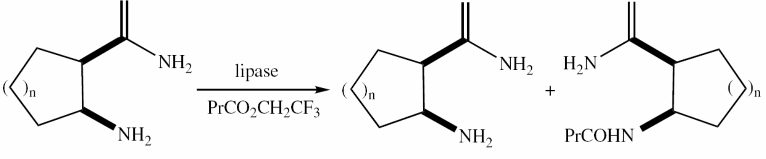
1. Introduction

Over the past few years, interest in the enantiomers of alicyclic β-amino acids has increased greatly from both pharmaceutical and chemical aspects.[1–3] The natural β-amino acid cispentacin [(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid] and some of its synthetic derivatives, in particular the 4-methylene derivative (BAY 1-8888/PLD-118), have been shown to exert strong antifungal activity against *Candida albicans*.[4–6] On the other hand, self-organizing β-peptides, and *cis*- and *trans*-2-aminocyclopentane- and -cyclohexanecarboxylic acid enantiomers have been shown to form interesting, stable secondary structure motifs depending on which enantiomers the peptide is made from.[1–3]

The enantiomers of several alicyclic $\beta^{2,3}$-amino acids have previously been resolved by lipases; examples are amino esters, β-lactams, *N*-hydroxymethylated β-lactam intermediates and β-aminonitriles.[7–11] In the present work, we introduce lipase catalysis for the asymmetric *N*-acylation of alicyclic β-amino amides *rac*-**1**–**4** (Schemes 1 and 2). The motivation was to create new chemoenzymatic possibilities for the preparation of β-amino acid enantiomers, and to learn more about the enantioselective behaviour of lipases towards small structural variations in their



rac-**1** (n=1)
rac-**2** (n=2)

(1*R*,2*S*)-**1** (n=1)
(1*R*,2*S*)-**2** (n=2)

(1*S*,2*R*)-**5** (n=1)
(1*S*,2*R*)-**6** (n=2)

Scheme 1. Transformations into the enantiomers of *cis*-β-aminocarboxamides **1** and **2**.

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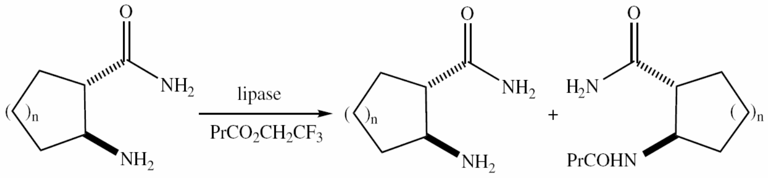

rac-**3** (n=1)
rac-**4** (n=2)

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

(1*R*,2*R*)-**7** (n=1)
(1*R*,2*R*)-**8** (n=2)

Scheme 2. Transformations into the enantiomers of *trans*-β-aminocarboxamides **3** and **4**.

substrates. Previously reported biological effects of various aminoamide derivatives in racemic and the enantiomerically pure forms furnished further motivation for the present work.[12–14] Thus, (1*S*,2*R*)-2-aminocyclohexanecarboxamide is needed as a terminal amino acid residue in small phosphotyrosine-containing peptides and in its mono-charged phosphinate isosters, prepared in order to inhibit tyrosine kinase in the treatment of cancer.[12]

Amino amides have not served as substrates for lipases previously, although the preparation of aminoamides as product enantiomers through the ammonolysis of amino esters was first described in 1993 by the groups of Gotor and Sheldon.[15,16] In the present work, ammonolysis could not be used because ethyl *cis*-2-aminocyclopentanecarboxylate is known to give a mixture of *cis*-2-aminocyclopentanecarboxamide **1** and the corresponding *trans* isomer **3**, under amidation conditions.[17] Thus, the unreacted amino ester enantiomer could not be prepared by ammonolysis without isomerization.

2. Results and discussion

On the basis of our earlier work, three lipases were selected in order to optimize the asymmetric *N*-acylation of amino amides *rac*-**1**–**4** (Schemes 1 and 2). The results are shown in Tables 1–3. Lipase PS from *Burkholderia cepacia* (adsorbed on Celite[18] or as commercial PS-C II) was selected because it has been effectively used for the (*R*)-selective *N*-acylation of the alicyclic β-amino ester and β-aminonitrile analogues of **1**–**4** in ether [Et_2O, $^iPr_2O$ (DIPE) and ^tBuOMe (TBME)] solutions.[7,11] Lipases A (CAL-A) and B (CAL-B) from *Candida antarctica* were also selected. CAL-A is known to display excellent (2*R*) enantioselectivity for the *N*-acylation of sterically hindered alicyclic racemic *cis*-β-amino

Table 1. *N*-Acylation of *rac*-**1** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) by lipase preparations (50 mg/mL) in solvent mixtures at 48 °C; reaction time 1 h

Entry	Enzyme	Solvent	Conversion (%)	$ee^{(1R,2S)-\mathbf{1}}$ (%)	$ee^{(1S,2R)-\mathbf{5}}$ (%)	*E*
1	CAL-B	TBME/TAA (1:1)	46	83	98	>200
2	CAL-B	TBME/TAA (3:1)	50	96	98	>200
3	CAL-B	TBME/TAA (4:1)	50	97	97	>200
4	CAL-B	TBME/MeCN (1:1)	47	85	95	108 ± 17
5	Lipase PS	TBME/TAA (1:1)	28	30	76	11 ± 0.3
6	Lipase PS-C II	TBME/TAA (1:1)	34	35	67	7 ± 0.1
				$ee^{(1S,2R)-\mathbf{1}}$	$ee^{(1R,2S)-\mathbf{5}}$	
7	CAL-A	TBME/TAA (1:1)	58	69	50	4 ± 0.3

Table 2. *N*-Acylation of *rac*-**2** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) by lipase preparations (50 mg/mL) in organic solvent mixtures at 48 °C

Entry	Enzyme	Solvent	Time (h)	Conversion (%)	$ee^{(1R,2S)-\mathbf{2}}$ (%)	$ee^{(1S,2R)-\mathbf{6}}$ (%)	*E*
1	CAL-B	TAA	24	30	41	95	61 ± 4
2	CAL-B	TBME/TAA (3:1)	6	31	42	91	37[c]
3	CAL-B	TBME/TAA (4:1)	6	42	66	91	40 ± 3
4	CAL-B	TBME/TAA (4:1)[a]	6	9	84	82	20 ± 5
5	CAL-B	TBME/THF (4:1)	6	59	79	56	8 ± 1
6	CAL-B	TBME/MeCN (4:1)	24	51	81	79	21 ± 1
					$ee^{(1S,2R)-\mathbf{2}}$	$ee^{(1R,2S)-\mathbf{6}}$	
7	CAL-A	TBME/MeCN (4:1)[b]	4	99	40	1	1 ± 0.2
8	Lipase PS-C II	TBME/MeCN (4:1)	24	50	19	19	2 ± 0.1
9	Lipase PS	TBME/TAA (4:1)	6	16	6	32	0.5 ± 0.1

[a] Reaction at room temperature (23 °C).
[b] An unknown side-reaction consumes the enantiomer product obtained.
[c] Calculated on the basis of one sample.

Table 3. *N*-Acylation of *rac*-**3** and *rac*-**4** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M) by lipase preparations (50 mg/mL) in TBME/TAA (1:1) at 48 °C

Entry	Substrate	Enzyme	Time (h)	Conversion (%)	ee$^{(1S,2S)\text{-}\mathbf{3}\text{ or }\text{-}\mathbf{4}}$ (%)	ee$^{(1R,2R)\text{-}\mathbf{7}\text{ or }\text{-}\mathbf{8}}$ (%)	*E*
1	**3**	CAL-B	5	50	99	>99	≫200
2	**3**	CAL-B[a]	5	43	76	>99	≫200
3	**3**	Lipase PS	2	38	50	81	16 ± 2
4	**3**	Lipase PS-C II	2	49	83	85	25 ± 8
5	**4**	CAL-B	2	47	88	>99	≫200
6	**4**	Lipase PS	2	56	5	4	1 ± 0.1
7	**4**	Lipase PS-C II	2	3	2	73	10 ± 0.4
8	**4**	CAL-A	2	1	—	66	6 ± 0.1

[a] CAL-B from Sigma under the trade name Lipolase.

esters, including the amino ester analogues of **1** and **2**.[7,19,20] Only moderate enantioselectivity of CAL-A has been observed with the corresponding aminonitrile substrates.[11] CAL-B, on the other hand, is known to display relatively high enantioselectivity in the case of racemic *cis*-2-aminocyclopentane and 2-aminocyclohexane carbonitriles.[11]

N-Acylation of *rac*-**1**–**4**, with the selected lipases, was expected to proceed with (2*R*) enantiopreference at the reaction centre as was observed for the corresponding amino esters and aminonitriles.[7,11] CAL-B-catalyzed reactions all fulfill this expectation. The specific rotation of the diamine obtained after $LiAlH_4$ reduction of (1*R*,2*S*)-**2** was consistent with the previously reported value.[11,22] However, the peaks in the GC chromatograms clearly reveal a change in enantiopreference for the CAL-A-catalyzed *N*-acylation of *rac*-**1** and for the CAL-A and lipases PS and PS-C II-catalyzed *N*-acylations of *rac*-**2**, where a slight (2*S*) enantiopreference is observed (Table 1, entry 7, and Table 2, entries 7–9, respectively).

The low solubilities of aminoamides *rac*-**1**–**4** in organic solvents other than alcohols, acetonitrile (MeCN) and tetrahydrofuran (THF) at first seemed to restrict the present enzymatic work. Primary or secondary alcohols as substrates for lipases could not be used as solvents. In the presence of CAL-B in *tert*-amyl alcohol (TAA), the *N*-acylation of *rac*-**2** with 2,2,2-trifluoroethyl butanoate proceeded slowly at 48 °C (Table 2, entry 1). Lipases react faster and are more selective for reactions in ether solutions (DIPE and TBME in particular) compared to reactions in many other solvents.[7] Accordingly, the studies were continued in solvent mixtures of TBME with TAA, acetonitrile and tetrahydrofuran. Screening of the lipases for the acylation of *rac*-**1**–**4** in these solvent mixtures indicated high enantioselectivity for CAL-B catalysis (Table 1, entries 1–4; Table 2, entries 1–6; Table 3, entries 1, 2 and 5). On the other hand, lipases PS, PS-C II and CAL-A could not be used for the present studies, because of the low enantioselectivities, the *E* values now varying between 1 and 25.

As it proved more demanding to resolve alicyclic *cis*-β-amino amides than the corresponding *trans* species, the optimization was continued by using *rac*-**1** and *rac*-**2** as substrates for CAL-B catalysis. The nature of the solvent mixture had a clear effect on the *N*-acylation (Tables 1 and 2). Thus, there was a drop in enantioselectivity for *rac*-**2**, when the mixture of TBME with TAA (4:1) was replaced by a mixture of TBME with THF or MeCN, as shown in Table 2 (entries 3, 4 and 5). The enantioselectivity for *rac*-**1** similarly dropped when TBME/TAA (1:1) was replaced by TBME/MeCN (1:1) (Table 1, entries 1 and 4). CAL-B-catalyzed *N*-acylation of *rac*-**1** proceeded with excellent enantioselectivity in various mixtures (1:1, 3:1 and 4:1) of TBME/TAA (Table 1, entries 1–3), while the enantioselectivity for *rac*-**2** in neat TAA was higher than that in TBME/TAA (3:1 and 4:1) (Table 2, entry 1, as compared with 2 and 3). On this basis, it was concluded that, for a practical resolution, the amount of TBME with respect to TAA should be as low as good reactivity allows.

Finally, the gram-scale resolutions of *rac*-**1**, *rac*-**3** and *rac*-**4** allowed the preparation of the enantiomers with CAL-B in a mixture of TBME/TAA (1:1) at 50% conversion. The kinetic resolution of *rac*-**2** was performed in TBME/TAA (4:1). As a consequence of the relatively moderate enantioselectivity, the kinetic resolution was performed in two stages. In the first stage, enantiopure (1*S*,2*R*)-**6** was isolated when the reaction was stopped at ca. 30% conversion. In the second enzymatic stage, the less reactive enantiomer (1*R*,2*S*)-**2** was purified enantiomerically. The reactions were performed at 45 °C, as dramatic decreases in reactivity and enantioselectivity were evident when carried out at room temperature (23 °C) (Table 2, entry 3, as compared with 4).

3. Conclusion

In conclusion, CAL-A, CAL-B and lipase PS and PS-C II-catalyzed *N*-acylations have been studied. Only CAL-B allowed the preparation of all enantiomers of *rac*-**1**–**4**. Interestingly, CAL-A and lipase PS were the most suitable lipases for the *N*-acylation of the corresponding amino esters,[7] while lipase PS and PS-C II were applicable for the corresponding aminonitriles.[11] This work demonstrates unexpected enantiopreference differences between CAL-A and lipase PS and PS-C II as compared with CAL-B for the *cis* substrates **1** and **2**.

4. Experimental

Lipases A (CAL-A) and B (CAL-B; Novozym 435) from *C. antarctica* were purchased from Novozyme. Novozym

435, known by the trade name Lipolase, was obtained from Sigma Aldrich. Lipases PS and PS-C II from *B. cepacia* (formerly *Pseudomonas cepacia*) were products of Amano Europe, England. Before use, CAL-A and lipase PS were adsorbed on Celite in the presence of sucrose, as described previously,[18] the final lipase preparation containing 20% (w/w) of the lipase. The solvents were of the highest analytical grade. 2,2,2-Trifluoroethyl butanoate was prepared from trifluoro ethanol and butanoyl chloride. Compounds *rac*-**1**–**4** were prepared as reported earlier.[11,17,21,22]

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at ambient temperature on a JEOL L400 or a Bruker AM400 spectrometer. Chemical shifts are given in δ (ppm); multiplicities were recorded as s (singlet), d (doublet), t (triplet), m (multiplet) or om (overlapping multiplet). Optical rotations were measured with a Perkin Elmer 341 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

In a typical small-scale experiment, one of the compounds *rac*-**1**–**4** (0.05 M) was dissolved in TAA or an appropriate solvent mixture (1 mL), followed by addition of the enzyme preparation (50 mg/mL). The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (0.1 M). The reaction mixture was shaken at 48 °C unless otherwise stated. The progress of the reaction and the ee values were determined by taking samples (0.1 mL) at intervals, filtering off the enzyme and analyzing them by gas chromatography on a Chrompack CP-Chirasil-DEX CB column (25 m). For good baseline separation, the unreacted amino group in the sample was acylated with acetic anhydride in the presence of pyridine containing 1% 4,4-dimethylaminopyridine. The determination of E was based on the equation $E = \ln[(1 - \mathrm{ee_S})/(1 + \mathrm{ee_S}/\mathrm{ee_P})]/\ln[(1 + \mathrm{ee_S})/(1 + \mathrm{ee_S}/\mathrm{ee_P})]$, where $c = \mathrm{ee_S}/(\mathrm{ee_S} + \mathrm{ee_P})$, with the use of linear regression, E being the slope of the line $\ln[(1 - c)(1 - \mathrm{ee_S})]$ versus $\ln[(1 - c)(1 + \mathrm{ee_S})]$, the subscripts referring to the less reactive substrate (S) and to the product formed (P).[23]

4.1. Gram-scale resolution of *cis*-2-aminocyclopentanecarboxamide, *rac*-1

Compound *rac*-**1** (0.3 g, 2.34 mmol) was dissolved in a mixture (47 mL) of TBME and TAA (1:1), and CAL-B (2.34 g, 50 mg/mL) was added. The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (0.7 mL, 4.7 mmol). The reaction mixture was then shaken at 45 °C. The reaction was stopped after 1.25 h, at 50% conversion ($\mathrm{ee}^{(1R,2S)\text{-}\mathbf{1}} = 97\%$, $\mathrm{ee}^{(1S,2R)\text{-}\mathbf{5}} = 98\%$) by filtering off the enzyme. After evaporation of the solvent, the residue was purified by column chromatography, using CH_2Cl_2/MeOH (9:1, then 1:1) to separate the resolution products.

Compound (1*R*,2*S*)-**1** (0.121 g, 0.94 mmol) was obtained as white crystals: mp 135–137 °C, ee = 97%, $[\alpha]_D^{20} = -70.4$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.15–2.10 (6H, om, 3 × CH_2), 1.93 (2H, s, CHNH_2), 2.24 (1H, m, $J = 9.30$ Hz, H-1), 3.22 (1H, m, $J = 7.04$ Hz, H-2), 5.25 (1H, s, $CONH_2$), 6.95 (1H, s, $CONH_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5 (C4), 23.3 (C5), 30.4 (C3), 45.6 (C2), 53.4 (C1), 181.7 ($CONH_2$) ppm. Anal. Calcd for $C_6H_{12}N_2O$: C, 56.23; H, 9.44; N 21.86. Found: C, 56.02; H, 9.11; N, 21.47.

Compound (1*S*,2*R*)-**5** (0.177 g, 0.90 mmol) was obtained as white crystals: mp 176–178 °C, ee = 99%, $[\alpha]_D^{20} = +92.2$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (3H, t, $J = 7.4$ Hz, CH_3), 1.41–1.86 (6H, om, 3 × CH_2), 1.70 (2H, m, $CH_3CH_2CH_2$), 2.00 (2H, t, $J = 7.4$ Hz, $CH_2CH_2CH_3$), 2.74 (1H, m, C*H*$CONH_2$), 4.24 (1H, m, C*H*NHCOPr), 6.72 (1H, s, NH_2), 7.10 (1H, s, NH_2), 7.46 (1H, d, $J = 8.0$ Hz, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.1 (CH_3), 19.2 ($CH_2CH_2CH_3$), 22.6 (C4), 27.8 (C5), 32.4 (C3), 37.9 ($CH_2CH_2CH_3$), 47.1 (C2), 52.3 (C1), 172.0 (NHCO), 175.3 ($CONH_2$) ppm. Anal. Calcd for $C_{10}H_{18}N_2O_2$: C, 60.58; H, 9.15; N 14.13. Found: C, 60.31; H, 8.95; N, 13.88.

4.2. Gram-scale resolution of *cis*-2-aminocyclohexanecarboxamide, *rac*-2

Compound *rac*-**2** (0.5 g, 3.5 mmol) was dissolved in a mixture (70 mL) of TBME and TAA (4:1), after which CAL-B (3.5 g, 50 mg/mL) was added. The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (1 mL, 7 mmol). The mixture was then shaken at 45 °C. The reaction was stopped after 5.75 h, at 27% conversion ($\mathrm{ee}^{(1R,2S)\text{-}\mathbf{2}} = 34\%$, $\mathrm{ee}^{(1S,2R)\text{-}\mathbf{6}} = 93\%$) by filtering off the enzyme. After evaporation, the residue was purified by a short column, using CH_2Cl_2/MeOH (9:1, then 1:1) as an eluent to separate the products.

In order to obtain the enantiomer substrate in an enantiopure form, the enantiomerically enriched substrate (0.347 g, $\mathrm{ee}^{(1R,2S)\text{-}\mathbf{2}} = 34\%$) was subjected to further enzymatic acylation under the above reaction conditions. The reaction was stopped after 150 h ($\mathrm{ee}^{(1R,2S)\text{-}\mathbf{2}} = 99\%$, $\mathrm{ee}^{(1S,2R)\text{-}\mathbf{6}} = 47\%$) by filtering off the enzyme and evaporation of the solvent. The substrate and the product were separated as described above.

Compound (1*R*,2*S*)-**2** (0.091 g, 0.64 mmol) was obtained as white crystals: mp 123–124 °C, ee = 99%, $[\alpha]_D^{20} = +10.1$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25–1.80 (8H, om, 4 × CH_2), 1.53 (2H, s, CHN*H*2), 2.21 (1H, m, $J = 10.16$ Hz, H-1), 3.12 (1H, m, $J = 4.46$ Hz, H-2), 6.69 (2H, s, $CONH_2$), 7.64 (1H, s, $CONH_2$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.2 (C4), 24.5 (C6), 25.2 (C5), 33.4 (C3), 47.6 (C2), 49.2 (C1), 177.2 ($CONH_2$) ppm. Anal. Calcd for $C_7H_{14}N_2O$: C, 59.13; H, 9.92; N 19.70. Found: C, 58.98; H, 9.65; N, 19.38.

Compound (1*S*,2*R*)-**6** (0.170 g, 0.80 mmol) was obtained as white crystals: mp 196.5–198 °C, ee = 95%, $[\alpha]_D^{20} = +14.6$ (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.82 (3H, t, $J = 7.38$ Hz, CH_3), 1.22–1.88 (8H, om, 4 × CH_2), 1.46 (2H, m, $CH_2CH_2CH_3$), 2.04 (2H, t, $J = 7.26$ Hz, $CH_2CH_2CH_3$), 2.41 (1H, m, C*H*$CONH_2$), 4.09 (1H, m, C*H*NHCOPr), 6.70 (1H, s, NH_2), 7.14 (1H, s, NH_2), 7.41 (1H, d, $J = 8.8$ Hz, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.0 (CH_3), 19.4 ($CH_2CH_2CH_3$), 22.0 (C4), 23.7 (C6), 25.1 (C5), 30.2 (C3), 37.8 ($CH_2CH_2CH_3$), 44.7 (C2), 47.3 (C1), 171.9 (NHCO), 175.6 ($CONH_2$) ppm.

Anal. Calcd for $C_{11}H_{20}N_2O_2$: C, 62.24; H, 9.50; N 13.02. Found: C, 61.95; H, 9.31; N, 12.73.

4.3. Gram-scale resolution of *trans*-2-aminocyclopentanecarboxamide, *rac*-3

Compound *rac*-**3** (0.098 mg, 0.76 mmol) was dissolved in a mixture (15.4 mL) of TBME and TAA (1:1), after which CAL-B (0.765 g, 50 mg/mL) was added. The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (0.23 mL, 1.52 mmol). The mixture was shaken at 45 °C. After 10 h, the enzyme was filtered off, at 50% conversion ($ee^{(1S,2S)\text{-}\mathbf{3}} = 99\%$, $ee^{(1R,2R)\text{-}\mathbf{7}} > 99\%$). The substrate and the product were separated as described above.

Compound (1*S*,2*S*)-**3** (0.023mg, 0.18 mmol) was obtained as white crystals: mp 123–124 °C, ee = 98%, $[\alpha]_D^{20} = +38.0$ (*c* 0.25, MeOH); ^1H NMR (400 MHz, DMSO-d_6): δ 0.95–1.90 (6H, om, $3\times CH_2$), 1.65 (2H, s, CHNH_2), 2.63 (2H, m, H-1 and H-2), 6.67 (1H, s, $CONH_2$), 7.28 (1H, s, $CONH_2$) ppm. Anal. Calcd for $C_6H_{12}N_2O$: C, 56.23; H, 9.44; N 21.86. Found: C, 55.97; H, 9.21; N, 21.79.

Compound (1*R*,2*R*)-**7** (0.031 mg, 0.16 mmol) was obtained as white crystals: mp 174–175 °C, ee >99%, $[\alpha]_D^{20} = -22$ (*c* 0.25, MeOH); ^1H NMR (400 MHz, DMSO-d_6): δ 0.84 (3H, t, $J = 7.37$ Hz, CH_3), 1.25–1.90 (6H, om, $3\times CH_2$), 1.45 (2H, m, $CH_3CH_2CH_2$), 2.01 (2H, t, $J = 7.30$ Hz, $CH_2CH_2CH_3$), 2.74 (1H, m, C*H*$CONH_2$), 4.10 (1H, m, C*H*NHCOPr), 6.72 (1H, s, NH_2), 7.28 (1H, s, NH_2), 7.78 (1H, d, $J = 7.28$ Hz, NH) ppm. Anal. Calcd for $C_{10}H_{18}N_2O_2$: C, 60.58; H, 9.15; N 14.13. Found: C, 60.14; H, 8.79; N, 14.00.

4.4. Gram-scale resolution of *trans*-2-aminocyclohexanecarboxamide, *rac*-4

Compound *rac*-**4** (0.4 g, 2.82 mmol) was dissolved in a mixture (56 mL) of TBME and TAA (1:1), after which CAL-B (2.8 g, 50 mg/mL) was added. The reaction was initiated by the addition of 2,2,2-trifluoroethyl butanoate (0.85 mL, 5.64 mmol). The mixture was shaken at 45 °C. The reaction was stopped after 21 h, at 50% conversion ($ee^{(1S,2S)\text{-}\mathbf{4}} = 98\%$ and $ee^{(1R,2R)\text{-}\mathbf{8}} > 99\%$) by filtering off the enzyme. After evaporation, the residue was purified by column chromatography, using CH_2Cl_2/MeOH (1:1).

Compound (1*S*,2*S*)-**4** (0.170 g, 1.2 mmol) was obtained as white crystals: mp 125–126.5 °C, ee = 98% $[\alpha]_D^{20} = +47.2$ (*c* 0.1, MeOH), in accordance with the literature[22] value given for (1*S*,2*S*)-(+)-2-aminocyclohexanecarboxamide ($[\alpha]_D^{20} = +70.1$ (*c* 0.107, MeOH)); ^1H NMR (400 MHz, $CDCl_3$): δ 0.98–1.83 (8H, om, $4\times CH_2$), 1.64 (2H, s, CHNH_2), 2.60–2.65 (2H, om, H-1 and H-2), 6.68 (1H, s, $CONH_2$), 7.29 (1H, s, $CONH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.8 (C4), 25.1 (C6), 29.0 (C5), 34.9 (C3), 51.26 (C2), 52.8 (C1), 176.6 ($CONH_2$) ppm. Anal. Calcd for $C_7H_{14}N_2O$: C, 59.13; H, 9.92; N 19.70. Found: C, 59.07; H, 9.60; N, 19.51.

Compound (1*R*,2*R*)-**8** (0.219 g; 1.04 mmol) was obtained as white crystals: mp 274–276 °C, ee >99% $[\alpha]_D^{20} = -12.0$ (*c* 1.0, MeOH); ^1H NMR (400 MHz, DMSO-d_6): δ 0.81 (3H, t, $J = 7.2$ Hz, CH_3), 1.04–1.75 (8H, om, $4\times CH_2$), 1.46 (2H, m, $CH_3CH_2CH_2$), 1.95 (2H, t, $J = 7.14$ Hz, $CH_2CH_2CH_3$), 2.09 (1H, m, C*H*$CONH_2$), 3.71 (1H, m, C*H*NHCOPr), 6.70 (1H, s, NH_2), 6.93 (1H, s, NH_2), 7.49 (1H, d, $J = 8.4$ Hz, N*H*COPr) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.9 (CH_3), 19.3 ($CH_2CH_2CH_3$), 25.0 (C4), 29.6 (C6), 33.1 (C5), 39.4 (C3), 40.0 ($CH_2CH_2CH_3$), 49.2 (C2), 49.6 (C1), 171.3 (NHCO), 175.8 ($CONH_2$) ppm. Anal. Calcd for $C_{11}H_{20}N_2O_2$: C, 62.24; H, 9.50; N 13.02. Found: C, 62.13; H, 9.27; N, 13.46.

4.5. Absolute configuration of (1*R*,2*S*)-2

Compound (1*R*,2*S*)-**2** (30 mg) was reduced with lithium aluminium hydride, to yield the corresponding diamine (17 mg) as a light-yellow oil: ee = 97%, $[\alpha]_D^{20} = +16.8$ (*c* 0.5, EtOH); ^1H NMR (400 MHz, $CDCl_3$): δ 1.22–2.05 (9H, om, H-2-6), 1.43 (2H, s, CH_2NH_2), 2.27 (2H, s, CH_2NH_2), 3.70 (1H, m, H-1), 5.00 (2H, s, CHNH_2) ppm. Rotation data correspond to the literature value for (1*S*,2*S*)-(+)-2-aminomethylcyclohexylamine {$[\alpha]_D^{20} = +21.8$ (*c* 2.18, EtOH) and +20.5 (*c* 1.0, EtOH)}.[11,22] The less reactive enantiomer thus has the (1*R*,2*S*) absolute configuration.

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Lipase-catalysed N-acylation of β²-amino esters

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Dedicated to Professor Gábor Bernáth on the occasion of his 75th birthday

Abstract—The *Candida antarctica* lipase A-catalysed N-acylation of ethyl 3-amino-2-ethylpropanoate *rac*-**3** and methyl 3-amino-2-isopropylpropanoate *rac*-**6** was performed with ethyl butanoate in *tert*-amyl alcohol at 4 °C. The resulting enantiomerically enriched derivatives were isolated as *N*-Boc-protected amino esters (*R*)-**9** and (*R*)-**10** and butyramides (*S*)-**7** and (*S*)-**8** (ee: 76–95%).

1. Introduction

Cyclic[1] and acyclic[2–5] β-amino acids are of key importance in the organic chemistry. These are the constituents of both naturally occurring and synthetic derivatives with valuable physiological effects,[6] for example, taxoids.[4,7] β-Peptides and α-peptides containing β-amino acids are not degradable by proteases, and may lead to protease inhibitors, which are currently important targets in pharmaceutical chemistry.[6] The folding properties and three-dimensional structures of β-peptides have already been explored,[3,8–10] while interest has recently turned to the preparation of enantiomerically pure β-amino acids and their derivatives;[1,11] many different methods have been described for the preparation of β²-amino acid enantiomers.[12–16]

Lipase-catalysed kinetic resolution has proven to be an excellent method for the preparation of β³- and β²,³-amino acid enantiomers, for which, unlike the β² derivatives, low enantioselectivities were described.[17] Enzymes can catalyse the transformations on both functional groups of amino esters: N-acylation[18–20] of the amino group, and ester hydrolysis[21] of the ester function. As far as we are aware, only three enzymatic resolutions of β²-amino esters have been reported.[22–24] *N*-Phenylacetylated α-methyl-β-alanine has been successfully hydrolysed with penicillin G amidase.[22] The enantiomers of α-methyl-β-alanine ethyl ester have been prepared through a two-step sequential resolution (N-acylation and interesterification) catalysed by CAL-A and CAL-B, respectively.[23] Various hydrolytic enzymes have been applied for the resolution of β²-amino acid esters, resulting in enantiomerically enriched β²-amino acids and esters.[24]

Herein, we report an N-acylation-based enzymatic resolution method for the preparation of 2-ethyl- and 2-isopropyl-3-aminopropanoic acid enantiomers.

2. Results and discussion

2.1. Syntheses of racemic β²-amino esters 3 and 6

Model racemic compounds were synthesised by a combination of literature procedures for the preparation of analogous α-substituted β-amino esters.[20,25,26] The addition of benzylamine to ethyl ethacrylate[27] **1** furnished the corresponding *N*-benzylamino ester **2**, which was transformed to ethyl 3-amino-2-ethylpropanoate *rac*-**3** by catalytic hydrogenolysis. The condensation of methyl cyanoacetate **4** and acetone under reductive conditions resulted in the isopropyl-substituted malononitrile derivative **5**,[28] the cyano group of which was reduced by catalytic hydrogenation to yield methyl 3-amino-2-isopropylpropanoate *rac*-**6** (Scheme 1).

2.2. Lipase-catalysed enantioselective N-acylation of (±)-3 and (±)-6

Enzyme screening was first performed on model compound (±)-**3**. Ethyl 3-amino-2-ethylpropanoate was subjected to

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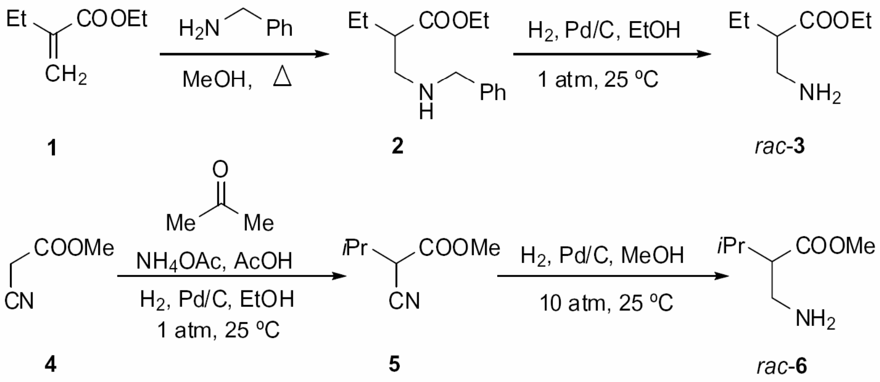


Scheme 1.

an enzyme-catalysed reaction in $i\mathrm{Pr_2O}$ (0.05 M solution) by the addition of vinyl acetate (2 equiv) in the presence of different lipase preparations (50 mg/mL) at 25 °C. The lipases tested were CAL-A, CAL-B (Lipolase), and lipases AY, AK, PS and PPL. CAL-A and CAL-B were previously used in the resolution of α-methyl-β-alanine ethyl ester[23] and β-substituted analogues;[19,29] CAL-B also catalyses N-acylation and interesterification. Our preliminary experiments indicated that the CAL-B-catalysed reaction led rapidly to multiple products, while the enantiomeric excess of the substrate remained low. During the enzyme screening, the enantioselectivities were under 2 in all the cases (conversions between 43% and 58%), except for CAL-A, which displayed a slightly higher enantioselectivity ($E = 4$), and accordingly was chosen for further optimisation.

In order to find a more appropriate acyl donor for the CAL-A-catalysed (50 mg/mL) resolution, six achiral esters (ethyl butanoate, butyl butanoate, vinyl butanoate, 2,2,2-trifluoroethyl butanoate, vinyl acetate and 2,2,2-trifluoroethyl chloroacetate) were tested. The reactions were started in a 0.05 M $i\mathrm{Pr_2O}$ solution of (±)-**3** at room temperature (25 °C) by the addition of 0.55 or 1 equiv of an acyl donor. The selectivities were low (E up to 6); the most promising E values were obtained when using butanoates. As a result of the low E values, new reactions were started at 4 °C (cold room) using 0.55 equiv of ethyl butanoate and 2,2,2-trifluoroethyl butanoate. The results led us to continue our studies with ethyl butanoate.

Table 1. CAL-A-catalysed (25 mg/mL) N-acylation of (±)-**3** (0.05 M) with ethyl butanoate (0.55 equiv) in different solvents at 4 °C

Solvent	Time (day)	ee$_s$[a] (%)	ee$_p$[b] (%)	Conv. (%)	E
MeCN	25	45	>99	26	>200
tert-AmOH	25	68	>99	40	>200
DCM	25	4	40	9	2
THF	25	8.7	45	16	2.9
$\mathrm{Et_2O}$	3	14	24	37	2
$i\mathrm{Pr_2O}$	10	52	58	47	6

[a] According to GC after derivatisation with trifluoroacetic anhydride in the presence of 4-dimethylaminopyridine and pyridine.

[b] Calculated by using an internal standard (octadecane).

We next optimised the solvent, with reactions in MeCN, *tert*-amyl alcohol (*tert*-AmOH), DCM, THF, $\mathrm{Et_2O}$ and $i\mathrm{Pr_2O}$, the most commonly used organic solvents for lipase-catalysed resolutions (Table 1). The reactions were highly enantioselective ($E > 200$), but extremely slow in polar solvents (MeCN and *tert*-amyl alcohol). The other solvents proved to be useless due to their low selectivity.

In an attempt to shorten the reaction time, a higher reaction temperature and an increased amount of enzyme were applied. At 25 °C, the selectivity of the reaction was lowered dramatically, both in *tert*-amyl alcohol and in MeCN. An increased amount of enzyme (50 mg/mL) accelerated the reaction while E decreased (Table 2).

Table 2. Effects of temperature and enzyme amount on CAL-A-catalysed resolution of (±)-**3**[a]

Temperature (°C)	Solvent	Enzyme amount (mg/mL)	Time (day)	ee$_s$[b] (%)	ee$_p$[c] (%)	Conv. (%)	E
4	MeCN	25	25	45	>99	26	>200
	tert-Amyl alcohol	25	25	68	>99	40	>200
		50	14	80	93	46	63
25	MeCN	25	21	30	67	32	17
	tert-Amyl alcohol	25	7	50	83	37	18
		50	3	49	77	39	12

[a] 0.05 M substrate, ethyl butanoate (0.55 equiv).

[b] According to GC after derivatisation with trifluoroacetic anhydride in the presence of 4-dimethylaminopyridine and pyridine.

[c] Calculated by using an internal standard (octadecane).

Another possibility to enhance the reaction rate is the use of various additives, which can also change the selectivity of lipase-catalysed reactions, especially in the case of lipase PS.[30–32] Selected additives ($CuCl_2$ 0.1 equiv; TEA 0.1 equiv; and 15-Crown-5, 0.33 equiv) were tested with lipase PS (25 mg/mL) under the above-described conditions in *tert*-amyl alcohol at 4 °C. However, both the reaction rate and the selectivity were low in all the cases (31–61% conversion after 10–28 days; E: 2–4). The same additives were next used in CAL-A-catalysed (25 mg/mL) reactions, when these lowered not only the selectivity, but also the reaction rate: the reactions reached 36–44% conversion after 35 days, while E was between 20 and 74. Unfortunately, LiCl (0.1 equiv) inactivated the CAL-A.

Following the preliminary results, the gram-scale resolutions of (±)-**3** and (±)-**6** were performed in *tert*-amyl alcohol (0.05 M) with 0.55 equiv of ethyl butanoate in the presence of 50 mg/mL CAL-A preparation at 4 °C. The less reactive enantiomers were transformed into their *N*-Boc-protected derivatives (*R*)-**9** and (*R*)-**10**, and separated from butyramides (*S*)-**7** and (*S*)-**8** by column chromatography.

Since the selectivity was relatively low ($E = 9$) in the case of (±)-**6**, the gram-scale reaction was stopped when the product ee was 78%, and the separated enantiomerically enriched substrate (ee = 58%) was then subjected to a second enzymatic reaction under the above conditions. The physical data on the enantiomers prepared are reported in Table 3.

Table 3. Physical data on enantiomers prepared

Compound	Yield (%)[a]	ee (%)	$[\alpha]_D^{25}$
(*S*)-**7**	36	95[b]	+26.0 (*c* 1.0, MeOH)
(*R*)-**9**	25	85[c]	−20.6 (*c* 0.5, MeOH)
(*S*)-**8**	38	78[c]	+26.4 (*c* 1.0, MeOH)
(*R*)-**10**	18	76[c]	−13.4 (*c* 1.0, MeOH)

[a] Maximum yield 50% at 50% conversion.

[b] From a comparison of the specific rotation with that of (*R*)-**7**, synthesised from unreacted (*R*)-**3** with ee = 88%.

[c] Determined by GC.

To determine the selectivity of the enzyme, *N*-Boc-protected amino ester **9** was hydrolysed with $LiOH{\cdot}H_2O$,[33] resulting in 3-*tert*-butoxycarbonylamino-2-ethylpropanoic acid **11**. The specific rotation for **11** was $[\alpha]_D^{25} = -13.2$ (*c* 1.0, CH_2Cl_2); the literature values for (*R*)-**11** {$[\alpha]_D^{26} = -17.3$ (*c* 1.3, MeOH);[33] $[\alpha]_D^{20} = -18.2$ (*c* 1.0, CH_2Cl_2), ee >95%[34]} confirmed the (*S*)-selectivity.

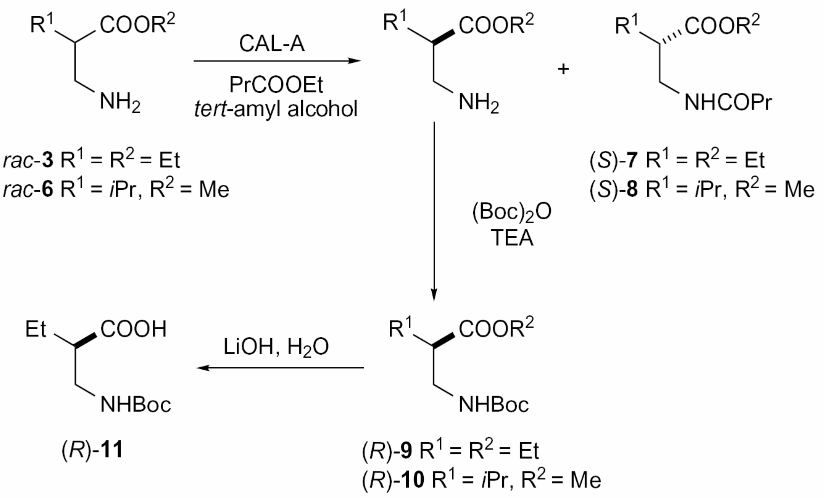
3. Conclusion

In conclusion, ethyl 3-amino-2-ethylpropanoate (±)-**3** and methyl 3-amino-2-isopropylpropanoate (±)-**6** were resolved via *Candida antarctica* lipase-A-catalysed (50 mg/mL) N-acylation in *tert*-amyl alcohol (0.05 M) with 0.55 equiv of ethyl butanoate at 4 °C (Scheme 2). The enzymatic reactions, followed by *N*-Boc protection of the less reactive amino ester enantiomers, led to butyramides (*S*)-**7** and (*S*)-**8** and *N*-Boc-protected amino esters (*R*)-**9** and (*R*)-**10**, which were separated by column chromatography with moderate to good ee values (ee: 76–95%) (Table 3).

4. Experimental

4.1. Materials and methods

CAL-A (*C. antarctica* lipase A, Chirazyme L-5) was purchased from Roche. Lipases AK (*Burkholderia* sp.) and PS (*Burkholderia cepacia*) were from Aldrich. Lipase AY (*Candida rugosa*) was from Amano Pharmaceuticals. CAL-B (from *C. antarctica*, Chyrazime L-2) immobilised on acrylic resin, known by the trade name Lipolase, was from Sigma Aldrich. Before use, CAL-A and lipases PS,



Scheme 2.

AK and AY were adsorbed on Celite in the presence of sucrose.[35] 2,2,2-Trifluoroethyl butanoate, butyl butanoate and 2,2,2-trifluoroethyl chloroacetate were prepared from the corresponding acid chlorides and alcohols. The solvents were of the highest analytical grade. ^1H NMR spectra were recorded on a Bruker AM 400 spectrometer. Optical rotations were measured with a Perkin Elmer 341 polarimeter.

4.2. Preparation of ethyl 3-amino-2-ethylpropanoate, (±)-3

A mixture of ethyl ethacrylate (**1**, 9.00 g, 70.2 mmol) and benzylamine (5.00 g, 46.7 mmol) was refluxed in EtOH (7 mL) for 4 h and then allowed to stand at room temperature for 2 days. The solvent was evaporated off and the residue was distilled to give **2** [7.36 g (31.3 mmol, 68%), bp: 120–135 °C (6 mm Hg)] as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.91 (3H, t, $J = 7.4$ Hz, $C\mathit{H}_3CH_2CH$), 1.26 (3H, t, $J = 7.1$ Hz, $OCH_2C\mathit{H}_3$), 1.51–1.72 (2H, m, $CH_3C\mathit{H}_2CH$), 2.53–2.62 (1H, m, $CH_3CH_2C\mathit{H}$), 2.72 (1H, dd, $J = 4.9$, 11.9 Hz, $C\mathit{H}_2NHCH_2$-Ph), 2.90 (1H, dd, $J = 8.9$, 11.9 Hz, $C\mathit{H}_2NHCH_2Ph$), 3.79 (1H, d, $J = 13.4$ Hz, $C\mathit{H}_2Ph$), 3.85 (1H, d, $J = 13.4$ Hz, $C\mathit{H}_2Ph$), 4.13–4.19 (2H, m, $OC\mathit{H}_2$–CH_3), 7.20–7.40 (5H, m, Ar).

A stirred mixture of ethyl 3-benzylamino-2-methylpropanoate (**2**, 11.00 g, 46.7 mmol), abs. EtOH (80 mL) and Pd catalyst (10 wt % on activated charcoal, 1.00 g) was hydrogenated at ambient temperature at atmospheric pressure for 20 h. The catalyst was then filtered off and washed with EtOH. The combined filtrates were evaporated and the residue was purified by distillation to give *rac*-**3** [3.60 g (24.8 mmol, 53%), bp: 85–90 °C (28 mm Hg)] as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.93 (3H, t, $J = 7.5$ Hz, $C\mathit{H}_3CH_2CH$), 1.27 (3H, t, $J = 7.1$ Hz, $OCH_2C\mathit{H}_3$), 1.50–1.73 (2H, m, $CH_3C\mathit{H}_2CH$), 2.36–2.45 (1H, m, $CH_3CH_2C\mathit{H}$), 2.85 (1H, dd, $J = 4.8$, 12.9 Hz, $C\mathit{H}_2NH$), 2.96 (1H, dd, $J = 8.4$, 12.9 Hz, $C\mathit{H}_2NH$), 4.13–4.22 (2H, m, $J = 7.1$ Hz, $OC\mathit{H}_2CH_3$).

4.3. Preparation of methyl 3-amino-2-isopropylpropanoate, (±)-6

A stirred mixture of methyl cyanoacetate **4** (10.00 g, 100.9 mmol), abs. acetone (8.1 mL, 109.8 mmol), NH_4OAc (0.78 g, 10.0 mmol), glacial AcOH (1.15 mL, 19.7 mmol) and EtOH (20 mL) was hydrogenated at ambient temperature and atmospheric pressure in the presence of Pd catalyst (5 wt % on activated charcoal, 0.40 g) for 5 h. The catalyst was then filtered off and the solvent evaporated off. The crude oily product **5** was purified by distillation [8.72 g (61.8 mmol, 61%), bp: 112–115 °C (28 mm Hg)]. The ^1H NMR spectrum of **5** was in accordance with the literature[36] data.

A stirred mixture of methyl 2-cyano-3-methylbutanoate **5** (8.70 g, 0.06 mol), MeOH (60 mL), 36% aqueous HCl (3.15 mL) and Pd catalyst (5 wt % on activated charcoal, 0.50 g) was hydrogenated at ambient temperature at 10 atm for 20 h. The catalyst was then filtered off and washed with MeOH and the filtrate was evaporated. The oily residue was dissolved in water (150 mL) and washed with EtOAc (3 × 30 mL). The aqueous phase was made alkaline with concd NH_3 solution and extracted with EtOAc (3 × 70 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated. The oily residue was converted to the crystalline hydrochloride salt *rac*-**6**·HCl by treatment with 22% ethanolic HCl and Et_2O. Recrystallisation of the crude product from a mixture of MeOH and Et_2O resulted in *rac*-**6**·HCl [1.34 g (7.4 mmol, 15%), mp: 144–145 °C] as colourless crystals. ^1H NMR (400 MHz, D_2O): δ (ppm) 0.96 (6H, d, $J = 6.9$ Hz, $(C\mathit{H}_3)_2CH$), 2.13 (1H, m, $(CH_3)_2C\mathit{H}$), 2.73 (1H, m, $(CH_3)_2CHC\mathit{H}$), 3.19 (1H, td, $J = 3.5$, 13.1, $C\mathit{H}_2NH$), 3.34 (1H, ddd, $J = 1.9$, 10.3, 13.1 Hz, $C\mathit{H}_2NH$), 3.79 (3H, s, $OC\mathit{H}_3$).

Amino ester base *rac*-**6** was obtained from the above hydrochloride salt by treatment with NH_3 solution, extraction (EtOAc) and evaporation.

4.4. Small-scale resolutions

In a typical small-scale experiment, the acyl donor (0.1 M) was added to a mixture of racemic amino ester (0.05 M) and enzyme preparation (25 or 50 mg/mL) in an organic solvent (1 mL), and the reaction mixture was then shaken at 4 °C (cold room) or at 25 °C (room temperature). The progress of the reaction and the ee values were determined by taking samples (0.05 mL) at intervals, filtering off the enzyme and analysing them by GC, on a Chrompack CP-Chirasil-DEX CB column (25 m). For a good baseline separation, the unreacted amino group was acylated with acetic or trifluoroacetic anhydride in the presence of pyridine containing 1% 4,4-dimethylaminopyridine.

Since the enantiomers of (±)-**3** could be separated by GC only as the trifluoroacetamide, but not as the *N*-acetyl, *N*-chloroacetyl, *N*-propionyl or *N*-butyryl derivatives, octadecane was used as the internal standard to follow the N-acylation of (±)-**3**.

4.5. Preparative-scale resolution of ethyl 3-amino-2-ethylpropanoate, (±)-3

Racemic **3** (0.80 g, 6.88 mmol) was dissolved in *tert*-amyl alcohol (70 mL). The CAL-A preparation (5.40 g) and ethyl butanoate (0.50 mL, 3.78 mmol) were added in order to start the reaction, with shaking at 4 °C. The reaction mixture was stopped after 42 days, at 52% conversion, by filtering off the enzyme. Evaporation of the solvent resulted in a mixture of (*R*)-**3** (ee = 88%) and (*S*)-**7** (ee = 95%).

Half of the residue was dissolved in MeCN (15 mL). Next TEA (0.14 g, 1.4 mmol, 0.19 mL) and di-*tert*-butoxycarbonyl (0.23 g, 1.05 mmol) were added and the reaction mixture was stirred at room temperature overnight. After evaporation and separation on silica [acetone/hexane (1:19)], butyramide (*S*)-**7** {260 mg (1.21 mmol, 36%); $[\alpha]_D^{25} = +26.0$ (*c* 1.0, MeOH); ee = 95%} and the *N*-Boc-protected amino ester (*R*)-**9** {210 mg (0.86 mmol, 25%); $[\alpha]_D^{25} = -20.6$ (*c* 0.5, MeOH); ee = 85%} were obtained as colourless oils. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) for (*S*)-**7**: 0.94 (6H, m, $C\mathit{H}_3CH_2CH + COCH_2CH_2C\mathit{H}_3$), 1.26 (3H, t, $J = 7.10$ Hz, $OCH_2C\mathit{H}_3$), 1.55–1.70 (4H, m, CH_3-

$CH_2CH + COCH_2CH_2CH_3$), 2.13 (2H, t, $J = 7.46$ Hz, $COCH_2CH_2CH_3$), 2.55 (1H, m, CH_3CH_2CH), 3.35 (1H, m, CH_2NH), 3.51 (1H, m, CH_2NH), 4.16 (2H, q, $J = 7.20$ Hz, OCH_2CH_3), 5.89 (1H, s, NH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for (*R*)-**9**: 0.94 (3H, t, $J = 7.46$ Hz, CH_3CH_2CH), 1.26 (3H, t, $J = 7.10$ Hz, OCH_2CH_3), 1.43 (9H, s, $(CH_3)_3O$), 1.53–1.69 (2H, m, CH_3CH_2CH), 2.52 (1H, m, CH_3CH_2CH), 3.23–3.35 (2H, m, CH_2NH), 4.17 (2H, q, $J = 7.13$ Hz, OCH_2CH_3), 4.85 (1H, br s, NH).

The other half of the residue was dissolved in EtOAc (30 mL) and extracted with 4% aqueous HCl (20 mL). The aqueous phase was basified with aqueous KOH (8%) and the free amino ester (*R*)-**3** was extracted with EtOAc (3 × 30 mL). The organic phase was dried over Na_2SO_4 and evaporated. This residue was used without further purification to synthesise butyramide (*R*)-**7**, the antipode of (*S*)-**3** obtained from enzymatic resolution. The butyrylation was carried out in pyridine (8 mL) with butanoic anhydride (1.80 mL, 11.2 mmol; 2 equiv). The reaction mixture was stirred overnight at room temperature. The next day, 20 mL of toluene was added to accelerate the evaporation. Purification by column chromatography [acetone/hexane (2:8)] gave (*R*)-**7** {260 mg (1.21 mmol, 89%); $[\alpha]_D^{25} = -24.0$ (*c* 1.0, MeOH); ee = 88%; ee did not decrease during the reaction} as a colourless oil. The comparison of the specific rotations of (*R*)-**7** and (*S*)-**7** confirmed the calculations involving the use of an internal standard, which gave ee (*S*)-**7** = 95%. The ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) data for (*R*)-**7** were similar to those for (*S*)-**7**.

4.6. Preparative-scale resolution of methyl 3-amino-2-isopropylpropanoate, (±)-6

Racemic **6** (0.60 g, 4.8 mmol) was dissolved in *tert*-amyl alcohol (80 mL). CAL-A preparation (4.80 g) and ethyl butanoate (0.36 mL, 2.7 mmol) were added and the reaction mixture was shaken at 4 °C. The reaction was stopped after 26 days, at 40% conversion (ee (*R*)-**6** = 52%, ee (*S*)-**8** = 78%), by filtering off the enzyme. After evaporation, the residue was dissolved in 4% aqueous HCl and extracted with EtOAc to obtain butyramide (*S*)-**8** {390 mg (1.8 mmol, 38%); $[\alpha]_D^{25} = +26.4$ (*c* 1.0, MeOH); ee = 78%} as a colourless oil. Aqueous KOH (8%) was added to the aqueous phase and the free amino ester (*R*)-**6** was extracted with EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for (*S*)-**8**: 0.94 (9H, m, $(CH_3)_2CH + COCH_2CH_2CH_3$), 1.64 (2H, m, $COCH_2CH_2CH_3$), 1.97 (1H, m, $(CH_3)_2CH$), 2.12 (2H, t, $J = 7.42$ Hz, $COCH_2CH_2CH_3$), 2.47 (1H, m, $(CH_3)_2CHCH$), 3.35 (1H, m, CH_2NH), 3.59 (1H, m, CH_2NH), 3.70 (3H, s, OCH_3), 5.87 (1H, br s, NH).

The enantiomerically enriched substrate (*R*)-**6** (250 mg, 1.7 mmol) was subjected to enzymatic reaction (without further purification) under the above conditions. After 24 days (ee (*R*)-**6** = 76%, ee (*S*)-**8** = 56%), the reaction was stopped by filtering off the enzyme. After evaporation, the free amino ester was transformed into the *N*-Boc-protected form by the method described above and (*R*)-**10** {210 mg (0.86 mmol, 18%); $[\alpha]_D^{25} = -13.4$ (*c*1.0, MeOH); ee = 76%} was separated from (*S*)-**8** by column chromatography [acetone/hexane (1:19)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for (*R*)-**10**: 0.96 (6H, d, $J = 6.88$ Hz, $(CH_3)_2CH$), 1.44 (9H, s, $(CH_3)_3CO$), 1.96 (1H, m, $(CH_3)_2CH$), 2.43 (1H, m, $(CH_3)_2CHCH$), 3.24 (1H, m, CH_2NH), 3.41 (1H, m, CH_2NH), 3.70 (3H, s, OCH_3), 4.81 (1H, br s, NH).

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