University of Szeged

Faculty of Pharmacy

Institute of Pharmaceutical Chemistry

ENZYMATIC RESOLUTION OF TETRAHYDROISOQUINOLINE DERIVATIVES IN BATCH AND CONTINUOUS-FLOW SYSTEMS

Ph.D. Thesis

László Schönstein

Supervisors:
Prof. Dr. Enikő Forró
Prof. Dr. Ferenc Fülöp

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Tetrahedron: Asymmetry **2011**, *22*, 1255-1260.

II. **L. Schönstein**, E. Forró, F. Fülöp
Continuous-flow enzymatic resolution strategy for the acylation of amino alcohols with a remote stereogenic centre: synthesis of calycotomine enantiomers
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Abbreviations

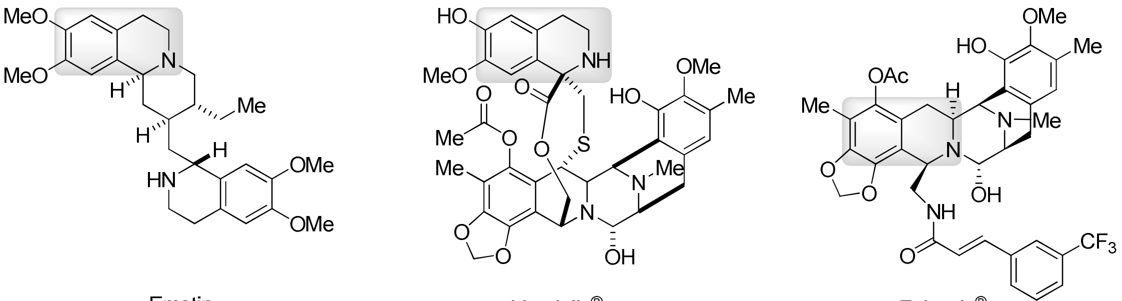
Ac	acetyl
AOL	*Aspergillus oryzae* lipase
atm.	atmosphere
Bn	benzyl
Boc	*tert*-butoxycarbonyl
Bz	benzoyl
CAL	*Candida antarctica* lipase
CAL-A	*Candida antarctica* lipase A
CAL-B	*Candida antarctica* lipase B
Cbz	carboxybenzoyl
CCL	*Candida cylindracea* lipase
CF	continuous-flow
CL-10	Chirazyme® L-10
Conv.	conversion
DMAP	4-dimethylaminopyridine
E	enantioselectivity
ee	enantiomeric excess
ee_p	enantiomeric excess of product
ee_s	enantiomeric excess of substrate
equiv.	equivalent
HPLC	high-performance liquid chromatography
*i*PA	isopropenyl acetate
lipase AK	*Pseudomonas fluorescens* lipase
lipase AY	*Candida rugosa* lipase
lipase PS	*Burkholderia (Pseudomonas) cepacia* lipase
lipase PS IM	*Burkholderia (Pseudomonas) cepacia* lipase
LQL	lipase QL from *Alcaligenes* species
Poc	phenyloxycarbonyl
PPL	porcine pancreas lipase
t	reaction time
T	temperature
VA	vinyl acetate
VB	vinyl butanoate
VD	vinyl decanoate
VL	vinyl laurate
VP	vinyl pivalate
VPr	vinyl propanoate

1. Introduction and aims

Tetrahydroisoquinoline derivatives are important members of the family of naturally-occurring alkaloids,[1] and are also of great importance in synthetic chemistry and drug research in view of their potential pharmaceutical activity.[2]

Some tetrahydroisoquinoline derivatives in enantiomerically pure form are important building blocks in drugs such as the antitussive noscapin,[3] the antitumour agent trabectedin (as Yondelis®)[4] and the expectorant emetin[5] (Figure 1), which is an *Ipecacuanha* alkaloid.[6]

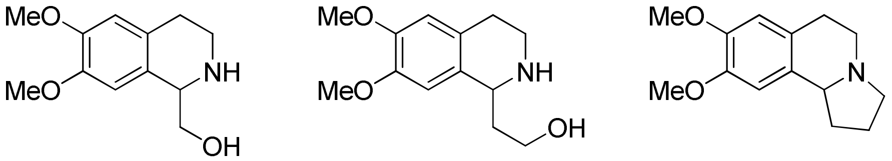
1-Methyl- and 1-phenyltetrahydroisoquinoline are drugs with an important role in the prevention of Parkinson's disease.[7] Another potential drug with a tetrahydroisoquinoline skeleton is Zalypsis® (Figure 1), which is currently undergoing Phase I clinical development for the treatment of solid tumours and haematological malignancies.[8]



Emetin Yondelis® Zalypsis®

Figure 1.

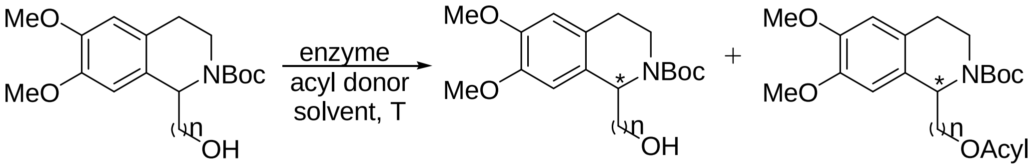
The primary aim of the research work described in my thesis was the development of a new enzymatic strategy for the preparation of both enantiomers of *calycotomine*, *homocalycotomine* and *crispine A* (Figure 2). Two of these compounds are naturally-occurring alkaloids: *calycotomine* was isolated from *Calycotome spinosa*[9] and *crispine A* from *Carduus crispus*.[10] *Crispine A* exhibits high biological activity against the human cancer cell lines SKOV3, KB and HeLa.[10,11] The third desired compound, *homocalycotomine*, is the key intermediate for the preparation of emetin.[6]



Calycotomine **1** *Homocalycotomine* **2** *Crispine A* **3**

Figure 2.

We planned to prepare the enantiopure intermediates of the desired compounds through enzymatic *O*-acylation of the corresponding *N*-*tert*-butoxycarbonyl (*N*-Boc)-protected primary alcohols [(±)-**4**–(±)-**6**] with a remote stereogenic centre in batch and continuous-flow (CF) systems. The CF technique has nowadays become a popular technique, thanks to its many advantages, such as a short reaction time (t), safer solvents, rapid heating and compression.[12,13]



(±)-**4**, n = 1 (*R*) or (*S*)-**4**, n = 1 (*R*) or (*S*)-**7**, n = 1
(±)-**5**, n = 2 (*R*) or (*S*)-**5**, n = 2 (*R*) or (*S*)-**8**, n = 2
(±)-**6**, n = 3 (*R*) or (*S*)-**6**, n = 3 (*R*) or (*S*)-**9**, n = 3

Scheme 1. Preparation of enantiopure compounds

Another aim was a systematic study of the effects of the remote stereogenic centre on the reaction rate and enantioselectivity through the enzyme-catalysed acylation of primary alcohols (±)-**4**–(±)-**6**, when the distance between the stereogenic and reaction centres was one [(±)-**4**], two [(±)-**5**] or three [(±)-**6**] carbon atoms (Scheme 1).

In order to determine the best reaction conditions for the preparative-scale resolutions of these model compounds, a number of preliminary small-scale reactions were planned in batch and CF systems. Thus, the effects of the enzyme, acyl donor, solvent, T and additives on the reaction rate and enantioselectivity (*E*) were investigated in batch reactions in an incubator shaker or in CF reactions in an H-Cube. Furthermore, the effect of pressure was investigated in the CF reactor.

2. Literature

The most important results of the enzymatic acylation of primary alcohols and the effect of the distance between the stereogenic and reaction centres (the latter an OH group) on the reaction rate and *E* are presented in this section. Enzymes commonly used for *O*-acylations are the lipases, which are serine hydrolases. The reaction mechanism, the *serine hydrolase mechanism* of lipase-catalysed *O*-acylation, reported by Brinck and co-workers[14], is outlined in Figure 3.

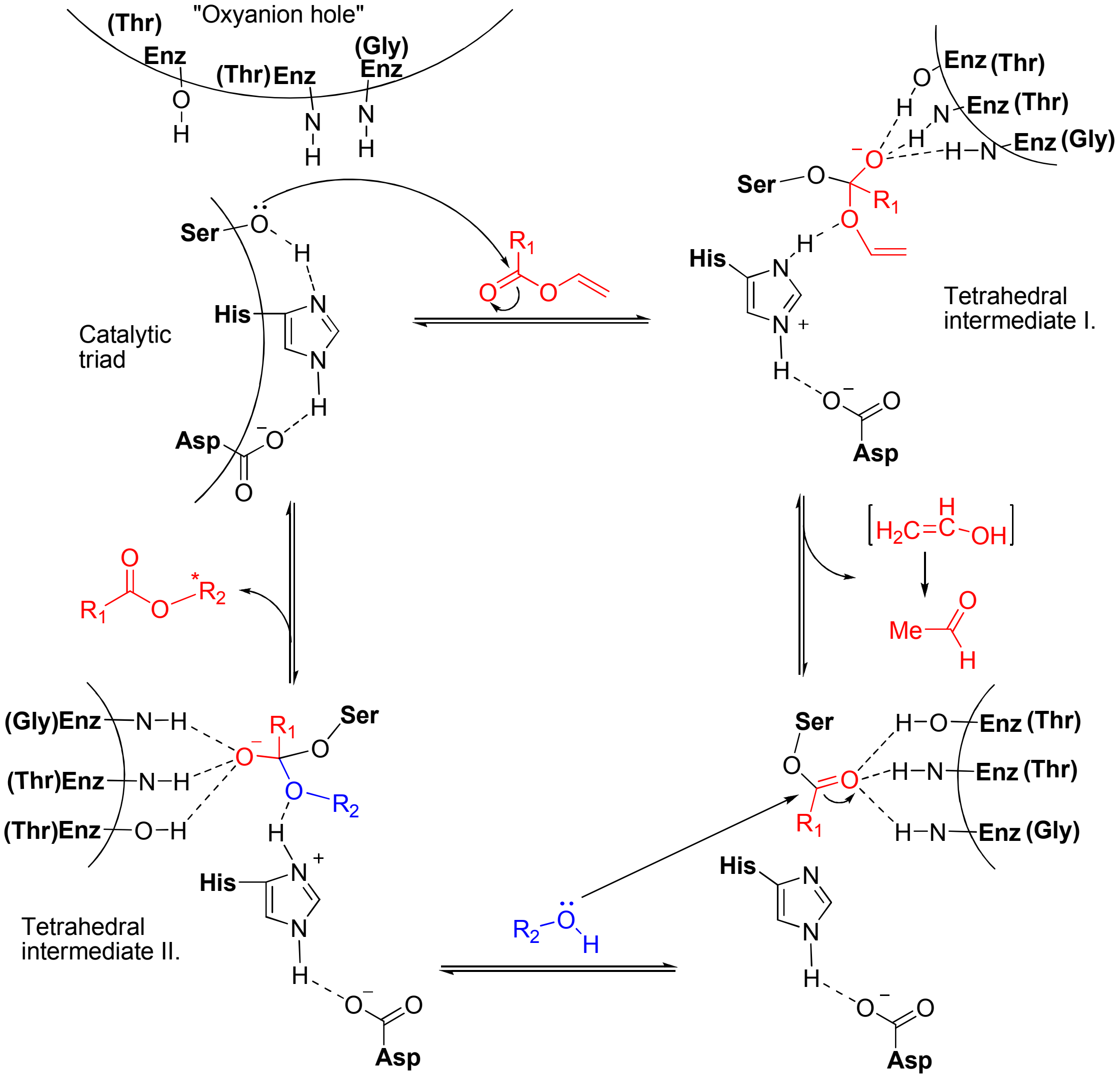


Figure 3. The reaction mechanism of lipase-catalysed *O*-acylation

The most important part of the active site of the lipases, called the catalytic triad, is formed from serine (Ser), histidine (His), asparagine (Asp) or glutamine (Glu) in case of

Geotrichum candidum lipase[15] and the „oxyanion hole”, which has an important role in stabilization of the transition states. In the first step of the enzymatic acylation, the OH group of serine initiates a nucleophilic attack on the C atom of the ester (a vinyl ester in Figure 3) carbonyl group and forms the first tetrahedral intermediate, which is stabilized in an oxyanion hole. Liberation of the leaving group (vinyl alcohol in Figure 3) leads to formation of the “acyl–enzyme” intermediate. Next, the nucleophile alcohol with the non-bonding electron pair attacks the electrophilic centre of the acyl–enzyme intermediate, and forms the second tetrahedral intermediate, which is followed by regeneration of the enzyme and release of the *O*-acylated product.

The enzyme-catalysed acylation of primary alcohols is of great interest and has been intensively studied, which has resulted in a large number of article publications, reviews and books.[16,17] In the literature, the most important enzymatic *O*-acylation reactions are collected, divided up as a function of the distance between the stereogenic and reaction centres.

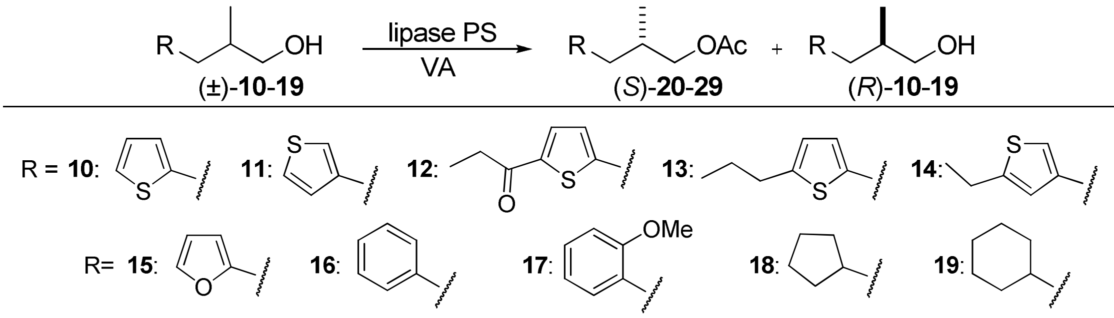
2.1. *Enzymatic O-acylation of primary alcohols with a one-carbon atom distance between the stereogenic and reaction centres*

2.1.1. *Burkholderia cepacia* lipase (lipase PS)

The enzyme most frequently used in the literature for the *O*-acylation of primary alcohols with a one-carbon atom distance between the stereogenic and reaction centres is lipase PS, for which good values of *E* are usually described.

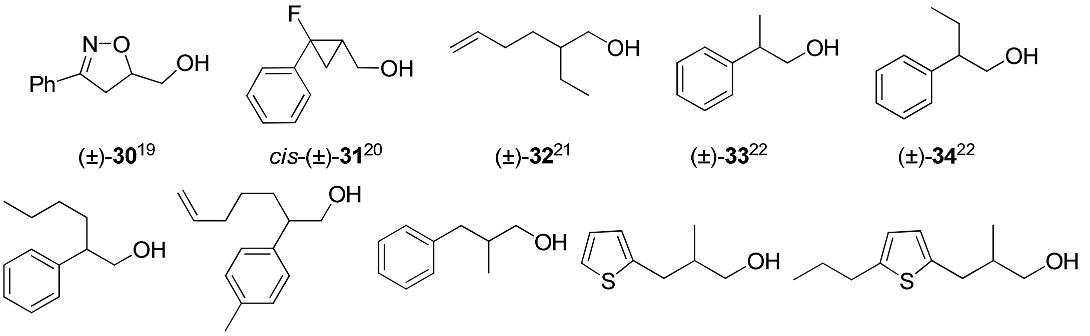
Nordin and co-workers[18] reported on a large study of the lipase PS-catalysed resolution of 3-substituted 2-methylpropan-1-ols [(±)-**10**–(±)-**19**] with vinyl acetate (VA). The reaction conditions and results obtained after 24 h are presented in Table 1. Compounds (±)-**10**–(±)-**17** were resolved with from good to high *E* values (*E* = 67–200), but in the cases of (±)-**18** and (±)-**19** the *E* values were much lower ($E \leq 18$).

Table 1. Lipase PS-catalysed acylation of 3-substituted 2-methylpropan-1-ols



Substrate	Solvent	ee_s (%)	ee_p (%)	Conv. (%)	*E*
(±)-**10**	*t*BuOMe	71.5	97.5	42.2	170
(±)-**11**	$CHCl_3$	61.3	96.6	38.9	108
(±)-**12**	*t*BuOMe	65.5	97.3	40.2	144
(±)-**13**	*t*BuOMe	76	98.5	43.5	> 200
(±)-**14**	*t*BuOMe	61	95.1	38.5	75
(±)-**15**	$CHCl_3$	44	97.1	31	105
(±)-**16**	$CHCl_3$	66.9	96.6	40.9	116
(±)-**17**	*t*BuOMe	66	94.2	41.2	67
(±)-**18**	*t*BuOMe	57.6	81.9	41.2	18
(±)-**19**	*t*BuOMe	53.4	83.2	39.4	18

Sakai et al.[19] presented a noteworthy ‘low-temperature method’ for the enzymatic resolution of 5-hydroxymethyl-3-phenyl-2-isoxazoline [(±)-**30**]. The reaction was performed in the presence of porous ceramic-immobilized lipase PS with VA at low T (-60 °C) in acetone. The reaction was characterised by high *E* (> 200) after 1 h. Further literature data have been found for lipase PS-catalysed resolutions with high enantioselectivity (*E* > 100): for monofluorinated phenylcyclopropane-substituted primary alcohol (±)-**31** and unsaturated primary alcohol (±)-**32**. Aryl-substituted primary alcohols (±)-**33**–**36** and aromatic primary alcohols (±)-**10**, (±)-**16** and (±)-**37** with a one-carbon atom distance from the stereogenic centre were also resolved with *E* > 100 (Figure 4).



(±)-**30**[19] *cis*-(±)-**31**[20] (±)-**32**[21] (±)-**33**[22] (±)-**34**[22]

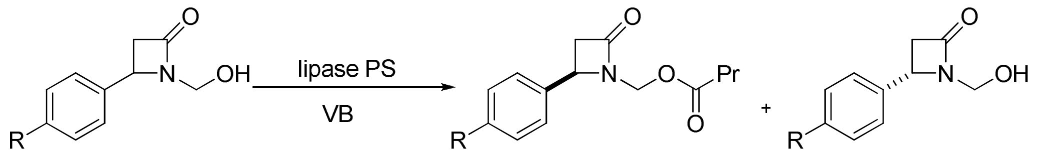
(±)-**35**[22] (±)-**36**[23] (±)-**16**[24] (±)-**10**[25,26,27] (±)-**37**[25]

Figure 4. Further lipase PS-catalysed *O*-acylations with *E* > 100

There have also been literature reports of the lipase PS-catalysed *O*-acylation of primary alcohols with moderate *E* (< 100).[28-42]

Another large group of primary alcohols is the family of *N*-hydroxymethylated β-lactams. The lipase PS-catalysed acylation of the *N*-hydroxymethylated β-lactams resulted in a relatively large range for *E*, depending on the type of subtrate. Forró and Fülöp[43] reported the lipase PS-catalysed *O*-acylation of 1-hydroxymethyl-4-phenyl-2-azetidinone (±)-**38** and 1-hydroxymethyl-4-(*p*-tolyl)-2-azetidinone (±)-**39** (Table 2). The reactions were performed with vinyl butanoate (VB) in toluene at 25 °C; after 1.5 h, the lipase PS-catalysed *O*-acylation of (±)-**38** was characterised by *E* > 200 and that of (±)-**39** by *E* = 57.

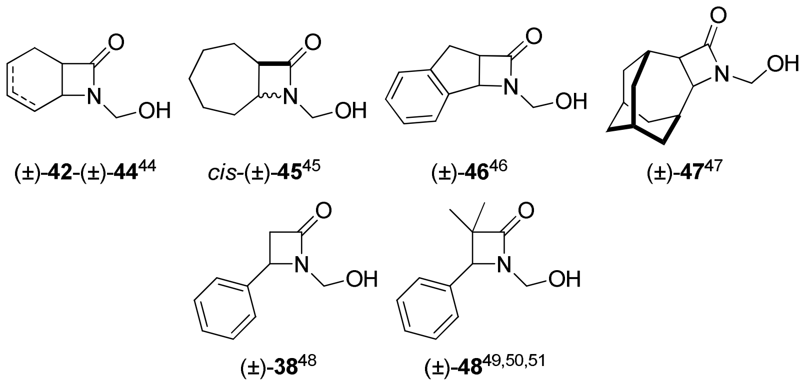
Table 2. Lipase PS-catalysed *O*-acylations



(±)-**38** and (±)-**39** (*R*)-**40** and (*R*)-**41** (*S*)-**38** and (*S*)-**39**

Substrate	R	t (h)	ee_s (%)	ee_p(%)	Conv. (%)	*E*
(±)-**38**	H	1.5	98	97	50	> 200
(±)-**39**	Me	1.5	95	88	52	57

Further lipase PS-catalysed *O*-acylations of carbocyclic *N*-hydroxymethylated β-lactams (±)-**42**–**47** and acyclic *N*-hydroxymethylated β-lactams (±)-**38** and (±)-**48** have also been described in the literature (Figure 5). The enzymatic resolutions of these compounds were performed with excellent *E* values (> 200).



(±)-**42**-(±)-**44**[44] *cis*-(±)-**45**[45] (±)-**46**[46] (±)-**47**[47]

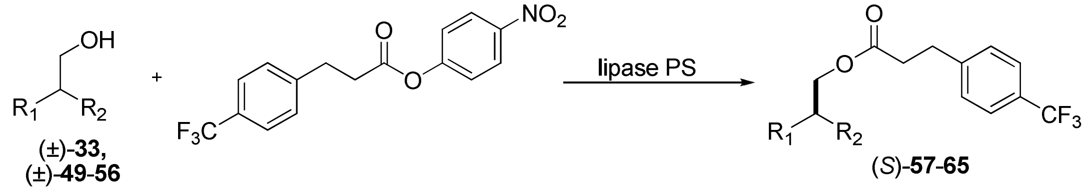
(±)-**38**[48] (±)-**48**[49,50,51]

Figure 5. β-Lactams subjected to lipase PS-catalysed *O*-acylation

More lipase PS-catalysed resolutions are to be found in the literature for *N*-hydroxymethylated β-lactams, with moderate *E* (< 94).[52-55]

A lipase PS-catalysed dynamic kinetic resolution method for primary alcohols (±)-**33** and (±)-**49–56** was presented by Bäckvall and co-workers.[56] The reactions were performed under an argon atmosphere in the presence of 4-nitrophenyl 3-[4-trifluoromethyl-phenyl]propanoate and Shvo's ruthenium catalyst in toluene at 80 °C. After 36 h, the product esters were obtained with good yield and high *ee* (Table 3).

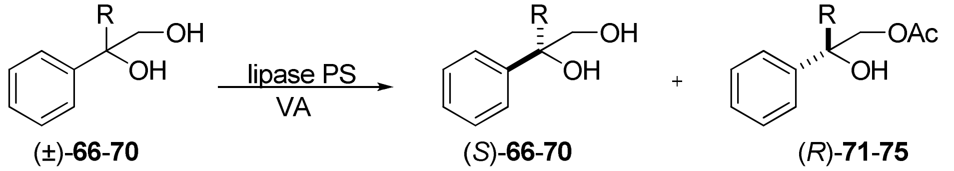
Table 3. Dynamic kinetic resolution of (±)-**33** and (±)-**49–56**



Substrate	R_1	R_2	Yield (%)	*ee* (%)	Substrate	R_1	R_2	Yield (%)	*ee* (%)
(±)-**33**	Ph	Me	87	93	(±)-**53**	4-$MeOC_6H_4$	Me	70	71
(±)-**49**	Ph	Et	80	78	(±)-**54**	4-$NO_2C_6H_4$	Me	85	67
(±)-**50**	Ph	*n*-Pr	70	86	(±)-**55**	4-BrC_6H_4	Me	81	70
(±)-**51**	2-$MeOC_6H_4$	Me	72	> 99	(±)-**56**	cyclohexyl	Me	84	84
(±)-**52**	3-$MeOC_6H_4$	Me	85	83					

Hof and Kellogg[57] reported the lipase PS-catalysed regioselective *O*-acylation of 1,2-diols (±)-**66–70**. The reactions were performed in *i*Pr_2O with VA at room T. For compounds (±)-**66–69**, the reactions were characterised by moderate *E* values, but in the case of (±)-**70** *E* was high (> 200) (Table 4).

Table 4. Lipase PS-catalysed regio- and enantioselective *O*-acylation of 1,2-diols (±)-**66–70**

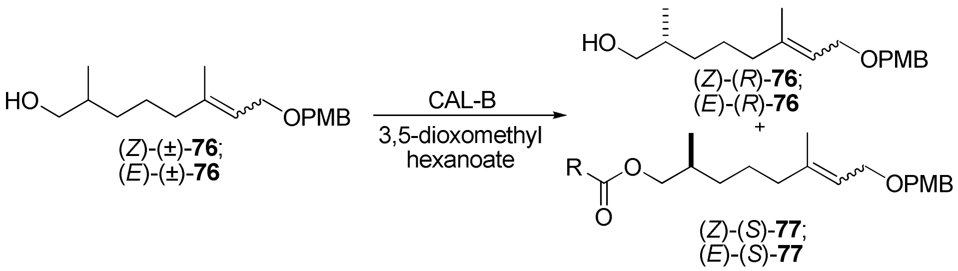


Substrate	R	t (day)	ee_s (%)	ee_p (%)	Conv. (%)	*E*
(±)-**66**	Et	1	1	7	13	1
(±)-**67**	*n*-Pr	1	rac.	rac.	8	1
(±)-**68**	$CH_2CH=CH_2$	7	76	78	49	19
(±)-**69**	$CH_2CH=CHCH_3$	3	94	65	59	16
(±)-**70**	Bn	2	99	93	52	> 200

The literature data on the lipase PS-catalysed *O*-acylation of primary alcohols reflect the good catalytic ability of this enzyme. It is not surprising, therefore, that lipase PS has been used for the *O*-acylation of a large number of compounds, e.g. aryl or alkyl-substituted primary alcohols, and carbocyclic or acyclic *N*-hydroxymethylated β-lactams, with high enantioselectivity (*E* > 100).

2.1.2. *Candida antarctica* lipase B (CAL-B)

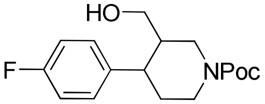
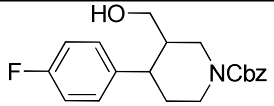
Nanda and Scott[58] developed a CAL-B-catalysed *O*-acylation method for the *E* and *Z* isomers of 8-(4-methoxybenzyloxy)-2,6-dimethyl-oct-6-en-1-ol [(*E*)- and (*Z*)-(±)-**76**] (Scheme 2). The enzymatic reactions were performed at room T, 10 Torr and under an argon atmosphere in the presence of 3,5-dioxomethyl hexanoate. The enantiomeric product alcohol and ester were obtained with *ee* > 93% and *E* = 138. The only difference between the *Z* and *E* isomers was that the *Z* isomer required a longer reaction (11-12 h) to reach the desired conversion of 50% *vs* its *E* counterpart, which needed 8 h for a conversion of 50%.



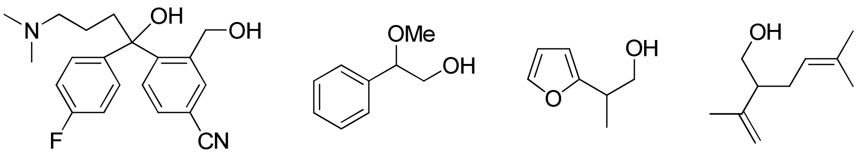
Scheme 2. Enzymatic acylation of (*E*)- and (*Z*)-8-(4-methoxybenzyloxy)-2,6-dimethyl-oct-6-en-1-ol

Gotor and co-workers[59,60,61] described the CAL-B-catalysed enzymatic resolution of *trans*-*N*-phenyloxycarbonyl-4-(4'-fluorophenyl)-3-hydroxymethylpiperidine (±)-**78**[59] and *trans*-*N*-benzyloxycarbonyl-4-(4'-fluorophenyl)-3-hydroxymethylpiperidine (±)-**79**[60] through acylation in toluene (Table 5). The resolution of (±)-**78** was performed in the presence of glutaric anhydride at 30 °C, and after 20 h the unreacted alcohol was obtained with *ee* = 74% and the ester with *ee* = 95%, at an *E* of 84. In the case of (±)-**79**, the acyl donor used was diallyl carbonate at 30 °C, and after 48 h the product ester was obtained with *ee* = 94% and the alcohol with *ee* = 28%, at an *E* of 42.

Table 5. CAL-B-catalysed resolution of *trans*-*N*-protected-4-(4'-fluorophenyl)-3-hydroxymethylpiperidine

(±)-**78**					(±)-**79**				
Acyl donor	*ee*$_s$ (%)	*ee*$_p$ (%)	Conv. (%)	*E*	Acyl donor	*ee*$_s$ (%)	*ee*$_p$ (%)	Conv. (%)	*E*
Glutaric anhydride	74	95	43	84	Diallyl carbonate	28	94	23	42

More literature data on the CAL-B-catalysed acylations of primary alcohols with a one-carbon atom distance between the stereogenic and reaction centres, but with somewhat lower enantioselectivities, are presented in Figure 6. It is noteworthy that in the case of (±)-**80**, which bears a primary and a tertiary OH group, the enzymatic *O*-acylation took place only at the primary OH group of the compound.



(±)-**80**[62,17] *E* = 70

(±)-**81**[63] *E* = 48

(±)-**82**[64] *E* = 20

(±)-**83**[65] *E* = 4.1

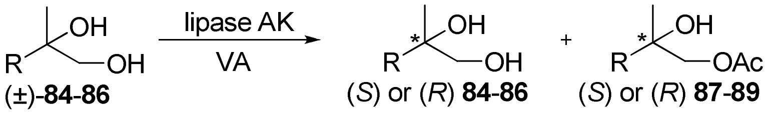
Figure 6. CAL-B-catalysed *O*-acylations

As a conclusion, the limited number of literature data relating to the CAL-B-catalysed *O*-acylation of primary alcohols prove that CAL-B is a less successful enzyme than lipase PS. Only one study has reported a high *E* (> 100) for the CAL-B-catalysed *O*-acylation of primary alcohols.

2.1.3. *Pseudomonas fluorescens* lipase (lipase AK)

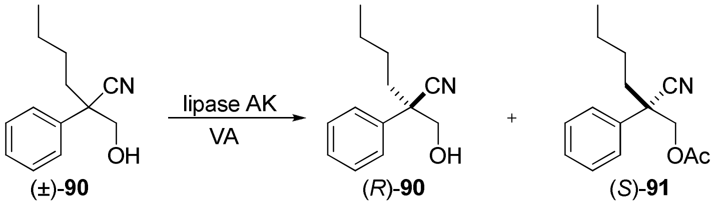
Ferraboschi and co-workers[66] presented the lipase AK-catalysed regio- and enantioselective *O*-acylation of diols (±)-**84**–**86**. The enzymatic reactions were performed under the same conditions: the enzyme was used at 7 mg mL^{-1}, with 4 equiv. of VA, and the reactions were performed in $CHCl_3$ at 30 °C. The regioselective acylation of (±)-**84** was not enantioselective (*E* = 1.3), but in the cases of (±)-**85** and (±)-**86** the reactions were characterised by good *E* (> 79) and the products were obtained with high *ee* values [*ee* = 92% for both (*R*)-**88** and (*R*)-**89,** *ee* = 98% for (*S*)-**85** and *ee* = 92% for (*S*)-**86** (Table 6)].

Table 6. Lipase AK-catalysed acylation of (±)-**84–86**



Substrate	R	Selectivity	t (h)	ee_s (%)	ee_p(%)	Conv. (%)	*E*
(±)-**84**	Ph	*S*	69	10	10	50	1.3
(±)-**85**	Bn	*R*	66	98	92	52	110
(±)-**86**	$(CH_3)_2C{=}CHCH_2$	*R*	15	92	92	50	79

Cheong and co-workers[67] described the lipase AK-catalysed highly enantioselective (*E* > 200) acylation of (±)-2-cyano-2-phenyl-1-hexanol (±)-**90** (Scheme 3). The reaction was performed with VA as acyl donor in anhydrous *n*-hexane/EtOAc (9:1) at 32–34 °C. Interestingly, although *E* was given as > 200, the authors stopped the reaction after 48 h at conversion of 22%, and the product acetate (*S*)-**91** (*ee* = 99%) and the alcohol (*R*)-**90** (*ee* = 28%) were separated.



Scheme 3. Lipase AK-catalysed acylation of (±)-**90**

There have been literature reports on a number of lipase AK-catalysed resolutions with high *E* (> 100), such as the acylation of epoxy alcohol (±)-**92**, *N*-substituted benzyl-*trans*-2-acetoxymethyl-5-(hydroxymethyl)pyrrolidine (±)-**93**, *cis*-4-hydroxymethyl-2-phenyl-1,3-dioxane (±)-**94**, *cis*-4-hydroxymethyl-2-(4-methoxyphenyl)-1,3-dioxane (±)-**95**, 2-methyl-1,3-propanediol monosilyl ethers [(±)-**96** and (±)-**97**], an α-vinyl derivative of alanine (±)-**98** and carbocyclic *N*-hydroxymethylated β-lactam (±)-**46** (Figure 7).

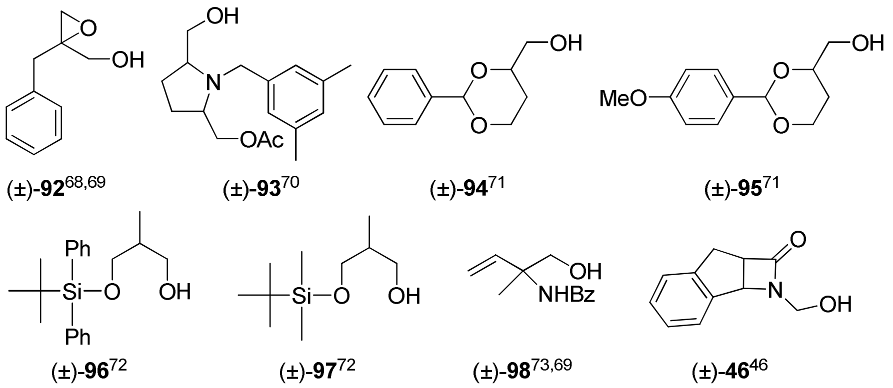

(±)-**92**[68,69] (±)-**93**[70] (±)-**94**[71] (±)-**95**[71]
(±)-**96**[72] (±)-**97**[72] (±)-**98**[73,69] (±)-**46**[46]

Figure 7. Lipase AK-catalysed *O*-acylations

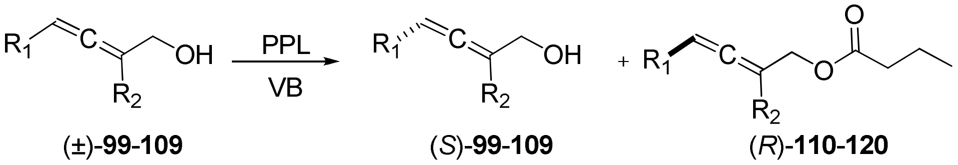
There are also literature data on lipase AK-catalysed resolutions with moderate enantioselectivity (E < 95).[74-79]

To summarize, lipase AK has been used most successfully for the acylation of the primary OH group in saturated heterocyclic substituted methanol derivatives.

2.1.4. Porcine pancreas lipase (PPL)

Only a few studies have been published on the PPL-catalysed acylation of a primary OH. Deska and Bäckwell[80] reported the PPL-catalysed acylation of axially chiral primary allenic alcohols (±)-**99–109** with VB in *i*Pr$_2$O at room T, with very different E values (Table 7). In the cases of (±)-**99**, (±)-**105**, (±)-**106** and (±)-**107**, E > 200.

Table 7. PPL-catalysed acylation of allenic alcohols (±)-**99–109**


(±)-**99-109** (*S*)-**99-109** (*R*)-**110-120**

Substrate	R$_1$	R$_2$	t (h)	ee_s (%)	ee_p (%)	Conv. (%)	E
(±)-**99**	Ph	Me	24	67	98	42	> 200
(±)-**100**	Ph	H	72	n.d.	76	25	9
(±)-**101**	Ph	Et	24	61	97	39	127
(±)-**102**	*o*-Tolyl	Me	30	21	94	19	40
(±)-**103**	*m*-Tolyl	Me	42	74	97	43	176
(±)-**104**	*p*-Tolyl	Me	24	82	95	46	102
(±)-**105**	*p*-ClPh	Me	28	96	97	49	> 200
(±)-**106**	*p*-CF$_3$Ph	Me	30	23	99	10	> 200
(±)-**107**	2-Naphthyl	Me	31	58	98	37	> 200
(±)-**108**	*i*Propenyl	Me	24	n.d.	81	30	13
(±)-**109**	*n*-Pentyl	Me	17	97	n.d.	59	23

Other literature data are to be found for PPL-catalysed resolutions with high enantioselectivity (*E* > 100), e.g. 2-(4-methoxyphenyl)propan-1-ol (±)-**121** and 2-amino-butan-1-ol (±)-**122** (Figure 8).

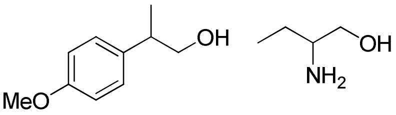

(±)-**121**[81] (±)-**122**[82]

Figure 8. PPL-catalysed acylations

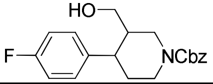
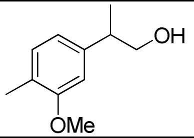
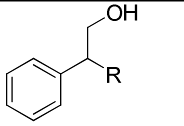
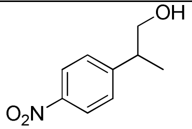
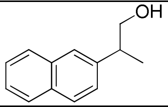
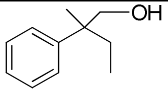
There have also been PPL-catalysed acylations of primary alcohols with somewhat lower enantioselectivities (*E* < 76), e.g. for 2-phenylpropan-1-ol.[83-86]

Thus, PPL has been used successfully with high *E* (> 100) in only a few acylations of a primary OH group.

2.1.5. Other enzymes

Table 8 presents literature results on the enzyme-catalysed acylation of primary alcohols with a one-carbon atom distance between the stereogenic and reaction centres, involving enzymes other than those featuring in sections 2.1.1–2.1.4.

Table 8. Enzyme (CAL-A, AOL and LQL)-catalysed acylations

Substrate		Enzyme	Acyl donor	t (h)	Solvent	ee_s (%)	ee_p (%)	Conv. (%)	*E*
	*trans*-(±)-**79**[87]	CAL-A	VA	10	toluene	88	96	48	143
	(±)-**123**[28]	AOL	VP	34	*t*BuOMe	98	72	58	27
	(±)-**33**[88] R=Me	LQL	*i*PA	0.3	*i*Pr$_2$O	24	14	63	2
	(±)-**124**[88] R=Et			0.8		60	62	49	8
	(±)-**125**[88] R=*i*Pr			2		59	82	42	18
	(±)-**126**[88] R=*n*-Bu			0.8		63	77	45	15
	(±)-**127**[88]	LQL	*i*PA	0.3	*i*Pr$_2$O	5	10	33	1.3
	(±)-**128**[88]	LQL	*i*PA	1.3	*i*Pr$_2$O	49	66	43	8
	(±)-**129**[88]	LQL	*i*PA	1.1	*i*Pr$_2$O	22	27	45	2

It may be seen that a high enantioselectivity ($E > 100$) was obtained only with CAL-A.

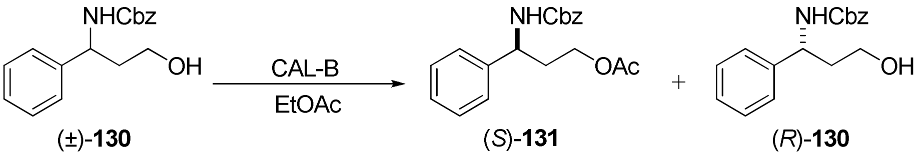
2.2. *Enzymatic O-acylation of primary alcohols with a two-carbon atom distance between the stereogenic and reaction centres*

Very few literature articles have dealt with the enzymatic acylation of primary alcohols with a remote stereogenic centre, i.e. when the distance between the stereogenic and reaction centres is two or more carbon atoms. The low number of such articles might be caused by the fact that the chiral recognition of the enzyme is lower in the case of primary alcohols with a remote stereocentre[16] and the enzymatic acylation proceeds with relatively low *E*.

The present section will deal with the enzymatic acylation of primary alcohols with a two-carbon atom distance between the stereogenic and reaction centres.

2.2.1. *Candida antarctica* lipase B (CAL-B)

Gotor and co-workers[89] described the CAL-B-catalysed *S*-selective acylation of benzyl-(3-hydroxy-1-phenylpropyl)carbonate [(±)-**130**] (Scheme 4). Although the authors performed a detailed optimization for the acylation of (±)-**130** (optimum conditions: EtOAc as acyl donor, *t*BuOMe as solvent and a reaction T of 30 °C), *E* was so low (no value was given) that the authors considered the reaction without practical use.

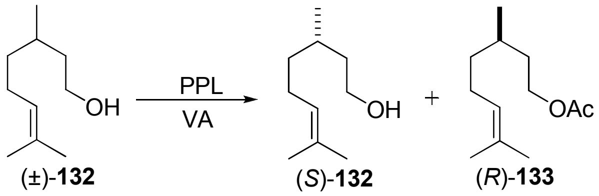


Scheme 4. CAL-B-catalysed *O*-acylation of (±)-**130**

2.2.2. Porcine pancreas lipase (PPL)

Enantiomerically pure citronellol (±)-**132** was prepared by Chattopadhyay and co-workers,[90] through the PPL-catalysed acylation of (±)-3,7-dimethyloct-6-en-1-ol [(±)-**132**] (Scheme 5). The reaction was performed with VA as acyl donor in *n*-hexane in two steps. In the first step, the reaction was stopped after 24 h at 30% conversion, with ee_S =

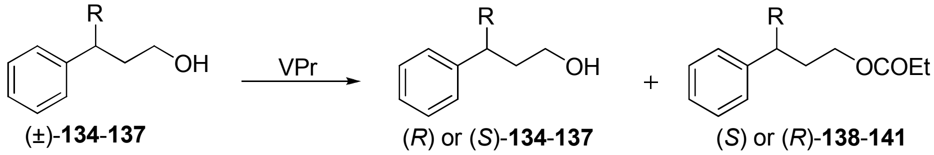
68%, ee_p = 93% and E = 56. The enzyme was filtered off and the product ester and the unreacted alcohol were separated by column chromatography. The enantiomerically enriched alcohol was subjected to a second enzymatic resolution under the same conditions until ee_s reached 92% after 42 h.



Scheme 5. PPL-catalysed *O*-acylation of (±)-**132**

Hedenström and co-workers[91] investigated the effect of lipase, acyl donor, solvent and T on the enzyme-catalysed acylation of primary alcohols (±)-**134–137** (Table 9). Under the optimized conditions [vinyl propanoate (VPr) as acyl donor, $i\mathrm{Pr}_2\mathrm{O}$ as solvent, temperature of -25 °C], the highest enantioselectivity (E = 11.6) for (±)-**137** was observed with PPL (entry 7), while in the cases of (±)-**134** and (±)-**135** the enantioselectivities were slightly higher when the authors used Chirazyme® L-10 (CL-10) (lipase from *Alcaligenes* species) instead of PPL (entries 1-4). The greatest difference in E was observed in the acylation of (±)-**136** [E = 11.3 for CL-10 (entry 6) *vs* E = 1.9 for PPL (entry 5)].

Table 9. Enzyme-catalysed *O*-acylation of (±)-**134–137**

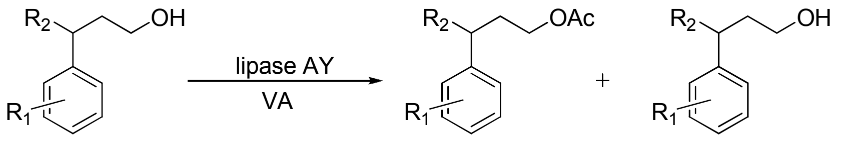


Entry	Substrate	R	Lipase	Enantio-preference	t (h)	ee_s (%)	ee_p (%)	Conv. (%)	E
1	(±)-**134**	Me	PPL	(*R*)-(–)	24	5.6	11	32.5	1.3
2			CL-10	(*S*)-(+)	2	35	44	44.2	3.6
3	(±)-**135**	Et	PPL	(*R*)-(+)	23	19	30	38.7	2.2
4			CL-10	(*S*)-(+)	6	40.9	47	46.5	4.1
5	(±)-**136**	*i*Pr	PPL	(*R*)-(+)	15	15	25	37.5	1.9
6			CL-10	(*R*)-(+)	1.8	39.2	77	33.7	11.3
7	(±)-**137**	*t*Bu	PPL	(*R*)-(+)	155	11.7	82.2	12.4	11.6
8			CL-10	(*R*)-(+)	24	31.6	43.4	42.1	3.4

2.2.3. *Candida rugosa* lipase (lipase AY)

Dogan and co-workers[92] studied the lipase AY-catalysed resolution of (±)-3-halo-3-arylpropanols [(±)-**142–148**] through acylation of the primary OH group (Table 10). Preliminary reactions were performed on the *O*-acylation of the model compound (±)-**142**. The authors determined that lipase AY was the best enzyme, VA the best acyl donor and cyclohexane the best solvent at 20-25 °C. A systematic study of the acylation of (±)-3-halo-3-arylpropanols was carried out under the optimised conditions. After 3 h, the *E* values were very similar (Table 10), even though the different substituents were situated at different positions of the aromatic ring. Bromide (entries 4 and 7) is larger than chloride (entry 3) but this had practically no effect on the enzyme selectivity.

Table 10. Enzymatic *O*-acylation of (±)-**142–148** with lipase AY

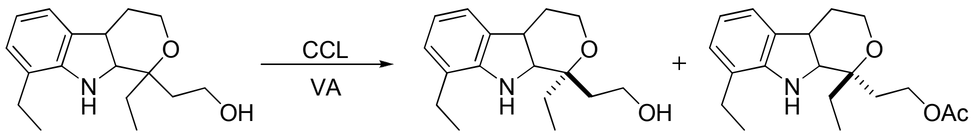


(±)-**142**-**148** (-)-**149**-**155** (+)-**142**-**148**

Entry	Substrate	R_1	R_2	ee_s (%)	ee_p (%)	Conv. (%)	*E*
1	(±)-**142**	H	Cl	29	33	44	2.6
2	(±)-**143**	*p*-F	Cl	21	32	40	2.4
3	(±)-**144**	*p*-Cl	Cl	21	37	39	2.6
4	(±)-**145**	*p*-Br	Cl	23	30	38	2.2
5	(±)-**146**	*o*-F	Cl	25	24	52	2.0
6	(±)-**147**	*o*-Br	Cl	43	36	52	3.1
7	(±)-**148**	H	Br	21	38	38	2.7

2.2.4. *Candida cylindracea* lipase (CCL)

In order to prepare enantiopure etodolac, which has anti-inflammatory and analgesic effects, Brenna and co-workers[93] carried out the CCL-catalysed acylation of (±)-**156**. The optimised reaction conditions for the highest enantioselectivity (*E* = 17) were CCL and VA in *t*BuOMe at room T (Scheme 6). The gram-scale resolution of (±)-**156** was performed under the optimum conditions and after 24 h the acetate (*R*)-**157** was isolated with *ee* = 59% and the unreacted alcohol with *ee* = 33%. To increase the *ee* of the enantiomerically enriched alcohol (*S*)-**156**, the authors performed a second enzymatic acylation of the enantiomerically enriched alcohol under the same conditions, and after 12 h the *ee* was increased to 90%.



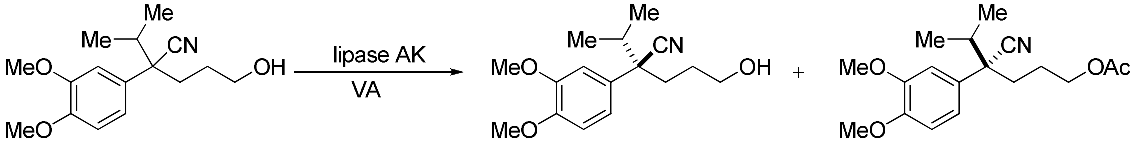
(±)-**156** (*S*)-**156** (*R*)-**157**

Scheme 6. Enzymatic *O*-acylation of (±)-**156**

2.3. ***Enzymatic O-acylation of primary alcohols with a three-carbon atom distance between the stereogenic and reaction centres***

We have found only one literature reference, to the work of Cheong and co-workers[94], in which the authors described the lipase AK-catalysed *O*-acylation of 4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol [(±)-**159**] (Table 11). The highest enantioselectivity (*E* = 13) was observed with lipase AK and VA in a solvent mixture of *n*-hexane/EtOAc 9:1 at 32–34 °C after 1.5 h. To increase *E* the authors investigated some additives (Table 11). When 18-crown-6 was used as additive for the (*S*)-selective *O*-acylation of (±)-**158**, *E* increased considerably (*E* = 49); the *ee* of the unreacted alcohol (*R*)-**158** was found to be 88%, while the *ee* of the product (*S*)-**159** reached 89% (entry 12).

Tabel 11. Effects of additives on the resolution of (±)-**158**[a]



(±)-**158** (*R*)-**158** (*S*)-**159**

Entry	Additives	t (h)	Conv. (%)	(*R*)-Alcohol *ee* (%)	(*S*)-Acetate *ee* (%)	*E*
1	Et_3N (35 µL)	3	30	37	82	14
2	Py (35 µL)	3	66	> 99	52	15
3	Piperidine (35 µL)	3	20	20	78	9.8
4	Piperazine (18 µL)	3	14	14	76	8.4
5	$i$$Pr_2NH$ (35 µL)	4	48	64	76	14
6	Imidazole (15µL)	21	30	12	52	3.6
7	K_2CO_3 (15µL)	2	67	> 99	52	16
8	LiCl (2.5 M, 35 µL)	25	7	6	32	2.1
9	Molecular sieve 4 Å (35 mg)	2	63	> 98	60	17
10	H_2O (35 µL)	21	10	8	84	12
11	NaOAc (15 mg)	2	45	62	74	12
12	18-Crown-6 (10 mg)	1.5	70	88	89	49

[a] lipase AK, VA, 32–34°C, in *n*-hexane

As stated earlier, the lipase-catalysed asymmetric *O*-acylation of primary alcohols with a remote stereogenic centre is a well-known reaction. The articles dealing with the enzymatic acylation of compounds with a one-carbon atom distance between the stereogenic and reaction centres describe high *E* values (usually $E > 200$ at 50% conversion) (section 2.1).[18,21,22,23] When the distance between the stereogenic centre and the OH group is two carbon atoms, the number of literature data is much lower.[89-93] The best result in the literature ($E = 56$) was observed for the PPL-catalysed acylation of (±)-**132** (section 2.2).[90] The number of literature data is extremely low (only one article) when the distance between the stereogenic and reaction centres is three carbon atoms. The best *E* value of 49 was obtained for the lipase AK-catalysed acylation of (±)**-158** (section 2.3).[94]

The literature revealed that lipase PS generally led to high *E* values for the acylation of primary alcohols when the OH group was at a distance of only one carbon atom from the stereogenic centre. CAL-B and PPL were mostly used in cases when the distance between the stereogenic and reaction centres was one or two carbon atoms, with relatively good *E* (> 100) for primary alcohols with a distance of one carbon atom between the stereogenic and reaction centres. Lipase AK was also used successfully for the acylation of primary alcohols when the distance between the stereogenic and reaction centres was one carbon atom. It is noteworthy that lipase AK also yielded relatively high *E* values in the acylation of primary alcohols in which the distance between the stereogenic and reaction centres was three carbon atoms.

A few additional articles are to be found in the literature on enzymatic *O*-acylation reactions in a CF reactor. Thus, Poppe and co-workers reported some lipase-catalysed *O*-acylation reactions of the secondary alcohols 1-phenylethanol,[95] 1-cyclohexylethanol,[95] 1-phenylpropan-2-ol[95] and 2-methylene-substituted cycloalkanols[96] by using a CF reactor. We have found no examples of the enzymatic acylation of primary alcohols in a CF system.

3. Materials and methods

3.1. *Materials and instruments*

The enzymes used were commercially available. CAL-B (lipase B from *Candida antarctica*) immobilized on acrylic resin (L47777) was from Sigma, lipase PS IM (from *Burkholderia cepacia*, immobilised on diatomaceous earth) was from Amano Enzyme Europe Ltd. Lipase PS (*Pseudomonas cepacia*) and PPL (porcine pancreas lipase type II) were from Sigma, and CAL-A (lipase A from *Candida antarctica*) from Novo Nordisk. Lipase AK (*Pseudomonas fluorescens*) was purchased from Amano Pharmaceuticals and lipase AY (*Candida rugosa*) was from Fluka. Before use, lipase PS, lipase AK, lipase AY and CAL-A (5 g) were dissolved in a Tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g). The lipase preparation thus obtained contained 20% (w/w) lipase.[97] The solvents were of the highest analytical grade.

The enzymatic reactions in the CF system were performed in a flow reactor (H Cube® by Thales Nano), while the batch reactions were performed in an incubator shaker (Innova 4000). The analytical measurements were carried out with a high-performance liquid chromatography (HPLC) system equipped with a Jasco PU-2089 Plus quaternary gradient pump and a Jasco MD-2010 Plus multiwavelength detector. The columns used were an amylase-based Chiralpak IA and a cellulose-based Chiralcel OD-H column. The optical rotations of the compounds were measured with a Perkin-Elmer 341 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. The elemental analyses were performed with a Perkin-Elmer 2400 CHNS elemental analyser.

3.2. *Enzymatic experiments*

In general, the enzymatic work was started with optimisation of the reaction conditions of the model compound on a small scale with the batch or the CF system. The preliminary experiments were begun with enzyme screening to determine the best catalyst. After selection of the most appropriate enzyme, the preliminary experiments were continued with determination of the best solvent, acyl donor, additive and T. The experiments performed in the CF system allow the possibility of testing the effects of pressure on *E* and the reaction rate. To verify the usability in a batch reaction of the optimised conditions determined in the CF system, a small-scale reaction was performed in an incubator shaker under the best conditions.

The preliminary experiments were followed by preparative-scale experiments. The product ester and the unreacted alcohol were separated by column chromatography. The product enantiomers were characterised with *ee*, optical rotation, absolute configurations, melting point, NMR and elemental analyses. The absolute configurations were determined by comparison of the optical rotations of the enantiomers or derivatives with the literature data.

3.3. *Analytical methods*

The enzymatic reactions were followed by analytical methods. HPLC with chiral columns was used to determine the *ee* values of the compounds from the reaction mixture (ee_s and ee_p). The conversion and *E* were then calculated via the following equations:

$$\boldsymbol{ee_s = (A_2 - A_1)/(A_1 + A_2)}$$

$$\boldsymbol{ee_p = (A_3 - A_4)/(A_3 + A_4)}$$

$$\textbf{Conv.} = \boldsymbol{ee_s/ee_s + ee_p}$$

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

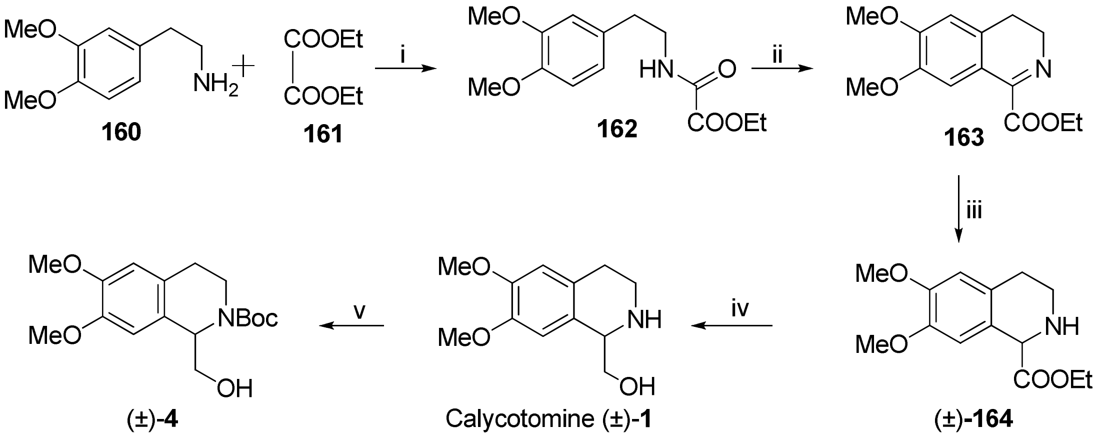
where A_1, A_2, A_3 and A_4 are the areas of peaks and $A_2 > A_1$, $A_3 > A_4$.

The *ee* values for *N*-Boc-protected amino alcohols (±)-**4**–(±)-**6**, esters (±)-**8**, (±)-**9**, (±)-**174** and (±)-**175**, and (±)-triisopropylsilyl-1-(2-hydroxyethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline [(±)-**177**] were determined by HPLC on Chiralpak IA column, while in the cases of (±)-**7** and *crispine A* enantiomers (*R*)-**3** and (*S*)-**3** a Chiralcel OD-H column was used for the enantioseparation.

The exact conditions concerning the eluent, flow rate, detection and retention times for the HPLC analyses can be found in the original papers.[I-IV]

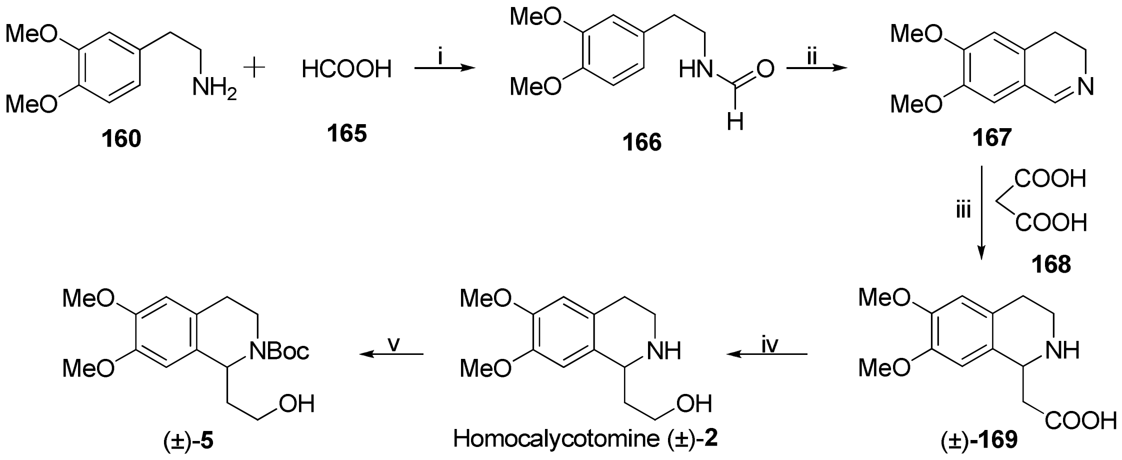
3.4. ***Syntheses of racemic starting materials*** [(±)-**4**–(±)-**6**]

The starting compounds used in the enzyme-catalysed kinetic resolutions were prepared according to literature methods.[98-101] Before the enzymatic acylation reactions, all the prepared amino alcohols (±)-**4**–(±)-**6** were *N*-Boc-protected to avoid the possibility of enzymatic acylation of the NH group. The *calycotomine* (±)-**1** intermediate *N*-Boc-protected 1-hydroxymethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (±)-**4** was prepared from β-(3,4-dimethoxyphenyl)ethylamine (**160**), which was reacted with diethyl oxalate (**161**). The product amide (**162**) was cyclised via a Bischler–Napieralski reaction to give ethyl 6,7-dimethoxy-3,4-dihydroisoquinoline-1-carboxylate (**163**), which was reduced first on Pt/C, and then on $LiAlH_4$. The NH group of the resulting 1-hydroxymethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline [(±)-**1**] was protected with Boc_2O and furnished the desired (±)-**4** (Scheme 7).



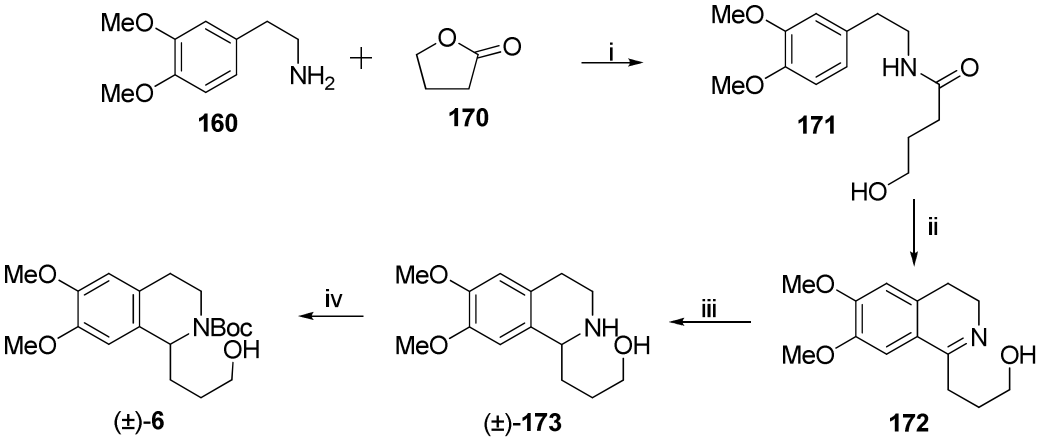
Scheme 7. Preparation of (±)-**4**
(i) 140 °C, 6 h; (ii) $POCl_3$, PhMe, 3.5 h; (iii) H_2, 5% Pt/C, EtOH, 25 atm. 25 °C; (iv) $LiAlH_4$, THF, 5 h; (v) Boc_2O, NaOH, dioxane/H_2O (4:1).

N-Boc-protected 1-(2-hydroxyethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (a *homocalycotomine* intermediate) was prepared according to the literature[100,101], starting with the reaction of homoveratrylamine (**160**) and HCOOH (**165**). The product formamide (**166**) was ring-closed in a Bischler–Napieralski reaction. The 6,7-dimethoxy-3,4-dihydroisoquinoline (**167**) obtained was then transformed into the amino acid (**169**) with malonic acid (**168**). The amino acid (**169**) was reduced with $LiAlH_4$ and the resulting amino alcohol [(±)-**2**] was transformed with Boc_2O to the desired (±)-**5** (Scheme 8).



Scheme 8. Preparation of (±)-**5**
(i) 170 °C, 5 h; (ii) $POCl_3$, PhMe, 1 h; (iii) 120 °C, 45 min;
(iv) $LiAlH_4$, THF, 8 h; (v) Boc_2O, NaOH, dioxane/H_2O (4:1).

The synthesis of (±)-**6** was achieved by a known literature method.[98,102] This started with the reaction of β-(3,4-dimethoxyphenyl)ethylamine (**160**) with γ-butyrolactone (**170**), and the resulting amide (**171**) was then subjected to Bischler–Napieralski cyclisation. The ring-closed 1-(3-hydroxypropyl)-6,7-dimethoxy-3,4-dihydroisoquinoline (**172**) was reduced with $NaBH_4$ to give 1-(3-hydroxypropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline [(±)-**173**]. Finally, *N*-Boc protection with Boc_2O furnished the desired (±)-**6** (Scheme 9).

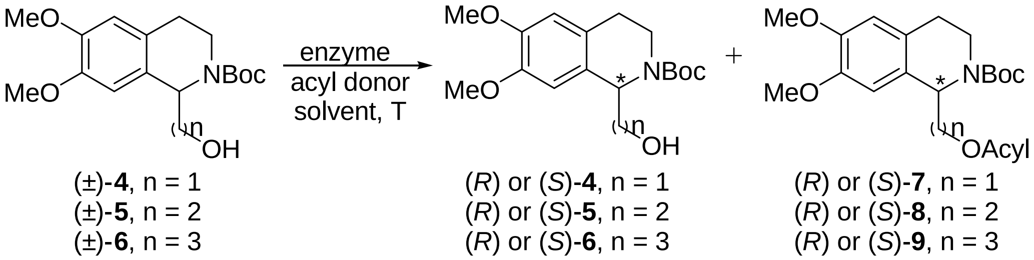


Scheme 9. Preparation of (±)-**6**
(i) 150 °C, 5 h; (ii) $POCl_3$, $CHCl_3$, 3 h; (iii) $NaBH_4$, MeOH, 10% NaOH, 3.5 h;
(iv) Boc_2O, NaOH, dioxane/H_2O (4:1).

4. Results and discussion

4.1. *Small-scale resolutions*

Small-scale preliminary experiments were carried out in order to optimise the lipase-catalysed enzymatic resolutions of (±)-**4**–(±)-**6** (Scheme 10). The preliminary experiments were performed either as reactions in a CF system or as batch reactions.



Scheme 10. Lipase-catalysed *O*-acylation of (±)-**4**–(±)-**6**

The preliminary experiments to determine the optimum conditions for the *O*-acylation of (±)-**4** and (±)-**5** were performed in a CF reactor in an H Cube system in ‘No H_2’ mode (Figure 9). The main components of the CF reactor were the HPLC pump and the heat- and pressure-resistant holder equipped with a 70-mm-long stainless-steel CatCart, filled with different enzymes. The HPLC pump pumped the reaction mixture [the substrate (±)-**4** or (±)-**5** and acyl donor (VA)] through the CatCart and the products were collected and analysed by HPLC.

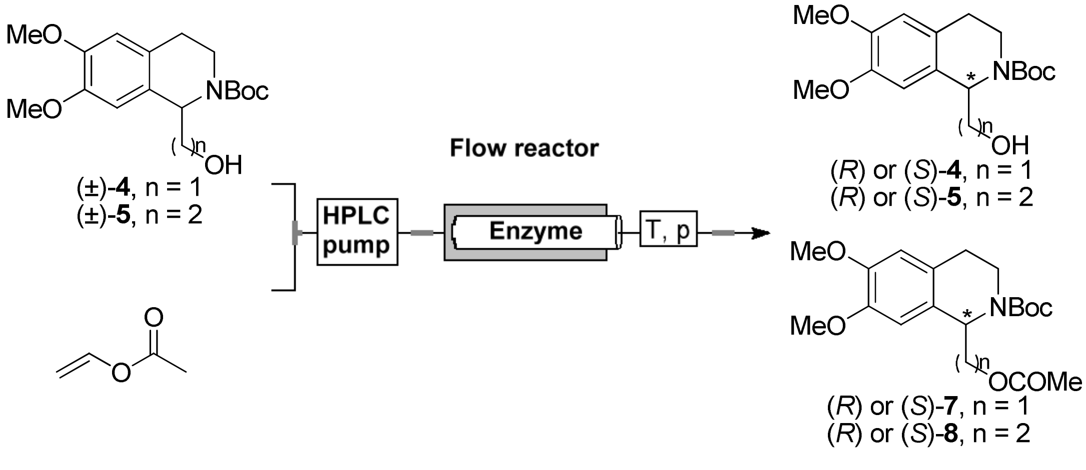
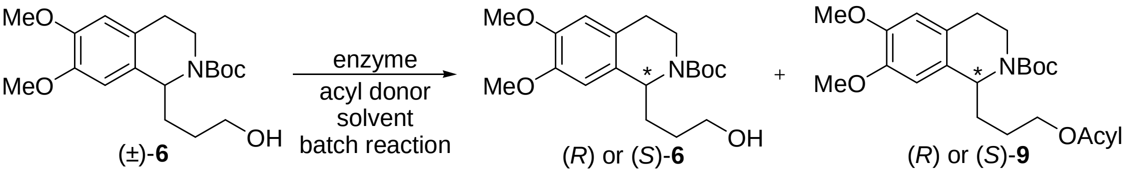


Figure 9. Enzymatic *O*-acylations of (±)-**4** and (±)-**5** in a CF reactor

To determine the best conditions for the enzymatic *O*-acylation of (±)-**6** (Scheme 11), the preliminary reaction was performed as a batch reaction in an incubator shaker.



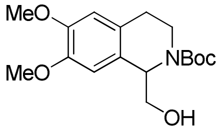
Scheme 11. Enzymatic *O*-acylation of (±)-**6**

In both types of reactions (CF and batch), systematic preliminary experiments were performed to investigate the effects of the enzyme, acyl donor, solvent, T and additives on the conversion and *E*.

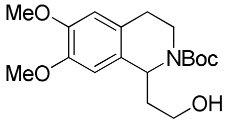
4.1.1. Effect of the enzyme

The preliminary reactions of (±)-**4** and (±)-**5** were started with enzyme screening at 80 bar, 0.1 mL min^{-1} flow rate and in the presence of VA as acyl donor (Table 12). PPL did not catalyse the reaction for either (±)-**4** or (±)-**5** (entry 1), while CAL-A catalysed the acylation of (±)-**4** without *E*, and no reaction was observed in the case of (±)-**5** (entry 2). Lipase PS catalysed the acylation of (±)-**4** with *E* = 3, but in the case of (±)-**5** no reaction was detected (entry 3). Lipase AY was also tried for the acylation of (±)-**4**, but *E* was also low (*E* = 3), and in the case of (±)-**5** lipase AY did not show any activity (entry 4). The best *E* values for both substrates were observed in the presence of CAL-B [*E* > 200 at 35% conversion for (±)-**4** and *E* = 5.2 at 42% conversion for (±)-**5**] (entry 5). The reactions were performed at 45 °C with PPL, CAL-A, lipase PS, lipase AY or lipase AK, and at 60 °C in the presence of CAL-B. Finally, CAL-B was chosen for further preliminary reactions.

Table 12. Enzyme screening for the acylation of (±)-**4**[a] and (±)-**5**[b]



(±)-**4**



(±)-**5**

Entry	Enzyme	(±)-**4** ee_s[c] (%)	(±)-**4** ee_p[c] (%)	(±)-**4** Conv. (%)	(±)-**4** *E*	(±)-**5** ee_s[c] (%)	(±)-**5** ee_p[c] (%)	(±)-**5** Conv. (%)	(±)-**5** *E*
1	PPL	No reaction				No reaction			
2	CAL-A	rac.	rac.	14	1	No reaction			
3	Lipase PS IM	6	54	10	3	No reaction			
4	Lipase AY	4	50	7	3	No reaction			
5	CAL-B	52	99	35	> 200	41	56	42	5.2

[a] 0.012 M substrate, 2 equiv. of VA, 80 bar, 0.1 mL min^{-1} flow rate, *n*-hexane, after one run. [b] 0.012 M substrate, 4 equiv. VA, 80 bar, 0.1 mL min^{-1} flow rate, toluene, after one run. [c] According to HPLC.

To determine the best enzyme for the *O*-acylation of (±)-**6** with VA, several enzymes (CAL-A, CAL-B, lipase AK, lipase AY, lipase PS and PPL) were tested in reactions in an incubator shaker. No reaction (no product was detected) was observed after 24 h with CAL-A, lipase AK, lipase AY or PPL (Table 13, entries 1-4), while with CAL-B the acylation was fast, but the product obtained was racemic and *E* was 1 (entry 5). *E* = 1.6 was observed at 15% conversion only in the case of lipase PS (entry 6). To increase *E*, the preliminary experiments were continued with lipase PS.

Table 13. Enzyme screening for the acylation of (±)-**6**[a]

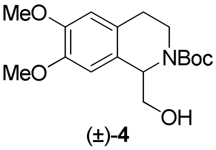
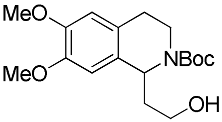
Entry	Enzyme	t (h)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	CAL-A	24	No reaction			
2	Lipase AK	24	No reaction			
3	Lipase AY	24	No reaction			
4	PPL	24	No reaction			
5	CAL-B	1	rac.	rac.	43	1
6	Lipase PS	1	4	22	15	1.6

[a] 0.0125 M substrate, 30 mg mL^{-1} enzyme, 1.1 equiv. of VA, 1 mL *i*Pr_2O, 45 °C. [b] According to HPLC.

4.1.2. Effect of the solvent

To improve the conversion without a decrease in *E* for the *calycotomine* intermediate (±)-**4** and the *homocalycotomine* intermediate (±)-**5,** the effects of solvents were tested (Table 14). In *t*BuOMe, *E* for both reactions was quite low (entry 1). When the acylation of (±)-**4** was performed in *n*-hexane, *E* was excellent (> 200 at 35% conversion), in contrast with (±)-**5**, in which case *E* remained very low (entry 2). In toluene, the conversion of the acylation of (±)-**4** reached 50% after one run (entry 3). The highest *E* for the acylation of (±)-**5** was obtained in toluene (*E* = 22 at 16% conversion) (entry 3). Toluene was, therefore chosen as the best of the solvents tested in the enzyme-catalysed *O*-acylation of (±)-**4** and (±)-**5**, which was in good agreement with literature data.[48]

Table 14. Solvent screening for the acylation of (±)-**4**[a] and (±)-**5**[b]

		 (±)-**4**				 (±)-**5**			
Entry	Solvent	ee_s[c] (%)	ee_p[c] (%)	Conv. (%)	*E*	ee_s[c] (%)	ee_p[c] (%)	Conv. (%)	*E*
1	*t*BuOMe	15	95	14	45	4	5	44	1.1
2	*n*-hexane	52	99	35	> 200	7	26	21	1.8
3	toluene	99	99	50	> 200	17	90	16	22

[a] 0.012 M substrate, CAL-B, 2 equiv. of VA, 80 bar, 60 °C, 0.1 mL min^{-1} flow rate, after one run.
[b] 0.012 M substrate, CAL-B, 4 equiv. of VA, 80 bar, 25 °C, 0.1 mL min^{-1} flow rate, after one run.
[c] According to HPLC.

In order to increase *E* for the lipase PS-catalysed acylation of (±)-**6** with vinyl decanoate (VD), several solvents were tested for the reactions performed as batch reactions: MeCN, toluene, *n*-hexane and *t*BuOMe (Table 15). No reaction was observed when the reaction was performed in MeCN (entry 1), while in toluene *E* = 5 was observed (entry 2). In *n*-hexane, *E* was 20 at a conversion of 23% (entry 4) and in the case of *t*BuOMe, in spite of a slower reaction, ee_p = 95% was achieved at a conversion of 10% and *E* = 43 (entry 5). As *t*BuOMe is considered a green solvent, the preliminary reactions for the acylation of (±)-**6** were continued in *t*BuOMe.

Table 15. Solvent screening for the acylation of (±)-**6**[a]

Entry	Solvent	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	MeCN	No reaction			
2	toluene	5	67	7	5
3	*i*Pr$_2$O	9	85	10	13
4	*n*-hexane	26	88	23	20
5	*t*BuOMe	10	95	10	43

[a] 0.0125 M substrate, 30 mg mL^{-1} lipase PS, 1.1 equiv. of VD, 1 mL solvent, 45 °C, after 1 h. [b] According to HPLC.

4.1.3. Effect of temperature

The effect of T on the conversion and *E* was also investigated. In the case of (±)-**4**, the tested T values were 60 °C, 40 °C and room T (~ 25 ºC). It was observed that *E* remained higher than 200, while the conversion decreased from 50% to 48%, and then to 42% with the decrease of T (Table 16 entries 1-3).

Table 16. Effects of T on *E* and the conversion in the acylation of (±)-**4**[a]

Entry	T (°C)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	60	99	99	50	> 200
2	40	92	99	48	> 200
3	25	71	99	42	> 200

[a] 0.012 M substrate, CAL-B, 2 equiv. of VA, 80 bar, 0.1 mL min^{-1} flow rate, toluene, after one run. [b] According to HPLC.

In an attempt to increase the *E* obtained for the CAL-B-catalysed acylation (VA) of (±)-**5**, the reactions were performed at different T values. The results obtained in this screening are presented in Table 17. *E* increased from 5.2 to 10, and then to 22 with the reduction of T from 60 °C to 45 °C and then to 25 °C, while the conversion decreased from 42% to 16% (entries 1-3). Finally, a reaction was performed as a batch reaction too in an incubator shaker at 3 °C. As supposed, a further decrease in the conversion (11%), but a considerable increase in *E* = 55 was observed (entry 4).

Table 17. T screening for the acylation of (±)-**5**[a,b]

Entry	T (°C)	ee_s[c] (%)	ee_p[c] (%)	Conv. (%)	*E*
1[a]	60	41	56	42	5.2
2[a]	45	17	80	18	10
3[a]	25	17	90	16	22
4[b]	3	12	96	11	55

[a] 0.012 M substrate, CAL-B, 4 equiv. of VA, toluene, 80 bar, 0.1 mL min^{-1} flow rate, toluene, after one run. [b] 0.012 M substrate, 30 mg mL^{-1} CAL-B, 4 equiv. of VA, 1 mL toluene, after 10 min. [c] According to HPLC.

In the investigation of the effect of T on the lipase PS-catalysed acylation (VA) of (±)-**6** in an incubator shaker, only slight differences were observed in the conversion, and there was no beneficial effect on *E* after 1 h (Table 18). In view of the reaction rate, further preliminary experiments were conducted at 45 °C (entry 1).

Table 18. Temperature screening for the acylation of (±)-**6**[a]

Entry	T (°C)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	45	4	22	15	1.6
2	30	4	26	13	1.8
3	2	3	29	9	1.9

[a] 0.0125 M substrate, 30 mg mL^{-1} lipase PS, 1.1 equiv. of VA, 1 mL solvent, 45 °C, after 1 h. [b] According to HPLC.

4.1.4. Effect of the acyl donor

In the CAL-B-catalysed *O*-acylation of (±)-**4** in a CF system in the presence of 2 equiv. VA, *E* was > 200 at 50% conversion after one run (Table 19, entry 1). When the quantity of the acyl donor was increased significantly (30 times more acyl donor), the same result was obtained, *E* remaining higher than 200 at 50% conversion (entry 2).

Table 19. Effects of VA equiv. on *E* and the conversion in the acylation of (±)-**4**[a]

Entry	VA (equiv.)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	2	99	99	50	> 200
2	60	98	99	50	> 200

[a] 0.012 M substrate, CAL-B, 80 bar, 60 °C, 0.1 mL min^{-1} flow rate, toluene, after one run. [b] According to HPLC.

To improve the low *E* obtained in the preliminary experiments for the asymmetric acylation of (±)-**6** with VA in an incubator shaker, the effects of the acyl donors were investigated (Table 20). With isopropenyl acetate (*i*PA), *E* was slightly higher than with VA (entry 2 *vs*. 1). With vinyl pivalate (VP), no reaction was observed (entry 4), but there was a slight increase in *E* when VB was used (entry 3 *vs* 1). The best *E* (13–14) were obtained with the use of long carbon chain acyl donors such as VD and vinyl laurate (VL) (entries 5 and 6). For further preliminary experiments, VD was used as acyl donor.

Table 20. Effects of acyl donor on *E* and the conversion in the acylation of (±)-**6**[a]

Entry	Acyl donor	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	VA	4	22	15	1.6
2	*i*PA	8	35	19	2.2
3	VB	12	71	14	7
4	VP	No reaction			
5	VD	9	85	10	13
6	VL	8	86	7	14

[a] 0.0125 M substrate, 30 mg mL^{-1} lipase PS, 1.1 equiv. of acyl donor, 1 mL of $i\mathrm{Pr_2O}$, 45 °C, after 1 h. [b] According to HPLC.

4.1.5. Effect of the additive

It is known from the literature data that additives potentially have an increasing effect on *E*.[94] In our enzymatic experiments, the effect of the additive was tested on *E* and the reaction rate. In the case of the acylation of (±)-**4**, the effect of an additive was not tested because of the excellent *E* > 200 obtained at 50% conversion.

In the case of the acylation (VA) of (±)-**5**, the best *E* achieved was 55 at 11% conversion. In order to increase *E* and the reaction rate, several additives were tested (Table 21) in reactions in an incubator shaker. Reactions performed with the use of a catalytic amount of alkali salt as additive, or with other additives such as $i\mathrm{Pr_2NEt}$ showed low *E* (entries 1-3), while on the use of $\mathrm{Et_3N}$ both the conversion and *E* were slightly increased (entry 5). The best result (*E* = 88 at 24% conversion when the reaction was carried out at 3 °C with VA in toluene) for the enzymatic acylation of (±)-**5** was obtained when catalytic amounts of $\mathrm{Et_3N}$ and $\mathrm{Na_2SO_4}$ were added to the reaction mixture (entry 5).

Table 21. Effects of additives on *E* and the conversion in the acetylation of (±)-**5**[a]

Entry	Additive	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	LiCl	15	83	15	12
2	KCl	14	86	14	15
3	$i\mathrm{Pr_2NEt}$	41	81	34	14
4	Et_3N	22	96	19	60
5	$Et_3N + Na_2SO_4$	30	97	24	88

[a] 0.012 M substrate, 30 mg mL^{-1}, CAL-B, 1 mL of toluene, 4 equiv. of VA, 3 °C, after 10 min.
[b] According to HPLC.

In the lipase PS-catalysed acylation of (±)-**6** with VD in the presence of Et_3N with Na_2SO_4 as additive, the amount of acyl donor was also tested (Table 22). With 1.1 equiv. of VD and a catalytic amount of additive, *E* was 45 at a conversion of 14% after 1 h (entry 1). When the amount of acyl donor was increased to 3 equiv., the conversion and *E* increased (entry 2). On the use of 4 equiv. of VD, a slight decrease in *E* was observed at 26% conversion (entry 3).

Table 22. Effects of the amount of acyl donor in the presence of $Et_3N + Na_2SO_4$ for the acylation of (±)-**6**[a]

Entry	Amount of acyl donor (equiv.)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	1.1	15	95	14	45
2	3	19	96	17	59
3	4	33	95	26	53

[a] 0.0125 M substrate, 30 mg mL^{-1} lipase PS, VD, 1 mL of solvent, 45 °C, after 1 h. [b] According to HPLC.

4.1.6. Effect of pressure

The use of a flow reactor allows the performance of preliminary reactions at different pressures and gives a possibility for tests of the effects of pressure on the conversion and *E*. The reactions were performed from atmospheric pressure up to 100 bar in 20 bar steps. When the acylation of (±)-**4** was performed in the presence of VA in toluene, *E* was > 200 at all pressures, and the conversion increased from 38% up to 50% at approximately 60 bar (Figure 10) after one run. At 80 and 100 bar, *E* remained excellent (> 200) without change in conversion (50%).

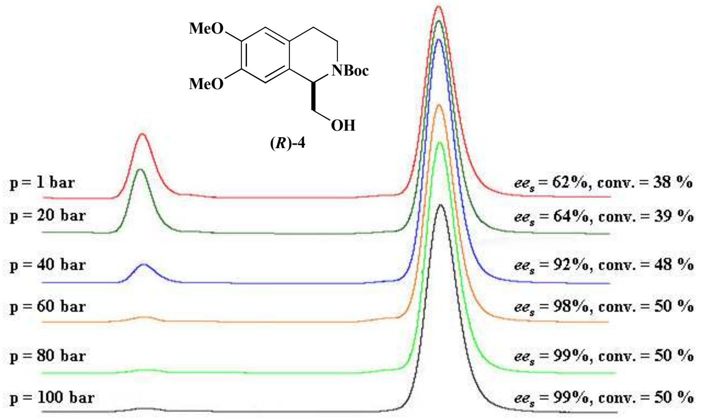


Figure 10. Dependence of *ee* and conversion on pressure

Unfortunately, the acylation of (±)-**5** at different pressures did not lead to an increased *E*, but resulted in similar moderate results (Table 23).

Table 23. Effects of pressure on *E* and the conversion in the acylation of (±)-**5**[a]

Entry	Pressure (bar)	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	1	18	89	17	20
2	20	17	90	16	22
3	40	16	91	15	24
4	60	17	90	16	22
5	80	17	90	16	22
6	100	16	89	15	20

[a] 0.012 M substrate, CAL-B, 4 equiv. of VA, 80 bar, 0.1 mL min^{-1} flow rate, toluene, after one run. [b] According to HPLC.

4.1.7. Other effects

The effects of the substrate concentration on *E* and the reaction rate were also tested in an attempt to increase the efficiency of the asymmetric acylation (VA) of (±)-**4** (Table 24). The acylation of the 5 (0.06 M) and 10 (0.12 M) times more concentrated substrate led in both cases to excellent *E* (> 200) at 50% conversion.

Table 24. Effects of substrate concentration on *E* and the conversion in the acylation of (±)-**4**[a]

Entry	Concentration	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	4 mg mL^{-1} (0.012 M)	99	99	50	> 200
2	20 mg mL^{-1} (0.06 M)	99	99	50	> 200
3	40 mg mL^{-1} (0.12 M)	99	99	50	> 200

[a] CAL-B, 2 equiv. of VA, 80 bar, 60 °C, 0.1 mL min^{-1} flow rate, toluene, after one run.
[b] According to HPLC.

Finally a preliminary small-scale enzymatic reaction of (±)-**4** (0.012 M) was performed with 30 mg mL^{-1} of CAL-B, 2 equiv. of VA in 1 mL of toluene at 60 °C in an incubator shaker, to verify that the optimum conditions found in the CF system were also valid in a batch reaction. The results obtained were very similar to those observed in the CF system. *E* was excellent (> 200) at 50% conversion after 1 h, and further optimization for the acylation of (±)-**4** was therefore not necessary. We decided to perform the preparative-scale resolution of (±)-**4** as a batch reaction.

The best conditions found in the preliminary experiments for the enzymatic *O*-acylation of (±)-**5** were as follows: CAL-B as enzyme in the presence of 4 equiv. of VA and a catalytic amount of Et_3N with Na_2SO_4, in toluene at 3 °C. We therefore planned to carry out the preparative-scale reaction of (±)-**5** under these conditions.

On the basis of the earlier optimization for the acylation of (±)-**6**, its preparative-scale reaction was planned with lipase PS, 4 equiv. of VD and a catalytic amount of Et_3N with Na_2SO_4 in *t*BuOMe at 45 °C.

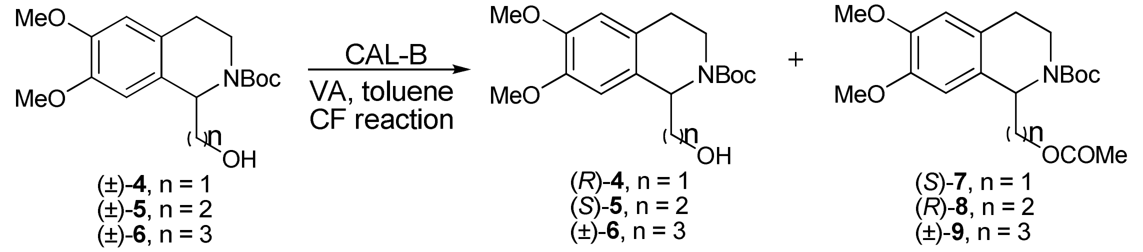
4.2. *Comparison of different distances between the stereogenic and reaction centres*

A systematic study was performed to investigate the effects of a remote stereogenic centre (n = 1, 2, 3) on the conversion and *E* in the enzyme-catalysed *O*-acylation of (±)-**4**–(±)-**6**.

When CAL-A, PPL, lipase AY or lipase AK was used as enzyme, it was practically impossible to distinguish any effect of the distance between the stereogenic and reaction centres (n = 1, 2, 3) either on *E* or on the reaction rate since the extremely slow reactions (7% conversion after one run) were characterized by very low *E* (< 3).

When CAL-B was used as enzyme under the best conditions (VA as acyl donor in toluene at 60 °C, 80 bar and 0.1 mL min^{-1} flow rate after one run) observed in the acylation of *calycotomine* intermediate (±)-**4** in a CF system, the results are presented in the Table 25 were obtained.

Table 25. Enzymatic acylation of (±)-**4**–(±)-**6**[a]



Entry	Substrate	*ee*$_s$[b] (%)	*ee*$_p$[b] (%)	Conv. (%)	*E*
1	(±)-**4**, n = 1	99	99	50	> 200
2	(±)-**5**, n = 2	34	88	28	21
3	(±)-**6**, n = 3	rac.	rac.	~64	1

[a] 0.012 M substrate, CAL-B, 2 equiv. of VA, toluene, 80 bar, 0.1 mL min^{-1} flow rate, after one run.
[b] According to HPLC.

As seen from Table 25, for the *calycotomine* intermediate [(±)-**4**], where the distance between the stereogenic and reaction centres is one carbon atom, *E* was excellent (> 200) at 50% conversion (entry 1) after one run. When the reaction was performed with the *homocalycotomine* intermediate [(±)-**5**], where the distance is two carbon atoms, *E* was significantly lower at a conversion of 28% (entry 2). The lowest *E* was obtained in the case of the greatest distance between the stereogenic centre and the OH group [(±)-**6**, n = 3] (entry 3).

The differences between the *ee*$_p$ and *ee*$_s$ data for the reactions were followed by HPLC and are illustrated in Figure 11.

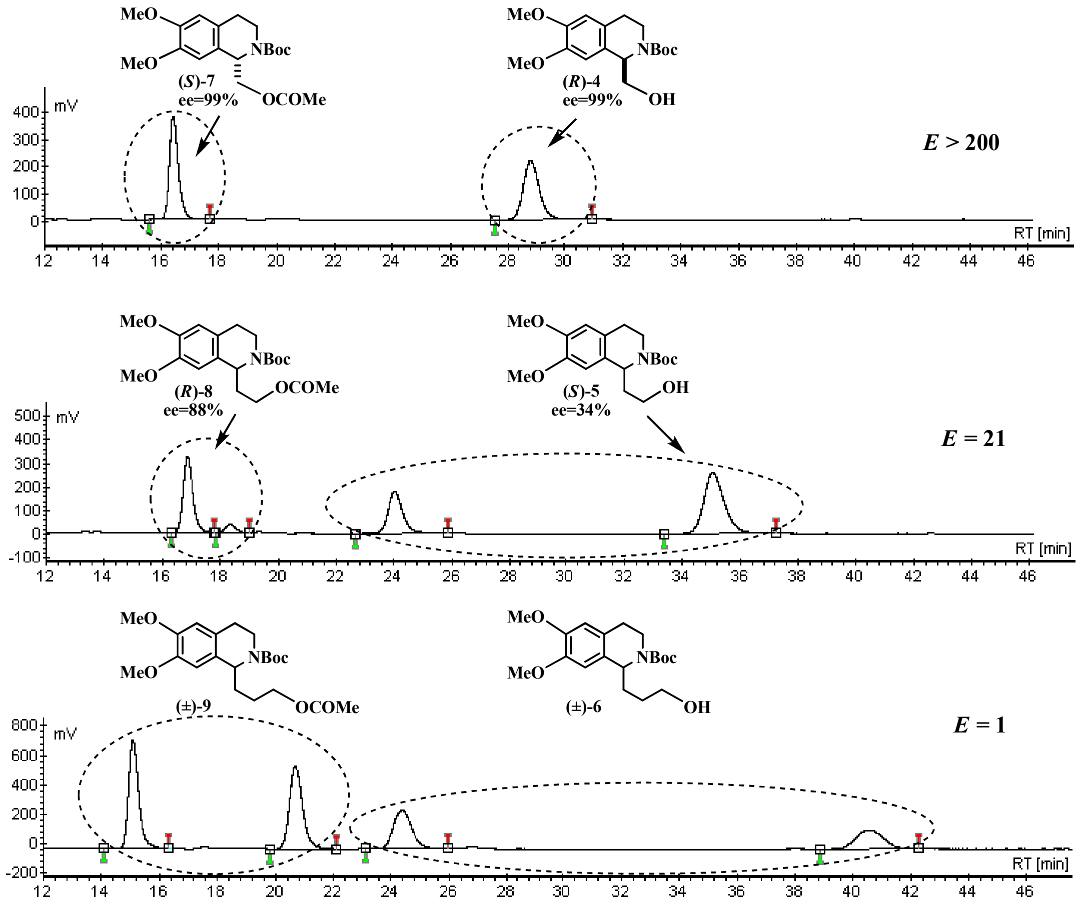
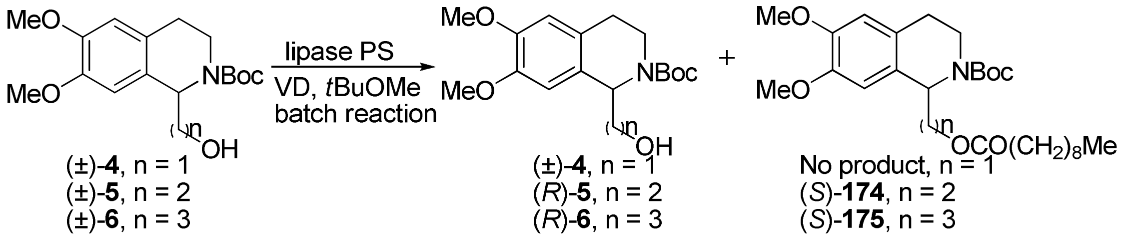


Figure 11. Progress of acylations of (±)-**4** to (±)-**6** under the same conditions

In the next part of the study on the effect of the remote reaction centre on the conversion and *E,* lipase PS was used for the *O*-acylation of (±)-**4**–(±)-**6**, under the best conditions obtained for the *O*-acylation of *crispine A* intermediate (±)-**6**. The reactions were performed as batch reactions with VD as acyl donor in *t*BuOMe at 45 °C and catalytic amounts of Et_3N and Na_2SO_4. The results are summarised in Table 26.

Table 26. Enzymatic acylation of (±)-**4**–(±)-**6**[a]



Entry	Substrate	ee_s[b] (%)	ee_p[b] (%)	Conv. (%)	*E*
1	(±)-**4**, n = 1	rac.	No reaction		
2	(±)-**5**, n = 2	5	64	7	4.8
3	(±)-**6**, n = 3	19	96	17	59

[a] 0.0125 M substrate, lipase PS, 4 equiv. of VD, *t*BuOMe, Et_3N and Na_2SO_4, 45 °C, after 1 h.
[b] According to HPLC.

The best *E* (= 59) was obtained when the distance between the stereogenic and reaction centres was three carbon atoms (±)-**6** (entry 3). At a two-carbon atom distance between the stereogenic centre and the OH group, *E* was 4.8 (entry 2). Finally, for the enzymatic acylation of *calycotomine* intermediate (±)-**4**, when the distance between the stereogenic and reaction centres is one carbon atom, no reaction was observed.

From the results obtained in the systematic study, we concluded that *E* is strongly dependent on the enzyme used. In the cases of CAL-A, PPL, lipase AY and lipase AK, the distance between the stereogenic and reaction centres did not have any effect on *E*, while in the case of CAL-B a significant decrease in *E* with increase of the distance was observed, in contrast with lipase PS, where a significant decrease in *E* was seen with decrease of the distance.

We plan to carry out a molecular modelling study in order to determine the relation between the structure of racemic (±)-**4**–(±)-**6** (the distance between the stereogenic and reaction centres) and the corresponding ***E*.**

4.3. ***Preparative-scale resolutions***

The preparative-scale resolutions of compounds (±)-**4**–(±)-**6** were performed under the optimum conditions obtained in the preliminary reactions. The *calycotomine* intermediate (±)-**4** was resolved with excellent *E* (> 200) in the presence of 25 mg mL^{-1} CAL-B and 2 equiv. of VA in toluene at 60 °C. After 1 h, the reaction was stopped at

50% conversion and the product ester (*S*)-**7** and the unreacted alcohol (*R*)-**4** were isolated with high *ee* values (ee_p = 99% and ee_s = 99%) in a good chemical yield (≥ 43%).

The optimum conditions for (±)-**5** were 30 mg mL^{-1} CAL-B and 4 equiv. of VA in toluene with catalytic amounts of Et_3N and Na_2SO_4 at 3 °C. After 10 min, the ester (*R*)-**8** was isolated with ee_p = 95% and the unreacted alcohol (*S*)-**5** with ee_s = 46% at 33% conversion. To increase the *ee* of the unreacted alcohol, a second enzymatic reaction was performed with the enantiomerically enriched alcohol. After 30 min, the reaction was stopped at ee_s = 94%.

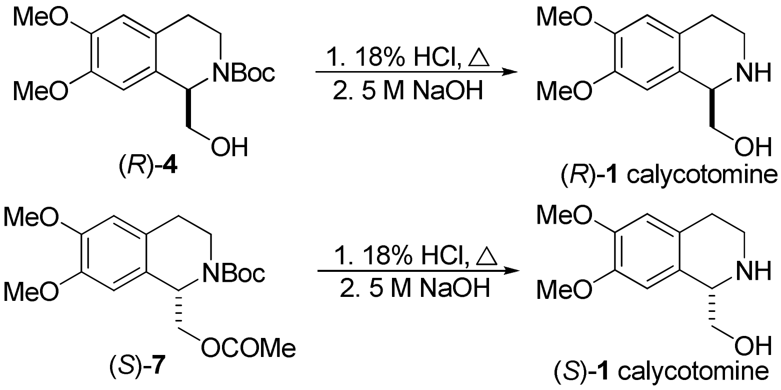
The resolution of racemic (±)-**6** was performed with 30 mg mL^{-1} lipase PS in *t*BuOMe by using 2 equiv. of VD, 0.1 equiv. of Et_3N and a catalytic amount of Na_2SO_4 at 45 °C. After 1 h, the reaction was stopped at 43% conversion by filtering off the enzyme, and the ester (*S*)-**178** was isolated with ee_p = 94% and the unreacted alcohol (*R*)-**6** with ee_s = 70%. To obtain a higher ee_s, the enantiomerically enriched alcohol (ee_s = 70%) was subjected to a second enzymatic resolution. After 3.5 h, the reaction was stopped and the alcohol was obtained with *ee* = 95%.

4.4. *Further transformations*

This section describes the preparation of the target *calycotomine*, *homocalycotomine* and *crispine A* enantiomers. The reactions were performed on the enantiomers obtained in preparative-scale resolutions (section 4.3).

4.4.1. Preparation of *calycotomine* enantiomers [(*R*)-1 and (*S*)-1]

The products obtained in the enzyme-catalysed reaction (section 4.3) were separated and (*R*)-**4** and (*S*)-**7** were transformed to the desired (*R*)-**1** and (*S*)-**1**. Deprotection of the amino group and hydrolysis of the ester were achieved with 18% HCl (Scheme 12). After refluxing for 5 h, 5 M NaOH was added to the product, and both enantiomers of *calycotomine*, (*R*)-**1** and (*S*)-**1**, were obtained with good *ee* (> 99%).



Scheme 12. Preparation of both enantiomers of *calycotomine*

The physical data on the compounds [(*R*)-**4** and (*S*)-**7**] obtained from the enzymatic reaction and on the *calycotomine* enantiomers [(*R*)-**1** and (*S*)-**1**] are presented in Table 27.

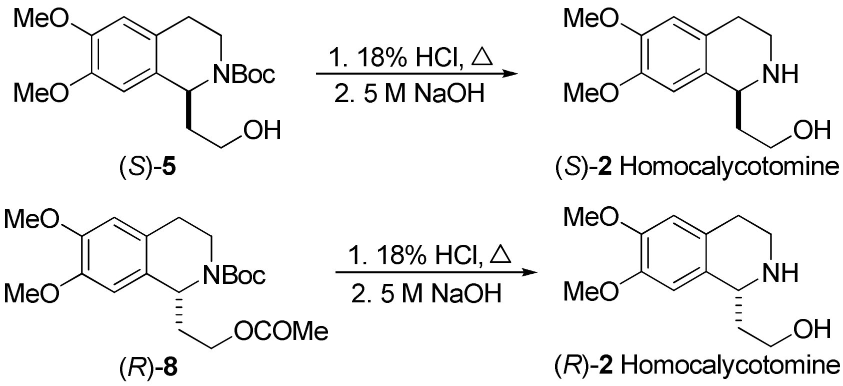
Table 27. Physical data on the prepared enantiomers

Compound	Yield (%)	*ee*[a] (%)	$[\alpha]_D^{25}$ (CHCl$_3$)
(*R*)-**4**	46	99	+82 (c 0.255)
(*S*)-**7**	43	99	-103 (c 0.24)
(*R*)-**1**	73	99	+16 (c 0.165)
(*S*)-**1**	76	99	-15 (c 0.18)

[a] According to HPLC.

4.4.2. Preparation of *homocalycotomine* enantiomers [(*R*)-2 and (*S*)-2]

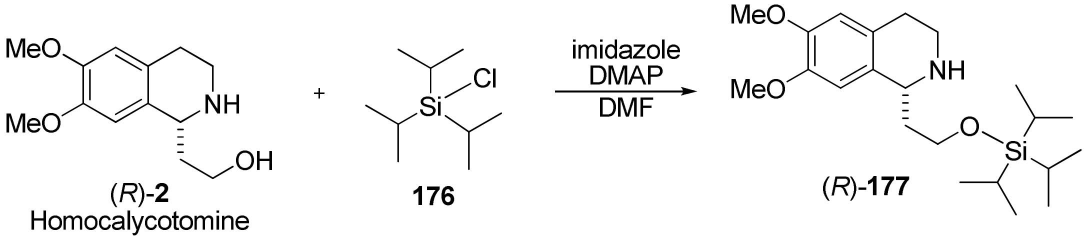
The *homocalycotomine* enantiomers [(*S*)-**2** and (*R*)-**2**] were prepared in a similar way as described in section 4.4.1, starting from (*S*)-**5** and (*R*)-**8** (Scheme 13).



Scheme 13. Preparation of both enantiomers of *homocalycotomine*

4.4.3. Preparation of (1*R*)-177

(1*R*)-Triisopropylsilyl-1-(2-hydroxyethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (*R*)-**177** was prepared by the reaction of (*R*)-*homocalycotomine* and triisopropylsilyl chloride (**176**) in the presence of imidazole and 4-dimethylaminopyridine (DMAP) in dimethylformamide (DMF) at room T (Scheme 14). The reaction was performed according to the method described by Tietze and co-workers.[6]



Scheme 14. Preparation of compound (*R*)-**177**

Physical properties of (*S*)-**5**, (*R*)-**8**, (*S*)-**2**, (*R*)-**2** and (*R*)-**177**, prepared in other transformations described in this section are presented in the Table 28.

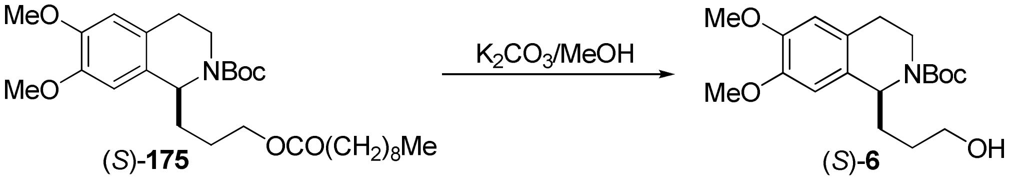
Table 28. Physical data on the prepared enantiomers

Compound	Yield (%)	*ee*[a] (%)	$[\alpha]_D^{25}$ ($CHCl_3$)
(*S*)-**5**	35	94	+95 (c 0.24)
(*R*)-**8**	32	95	-49 (c 0.2)
(*S*)-**2**	71	94	+9 (c 0.18)
(*R*)-**2**	74	95	-10 (c 0.2)
(*R*)-**177**	71	94	+10.6 (c 0.44)

[a] According to HPLC.

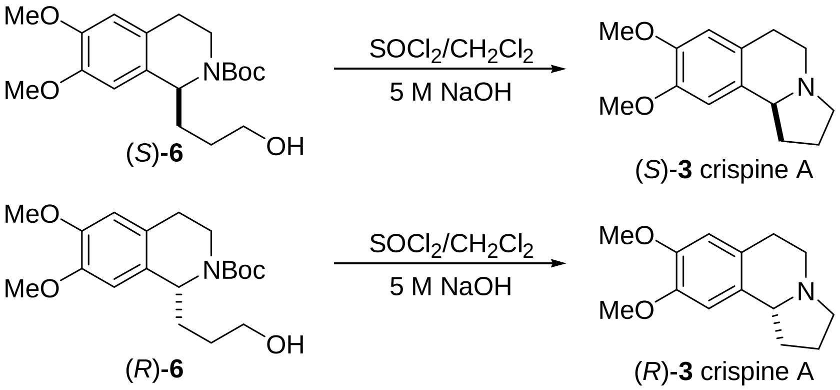
4.4.4. Preparation of *crispine A* enantiomers [(*R*)-3 and (*S*)-3]

In the preparation of the *crispine A* enantiomers, the first step was the hydrolysis of the ester group of (*S*)-**175** (*ee* = 94) (Scheme 15). The reaction was performed in the presence of K_2CO_3 in MeOH. After 8 h, the product (*S*)-**6** was obtained without a decrease in *ee* (= 94%).



Scheme 15. Hydrolysis of (*S*)-**175**

Both enantiomers of *crispine A* were prepared from the corresponding *N*-Boc-protected amino alcohols. Deprotection of the amino group and cyclization of the enantiomers (*R*)-**6** and (*S*)-**6** were performed in one step in the presence of $SOCl_2$ in CH_2Cl_2 at 40 °C (Scheme 16). After 2 h, NaOH was added to the reaction mixture and the *crispine A* enantiomers were obtained without a decrease in *ee* ($\geq$ 95%).



Scheme 16. Preparation of *crispine A* enantiomers

The physical properties of the enantiomeric compounds obtained through the enzymatic acylation of (±)-**6** followed by different further transformations are presented in Table 29.

Table 29. Physical data on the prepared enantiomers.

Compound	Yield (%)	*ee*[a] (%)	$[\alpha]_D^{25}$ ($CHCl_3$)
(*S*)-**175**	31	94	+52 (c 0.2)
(*S*)-**6**	89	94	+60 (c 0.31)
(*R*)-**6**	31	95	-60 (c 0.5)
(*S*)-**3**	78	96	-61 (c 0.28)
(*R*)-**3**	83	95	+60 (c 0.255)

[a] According to HPLC.

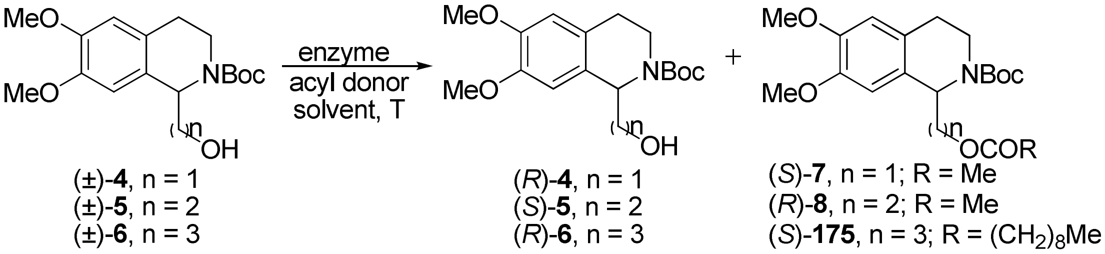
4.5. *Absolute configurations*

The absolute configurations of the *calycotomine* and *crispine A* enantiomers were determined by comparing the specific rotations of the enantiomers prepared with the rotations known from the literature data [for (*S*)-*calycotomine*, lit.[103] $[\alpha]_D^{25}$ = -13.1 (c 0.31, $CHCl_3$), and for the enantiomeric *calycotomine* obtained through the enzyme-catalysed *O*-acylation of (±)-**4** $[\alpha]_D^{25}$ = - 15 (c 0.18, $CHCl_3$); for (*R*)-*crispine A*, lit.[104] $[\alpha]_D^{23}$ = +96.9 (c 1.1, $CHCl_3$), and for the enantiomeric *crispine A* obtained through the enzyme-catalysed *O*-acylation of (±)-**6** $[\alpha]_D^{25}$ = +60 (c 0.255, $CHCl_3$)]. The CAL-B-catalysed asymmetric *O*-acylation of (±)-**4** and the lipase PS-catalysed acylation of (±)-**6** therefore both displayed *S E*.

To determine the stereochemistry of the *homocalycotomine* enantiomers, enantiomeric triisopropylsilyl-1-(2-hydroxyethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**177**) was prepared from the enantiomeric alcohol **2**, according to the method described by Tietze and co-workers.[6] The α value of enantiomeric **177** $[\alpha]_D^{25}$ = +10.6 (c 0.44, $CHCl_3$), obtained through the enzyme-catalysed *O*-acylation of (±)-**5**, was compared with the literature data[6] $[\alpha]_D^{25}$ = -12.9 (c 0.58, $CHCl_3$) for the *S* enantiomer of triisopropylsilyl-1-(2-hydroxyethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline. It was concluded that the CAL-B-catalysed asymmetric *O*-acylation of (±)-**5** proceeded with *R E*.

5. Summary

Racemic primary alcohols [(±)-**4**–(±)-**6**] were resolved through enzyme-catalysed asymmetric *O*-acylation (Scheme17).



Scheme 17. Lipase-catalysed *O*-acylation of (±)-**4**–(±)-**6**

A new enzymatic method was developed for the CAL-B-catalysed *S*-selective *O*-acylation of *N*-Boc-protected 1-hydroxymethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline [(±)-**4**] in a CF reactor. The optimized conditions in the CF system were valid in a batch reaction and the preparative enzymatic acylation was performed with excellent *E* (*E* > 200) by using CAL-B as enzyme, and VA as acyl donor in toluene at 60 °C. After 1 h, the product ester (*S*)-**7** and the unreacted alcohol (*R*)-**4** were isolated with high *ee* values (> 99%) at 50% conversion.

The optimum conditions for the *R*-selective *O*-acylation of *N*-Boc-protected 1-(2-hydroxyethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline [(±)-**5**] were determined with a combination of the batch and a CF system. In the CF system, the best *E* was *E* = 22 in the presence of lipase PS as enzyme, with VA as acyl donor in toluene at 24 °C. The best conditions obtained in the CF system were enhanced in the batch reactions by using catalytic amounts of Et_3N and Na_2SO_4 as additives at 3 °C. The best *E* obtained was 88 at 24% conversion.

We have developed the first total synthetic route for the preparation of *crispine A* enantiomers, which involves an enzymatic key step, the *S*-selective *O*-acylation of *N*-Boc-protected 1-(3-hydroxypropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroiso-quinoline [(±)-**6**] (*E* = 59). The reaction was performed with lipase PS as enzyme, vinyl decanoate as acyl donor, and Et_3N and Na_2SO_4 as additives in *t*BuOMe at 45 °C.

The preparative-scale resolutions of (±)-**4**–(±)-**6** were performed in batch reactions under the optimum conditions. Amino alcohol (±)-**4** was resolved with *E* > 200 and the resulting unreacted alcohol (*R*)-**4** and the ester (*S*)-**7** were obtained with excellent *ee* (≥ 99%). The preparative-scale resolution of [(±)-**5**] was performed in two steps to obtain the unreacted alcohol and the ester enantiomers with high *ee* values. In the first step, the reaction was stopped after 10 min at 33% conversion, and the ester (*R*)-**8** was obtained with *ee* = 95% and the unreacted alcohol (*S*)-**5** with *ee* = 46%. After separation of the products with the enantiomerically enriched alcohol a second enzymatic reaction was performed under the above-described conditions, when the alcohol (*S*)-**5** was obtained with *ee* = 94%. The enzymatic resolution of [(±)-**6**] was also performed in a two-step preparative-scale acylation under the optimum conditions. First, the reaction was stopped after 1 h at 43% conversion, and the ester (*S*)-**175** was obtained with ee_p = 94%. The enantiomerically enriched alcohol (*R*)-**6** with ee_s = 70% was then subjected to a second enzymatic reaction, which after 3.5 h furnished the unreacted alcohol with *ee* = 95%.

Both enantiomers of *calycotomine* and *homocalycotomine* were prepared through Boc deprotection and ester hydrolysis of the enantiomeric products (*R*)-**4**, (*S*)-**7**, (*S*)-**5**, and (*R*)-**8**, obtained in the preparative-scale enzymatic resolutions of (±)-**4** and (±)-**5**. In the cases of (*R*)-**6** and (*S*)-**6**, the Boc deprotection and the cyclisation were performed in one step and resulted in both *crispine A* enantiomers.

We performed a systematic study to investigate the effects of the remote stereogenic centre (n = 1, 2, 3) on the conversion and *E* with six different enzymes. When CAL-A, PPL, lipase AY or lipase AK was used, the distance between the stereogenic and reaction centres did not have an effect on *E*. In the presence of lipase PS, *E* increased as the distance between the stereogenic centre and the OH group increased from one carbon atom (no reaction) to two carbon atoms (*E* = 4.8) and then to three carbon atoms (*E* = 59), while with CAL-B *E* decreased from 200 to 21 and then to 1 as the distance increased from one to three carbon atoms.

All the enantiomers obtained were characterized by HPLC measurements, optical rotation, ^{1}H NMR, ^{13}C NMR, elemental analysis and melting point measurement. The absolute configurations were determined by comparing the optical rotations of the enantiomers or their derivatives with optical rotations described in the literature.

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References

1. Miyazaki, M.; Ando, N.; Sugai, K.; Seito, Y.; Fukuoka, H.; Kanemitsu, T.; Nagata, K.; Odanaka, Y.; Nakamura, K. T.; Itoh, T. *J. Org. Chem.* **2011**, *76*, 534-542.
2. Chrzanowska, M.; Rozwadowska, M. D. *Chem. Rev*. **2004**, *104*, 3341-3370.
3. Al-Yahya, M. A.; Hassan, M. M. A. *Anal. Profiles Drug Subst.* **1982**, *11*, 407-461.
4. Cuevas, C.; Francesch, A. *Nat. Prod. Rep.* **2009**, *26*, 322-337.
5. Boyd, E. M.; Knight, L. M. *J. Pharm. Pharmacol*. **1964**, *16*, 118-124.
6. Tietze, L. F.; Rackelmann, N.; Müller, I. *Chem. Eur. J.* **2004**, *10*, 2722-2731.
7. Taniyama, D.; Hasegawa, M.; Tomioka, T. *Tetrahedron: Asymmetry* **1999**, *10*, 221-223.
8. Leal, J. F. M.; García-Hernández, V.; Moneo, V.; Domingo, A.; Bueren-Calabuig, J. A.; Negri, A.; Gago, F.; Guillén-Navarro, M. J.; Avilés, P.; Cuevas, C.; García-Fernández, L. F.; Galmarini, C. M. *Biochem. Pharmacol.* **2009,** *78*, 162-170.
9. White, E. P. N. Z. *J. Sci. Technol.* **1944**, *25B*, 137-162.
10. Zhang, Q.; Tu, G.; Zhao, Y.; Cheng, T. *Tetrahedron* **2002**, *58*, 6795-6798.
11. Szawkalo, J.; Zawadzka, A.; Wojtasiewicz, K.; Leniewski, A.; Maurin, J. K.; Drabowicz, J.; Czarnocki, Z. *Tetrahedron: Asymmetry* **2005**, *16*, 3619-3621.
12. Wegner, J.; Ceylan, S.; Kirschning, A. *Chem. Commun*. **2011**, *47*, 4583-4592.
13. Wiles, C.; Watts, P. *Green Chem*. **2012**, *14*, 38-54.
14. Hu, C.-H.; Brinck, T.; Hult, K. *Int. J. Quantum Chem.* **1998**, *69*, 89-103.
15. Schrag, J. D.; Li, Y.; Wu, S.; Cygler, M. *Nature*, **1991**, *351*, 761-764.
16. Drauz, K.; Gröger, H.; May, O. *Enzyme catalysis in organic synthesis*, **2012**, Wiley-VCH Verlag & Co. KGaA.
17. Ghanem, A.; Aboul-Enein, H. Y. *Chirality* **2005**, *17*, 1-15.
18. Nordin, O.; Nguyen, B. V.; Vörde, C.; Hedenström, E.; Högberg, H. E. *J. Chem. Soc*., *Perkin Trans. 1* **2000**, 367-376.
19. Sakai, T.; Mitsutomi, H.; Korenaga, T.; Ema, T. *Tetrahedron: Asymmetry* **2005**, *16*, 1535-1539.
20. Rosen, T. C.; Haufe, G. *Tetrahedron: Asymmetry* **2002**, *13*, 1397-1405.
21. Baczko, K.; Larpent, C. *J. Chem. Soc*., *Perkin Trans. 2*, **2000**, 521-526.
22. Kawasaki, M.; Goto, M.; Kawabata, S.; Kometani, T. *Tetrahedron: Asymmetry* **2001**, *12*, 585-596.
23. Kawasaki, M.; Hayashi, Y.; Kakuda, H.; Toyooka, N.; Tanak, A.; Goto, M.; Kawabata, S.; Kometani, T. *Tetrahedron: Asymmetry* **2005**, *16*, 4065-4072.
24. Santaniello, E.; Casati, S.; Ciuffreda, P.; Meroni, G.; Pedretti, A.; Vistoli, G. *Tetrahedron: Asymmetry* **2009**, *20*, 1833-1836.
25. Nguyen, B. V.; Nordin, O.; Vörde, C.; Hedenström, E.; Högberg, H. E. *Tetrahedron: Asymmetry* **1997**, *8*, 984-986.
26. Bracher, F.; Papke, T. *Tetrahedron: Asymmetry* **1994**, *5*, 1653-1656.
27. Weissfloch, A. N. E.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 6959-6969.
28. Delinck, D. L.; Margolini, A. L. *Tetrahedron Lett.* **1990**, *31*, 6797-6798.
29. Santanieilo, E.; Ferraboschi, P.; Grisenti, P. *Enzyme Microb. Technol.* **1993**, *15*, 367-382.
30. Miyazawa, T.; Yukawa, T.; Koshiba, R.; Sakamoto, H.; Ueji, S.; Yanagihara, R.; Yamada, T. *Tetrahedron: Asymmetry* **2001**, *12*, 1595-1602.
31. Barth. S.; Effenberger, F. *Tetrahedron: Asymmetry* **1993**, *4*, 823-833.
32. Ghanem, A.; Aboul-Enein, H. Y. *Tetrahedron: Asymmetry* **2004**, *15*, 3331-3351.
33. Hirose, K.; Naka, H.; Yano, M.; Ohashi, S.; Naemura, K.; Tobe, Y. *Tetrahedron: Asymmetry* **2000**, *11*, 1199-1210.
34. Shafioul, A. S. M.; Cheong, C. S. *J. Mol. Catal. B: Enzym*. **2012**, *74*, 199-203.
35. Bencze, L. C.; Paizs, C.; Tosa, M. I.; Irimie, F. D. *Tetrahedron: Asymmetry* **2011**, *22*, 675-683.

36. Sakai, T.; Liu, Y.; Ohta, H.; Korenaga, T.; Ema, T. *J. Org. Chem.* **2005**, *70*, 1369-1375.
37. Sakai, T.; Kawabata, I.; Kishimoto, T.; Ema, T.; Utaka, M. *J. Org. Chem.* **1997,** *62*, 4906-4907.
38. Sakai, T. *Tetrahedron: Asymmetry* **2004**, *15*, 2749-2756.
39. Kawanami, Y.; Honnma, A.; Ohta, K.; Matsumoto, N. *Tetrahedron* **2005**, *61*, 693-697.
40. Goswami, A.; Howell, J. M.; Hua, E. Y.; Mirfakhrae, K. D.; Soumeillant, M. C.; Swaminathan, S.; Qian, X.; Quiroz, F. A.; Vu, T. C.; Wang, X.; Zheng, B.; Kronenthal, D. R.; Patel, R. N. *Org. Process. Res. Dev.* **2001**, *5*, 415-420.
41. Patel, R. N. *Coord. Chem. Rev.* **2008**, *252*, 659-701.
42. Patel, R. N. *Food Technol. Biotechnol.* **2004**, *42*, 305-325.
43. Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 2351-2358.
44. Kámán, J.; Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2000**, *11*, 1593-1600.
45. Gyarmati, Z. G.; Liljeblad, A.; Rintola, M.; Bernáth, G.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2003**, *14*, 3805-3814.
46. Fülöp, F.; Palkó, M.; Kámán, J.; Lázár, L.; Sillanpää, R. *Tetrahedron: Asymmetry* **2000**, *11*, 4179-4187.
47. Gyarmati, Z. G.; Liljeblad, A.; Argay, G.; Kálmán, A.; Bernáth, G.; Kanerva, L. T. *Adv. Synth. Catal.* **2004**, *346*, 566-572.
48. Li, X. G.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2007**, *18*, 2468-2472.
49. Nagai, H.; Shiozawa, T.; Achiwa, K.; Terao, Y. *Chem. Pharm. Bull.* **1993**, *41*, 1933-1938.
50. Jouglet, B.; Rousseau, G. *Tetrahedron Letters* **1993**, *34*, 2307-2310.
51. Nagai, H.; Shiozawa, T.; Achiwa, K.; Terao, Y. *Chem. Pharm. Bull.* **1992**, *40*, 2227-2229.
52. Oumoch, S.; Rousseau, G. *Bioorg. Med. Chem.Lett.* **1994**, *4*, 2841-2844.
53. Li, X. G.; Kanerva, L. T. *Adv. Synth. Catal.* **2006**, *348*, 197-205.
54. Yamanaka, T.; Ohkubo, M.; Takahashi, F.; Kato, M. *Tetrahedron Letters* **2004**, *45*, 2843-2845.
55. Forró, E.; Árva, J.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 643-649.
56. Strübing, D.; Krumlinde, P.; Piera, J.; Bäckvall, J. B. *Adv. Synth. Catal.* **2007**, *349*, 1577-1581.
57. Hof, R. P.; Kellogg, R. M. *Tetrahedron: Asymmetry* **1994**, *5*, 565-568.
58. Nanda, S.; Scott, A. I. *Tetrahedron: Asymmetry* **2004**, *15*, 963-970.
59. de Gonzalo, G.; Brieva, R.; Sánchez, V. M.; Bayod, M.; Gotor, M. *J. Org. Chem.* **2003,** *68*, 3333-3336.
60. de Gonzalo, G.; Brieva, R.; Sánchez, V. M.; Bayod, M.; Gotor, V. *Tetrahedron: Asymmetry* **2003**, *14*, 1725-1731.
61. Gotor-Fernández, V.; Brieva, R.; Gotor, V. *J. Mol. Catal. B: Enzym.* **2006**, *40*, 111-120.
62. Solares, L. F.; Brieva, R.; Quirós, M.; Llorente, I.; Bayod, M.; Gotor, V. *Tetrahedron: Asymmetry* **2004**, *15*, 341-345.
63. Monterde, M. I.; Brieva, R.; Sánchez, V. M.; Bayod, M.; Gotor, V. *Tetrahedron: Asymmetry* **2002**, *13*, 1091-1096.
64. Bierstedt, A.; Stölting, J.; Fröhlich, R.; Metz, P. *Tetrahedron: Asymmetry* **2002**, *12*, 3399-3407.
65. Cross, H.; Marriott, R.; Grogan, G. *Biotechnol. Lett.* **2004**, *26*, 457-460.
66. Ferraboschi, P.; Casati, S.; Grisenti, P.; Santaniello, E. *Tetrahedron: Asymmetry* **1994**, *5*, 1921-1924.
67. Im, D. S.; Cheong, C. S.; Lee, S. H.; Park, H.; Youn, B. H. *Tetrahedron: Asymmetry* **2000**, *56*, 1309-1314.
68. Ferraboschi, P.; Brembilla, D.; Grisenti, P.; Santaniello, E. *J. Org. Chem.* **1991**, *56*, 5478-5480.
69. Pogorevc, M.; Faber, K. *J. Mol. Catal. B: Enzym.* **2000**, *10*, 357-376.
70. Kawanami, Y.; Iizuna, N.; Maekawa, K.; Maekawa, K.; Takahashi, N.; Kawada, T. *Tetrahedron* **2001**, *57*, 3349-3353.
71. Herradón, B.; Valverde, S. *Tetrahedron: Asymmetry* **1994**, *5*, 1479-1500.

72. Grisenti, P.; Ferraboschi, P.; Manzocchi, A.; Santaniello, E. *Tetrahedron: Asymmetry* **1992**, *48*, 3827-3834.
73. Berkowitz, D. B.; Pumphrey, J. A.; Shen, Q. *Tetrahedron Letters.* **1994**, *35*, 8743-8746.
74. Grisenti, P.; Ferraboschi, P.; Casati, S.; Santaniello, E. *Tetrahedron: Asymmetry* **1993**, *4*, 997-1006.
75. Naemura, K.; Fukuda, R.; Murata, M.; Konishi, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1995**, *6*, 2385-2394.
76. Haddad, M.; Larchevêque, M. *Tetrahedron: Asymmetry* **2005**, *16*, 2243-2247.
77. Vänttinen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1997**, *8*, 923-933.
78. Wirz, B.; Walther, W. *Tetrahedron: Asymmetry* **1992**, *3*, 1049-1054.
79. Csomós, P.; Kanerva, L. T.; Bernáth, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1789-1796.
80. Deska, J.; Bäckvall, J. E. *Org. Biomol. Chem.* **2009**, *7*, 3379-3381.
81. Serra, S. *Tetrahedron: Asymmetry* **2011**, *22*, 619-628.
82. Chen, C. S.; Sih, C. J. *Angew. Chem. lnt. Ed. Engl.* **1989**, *28*, 695-707.
83. Abate, A.; Brenna, E.; Negri, C. D.; Fuganti, C.; Serra, S. *Tetrahedron: Asymmetry* **2002**, *13*, 899-904.
84. Faigl, F.; Thurner, A.; Battancs, M.; Farkas, F.; Poppe, L.; Bódai, V.; Kmecz, I.; Simándi, B. *Tetrahedron: Asymmetry* **2005**, *16*, 3841-3847.
85. Abate, A.; Brenna, E.; Fuganti, C.; Gatti, F. G.; Giovenzana, T.; Malpezzi, L.; Serra, S. *J. Org. Chem.* **2005**, *70*, 1281-1290.
86. Kawasaki, M.; Goto, M.; Kawabata, S.; Kodama, T.; Kometani, T. *Tetrahedron Lett.* **1999**, *40*, 5223-5226.
87. de Gonzalo, G.; Brieva, R.; Sánchez, V. M.; Bayod, M.; Gotor, M. *J. Org. Chem.* **2001,** *66*, 8947-8953.
88. Naemura, K.; Murata, M.; Tanaka, R.; Yano, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1996**, *7*, 3285-3294.
89. Torre, O.; Gotor-Fernández, V.; Gotor, V. *Tetrahedron: Asymmetry* **2006**, *17*, 860-866.
90. Sankaranarayanan, S.; Sharma, A.; Chattopadhyay, S. *Tetrahedron: Asymmetry* **2002**, *13*, 1373-1378.
91. Sabbani, S.; Hedenström, E.; Andersson J. *Tetrahedron: Asymmetry* **2007**, *18*, 1712-1720.
92. Isleyen, A.; Tanyeli, C.; Dogan, Ö. *Tetrahedron: Asymmetry* **2006**, *17*, 1561-1567.
93. Brenna, E.; Fuganti, C.; Fuganti, D.; Grasselli, P.; Malpezzi, L.; Pedrocchi-Fantoni, G. *Tetrahedron* **1997**, *53*, 17769-17780.
94. Im, D. S.; Cheong, C. S.; Lee, S. H.; Park, H.; Youn, B. H. *Tetrahedron: Asymmetry* **1999**, *10*, 3759-3767.
95. Csajági, C.; Szatzker, G.; Tőke, E. R.; Ürge, L.; Davas, F.; Poppe. L. *Tetrahedron Asymmetry* **2008**, *19*, 237-246.
96. Tomin, A.; Hornyánszky, G.; Kupai, K.; Dorkó, Z.; Ürge, L.; Darvas, F.; Poppe, L. *Proc. Biochem.* **2010**, *45*, 859-865.
97. Kanerva, L. T.; Sundholm, O. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407-2410.
98. Lázár, L.; Fülöp, F.; Bernáth, G.; Mattinen, J. *Org. Prep. Proc. Int.* **1993**, *25*, 91-97.
99. Zalán, Z.; Martinek, T. A.; Lázár, L.; Sillanpää, R.; Fülöp, F. *Tetrahedron* **2006**, *62*, 2883-2891.
100. Kóbor, J.; Fülöp, F.; Bernáth, G. *Heterocycles* **1986**, *24*, 2227-2231.
101. Fülöp, F.; Forró, E.; Martinek, T.; Günther, G.; Sillanpää, R. *J. Mol. Struct.* **2000**, *554*, 119-125.
102. Campbell, J. A.; Lee, W. K.; Rapoport, H. *J. Org. Chem.* **1995**, *60*, 4602-4616.
103. Pedrosa, R.; Andrés, C.; Iglesias, J. M. *J. Org. Chem.* **2001**, *66*, 243-250.
104. Gurram, M.; Gyimothy, B.; Wang, R.-F.; Lam, S.-Q.; Ahmed, F.; Herr, R. J. *J. Org. Chem.* **2011**, *76*, 1605-1613.

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