University of Szeged

Faculty of Pharmacy

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PREPARATION OF PHARMACEUTICALLY IMPORTANT ENANTIOMER COMPONENTS THROUGH LIPASE CATALYSED ACYLATION AND HYDROLYSIS

Ph.D. Thesis

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Publications

Papers related to the thesis

I. Forró, E., Galla, Z. and Fülöp, F.
Candida antarctica lipase B catalysed reactions of *β*-hydroxy esters: Competition of acylation and hydrolysis
J. Mol. Catal. B: Enzym. **2013**, *98*, 92-97. **IF:2.745**

II. Forró, E., Galla, Z., Nádasdi, Z., Árva, J. and Fülöp, F.
Novel chemo-enzymatic route to a key intermediate for the taxol side-chain through enantioselective *O*-acylation. Unexpected acyl migration
J. Mol. Catal. B: Enzym. **2015**, *116*, 101-105. **IF:2.128**

III. Galla, Z., Beke, F., Forró, E. and Fülöp, F.
Enantioselective hydrolysis of 3,4-disubstituted *β*-lactams. An efficient enzymatic method for the preparation of a key Taxol side-chain intermediate
J. Mol. Catal. B: Enzym. **2016**, *123*, 107-112. **IF:2.128***

IV. Forró, E., Galla, Z. and Fülöp, F.
The *N*-hydroxymethyl group as a traceless activating group for the CAL-B catalysed ring cleavage of *β*-lactams: A type of two-step cascade reaction
Eur. J. Org. Chem. **2016**, *15*, 2647-2652. **IF:3.068***

V. Galla, Z., Forró, E. and Fülöp, F.
Enhanced enzymatic synthesis of the enantiopure intermediate for the blockbuster drug intermediate abacavir through a two-step enzymatic cascade reaction
Tetrahedron: Asymmetry **2016**, *27*, 729-731. **IF:2.108***

Other paper

VI. Nagy, B., Galla, Z., Forró, E., Csaba, L., Monica, B., Toşa, I.. Paizs, C. and Fülöp, F.
Covalently immobilized lipases are efficient stereoselective catalysts for the kinetic resolution of novel *rac*-(5-phenylfuran-2-yl)-*β*-alanine-ethyl ester hydrochlorides
Eur. J. Org. Chem. **2017**, accepted manuscript. **IF:3.068***

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Lectures

a. **Galla Zsolt**, Forró Enikő, Fülöp Ferenc
Hidroxiészterek Candida antarctica B-lipáz katalizált reakciói: Párhuzamosan lejátszódó acilezés és hidrolízis
Hajdúszoboszlói Vegyészkonferencia, 26-28 June, 2013, Hajdúszoboszló, Hungary, poster presentation.

b. **Zsolt Galla**, Enikő Forró, Ferenc Fülöp
Enzymatic hydrolysis of 3,4-disubstituted β-lactams
Ist EuCheMS Congress on Green and Sustainable Chemistry, 13-15 October, 2013, Budapest, Hungary, poster presentation.

c. **Galla Zsolt**, Forró Enikő, Fülöp Ferenc
3,4-Diszubsztituált β-laktámok enzimatikus hidrolízise
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d. **Galla Zsolt**, Forró Enikő, Fülöp Ferenc
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e. **Zsolt Galla**, Enikő Forró, Zala Nádasdi, Ferenc Fülöp
A new enzymatic route for the preparation of Taxil side-chain key intermediate. Unexpected acyl migration.
Biotrans 2015, 26-30 July, 2015, Vienna, Austria, poster presentation.

f. Botond Nagy, **Zsolt Galla**, Csaba Paizs, Enikő Forró, Ferenc Fülöp
Synthesis and enzymatic kinetic resolution of novel 3-amino-3-(5-phenylfuran-2-yl)propanoic acid ethyl esters
ISySyCat 2015, 2-4 September, 2015, Évora, Portugal, poster presentation.

g. Enikő Forró, **Zsolt Galla**, Ferenc Fülöp
A traceless activating group for the CAL-B catalysed ring cleavage of β-lactams
Chirality 2016, 24-27 July, 2016, Heidelberg, Germany, poster presentation.

Abbreviations

2,2,2-TFB	2,2,2-trifluoroethyl butyrate
2-Me-THF	2-methyltetrahydrofuran
Boc	*tert*-butoxycarbonyl
BnO	benzoyloxy
CAL-A	*Candida antarctica* lipase A
CAL-B	*Candida antarctica* lipase B
CAN	ceric ammonium nitrate
Cbz	carboxybenzoyl
Conv.	conversion
E	enantioselectivity
ee	enantiomeric excess
equiv.	equivalent
er	enantiomeric ratio
GABA	gamma-aminobutyric acid
GC	gas chromatography
GLP-1	glucagon-like peptide-1
HPLC	high-performance liquid chromatography
iPr$_2$O	diisopropyl ether
Lipase AK	*Pseudomonas fluorescens* lipase
Lipase AY	*Candida rugosa* lipase
Lipase PS	*Burkholderia (Pseudomonas) cepacia* lipase
m	mass
MTBE	methyl *tert*-butyl ether
NMR	nuclear magnetic resonance
PhO	phenyloxy
PPL	porcine pancreas lipase
RNA	ribonucleic acid
RSV	respiratory syncytial virus
T	temperature
t	reaction time
t-BuOH	*tert*-butyl alcohol
THF	tetrahydrofuran
VA	vinyl acetate
VB	vinyl butyrate

1. Introduction and aims

A large number of enantiomerically pure *β*- and *γ*-lactams as well as *β*- and *γ*-amino acids have chemical and pharmaceutical importance.[1-5] They may be effective by themselves or can be key intermediates of some complex molecules due to their bifunctional structures (Figure 1). *cis*-Hydroxycarbocyclic acids may also be important building blocks of biologically active molecules.[6,7]

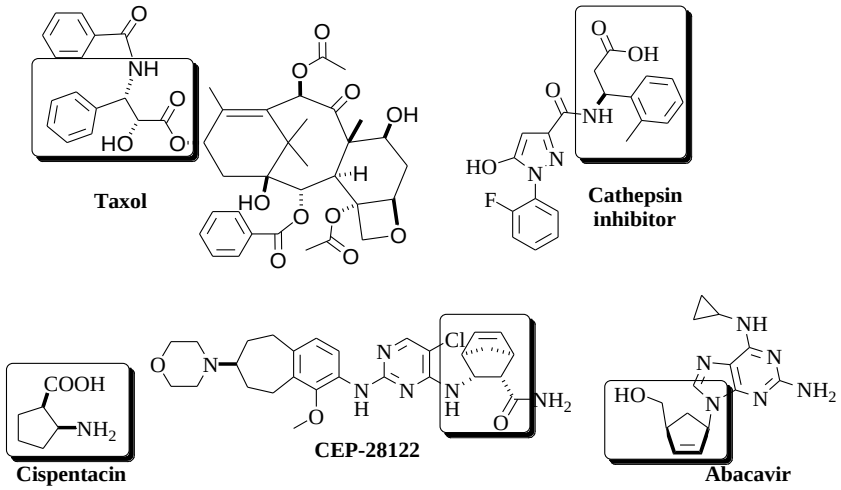


Figure 1

Taxol (Figure 1) and **Taxotere** its analogue are considered to be among the most efficient drugs in cancer chemotherapy.[8,9] The mechanism of action involves interference with the normal breakdown of microtubules during cell division.[10] For instance, the (2*R*,3*S*)-3-phenylisoserine side-chain is essential for antitumor activity of these chemotherapeutic agents.

Cathepsin A is a serine carboxypeptidase.[11] The inhibition of this peptide has potential effects in cardiovascular diseases, among others the salt-induced hypertension. Some *β*-amino acid derivatives, which contain an (*R*)-3-amino-3-(*o*-tolyl)propanoic acid subunit, show pharmacokinetic profiles of the inhibition of **Cathepsin A**. One of these inhibitors is currently undergoing phase I clinical trials (Figure 1).[12]

(1*R*,2*S*)-2-Aminocyclopentanecarboxylic acid (**Cispentacin**) (Figure 1),[13,14] the simplest biologically active *β*-amino acid and its methylene derivative (1*R*,2*S*)-2-amino-4-

methylenecyclopentane-carboxylic acid (**Icofungipen**, **PLD-118**)[15] can themselves exhibit pharmaceutical activity. Furthermore, they can be found in the structures of certain natural products, *e.g.* in the antibiotic amipurimycin.

(1*S*,4*R*)-4-Aminocyclopent-2-ene-1-carbocyclic acid a *γ*-amino acid is a key intermediate for the blockbuster **Abacavir**[5] (Figure 1), which is a nucleoside analogue reverse transcriptase inhibitor. The importance of **Abacavir** is also accentuated in the *WHO's List of Essential Medicines*, a collection of the most important medications needed in a basic health system.

Anaplastic lymphoma kinase is activated in a number of human cancer types due to chromosomal translocations, point mutations and gene amplification, and it has an excellent molecular target for cancer therapy. (1*R*,2*R*,5*S*,6*S*)-3-Azatricyclo[4.2.1.$0^{2.5}$]non-7-en-4-one is used for the preparation of **CEP-28122** (Figure 1), a selective orally active anaplastic lymphoma kinase inhibitor.[16]

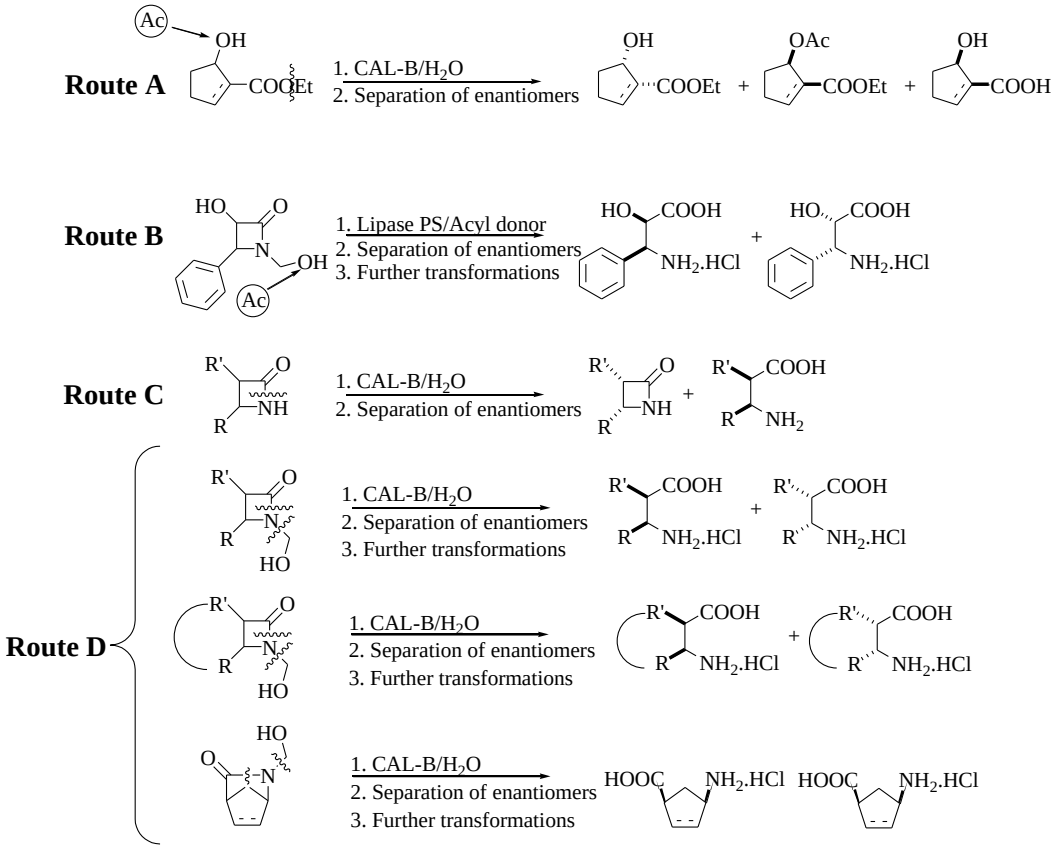
Molecules containing a 2-azetidinone ring may possess antibacterial activity. **Carumonam** is a *β*-lactamase-resistant monobactam antibiotic,[17] while others containing a *cis*-3,4-disubstituted *β*-lactam ring may display PPAR α/γ agonist,[18] vasopressin VIa agonist[19] or anticancer activity.[20,21] The enantiomers of 2-azabicyclo[2.2.1]hept-5-en-3-one are building blocks of pharmaceutically important molecules exhibiting antiviral or antibacterial activity.[4, 22]

5-Hydroxycyclopent-1-enecarboxylic acid has a considerable synthetic potential as a versatile chiral building block, *e.g.* for the synthesis of the naturally occurring mitsugashiwalactone and dolichodial or an intermediate of **Vincamine**.[6,7]

Consequently, there are numerous reasons to prepare molecules in enantiomerically pure or enantiomerically enriched form. The synthesis of pharmaceutically important enantiomers is a difficult and complex problem.[23] A method frequently used for the preparation of enantiomers is enzymatic kinetic resolution. Some commercially available enzymes such as CAL-B and lipase PS have great importance in the pharmaceutical industry.[24-26]

One of our aims was to devise a new method for the enantioseparation of *β*-hydroxy esters through enzyme-catalysed hydrolysis or *O*-acylation and demonstrate the occurrence of hydrolysis of the C1 ester function during *O*-acetylation with CAL-B as catalyst[I] (Scheme 1, Route A). We also intended to develop a novel chemo-enzymatic route to a key intermediate for the **Taxol** side-chain through enantioselective *O*-acylation[II] (Scheme 1, Route B). The third aim was to develop an enantioselective CAL-B catalysed route for the ring opening of 3,4-disubstituted *β*-lactams[III] (Scheme 1, Route C).

The final aim was to devise an enzymatic ring-opening method for activated *β*- and *γ*-lactams where the hydroxymethyl moiety acts as a traceless activating group[IV, V] (Scheme 1, Route D). In order to achieve the best reaction conditions for preparative-scale resolutions, the amounts of enzymes, nucleophiles and acyl donors as well as the effect of solvents and temperature was investigated.



Scheme 1

2. Literature

In this section, we give a brief insight into the most important CAL-B and lipase PS-catalysed strategies for the preparation of pharmaceutically important enantiomeric components. We focus on their use in the pharmaceutical and chemical industries in the past few decades. These methods, without completeness are classified as ester hydrolytic, *O*-acyl hydrolytic, ring opening and *OH*-acylation strategies. Values of *ee*, *wt*, *er* or *purity* of the product, m of the substrate and lipase (according to the reviewed publications) were used for the characterisation of the enzymatic steps of the reactions.

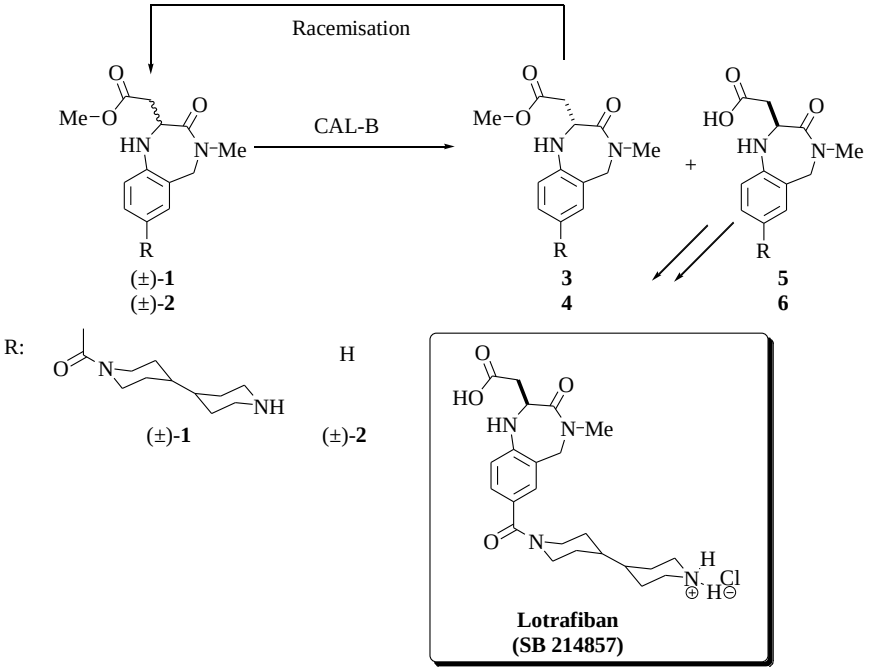
CAL-B and lipase PS have clear advantages compared to most lipases. These include, for instance, broad substrate specificity, commercial availability and high chemo-, regio-, and enantioselectivity, which make them useful in pharmaceutical chemistry. The use of these lipases improves a wide range of production processes, reduces energy consumption, may eliminate chemical synthetic steps and generates less waste and toxic side-products.

2.1. Application of CAL-B lipase in the pharmaceutical industries

2.1.1. CAL-B catalysed ester hydrolysis

Lotrafiban (**SB-214857**) is an orally active GPIIb/IIIa fibrinogen receptor antagonist, which was developed to provide additional benefits for the prevention of thrombosis in chronic treatment, especially in high-risk patients.[27,28] The molecule with (*S*) absolute configuration is the active enantiomer. In the original medicinal chemistry synthesis[29] (*S*)-aspartic acid was used as the source of chirality. Walsgrove et al.[30] described the preparation of **Lotrafiban** using an immobilised form of CAL-B (Boehringer L-2) as catalyst in water (pH = 6.2) at 30 °C (Scheme 2). The separation of the unreacted ester (**3**) from the product was carried out with the use of Cbz chloride. The (*R*)-*N*-Cbz isomer could be racemised after deprotection to regenerate the substrate. 42% overall yield and 99% *ee* were determined for the (*S*) isomer (**5**) (Table 1). Atkins and co-workers[31] optimized the synthetic route from (±)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid methyl ester [(±)-**2**], using Novozym 435, which was designed for anhydrous systems. Under optimised conditions,

t-BuOH/H_2O (22:3, pH = 7.0) was used at 50 °C (Scheme 2) to provide an overall product yield of 43% and an *ee* of 99.8% (Table 1). The unreacted ester was racemised with the use of dimethyl carbonate and sodium methoxide in methanol.



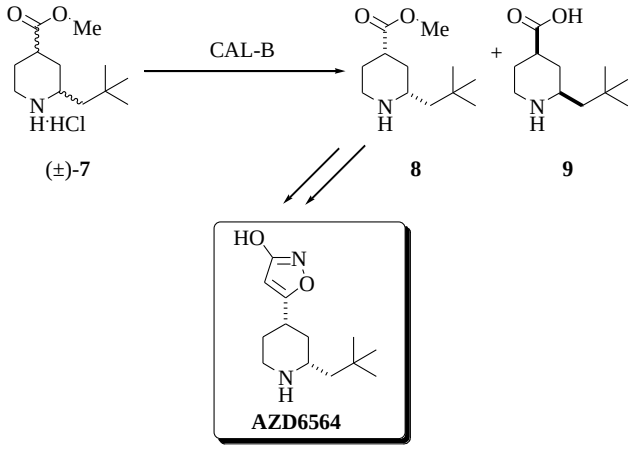
Scheme 2

Table 1. Synthesis of **Lotrafiban**, enzymatic steps

Substrate	t (h)	$m_{substrate}$ (kg)	m_{enzyme} (kg)	yield (%)	$ee_{product}$ (%)
(±)-**1**	2.5	76.3	6	42	99
(±)-**2**	10	246	74	43	99.8

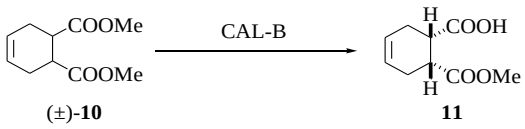
The inhibition of fibrinolysis is a major problem in the clinical practice. **Tranexamic acid** and **ε-Aminocaproic acid**[32] are front-line drugs for inhibiting the activation of plasminogen by reversibly blocking lysine binding sites on plasminogen, but their pharmacokinetics are unfavourable. **AZD6564** is an oral fibrinolysis inhibitor with good metabolism and pharmacokinetic properties.[33,34] Cheng and co-workers published the medicinal chemistry route[34] for the preparation of **AZD6564**. Andersen et al.[35] described a kilogram-scale total synthetic route using an enzymatic step for the enantioseparation of (±)-methyl *cis*-2-neopentylpiperidine-4-carboxylate hydrochloride (±)-**7**. They used an

immobilised lipase from Chiralvision (Immozyme CAL-B-T3-150) in H_2O/K_2HPO_4 buffer (pH = 8.0) at 35 °C (Scheme 3). 99% conversion was observed after 40 h and the *wt*% of the product after five steps were 98.9% (Table 2).



Scheme 3

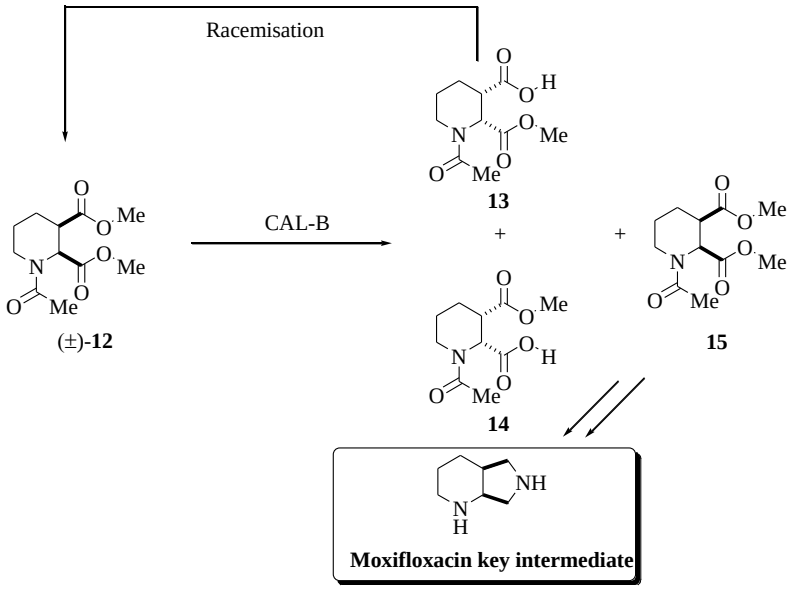
Enzymatic desymmetrization is a simple, widely-used method for the preparation of enantiomers with excellent yields.[36] The reactions usually require mild conditions. (1*S*,2*R*)-2-(Methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid [**11**] is a key chiral intermediate for the preparation of a G-protein-coupled chemokine receptor modulator.[37,38] A number of publications have reported the preparation of **11**.[39-42] Goswami and Kissick[43] described an excellent desymmetrization route from dimethyl cyclohex-4-ene-*cis*-1,2-dicarboxylate [**10**] catalysed by *Candida antarctica* lipase B, which gave **11** with high enantioselectivity (*ee* >99.9%) and in almost quantitative yield (98.1%). They used Novozym 435 in H_2O, K_2HPO_4 buffer (pH = 8.5) at 50 °C (Scheme 4, Table 2).



Scheme 4

Moxifloxacin is a fourth-generation, broad spectrum fluoroquinolone antibacterial agent developed by Bayer AG. Its antibacterial spectrum includes enteric Gram-(-), gram-(+) bacteria and anaerobes.[44] (*S*,*S*)-2,8-Diazobicyclo[4.3.0]nonane is a key chiral intermediate of **Moxifloxacin**. Several synthetic routes have been reported for its preparation. For the

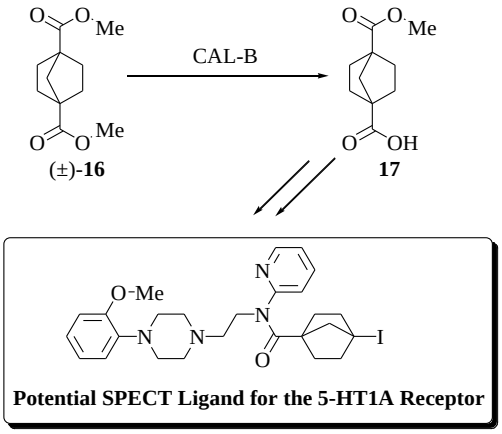
resolvation step Uwe et al.[45] used L-tartaric acid, whereas Fey and co-workers[46] applied camphor sulfonic acid or (-)-2,3,4,6-di-*O*-isopropylidine-2-keto-L-gulonic acid. Fadnavis, Ramesh and Harini[47] described an efficient enzymatic method with soluble Candida antarctica lipase B (Addyzme) (Scheme 5) for the preparation of **15**. Under optimised condition [H_2O, 0.01 M sodium phosphate buffer (pH = 7.5), 45 °C], the reaction appears to be completely stereo- and regioselective. The *ee* of unreacted diester (**15**) was >99% besides 46% yield (Table 2). The product monoesters (**13** and **14**) were converted back into the corresponding diesters in a five-step reaction route.



Scheme 5

4-(Methoxycarbonyl)bicyclo[2.2.1]heptane-1-carboxylic acid (**17**) is a building block of many therapeutic candidates, including 4-iodo-*N*-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-*N*-(pyridine-2-yl)cubyl-1-carboxamide, which is a 5-hydroxytryptamine-1A (5-HT_{1A}) receptor ligand.[48] 5-HT_{1A} receptors mediate various serotonergic functions[49,50] and may be implicated in depressive and anxiety disorders, Alzheimer's disease[51,52] and schizophrenia. Guo et al.[53] developed an enzyme-catalysed hydrolytic route for the preparation of **17**. They screened more than 100 hydrolytic enzymes for the hydrolysis of dimethyl bicyclo[2.2.1]heptane-1,4-dicarboxylate [(±)-**16**]. PS-SD (Lipase PS "Amano" SD) and CAL-B showed the highest activities and selectivities. The CAL-B catalysed scale-up process was

carried out in sodium phosphate buffer (pH = 7.0) at 55 °C (Scheme 6). The yield was 91% and the ratio of monoester/diester/diacid was 97.9 : 1.5 : 0.6 (Table 2).



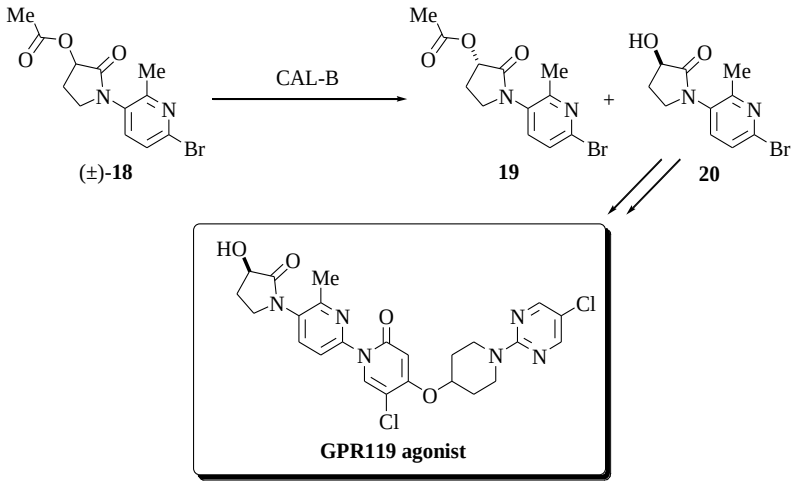
Scheme 6

Table 2. Synthesis of **AZD6564**, **chemokine receptor modulator**, **Moxifloxacin key intermediate** and **SPECT ligand**, enzymatic steps

Substrate	t (h)	$m_{substrate}$ (kg)	m_{enzyme} (kg)	yield (%)	$ee_{product}$ (%)	$wt_{product}$ (%)	$er_{product}$ (%)
(±)-**7**	40	13,1	3,95	31 (5 steps)	n.d.	98.9 (after 5 steps)	n.d.
(±)-**10**	27	1.73	1.5×10^{-1}	98.1	> 99.9	n.d.	n.d.
(±)-**12**	16	8×10^{-2}	4×10^{-2}	46	> 99	n.d.	n.d.
(±)-**16**	31	5×10^{-1}	1×10^{-2}	91	n.d.	n.d.	97.9

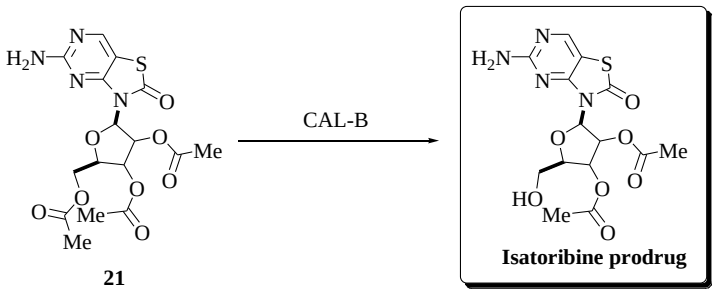
2.1.2. CAL-B catalysed O-acyl hydrolysis

G Protein-coupled receptor 119 (**GPR119**) agonists may improve glucose control with low added risk of hypoglycemia, slow down diabetes progression, reduce food intake and constitute small synthetic molecules, which could be taken orally unlike GLP-1 analogs requiring parenteral administration.[54-56] Singh and co-workers[57] described an enzyme-catalysed hydrolytic route for the preparation of (*R*)-1-(6-bromo-2-methylpyridin-3-yl)-3-hydroxypyrrolidin-2-one (**20**), which is a key chiral intermediate for a **GPR119 agonist** compound.[58] They used CAL-B (Novozym 435) in H_2O/MeCN mixture at 25 °C (Scheme 7). The reaction was selective [conv. = 48.5%, *ee* = 99.7% (*R*-alcohol)] with a yield of 37.1% (Table 3).



Scheme 7

(2*R*,3*R*,4*R*,5*R*)-2-(Hydroxymethyl)-5-(5-amino-2-oxothiazolo[4,5-d]pyrimidin-3(2*H*)-yl)tetrahydrofuran-3,4-diyl diacetate is an oral prodrug of **Isatoribine**, which is a potentially useful nucleoside analogue for the treatment of chronic hepatitis C and viral infections.[59] Gallou et al.[60] demonstrated a practical and robust synthetic process for its preparation. In the last regioselective hydrolytic step they used CAL-B (Novozym 435) in a biphasic mixture (2-Me-THF and phosphate buffer, pH = 6.3–6.5, room temperature) (Scheme 8). Both the yield (86%) and the *purity* (96.1%) of the product enantiomer were excellent (Table 3).



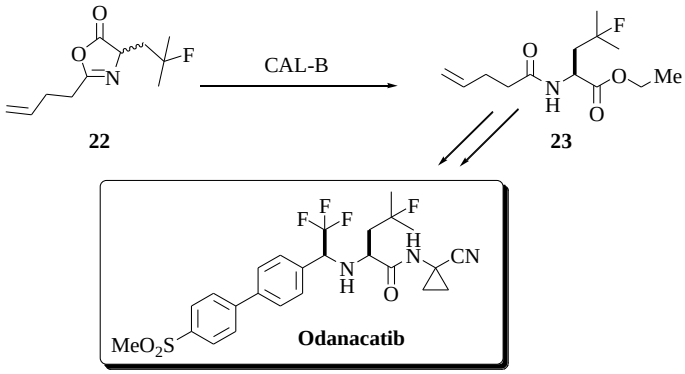
Scheme 8

Table 3. Synthesis of **GPR119 agonist** and **Isatoribine prodrug**, enzymatic steps

Substrate	t (h)	$m_{substrate}$ (kg)	m_{enzyme} (kg)	yield (%)	$ee_{product}$ (%)	$purity_{product}$ (%)
(±)-**18**	28	5×10^{-2}	1.25×10^{-2}	37.1	99.7	n.d.
21	4	1	3.7×10^{-1}	86	n.d.	96.1

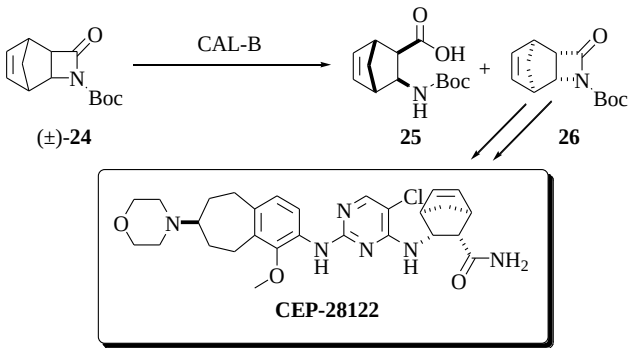
2.1.3. CAL-B catalysed ring-opening reactions

Odanacatib an orally active selective cathepsin K inhibitor has been developed for the treatment of high bone turnover diseases such as osteoporosis.[61] *N*-Protected (*S*)-γ-fluoroleucine ethyl ester (**23**) is a key intermediate in the synthesis of **Odanacatib**. Both Truppo[62] and Limanto et al.[63] worked out efficient chemoenzymatic dynamic kinetic approaches to prepare the (*S*)-enantiomer. In the latter method, CAL-B (Novozym 435) was used in MTBE with 5 equiv. of EtOH and 0.2 equiv. Et_3N at room temperature (Scheme 9). The reaction is (*S*)-selective giving the product in 80% yield with 84% *ee*. Truppo and Hughes[64] developed a method with a new immobilized CAL-B preparation (MRK-CALB-EXE120) and performed repeated 100-kg-scale runs (>90% yield, 88% *ee*) (Table 4). With this catalyst, the process was less expensive than with Novozym 435.



Scheme 9

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase member of the insulin receptor superfamily and constitutively activated in a variety of human cancers.[65] **CEP-28112** is a selective, potent ALK inhibitor.[66] The medicinal chemistry route of **CEP-28112** involved coupling three key structures, the benzocycloheptane unit (A-ring), the diaminopyrimidine central core (B-ring) and the bicyclic amino amide fragment (C-ring). Allwein and co-workers[16] described an efficient, scalable eight-step process for the preparation of **CEP-28112** using CAL-B (Novozym 435) as catalyst in a stereoselective step[67,68] (MTBE with 1 equiv. of H_2O, 50 °C) (Scheme 10). The *ee* and yield are, respectively, 98.3% and 48% (Table 4).



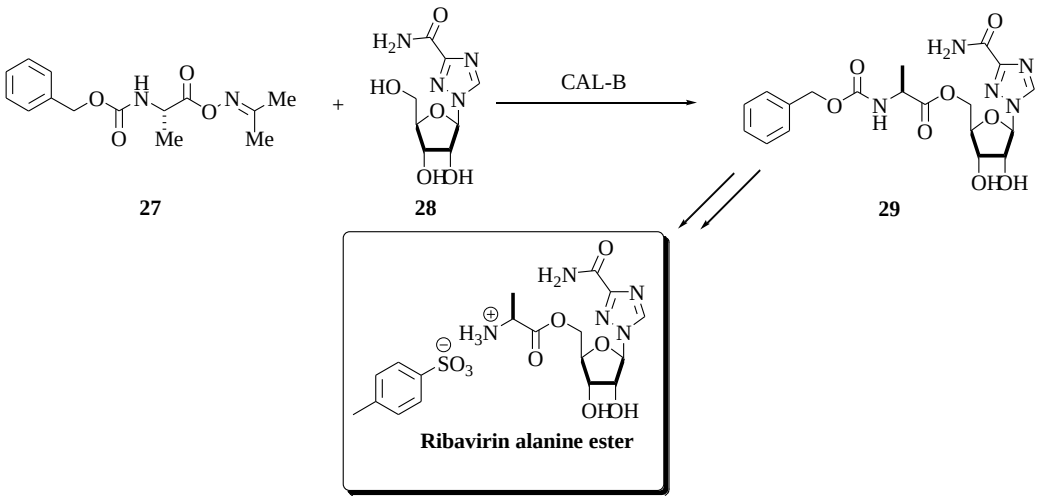
Scheme 10

Table 4. Synthesis of **Odanacatib** and **CEP-28112**, enzymatic steps

Substrate	t (h)	$m_{substrate}$ (kg)	m_{enzyme} (kg)	yield (%)	$ee_{product}$ (%)
22	4	4	4	80	84
(±)-**24**	144	12.5	8.8	48	98.3

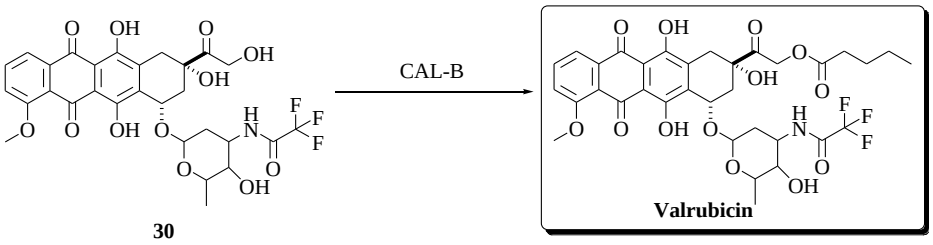
2.1.4. CAL-B catalysed OH-acylations

Ribavirin is an antiviral agent used in combination with peginterferon alfa-2*β* or peginterferon alfa-2*α* to treat hepatitis C or severe RSV infections.[69-71] It has highly variable pharmacological characteristics, which may cause several serious side effects (anemia, hemolysis) or low response due to the low bioavailability. The alanine ester of ribavirin may have more favourable pharmacokinetic profile and may reduce the side effects. Tamarez et al.[72] described a regioselective route for the preparation of a prodrug intermediate of **Ribavirin** through a primary *OH*-acylation process. The reaction was performed with CAL-B (Chirazyme L-2) using *O*-(*N*-benzyloxycarbonyl-L-alanyl)acetoxime as acyl donor in THF at 60 °C (Scheme 11). High regioselectivity (*de* = 99.9%) and good isolated yields (an average of 82%) were achieved in three batches (Table 5).



Scheme 11

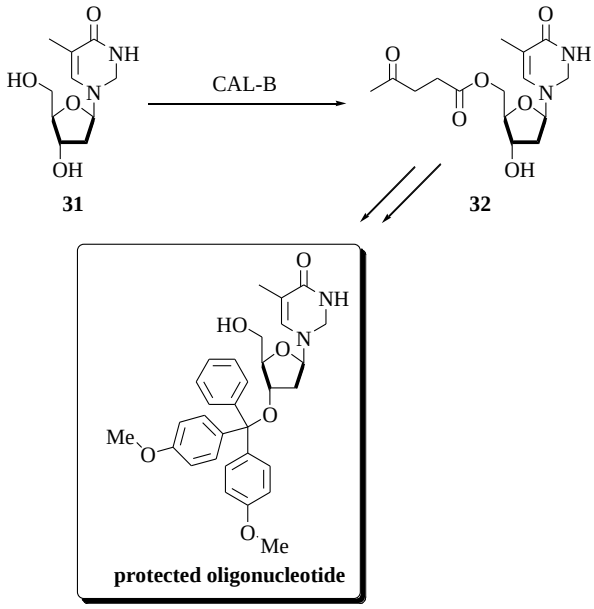
Valrubicin is an *N*-trifluoroacetylated doxorubicin analogue used in the treatment of bladder carcinoma, as topoisomerase II and RNA synthesis inhibitor.[73,74] A few synthetic routes were published for the preparation of **Valrubicin**.[75,76] In addition, Cotterill and Rich[77] worked out an efficient two-step, CAL-B catalysed regioselective synthesis through a primary *OH*-acylation step (Scheme 12). The enzymatic reaction was performed in a mixture of MTBE/2-butanone (3:1) in the presence of valeric acid as acyl donor at 58 °C. The two-step process is regioselective with an overall yield of 79% (Table 5).



Scheme 12

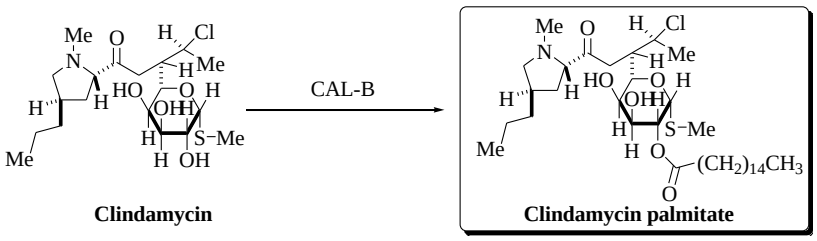
Oligonucleotide drugs are promising therapeutic agents for the treatment of cardiovascular, metabolic and infection diseases, and a variety of cancers.[78] Two excellent examples for oligonucleotide structures of high importance are the antiviral **Vitravene®** and the anti-angiogenic **Macugen®**. Díaz-Rodríguez and co-workers[79] described an easy, efficient and scalable chemoenzymatic route for the regioselective synthesis of 3'-*O*-dimethoxytritylthymidine successfully addressing the challenging problem of selective protection of a multifunctional molecule. The key step is a CAL-B catalysed enzymatic

primary *OH*-acylation in anhydrous THF under nitrogen in a suspension of thymidine and acetonoximelevulinate (Scheme 13). The *purity* of the product was excellent (>98%) with an overall yield of 73% (Table 5).



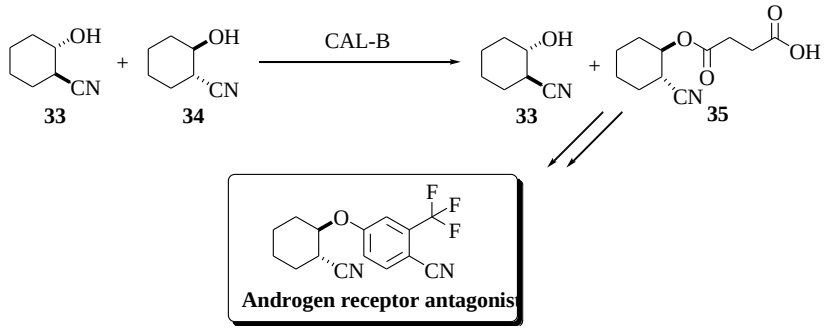
Scheme 13

Clindamycin palmitate is a prodrug of **Clindamycin**, which is effective against Gram positive organisms, Gram negative anaerobes,[80] and used in the treatment of a wide variety of serious bacterial infections including infections of the respiratory tract, skin and soft tissue. The chemical synthesis process of **Clindamycin palmitate** from **Clindamycin** is a three-step reaction providing a yield below 50%. Li et al.[81] developed a lipase-catalysed (Novozym 435) one-step reaction, which simplifies the preparation of **Clindamycin palmitate** and affords a much higher yield (~90%). The enzymatic reaction was performed in toluene in the presence of vinyl palmitate as acyl donor at room temperature (Scheme 14). The overall yield was 90% and the reaction was regioselective at the 2-*OH* position (Table 5).



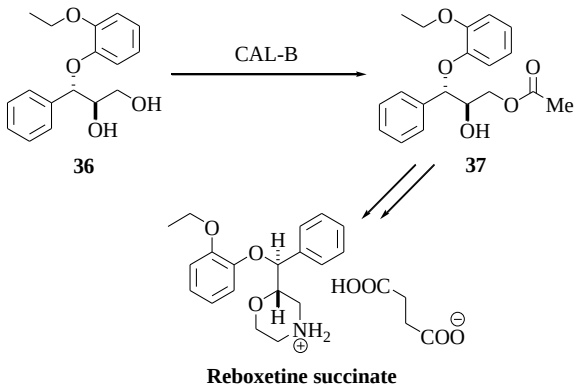
Scheme 14

4-[(1*R*,2*S*)-2-Cyanocyclohexyloxy]-2-(trifluoromethyl)benzonitrile is an androgen receptor antagonist molecule which was developed for the treatment of alopecia and excess sebum.[82,83] Synthetic processes affording the highest selectivities were either unprofitable or had low *E*.[84] Vaidanathan and co-workers[85] improved the first-generation synthetic route with an enzymatic step. Several enzymes were tested and the best selectivity was observed with CAL-B (Novozym 435) in the presence of succinic anhydride in MTBE at 50 °C (Scheme 15). The reaction was selective with a 38% yield and >99.9% *ee* was determined for the product enantiomer (**35**) (Table 5).



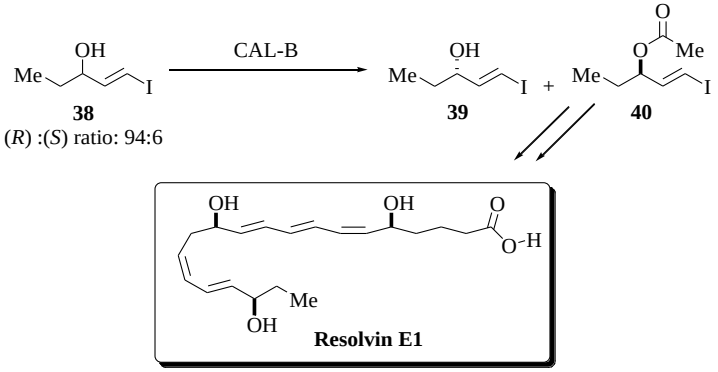
Scheme 15

Reboxetine mesylate is a selective norepinephrine reuptake inhibitor (**Edronax®**). The (*S*,*S*)-enantiomer is significantly more active than either the (*R*,*R*)-enantiomer or the racemate.[86-88] (*S*,*S*)-**Reboxetine succinate** is a potential drug candidate for the treatment of fibromyalgia. There are several published synthetic routes for the preparation (*S*,*S*)-**Reboxetine**.[89,90] Hayes et al.[91] worked out a synthesis for the preparation of **Reboxetine succinate**, which included a one-kg-scale regioselective step catalysed by CAL-B (Novozym 435) (Scheme 16). They used isopropenyl acetate for the acylation of the primary *OH* of (2*R*,3*S*)-3-(2-ethoxyphenoxy)-3-phenylpropane-1,2-diol (**36**) in toluene at 30 °C. Both the yield (>99%) and the *ee* (>98%) were excellent (Table 5).



Scheme 16

Resolvin E1 (**RevE1**) is a member of a family of endogenous small molecules, which are key mediators of the resolution of inflammation. Due to these anti-inflammatory properties, **RevE1** might be a potential therapeutic agent of conditions involving uncontrolled inflammation such as allograft rejection, asthma and inflammatory pain.[92,93] Numerous synthetic routes have been reported for the preparation of **RevE1**.[94,95] Amin and co-workers[96] described a revised multi-kilogram process of **RevE1** chiral key intermediate. The starting material of the enzymatic step was (*E*)-1-iodopent-1-en-3-ol (**38**) [(*R*)/(*S*) ratio= 94 : 6]. The best selectivity of several lipases was observed with CAL-B (Novozym 435) in the presence of vinyl acetate in MTBE at 20 °C (Scheme 17). The overall yield was 75% with high enantioselectivity (*ee* >99%) (Table 5).



Scheme 17

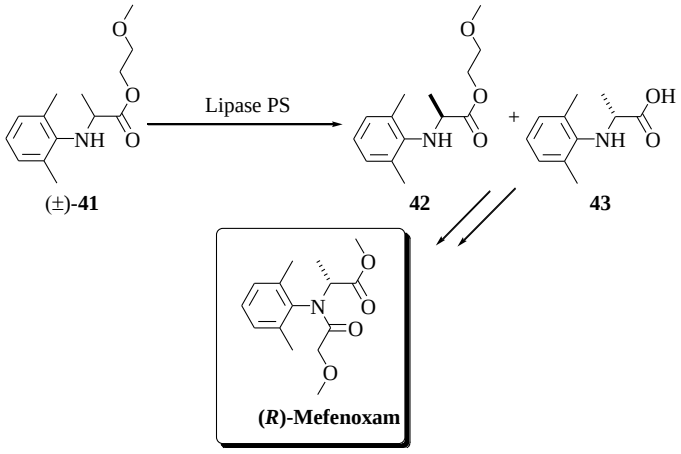
Table 5. Synthesis of **Ribavirin alanine ester**, **Valrubicin**, **a protected oligonucleotide**, **Clindamycin palmitate**, **CEP-28112**, **androgen receptor antagonist**, **Reboxetine succinate** and **Resolvin E1**, enzymatic steps

Substrate	t (h)	$m_{substrate}$ (kg)	m_{enzyme} (kg)	yield (%)	$ee_{product}$ (%)	$de_{product}$ (%)	$purity_{product}$ (%)
27	24	18	14	83	n.d.	99.9[a]	n.d.
30	8	4.5×10^{-4}	2×10^{-3}	90	n.d.	n.d.	n.d.
31	23	2.5×10^{-2}	2.5×10^{-2}	73	n.d.	n.d.	> 98%
Clindamycin	0.5	1.06×10^{-3}	1.2×10^{-3}	90	n.d.	n.d.	n.d.
33, **34**	24	22.4	22.4	38	> 99.9	n.d.	n.d.
36	17	1	2×10^{-2}	> 99	> 98%	n.d.	n.d.
38	15	3.1	2×10^{-1}	75	> 99	n.d.	n.d.

2.2. Application of lipase PS in the pharmaceutical industries

2.2.1. Lipase PS-catalysed ester hydrolysis

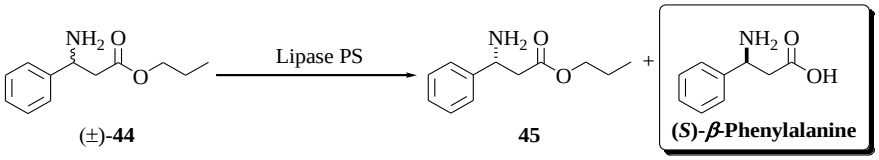
Mefenoxam is a widely used pesticide, which has fungicide effect.[97] In the asymmetric synthetic route of **Mefenoxam** chiral catalysts are used, but the scale-up of the reaction is problematic. Park et al[98] described a method for the preparation of methyl (*R*)-*N*-(2,6-dimethylphenyl)alaninate (**43**) a key chiral intermediate of **Mefoxam**. They applied lipase PS (PS on Sepabead EC-butyl) as catalyst in phosphate buffer (pH = 7.0) at 25–30 °C (Scheme 18). The reaction was (*R*)-selective (*ee* = 96%, yield = 34%) (Table 6).



Scheme 18

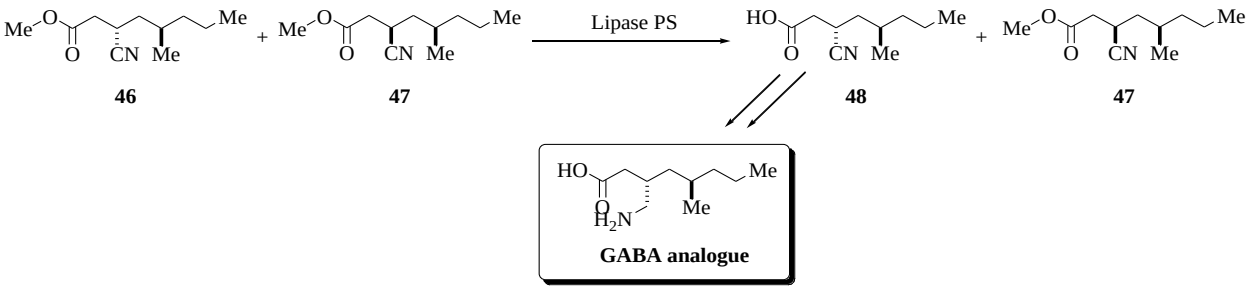
β-Amino acids and some of their derivatives are widely used in combinatorial, peptide, organic and medicinal chemistry.[3, 99-101] **(*S*)-*β*-Phenylalanine** is a key intermediate in the

preparation of the antibiotic **Pyloricidin A** and **Moiramide A**. The synthesis of **(*S*)-*β*-Phenylalanine** has been published as part of the synthesis of maraviroc,[102] but it was not suitable for development to full manufacturing scale. Grayson and co-workers[103] reported a number of laboratory routes for the preparation of **(*S*)-*β*-Phenylalanine** in scales of 400 kg. They used lipase PS (Amano) for the stereoselective step in a biphasic mixture (MTBE/H_2O, pH = 8.2) at 30 °C (Scheme 19). A yield of 41.3% and an excellent *ee* of 99% were reported (Table 6).



Scheme 19

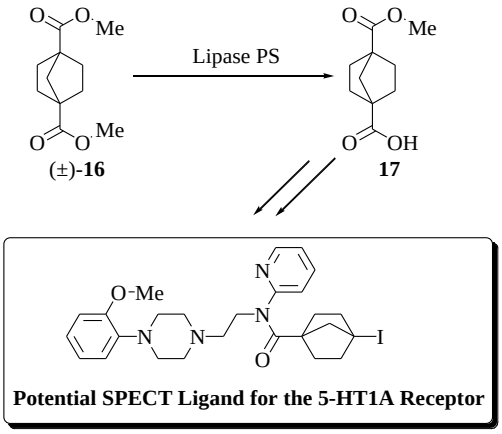
(3*S*,5*R*)-3-(Aminomethyl)-5-methyloctanoic acid is a lipophilic γ-aminobutyric acid analogue nominated for development to treat interstitial cystitis.[104] Murtagh et al.[105] developed the modified medicinal chemistry route for the preparation of this compound from methyl (5*R*)-3-cyano-5-methyloctanoate (**46**, **47**) using PS-SD as chiral catalyst in H_2O/sodium bicarbonate/toluene mixture at 45°C (Scheme 20). The yield was 45.6% and the *de* of the product enantiomer was 99.4% (Table 6).



Scheme 20

4-(Methoxycarbonyl)bicyclo[2.2.1]heptane-1-carboxylic acid (**17**) is a building block of many therapeutic candidates, including 4-iodo-*N*-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-*N*-(pyridine-2-yl)cubyl-1-carboxamide, which is a 5-hydroxytryptamine-1A (5-HT_{1A}) receptor ligand. Guo et al.[53] developed an enzyme-catalysed scale-up process (see also Chapter 2.1.1). PS-SD was applied in sodium phosphate buffer (pH = 7.0) at 48 °C (Scheme

21). The yield was 94% and the ratio of monoester/diester/diacid was 97.4 : 2.6 : 0.0 (Table 6).



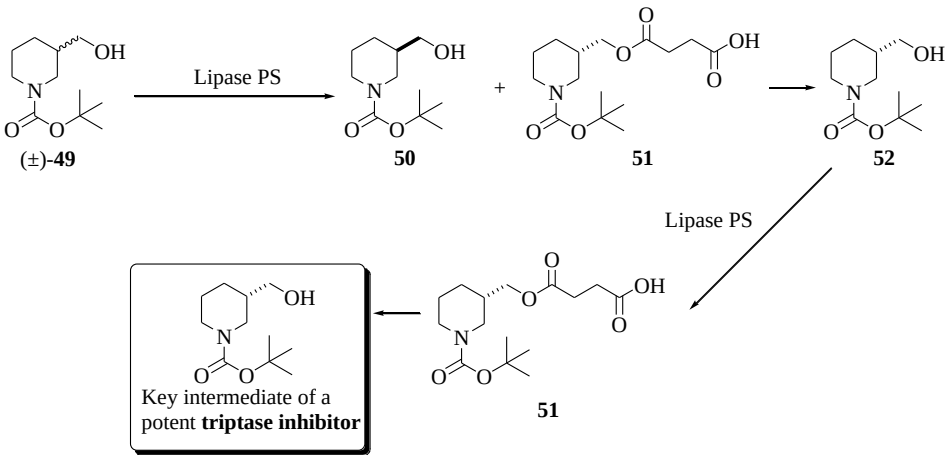
Scheme 21

Table 6. Synthesis of **Mefenoxam**, **(*S*)-*β*-Phenylalanine**, **GABA analogue** and **SPECT ligand**, enzymatic steps

Substrate	t (h)	$m_{substrate}$ (kg)	m_{enzyme} (kg)	yield (%)	$ee_{product}$ (%)	$de_{product}$ (%)	$er_{product}$ (%)
(±)-**41**	41	4	0.96	34	96	n.d.	n.d.
(±)-**44**	15	430	21	41.3	99	n.d.	n.d.
46, **47**	48	54	10.8	45.6	n.d.	99.4	n.d.
(±)-**16**	25	2×10^{-2}	3×10^{-2}	94	n.d.	n.d.	95.82

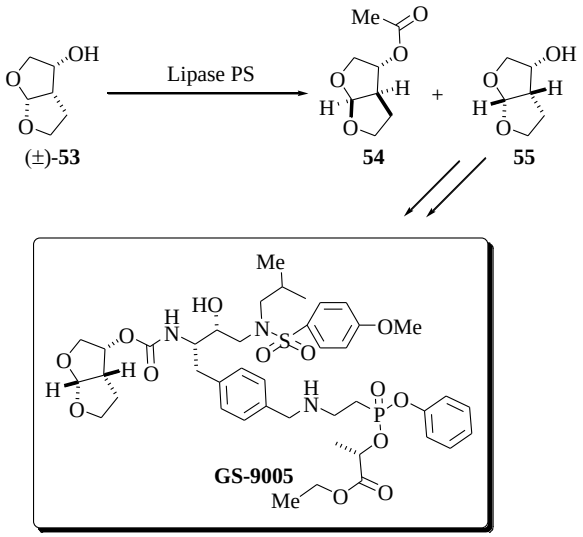
2.2.2. Lipase PS-catalysed OH-acylations

Tryptase inhibitors are a class of anti-inflammatory drugs.[106] *S-N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine is a key intermediate in the preparation of a potent tryptase inhibitor. Goswani and co-workers[107] described a lipase PS-catalysed two-step route for the synthesis from (*R*,*S*)-*N*-(*tert*-butoxycarbonyl)-3-hydroxymethylpiperidine (**49**) in toluene in the presence of succinic anhydride at room temperature (Scheme 22). The overall yield of the two steps was 32% and the *ee* was 98.9% (Table 7).



Scheme 22

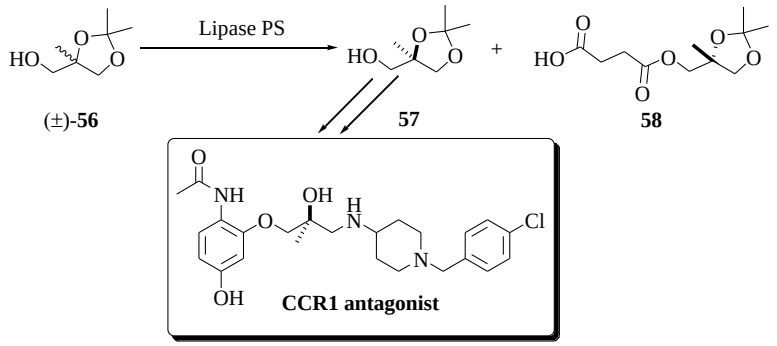
Human immunodeficiency virus (HIV) is a retrovirus, which causes HIV infection and acquired immunodeficiency syndrome (AIDS).[108,109] (3a*R*,3a*S*,6a*R*)-Hexahydrofuro[2,3-b]furan-3-ol moiety (**GS-9005**) is a potent HIV protease inhibitor candidate. There are several synthetic routes published for its preparation.[110,111] Yu et al.[112] described a lipase PS (PS-C1) catalysed flow-chemistry route for the synthesis of **55** in ethylene glycol dimethyl ether at 25–30 °C (flow rate: 2 L/min) (Scheme 23). A yield of 33% and an *ee* of 97% were achieved with acetic anhydride as acyl donor (Table 7).



Scheme 23

Chemokine receptors (CCR) are important in the occurrence of various diseases and disorders with immune or inflammatory response (asthma, rheumatoid arthritis and

atherosclerosis). *N*-[2-((2*S*)-3-[(1-(4-Chlorobenzyl)piperidin-4-yl)amino]-2-hydroxy-2-methylpropyl)oxy)-4-hydroxyphenyl] acetamide is a potent **CCR1 antagonist**.[113] Ainge and co-workers[114] modified the medicinal chemistry route and described a scale-up synthesis. They used lipase PS (Lipase PS Amano IM) for the stereoselective step in TBME at 20 °C with succinic anhydride as acyl donor (Scheme 24). The yield of the enzymatic step and the *purity* of the product, respectively, were 27% and 95% (Table 7).



Scheme 24

Table 7. Synthesis of **a potent tryptase inhibitor**, **GS-9005** and **CCR1 antagonist**, enzymatic steps

Substrate	t (h)	$m_{substrate}$ (kg)	m_{enzyme} (kg)	yield (%)	$ee_{product}$ (%)	$purity_{product}$ (%)
(±)-**49**	2	2.55×10^{-1}	6.4×10^{-3}	48	85.7	n.d.
(±)-**53**	n.d.	4.85	0.36	33	97	n.d.
(±)-**56**	6	1.13×10^{-1}	1.25×10^{-2}	27	n.d.	95

3. Materials and methods

3.1. Materials and instruments

The enzymes used in this work were commercially available. Thus, Lipase PS (immobilized on diatomaceous earth) was from Amano Enzyme Europe Ltd. CAL-B, produced by the submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin (Catalogue No. L4777), was from Sigma-Aldrich. PPL was also a product from Sigma–Aldrich, while CAL-A was purchased from Roche Diagnostics Corporation. Lipase AK was from Amano Pharmaceuticals, and lipase AY was a product from Fluka. Solvents were of the highest analytical grade.

Batch reactions were performed in an incubator shaker (Innova 4000). 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 or gas chromatography (GC). An amylase-based Chiralpak IA column was used for HPLC analysis. An L-Val and a Chrompack Chirasil-Dex CB column were used for GC measurements. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. All melting points were measured on an X-4 melting-point apparatus with a microscope. The elemental analyses were performed with a Perkin-Elmer 2400 CHNS elemental analyser.

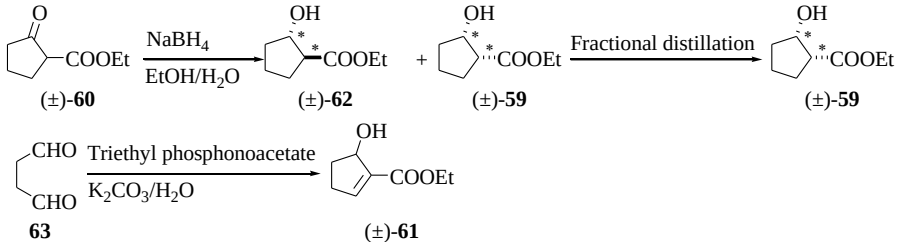
3.2. Enzymatic experiments

The enzymatic works were started with optimisation of the reaction conditions of model compounds. These small-scale reactions were performed as batch reactions. First, an enzyme screening was carried out to identify the best catalyst. Then preliminary experiments were conducted in different solvents, in the presence of additives, at different temperatures. In a typical small-scale experiment the enzyme (30 mg mL^{-1}) was added to the racemic compound (0.007 M, 0.015 M or 0.05 M solutions) in a solvent (1 mL) followed by adding the additive (0, 1, 1.2, 2, 3, 4, 5, 6, 10, 12, 15, 20, 25, 50 or 100 equiv.). The mixture was shaken at 2, 4, 25, 30, 45, 50, 60, 70 or 80 °C.

Preparative-scale reactions were performed under the optimised conditions. The formed enantiomers were separated by adequate methods; for example, extraction or column chromatography (details are in the annexed articles). The separated enantiomers were characterized by NMR, elemental analysis, specific rotation and melting point. Absolute configurations were determined by comparing the optical rotations of the enantiomers or their derivatives with literature data.

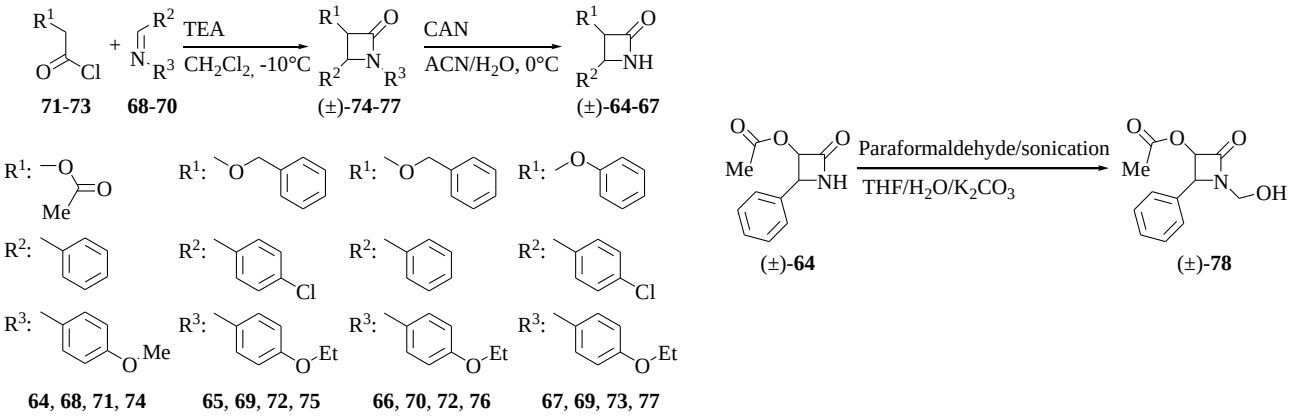
3.3. Syntheses of starting materials

Ethyl *cis*-(±)-2-hydroxycyclopentane-1-carboxylate [(±)-**59**] was synthetized from ethyl 2-oxocyclopentane-1-carboxylate [(±)-**60**] by $NaBH_4$ reduction, followed by separation of the resulting *cis* and *trans* diastereomers, according to a literature method.[115] A literature process[116] was also applied for the synthesis of ethyl (±)-5-hydroxycyclopent-1-enecarboxylate [(±)-**61**] starting from succinaldehyde and triethylphosphonoacetate in aqueous potassium carbonate solution (Scheme 25).



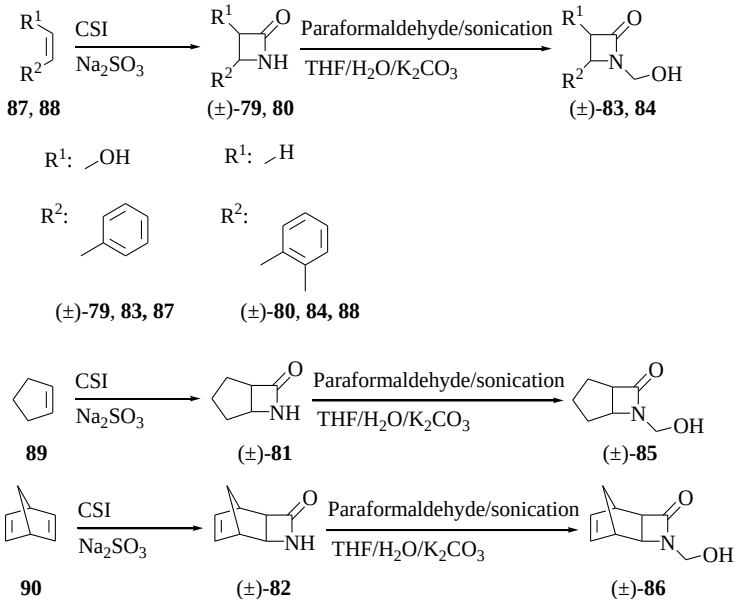
Scheme 25

Racemic *cis*-3-acetoxy-4-phenylazetidin-2-one [(±)-**64**],[117,118] racemic *cis*-3-benzyloxy-4-(4-chlorophenyl)azetidin-2-one [(±)-**65**], *cis*-3-benzyloxy-4-phenylazetidin-2-one [(±)-**66**] and *cis*-4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(±)-**67**] were synthesized according to literature methods.[119] A mixture of *p*-ethoxyaniline and the appropriate aldehyde furnished the corresponding Schiff bases [**68**–**70**] which, through cycloadditions in the presence of the appropriate acyl chlorides [**71**–**73**], resulted in the formation of *N*-protected *β*-lactams (±)-**74**–**77**. CAN-mediated oxidative removal of the 4-ethoxyphenyl group gave the desired *β*-lactams [(±)-**64**–**67**]. (±)-**64** was then transformed with paraformaldehyde under sonication[120,121] into *N*-hydroxymethyl-*cis*-3-acetoxy-4-phenylazetidin-2-one (±)-**78** (Scheme 26).



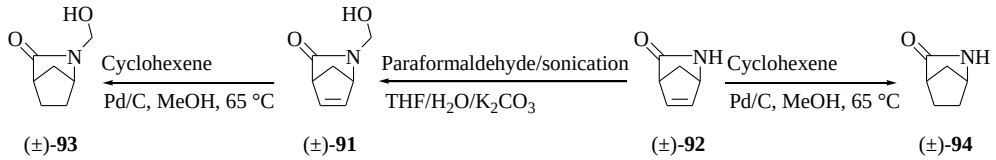
Scheme 26

Racemic *cis*-3-hydroxy-4-phenylazetidin-2-one [(±)-**79**], 4-(*o*-tolyl)azetidin-2-one [(±)-**80**], 6-azabicyclo[3.2.0]heptan-7-one [(±)-**81**] and *exo*-3-azatricyclo[4.2.1.0$^{2.5}$]non-7-en-4-one [(±)-**82**] were prepared according to the literature.[118] Then the products were transformed with paraformaldehyde under sonication[121] to *N*-hydroxymethyl-*cis*-3-hydroxy-4-phenylazetidin-2-one [(±)-**83**], *N*-hydroxymethyl-4-(*o*-tolyl)azetidin-2-one [(±)-**84**], *N*-hydroxymethyl-6-azabicyclo [3.2.0] heptan-7-one [(±)-**85**] and *N*-hydroxymethyl-*exo*-3-azatricyclo[4.2.1.0$^{2.5}$]non-7-en-4-one [(±)-**86**] (Scheme 27).



Scheme 27

Racemic *N*-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-**91**] was synthesized from commercially available 2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-**92**] with paraformaldehyde under sonication.[122] Catalytic transfer hydrogenation of (±)-**91** and (±)-**92** in the presence of cyclohexene as a hydrogen donor gave, respectively, racemic *N*-hydroxymethyl-2-azabicyclo[2.2.1]heptan-3-one [(±)-**93**] and 2-azabicyclo[2.2.1]heptan-3-one [(±)-**94**] (Scheme 28).[123]



Scheme 28

3.4. Analytical methods

The progress of the reactions was monitored by analysing samples directly or after derivatisation by GC or HPLC equipped with a chiral column taken from the reaction mixture at intervals. From the chromatograms, *ee*, conversion and *E* were calculated via the following equations:

$$ee_s = (A_2 - A_1) / (A_1 + A_2)$$

$$ee_p = (A_3 - A_4) / (A_3 + A_4)$$

$$\text{Conv.} = ee_s / (ee_s + ee_p)$$

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

When an internal standard was used, the *ee* and conversion were calculated[124] via the following equations:

$$k^0 = A_{st}^0 / (A_1^0 + A_2^0)$$

$$k = A_{st} / (A_1 + A_2)$$

$$\text{Conv.} = 100 \times (k - k^0) / k$$

where A_1, A_2, A_3 A_4 and A_{st} are peak areas where $A_2 > A_1$, $A_3 > A_4$.

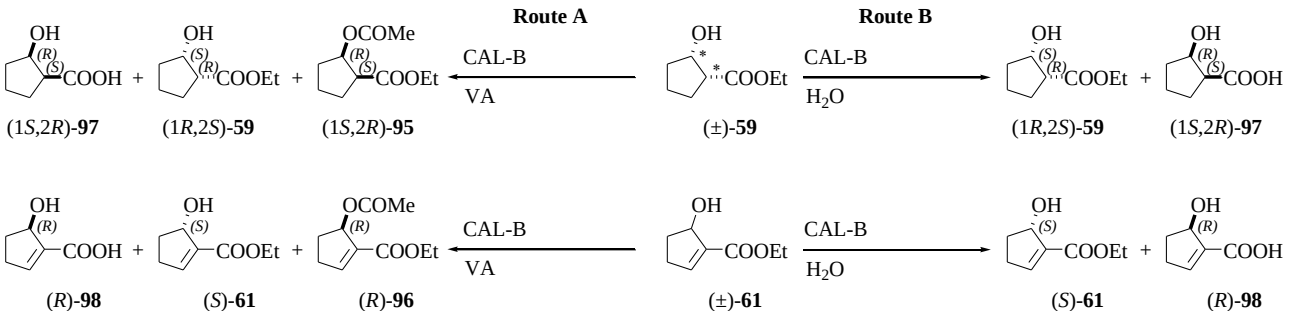
The *ee* values for *β*-hydroxy esters [**59**, **61**] and the *O*-acylated enantiomers [**95**, **96**] were determined by GC on a Chrompack Chirasil-Dex CB column. Samples were injected directly into the column. The *ee* values for *β*-hydroxy acids [**97**, **98**] were determined by GC on a Chrompack Chirasil-Dex CB column after a simple derivatization with CH_2N_2. The *ee* value for *β*-lactam **78** was determined by GC on a Chirasil L-Valine column. The samples were injected directly into the column. The *ee* values for the *N*-hydroxymethylated *β*-lactams [**83**–**86**, **99**, **100**] were determined by GC on a Chrompack Chirasil-Dex CB column. The samples were injected directly into the column. The *ee* values for the *N*-hydroxymethylated *γ*-lactams [**91**, **93**] were determined by GC on a Chrompack Chirasil-Dex CB column. The samples were injected directly into the column. The *ee* values for *β*-lactams [**64**–**67**] were determined by HPLC on a Chiralpak IA column. The samples were injected directly into the column. The *ee* value for *β*-amino acid **101** was determined by GC on a Chrompack Chirasil-Dex CB column after a simple and rapid double derivatization[125] with (i) CH_2N_2 and (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine. The *ee* values for *β*-amino acids [**102**–**104**] were determined by GC on a Chirasil L-Valine column after a simple and rapid double derivatization[125] with (i) CH_2N_2 and (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine. The *ee* values for *β*-amino acids [**105**–**107**] were determined by HPLC on a Chiralpak IA column after a simple derivatization with CH_2N_2. The *ee* values for *γ*-amino acids [**108**, **109**] were determined by GC on a Chrompack Chirasil-Dex CB column after a simple and rapid double derivatization[125] with (i) CH_2N_2 and (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine. Analysis conditions and retention times for the HPLC and GC analyses can be found in the original papers.[I-V]

4. Results and discussion

4.1. CAL-B catalysed reactions of *β*-hydroxy esters. New routes for the preparation of pharmaceutically active key intermediates.[I]

CAL-B is one of the most useful enzymes for the preparation of enantiomerically enriched amines, carboxylic acids and alcohols due to its broad substrate specifity.[126,127] It is a widely-used catalyst for various enantio- and regioselective reactions such as acylation[128], transesterification[129] and hydrolysis.[130] Some of the reported substrates to be acylated contain a COOR function next to the reactive OH or NH group.[131-133] Here, the question arises whether the ester group undergoes hydrolysis. This assumption also emerged from the results published by Gotor et al.[132] about the CAL-B catalysed enantioselective *O*-acylation of some cyclic *cis*-*β*-hydroxy esters, such as ethyl *cis*-(±)-2-hydroxycyclopentane-1-carboxylate [(±)-**59**)]. The authors described excellent *E* (>200) for the *R*-selective acylation of (±)-**59** with 3 equiv. of VA in TBME at 30 °C, although the *O*-acylated enantiomer was obtained in a relatively modest yield of 35%. This low yield is not surprising if hydrolysis of the C1 ester function occured as a side-reaction.

In our work, the main challenge was to demonstrate the occurrence of hydrolysis of the C1 ester function during the CAL-B catalysed *O*-acetylation of (±)-**59** and (±)-**61** with 2 equiv. of VA in TBME at 30 °C (Scheme 29, Route A). If hydrolysis was demonstrated, we wanted to devise a new enzymatic route for the resolution of (±)-**59** and (±)-**61** through CAL-B catalysed hydrolysis (Scheme 29, Route B).



Scheme 29

4.1.1. Small-scale resolutions

First, acetylation of (±)-**59** was performed with CAL-B in TBME, 3 equiv. of VA at 30 °C (conditions used by Gotor's group). After a 30-min reaction, in addition to the *O*-acetylated product [(1*S*,2*R*)-**95**, *ee* >98%] and the unreacted *β*-hydroxy ester [(1*R*,2*S*)-**59**, *ee* ~35%], a significant amount of hydroxy acid [(1*S*,2*R*)-**97**, *ee* >98%] was detected. A conversion of ~30% was calculated[124] with the use of *n*-heptadecane as an internal standard.

When acetylation of (±)-**61** was carried out under the above conditions (CAL-B, 3 equiv. of VA, TBME, 30 °C), the hydrolysis of the C1 ester group occurred just as in the case of (±)-**59**. After a reaction time of 1 h, the desired products were obtained with relatively low enantioselectivities [(*S*)-**61**, *ee* = 20%, (*R*)-**96**, *ee* >98%, (*R*)-**98**, *ee* = 40%] and the conversion for the hydrolysis reached 30% (calculated with the use of (1*S*,6*R*)-7-azabicyclo[4.2.0]oct-3-en-8-one[68] as an internal standard). When VA was used in a higher excess (5, 10, 25 or 50 equiv.), the hydrolysis of (±)-**61** could be limited (after a reaction time of 1 h, conversion ~9% with 3 equiv. and <1% with 5 equiv. *vs.* ~30% with 2 equiv. of VA).

As hydrolysis during acylation demonstrated, our attention turned to optimize the hydrolysis of (±)-**59**. On the basis of earlier observations of the lipase-catalysed hydrolysis of *β*-amino esters[134], we started preliminary experiments with enzyme screening, performing the reactions in TBME at 45 °C with 4 equiv. of added H_2O as nucleophile. Besides CAL-B, a number of lipases (PS, AK, AY, PPL and CAL-A) were tested (Table 8).

Table 8. Enzyme screening for the hydrolysis of (±)-**59**[a].

Entry	Enzyme (30 mg mL^{-1})	$ee_{substrate}$[b] (%)	$ee_{product}$[c] (%)	Conv. (%)	*E*
1	Lipase PS	34	8	80	1
2	Lipase AK	15	35	30	2
3	Lipase AY	23	14	62	2
4	PPL	3	35	8	2
5	CAL-A	5	49	9	3
6	CAL-B	29	93	24	36

[a] 0.05 M substrate, 30 mg mL^{-1} CAL-B, TBME, 45 °C, after 30 min. [b] According to GC. [c] According to GC, after derivatization with CH_2N_2.

All lipases tested showed some activity, but the best *E* was observed with CAL-B (entry 6), which was chosen as the catalyst for further experiments. Next, the effects of solvents and increased amounts of CAL-B on the *E* and reaction rate were tested (Table 9). The moderately enantioselective hydrolysis of (±)-**59** was found to be relatively slow in *n*-hexane, THF and 1,4-dioxane (entries 6–8), but slightly faster in toluene (entry 5) and CH_2Cl_2 (entry 9). Reactions were relatively fast in ether-type solvents (entries 1 and 4). In spite of the lower *E* (36 *vs*. 40), TBME as a green solvent was chosen for use in further experiments. As the amount of enzyme was increased from 30 to 50 and then 75 mg mL^{-1} (entries 1–3), reaction rates increased slightly, while *E* decreased progressively. Thus, 30 mg mL^{-1} enzyme was used in subsequent experiments.

Table 9. Conversion and *E* of the hydrolysis of (±)-**59**[a].

Entry	Solvent	CAL-B (mg mL^{-1})	$ee_{substrate}$[b] (%)	$ee_{product}$[c] (%)	Conv. (%)	*E*
1	TBME	30	29	93	24	36
2	TBME	50	31	91	25	28
3	TBME	75	35	86	29	18
4	Et_2O	30	21	94	18	40
5	Toluene	30	8	83	9	12
6	*n*-Hexane	30	4	72	5	6
7	THF	30	3	78	4	8
8	1,4-Dioxane	30	3	85	3	13
9	CH_2Cl_2	30	8	43	16	3

[a] 0.05 M substrate, 4 equiv. of added H_2O, TBME, 45 °C, after 20 min. [b] According to GC. [c] According to GC, after derivatization with CH_2N_2.

Both conversion and *E* found with CAL-B in TBME at 45 °C were affected by the amount of H_2O added to the reaction mixture. As the concentration was increased from 0 to 15 equivalents, the reaction rate increased, but *E* showed a maximum of 22 at 10 equiv. of H_2O. Therefore, 10 equiv. of added H_2O was used for the preparative-scale reaction. Note that in accordance with earlier observations,[124, 135] the reaction proceeded even without the addition of H_2O (entry 1). The quantity of H_2O present in the reaction medium (<0.1%) or in the enzyme preparation (2–5%) was still sufficient for the hydrolysis. It is known, that the temperature can influence both the *E* and the reaction rate of a lipase-catalysed reaction.[136,137] Therefore, reactions were performed at 2, 25 and 30 °C. The *E* and the reaction rate increased

slightly with increasing temperature. However, further increases in temperature (45, 60, 70, 80 °C) were accompanied by decreases in both *E* and the reaction rate (data not shown).

When the hydrolysis of (±)-**61** was performed under conditions optimized for the hydrolysis of (±)-**59** (CAL-B, 10 equiv. of added H_2O, TBME, 30 °C), the results obtained were modest ($ee_{(S)\text{-}\mathbf{61}}$ = 22% and $ee_{(R)\text{-}\mathbf{98}}$= 49%, conv. = 34% after 1 h, *E* = 4) similar to those found in the case of (±)-**59**.

4.1.2. Preparative–scale resolutions

Since the CAL-B catalysed asymmetric acetylation of (±)-**61** has not yet been described, a preparative-scale acetylation of (±)-**61** was performed with 5 equiv. of VA. The *E* was excellent (E >200) and the enantiomers were isolated in good yields (>43%) and with high enantiomeric excess values (*ee* >98%). The preparative-scale hydrolysis of (±)-**59** and (±)-**61** were performed with CAL-B (30 mg mL^{-1}) in TBME with 10 equiv. of added H_2O at 30 °C. The results are presented in Table 10. Both hydroxy acids [(1*S*,2*R*)-**97** and (*R*)-**98**] proved to be quite unstable allowing to record the ^{1}H NMR spectrum only for (1*S*,2*R*)-**97**. (Table 10).

Table 10. Preparative-scale resolutions of (±)-**59** and (±)-**61**[a].

Substrate	VA or H_2O	t (h)	Conv. (%)	Product enantiomer				Unreacted enantiomer			
				Yield (%)	Isomer	$ee_{product}$[b] (%)	$[\alpha]_D^{25}$	Yield (%)	Isomer	$ee_{substrate}$[c] (%)	$[\alpha]_D^{25}$
(±)-**59**	H_2O	1	22	16	(1*S*,2*R*)-**97**	90	-23[d]	78	(1*R*,2*S*)-**59**	26	+8[e]
(±)-**61**	VA	1.1	50	46	(*R*)-**96**	>98	+2,5[f]	43	(*S*)-**61**	>98	-34[g]
(±)-**61**	H_2O	6	34	28	(*R*)-**98**	47	+5[h]	60	(*S*)-**61**	24	-6[i]

[a] 0.05 M substrate, 5 equiv. of VA as acyl donor or 10 equiv. of added H_2O as nucleophile, TBME, 30 °C. [b] According to GC, after derivatization with CH_2N_2. [c] According to GC. [d] c = 0.15; H_2O. [e] c = 0.25; EtOH. [f] c = 0.4; $CHCl_3$. [g] c = 0.31; $CHCl_3$. [h] c = 0.2; EtOH. [i] c = 0.18; $CHCl_3$

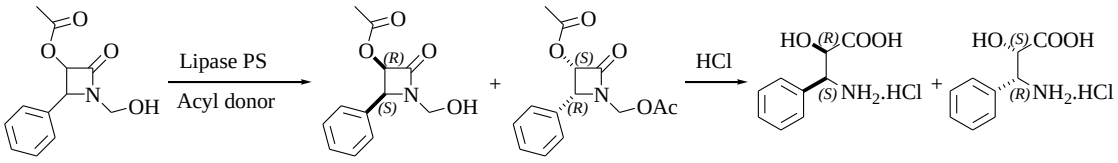
4.1.3. Absolute configurations

Absolute configurations were measured by comparing the [α] value of the unreacted (*S*)-**61** $[\alpha]_D^{25} = -34$ (c = 0.31; $CHCl_3$) with the literature value[138] for (*S*)-**61** $[\alpha]_D^{31} = -34.5$ (c = 1.10; $CHCl_3$); thus, the CAL-B catalysed acetylation of (±)-**61** displayed *R* selectivity.

4.2. Lipase PS-catalysed *O*-acylation. A new route for the preparation of pharmaceutically active key intermediate.[II]

Enantiopure *β*-amino acids and their derivatives are important building blocks of pharmaceutically and chemically important molecules.[2, 139-141] They have been recognized as an important class of compounds for the synthesis of pharmaceutical drugs, for example, **Taxol** and its analogue **Taxotere**.[9, 142] Since the total synthesis of **Taxol** is a very lengthy and expensive process, chemists are continuously working on the development of semi-synthetic methods, which involve the coupling of the corresponding side-chain to the C(13)-*O* of baccatin III derivatives. We have published very efficient enzyme-catalysed direct and indirect strategies for the preparation of (2*R*,3*S*)-3-phenylisoserine [(2*R*,3*S*)-**101**], either through lipase PS-catalysed ester hydrolysis of racemic ethyl 3-amino-3-phenyl-2-hydroxypropionate [H_2O as nucleophile in TBME or iPr_2O, 50 or 60 °C (E >200)][143], CAL-B catalysed ring cleavage of racemic *cis*-3-hydroxy-4-phenylazetidin-2-one [H_2O in TBME, 60 °C (*E* >200)][144] and a new type of enzymatic two-step hydrolytic cascade reaction of racemic *cis*-3-acetoxy-4-phenylazetidin-2-one [H_2O in iPr_2O, 60 °C].[144]

With regard to previous results on enzymatic acylation of primary alcohols with a remote stereocentre[145], our aim was to develop an enantioselective method for the preparation of (2*R*,3*S*)-**101**, through the enzymatic *O*-acylation of racemic *N*-hydroxymethylated *cis*-3-acetoxy-4-phenylazetidin-2-one [(±)-**78**] (Scheme 30).



(±)-**78** (3*S*,4*R*)-**78** (3*R*,4*S*)-**99** (3*R*,4*S*)-**100** (2*R*,3*S*)-**101.HCl** (2*S*,3*R*)-**101.HCl**

Scheme 30

4.2.1. Small-scale resolutions

On the basis of earlier results on the enzymatic acylations of cyclic[120] and acyclic[146] *N*-hydroxymethylated *β*-lactams, first, we carried out the acylation of (±)-**78** with VA (1.2 equiv.) as acyl donor in the presence of lipase PS (30 mg mL^{-1}) in *i*Pr_2O at 50 °C. An unexpected acyl migration was observed resulting from the proximity of the *OH* and *O*-acyl groups and traces of H_2O present in the reaction medium. As a result, a significant amount of the undesirable diol [(3*S*,4*R*)-**83**] (~4% mole fraction, *ee* = 46%) was formed besides the desired diacetate (3*R*,4*S*)-**99** (6% mole fraction, apparent *ee* = 45%) and unreacted (3*S*,4*R*)-**78** (81% mole fraction, *ee* = 10%) in 24 h (mole fractions were determined with *n*-heptadecane as internal standard by GC).

Taking into account our previous results with *β*-hydroxy esters exhibiting a competition of acylation and hydrolysis[I], the enzyme screening for the acetylation of (±)-**78** was performed with 6 equiv. of VA. Only traces of the enantiomerically enriched (3*S*,4*R*)-**83** were detected after 10 min. at a conversion of 53% (*ee* = 91% [(3*S*,4*R*)-**78**], apparent *ee* = 80% [(3*R*,4*S*)-**99**], *E* = 28). No changes were observed when a catalytic amount of Et_3N was added to the reaction mixture under the same conditions.

These preliminary experiments were followed by enzyme screening. Besides lipase PS and CAL-A, lipase AK and AY were tested for the acetylation of (±)-**78** under the above conditions (0.015 M substrate, 30 mg mL^{-1} enzyme, 6 equiv. of VA, *i*Pr_2O, 50 °C). The enzymes tested catalysed only the acetylation reactions (Table 11), but the undesirable, enantiomerically enriched (3*S*,4*R*)-**83** (formed as a result of acyl migration) appeared in all reaction mixtures. Finally, lipase PS was selected for further preliminary reactions.

Table 11. Enzyme screening for the acetylation of (±)-**78**[a].

Entry	Enzyme (30 mg mL^{-1})	**78** (mol%)	*ee* (%) (3*S*,4*R*)-**78**[b]	**99** (mol%)	*ee* (%) (3*R*,4*S*)-**99**[b,c]	**83** (mol%)	*ee* (%) (3*S*,4*R*)-**83**[b]
1	CAL-A	79	16	14	40	7	42
2	Lipase AK	46	91	48	80	6	67
3	Lipase AY	91	1	7	17	2	15

[a] 0.015 M substrate, 6 equiv. of VA, *i*Pr_2O, 50 °C, after 30 min. [b] According to GC. [c] Apparent values.

To suppress acyl migration in the lipase PS-catalysed acetylation of (±)-**78**, several solvents, such as toluene, TBME and 2-Me-THF were tested (Table 12). Unfortunately, no significant beneficial effect was observed [2–4% mole fractions of (3*S*,4*R*)-**83** were still detected].

Table 12. Solvent screening for the acetylation of (±)-**78**[a].

Entry	Solvent	Conv.[b] (%)	*ee* (%) (3*S*,4*R*)-**78**[c]	*ee* (%) (3*R*,4*S*)-**99**[c,d]	Apparent *E*
1	Toluene	53	93	81	32
2	TBME	56	90	70	17
3	2-Me-THF	40	50	76	12

[a] 0.015 M substrate, 6 equiv. of VA, lipase PS 30 mg mL^{-1}, 50 °C, after 1 h. [b] Conv. values calcd. from *ee* unreacted substrate and *ee* product values. [c] According to GC. [d] Apparent values.

In order to avoid the inaccuracy of *ee* measurements, two other acyl donors, VB and 2,2,2-TFB (6 equiv.) were tested for the acylation of (±)-**78**. In these cases, racemic (±)-**99** (~4% mole fraction) and enantiomerically enriched (3*S*,4*R*)-**83** (*ee* ≤54%) (~3% mole fraction) were also detected in the reaction mixtures besides the required enantiomers. The *E* of the reactions was excellent at 50% conversion (>200) (Table 13, entries 1, 2). Solvent screening (toluene, TBME and 2-Me-THF) was also performed (Table 13, entries 3–5). In view of the best combination of *E* and reaction rate, *i*Pr_2O was chosen as reaction medium for further experiments. The effect of temperature was also studied in the lipase PS-catalysed butyrylation of (±)-**78** with VB. By lowering the reaction temperature from 50 to 25 °C and then to 4 °C, a significant decrease in reaction rate was observed without a drop in *E* (Table 13, entries 6, 7). Unfortunately, (±)-**99** and enantiomerically enriched (3*S*,4*R*)-**83** were still present in the reaction mixtures (≤7% mole fractions). Based on these results, 25 °C was selected as reaction temperature for the preparative-scale reaction.

Table 13. Effects of acyl donor, solvent and temperature of butyrylation of (±)-**78**[a].

Entry	Acyl donor (6 equiv.)	Solvent	t (min)	Temperature (°C)	Conv.[b] (%)	*ee* (%) (3*S*,4*R*)-**78**[c]	*ee* (%) (3*R*,4*S*)-**100**[c]	Apparent *E*
1	VB	*i*Pr_2O	5	50	48	92	99	>200
2	2,2,2-TFB	*i*Pr_2O	5	50	26	34	99	>200
3	VB	Toluene	5	50	26	34	99	>200
4	VB	MTBE	5	50	48	87	93	78
5	VB	2-Me-THF	5	50	23	28	96	64
6	VB	*i*Pr_2O	10	25	46	86	99	>200
7	VB	*i*Pr_2O	30	4	41	70	99	>200

[a] 0.015 M substrate, lipase PS 30 mg mL^{-1}, in the solvent tested, 50 °C, after 5 min. [b] Conv. values calcd. from *ee* unreacted substrate and *ee* product values. [c] According to GC.

Finally, the amounts of acyl donor VB (10 and 20 *vs*. 6 equiv.) were tested [10 equiv.: (3*S*,4*R*)-**78** = 95%, (3*R*,4*S*)-**100** = 98%, conv. = 49%, E >200, in 30 min; 20 equiv.: (3*S*,4*R*)-**78** = 97%, (3*R*,4*S*)-**100** = 97%, conv. = 50%, E >200, in 20 min], and in view of these results, 10 equiv. of VB was selected for the preparative-scale butyrylation.

4.2.2. Preparative-scale resolutions

The lipase PS-catalysed gram-scale acetylation of racemic (±)-**78** was carried out with 6 equiv. of VA in *i*Pr_2O at 25 °C. The resulting (3*R*,4*S*)-**99** was crystallized and then recrystallized three times from Et_2O (yield = 22%, *ee* = 99%). The unreacted (3*S*,4*R*)-**78** (yield = 38%) enantiomer was isolated with a high enantiomeric excess (*ee* = 91%) (Table 14, entry 1). A preparative-scale acylation of (±)-**78** with 10 equiv. of VB was also performed under the optimised conditions (30 mg mL^{-1} lipase PS, *i*Pr_2O, 25 ºC). The resulting *β*-lactam enantiomers were isolated with high enantiomeric excesses (*ee* ≥ 98%) and good yields (46%) (Table 14, entry 2).

Table 14. Preparative-scale resolutions of (±)-**78**[a,b].

Entry	Acyl donor	t (min)	Conv.[c] (%)	Product enantiomer				Unreacted enantiomer			
				Yield (%)	Isomer	$ee_{product}$[d] (%)	$[\alpha]_D^{25}$ EtOH	Yield (%)	Isomer	$ee_{substrate}$[d] (%)	$[\alpha]_D^{25}$ EtOH
1	VA	30	48 (54)[e]	22	(3*R*,4*S*)-**99**	99[f]	+27[g]	38	(3*S*,4*R*)-**78**	91	-36[h]
2	VB	20	50	46	(3*R*,4*S*)-**100**	99	+35[i]	46	(3*S*,4*R*)-**78**	98	-46[j]

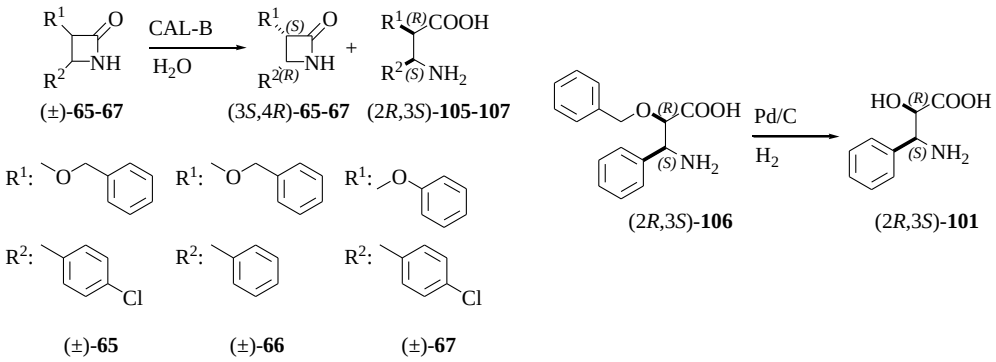
[a] 6 equiv. of VA; 10 mg mL^{-1} lipase PS, *i*Pr_2O, 25 ºC. [b] 10 equiv. of VB; 30 mg mL^{-1} lipase PS, *i*Pr_2O, 25 ºC. [c] Values calcd. from *ee* (unreacted substrate) and *ee* (product) values. [d] Determined by GC. [e] Overall Conv. [f] Recrystallized 3 times from Et_2O. [g] c = 0.30. [h] c = 0.26. [i] c = 0.30. [j] c = 1.325.

4.2.3. Further transformations and absolute configurations

The ring opening of *β*-lactams (3*S*,4*R*)-**78**, (3*R*,4*S*)-**99** and (3*R*,4*S*)-**100** with 18% aqueous HCl resulted in enantiomeric *β*-amino acid hydrochlorides [(2*R*,3*S*)-**101.HCl**, (2*S*,3*R*)-**101.HCl**] (Scheme 30). Absolute configurations were determined by comparing [α] values with literature data.[144] The lipase PS-catalysed acylation of (±)-**78** was found to give a product with 4*S* selectivity.

4.3. CAL-B catalysed ring-opening reactions of 3,4-disubstituted *β*-lactams. New routes for the preparation of pharmaceutically active key intermediates.[III]

In addition to the lipase PS-catalysed *O*-acylation[II] method, a CAL-B catalysed ring-opening procedure was also developed for the preparation of (2*R*,3*S*)-**101**, the key intermediate of the **Taxol** side-chain. Furthermore, to extend the substrate scope and analyse the influence of ligands with different sizes on C3 or C4 in the ring-cleavage reaction, racemic 3,4-disubstituted *β*-lactams (±)-**65**–**67** were also investigated (Scheme 31).



Scheme 31

4.3.1. Small-scale resolutions

CAL-B proved to be an excellent catalyst for the enantioselective (E >200) ring opening of both 4-aryl-substituted[147] and carbocyclic β-lactams.[124] Therefore, the reaction was carried out with 1 equiv. of H_2O in iPr$_2$O at 60 °C with CAL-B as catalyst. The model compound of the preliminary experiments was (±)-**65** (Table 15, entry 1). In order to find the best conditions for the preparative-scale resolutions of β-lactams and determine the effects on E and the reaction rate, first solvent screening was performed (Table 15, entries 1–6). No reaction was detected after 65 h in reactions carried out in THF (Table 15, entry 4) or 2-Me-THF (Table 15, entry 5). In TBME and iPr$_2$O, the ring opening was slow, but highly enantioselective (conv. = 5–8%, E >200 in 65 h) (Table 15, entries 1 and 6). In turn, in toluene (conv. = 15%, E = 32 after 65 h) (Table 15, entry 2) and n-hexane (conv. = 17%, E = 39 after 65 h) (Table 15, entry 3), we observed slightly faster reactions without satisfactory selectivity. In view of the results, TBME was chosen for further preliminary experiments.

Water as a nucleophile is essential for the ring-opening reaction, but its quantity can significantly affect the enzymatic activity.[148] Therefore, different quantities of added H_2O (Table 15, entries 7–10 and 12–15) were tested. On increasing the amount of H_2O up to 25 equiv., the reactions became faster without a drop in E (entries 8–10), but further increases in the H_2O content resulted in considerable decreases in E (Table 15, entries 12–15). Finally, 25 equiv. of H_2O was chosen for the further experiments.

Table 15. Conv. and *E* of the ring opening of (±)-**65**[a].

Entry	H_2O (equiv.)	Solvent	Temperature (°C)	Conv. (%)	*ee* (%) (3*S*,4*R*)-**65**[b]	*ee* (%) (2*R*,3*S*)-**105**[c]	*E*
1	1	*i*Pr_2O	60	5	5	99	>200
2	1	toluene	60	15	16	93	32
3	1	*n*-hexane	60	17	20	94	39
4	1	TBME	60		No reaction		
5	1	TBME	60		No reaction		
6	1	TBME	60	8	9	99	>200
7	0	TBME	60	5	5	99	>200
8	2	TBME	60	9	10	99	>200
9	10	TBME	60	18	21	99	>200
10	25	TBME	60	27	36	99	>200
11	25	TBME	70	35	54	99	>200
12	50	TBME	60	41	67	96	133
13	100	TBME	60	30	41	96	73
14	1850	TBME	60	32	45	95	61
15	-	H_2O	60	27	35	95	55

[a] 0.015 M substrate, 30 mg mL^{-1} CAL-B, after 65 h. [b] According to HPLC. [c] According to HPLC after derivatization.

Increasing the temperature from 60 °C (Table 15, entry 10) to 70 °C, the reaction rate increased too without any decrease in *E* (Table 15, entry 11). Consequently, 70 °C was chosen for the preparative-scale resolution.

The ring opening of (±)-**66** and (±)-**67** was then carried out under the optimized reaction conditions discussed above (25 equiv. of H_2O, 1 mL TBME, at 70 °C). For (±)-**66** an excellent enantioselectivity (*E* >200) but a very poor *E* (5) for (±)-**67** were found. We therefore continued the optimizations for (±)-**67** with a new solvent screening, changing the amount of added H_2O as well as the temperature of the reaction (data not shown). In toluene and *n*-hexane the reactions proceeded slowly with low *E* while in MeCN and THF the enzyme did not display activity even after 65 h. A slightly increased *E* (8) was observed in *i*Pr_2O *vs.* TBME (*E* = 2). Variation of the quantity of H_2O (from 2 to 100 equiv.) and temperature (50 and 70 °C) led to the same results as observed earlier for (±)-**65**. *E* increased slightly (*E* = 14) when the reaction was carried out with 25 equiv. of H_2O in *i*Pr_2O at 70 °C.

4.3.2. Preparative-scale resolutions

CAL-B catalysed preparative-scale ring-opening reactions of (±)-**65** and (±)-**66** were performed with 25 equiv. of H_2O in TBME at 70 °C, according to the preliminary results. The unreacted *β*-lactams and the *β*-amino acid enantiomers were isolated in good yields (30–48%) and excellent *ee* (≥98%) (Table 16). The preparative-scale resolution of (±)-**67** was performed under the conditions described above (25 equiv. of H_2O, *i*Pr_2O, 70 °C). In order to obtain (3*S*,4*R*)-**67** with a good *ee* value, the reaction was overrun to 66% conversion The unreacted *β*-lactam (3*S*,4*R*)-**67** (*ee* = 98%) and *β*-amino acid (*ee* = 50%) were obtained in acceptable yields (16 and 61%) (Table 16).

Table 16. Preparative-scale resolutions of (±)-**65-67**.

Substrate	t (h)	Conv. (%)	Product enantiomer				Unreacted enantiomer			
			Yield (%)	Isomer	$ee_{product}$ [c] (%)	$[\alpha]_D^{25}$	Yield (%)	Isomer	$ee_{substrate}$ [d] (%)	$[\alpha]_D^{25}$
(±)-**65**[a]	144	50	30	(2*R*,3*S*)-**105**	99	+38[e]	35	(3*S*,4*R*)-**65**	98	-20[f]
(±)-**66**[a]	24	50	47	(2*R*,3*S*)-**106**	99	+70[g]	48	(3*S*,4*R*)-**66**	98	-15[h]
(±)-**67**[b]	336	66	61	(2*R*,3*S*)-**107**	50	+11[i]	16	(3*S*,4*R*)-**67**	98	+45[j]

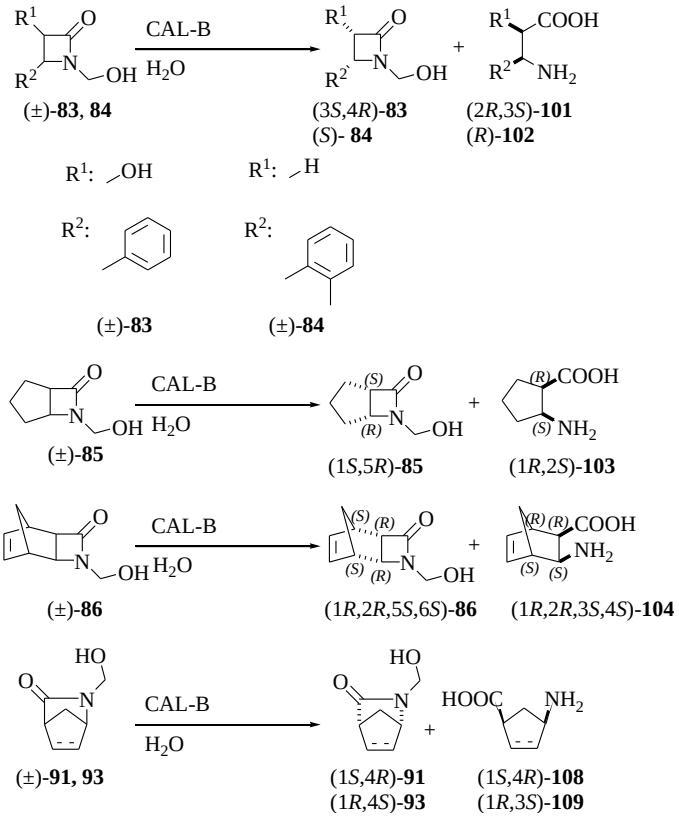
[a] 0.015 M substrate, 25 equiv. of added H_2O, 30 mg mL^{-1} CAL-B, TBME, 70 ºC. [b] 0.015 M substrate, 25 equiv. of added H_2O, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 70 ºC. [c] According to HPLC after derivatization. [d] According to HPLC. [e] *c* = 0.10; MeOH. [f] *c* = 0.30; $CHCl_3$. [g] *c* = 0.30; EtOH. [h] *c* = 0.21; $CHCl_3$. [i] *c* = 0.20; MeOH. [j] *c* = 0.21; $CHCl_3$.

4.3.3. Further transformations and absolute configurations

To prepare (2*R*,3*S*)-**101**, the key intermediate of **Taxol**, the debenzylation of (2*R*,3*S*)-**105** (*ee* = 99%) was performed in a continuous flow system (H-CUBE®) by using a CatCart® filled with 10% Pd/C, operating at a flow rate of 0.1 mL/min, 50 bar, 40 °C (Scheme 31). Thus, (2*R*,3*S*)-**101** was obtained with good *ee* (99%) and in nearly quantitative yield (93%). The absolute configuration of (2*R*,3*S*)-**101** obtained was determined by comparing the optical rotation with the literature[144] [α] value. Thus, CAL-B catalysed the ring-opening of (±)-**66** with (2*R*,3*S*) selectivity, while for (±)-**65** and (±)-**67** the analysed chromatograms indicated the same enantiopreference for CAL-B.

4.4. CAL-B catalysed two-step cascade reactions of *β*- and *γ*-lactams. New routes for the preparation of pharmaceutically active key intermediates.[IV, V]

Enantiopure cyclic and acyclic *β*- and *γ*-amino acids, *β*- and *γ*-lactams and their derivatives are of great interest from both pharmaceutical and chemical perspectives.[2, 4,5] A relatively large number of syntheses including enzymatic routes have been developed for the preparation of enantiomeric *β*- and *γ*-amino acids and *β*- and *γ*-lactams [123, II, III] and new synthetic methods continuously appear. Ring opening of *N*-activated *β*-lactams has also been analysed. For example, Kanerva et al.[149] examined the effects of *N*-substitution such as *N*-acetyl, *N*-chloroacetyl and *N*-tert-butoxycarbonyl groups on the lipase-catalysed ring-opening reaction of racemic 4-phenylazetidin-2-one in dry organic solvents using MeOH as nucleophile. These results showed that the ring-opening reaction is sensitive to the structure of the nitrogen-protecting group. Barbier et al.[150] used electron-withdrawing groups (amide, sulfonyl-based groups or *Boc*) for the *N*-activation of *β*-lactams to prepare 1,3-oxazinan-6-ones. Chu and co-workers[118] described a three-step synthesis of (±)-pregabalin using traceless activating groups. These procedures inspired us to find an activating group for the CAL-B catalysed ring cleavage of *N*-activated *β*- and *γ*-lactams (±)-**83**–**86**, **91**, **92**, which after reaction might undergo traceless removal furnishing the desired *β*- and *γ*-amino acids (Scheme 32). Transformations of the enantiomeric *N*-activated *β*- and *γ*-lactams into the desired inactivated *β*- and *γ*-lactams and *β*- and *γ*-amino acid hydrochlorides were also carried out.



Scheme 32

4.4.1. Small-scale resolutions

The model compound for preliminary experiments was (±)-**83**. The observation that the lactam ring of (±)-**78** was partially opened during CAL-B (30 mg mL^{-1}) catalysed *O*-acetylation (0.015 M substrate, 6 equiv. of vinyl-acetate, *i*Pr_2O, 50 °C)[II], combined with the results on the CAL-B catalysed ring cleavage of unactivated *β*-lactams[III], suggested the possibility of CAL-B catalysed ring opening of (±)-**83**. In view of earlier results, the ring cleavage was first carried out in *i*Pr_2O at 60 °C with 0.5 equiv. of added H_2O (Table 17, entry 1). Surprisingly, in addition to the highly enantioselective (*E* >200) ring opening of (±)-**83**, in situ degradation of the *N*-hydroxymethyl group of the ring-opened amino acid also occurred. Two-step enzymatic transformations, where the reaction sequence is triggered by a biocatalyst and combined with a chemical reaction to furnish a domino sequence, are well known in the literature.[144, 151] The recent two-step cascade reaction of (±)-**83** consists of two consecutive transformations, without any intervention and requires a fast initial enantioselective (*E* >200)

enzymatic step [cleavage of the lactam at N1–C2] followed by a second chemical step [spontaneous removal of the hydroxymethyl group]. The accelerator effect of the activating group was demonstrated by comparing the reaction rates of *N*-hydroxymethyl lactam (±)-**83** and the corresponding inactivated lactam (±)-**79** under the same conditions (Table 17, entry 1 *vs*. entry 2). The preparative-scale ring-opening reaction of (±)-**83** furnished products with *ee* >99%, but the poor yield for unreacted (3*S*,4*R*)-**83** (14%) strongly indicated the involvement of the formaldehyde-induced polymerization of the lactam. *n*-Heptadecane was added to the reaction as an internal standard, in order to follow the progress of the reaction and investigate possible side-reactions (Table 17, entry 3). Next, to minimize undesired side-reactions, the effect of benzylamine (1 equiv.) was explored. Its role was to serve as a nucleophilic capture agent capable of consuming formaldehyde generated in situ (Table 17, Entry 4). A faster ring-opening reaction without any evidence of side-reactions appeared to take place (Table 1, entry 4 *vs*. entry 2). It was hypothesized that this results from the fact that the enzyme surface was no longer coated, and thus inhibited by the products of a putative polymerization reaction. Finally, the catalytic activity of benzylamine in the reaction medium was investigated (Table 17, entry 5 *vs*. entry 2). In addition to the above observation, benzylamine did not show catalytic activity.

With regard to the excellent results obtained for (±)-**83**, the ring-opening reactions of racemic *β*- and *γ*-lactams [(±)-**80**-**82**, (±)-**84**-**86** and (±)-**91**-**94**] were also studied under the same conditions (Table 17, entries 6-18). Reactions were enantioselective (*E* >200) and the degradation of the *N*-hydroxymethyl group of the ring-opened amino acids were observed (Table 17, entries 6, 9, 12, 15 and 17). As compared with the results obtained for the ring-opening reactions of the corresponding inactivated lactams, there was again evidence of the advantageous accelerator effect of the activating group (Table 17, entries 6, 9, 12, 15 and 17 *vs*. entries 7, 10, 13, 16 and 18).

Table 17. CAL-B catalysed ring opening of (±)-**79**–**86** and (±)-**91**–**94**.

Entry	Substrate	t (h)	Conv.[a] (%)	*ee* (%) (3*S*,4*R*)-**83**[b]	*ee* (%) (2*R*,3*S*)-**101**[b]	*E*
1	(±)-**83**[c]	0.5	48	90	99	>200
2	(±)-**79**[c]	1	42	72	99	>200
3	(±)-**83**[c]	0.5	83	90	99	>200
4	(±)-**83**[e]	1	50	98	99	>200
5	(±)-**79**[e]	1	42	72	99	>200
6	(±)-**84**[e]	15	45	82	99	>200
7	(±)-**80**[c]	15	38	62	99	>200
8	(±)-**80**[e]	15	38	60	99	>200
9	(±)-**85**[e]	22	49	96	99	>200
10	(±)-**81**[c]	22	17	20	99	>200
11	(±)-**81**[e]	22	17	20	99	>200
12	(±)-**86**[e]	19	50	98	99	>200
13	(±)-**82**[c]	19	11	12	99	>200
14	(±)-**82**[e]	19	10	11	99	>200
15	(±)-**91**[e]	0.5	45	80	99	>200
16	(±)-**92**[c]	0.5	33	50	99	>200
17	(±)-**93**[e]	48	41	70	99	>200
18	(±)-**94**[c]	48	33	50	99	>200

[a] Calculated from *ee*. [b] According to GC. [c] 0.015 M substrate, 0.5 equiv. of added H_2O, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 60 °C. [d] Determined by using *n*-heptadecane as internal standard. [e] 0.015 M substrate, 0.5 equiv. of added H_2O, 1 equiv. of benzylamine, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 60 °C.

4.4.2. Preparative-scale resolutions

On the basis of the preliminary results, preparative-scale resolutions of racemic (±)-**83**–**86** and (±)-**91**, (±)-**93** were performed with 0.5 equiv. of H_2O, 1 equiv. of benzylamine in *i*Pr_2O at 60 °C. The reactions were stopped by filtering off the enzyme at ~50% overall conversion. The unreacted lactams and amino acids were isolated in good yields (42–49%) and excellent *ee* (≥ 95%) (Table 18).

Table 18. Preparative-scale resolutions of (±)-**83**[a], (±)-**84**[b], (±)-**85**[c], (±)-**86**[d], (±)-**91**[e] and (±)-**93**[f].

Substrate	t (h)	Conv. (%)	Product enantiomer				Unreacted enantiomer			
			Yield (%)	Isomer	$ee_{product}$[g] (%)	$[\alpha]_D^{25}$ H_2O	Yield (%)	Isomer	$ee_{substrate}$[g] (%)	$[\alpha]_D^{25}$ $CHCl_3$
(±)-**83**	3	49	48	(2*R*,3*S*)-**101**	99	-7.1[h]	42	(3*S*,4*R*)-**83**	95	-225[i]
(±)-**84**	24	50	47	(*R*)-**102**	99	+25[j]	47	(*S*)-**84**	97	-275[k]
(±)-**85**	48	50	46	(1*R*,2*S*)-**103**	99	-9[l]	45	(1*S*,5*R*)-**85**	99	-35.7[m]
(±)-**86**	19	50	49	(1*R*,2*R*,3*S*,4*S*)-**104**	99	-12.4[n]	48	(1*R*,2*R*,5*S*,6*S*)-**86**	99	+82.1[o]
(±)-**91**	2	50	49	(1*S*,4*R*)-**108**	99	-240[p]	49	(1*S*,4*R*)-**91**	99	+342[q]
(±)-**93**	55	49	43	(1*R*,3*S*)-**109**	99	-11[r]	44	(1*R*,4*S*)-**93**	96	+49.8[s]

[a] 0.039 M substrate, 0.5 equiv. of added H_2O, 1 equiv. of benzylamine, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 60 °C. [b] 0.039 M substrate, 0.5 equiv. of added H_2O, 1 equiv. of benzylamine, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 60 °C. [c] 0.05 M substrate, 0.5 equiv. of added H_2O, 1 equiv. of benzylamine, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 60 °C. [d] 0.049 M substrate, 0.5 equiv. of added H_2O, 1 equiv. of benzylamine, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 60 °C. [e] 0.072 M substrate, 0.5 equiv. of added H_2O, 1 equiv. of benzylamine, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 60 °C. [f] 0.071 M substrate, 0.5 equiv. of added H_2O, 1 equiv. of benzylamine, 30 mg mL^{-1} CAL-B, *i*Pr_2O, 60 °C. [g] Determined by GC. [h] c = 0.34. [i] c = 0.51. [j] c = 0.15. [k] c = 0.30. [l] c = 0.34. [m] c = 0.40. [n] c = 0.32. [o] c = 0.97. [p] c = 0.30. [q] c = 0.50. [r] c = 0.30. [s] c = 0.72.

4.4.3. Further transformations and absolute configurations

The ring-opened *β*- and *γ*-lactams [(3*S*,4*R*)-**83**, (*S*)-**84**, (1*S*,5*R*)-**85**, (1*R*,2*R*,5*S*,6*S*)-**86**, (1*S*,4*R*)-**91**, (1*R*,4*S*)-**93**] with 18% aqueous HCl gave the corresponding enantiomeric *β*- and *γ*-amino acid hydrochlorides. *N*-Deprotection reactions of *β*- and *γ*-lactam enantiomers from the hydroxymethylated counterparts (with no drop in *ee*) with $KMnO_4$ in a mixture of acetone and H_2O[152] or NH_4OH and MeOH[153] are well known in the literature. The reactions of *N*-hydroxymethyl-lactam enantiomers [(3*S*,4*R*)-**83**, (*S*)-**84**, (1*S*,5*R*)-**85**, (1*R*,2*R*,5*S*,6*S*)-**86**, (1*S*,4*R*)-**91**, (1*R*,4*S*)-**93**] with NH_4OH/MeOH afforded the non-activated lactam enantiomers. The absolute configurations were determined by comparing the specific rotation values with literature data.[67, 123,124, 144, 146, 154]

5. Summary

CAL-B catalysed *O*-acylation reactions of ethyl *cis*-(±)-2-hydroxycyclopentane-1-carboxylate [(±)-**59**] and ethyl (±)-5-hydroxycyclopent-1-enecarboxylate [(±)-**61**] were highly enantioselective (*E* >200) with 5 equiv. of VA in TBME at 30 °C. Hydrolysis as side-reaction, due to the presence of H_2O on the surface of the enzyme, was found to take place in addititon to the acylation reaction. Because the optimization of the hydrolyses of (±)-**59** and (±)-**61** (CAL-B, 10 equiv. of H_2O, TBME, 30 °C) did not lead to an efficient strategy for the preparation of the corresponding (1*S*,2*R*)-**97** and (*R*)-**98** derivatives, a new method has been devised for the enantioseparation of *β*-hydroxy esters through enzyme-catalysed hydrolysis. Note that the possibility of hydrolysis needs to be carefully investigated for substrates containing hydrolysable function.

When lipase PS-catalysed (*S*)-selective *O*-acylation of *N*-hydroxymethyl-*cis*-3-acetoxy-4-phenylazetidin-2-one [(±)-**78**] was performed in *i*Pr_2O with 10 equiv. of VB at 25 °C (*E* >200), the unreacted and product *β*-lactam enantiomers were obtained with excellent *ee* (≥98%). The *β*-lactam enantiomers were transformed into the corresponding *β*-amino acid hydrochlorides (*ee* ≥98%) with 18% HCl. It is noteworthy that the possibility of intramolecular acyl migration during an enzymatic transformation of substrates containing both OCOR and OH functions needs to be carefully investigated. The method proved to be suitable for the preparation of (2*R*,3*S*)-3-phenylisoserine a key **Taxol** intermediate [(2*R*,3*S*)-**101**].

High enantioselectivities (*E* >200) were obtained for the CAL-B catalysed ring opening of *cis*-3-benzyloxy-4-(4-chlorophenyl)azetidin-2-one [(±)-**65**] and *cis*-3-benzyloxy-4-phenylazetidin-2-one [(±)-**66**] when CAL-B was used as catalyst, with 25 equiv. of H_2O as nucleophile, in TBME at 70 °C. In contrast, while a relatively modest *E* (12) was obtained for *cis*-4-(4-chlorophenyl)-3-phenoxyazetidin-2-one [(±)-**67**] in *i*Pr_2O with 25 equiv. of H_2O at 70 °C. The differences in *E* for (±)-**65** and (±)-**66** *vs*. (±)-**67** are presumably result from the very different steric hindrance of BnO *vs*. PhO, which influences the accommodation of the enantiomers in the active site of CAL-B. The products could be easily separated through extraction. The debenzylation of (2*R*,3*S*)-**106** (*ee* = 99%) performed in a continuous flow

system (H-CUBE®) (CatCart® filled with 10% Pd/C, flow rate of 0.1 mL/min, 50 bar, 40 °C) resulted in the formation of the desired (2*R*,3*S*)-**101** with an excellent *ee* of 99%. The method proved also to be suitable for the preparation of **Taxol** key intermediate (2*R*,3*S*)-**101**.

The CAL-B catalysed two-step cascade reactions of *N*-hydroxymethyl-*cis*-3-hydroxy-4-phenylazetidin-2-one [(±)-**83**], *N*-hydroxymethyl-4-(*o*-tolyl)azetidin-2-one [(±)-**84**], *N*-hydroxymethyl-6-azabicyclo[3.2.0]heptan-7-one [(±)-**85**], *N*-hydroxymethyl-*exo*-3-azatricyclo[4.2.1.$0^{2,5}$]non-7-en-4-one [(±)-**86**], *N*-hydroxymethyl-2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-**91**] and *N*-hydroxymethyl-2-azabicyclo[2.2.1]heptan-3-one [(±)-**93**] were performed in *i*Pr_2O with H_2O (0.5 equiv) in the presence of benzylamine (1 equiv) at 60 °C. Reactions were highly enantioselective (*E* >200) and afforded the unreacted lactams with excellent enantioselectivity (*ee* ≥95%). Immediately after ring opening, the *N*-hydroxymethyl group of the amino acids formed underwent spontaneous degradation to give un-activated amino acid enantiomers. The amino acid and lactam enantiomers could be easily separated through extraction. Transformations of the unreacted *N*-hydroxymethyl lactam via NH_4OH or acidic hydrolysis resulted in the desired lactam or amino acid hydrochlorides, without a loss in *ee*. The method proved to be suitable for the preparation of **Taxol** key intermediate (2*R*,3*S*)-**101**, **Cathepsin A** inhibitor subunit (*R*)-**102**, **Cispentacin** (1*R*,2*S*)-**103**, **CEP-28122** key intermediate (1*R*,2*R*,5*S*,6*S*)-**82** and (1*S*,4*R*)-**108**, a key intermediate for the blockbuster **Abacavir**.

All synthesized enantiomers were characterized by GC or HPLC measurements, optical rotation, ^{1}H NMR, elemental analysis and melting point measurement.[I-V]

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ANNEX