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

Ph.D. Thesis

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2017
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I. Forró, E., Galla, Z. and Fülöp, F. 
*Candida antarctica* lipase B catalysed reactions of β-hydroxy esters: Competition of acylation and hydrolysis  
**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  
**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  
**IF:** 2.128*

IV. Forró, E., Galla, Z. and Fülöp, F. 
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**IF:** 3.068*

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**IF:** 2.108*

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**IF:** 3.068*

Cumulative impact factor: 15.245

*The impact factors for the year 2015 are presented.*
Lectures

a. **Galla Zsolt**, Forró Enikő, Fülöp Ferenc
   *Hidroxiészterek Candida antarctica B-lipáz katalizált reakciói: Párhuzamosan lejátszódó acilezés és hidrolízis*

b. **Zsolt Galla**, Enikő Forró, Ferenc Fülöp
   *Enzymatic hydrolysis of 3,4-disubstituted β-lactams*

c. **Galla Zsolt**, Forró Enikő, Fülöp Ferenc
   *3,4-Diszubsztituált β-laktámok enzimatikus hidrolízise*

d. **Galla Zsolt**, Forró Enikő, Fülöp Ferenc
   *Új enzimes út a Taxol kulcsintermedierjének szintézisére*
   XX. Szent-Györgyi Napok, 14-17 November, 2013, Szeged, Hungary, oral presentation.

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*
Abbreviations

2,2,2-TFB 2,2,2-trifluoroethyl butyrate
2-Me-THF 2-methyltetrahydrofuran
Boc tert-butoxycarbonyl
BnO benzyloxy
CAL-A Candida antarctica lipase A
CAL-B Candida antarctica lipase B
CAN ceric ammonium nitrate
Chz carboxybenzoyl
Conv. conversion
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
iPr2O 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). \(\text{cis}\)-Hydroxycarbocyclic acids may also be important building blocks of biologically active molecules.\(^6\)\(^,\)\(^7\)

![Figure 1](image1)

**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 \((2R,3S)\)-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).\(^12\)

\((1R,2S)\)-2-Aminocyclopentanecarboxylic acid (**Cispentacin**) (Figure 1),\(^13\)\(^,\)\(^14\) the simplest biologically active β-amino acid and its methylene derivative \((1R,2S)\)-2-amino-4-
methylene cyclopentane-carboxylic acid (Icofungipen, PLD-118)\textsuperscript{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\text{S},4\text{R})-4-Aminocyclopent-2-ene-1-carboxylic acid a $\gamma$-amino acid is a key intermediate for the blockbuster \textbf{Abacavir}\textsuperscript{5} (Figure 1), which is a nucleoside analogue reverse transcriptase inhibitor. The importance of \textbf{Abacavir} is also accentuated in the \textit{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\text{R},2\text{R},5\text{S},6\text{S})-3-Azatricyclo[4.2.1.0$^2$]2.5]non-7-en-4-one is used for the preparation of \textbf{CEP-28122} (Figure 1), a selective orally active anaplastic lymphoma kinase inhibitor.\textsuperscript{16}

Molecules containing a 2-azetidinone ring may possess antibacterial activity. \textbf{Carumonam} is a $\beta$-lactamase-resistant monobactam antibiotic,\textsuperscript{17} while others containing a cis-3,4-disubstituted $\beta$-lactam ring may display PPAR $\alpha/\gamma$ agonist,\textsuperscript{18} vasopressin V1a agonist\textsuperscript{19} or anticancer activity.\textsuperscript{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.\textsuperscript{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 mitsugashiiwala lactone and dolichodial or an intermediate of \textbf{Vincamine}.\textsuperscript{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.\textsuperscript{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.\textsuperscript{24-26}
One of our aims was to devise a new method for the enantioseparation of \( \beta \)-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\(^1\) (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\(^\text{II} \) (Scheme 1, Route B). The third aim was to develop an enantioselective CAL-B catalysed route for the ring opening of 3,4-disubstituted \( \beta \)-lactams\(^\text{III} \) (Scheme 1, Route C).

The final aim was to devise an enzymatic ring-opening method for activated \( \beta \)- and \( \gamma \)-lactams where the hydroxymethyl moiety acts as a traceless activating group\(^\text{IV, V} \) (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**

![Scheme 1](image)
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. The molecule with (S) absolute configuration is the active enantiomer. In the original medicinal chemistry synthesis (S)-aspartic acid was used as the source of chirality. Walsgrove et al. 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 optimized the synthetic route from (±)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid methyl ester [(±)-2], using Novozym 435, which was designed for anhydrous systems. Under optimised conditions,
$t$-BuOH/H$_2$O (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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$t$ (h)</th>
<th>$m_{\text{substrate}}$ (kg)</th>
<th>$m_{\text{enzyme}}$ (kg)</th>
<th>Yield (%)</th>
<th>$ee_{\text{product}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-1</td>
<td>2.5</td>
<td>76.3</td>
<td>6</td>
<td>42</td>
<td>99</td>
</tr>
<tr>
<td>(±)-2</td>
<td>10</td>
<td>246</td>
<td>74</td>
<td>43</td>
<td>99.8</td>
</tr>
</tbody>
</table>

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 H₂O/K₂HPO₄ 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).

Enzymatic desymmetrization is a simple, widely-used method for the preparation of enantiomers with excellent yields. The reactions usually require mild conditions. (1S,2R)-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. A number of publications have reported the preparation of 11. Goswami and Kissick 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 H₂O, K₂HPO₄ buffer (pH = 8.5) at 50 °C (Scheme 4, Table 2).

Moxifloxacin is a fourth-generation, broad spectrum fluoroquinolone antibacterial agent developed by Bayer AG. Its antibacterial spectrum includes enteric Gram-(−), gram-(+) bacteria and anaerobes. (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. used l-tartaric acid, whereas Fey and co-workers applied camphor sulfonic acid or (-)-2,3,4,6-di-O-isopropylidene-2-keto-l-gulonic acid. Fadnavis, Ramesh and Harini described an efficient enzymatic method with soluble Candida antarctica lipase B (Addyzme) (Scheme 5) for the preparation of 15. Under optimised condition [H2O, 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-HT1A) receptor ligand. 5-HT1A receptors mediate various serotonergic functions and may be implicated in depressive and anxiety disorders, Alzheimer’s disease and schizophrenia. Guo et al. 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).

![Potential SPECT Ligand for the 5-HT1A Receptor](image)

**Scheme 6**

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>t (h)</th>
<th>m_substrate (kg)</th>
<th>m_enzyme (kg)</th>
<th>yield (%)</th>
<th>ee_product (%)</th>
<th>wt_product (%)</th>
<th>er_product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-7</td>
<td>40</td>
<td>13.1</td>
<td>3.95</td>
<td>31</td>
<td>n.d.</td>
<td>98.9</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5 steps)</td>
<td>(after 5 steps)</td>
<td>n.d.</td>
</tr>
<tr>
<td>(±)-10</td>
<td>27</td>
<td>1.73</td>
<td>1.5×10⁻¹</td>
<td>98.1</td>
<td>&gt; 99.9</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>(±)-12</td>
<td>16</td>
<td>8×10⁻²</td>
<td>4×10⁻²</td>
<td>46</td>
<td>&gt; 99</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>(±)-16</td>
<td>31</td>
<td>5×10⁻¹</td>
<td>1×10⁻²</td>
<td>91</td>
<td>n.d.</td>
<td>n.d.</td>
<td>97.9</td>
</tr>
</tbody>
</table>

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. Singh and co-workers described an enzyme-catalysed hydrolytic route for the preparation of (R)-1-(6-bromo-2-methylpyridin-3-yl)-3-hydroxypryrolidin-2-one (20), which is a key chiral intermediate for a GPR119 agonist compound. They used CAL-B (Novozym 435) in H₂O/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).
(2R,3R,4R,5R)-2-(Hydroxymethyl)-5-(5-amino-2-oxothiazolo[4,5-d]pyrimidin-3(2H)-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. Gallou et al. 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).

Table 3. Synthesis of GPR119 agonist and Isatoribine prodrug.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>t (h)</th>
<th>m_{substrate} (kg)</th>
<th>m_{enzyme} (kg)</th>
<th>yield (%)</th>
<th>ee_{product} (%)</th>
<th>purity_{product} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-18</td>
<td>28</td>
<td>5 × 10^{-2}</td>
<td>1.25 × 10^{-2}</td>
<td>37.1</td>
<td>99.7</td>
<td>n.d.</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>1</td>
<td>3.7 × 10^{-1}</td>
<td>86</td>
<td>n.d.</td>
<td>96.1</td>
</tr>
</tbody>
</table>
2.1.3. CAL-B catalysed ring-opening reactions

**Odanacatib** is an orally active selective cathepsin K inhibitor that has been developed for the treatment of high bone turnover diseases such as osteoporosis.\(^{61}\) N-Protected (S)-\(\gamma\)-fluoroleucine ethyl ester (23) is a key intermediate in the synthesis of **Odanacatib**. Both Truppo\(^{62}\) and Limanto et al.\(^{63}\) have 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. of Et\(_3\)N 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](image)

**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 (MTBE with 1 equiv. of H\(_2\)O, 50 °C) (Scheme 10). The ee and yield are, respectively, 98.3% and 48% (Table 4).
### Scheme 10

![Scheme 10](image)

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>t (h)</th>
<th>m_substrate (kg)</th>
<th>m_enzyme (kg)</th>
<th>yield (%)</th>
<th>ee_product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>80</td>
<td>84</td>
</tr>
<tr>
<td>(±)-24</td>
<td>144</td>
<td>12.5</td>
<td>8.8</td>
<td>48</td>
<td>98.3</td>
</tr>
</tbody>
</table>

2.1.4. CAL-B catalysed OH-acylations

Ribavirin is an antiviral agent used in combination with peginterferon alfa-2β or peginterferon alfa-2α 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).
Valrubicin is an N-trifluoroacetylated doxorubicin analogue used in the treatment of bladder carcinoma, as topoisomerase II and RNA synthesis inhibitor.\textsuperscript{73,74} A few synthetic routes were published for the preparation of Valrubicin.\textsuperscript{75,76} In addition, Cotterill and Rich\textsuperscript{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-butanol (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).

Oligonucleotide drugs are promising therapeutic agents for the treatment of cardiovascular, metabolic and infection diseases, and a variety of cancers.\textsuperscript{78} Two excellent examples for oligonucleotide structures of high importance are the antiviral Vitravene\textsuperscript{®} and the anti-angiogenic Macugen\textsuperscript{®}. Díaz-Rodríguez and co-workers\textsuperscript{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](image)

**Scheme 13**

Clindamycin palmitate is a prodrug of Clindamycin, which is effective against Gram positive organisms, Gram negative anaerobes, 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. 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](image)

**Scheme 14**
4-[(1R,2S)-2-Cyanocyclohexyloxy]-2-(trifluoromethyl)benzonitrile is an androgen receptor antagonist molecule which was developed for the treatment of alopecia and excess sebum.\textsuperscript{82,83} Synthetic processes affording the highest selectivities were either unprofitable or had low $E$.\textsuperscript{84} Vaidanatha and co-workers\textsuperscript{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).

\[
\begin{align*}
\text{CAL-B} & \quad \begin{array}{c}
\text{CN} \\
\text{33}
\end{array} + \\
\begin{array}{c}
\text{OH} \\
\text{34}
\end{array} & \quad \begin{array}{c}
\text{CN} \\
\text{33}
\end{array} + \\
\begin{array}{c}
\text{CN} \\
\text{35}
\end{array} \quad \text{sodium cyanide}
\end{align*}
\]

\textbf{Scheme 15}

\textit{Reboxetine mesylate} is a selective norepinephrine reuptake inhibitor (\textit{Edronax}®). The (S,S)-enantiomer is significantly more active than either the (R,R)-enantiomer or the racemate.\textsuperscript{86-88} (S,S)-\textit{Reboxetine succinate} is a potential drug candidate for the treatment of fibromyalgia. There are several published synthetic routes for the preparation (S,S)-\textit{Reboxetine}.\textsuperscript{89,90} Hayes et al.\textsuperscript{91} worked out a synthesis for the preparation of \textit{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 \textit{OH} of (2R,3S)-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).
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.\textsuperscript{92,93} Numerous synthetic routes have been reported for the preparation of RevE1.\textsuperscript{94,95} Amin and co-workers\textsuperscript{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).
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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>t (h)</th>
<th>msubstrate (kg)</th>
<th>menzyme (kg)</th>
<th>yield (%)</th>
<th>ee product (%)</th>
<th>de product (%)</th>
<th>purity product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>24</td>
<td>18</td>
<td>14</td>
<td>83</td>
<td>n.d.</td>
<td>99.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>4.5×10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>2×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>90</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>31</td>
<td>23</td>
<td>2.5×10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>2.5×10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>73</td>
<td>n.d.</td>
<td>n.d.</td>
<td>&gt; 98%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.5</td>
<td>1.06×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>1.2×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>90</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>33, 34</td>
<td>24</td>
<td>22.4</td>
<td>22.4</td>
<td>38</td>
<td>&gt; 99.9</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>36</td>
<td>17</td>
<td>1</td>
<td>2×10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>&gt; 99</td>
<td>&gt; 98%</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>38</td>
<td>15</td>
<td>3.1</td>
<td>2×10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>75</td>
<td>&gt; 99</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

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.<sup>97</sup> In the asymmetric synthetic route of Mefenoxam chiral catalysts are used, but the scale-up of the reaction is problematic. Park et al.<sup>98</sup> 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).

\[ \text{Lipase PS} \]

\[ \text{Mefenoxam} \]

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

\[ \text{Scheme 18} \]
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, but it was not suitable for development to full manufacturing scale. Grayson and co-workers 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$_2$O, pH = 8.2) at 30 °C (Scheme 19). A yield of 41.3% and an excellent ee of 99% were reported (Table 6).

\[ \text{Lipase PS (Amano)} \rightarrow \begin{array}{c} \text{NH}_2 \text{O} \end{array} + \begin{array}{c} \text{NH}_2 \text{O} \end{array} \]

\[ (\pm)-44 \rightarrow \begin{array}{c} \text{NH}_2 \text{O} \end{array} \rightarrow \begin{array}{c} \text{NH}_2 \text{O} \end{array} + \begin{array}{c} \text{NH}_2 \text{OH} \end{array} \]

\[ (S)-\beta-\text{Phenylalanine} \]

Scheme 19

(3S,5R)-3-(Aminomethyl)-5-methyloctanoic acid is a lipophilic γ-aminobutyric acid analogue nominated for development to treat interstitial cystitis. Murtagh et al. developed the modified medicinal chemistry route for the preparation of this compound from methyl (5R)-3-cyano-5-methyloctanoate (46, 47) using PS-SD as chiral catalyst in H$_2$O/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).

\[ \text{Lipase PS (PS-SD)} \rightarrow \begin{array}{c} \text{HO} \text{O} \end{array} + \begin{array}{c} \text{HO} \text{O} \end{array} \]

\[ 46 \rightarrow \begin{array}{c} \text{HO} \text{O} \end{array} + \begin{array}{c} \text{HO} \text{O} \end{array} \]

\[ \text{GABA analogue} \]

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. 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 20).
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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>t (h)</th>
<th>msubstrate (kg)</th>
<th>menzyme (kg)</th>
<th>yield (%)</th>
<th>ee&lt;sub&gt;product&lt;/sub&gt; (%)</th>
<th>de&lt;sub&gt;product&lt;/sub&gt; (%)</th>
<th>er&lt;sub&gt;product&lt;/sub&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-41</td>
<td>44</td>
<td>25</td>
<td>0.96</td>
<td>34</td>
<td>96</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>(±)-44</td>
<td>15</td>
<td>430</td>
<td>21</td>
<td>41.3</td>
<td>99</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>46, 47</td>
<td>48</td>
<td>2×10^{-2}</td>
<td>54</td>
<td>10.8</td>
<td>99.4</td>
<td>n.d.</td>
<td>95.82</td>
</tr>
<tr>
<td>(±)-16</td>
<td>25</td>
<td>3×10^{-2}</td>
<td>4</td>
<td>94</td>
<td>n.d.</td>
<td>n.d.</td>
<td>-</td>
</tr>
</tbody>
</table>

2.2.2. Lipase PS-catalysed OH-acylations

Tryptase inhibitors are a class of anti-inflammatory drugs. S-N-(tert-butoxycarbonyl)-3-hydroxymethylpiperidine is a key intermediate in the preparation of a potent tryptase inhibitor. Goswani and co-workers 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).
Human immunodeficiency virus (HIV) is a retrovirus, which causes HIV infection and acquired immunodeficiency syndrome (AIDS).\textsuperscript{108,109} (3a\text{R},3a\text{S},6a\text{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.\textsuperscript{110,111} Yu et al.\textsuperscript{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).

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-((2S)-3-[(1-(4-Chlorobenzyl)piperidin-4-yl)amino]-2-hydroxy-2-methylpropyl]oxy)-4-hydroxyphenyl] acetamide is a potent CCR1 antagonist.\textsuperscript{113} Ainge and co-workers\textsuperscript{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 \textit{purity} of the product, respectively, were 27% and 95% (Table 7).

![Scheme 24](image)

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>t (h)</th>
<th>m\text{substrate} (kg)</th>
<th>m\text{enzyme} (kg)</th>
<th>yield (%)</th>
<th>ee\text{product} (%)</th>
<th>purity\text{product} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-49</td>
<td>2</td>
<td>2.55×10\textsuperscript{-1}</td>
<td>6.4×10\textsuperscript{-3}</td>
<td>48</td>
<td>85.7</td>
<td>n.d.</td>
</tr>
<tr>
<td>(±)-53</td>
<td>n.d.</td>
<td>4.85</td>
<td>0.36</td>
<td>33</td>
<td>97</td>
<td>n.d.</td>
</tr>
<tr>
<td>(±)-56</td>
<td>6</td>
<td>1.13×10\textsuperscript{-1}</td>
<td>1.25×10\textsuperscript{-2}</td>
<td>27</td>
<td>n.d.</td>
<td>95</td>
</tr>
</tbody>
</table>
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 NaBH₄ reduction, followed by separation of the resulting cis and trans diastereomers, according to a literature method. A literature process 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](image)

Racemic cis-3-acetoxy-4-phenylazetidin-2-one [(±)-64], 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-phenoxazetidin-2-one [(±)-67] were synthesized according to literature methods. 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 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. Then the products were transformed with paraformaldehyde under sonication 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](image)

### 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:

\[
ee_s = \frac{(A_2 - A_1)}{(A_1 + A_2)}
\]

\[
ee_p = \frac{(A_3 - A_4)}{(A_3 + A_4)}
\]

\[\text{Conv.} = \frac{\ee_s}{(\ee_s + \ee_p)}\]

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

When an internal standard was used, the \( ee \) and conversion were calculated\(^{124}\) via the following equations:

\[
k^0 = \frac{A_{st}^0}{(A_1^0 + A_2^0)}
\]

\[
k = \frac{A_{st}}{(A_1 + A_2)}
\]

\[\text{Conv.} = 100 \times \frac{(k - k^0)}{k}\]

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 CH₂N₂. 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 derivatization¹²⁵ with (i) CH₂N₂ and (ii) Ac₂O 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 derivatization¹²⁵ with (i) CH₂N₂ and (ii) Ac₂O 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 CH₂N₂. 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 derivatization¹²⁵ with (i) CH₂N₂ and (ii) Ac₂O 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.¹⁻⁵
4. Results and discussion

4.1. CAL-B catalysed reactions of β-hydroxy esters. New routes for the preparation of pharmaceutically active key intermediates.¹

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 specificity.¹²⁶,¹²⁷ It is a widely-used catalyst for various enantio- and regioselective reactions such as acylation¹²⁸, transesterification¹²⁹ 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 occurred 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-acylation 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](image)
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 [(1S,2R)-95, ee >98%] and the unreacted β-hydroxy ester [(1R,2S)-59, ee ~35%], a significant amount of hydroxy acid [(1S,2R)-97, ee >98%] was detected. A conversion of ~30% was calculated 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 (1S,6R)-7-azabicyclo[4.2.0]oct-3-en-8-one 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, we started preliminary experiments with enzyme screening, performing the reactions in TBME at 45 °C with 4 equiv. of added H2O as nucleophile. Besides CAL-B, a number of lipases (PS, AK, AY, PPL and CAL-A) were tested (Table 8).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme (30 mg mL⁻¹)</th>
<th>ee_{substrate} (%)</th>
<th>ee_{product} (%)</th>
<th>Conv. (%)</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lipase PS</td>
<td>34</td>
<td>8</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Lipase AK</td>
<td>15</td>
<td>35</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Lipase AY</td>
<td>23</td>
<td>14</td>
<td>62</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>PPL</td>
<td>3</td>
<td>35</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>CAL-A</td>
<td>5</td>
<td>49</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>CAL-B</td>
<td>29</td>
<td>93</td>
<td>24</td>
<td>36</td>
</tr>
</tbody>
</table>

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 CH₂N₂.
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 \((\pm)-59\) 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\(_2\)Cl\(_2\) (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>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>CAL-B (mg mL(^{-1}))</th>
<th>( ee_{\text{substrate}} ) (%)</th>
<th>( ee_{\text{product}} ) (%)</th>
<th>Conv. (%)</th>
<th>( E )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TBME</td>
<td>30</td>
<td>29</td>
<td>93</td>
<td>24</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>TBME</td>
<td>50</td>
<td>31</td>
<td>91</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>TBME</td>
<td>75</td>
<td>35</td>
<td>86</td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>Et(_2)O</td>
<td>30</td>
<td>21</td>
<td>94</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Toluene</td>
<td>30</td>
<td>8</td>
<td>83</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>( n )-Hexane</td>
<td>30</td>
<td>4</td>
<td>72</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>THF</td>
<td>30</td>
<td>3</td>
<td>78</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>1,4-Dioxane</td>
<td>30</td>
<td>3</td>
<td>85</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>CH(_2)Cl(_2)</td>
<td>30</td>
<td>8</td>
<td>43</td>
<td>16</td>
<td>3</td>
</tr>
</tbody>
</table>

\( a \) 0.05 M substrate, 4 equiv. of added H\(_2\)O, TBME, 45 °C, after 20 min.
\( b \) According to GC.
\( c \) According to GC, after derivatization with CH\(_2\)N\(_2\).

Both conversion and \( E \) found with CAL-B in TBME at 45 °C were affected by the amount of H\(_2\)O 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\(_2\)O. Therefore, 10 equiv. of added H\(_2\)O 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\(_2\)O (entry 1). The quantity of H\(_2\)O 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$_2$O, TBME, 30 °C), the results obtained were modest ($ee_{(S)-61} = 22\%$ and $ee_{(R)-98} = 49\%$, 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$_2$O at 30 °C. The results are presented in Table 10. Both hydroxy acids [((1S,2R)-97 and (R)-98] proved to be quite unstable allowing to record the $^1$H NMR spectrum only for (1S,2R)-97. (Table 10).

#### Table 10. Preparative-scale resolutions of (±)-59 and (±)-61$^a$.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>VA or H$_2$O</th>
<th>t (h)</th>
<th>Conv. (%)</th>
<th>Yield (%)</th>
<th>Product enantiomer</th>
<th>Isomer</th>
<th>ee$_{product}^b$ (%)</th>
<th>[α]$_D^{25}$</th>
<th>Yield (%)</th>
<th>Isomer</th>
<th>ee$_{substrate}^c$ (%)</th>
<th>[α]$_D^{25}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-59</td>
<td>H$_2$O</td>
<td>1</td>
<td>22</td>
<td>16</td>
<td>(1S,2R)-97</td>
<td>(R)-97</td>
<td>&gt;98</td>
<td>-23$^d$</td>
<td>78</td>
<td>(1R,2S)-59</td>
<td>(S)-98</td>
<td>-8$^e$</td>
</tr>
<tr>
<td>(±)-61</td>
<td>VA</td>
<td>1.1</td>
<td>50</td>
<td>46</td>
<td>&gt;98</td>
<td>(R)-98</td>
<td>47</td>
<td>+5$^b$</td>
<td>60</td>
<td>24</td>
<td>-6$^i$</td>
<td></td>
</tr>
<tr>
<td>(±)-61</td>
<td>H$_2$O</td>
<td>6</td>
<td>34</td>
<td>28</td>
<td>&gt;98</td>
<td>(S)-61</td>
<td>78</td>
<td>+2.5$^f$</td>
<td>43</td>
<td>26</td>
<td>+5$^g$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ 0.05 M substrate, 5 equiv. of VA as acyl donor or 10 equiv. of added H$_2$O as nucleophile, TBME, 30 °C. $^b$ According to GC, after derivatization with CH$_2$N$_2$. $^c$ According to GC. $^d$ c = 0.15; H$_2$O. $^e$ 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 $(\pm)$-61 displayed $R$ selectivity.

4.2. Lipase PS-catalysed $O$-acylation. A new route for the preparation of pharmaceutically active key intermediate.$^{11}$

Enantiopure $\beta$-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 $(2R,3S)$-3-phenylisoserine $[(2R,3S)$-101], either through lipase PS-catalysed ester hydrolysis of racemic ethyl 3-amino-3-phenyl-2-hydroxypropionate [H$_2$O as nucleophile in TBME or iPr$_2$O, 50 or 60 °C (E >200)]$^{143}$, CAL-B catalysed ring cleavage of racemic cis-3-hydroxy-4-phenylazetidin-2-one [H$_2$O 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$_2$O in iPr$_2$O, 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 $(2R,3S)$-101, through the enzymatic $O$-acylation of racemic $N$-hydroxymethylated cis-3-acetoxy-4-phenylazetidin-2-one $[(\pm)$-78] (Scheme 30).

\[
\begin{align*}
(\pm)$-78 & \xrightarrow{\text{Lipase PS}} (3S,4R)-78 \\
(3S,4R)-78 & \xrightarrow{\text{HCl}} (2R,3S)-101.HCl \\
(3R,4S)-99 & \xrightarrow{\text{HCl}} (2S,3R)-101.HCl \\
(3R,4S)-100 & \xrightarrow{\text{HCl}} (2S,3R)-101.HCl
\end{align*}
\]

Scheme 30
4.2.1. Small-scale resolutions

On the basis of earlier results on the enzymatic acylations of cyclic\textsuperscript{120} and acyclic\textsuperscript{146} N-hydroxymethylated \(\beta\)-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\textsuperscript{-1}) in \(iPr_2O\) 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] (\(\sim\)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 \(\beta\)-hydroxy esters exhibiting a competition of acylation and hydrolysis\textsuperscript{1}, 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\textsuperscript{-1} enzyme, 6 equiv. of VA, \(iPr_2O\), 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\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Enzyme (30 mg mL\textsuperscript{-1})</th>
<th>78 (mol%)</th>
<th>(ee) (%) (3(S),4(R))-78\textsuperscript{b}</th>
<th>99 (mol%)</th>
<th>(ee) (%) (3(R),4(S))-99\textsuperscript{b,c}</th>
<th>83 (mol%)</th>
<th>(ee) (%) (3(S),4(R))-83\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CAL-A</td>
<td>79</td>
<td>16</td>
<td>14</td>
<td>40</td>
<td>7</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>Lipase AK</td>
<td>46</td>
<td>91</td>
<td>48</td>
<td>80</td>
<td>6</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>Lipase AY</td>
<td>91</td>
<td>1</td>
<td>7</td>
<td>17</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 0.015 M substrate, 6 equiv. of VA, \(iPr_2O\), 50 °C, after 30 min. \textsuperscript{b} According to GC. \textsuperscript{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 (3S,4R)-83 were still detected].

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

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Conv. (%)</th>
<th>ee (%) (3S,4R)-78</th>
<th>ee (%) (3R,4S)-99</th>
<th>Apparent E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toluene</td>
<td>53</td>
<td>93</td>
<td>81</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>TBME</td>
<td>56</td>
<td>90</td>
<td>70</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>2-Me-THF</td>
<td>40</td>
<td>50</td>
<td>76</td>
<td>12</td>
</tr>
</tbody>
</table>

*a 0.015 M substrate, 6 equiv. of VA, lipase PS 30 mg mL⁻¹, 50 °C, after 1 h. b Conv. values calc. 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 (3S,4R)-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, iPr₂O 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 (3S,4R)-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 (±)-78a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acyl donor (6 equiv.)</th>
<th>Solvent</th>
<th>t (min)</th>
<th>Temperature (°C)</th>
<th>Conv. b (%)</th>
<th>ee (%) (3S,4R)-78c</th>
<th>ee (%) (3R,4S)-100c</th>
<th>Apparent E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VB</td>
<td>iPr2O</td>
<td>5</td>
<td>50</td>
<td>48</td>
<td>92</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>2</td>
<td>2,2,2-TFB</td>
<td>iPr2O</td>
<td>5</td>
<td>50</td>
<td>26</td>
<td>34</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>3</td>
<td>VB</td>
<td>Toluene</td>
<td>5</td>
<td>50</td>
<td>26</td>
<td>34</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>4</td>
<td>VB</td>
<td>MTBE</td>
<td>5</td>
<td>50</td>
<td>48</td>
<td>87</td>
<td>93</td>
<td>78</td>
</tr>
<tr>
<td>5</td>
<td>VB</td>
<td>2-Me-THF</td>
<td>5</td>
<td>50</td>
<td>23</td>
<td>28</td>
<td>96</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>VB</td>
<td>iPr2O</td>
<td>10</td>
<td>25</td>
<td>46</td>
<td>86</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>7</td>
<td>VB</td>
<td>iPr2O</td>
<td>30</td>
<td>4</td>
<td>41</td>
<td>70</td>
<td>99</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

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.: (3S,4R)-78 = 95%, (3R,4S)-100 = 98%, conv. = 49%, E >200, in 30 min; 20 equiv.: (3S,4R)-78 = 97%, (3R,4S)-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 iPr2O at 25 °C. The resulting (3R,4S)-99 was crystallized and then recrystallized three times from Et₂O (yield = 22%, ee = 99%). The unreacted (3S,4R)-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, iPr2O, 25 °C). 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 (±)-78ab.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acyl donor</th>
<th>t (min)</th>
<th>Conv. (%)</th>
<th>Product enantiomer</th>
<th>Unreacted enantiomer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yield (%)</td>
<td>Isomer</td>
</tr>
<tr>
<td>1</td>
<td>VA</td>
<td>30</td>
<td>48 (54)c</td>
<td>22 (3R,4S)-99</td>
<td>99&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>VB</td>
<td>20</td>
<td>50</td>
<td>46 (3R,4S)-100</td>
<td>99&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* 6 equiv. of VA; 10 mg mL<sup>-1</sup> lipase PS, iPr<sub>2</sub>O, 25 °C. b 10 equiv. of VB; 30 mg mL<sup>-1</sup> lipase PS, iPr<sub>2</sub>O, 25 °C. c Values calc'd from ee (unreacted substrate) and ee (product) values. d Determined by GC. e Overall Conv. f Recrystallized 3 times from Et<sub>2</sub>O. 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 (3S,4R)-78, (3R,4S)-99 and (3R,4S)-100 with 18% aqueous HCl resulted in enantiomeric β-amino acid hydrochlorides [(2R,3S)-101.HCl, (2S,3R)-101.HCl] (Scheme 30). Absolute configurations were determined by comparing [α] values with literature data. The lipase PS-catalysed acylation of (±)-78 was found to give a product with 4S 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.

In addition to the lipase PS-catalysed O-acylation method, a CAL-B catalysed ring-opening procedure was also developed for the preparation of (2R,3S)-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).
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\(_2\)O 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\(_2\)O (Table 15, entries 7–10 and 12–15) were tested. On increasing the amount of H\(_2\)O up to 25 equiv., the reactions became faster without a drop in *E* (entries 8–10), but further increases in the H\(_2\)O content resulted in considerable decreases in *E* (Table 15, entries 12–15). Finally, 25 equiv. of H\(_2\)O was chosen for the further experiments.
Table 15. Conv. and E of the ring opening of (±)-65.

<table>
<thead>
<tr>
<th>Entry</th>
<th>H₂O (equiv.)</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Conv. (%)</th>
<th>ee (%) (3S,4R)-65</th>
<th>ee (%) (2R,3S)-105</th>
<th>E</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>1</td>
<td>iPr₂O</td>
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<td>5</td>
<td>99</td>
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</tr>
<tr>
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<td>1</td>
<td>toluene</td>
<td>60</td>
<td>15</td>
<td>16</td>
<td>93</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>n-hexane</td>
<td>60</td>
<td>17</td>
<td>20</td>
<td>94</td>
<td>39</td>
</tr>
<tr>
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<td>1</td>
<td>TBME</td>
<td>60</td>
<td></td>
<td>No reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>TBME</td>
<td>60</td>
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<td>8</td>
<td>9</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>TBME</td>
<td>60</td>
<td>5</td>
<td>5</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>TBME</td>
<td>60</td>
<td>9</td>
<td>10</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>TBME</td>
<td>60</td>
<td>18</td>
<td>21</td>
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<td>&gt;200</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>TBME</td>
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<td>36</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>TBME</td>
<td>70</td>
<td>35</td>
<td>54</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
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<td>100</td>
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<td>41</td>
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<td>1850</td>
<td>TBME</td>
<td>60</td>
<td>32</td>
<td>45</td>
<td>95</td>
<td>61</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>H₂O</td>
<td>60</td>
<td>27</td>
<td>35</td>
<td>95</td>
<td>55</td>
</tr>
</tbody>
</table>

* 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 H₂O, 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 H₂O 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 iPr₂O vs. TBME (E = 2). Variation of the quantity of H₂O (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 H₂O in iPr₂O 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 H₂O 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 H₂O, iPr₂O, 70 °C). In order to obtain (3S,4R)-67 with a good ee value, the reaction was overrun to 66% conversion. The unreacted β-lactam (3S,4R)-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.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>t (h)</th>
<th>Conv. (%)</th>
<th>Product enantiomer</th>
<th>Yield (%)</th>
<th>Isomer</th>
<th>ee_product</th>
<th>[α] D25</th>
<th>Unreacted enantiomer</th>
<th>Yield (%)</th>
<th>Isomer</th>
<th>ee_substrate</th>
<th>[α] D25</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-65</td>
<td>144</td>
<td>50</td>
<td>(2R,3S)-105</td>
<td>30</td>
<td>(2R,3S)</td>
<td>99</td>
<td>+38°</td>
<td>(3S,4R)-65</td>
<td>35</td>
<td>(2R,3S)</td>
<td>98</td>
<td>-20°</td>
</tr>
<tr>
<td>(+)-66</td>
<td>24</td>
<td>50</td>
<td>(2R,3S)-106</td>
<td>47</td>
<td>(2R,3S)</td>
<td>99</td>
<td>+70°</td>
<td>(3S,4R)-66</td>
<td>48</td>
<td>(2R,3S)</td>
<td>98</td>
<td>-15°</td>
</tr>
<tr>
<td>(+)-67</td>
<td>336</td>
<td>66</td>
<td>(2R,3S)-107</td>
<td>61</td>
<td>(2R,3S)</td>
<td>50</td>
<td>+11°</td>
<td>(3S,4R)-67</td>
<td>16</td>
<td>(2R,3S)</td>
<td>98</td>
<td>+45°</td>
</tr>
</tbody>
</table>

a 0.015 M substrate, 25 equiv. of added H₂O, 30 mg mL⁻¹ CAL-B, TBME, 70 °C. b 0.015 M substrate, 25 equiv. of added H₂O, 30 mg mL⁻¹ CAL-B, iPr₂O, 70 °C. c According to HPLC after derivatization. d According to HPLC. e c = 0.10; MeOH. f c = 0.30; CHCl₃. g c = 0.30; EtOH. h c = 0.21; CHCl₃. i c = 0.20; MeOH. j c = 0.21; CHCl₃.

4.3.3. Further transformations and absolute configurations

To prepare (2R,3S)-101, the key intermediate of Taxol, the debenzylation of (2R,3S)-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, (2R,3S)-101 was obtained with good ee (99%) and in nearly quantitative yield (93%). The absolute configuration of (2R,3S)-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 (2R,3S) 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.\textsuperscript{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.\textsuperscript{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 \textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{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 (±)\textsuperscript{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.
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, \(\text{iPr}_2\text{O}, 50 \, ^\circ\text{C}\))\(^{\text{II}}\), combined with the results on the CAL-B catalysed ring cleavage of unactivated \(\beta\)-lactams\(^{\text{III}}\), suggested the possibility of CAL-B catalysed ring opening of (±)-83. In view of earlier results, the ring cleavage was first carried out in \(\text{iPr}_2\text{O}\) at 60 \, ^\circ\text{C} with 0.5 equiv. of added H\(_2\text{O}\) (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 (3S,4R)-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.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>t (h)</th>
<th>Conv.(^a) (%)</th>
<th>ee (%) ((3S,4R)-83)^b</th>
<th>ee (%) ((2R,3S)-101)^b</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(±)-83(^c)</td>
<td>0.5</td>
<td>48</td>
<td>90</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>2</td>
<td>(±)-79(^c)</td>
<td>1</td>
<td>42</td>
<td>72</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>3</td>
<td>(±)-83(^c)</td>
<td>0.5</td>
<td>83</td>
<td>90</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>4</td>
<td>(±)-79(^e)</td>
<td>1</td>
<td>50</td>
<td>98</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>5</td>
<td>(±)-84(^e)</td>
<td>1</td>
<td>42</td>
<td>72</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>6</td>
<td>(±)-80(^e)</td>
<td>15</td>
<td>38</td>
<td>62</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>7</td>
<td>(±)-80(^e)</td>
<td>15</td>
<td>38</td>
<td>60</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>8</td>
<td>(±)-85(^e)</td>
<td>22</td>
<td>49</td>
<td>96</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>9</td>
<td>(±)-81(^c)</td>
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<td>17</td>
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<td>19</td>
<td>50</td>
<td>98</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
<td>12</td>
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<td>12</td>
<td>99</td>
<td>&gt;200</td>
</tr>
<tr>
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<td>11</td>
<td>99</td>
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</tr>
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<td>(±)-92(^e)</td>
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<td>&gt;200</td>
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<td>(±)-92(^e)</td>
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<td>&gt;200</td>
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<td>(±)-93(^e)</td>
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</tr>
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<td>(±)-94(^e)</td>
<td>48</td>
<td>33</td>
<td>50</td>
<td>99</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

\(^a\)Calculated from ee. \(^b\)According to GC. \(^c\)0.015 M substrate, 0.5 equiv. of added H\(_2\)O, 30 mg mL\(^{-1}\) CAL-B, iPr\(_2\)O, 60 °C. \(^d\)Determined by using n-heptadecane as internal standard.

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\(_2\)O, 1 equiv. of benzylamine in iPr\(_2\)O 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, (±)-84, (±)-85, (±)-86, (±)-91 and (±)-93.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>t (h)</th>
<th>Conv. (%)</th>
<th>Yield (%)</th>
<th>Isomer</th>
<th>ee_{product} (%)</th>
<th>[α]_D^{25} H_2O</th>
<th>Product enantiomer</th>
<th>Unreacted enantiomer</th>
<th>Yield (%)</th>
<th>Isomer</th>
<th>ee_{substrate} (%)</th>
<th>[α]_D^{25} CHCl_3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-83</td>
<td>3</td>
<td>49</td>
<td>48</td>
<td>(2R,3S)-101</td>
<td>99</td>
<td>-7.1^b</td>
<td>42</td>
<td>(3S,4R)-83</td>
<td>95</td>
<td>-225^i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(±)-84</td>
<td>24</td>
<td>50</td>
<td>47</td>
<td>(R)-102</td>
<td>99</td>
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<td>47</td>
<td>(S)-84</td>
<td>97</td>
<td>-275^k</td>
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<tr>
<td>(±)-85</td>
<td>48</td>
<td>50</td>
<td>46</td>
<td>(1R,2S)-103</td>
<td>99</td>
<td>-9^l</td>
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<td>(1S,5R)-85</td>
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<td>-35.7^n</td>
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<tr>
<td>(±)-86</td>
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<td>49</td>
<td>(1S,4R)-104</td>
<td>99</td>
<td>-12.4^n</td>
<td>48</td>
<td>(1S,4R)-86</td>
<td>99</td>
<td>+82.1^o</td>
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<td>(±)-91</td>
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<td>50</td>
<td>49</td>
<td>(1R,3S)-108</td>
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<td>-240^p</td>
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<td>(1R,4S)-91</td>
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<td>+34.2^d</td>
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<tr>
<td>(±)-93</td>
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<td>49</td>
<td>43</td>
<td>(1R,3S)-109</td>
<td>99</td>
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<td>(1R,4S)-93</td>
<td>96</td>
<td>+49.8^o</td>
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</tbody>
</table>

* 0.039 M substrate, 0.5 equiv. of added H_2O, 1 equiv. of benzylamine, 30 mg mL^{-1} CAL-B, iPr_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, iPr_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, iPr_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, iPr_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, iPr_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 [(3S,4R)-83, (S)-84, (1S,5R)-85, (1R,2R,5S,6S)-86, (1S,4R)-91, (1R,4S)-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 or NH_4OH and MeOH are well known in the literature. The reactions of N-hydroxymethyl-lactam enantiomers [(3S,4R)-83, (S)-84, (1S,5R)-85, (1R,2R,5S,6S)-86, (1S,4R)-91, (1R,4S)-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-ene-1-carboxylate [(±)-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$_2$O on the surface of the enzyme, was found to take place in addition to the acylation reaction. Because the optimization of the hydrolyses of (±)-59 and (±)-61 (CAL-B, 10 equiv. of H$_2$O, TBME, 30 °C) did not lead to an efficient strategy for the preparation of the corresponding (1S,2R)-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 iPr$_2$O 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 (2R,3S)-3-phenylisoserine a key Taxol intermediate [(2R,3S)-101].

High enantioselectivities (E > 200) were obtained for the CAL-B catalysed ring opening of cis-3-benzylxy-4-(4-chlorophenyl)azetidin-2-one [(±)-65] and cis-3-benzylxy-4-phenylazetidin-2-one [(±)-66] when CAL-B was used as catalyst, with 25 equiv. of H$_2$O 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 iPr$_2$O with 25 equiv. of H$_2$O 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 (2R,3S)-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 (2R,3S)-101 with an excellent ee of 99%. The method proved also to be suitable for the preparation of Taxol key intermediate (2R,3S)-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[4.2.1.02,5]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 iPr₂O with H₂O (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 NH₄OH 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 (2R,3S)-101, Cathepsin A inhibitor subunit (R)-102, Cispentacin (1R,2S)-103, CEP-28122 key intermediate (1R,2R,5S,6S)-82 and (1S,4R)-108, a key intermediate for the blockbuster Abacavir.

All synthesized enantiomers were characterized by GC or HPLC measurements, optical rotation, ¹H NMR, elemental analysis and melting point measurement.¹Ⅴ
Acknowledgements

This work was carried out in the Institute of Pharmaceutical Chemistry, University of Szeged during the years 2013–2016.

I would like to express my deep gratitude to my supervisors, Professor Ferenc Fülöp and Professor Enikő Forró, for their scientific guidance of my work, their valuable advice and constructive criticism, whereby they provided professional attitude with a proper scientific outlook and taught me to solve scientific problems.

Above all, I express my heartfelt thanks to Dr. Enikő Forró for introducing me to the enzymatic work in addition to her kindness, which has helped me through many difficulties. Special thanks are due to my colleagues at the Institute of Pharmaceutical Chemistry, and especially the former and current staff of the Enzymatic Laboratory, for their friendship and a pleasant working atmosphere.

I am also grateful to Mrs. Judit Árva and Ferenc Beke for the synthesis of the starting substrates. Finally, I express my warmest thanks to my family and my friends, for the love, support and understanding they have displayed during these years.
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