Chemoenzymatic synthesis of enantiopure 1,4-dihydropyridine derivatives

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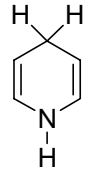
Chapter 1

Introduction

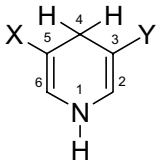
1 General introduction

1.1 Chemical characteristics of 1,4-dihydropyridines

1,4-Dihydropyridines (DHPs) belong to the class of nitrogen-containing heterocycles having a 6-membered ring (dihydroderivatives of azines). The parent 1,4-DHP was prepared as an unstable substance by Cook and Lyons in 1965.[1] Every 3,5-disubstitution at the skeleton of 1,4-DHP **1** by electron-withdrawing substituents X and Y such as COR, COOR, CN and NO_2 enhances their chemical stability, whereas electron donating groups like SC_6H_5 and OC_6H_5 have a destabilising effect. 1,4-DHP-3,5-dicarboxylates are called Hantzsch dihydropyridines or Hantzsch esters and have been widely investigated.



1,4-DHP



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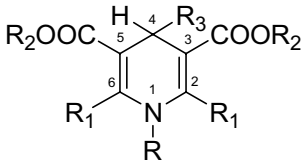


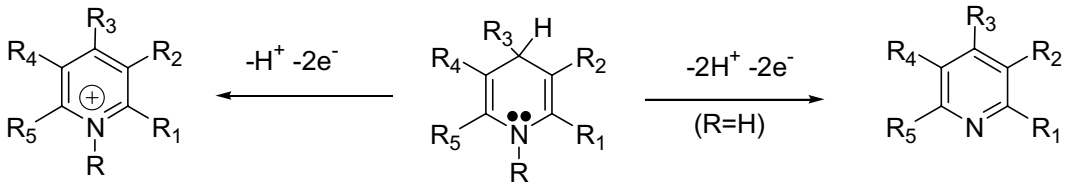
Hantzsch-type 1,4-DHP

2a R=H; R_1=Me; R_2=Et, R_3=H
2b R=H; R_1=Me; R_2=Et, R_3=Ph

The three-dimensional structure of 1,4-DHP-3,5-dicarboxylates is important for the estimation of their reactivity. The molecular geometry of Hantzsch type 1,4-DHPs seems to be planar for 4-unsubstituted compounds (**2a**) and boat-like for 4-substituted derivatives (**2b**).[2] However, the degree of ring puckering varies among the 4-aryl substituted 1,4-DHPs.[3] The interatomic distances between N-C_2=C_3-C_4 indicate conjugation in the β-aminovinylcarbonyl system, leading to an sp^2-N_1 atom. X-ray diffraction analyses performed in our lab confirm the delocalisation of the formal double bonds of the C=C-N-C=C fragment.[4]

1.1.1 Oxidation of the 1,4-dihydropyridine ring

One of the typical reactions of the dihydropyridine ring is aromatisation of the 1,4-DHP ring to a pyridine ring. Formally, the oxidation of 1,4-DHPs goes *via* loss of 1 or 2 protons and 2 electrons (Scheme 1).

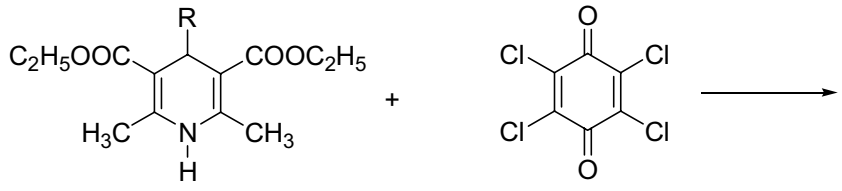


Scheme 1

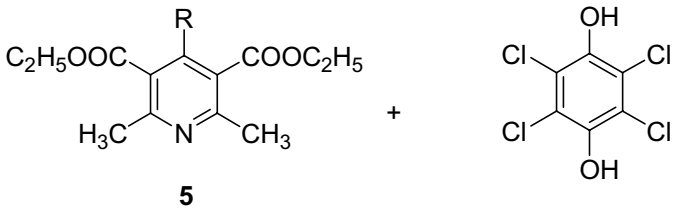
The use of organic and inorganic reagents such as hydrogen peroxide, ozone, halogens, potassium permanganate, nitric acid, sodium nitrite, chromium oxides, chloranil, tetrachloromethane, nitrosobenzene, lead tetraacetate, tin tetrachloride and other newly introduced reagents for oxidations of 1,4-DHPs has been reported.[2,5-7] Catalytic and thermal dehydrogenation as well as electrochemical and enzymatic oxidation[8] have been employed for the aromatisation of 1,4-DHPs.

The aromatisation is dependent on a combination of steric and electronic effects of the substituents of the dihydropyridine ring.[2] Introduction of a methyl or a phenyl group in the 4-position of 1,4-DHP **3** decreases the rate constant of the reaction with chloranil **4** 214 and 250 times, respectively (Scheme 2). The combination of electronic and steric effects of influence of 3,5-substitution on the rate of oxidation of 1,4-DHPs with chloranil (**4**) is shown on the next page for 4-unsubstituted 1,4-DHPs **6a**, 4-methyl-1,4-DHPs **6b**, and for 4-phenyl-1,4-DHPs **6c**.

Additional structure-reactivity relationships were established for enzymatic oxidations with the peroxidase-H_2O_2 system,[8] and electrochemical oxidations.[9] The influence of the substituent in the 4-position is depicted for 1,4-DHP **7** on the next page. 4-Unsubstituted 1,4-DHPs react more readily than the corresponding 4-substituted analogues. Generally, the influence of the substituents in the 3-, 4- and 5-position have similar trends: the most reactive compounds are the ones with the most electron-withdrawing substituents.



3a R=H; **3b** R=CH_3; **3c** R=C_6H_5 **4**



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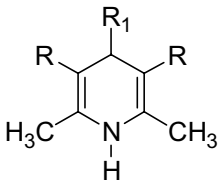
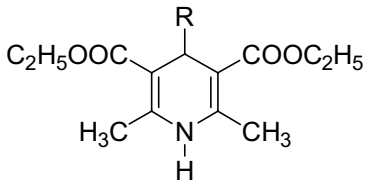
Scheme 2



6a R_1= H, **6b** R_1=CH_3; **6c**; R_1=C_6H_5

the rate of oxidation with chloranil decreases in the following order:
6a with R: $COOC(CH_3)_3$ > $COCH_3$ > $COOC_2H_5$ > COC_6H_5 > CN
6b with R: COC_6H_5 > $COCH_3$ > $COOC_2H_5$
6c with R: $CONHC_6H_5$ > $COOC_2H_5$



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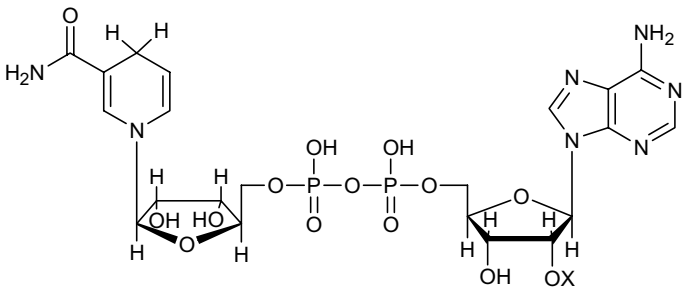
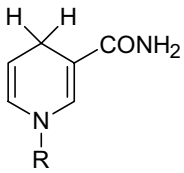
the rate of oxidation with peroxidase/H_2O_2 decreases in the following order: R: H > COONa > $CH{=}CHC_6H_5$ > $CH_2C_6H_5$ > CH_3 > C_6H_5 > 3-pyridyl > $COOCH_3$ > $COOC_2H_5$

The co-enzymes dihydronicotinamide adenine dinucleotide (NADH) and its phosphate (NADPH) have the unique ability in biological systems to reduce unsaturated functionalities (carbonyls, conjugated olefins, etc).



X=H (NADH); X=PO_3H_2 (NADPH)

Some NADH models:



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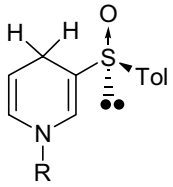
R= C_3H_7, $C_6H_5CH_2$, $(CH_2)_{11}CH_3$, $(CH_2)_2N(C_2H_5)_2$



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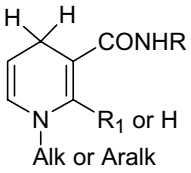
R=$CH_2C_6H_5$, C_3H_7, CH_3



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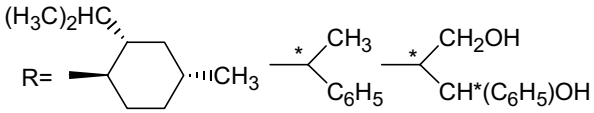
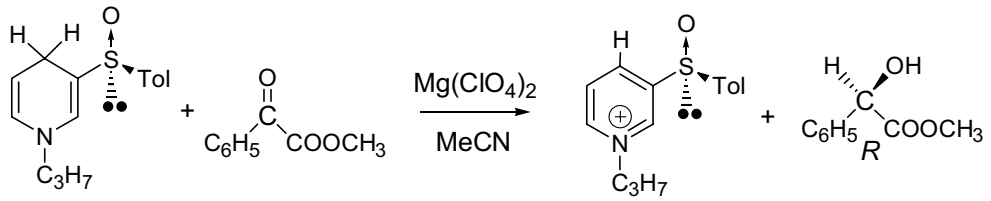


The position of the chiral centre is indicated by an asterisk

The synthesis of the 1,4-dihydronicotinamide part of NADH (NADPH) analogues **8**-**10** and the mechanisms of hydrogen transfer in different biomimetic systems by NADH analogues have been widely investigated.[2,5-7,10] The great majority of the known NADH-mimics **8**-**10** have a carbonyl group (*e.g.* an amide or an ester) at the 3-position of the 1,4-DHP ring and this functionality plays an important role in determining the stability and reactivity of the labile 1,4-DHP moiety.[11]



Scheme 3

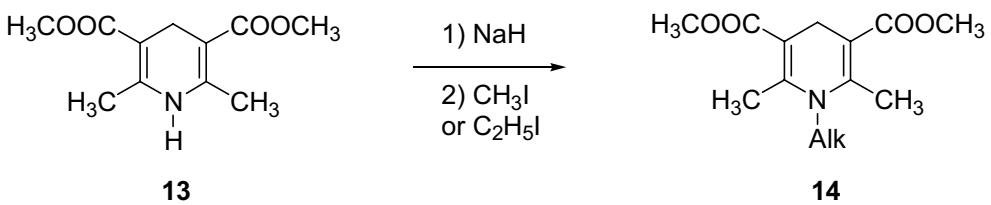
Chiral NADH model compounds of different structures have been used for asymmetric ketone reductions, *e.g.* with chiral groups at the C(3)-amido nitrogen or the *p*-tolylsulfinyl group **9**.[11-13] For instance, methyl benzoylformate **11** was successfully reduced to methyl (*R*)-mandelate **12** in 75% yield with 97% e.e. by the NADH mimicking compound **9** containing the *p*-tolylsulfinyl group (Scheme 3).[14,15]

1.1.2 Reduction of the 1,4-dihydropyridine ring

Catalytic hydrogenation of 1,2- and 1,4-DHPs on palladium gives the corresponding tetrahydroderivatives or piperidines.[7] Partial hydrogenation seems to be possible only if one of the double bonds of DHP ring is conjugated to a π-system of a substituent.[2] Bulky substituents in the 4-position of the 1,4-DHP ring inhibit catalytic hydrogenation.[5] Some 1,2- and 1,4-DHPs have been reduced with hydrides (*e.g.* sodium borohydride) or sodium dithionite, as well as electrochemically, giving tetrahydropyridines or piperidines.

1.1.3 Alkylation and acylation of the N-H group of the 1,4-dihydropyridine ring

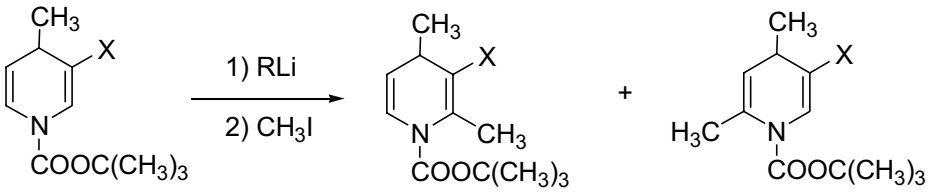
Ring substitution reactions with various electrophilic reagents are characteristic for 1,4-DHPs **13**. Most of 1,4-DHPs are very weak acids, and for cleavage of the N-H bond it is necessary to use sodium hydride or potassium hydroxide for the formation of the anion. N-alkylated or N-acylated 1,4-DHPs **14** result when the anions are treated with alkyl or acyl halides (Scheme 4). This has already become a standard procedure.[2,5-7,10]



Scheme 4

1.1.4 Electrophilic additions to the ring carbons

2,6-Unsubstituted 1,4-DHPs **15** react with strong metalating reagents, in most cases forming vinyl carbanions that readily react with a variety of electrophilic reagents such as alkyl iodides and bromides, acyl chlorides, carboxylic esters, isocyanates, diethyl chlorophosphate and others. For example (Scheme 5), alkyl groups were introduced into the 2-position of 1,4-DHP **15** *via* lithium derivatives. The 2-methyl derivative **16** predominates if X=Cl, Br, or $OCONEt_2$, while the 6-methyl isomer **17** prevails if X is OMe or Me; their ratio also depends on the organolithium compound (R= *n*-Bu, *sec*-Bu, Ph, mesityl).[16]

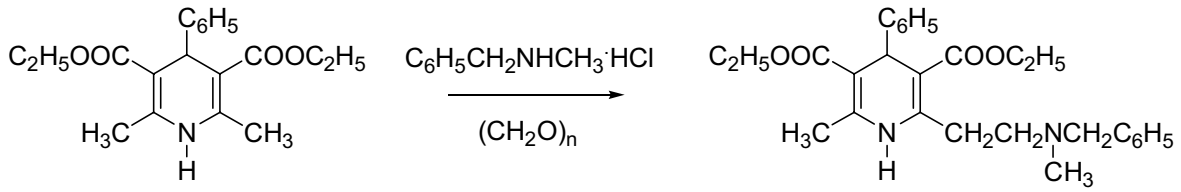


15 **16** **17**

Scheme 5

1.1.5 Reactions of the methyl groups at the 2- and 6-positions of the 1,4-dihydropyridine ring

2,6-Disubstituted 1,4-DHPs are common products of the Hantzsch synthesis because of the availability of starting materials (see section 1.4). The methyl groups in the positions 2 and 6 of Hantzsch type 1,4-DHPs can be involved in Mannich reactions. 1,4-DHPs **18** form conventional products of aminomethylation **19** when treated with paraformaldehyde and secondary amine hydrochlorides (Scheme 6). Mannich reactions with primary amines give a variety of polycyclic heterocycles.[2,7,17]

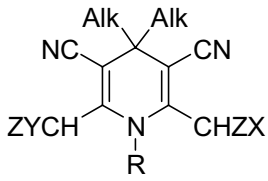


18 **19**

Scheme 6

Bromination with bromine, N-bromosuccinimide or pyridinium bromide perbromide ($C_5H_5NHBr_3$) leads to 2-bromomethyl or other products of bromination **20**. Addition of chlorine to the ring double bonds occurs as a side reaction to substitution of the 2,6-methyl groups during the chlorination. The

halogen atoms in the 2-CH_2Br and 2-CH_2Cl groups can be displaced with appropriate nucleophilic reagents to I, OH, OR, SR, NR_2, N^+R_3, N_3, $CHRR_1$, SeR, P^+R_3, as well as N-heterocycles.[2,7,18]



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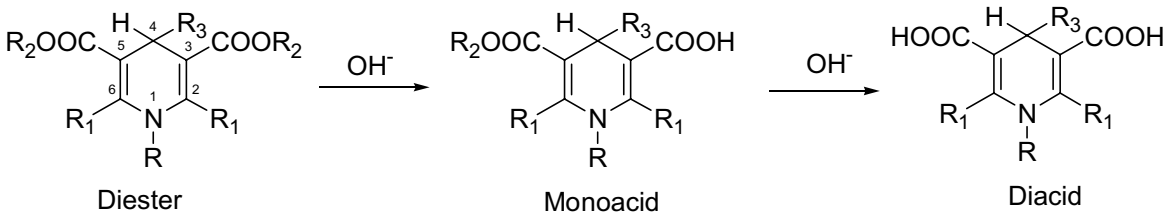
X=Br Y, Z=H; X=Y=Br, Z=H; X, Y, Z=Br

1.1.6 Chemical hydrolysis of 1,4-dihydropyridine-3,5-dicarboxylates



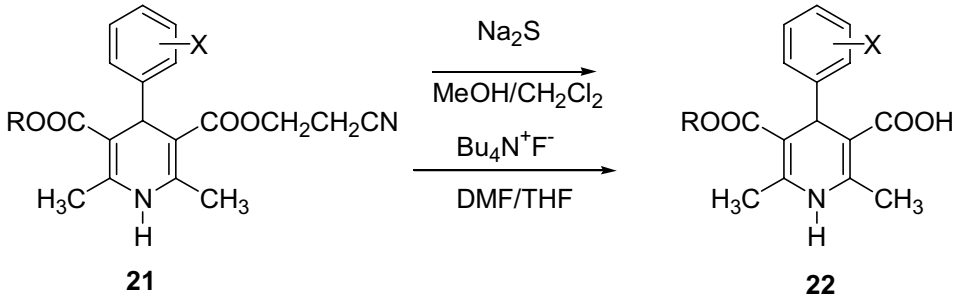
Scheme 7

Alkyl ester groups on positions 3 and 5 of 1,4-DHPs having substituents in the positions 2/6 and 4 are exceptionally stable upon the treatment with nucleophilic reagents. Protection of the N-H group and lack of substituents in the positions 2/6 or 4 enables the hydrolysis or transesterification of alkyl esters at the 3- and 5-position under strongly basic conditions (Scheme 7).[19,20] A combination of steric and electronic factors are the reason for these differences in hydrolysis rate of 1,4-DHPs with different substitution of the dihydropyridine ring. In contrast to 3,5-dialkoxycarbonyl-1,4-DHPs, with a conjugated β-aminovinylcarbonyl system, the corresponding 3,5-dialkoxycarbonylpyridines undergo hydrolysis without difficulties.[21,22] Besides the conjugation of the β-aminovinylcarbonyl system, steric factors formed by substituents next to the ester group interfere with the attack by nucleophilic reagents. Facilitation of the hydrolysis of N-protected 4-substituted 3,5-dialkoxycarbonyl-1,4-DHPs can be explained by a decrease of the conjugation of the esters with the 1,4-DHP ring.[19] The 3,5-diester groups of 2,6-dimethyl-1,4-DHPs are rotated out of the 1,4-DHP plane when the nitrogen atom is substituted with an aryl, alkyl, aralkyl or acyl group,[20] probably because the substituents cannot be accommodated in a plane (the so-called buttressing effect).

4-Unsubstituted 1,4-DHP diesters undergo transesterification with the more nucleophilic alkoxide ion more easily than hydrolysis.[23] Instead of hydrolysis, the transesterification of bis(ethyl) 2,6-

dimethyl-4-(*m*-nitrophenyl)-1,4-DHP-dicarboxylate was observed in MeOH with KOH, in moderate yields.[20]

The cleavage of esters of 4-aryl-2,6-dimethyl-1,4-DHP-3,5-dicarboxylates can be easy performed when ester moieties with electron-withdrawing groups are introduced at the β-position, so that they can undergo β-eliminations instead of hydrolysis.[2,7] Frequently 3,5-dicarboxylic acids or 3-monocarboxylic acids of 1,4-DHPs **22** have been prepared from the corresponding 2-cyanoethyl esters of 1,4-DHPs **21** and potassium hydroxide.[7,24-26] Smooth cleavage of the 2-cyanoethyl esters was also achieved using sodium sulfide or tetrabutylammonium fluoride (Scheme 8).[27]



X= 3-NO_2, 3-Cl, 2,3-diCl, 2-F, 2-CF_3
R= Alk; $C_2H_4OCH_3$, $C_2H_4SCH_3$, $C_2H_4ONO_2$, $C_3H_6ONO_2$, $C_2H_4NHCOPy$-3

Scheme 8

Electron-withdrawing substituents might decrease the influence of conjugation and can therefore be another reason for the enhanced reactivity towards hydrolysis. Thus, the facile cleavage of CH_2CH_2SOPh, $CH_2CH_2SO_2Me$, $CH_2CH_2SO_2Ph$,[28] $CH(CH_2OH)CH_2SO_2C_6H_4$-CH_3-*p*,[29] $CH_2CH_2S^+Me_2$[30] and $CH_2CH_2N^+Me_3$[31] esters by KOH has been reported.[7]

The reactions of 1,4-DHPs that are the most characteristic and relevant to this thesis have been briefly mentioned above. Other reactions of 1,4-DHPs, such as addition reactions to the dihydropyridine double bond, photochemical reactions, cyclisations, rearrangements, isomerisations, cleavage of the DHP ring, reactions of substituents of the 1,4-DHPs, as well as additional information concerning NADH mimicking features can be found in several good reviews and in citations therein.[2,5-7,10]

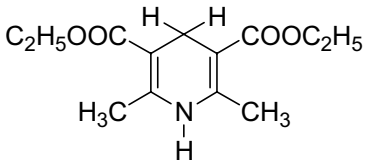
1.2 Biological activities of 1,4-dihydropyridines

Among the different dihydropyridine isomers, 1,4-DHPs merit special attention, not only because of the general interest in their chemistry, but particularly because of their increasing pharmaceutical importance. The dihydropyridine structure is involved in biological redox processes as the reduced

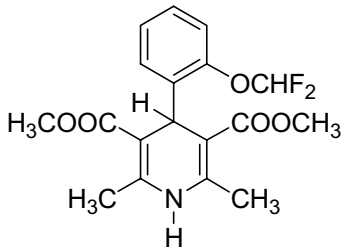
forms of the co-enzymes NADH and NAD(P)H, and the pharmacological properties of 1,4-DHPs have been extensively studied for several decades.[32]

The antioxidative effects of dihydropyridine derivatives have been widely investigated because this property is thought to contribute to the pharmacological actions of 1,4-DHPs.[33-36] Charged amphiphilic dihydropyridines may have potential as efficient and safe agents for gene delivery in cells.[37,38] 4-Aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates are widely used for the treatment of cardiovascular diseases (hypertension, angina pectoris and infarction).[39-41] 1,4-DHPs possess a broad range of other biological activities, such as hepatoprotective,[42,43] photosensitising,[44] antitumor,[45,46] antimutagenic,[47] geroprotective,[48] as well as antidiabetic,[49] anti-inflammatory[50] and antibacterial[51] properties. 1,4-DHPs were found capable of prevention of alcohol-induced neurodegenerative disorders.[52-54]

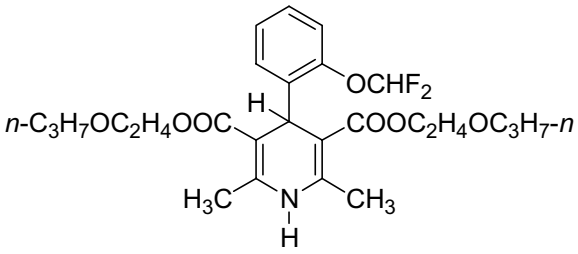
Diludine, foridone, cerebrocrast and glutapyrone have been developed in the Laboratory of Membrane Active Compounds and β-diketones of the Latvian Institute of Organic Synthesis.



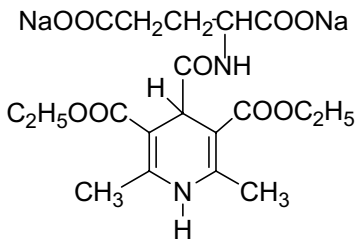
Diludine



Foridone



Cerebrocrast



Glutapyrone

Diludine possesses free radical scavenging[55] and antioxidative[56] properties. In agriculture diludine is used as growth promoter and feed protector for cattle. Due to its radioprotective properties diludine (trade name of ointment for medical use is diethone) is currently used to diminish the side effects of radiotherapy in the treatment of cancer patients.[57-59]

Foridone (riodipine) is an antihypertensive and antianginal drug, particularly in the cases where the cardiovascular diseases are accompanied by bronchial asthma, and pulmonary hypertension.[60,61]

Cerebrocrast differs substantially from the typical calcium antagonist drugs. It has nootropic activities (memory–improving, antiamnestic), and also anticonvulsant, antihypoxic and antidiabetic

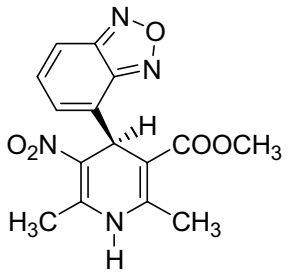
action.[49,53] It is capable to prevent neurodegenerative disorders, *e.g.*, in the animal model of alcohol-induced accelerated aging,[54] and to increase corticosterone concentration in plasma.[62] At very low doses it selectively dilates vertebral artery blood flow. Thus, at a dose of 0.001 mg/kg cerebrocrast increased blood flow by 90% in the vertebral and by 60% in the carotid artery, whereas in the femoral artery only by 10%.[63] Moreover, anti-inflammatory effects of cerebrocrast have been reported recently.[50]

Glutapyrone is the representative of a novel class of amino acid-containing 1,4-DHPs. Glutapyrone is a water-soluble, extremely low toxic (LD_{50} >3,000 mg/kg, *i.v.*; >8,000 mg/kg, *i.p.*; >10,000 mg/kg, *per os*) non-calcium antagonist derivative of 1,4-dihydroisonicotinic acid possessing neuromodulatory and neuroregulatory action. It is an anticonvulsant, stress-protective, antiarrhythmic, cognition-enhancing compound of long-term activity.[64-67]

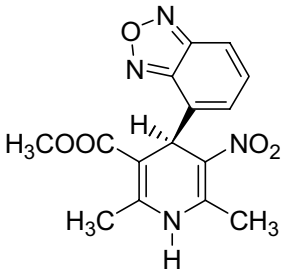
1.3 The impact of chirality on drug development and use

1.3.1 The pharmacological activities of unsymmetrical 1,4-dihydropyridines

When substituents on the left side differ from those on the right side of a 1,4-DHP, the molecule becomes chiral, with a substituted C(4) as the stereogenic centre. Many of the synthesised 1,4-DHP calcium antagonists are asymmetric. The ester groups at the positions 3 and 5 have a crucial influence on the pharmacological activities of 1,4-DHPs.[39] Thus, the enantiomers of unsymmetrical 1,4-DHPs usually differ in their biological activities[25,68] and could have even an opposite action profile[69] (calcium antagonist-calcium agonist; hypotensive activity-hypertensive activity):



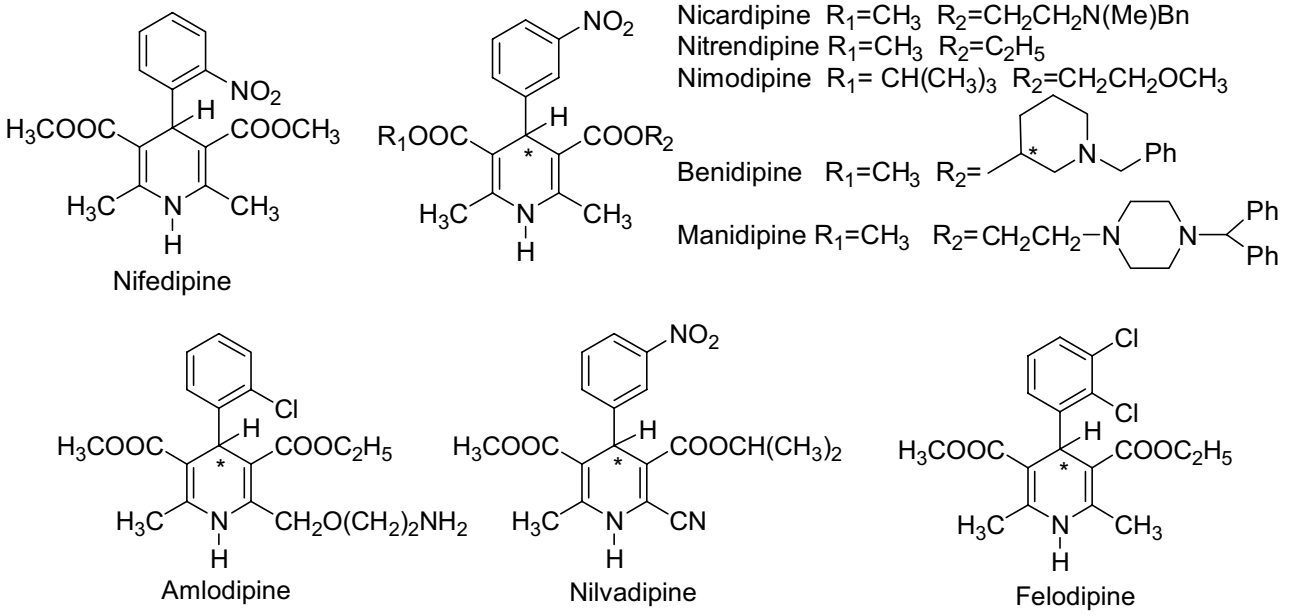
(+)-(*S*)-202-791
Calcium channel agonist



(-)-(*R*)-202-791
Calcium channel antagonist

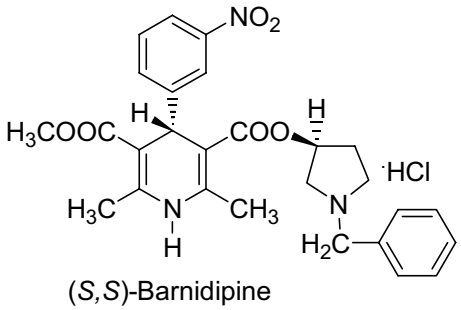
The dihydropyridines are among the most effective calcium antagonists or calcium channel blockers.[41] The most common side effects caused by calcium antagonists are due to excessive vasodilatation. Since nifedipine (Adalat®) was introduced in Germany in 1975 by Bayer AG[70] many

other products such as nicardipine, nilvadipine, nitrendipine, foridone, nimodipine, benidipine, manidipine, amlodipine, felodipine and many others have appeared on the market.[39]



The position of the chiral centre is indicated by an asterisk

Some of the above mentioned drugs possess longer lasting antihypertensive activities, better tissue selectivity and gradual onset of the pharmacological effect, than the parent nifedipine.[71] Yamanouchi (Japan) was the first to introduce the most potent diastereomer of barnidipine[72,73] in Japan in 1992 and in the Netherlands in 2000, however other calcium antagonists are still marketed as racemic mixtures to this day.



The different activities of enantiomers and diastereomers of 1,4-DHPs could cause problems in drug development and promotion in the future.

1.3.2 The role of regulatory authorities in the process of development of chiral pharmaceuticals

Recognition of potential differences in pharmaceutical properties of enantiomers has led to increased attention by regulatory agencies with respect to such compounds.[74-76] The Food and Drug Administration (FDA)[77] in USA and the Committee for Proprietary Medicinal Products (CPMP)[78] in Europe have published specific rules regulating the development of new racemic and enantiomeric drugs. FDA recommendations include also switching to a single enantiomer for an approved racemate, where appropriate.[74,75]

The racemates developed as drugs will be considered as fixed combination or fixed dosage products.[79] The chemistry, toxicology, pharmacokinetics and pharmacodynamics of both enantiomers of the racemate must have been studied independently, and new studies on the racemate must be submitted for approval.

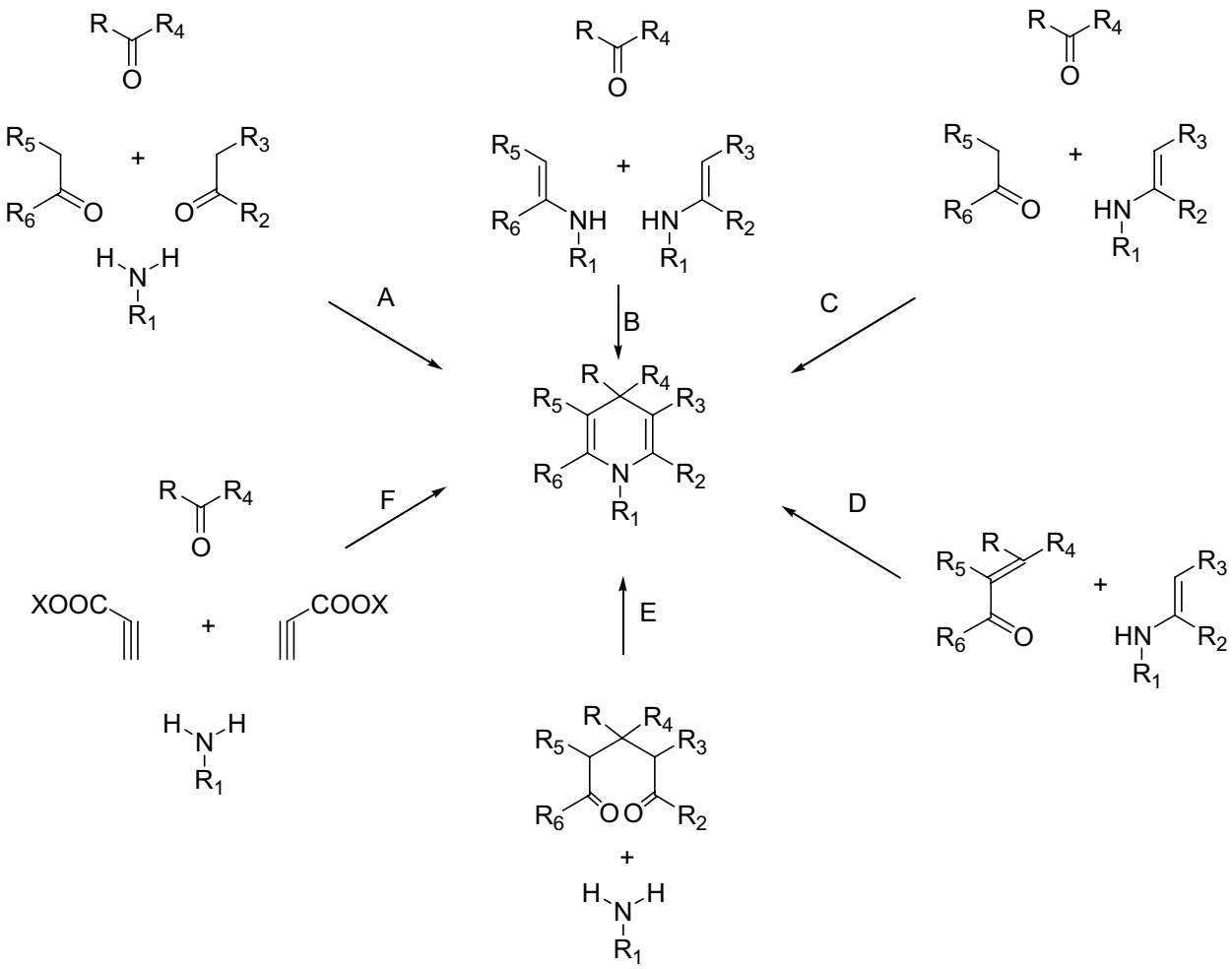
Since the number of chiral medicines annually increases, synthetic strategies have to be developed that exhibit fine stereocontrol and can be efficiently performed on a large scale.[76,80]

1.4 Synthesis of 1,4-dihydropyridines

Several good review articles cover the scope and limitations of methods of synthesis and chemical properties of hydrogenated pyridine derivatives from 1881 up to now.[2,5-7,18,81,82] Only the main features of the diverse synthetic approaches will be mentioned here, with special emphasis on the synthesis of chiral 1,4-DHPs.

1.4.1 Hantzsch synthesis and related cyclocondensations

The first synthesis of a dihydropyridine was performed by Arthur Hantzsch in 1881.[82-84] The synthesis, which now bears his name, consists of the cyclocondensation of an aldehyde with an active methylene carbonyl compound (*e.g.* ethyl acetoacetate) and ammonia or a primary amine (Pathway A, Scheme 9).[85,86] The Hantzsch synthesis remains up to now the most common method for the synthesis of a wide variety of 1,4-dihydropyridines. Various modifications of this method have been used for the preparation of mono- and polycyclic 1,4-DHPs; the principal pathways are outlined in Scheme 9.



Scheme 9

The same or structurally related 1,4-DHPs can be frequently obtained *via* different synthetic pathways, *e.g.* 3-aminocrotonate can be replaced by ethyl acetoacetate and ammonia (or a primary amine) (Method B).[63] The use of different enamines permits the isolation of unsymmetrical dihydropyridines.[87,88] Enamines in combination with an active methylene carbonyl compound can be used for the synthesis of unsymmetrical dihydropyridines (Method C).[27] Aldehydes may be condensed with active methylene compounds to give α,β-unsaturated ketones. The latter can react with an enamine, or a ketone and ammonia, to give an unsymmetrical 1,4-DHP (Method D).[89] Sometimes this method gives good results when the usual Hantzsch synthesis fails. Since many active methylene compounds react with aldehydes to give 1,5-diketones, this behaviour was exploited in another variation on the Hantzsch synthesis (Method E).[20] The use of acetylenic derivatives with aldehydes and ammonia or a primary amine is another way to synthesise 2,6-unsubstituted 1,4-DHPs. In the latter case, the nitrogen atom can be substituted or not (Method F).[90]

1.4.2 Preparation of hydrogenated pyridines from pyridine derivatives

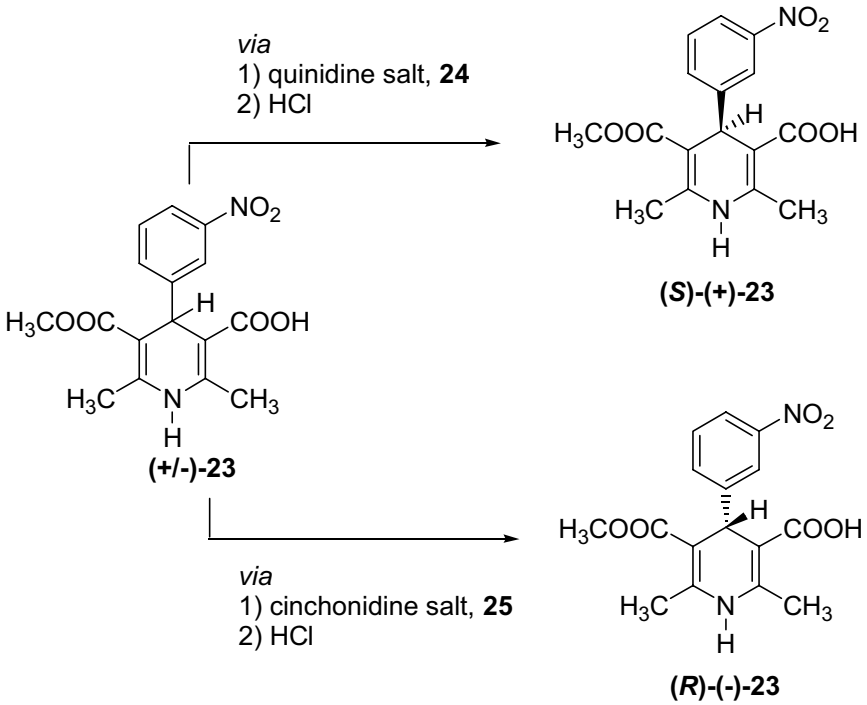
1,4-Dihydropyridines can be synthesised also *via* the reduction of pyridines or quaternary pyridinium salts. The reduction of pyridines and pyridinium salts with metal hydrides usually leads to a mixture of 1,4- and 1,2- isomers and to 1,2,3,4-tetrahydropyridines. The product ratio can be altered by changing the reaction conditions and/or the reducing agent. Dihydropyridines have also been prepared by the reaction of organometallic reagents (organolithiums or Grignard reagents) with pyridines or pyridinium salts. The reaction of the cyanide anion with pyridinium salts predominantly takes place at the 4-position giving the corresponding 4-cyano-1,4-DHPs.[91] Reduction with sodium dithionite of pyridinium salts with electron withdrawing groups at the positions 3 and 5 exclusively leads to the corresponding 1,4-DHPs.
Metals such as sodium, sodium amalgam, zinc, or activated aluminium have been employed for the reduction of pyridines and pyridinium salts. Electrolytic reduction of pyridinium salts and catalytic hydrogenation of pyridines have also been used for the synthesis of hydrogenated pyridines. Information concerning the preparation of 1,4-DHPs *via* reduction of pyridines and quaternary pyridinium salts can be found in several literature reviews.[2,5,6,18]

1.5 Stereoselective synthesis of 1,4-dihydropyridines

The classical Hantzsch synthesis produces racemic mixtures of unsymmetrical 1,4-DHPs. This is the reason for intensive research interest for the preparation of enantiomerically pure 1,4-DHPs such as stereoselective synthesis, resolution or asymmetrisation, among others *via* biocatalytic processes.[39,92,93] The principle of formation of diastereomers and their separation by means of crystallisation or preparative chromatography is used for the resolution of 1,4-DHP-3-carboxylic acids *via* diastereomeric salts or diastereomeric esters. Chiral aldehydes have been applied for the synthesis of pure diastereomers of 1,4-DHPs. Diastereoselective reactions of nucleophiles with chiral pyridines have been also described. Enantioselective synthesis using chiral auxiliaries attached to the nitrogen atom has been developed and proved to be practically useful. Enzyme-catalysed hydrolysis or transesterification of ester or alcohol derivatives of 1,4-DHPs has also become a powerful method for the preparation of enantiopure 1,4-DHPs. The next sections give an overview; the latter technique will be discussed in more detail.

1.5.1 Resolution of racemic 1,4-dihydropyridinecarboxylic acids

Many optical resolutions of racemic dihydropyridinecarboxylic acids with various structures *via* diastereomeric salts were performed using chiral amine bases as resolving agents.[94] The presently most preferred resolving agents are cinchonidine,[25,26,95] cinchonine[26,95] and quinidine.[25] Common in these separations is that a racemic acid *e.g.* **23** is treated with a chiral base (*e.g.* quinidine[25]), which forms a crystalline diastereomeric salt **24** (Scheme 10). The diastereomeric purity of this salt is improved by repeated crystallisation and at the end the enantiomerically pure acid **23** is set free by acidification.

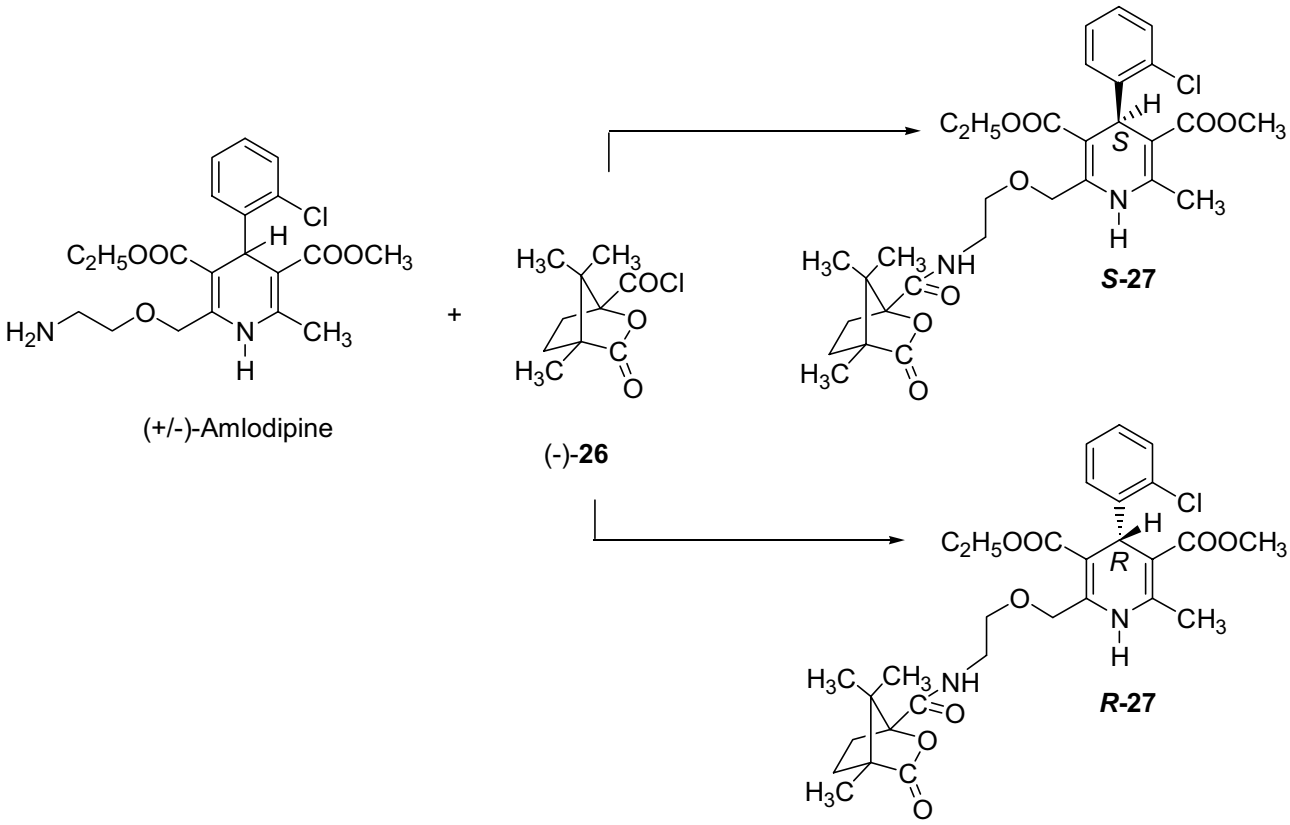


Scheme 10

The recovered monoacid from the mother liquor is treated with another chiral base (*e.g.* cinchonidine[25]) which forms the crystalline optical antipode **25** and after repeated crystallisation the second enantiomer can be obtained after acidification. A restriction of the method is that both enantiomers can be obtained only with those chiral bases which form crystalline salts. Furthermore, crystallisation, which is lowering the yields, is essential to obtain a high e.e. of the enantiomers.

1.5.2 Resolution of racemic basic dihydropyridine derivatives

Chiral acids such as camphorsulphonic acid and substituted tartaric acids have been used to separate the enantiomers of basic dihydropyridine derivatives.[39] The resolution of racemic amlodipine was carried out using (—)-(1*S*)-camphanic acid chloride **26**. The diastereomeric amides **27** were separated chromatographically (Scheme 11).[96] This method of enantiomer separation is suitable for many dihydropyridines with basic substituents at the 2-position.

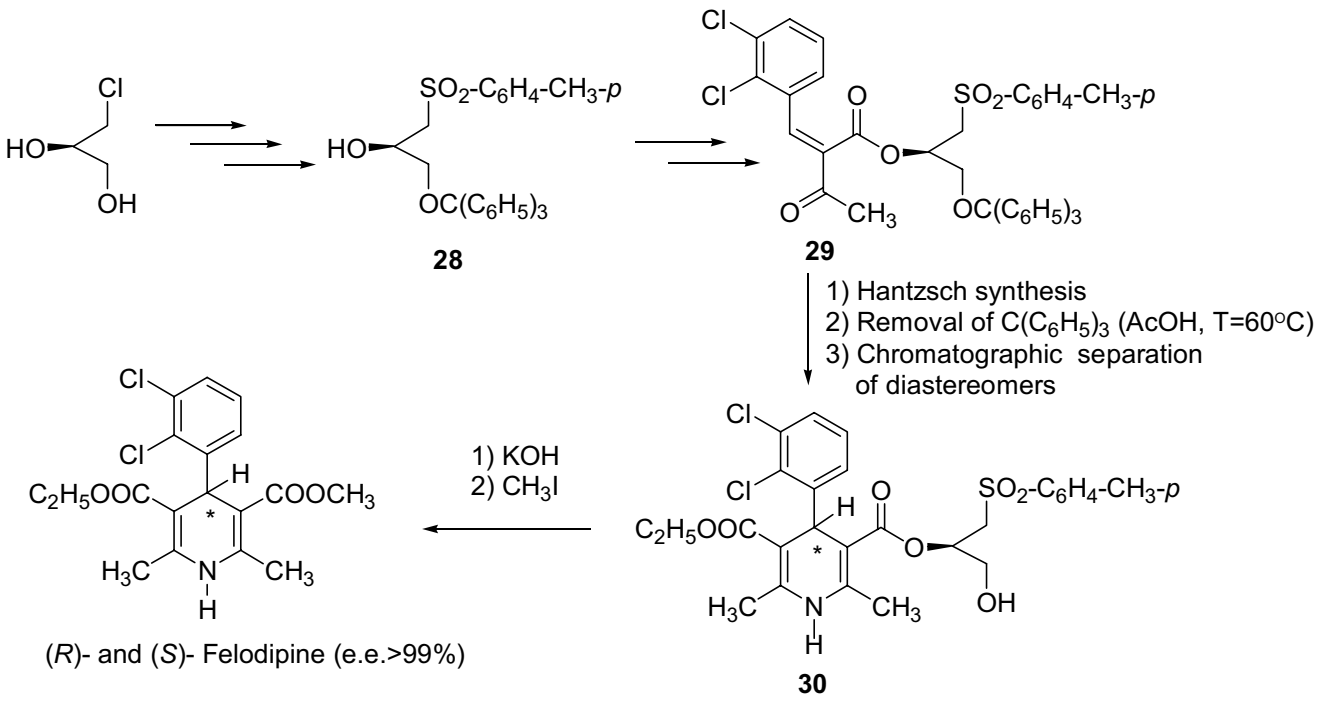


Scheme 11

1.5.3 Separation *via* diastereomeric esters of 1,4-dihydropyridines

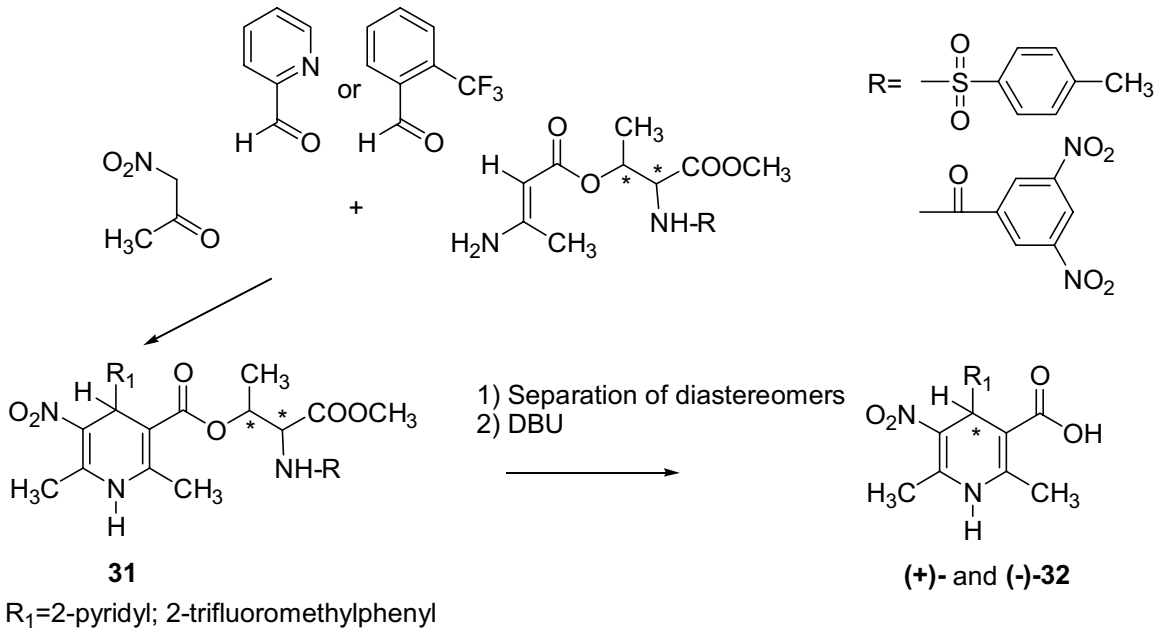
This synthetic strategy involves the synthesis of diastereomeric dihydropyridines with an easily removable chiral auxiliary, followed by separation of the diastereomers by means of chromatography or fractional crystallisation, and regioselective removal of the ester group containing the chiral auxiliary (Scheme 12).

The introduction of the chiral ester group is often combined in an elegant way with the synthesis of the DHP ring. For instance, the enantiomers of felodipine were obtained *via* a multi-step synthesis starting from (*R*)-1-(*p*-toluenesulfonyl)-3-trityloxypropan-2-ol **28** (**28→29→30**) and chromatographic separation of the diastereomeric esters **30**.[29]



Scheme 12

In a similar way the synthesis of both enantiomers of 4-(2-pyridyl)- and 4-(2-trifluoromethylphenyl)-1,4-DHP derivatives **32** with high e.e. was performed *via* conventional chromatographical separation of the diastereomeric esters **31** with threonine derivatives, which can be removed with bases like DBU (Scheme 13).[97,98]

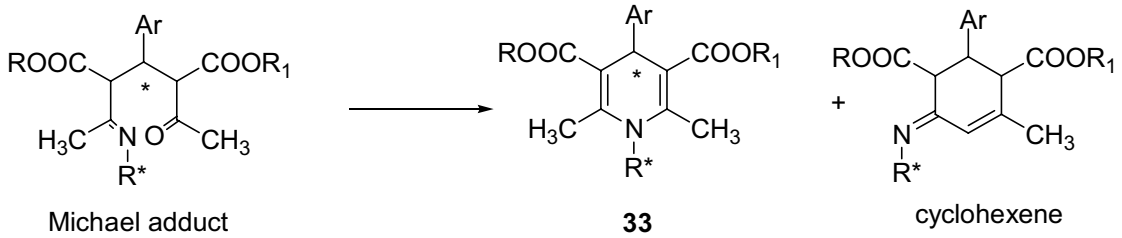


Scheme 13

In both syntheses the carboxyl group was set free using β-elimination; no hydrolysis was involved.

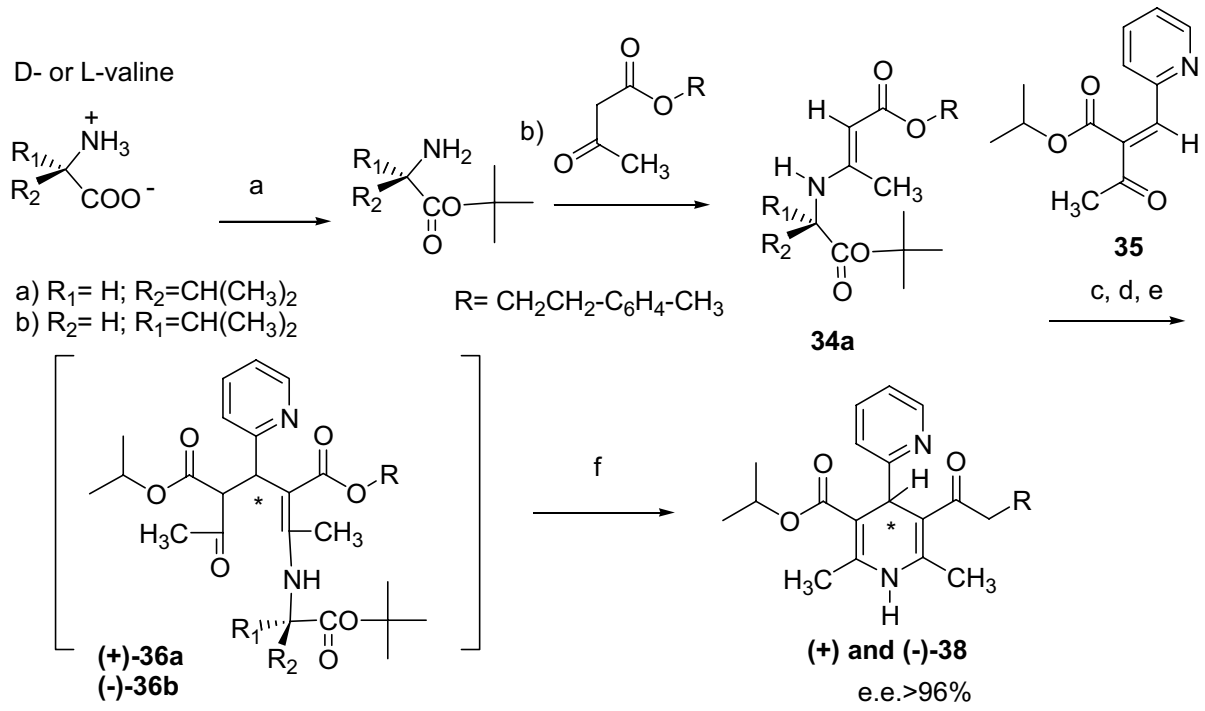
1.5.4 Enantioselective synthesis of 1,4-dihydropyridines starting from chiral enamines

Enantiomerically pure 1,4-DHPs can be also synthesised *via* derivatives with the chiral auxiliary attached to the nitrogen. However, low yields of N-substituted 1,4-DHPs **33** and formation of cyclohexenes as by-products were observed.[39] Moreover, the chiral auxiliary has to be removed afterwards (Scheme 14).

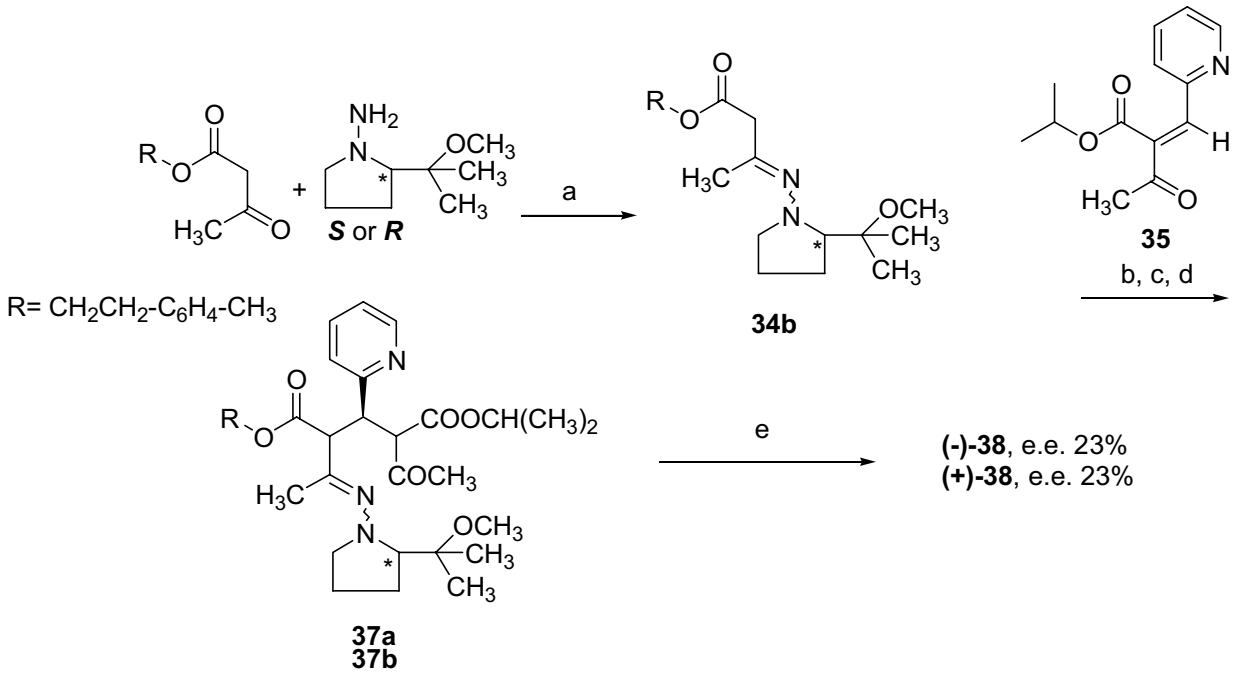


Scheme 14

One drawback of the method can be avoided if the chiral auxiliary on the nitrogen (compound **36a,b** and **37a,b**, Scheme 15) is replaced by ammonia at the stage of the Hantzsch cyclisation, after diastereoselective Michael addition of the chiral enamine to the isopropyl 2-(3-pyridylylidene) acetoacetate (Scheme 15). The stereoselective step in this procedure is the asymmetric Michael addition of a metallated chiral aminocrotonate **34**, derived from D- or L- valine, respectively, to the isopropyl 2-(3-pyridylylidene) acetoacetate **35**.[99] Beside amino acid derivatives as chiral auxiliary amines, chiral hydrazones (compound **37a,b**) can be applied for the synthesis of chiral 1,4-DHPs **38a,b** (Scheme 15). However, high enantiomeric accesses were reached only for methoxy-substituted aromatic compounds and bulky *t*-butyl esters.[100]



a) *t*-butyl acetate, 70% $HClO_4$, rt, 4 days; b) 4-methylphenethyl acetoacetate, MeOH, rt, 1 day;
c) lithium diisopropylamide (LDA), THF, -78°C; d) **35**, THF, -78°C; e) saturated aqueous NH_4Cl, rt;
f) NH_4OAc, MeOH, H_2O, reflux, 1 h.

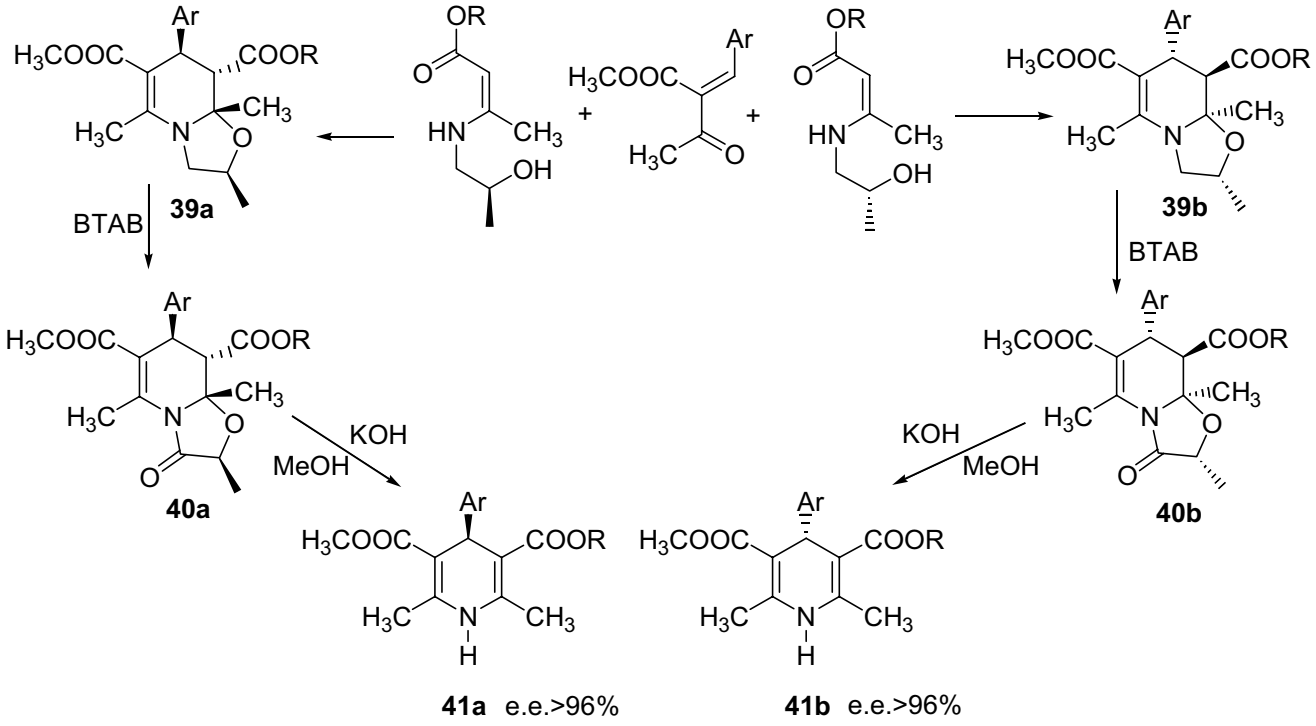


a) benzene, reflux 3 h, Dean-Stark adapter; b) *n*-BuLi, THF, -78°C; c) **35**, THF, -78°C;
d) saturated aqueous NH_4Cl; e) NH_4Cl, MeOH, 65°C.

Scheme 15

A different approach is based on the indirect transformation of 2-alkyl-2,3,8,8a-tetrahydro-7*H*-oxazolo[3,2-*a*]pyridines **39a,b** with known stereochemistry to 1,4-DHPs **41a,b**. The enantiopure bicycles **39a,b** were prepared from chiral enamines obtained from chiral aminoalcohols.[101] Basic

treatment of the oxazolidine moiety of compound **39** proved to be ineffective for the direct transformation into 1,4-DHP **41** (Scheme 16). The oxazolidine ring of **39** was converted by oxidation into a more reactive oxazolidinone **40**, which could be hydrolysed to the 1,4-DHP **41**. In this way both enantiomers of several 1,4-DHPs were synthesised from the pure stereoisomers **40a,b**.[102]

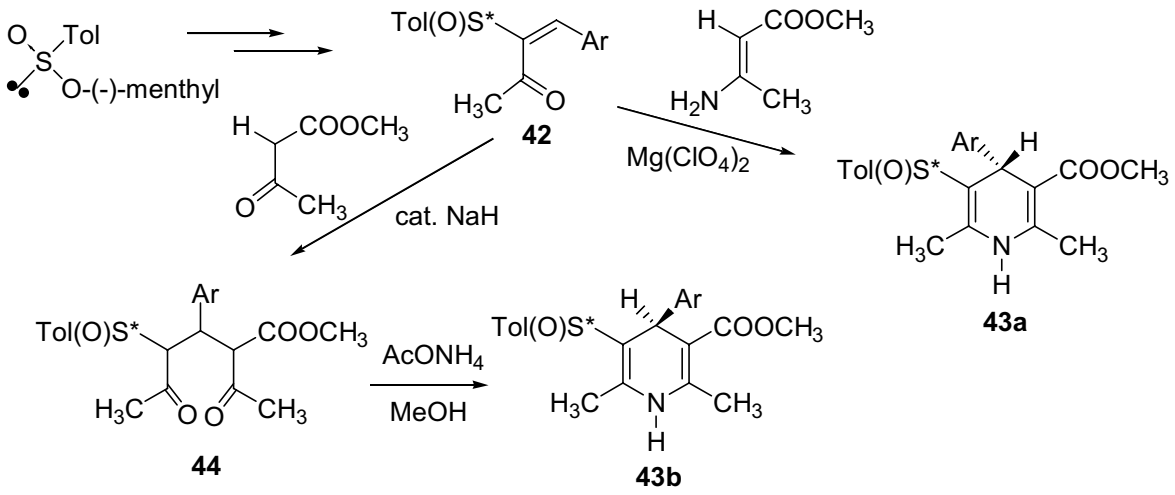


Scheme 16

1.5.5 Diastereoselective synthesis of *p*-tolylsulfinyl substituted 1,4-dihydropyridines *via* chiral sulfoxides

The stereoselective Hantzsch synthesis of 1,4-DHPs with chiral ester groups is uncommon, usually the diastereomeric mixtures are separated by means of chromatography or fractional crystallisation (see section 1.5.3). Only a few examples of diastereoselective syntheses of 1,4-DHPs having a *p*-tolylsulfinyl group are known. The diastereoselective synthesis of 1,4-DHPs with the *p*-tolylsulfinyl group was performed starting from the α-sulfinyl enones **42**, which were obtained from (—)-menthyl (*S*)-*p*-tolylsulfinate (Scheme 17). Two synthetic strategies were used to prepare the 1,4-DHPs: the reaction of enones **42** with methyl 3-aminocrotonate in the presence of magnesium perchlorate gave single diastereomers of 1,4-DHP **43a**, alternatively enones were treated with

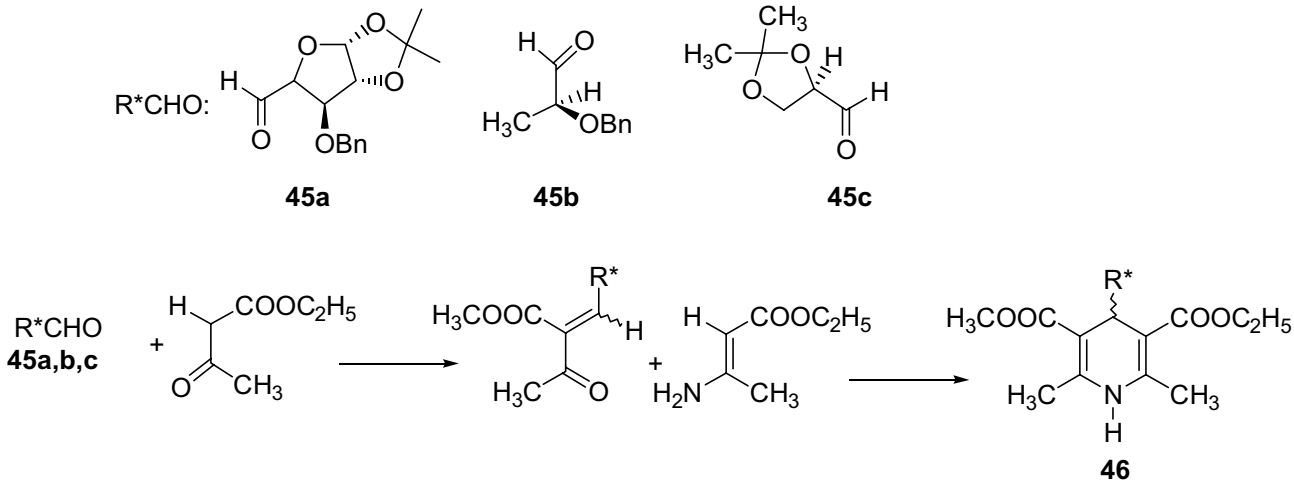
methyl acetoacetate to give the corresponding diketones **44**, which were condensed with ammonium acetate to give both diastereomers of 1,4-DHP **43a** and **43b** in a ratio of 1:1.5-2.[103]



Scheme 17

1.5.6 Diastereoselective synthesis of 1,4-dihydropyridines from chiral aldehydes

This synthetic methodology is based on the Michael addition of β-aminocrotonates to chiral α-acetylacrylates, which are readily available from condensation of chiral aldehydes **45** with acetoacetates (Scheme 18).[104] These additions lead to 1,4-DHPs like **46** in a d.e. of 80-99%.



Scheme 18

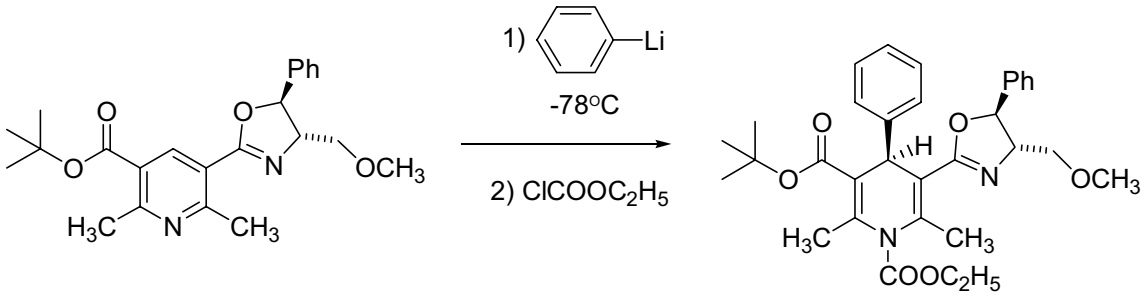
1.5.7 Chromatographic separation of enantiomers of 1,4-dihydropyridines

Chiral resolution of enantiomers by HPLC can be performed *via* derivatisation to the corresponding diastereomers using a chiral reagent (this approach is discussed in 1.5.2, 1.5.3), followed by

conventional stationary phase chromatography. Chiral mobile phase additives (CMPAs) have been widely used for separation of enantiomers on achiral stationary phases. Alternatively, enantiomers can be directly analysed on chiral stationary phases (CSPs).[40] The direct chromatographic enantioseparation of unsymmetrical 1,4-DHPs on CSPs has been widely used for the determination of enantiomeric purity,[105] for preparation of small quantities of enantiomers,[106] as well as diastereomers,[68] for biological investigations and for detection of enantiomers in human plasma and tissue.[107,108] Column selection is an empirical process based on what has been shown to work, or by exploring column utility for related compounds, or by trial and error. Pirkle-type,[109] α_1-acid glycoprotein (AGP),[110] β-cyclodextrines,[111] polysaccharide (cellulose and amylose) derivatives[106,112] and others CSPs with different types of chiral recognition mechanisms have been applied for enantioseparations of 1,4-DHPs.

1.5.8 Diastereoselective reactions of nucleophiles with chiral pyridines

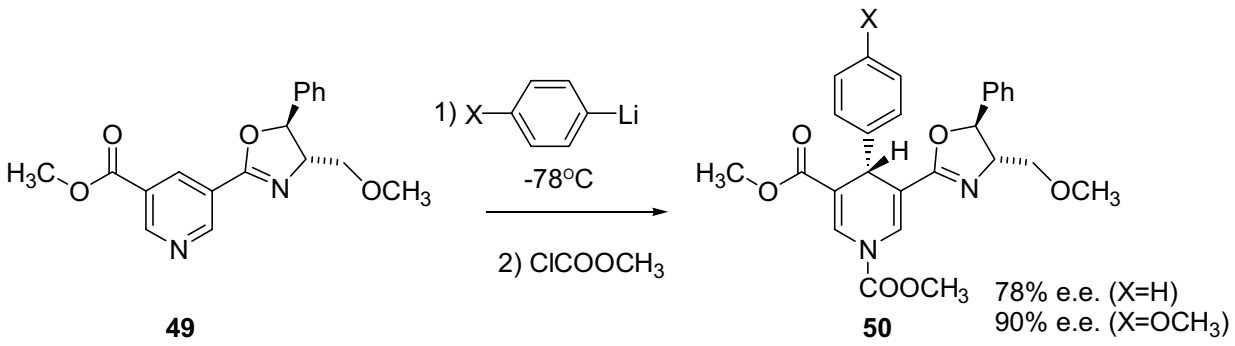
The drawback of diastereoselective reduction of 4-aryl substituted pyridines with bulky complex hydrides is the lack of regioselectivity leading to the formation of 1,4- and 1,2-DHPs, as well as reduction of the ester group to the alcohol as a competitive reaction.[113,114] In the case of 4-unsubstituted 1,4-DHP **47** the addition of aryl anions in the 4-position has been successfully performed with a d.e. of 67% (compound **48**). The ester groups in the 3- and 5-positions should be protected against nucleophilic attack of PhLi by using bulky esters (Scheme 19).[22]



47 **48**

Scheme 19

Higher diastereomerical purities of 4-phenyl-1,4-DHPs can be achieved for 2,6-unsubstituted pyridines **49** (Scheme 20), but the products **50** are pharmacologically less important.[115]



Scheme 20

1.6 The use of enzymes in synthetic chemistry

Enzymes are biocatalysts, *i.e.* they are a subset of proteins which catalyse the chemistry of life. Biocatalysts are being used through the ages, especially in the form of whole cells/microorganisms. The use of enzymes for the transformation of non-natural compounds has been reported more than hundred years ago and is steadily increasing, especially in the last two decades.[116] Recent investigations show the usefulness of enzymes and whole cells in organic synthesis as well as for the degradation of organic and inorganic pollutants.[117] Enzymes can efficiently catalyse a broad spectrum of reactions, they accept different kinds of man-made substrates and they can be used in combination with other enzymes.[116] They are chemoselective and react only with only one type of functional groups and do not affect other functional groups, which diminishes the formation of by-products.[116,118] Respective to the type of catalysed reaction, enzymes can be divided into oxidoreductases, transferases, hydrolases (*e.g.* lipases, esterases and proteases), lyases, isomerases and ligases.[116] The three-dimensional structure of enzymes is the reason of their positional selectivity. Enzymes can distinguish between the same reacting groups at different places of the substrate. As enzymes are chiral by themselves they react enantioselectively with prochiral and racemic substrates. Stereoselectivity is the most exploited advantage of the enzymes, because of the growing interest in enantiopure compounds.[116,118] Enzymes in synthetic chemistry can be used without the tedious blocking and deblocking procedures, which are common in stereoselective synthesis, because of their positional (regio-) and stereoselectivity.[119] Additionally they are active under mild conditions and are environmentally acceptable. However, the sensitivity of enzymes to high temperatures, inhibition at high substrate and product concentrations as well as inactivation by some organic solvents should be considered.

Quite recently it was established that enzymes are able to function in organic solvents.[120] Most of the substrates have better solubility in organic solvents than in water. Enzymes in organic solvents often show higher stability and enhanced selectivities, which can be affected by changing the

solvent system.[119-122] Reactions like esterification and transesterification, which are impossible in water, can be performed in organic solvents. Very often the transition from an aqueous medium to neat (pure) organic solvents significantly decreases the activity of enzymes. The selectivity and reactivity of enzymes can be enhanced by giving them a 'pH memory' by lyophilisation (freeze-drying) from aqueous solution with a certain pH. Lyophilisation from non-buffer salt solutions or adding additives to the reaction medium is also used for reaction rate enhancements.[119,120,123]

Enzymes are mainly used in crude form. Purified enzymes are less spread because of their high price. A tool for the improvement of the lifetime of enzymes is immobilisation. Enzymes can be attached to a solid support by means of adsorption, ionic binding or covalent attachment. CLEC's or CLEA's are cross linked enzyme crystals or aggregates formed by covalent attachment of enzyme molecules to each other. Enzymes can be entrapped into gels, in membrane reactors, vesicles and reversed micelles. The immobilised catalysts can be recovered after performing the reaction in an aqueous or organic medium and can be reused without significant loss of activity during several cycles. Many immobilised catalysts are characterised by better operational and thermal stability and improved enantioselectivity, but immobilisation often leads to reduced enzyme activity due to diffusional limitation.

The difficulties which can not be fixed by tuning of reaction conditions can be solved by protein engineering *via* directed evolution and gene-shuffling techniques which may give catalysts with higher activities, better selectivities and enhanced stabilities.[119]

The main sources of enzymes are fungi and bacteria. Enzymes from mammals have also found some applications in biocatalysis. Enzymes from plants are less common.[116,118] Many lipases and proteases are used as additives to detergents, in beer brewing, dairy and food industry. Also the chemical and pharmaceutical industry is now implementing these enzymes in their synthetic routes. The synthetically most used class of enzymes is lipases, as they are extremely stable and rather active, also in nonaqueous systems. The natural function of lipases is the hydrolysis of water-insoluble esters such as triglycerides. Lipases bind to the water-organic interface and catalyse the hydrolysis there.[118] Lipases differ in their amino acid sequences and the size, which varies from 35 to 65 kDa. The 3-D structures of a number of lipases from four family types such as mammalian pancreatic lipases, the *Candida rugosa* family, the *Rhizomucor* family and the *Pseudomonas* family have been established recently and they were found to fold in similar ways. This fold is called as the α/β-hydrolase fold. The fold consists of a core of eight parallel β-sheets, surrounded by α-helices on both sides (Figure 1). The linkage of the sheets and helices is similar in all α/β-hydrolases. The catalytic domain consists of a triad of Ser, His, and Asp(Glu) and several oxyanion stabilising residues. The catalytically active serine residue is situated in a loop between an α-helix and a β-

sheet. The specific arrangement of the catalytic triad entails a lowering of the pK_a of the serine residue facilitating its nucleophilic attack on the carbonyl group of the substrate.[118]

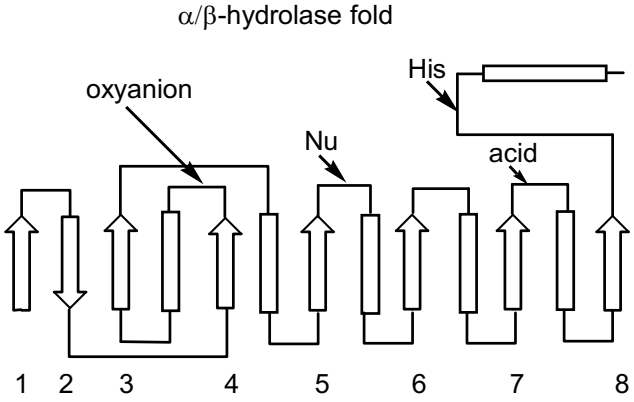
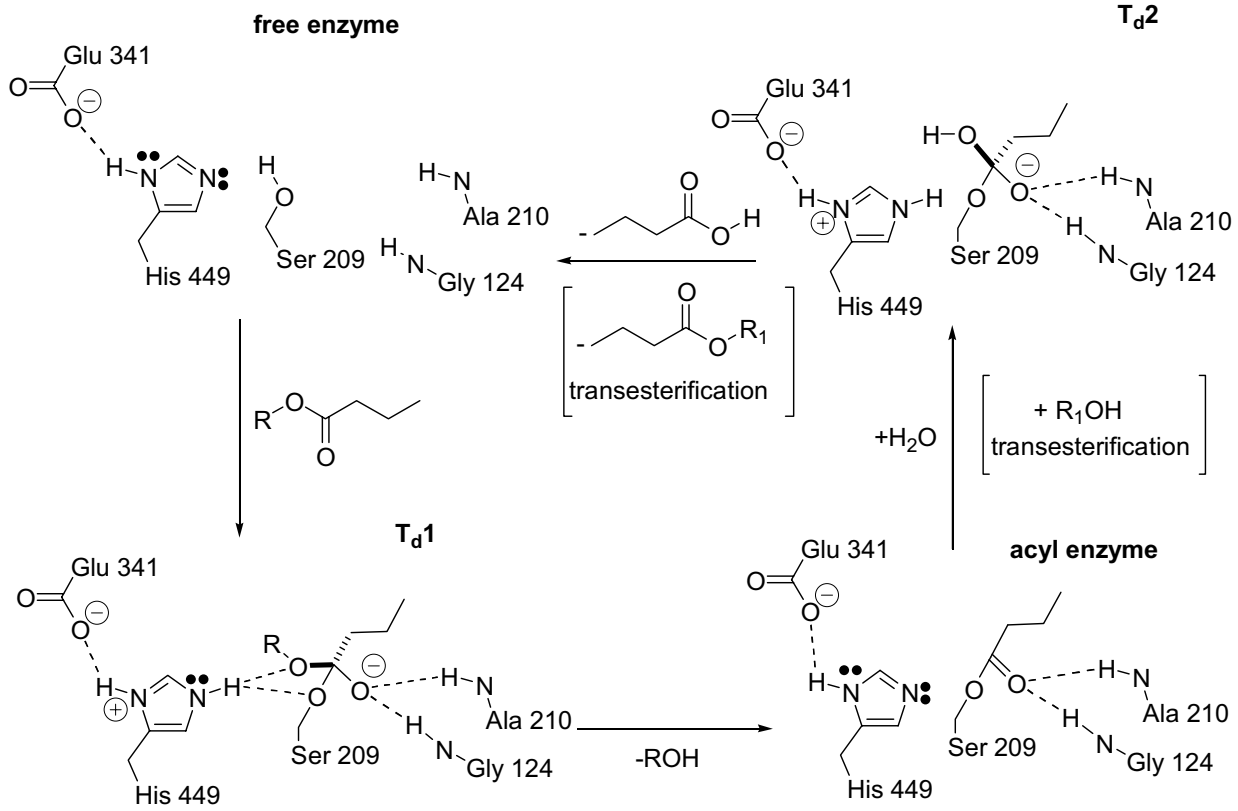


Figure 1. Schematic structure of the α/β-hydrolase fold, where rectangles are α-helices, arrows are β-sheets, oxyanion are residues which stabilise the oxyanion, Nu is nucleophilic residue (Nu is Ser for lipases, esterases and proteases), acid is Asp or Glu.[118,124]



The amino acid numbering corresponds to the active site of *Candida rugosa* lipase.[118]

Scheme 21

The catalytic mechanism for the lipase- or esterase-catalysed hydrolysis is schematically depicted in Scheme 21. At the beginning, the substrate (*e.g.*, a butyric acid ester: C_3H_7COOR) binds to the

lipase and the catalytic serine attacks the carbonyl forming a tetrahedral intermediate (**T_d1**). Collapse of this tetrahedral intermediate liberates the alcohol (ROH) and leaves an acyl enzyme intermediate. In a hydrolysis reaction, water attacks this acyl enzyme and a second tetrahedral intermediate (**T_d2**) is formed. Collapse of this intermediate liberates the acid (C_3H_7COOH). Another nucleophile such as an alcohol (R_1OH) can attack the acyl enzyme yielding a new ester ($C_3H_7COOR_1$) *via* a transesterification reaction. After hydrolysis or transesterification reaction the free lipase is recovered.

As mentioned earlier, in the last decade the X-ray structures of some synthetically useful lipases have been established. The structure analyses show that Ser-His-Asp catalytic triad of lipases are covered by short helical segment, called as lid or flap.[118,125] Upon the binding to a hydrophobic interface such as a lipid droplet, the lid opens and the catalytic activity of the lipase increases. Moreover, the opening of the lid places one of the oxyanion-stabilising residues into the catalytic orientation. Cutinase and acetylcholine esterase, which show no interfacial activation, lack a lid and contain a pre-formed oxyanion hole. Lipases with small lids such as *Candida antarctica* lipase B do not show interfacial activation. The lipase from *Staphylococcus hyicus* shows interfacial activation with some substrates, but not with others.[118]

Lipases and esterases have distinct substrate binding sites for the alcohol and acid part of esters, which are responsible for the stereoselectivity toward substrates. The alcohol binding site is similar in all investigated lipases. It is a crevice containing two regions – a large hydrophobic pocket, which is open to the solvent, and a small pocket that faces the floor of the crevice. The shape of this pocket sets the stereoselectivity of the lipase toward the secondary alcohol. The lipase's binding site for the acid part of the ester is unique for every lipase and can have the shape of a tunnel, a funnel or a crevice located near the protein surface.[118]

Proteases usually cleave peptide or amide bonds. The synthetically most important class of proteases is the serine proteases, which contains among others subtilisin and related enzymes, chymotrypsin, and penicillin amidase. There are also cysteine proteases, like papain, and metalloproteases like acylase, thermolysin and aminopeptidase. Aspartic proteases are uncommon in organic synthesis. Proteases have two major applications in synthetic chemistry. The first one is the enantioselective hydrolysis of natural and unnatural α-amino acid esters and other carboxylic acid esters and the second is the synthesis of di- and oligopeptides by coupling of N-protected amino acid esters and peptide esters. They are also used for the enantioselective hydrolysis of esters of secondary alcohols and for regioselective reactions of sugars or sugar derivatives. The binding site of a protease usually has the shape of a channel and is located close to the surface. The different regions of the binding site of proteases can be numbered according to the place where the enzyme reacts with the substrate (Figure 2).

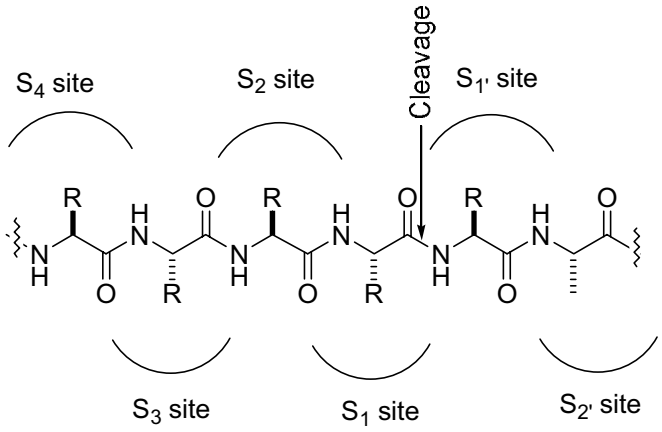


Figure 2. Naming of the binding site of proteases.[126]

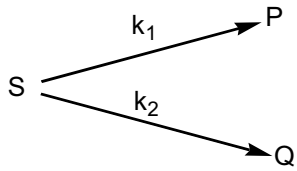
The acyl part of the amide link undergoing the cleavage by the protease lies in the S_1, S_2, S_3, etc. binding sites, whereas the amino part of the amide link undergoing the cleavage by the protease lies in the S_1', S_2', etc. binding sites. The substrate residues are called P_1, P_2, P_3, etc., according to their location with respect to the place of cleavage.[118]

Structurally serine proteases are different from lipases which is clearly shown for subtilisin and chymotrypsin. They have a similar 3-D arrangement of catalytic residues, which form similar active sites, but their protein folds are not related. Chymotrypsin has a β/β fold, formed by two antiparallel β-barrel domains, while subtilisin, like lipases and esterases, has an α/β fold, formed by a core of parallel β-sheets surrounded by four α-helices. However this fold is not the same as the α/β-fold of lipases.

For more details concerning the structure of lipases, proteases and esterases the reader is referred to the excellent book of Bornscheuer and Kazlauskas[118] and other sources.[116,117,119]

1.6.1 Kinetic aspects of enzyme-catalysed asymmetrisations of prochiral substrates and kinetic resolutions of racemic substrates

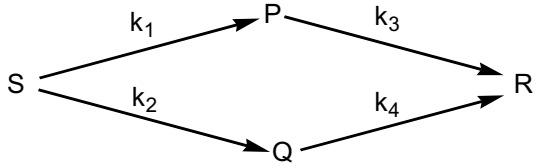
In catalytic single-step asymmetrisation reactions a prochiral substrate is transformed into two enantiomeric products P and Q at different rates determined by the apparent first-order rate constants k_1 and k_2, respectively (Figure 3).[116,127] The selectivity of the reaction α (selectivity factor) is controlled by the ratio of k_1/k_2, which is constant during the reaction. The enantiomeric excess of the reaction product is not dependent on the degree of conversion.



$$\alpha = \frac{k_1}{k_2} \qquad e.e. = \frac{\alpha-1}{\alpha+1} = \frac{P-Q}{P+Q}$$

Figure 3. Single-step kinetics

Asymmetrisation of bifunctional prochiral substrates often proceeds through two consecutive steps (Figure 4).[116,127] The reaction of substrate (S) with the enzyme does not terminate at the stage of the chiral intermediate giving products P and Q, but is followed by kinetic resolution of the intermediate (P+Q) yielding an achiral product (R). The enantiomeric excess of the product depends on all four rate konstants k_1-k_4 and becomes a function of the conversion of the reaction. The e.e. of chiral intermediate (P+Q) increases during the reaction, when the stereopreferences of two consecutive steps are opposite to each other (*i.e.* if $k_1 > k_2$, and $k_4 > k_3$). Usually, the enzymes have a continuous preference for reactive groups with the same stereochemistry, which means that selectivity of the second hydrolytic step is indeed opposite compared to that of the first step ($k_1 > k_2$ and $k_4 > k_3$).[116,127] The best e.e. of the chiral intermediate (P+Q) can be reached at a higher degree of conversion, albeit at the expense of the chemical yield. The computer program 'SeKiRe' for the prediction of the e.e. of the chiral intermediate (P+Q) was developed by the group of Faber.[127]

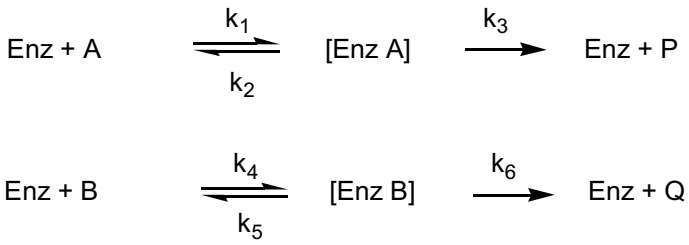


$$\alpha = \frac{k_1}{k_2} \qquad e.e. = \frac{P-Q}{P+Q}$$

$$E_1 = \frac{k_3}{k_1+k_2} \qquad E_2 = \frac{k_4}{k_1+k_2}$$

Figure 4. Double-step kinetics of a prochiral substrate

In kinetic resolutions, the enantiomeric purity of the reaction product and the remaining substrate is not constant and varies during the reaction. Irreversible reactions can be described by the Michaelis-Menten model of competitive inhibition.



Enz - Enzyme; A and B - enantiomeric substrates; P and Q - enantiomeric products; k_1 through k_6 - rate constants

Figure 5. Enzymatic kinetic resolution of a racemic mixture (irreversible reaction)

Instead of determining all individual rate constants for each of the enantiomers, a more convenient way for the comparison of enantioselectivity of kinetic resolutions was introduced by the group of Sih.[128] The enantiomeric ratio (*E* value) remains constant throughout the reaction and characterises the ability of enzyme to distinguish between two enantiomers.

$$E_p = \frac{\ln[1-c(1+e.e._p)]}{\ln[1-c(1-e.e._p)]} \qquad E_s = \frac{\ln[1-c(1+e.e._s)]}{\ln[1-c(1-e.e._s)]} \qquad E_{tot} = \frac{\ln \frac{[e.e._p(1-e.e._s)]}{(e.e._p+e.e._s)}}{\ln \frac{[e.e._p(1+e.e._s)]}{(e.e._p+e.e._s)}}$$

Figure 6.

The *E* value is mathematically linked to the conversion (c) and the enantiomeric excess of the product ($e.e._p$) or the substrate ($e.e._s$) and is expressed in equations for E_p or E_s (Figure 6). When the degree of conversion (c) cannot be accurately determined the equation for E_{tot} should be used.[129]

A nonselective reaction has *E* value of 1. *E* values below 15 are considered as unacceptable for practical purposes. They can be regarded as moderate to good from 15 to 30 and above 30 they are regarded as excellent. The values of $E \geq 100$ cannot be accurately measured due to the fact that even very small variations of $e.e._s$ and $e.e._p$ cause a significant change in the enantiomeric ratio. Disregarding the fact that these equations include assumptions such as an irreversible reaction, (pseudo) first order kinetics, and the absence of product inhibition, they are reliable in many cases, and are therefore especially suited for screening studies. Several computer programs for the calculation of enantiomeric ratio's and other parameters of enzyme-catalysed reactions have been developed.[116,127,130-132]

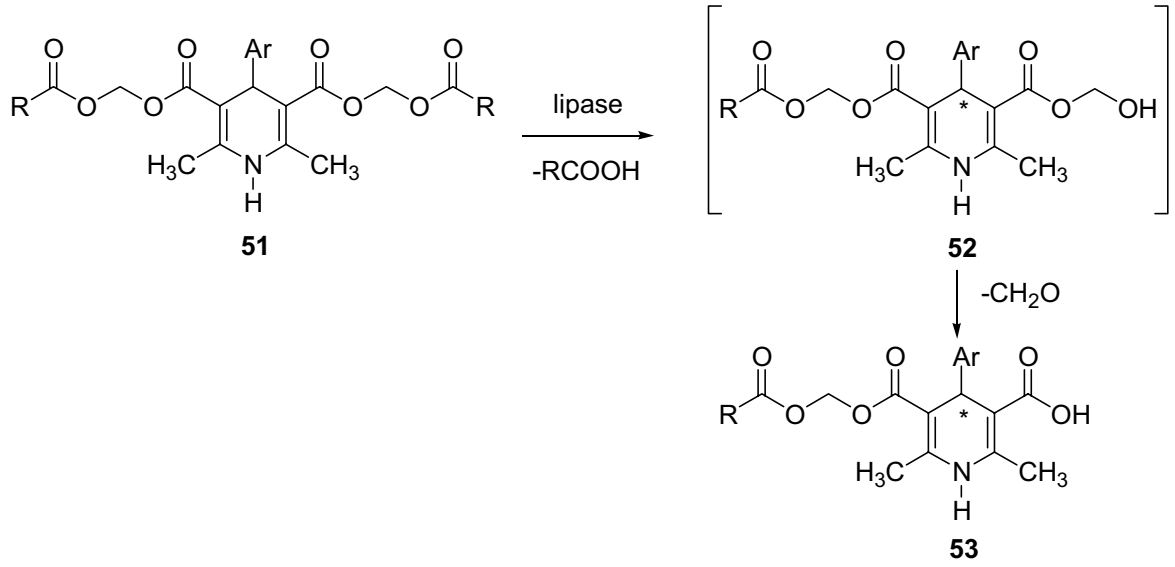
Additional information about enzyme-catalysed asymmetrisations of prochiral substrates and kinetic resolutions of racemic substrates as well as kinetic aspects of more complicated cases of kinetic resolutions can be found in the book of Faber[116] and other sources.[127,128,130,132,133]

1.7 Chemoenzymatic synthesis of enantiopure 1,4-dihydropyridines

Amongst the many chemical methods for preparing enantiopure compounds, the biotechnological approach based on enzyme-catalysed enantiomeric differentiation has become a promising way for the synthesis of enantiopure 1,4-dihydropyridines. Since a number of 1,4-dihydropyridines have esters moieties at the 3- and 5-positions, hydrolases seem to be the most suitable class of enzymes

for the preparation of pure enantiomers. Hydrolases can be used to asymmetrise or resolve both prochiral and racemic substrates.

Commercially available hydrolases are not capable of hydrolysing alkyl and phenyl esters at the 3- and 5-positions of 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates because of steric and electronic reasons.[134-136] To circumvent this obstacle, a spacer containing a hydrolysable group was attached to the hindered carboxylic acid. In this way, the hindered compound is turned into a substrate for hydrolytic enzymes. This spacer should have such structural characteristics that after hydrolysis of the ester group the spacer is split off in a spontaneous reaction. To achieve this, acyloxymethyl esters **51** were introduced by the groups of Sih[134] and Achiwa[135] and proved to be efficient for lipase-catalysed asymmetric hydrolysis of chiral 1,4-DHPs. After enzymatic hydrolysis of the terminal ester bond, the hemiacetal intermediate **52** spontaneously loses formaldehyde to give the desired monoacid **53** (Scheme 22). The reaction centre (*i.e.*, the place where the enzyme attacks) is six bonds away from the chiral centre, which is a drawback of the method, however it proves to be still possible to achieve stereoselective enzymatic hydrolysis.



Scheme 22

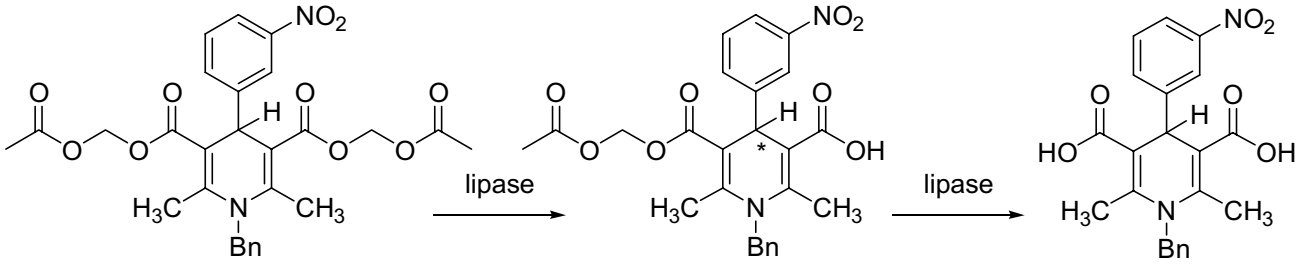
In the following sections, an overview will be given of the lipase- and protease-mediated preparation of enantiopure 1,4-DHPs.

1.8 Asymmetrisation of prochiral 1,4-dihydropyridines

1.8.1 Lipase-catalysed asymmetrisation of bis(acyloxymethyl) 4-aryl-1,4-dihydropyridine-3,5-dicarboxylates

The N-protected acyloxymethyl esters of 1,4-DHPs were subjected to lipase-catalysed hydrolysis first, as they were prepared via hydrolysis and esterification of alkyl esters of N-protected 2,6-dimethyl-1,4-DHPs. Only N-protection makes chemical hydrolysis of 3,5-diesters possible.[19,20]
Initially the hydrolysis of N-benzyl substituted bis(acetoxymethyl) esters of 4-(3-nitrophenyl)-1,4-DHP **54** was carried out in phosphate buffer at pH 7.0 with different lipases, however the reaction led to low e.e.'s and formation of side products. When the reaction medium was changed to *n*-butanol-water (10:1) the lipase-catalysed hydrolysis proceeded with moderate to good enantioselectivity (Table 1). Taking into the account that the hydrolysis of diesters **54** is in fact a two-step process leading at the end to achiral diacids **56**, prolongation of the reaction time offers a tool for controlling the e.e. of the produced monoacids **55**.[134,136] It was shown that the *Pseudomonas* lipases AK, P-30 and K-10 all have the same pro-*R* stereopreference whereas *Candida rugosa* lipase preferred pro-*S* chirality towards substrates **54**.

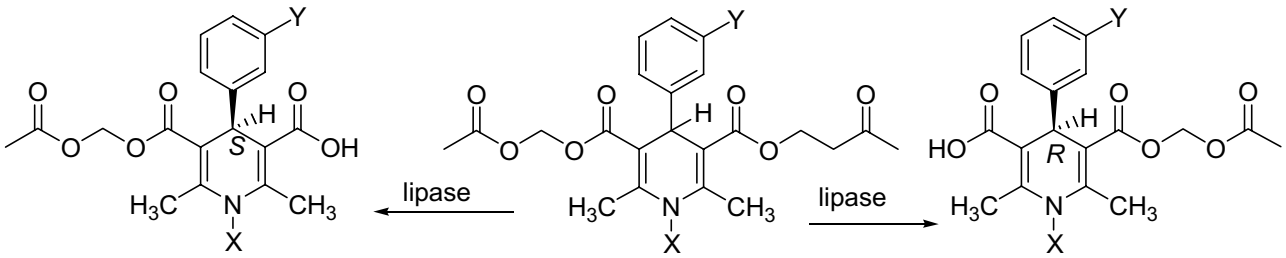
Table 1. Reaction of N-benzyl-1,4-DHP **54** with lipases in *n*-BuOH/H_2O



54 **55** **56**

Lipase	Time, h	Isolated yields, %			Abs. Conf.	e.e., %
		54	**55**	**56**		
P-30 (*Pseudomonas cepacia*)	49	5	21	34	*S*	88
K-10 (*Pseudomonas sp.*)	49	32	14	20	*S*	63±5
OF-360 (*Candida rugosa)*	49	46	1	24	*R*	73±3
AK (*Pseudomonas sp.*)	7.5	0	27	29	*S*	77
AK	16.5	0	37	16	*S*	89
AK	22	0	31	8	*S*	97

Using *t*-butyl methyl ether as the solvent and methanol as the nucleophile for *Pseudomonas* lipases and water for *Candida rugosa* lipase (CRL), the transformations of **57a-d** were performed (Scheme 23).[137] It was found that CRL always shows pro-*S* stereopreference towards all substrates **57a-d** (giving *S*-**58**), whereas loss of enantioselectivity was found for N-H derivative **57d** (giving *S*-**58d** with 8% e.e.). The stereochemical preferences of the lipases P-30 and K-10 were found to be always pro-*R* (giving *R*-**58**). These enzymes were enantioselective in the case of N-H (95-99% e.e.) and N-methoxymethyl (N-MOM) (85-95% e.e.) dihydropyridines. In contrast, the prochiral preference of lipase AK varied depending on the substituents on the dihydropyridine ring. The N-methoxymethyl derivatives afforded the 4*S* isomers (95% e.e.) whereas N-H compounds yielded the 4*R* isomers (50-70% e.e.).



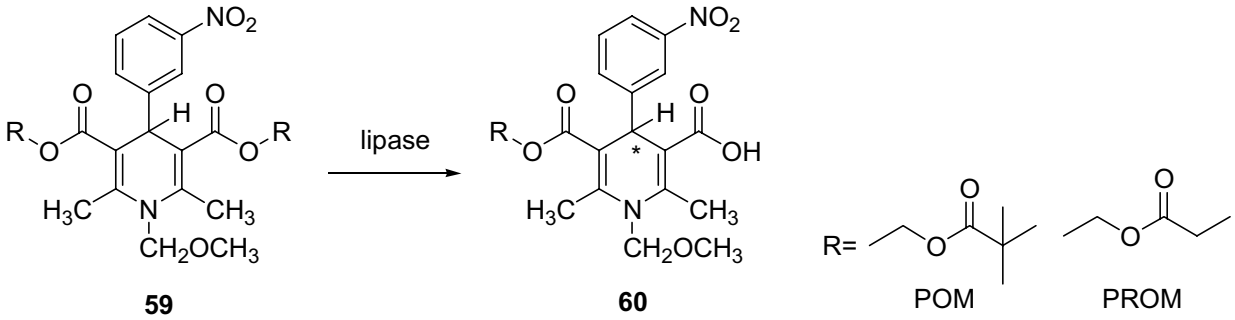
S-**58** **57** *R*-**58**

a) X=CH_2OCH_3 (MOM), Y=H; b) X=Y=H; c) X=MOM, Y=NO_2; d) X=H, Y=NO_2

Scheme 23

Achiwa *et al.* hydrolysed bis(pivaloyloxymethyl) (POM) and bis(propionyloxymethyl) (PROM) esters of N-MOM-protected 1,4-DHP **59** (Table 2). First, the reaction was carried out in phosphate buffer (pH 8) containing 10% of acetone (entry 1). Although the e.e. was high, slow reaction rates, solubility problems and a difficult extraction procedure made this process unpractical. A change of the solvent to water-saturated IPE facilitated the reaction work-up (entries 2-5).[92,135,138] Using 2 different lipases it was possible to produce both enantiomers of 4-(3-nitrophenyl) substituted dihydropyridines **60**.

Table 2. Lipase-catalysed enantioselective hydrolysis of N-MOM 1,4-DHPs **59**



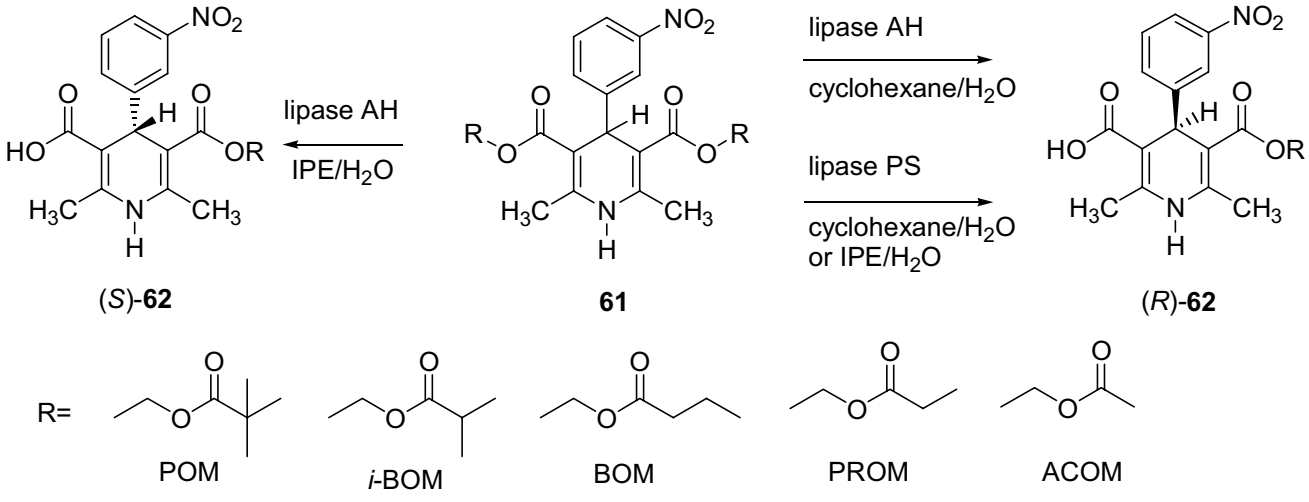
59 **60**

Entry	R	Lipase	Solvent	Time, h	T, °C	Products		
						Yield, %	e.e., %	Abs. Conf.
1	POM	AH	Buffer (pH 8)	24	rt	80	>99	*S*
2	POM	AH	IPE/H_2O	4	rt	95	>99	*S*
3	PROM	AH	IPE/H_2O	8	0-5	71	>99	*S*
4	POM	PS	IPE/H_2O	72	rt	44	69	*R*
5	PROM	PS	IPE/H_2O	10	rt	78	88	*R*

AH –lipase AH (from *Pseudomonas sp*.); PS – lipase PS (from *Pseudomonas cepacia*)

The N-unprotected prochiral POM ester **61** (entries 1,6, Table 3) was hydrolysed slower by lipase AH and PS[92,122,138] than the corresponding N-MOM derivative **59** (entries 2,4, Table 2). The authors give no explanations for this fact; most likely the reason lies in the different three-dimensional structures and the orientations of the substituents at the positions 3 and 5 of the N-protected and N-H derivatives.

Table 3. Solvent influence and the effect of steric hindrance of the acyloxymethyl group on the enantioselectivity of the lipase AH and PS-catalysed enantioselective hydrolysis of compound **61**



(*S*)-**62** **61** (*R*)-**62**

Entry	Substrate	Lipase	IPE/H_2O				Cyclohexane/H_2O			
			Time, h	Yield, %	e.e., %	Abs. Conf.	Time, h	Yield, %	e.e., %	Abs. Conf.
1	POM	AH	48	87	99	*S*	48	88	88.8	*R*
2	*i*-BOM	AH	24	83	89	*S*	48	71	91	*R*
3	BOM	AH	24	71	89	*S*	48	62	91	*R*
4	PROM	AH	1	83	68	*S*	17	57	91	*R*
5	ACOM	AH	5	77	42	*S*	17	32	88	*R*
6	POM	PS	200	8	73	*R*	200	23	97	*R*
7	*i*-BOM	PS	20	34	86	*R*	48	32	99	*R*
8	BOM	PS	20	91	96	*R*	48	29	99	*R*
9	PROM	PS	24	86	99	*R*	72	31	92	*R*
10	ACOM	PS	24	87	99	*R*	72	28	99	*R*

According to Table 3 it is evident that in water saturated IPE the bulkiness of the acyloxymethyl moiety affected the enantioselectivity of lipases. The increase of the steric hindrance of the acyloxymethyl group went together with the increase of the enantioselectivity of lipase AH, whereas the reversed effect was found for lipase PS in IPE. This effect was not so clear when the hydrolysis was carried out in water saturated cyclohexane. A striking solvent effect has been described for lipase AH: it converts prochiral bis(acyloxymethyl) esters **61** into (*S*)-monoacids **62** in IPE, diethylether, 2,5-dimethyl-THF and THF, whereas it gives (*R*)-monoacids **62** in cyclohexane and cycloheptane. This phenomenon can not be explained on the basis of the hydrophobicity of the solvent and might be due to the interaction between this enzyme and the solvent.[92,122] In contrast, lipase PS converts the substrates **61** into (*R*)-monoacids **62** in both IPE and cyclohexane.

The influence of the water concentration in IPE and cyclohexane was studied. The best selectivity for lipase AH was obtained when the water concentration in IPE was maximal (saturated, around 4000 mg/L), whereas the best results in cyclohexane were obtained at a water concentration of around 35 mg/L.[92]

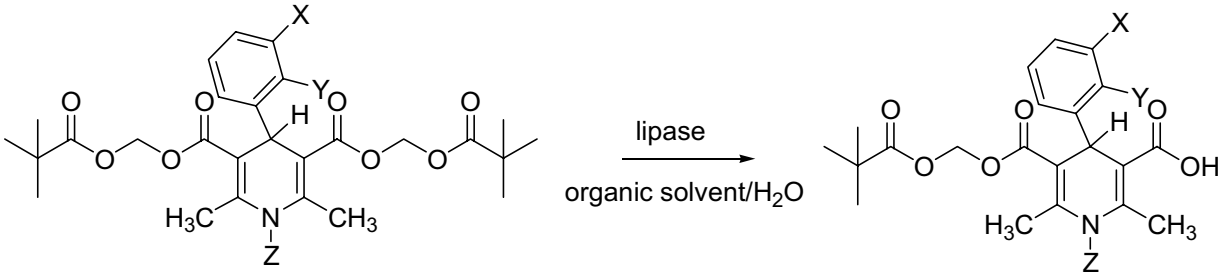
Table 4. Effect of the temperature on enzyme-catalysed enantioselective hydrolysis of 1,4-DHPs **61**

Substrate	Lipase	T, °C	Time, h	Yield, %	e.e., %	Abs. Conf.
POM	AH	20	48	87	99	*S*
POM	AH	40	17	73	92	*S*
POM	AH	68.5	6	67	75	*S*
PROM	PS	20	24	86	99	*R*
PROM	PS	40	24	84	99	*R*
PROM	PS	68.5	8	80	99	*R*

The effect of temperature on the enzyme-catalysed enantioselective hydrolysis was also studied (Table 4). The reaction time of lipase AH is shorter but the chemical yield and enantiomeric excess are lower at higher reaction temperature. For the case of lipase PS, the reaction time seems to be shorter and the chemical yield and enantiomeric excess are similar despite the rise of the reaction temperature. From these points of view, it supposed that lipase AH has a quite flexible structure while lipase PS is more rigid.

Dihydropyridines with structural variations in the 4-aryl substituent were also hydrolysed by lipase AH and PS. Lipase AH-catalysed hydrolysis of N-protected and non-protected substrates **63** in IPE occurred giving good or excellent enantiomeric excesses of monoacids **64** (entries 1-3, 5,6, Table 5).[92,135] Lipase AH also shows opposite stereopreference in different solvents towards 4-(2,3-dichlorophenyl) derivative **63** (entries 6,7, Table 5), which is suppressed by N-protection of the substrate (entries 3,5, Table 5).[139]

Table 5. Lipase-catalysed hydrolysis of 4-aryl-1,4-DHPs **63**



63 **64**

Entry	Z	X	Y	Lipase	Solvent	Time, h	1,4-DHP-3-carboxylic acids **64**		
							Yield, %	e.e., %	Abs. Conf.
1	MOM	H	H	AH	IPE	5	76	>99	?
2	MOM	H	CF_3	AH	IPE	7	83	>99	*S*
3	MOM	Cl	Cl	AH	IPE	6	81	96	*S*
4	MOM	Cl	Cl	PS	IPE	300	19	81	*S*
5	MOM	Cl	Cl	AH	CH	80	62	93	*S*
6	H	Cl	Cl	AH	IPE	360	78	>99	*S*
7	H	Cl	Cl	AH	CH	312	88	47[1]	*R*

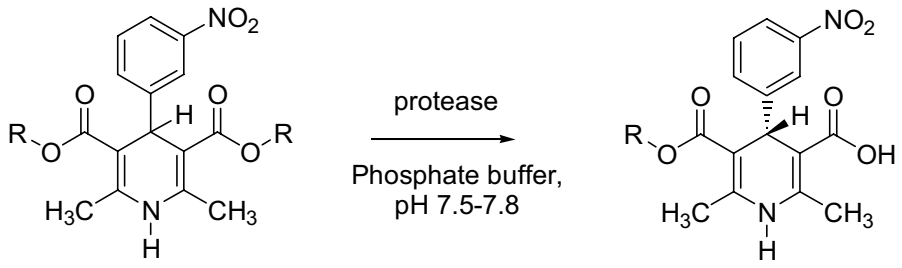
CH- cyclohexane

[1]In another publication,[140] 64% e.e. in 312 h reaction time with lipase AH in cyclohexane was reported.

1.8.2 Enantioselective hydrolysis and transesterification reactions of prochiral 4-aryl-1,4-dihydropyridine derivatives by proteases

Activated esters of 1,4-DHPs that are easily hydrolysed by base like cyanoethyl esters could be susceptible to enzymatic hydrolysis. Several 1,4-DHP-3,5-dicarboxylates were screened for this transformation with different hydrolytic enzymes.[92,141] It was reported that nine kinds of these substrates were found suitable for enzymatic hydrolysis (Table 6). No lipases or esterases hydrolysed these compounds but enzymatic hydrolysis was observed using some proteases, like seaprose S (*Aspergillus melleus*), protease P6 (*Aspergillus melleus*),[142] protease A (*Aspergillus oryzae*), proleather (*Bacillus subtilis*), deamizyme (*Aspergillus sp.*), acylase 30,000 (*Aspergillus sp.*), subtilisin Carlsberg (*Bacillus licheniformis*),[142] and other less active enzymes.[92,141] In contrast to lipase-catalysed hydrolysis, where lipases were active in both aqueous and organic medium, the proteases only show activity in aqueous solutions in a certain range of pH values. A large amount of enzyme is needed to achieve a good yield of hydrolysis products. The best results were obtained with seaprose S and protease P6.[141,142] High enantioselectivity of proteases was found towards all substrates, but the reaction rates and chemical yields differ quite a lot (Table 6).

Table 6. Protease-catalysed asymmetric hydrolysis of 1,4-DHPs **65**



65 (-)-**66**

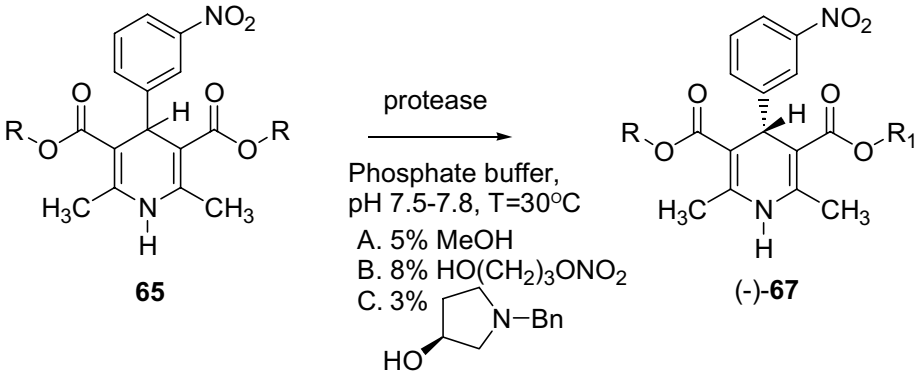
R	Enzyme	T, °C	Time, h	Yield, %	e.e., %	Abs. Conf.
CH_2CH_2CN	seaprose S	rt	72	87	>99	*R*
$CH_2CH_2SO_2CH_3$	seaprose S	rt	72	50	>99	*R*
CH_2CONH_2	seaprose S	rt	20	83	>99	*R*
$CH_2COOC_2H_5$	seaprose S	rt	72	60	>99	*R*
CH_2OCH_3	seaprose S	rt	72	54	98	*R*
CH_2CH_2NHCO-3-Py	protease P6	30	27	79	>99	*R*
$CH_2CH_2N(CH_3)CH_2C_6H_5$	seaprose S			screening results[1]		
$CH_2CH_2N=(CH_2)_5$	seaprose S			screening results[1]		
$CH_2CH_2N=(CH_2)_4=O$	seaprose S			screening results[1]		

[1] The substrate reacted with seaprose S; no quantitative data were given.[92]

The attempts to hydrolyse bis(cyanoethyl) or bis(methylsulfoneethyl) esters of 4-methyl-1,4-dihydropyridines with seaprose S were unsuccessful. These results suggested that the aryl group at the 4 position was necessary for seaprose S catalysed hydrolysis. Seaprose S catalysed the hydrolysis or transesterification of ethoxycarbonylmethyl esters of 4-(3-nitrophenyl)-1,4-DHP **65** with the splitting of 'outer' and 'inner' ester groups to the corresponding carboxylic acid **66**. Protease P6 converts bis[(2-nicotinoylamino)ethyl] ester **65** to the corresponding monoacid **66** without touching the amide bond. It can be concluded that seaprose S and protease P6 attack the carboxyl group of 1,4-DHP that is directly attached to the ring, giving monoesters in high yield and e.e.

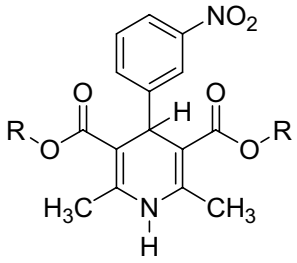
Transesterification is an interesting reaction since it converts a prochiral ester into an enantiopure ester in one step (Table 7). As in the case of hydrolysis, protease P6 and seaprose S were shown to have the best transesterification activity toward the 4-aryl-1,4-DHPs **65**.[92,142,143] Transesterification reactions instead of hydrolysis proceeded in phosphate buffer containing 3-8% of alcohol to give the corresponding enantiopure diesters **67** with e.e.'s above 99%, without a change of enantiopreference of the protease compared to the hydrolysis of these substrates. The amount of alcohol in the reaction medium should be adjusted in order to make transesterification predominant over hydrolysis.[142]

Table 7. Enantioselective transesterification of **65** with various alcohols by proteases

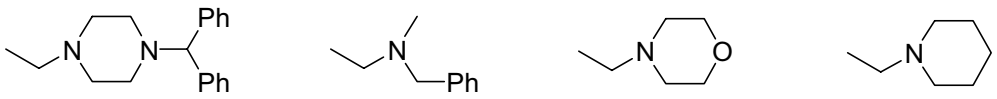


R	Enzyme	R_1OH	Time, h	Yield, %	e.e., %	Abs. Conf.
CH_2CH_2CN	seaprose S	A	120	35	99	*R*
$CH_2CH_2SO_2CH_3$	seaprose S	A	120	48	99	*R*
CH_2CONH_2	seaprose S	A	20	83	99	*R*
CH_2CONH_2	seaprose S	C		62	99	*S*
$CH_2COOC_2H_5$	seaprose S	A	48	52	99	*R*
CH_2OCH_3	seaprose S	A	120	26	99	*R*
CH_2CH_2NHCO-3-Py	protease P6	B	27	79	99	*R*

It was also reported by Achiwa *et. al.*[92] that seaprose S was able to catalyse the transesterification of 4-(3-nitrophenyl) derivatives **65** having the following substituents in the positions 3 and 5 of the 1,4-DHP ring:



65

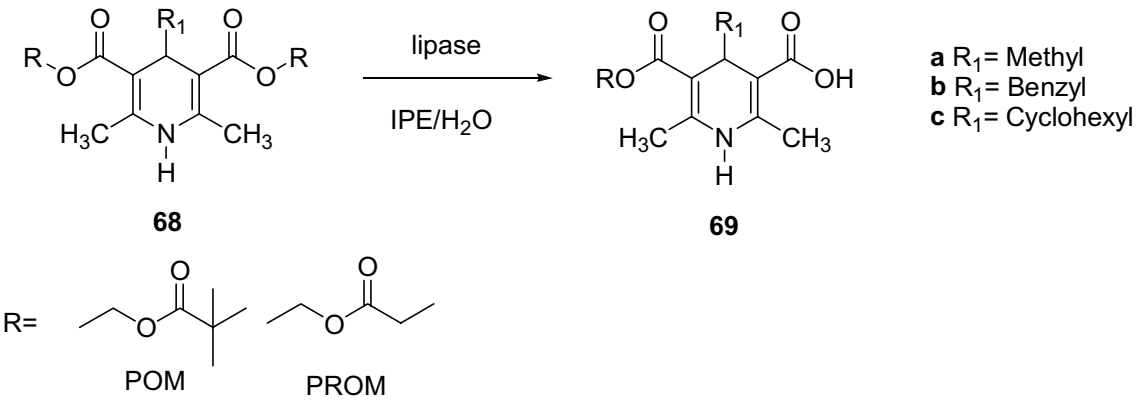
R= 

These unique features make this enzyme a highly interesting biocatalyst for the preparation of chiral 1,4-DHPs. Unfortunately, seaprose S is not commercially available in Europe.

1.8.3 Lipase-catalysed hydrolysis of 4-methyl-, benzyl- and cyclohexyl-1,4-dihydropyridines

The hydrolysis with lipase B from *Pseudomonas fragi* of 4-methyl substituted derivative **68a** proceeded with 91% e.e. to give the (+)-enantiomer of **69**, though the enantioselectivity of enzymes towards the 4-benzyl derivative **68b** was quite moderate (Table 8). In 4-cyclohexyl derivatives **68c**, the steric hindrance of the cyclohexyl group seemed to interfere with the hydrolysis of the acyloxymethyl group. These results showed that the substituent at the position 4 affects the reactivities and enantioselectivities of the enzymes.[144]

Table 8. Lipase-catalysed hydrolysis of 4-methyl-, 4-benzyl- and 4-cyclohexyl-1,4-DHPs **68**



R	R_1	Lipase (mg/mmol)	Time, h	Yield, %	e.e., %	$[\alpha]_D$
POM	Me	B (100)	8	76	91	+25.8
POM	Bn	B (200)	151	67	0	+2.6
PROM	Bn	B (50)	4	56	48	–27.2
PROM	Bn	P (100)	48	71	91	–42.9
POM	Cy	B (200)	96	62	56	+11.5
PROM	Cy	B (50)	89	16	20	–13.4
PROM	Cy	P (100)	118	29	26	–16.3

Bn – Benzyl; lipase B (from *Pseudomonas fragi*); lipase P (from *Pseudomonas fluorescens*)

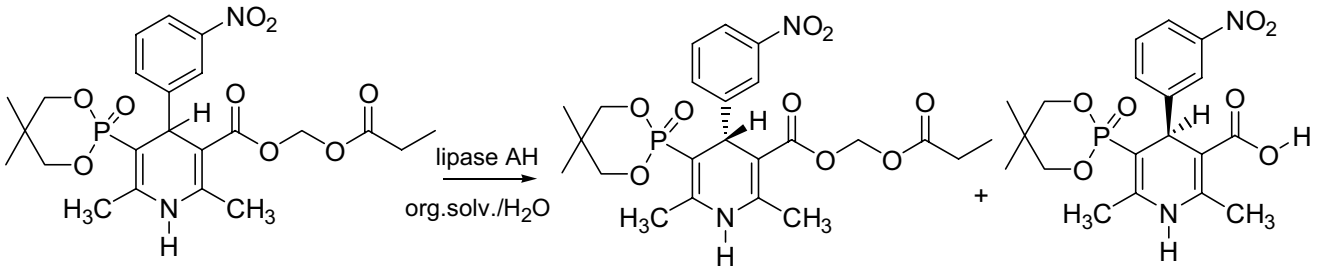
1.9 Kinetic resolution of racemic 1,4-dihydropyridines

Kinetic resolution of a racemate gives only 50% yield. However, this method has some advantages over asymmetisation: the remaining substrate can almost always be obtained in very high e.e. and the structure of the product is often closer to the final compound. An overview of kinetic resolutions of racemic 1,4-DHPs is given in the next sections.

1.9.1 Lipase-catalysed kinetic resolution of racemic 3-acyloxymethyl esters of 2,6-dimethyl-1,4-dihydropyridines

Unsymmetrical 3-propionyloxymethyl 5-(5,5-dimethyl-2-oxo-1,3-dioxaphosphorinan-2-yl)-1,4-DHP-3-carboxylate **70** was hydrolysed by lipase AH with moderate enantioselectivity to give the carboxylic acid (*S*)-**71** and remaining ester (*R*)-**70** (Table 9).[145] It is noteworthy that, in contrast to the prochiral analogues, the pivaloyloxymethyl ester of **70** is not reactive at all with lipase AH.

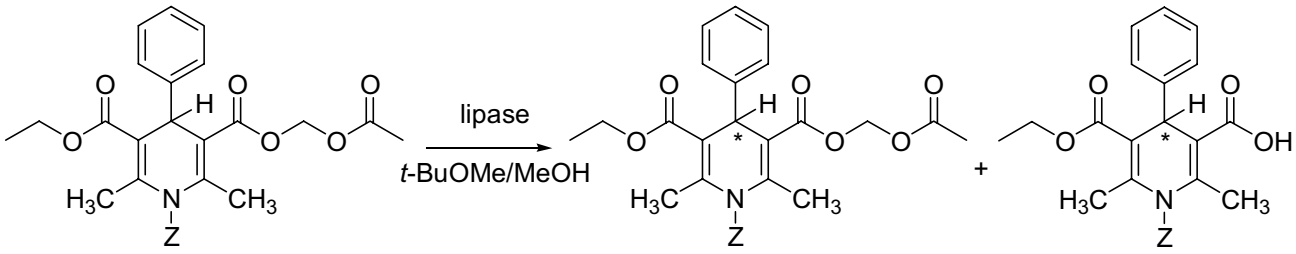
Table 9. Lipase AH-catalysed kinetic resolution of racemic 1,4-DHP **70**



R	Solvent	Time	Ester (*R*)-**70**		Carboxylic acid (*S*)-**71**	
			Yield, %	e.e., %	Yield, %	e.e., %
POM	IPE	7 days	>99	0	0	0
PROM	IPE	6 hours	50	60	40	70
PROM	2,5-di-CH_3-THF	6 hours	50	69	40	82

The methanolysis of the acyloxymethyl ester of racemic 1,4-DHP **72** with lipase AK (from *Pseudomonas fluorescens*) and lipase PS was also performed (Table 10).[146] The nitrogen substituent influenced the enantiopreference of lipase AK. Whereas the resolution of N-protected **72** was preferential towards the (*R*)-enantiomer, the opposite *S* preference was observed for the N-H derivative **72**. On the other hand, the enantiopreference of lipase PS was similar toward both substrates, showing the best resolution for the N-H compound. These results are in accordance with the enzymatic resolution of the prochiral compounds.[137] The influence of other solvents was also studied, however it is difficult to compare the results as the observations were made on isolated yields of the reaction product and the remaining substrate.

Table 10. Methanolysis of racemic 1,4-DHPs **72** with lipase AK and lipase PS

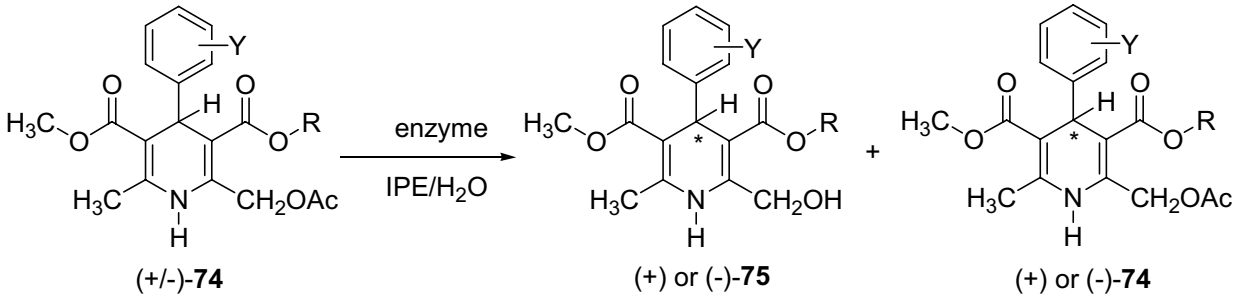


Z	Lipase	Time, h	Ester **72***		Carboxylic acid **73**	
			Yield, %	e.e., %	Yield, %	e.e., %
H	AK	26.5	50	15 (*R*)	30	20 (*R*)
H	PS	24	51	70 (*R*)	39	70 (*R*)
MOM	AK	7	42	95 (*S*)	51	63 (*S*)
MOM	PS	11	39	18 (*R*)	39	24 (*R*)

1.9.2 Enzymatic kinetic resolution of racemic 2-hydroxymethyl-1,4-dihydropyridines or derivatives thereof

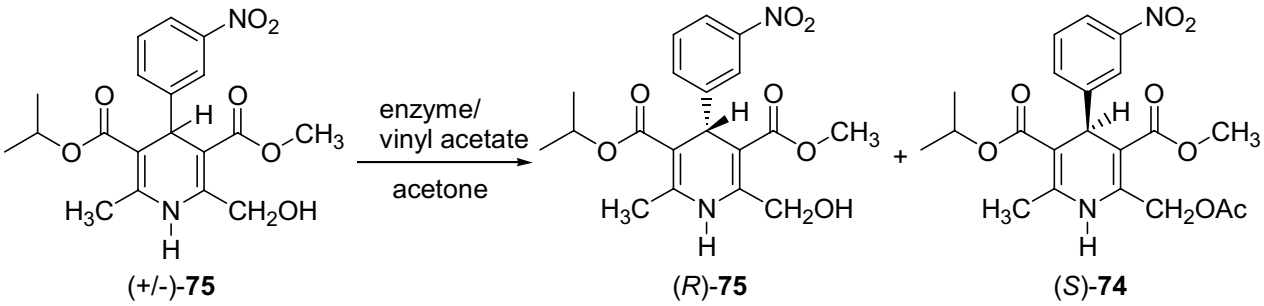
Lipase AH, lipase PS and cholesterol esterase (CHE) were found effective for the hydrolysis of 2-acetoxymethyl-1,4-DHPs **74** and for the transesterification of 2-hydroxymethyl-1,4-DHPs **75** with vinyl acetate.[105,147] The enantioselectivity of the enzymes was dependent on the aryl and ester substituents of the substrate (Table 11). Lipase AH was found very selective in the case of the 2-chlorophenyl derivative (entry 5, Table 11), whereas CHE showed excellent enantioselectivity toward the 2,3-dichlorophenyl derivative (entry 1, Table 11). Fine tuning of the reaction conditions of the lipase PS-mediated kinetic resolution of the 3-nitrophenyl derivative **75** with vinyl acetate led to a good e.e. of the product (Table 12). However, it is difficult to compare the results as the reactions were interrupted at a degree of conversion that was different from 50% and the data that could characterise the enantioselectivity (*e.g.* the *E* value[128]) were not estimated.

Table 11. Enzymatic resolution of racemic 2-acetoxymethyl-1,4-DHP derivatives **74**



Entry	Y	R	Enzyme	Time, h	**75**			**74**		
					Yield, %	e.e., %	$[\alpha]_D$	Yield, %	e.e., %	$[\alpha]_D$
1	2,3-diCl	*i*-Pr	CHE	3	42	92	+34.4	50	98	–37.6
2	2,3-diCl	*i*-Pr	lipase AH	11	11	11	–2.0	75	3	+1.2
3	2,3-diCl	*i*-Pr	lipase PS	11	9	63	–14.7	78	6	+2.2
4	2-Cl	Et	CHE	4	50	75	+11.2	50	75	–23.8
5	2-Cl	Et	lipase AH	4	50	91	+14.3	50	98	–29.1
6	2-Cl	Et	lipase PS	9	36	55	+8.1	54	41	–12.9
7	3-NO_2	*i*-Pr	lipase PS	96	36	81(*S*)		41	74 (*R*)	
8	3-NO_2	*i*-Pr	CHE	24	50	25 (*S*)		32	39 (*R*)	

Table 12. Enzyme-catalysed kinetic resolution of racemic 2-hydroxymethyl-4-(3-nitrophenyl)-1,4-DHP **75**

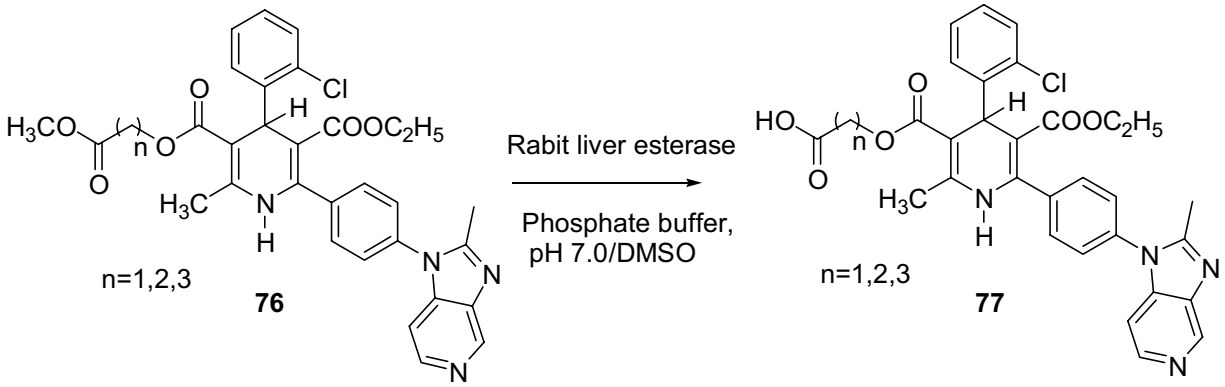


(+/-)-**75** (*R*)-**75** (*S*)-**74**

Entry	Enzyme	T, °C	Time, h	(R)-(+)-**75**		(S)-(—)-**74**	
				Yield, %	e.e., %	Yield, %	e.e., %
1	lipase AH	rt	33	49	68	46	90
2	CHE	rt	19	49	29	41	35
3	lipase PS	rt	46	46	78	40	89
4	lipase PS	40	44	42	97	55	72

1.9.3 Resolution of racemic methoxycarbonylalkyl esters with rabbit liver esterase

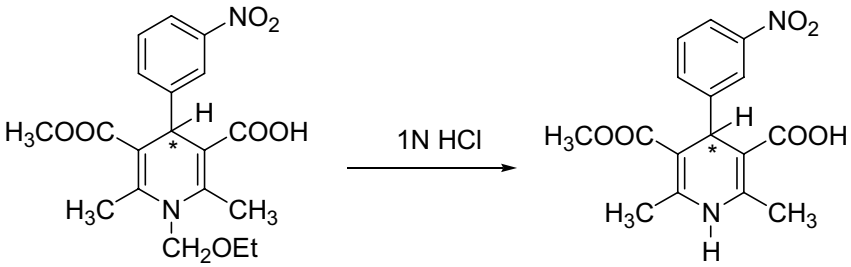
Rabbit liver esterase was able to hydrolyse the 1,4-DHPs **76** with different substituents in the positions 2, 6, 3 and 5, with low selectivity (Scheme 24). An *E* value of 3 was determined for the methoxycarbonylmethyl ester. The enzymatic hydrolysis of higher homologues (n=2,3) proceeded with almost no enantioselectivity. As the length of the alkyl spacer arm increases, there are fewer interactions between the enzyme and the chiral centre, and hence the factors governing selectivity are diminished.[148] However, it is revealed by other research groups that with the right choice of enzyme it is still possible to reach rather high enantioselectivity in hydrolysis reaction where the place of enzymatic attack is quite remote from the chiral centre.[134,135,138,141]



Scheme 24

1.10 Derivatisation of enantiomerically pure 1,4-dihydropyridines

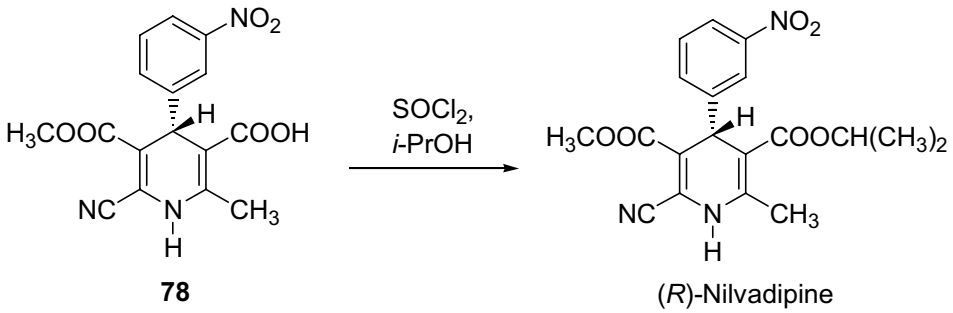
Enantioselective synthesis of chiral 1,4-DHPs in many cases does not lead directly to biologically active dihydropyridines and further derivatisation is required. If protection of the N-H group with the methoxymethyl (MOM) or ethoxymethyl group is used in order to perform stereoselective synthesis, the N-H group can be easily deprotected by hydrochloric acid (Scheme 25).[95] This is necessary since the free N-H moiety is essential for most of the biologically active 1,4-DHPs.



73 **23**

Scheme 25

As was described above for numerous unsymmetrical analogues of nimodipine, the ester moieties of 1,4-DHP-3,5-dicarboxylates play an important role in the biological actions of dihydropyridines. In many cases the product of enantioselective synthesis is a monocarboxylic acid, with or without an exchangeable function on the other side. The desired ester moieties in the positions 3 and 5 of 1,4-DHP ring can be introduced *via* esterification of the chiral monoacids (*e.g.* **78**) by known methods (Scheme 26).[26,95,105,145]



78 (*R*)-Nilvadipine

Scheme 26

Functionalisations of chiral 2-hydroxymethyl-1,4-DHPs as well as 2,6-dimethyl-1,4-DHP-3,5-dicarboxylates lead to other types of chiral 1,4-DHPs with different substituents in the 2-position, like amlodipine, and nilvadipine.[105]

1.11 Scope of this thesis

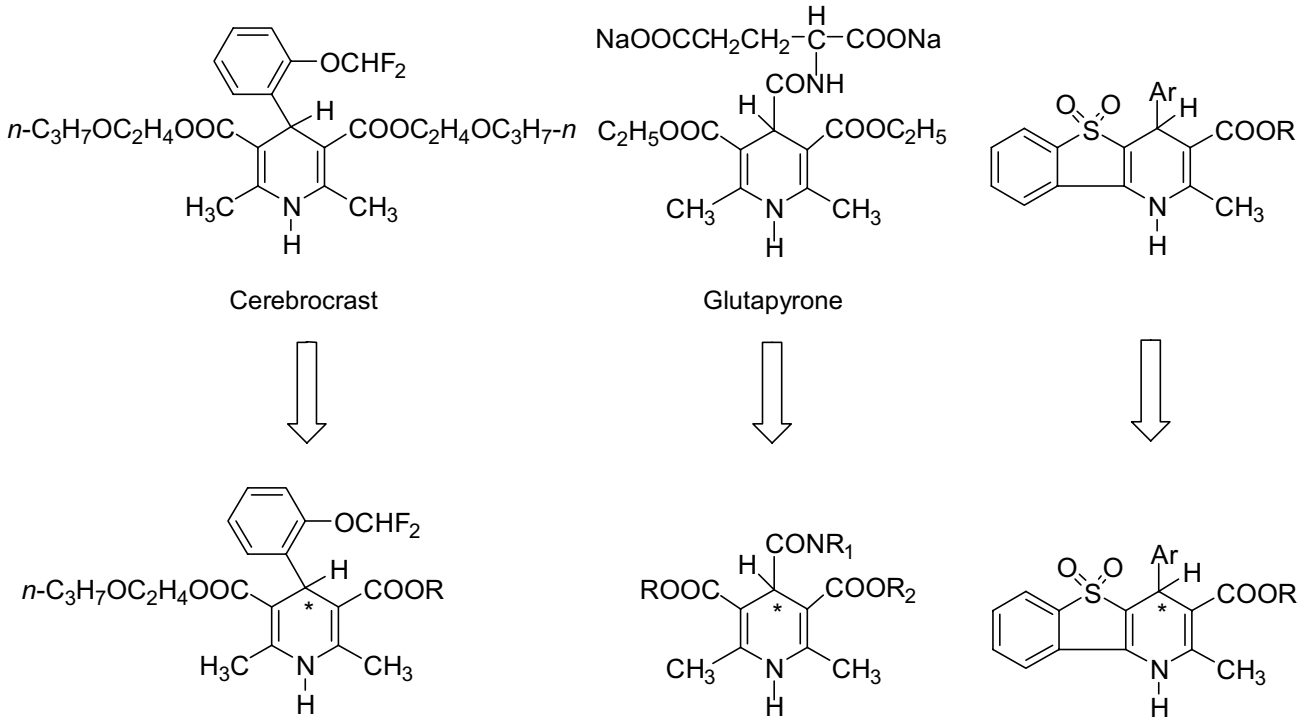
Previous experience in biological activity tests have proven that enantiomers of chiral drugs should be regarded as two individual, separate compounds.[80] Pharmacological evaluations of enantiomers confirm that they usually have different activity and even can have opposite action profiles. In addition, the kinetics and metabolism of enantiomers can be significantly different when administered alone or in combination. Therefore, the regulatory authorities have determined that administration of a racemate should be viewed in the same way as a combination therapy and it is necessary to show the differences and similarity of the pharmacology of each enantiomer as well as of their combination.[74,75,77-79,149]

Most of the known investigations described in literature in the field of chiral dihydropyridines have been devoted to the synthesis of calcium antagonists. The synthesis of a number of structural analogues of nifedipine *via* a chemoenzymatic approach led to the development of a new generation of highly selective calcium antagonist preparations.

The aim of the research described in this thesis is to elaborate adequate stereoselective biotechnological methods for the synthesis of 1,4-dihydropyridine and 1,4-dihydroisonicotinic acid derivatives in enantiopure form as key intermediates for the synthesis of unsymmetrical potentially biologically active compounds (Scheme 27). The target unsymmetrical compounds are structurally related to cerebrocrast and glutapyrone, two highly active compounds with an unusually broad spectrum of biological activities, not related to the conventional cardiovascular activity. The synthesis of enantiopure unsymmetrical structural analogues of these compounds is necessary for the elucidation of their pharmacological mechanism of action, which should result in more efficient and more selective drugs.

While methods of synthesis of enantiomerically pure 4-aryl-1,4-DHPs have been widely studied, 1,4-dihydroisonicotinic acid derivatives and polycyclic dihydropyridines were not synthesised until recently, and even less information is available on their enantiomerically pure derivatives.

The research described here consists of the chemical synthesis of symmetrical or racemic 1,4-DHPs and the enantioselective enzymatic asymmetrisation or resolution of these compounds. An extended set of 1,4-DHP derivatives was prepared and screened against hydrolytic enzymes. For this purpose hydrolytic enzymes like lipases, proteases and esterases taken from different commercial sources were used. The enantioselectivity of lipases in relation to structural variation in the substrates was studied in detail. In addition, other parameters, like organic solvent, temperature, pH of the aqueous medium and co-solvents of the aqueous medium were studied in depth. The synthesis of the first representative enantiopure target compounds was performed. The absolute configuration of some enantiopure products of the reactions was established.



Scheme 27

-In **chapter 1**, a literature review is given about 1,4-dihydropyridine derivatives and their biological activities. The synthesis of 1,4-DHPs by cyclocondensation reactions is described. Special attention is paid to stereoselective chemical and biotechnological methods for the synthesis of enantiopure 1,4-DHPs.

-In **chapter 2**, the *Candida antarctica* B lipase-catalysed enantioselective hydrolysis of prochiral bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates with different substituents at the 4-position is described.

-In **chapter 3** the synthesis of both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate is described, using *Candida rugosa* lipase-catalysed hydrolysis of an (isobutyryloxy)methyl spacer. The effect of acyl chain length and branching on the enantioselectivity of *Candida rugosa* lipase in the kinetic resolution of 4-(2-difluoromethoxyphenyl) substituted 1,4-dihydropyridine 3,5-diesters is studied.

-In **chapter 4** the highly stereoselective *Candida rugosa* lipase-catalysed enantioselective asymmetrisation is reported of the prochiral bis [(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate. This compound was used to prepare (—) 3-methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, an asymmetric analogue of cerebrocrast.

-In **chapter 5** the enantioselective lipase-catalysed kinetic resolution of acyloxymethyl and ethoxycarbonylmethyl esters of 1,4-dihydroisonicotinic acid derivatives is described.

-In **chapter 6** the *Candida rugosa* lipase-catalysed kinetic resolution of 3-(isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno[3,2-*b*]pyridine-3-carboxylate is reported.

-In **chapter 7**, discussion and conclusions about the lipase-catalysed synthesis of enantiopure 1,4-dihydropyridine derivatives are given, followed by a summary in Dutch, English and Latvian.

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Chapter 2

Candida antarctica lipase-catalysed hydrolysis of 4-substituted bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates as the key step in the synthesis of optically active dihydropyridines*

Abstract—Prochiral bis(ethoxycarbonylmethyl) substituted 4-aryl-1,4-dihydropyridine-3,5-dicarboxylates were hydrolysed enantioselectively by *Candida antarctica* lipase B (Novozym 435®). The enantiomeric excesses varied from 68 to 93%, depending on the substituent at position 4. In some cases, the e.e. could be significantly increased by changing the solvent system.

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2.1 Introduction

4-Aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1,4-DHP) derivatives are widely used for the treatment of cardiovascular diseases (hypertension, angina pectoris, infarction).[1] 1,4-DHPs having different ester groups at the 3- and 5- positions possess an stereogenic carbon at the 4 position in the 1,4-DHP nucleus, and enantiomers often show different biological activities.[2] The first optically active DHP derivative appeared on the market in 1992; however, at present, almost all chiral DHPs are still sold as racemates.[3]

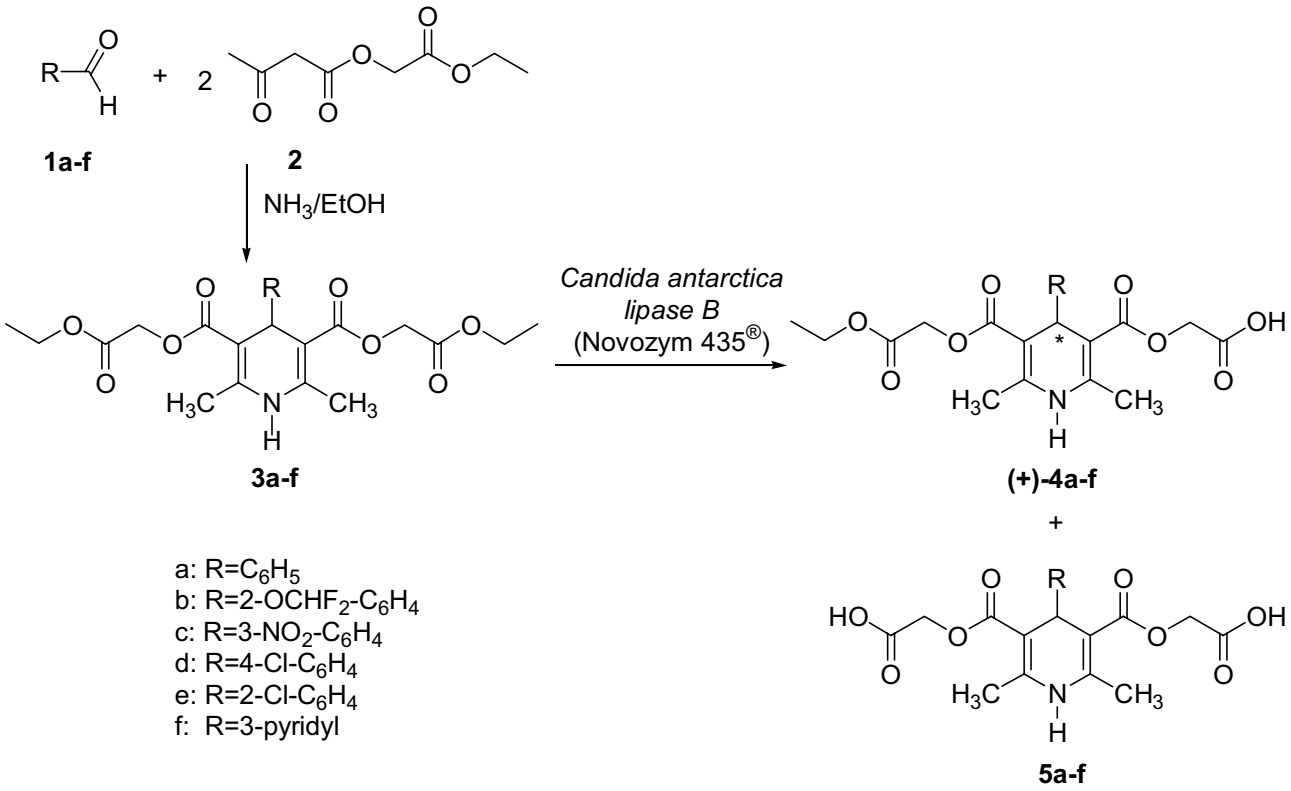
Most investigations in the field of chiral dihydropyridines have been devoted to the synthesis of calcium antagonists.[4] We are focusing on novel activities of 1,4-DHPs such as antidiabetic, nootropic, neuromodulatory, regulatory mode action and neuropeptide mimicking effects.[1,5] These activities have been found for some 4-pyridyl and 2-difluoromethoxyphenyl substituted dihydropyridines.[2,5,6] Derivatives of bis(ethoxycarbonylmethyl) substituted 1,4-dihydropyridine-3,5-dicarboxylates possess antimetastatic properties in combination with a low toxicity.[7a] Bis(carboxymethyl) 2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate (carbatone) is active against the Herpes simplex virus including acyclovir-resistant stains.[7b] Carbatone has been also reported to elevate free plasma corticosterone.[7c]

Amongst the many chemical methods for preparing enantiopure compounds, the biotechnological approach based on enzyme-catalysed enantiomeric differentiation has become a promising way for the synthesis of enantiopure 1,4-dihydropyridines.[8] The use of biocatalysts shows a number of distinct advantages as compared to other methods: enzymes are active under mild conditions and they often exhibit high stereoselectivity, combined with a broad substrate tolerance. In the case of 1,4-DHPs, enzymes are not capable to hydrolyse alkyl esters at the position 3- and 5- of 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates.[8] Modification of the alkyl esters to hydrolysable groups on spacers makes the hydrolysis possible, in some cases with high stereoselectivity.[9] When substituted alkyl ester chains were introduced at the positions 3- and/or 5- of the dihydropyridine ring, rabbit liver esterase showed the hydrolysis of the more distant ester group with low selectivity.[10] Seaprose S (*Aspergillus melleus*) catalysed the hydrolysis or transesterification of ethoxycarbonylmethyl esters of 1,4-DHP with splitting of both ‘outer’ and ‘inner’ ester groups to give the corresponding carboxylic acid or methyl ester.[11,12] It has also been reported that the stereoselectivity of lipase AH (*Pseudomonas sp.*) toward the same 1,4-DHPs can be changed or even reversed by changing the solvent.[13]

In this chapter, the *Candida antarctica* B lipase-catalysed enantioselective hydrolysis of prochiral bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates **3a-f** with different substituents at the position 4 is described.

2.2 Results and discussion

Bis(ethoxycarbonylmethyl) substituted 1,4-dihydropyridine-3,5-dicarboxylates **3a-f** were prepared by Hantzsch cyclisation of ethoxycarbonylmethyl acetoacetate **2** with aromatic aldehydes **1a-f** in ethanol with 42-67% yields (Scheme 1). In the case of aryl substituted 1,4-DHP **3a-e**, gradual addition of ammonia to the reaction mixture increases the yield by compensating for the loss of ammonia due to evaporation.



Scheme 1

The first enzymatic asymmetrisations of the substrates **3a-f** were performed in phosphate buffer pH 7.5, modified with 15% of acetonitrile, at 45°C using Protease P6 (*Aspergillus melleus*) and Acylase 30,000 (*Aspergillus sp.*). Both enzymes readily hydrolysed the 'outer' ester group on both sides of the substrates, eventually leading to the diacids **5a-f**. This contrasts the earlier reports where these enzymes and Seaprose S hydrolysed the 'inner' ester.[11,12] Furthermore, alkaline hydrolysis only takes place at the 'outer' ester groups[7] because of steric and electronic factors.

Long reaction times and low enantioselectivity of protease P6 (*Aspergillus melleus*) and acylase 30,000 (*Aspergillus sp.*) in the given reaction conditions led us to investigate other possibilities of conversion of substrates **3a-f**. Initial studies of the enantioselectivity of *Candida rugosa* lipase and *Rhizomucor miehei* lipase towards the substrates **3a-f** showed that these enzymes have also low enantioselectivity under the given reaction conditions.

Candida antarctica lipase B (CAL-B) is a very efficient catalyst for the enantioselective transformations of different kinds of substrates and is widely used in practice.[14] When *Candida antarctica* B lipase was used for the asymmetrisation of **3a-f** in phosphate buffer pH 7.5 (modified with acetonitrile, at 45°C; Table 1), a rather high enantioselectivity was reached together with shorter reaction times, so this enzyme was used for all subsequent experiments. The reaction should be carefully monitored by HPLC because of the subsequent hydrolysis to the diacid. The limiting factor of the hydrolysis of the aryl substituted **3a-e** is their insolubility in aqueous medium. Mixing the phosphate buffer with acetonitrile enhances the solubility of the substrates **3a-e**, but the reaction mixture remains heterogeneous in the beginning. The fact that the substrates dissolve during the reaction makes it difficult to directly compare reaction rates for the different substrates. Only in the case of 4-pyridyl substituted 1,4-DHP **3f** good solubility in buffer with just 5% of acetonitrile was observed.

Table 1. CAL B-catalysed hydrolysis of **3a-f** in phosphate buffer, pH 7.5 modified with acetonitrile at 45°C

Entry	Substrate	Reaction medium[a]	Time, h	Chemical Yield, %	Enantiomeric excess (e.e.), %	Optical rotation, $[\alpha]_D^{20}$
1	**3a**	A	19	87	93	+49.9
2	**3b**	A	48	44	79	+44.7
3	**3c**	A	20	66	77	+52.5
4	**3d**	A	96	31	68	+5.5
5	**3e**	A	48	29	72	+15.1
6	**3f**	B	18	58	82	+23.7

[a] (A) 15% solution of acetonitrile in 20 mM K-phosphate buffer, pH 7.5; (B) 5% solution of acetonitrile in 20 mM K-phosphate buffer, pH 7.5.

The above mentioned solubility difficulties of the substrates such as **3d,e** and insufficient enantioselectivity of CAL-B under the given reaction conditions towards substrates **3b-f** forced us to search for more suitable reaction conditions. It is known that the stereoselectivity of an enzyme can change considerably and sometimes even can reverse on transition from one solvent to another.[13,15]

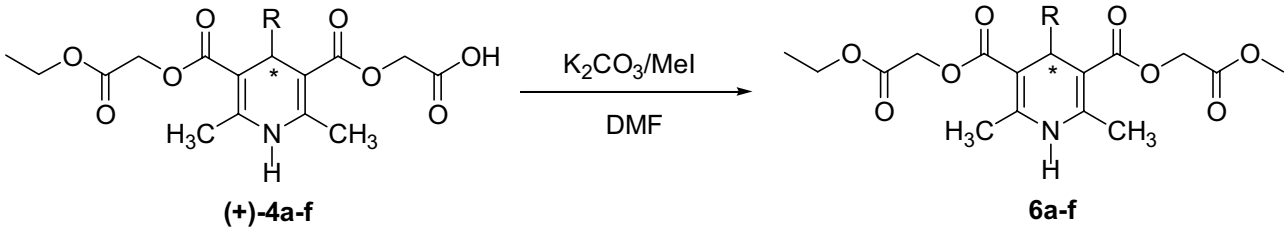
Altering the reaction media led in some cases to a higher enantioselectivity of CAL B towards substrates **3**; some examples are given in Table 2. From this table it is clear that the e.e. of **(+)-4b** was remarkably improved when water-saturated diisopropylether (IPE) was used, albeit at the expense of the reaction rate. However, in other cases changing the reaction medium to IPE did not have a significant influence on the enantioselectivity of enzyme. Other organic solvents like acetone, tetrahydrofuran, *t*-butanol and dimethylsulfoxide were used instead of acetonitrile, but in all cases (including acetonitrile) an increase of the amount of organic solvent in reaction mixture led to longer reaction times and a decreased e.e. of the product.

Table 2. Some examples of CAL B-catalysed hydrolysis of **3a-f** in different reaction media at 45°C

Entry	Substrate	Reaction medium[a]	Time, h	Chemical yield of **(+)-4**, %	Enantiomeric excess (e.e.), %
1	**3a**	C	48	21[b]	88
2	**3a**	D	22	49[b]	55
3	**3b**	C	282	47	97
4	**3c**	C	168	15[b]	67
5	**3d**	D	48	27[b]	68
6	**3d**	E	48	35[b]	69
7	**3e**	D	200	40	1
8	**3e**	E	24	39[b]	70
9	**3e**	F	28	55	92
10	**3e**	G	138	39	93

[a] Reaction medium: (C) water-saturated IPE ; (D) 15% solution of *t*-butanol in 20 mM phosphate buffer pH 7.5; (E) 15% solution of acetone in 20 mM phosphate buffer pH 7.5; (F) 1% DMSO solution in 20 mM phosphate buffer pH 7.5; (G) 20% of DMSO in 20 mM phosphate buffer pH 7.5. [b] Chemical yields were determined by HPLC.

The structure of the products was proven by mass spectrometry. Since mass spectra of the enzymatic products **(+)-4a-f** did not show molecular ions, the acids were converted into the corresponding esters **6a-f** by esterification with MeI in DMF (Scheme 2). Other ester derivatives of compound **3b** which could be suitable for the determination of the absolute configuration by X-ray analysis were synthesised using different approaches. Attempts to obtain suitable crystals failed, despite the fact that racemic analogues of the synthesised esters are often crystalline materials, as for example in the case of **6d,e**. It is already known[16] that problems with crystallisation of stereoisomers may occur even if the corresponding racemate is a crystalline substance.



Scheme 2

2.3 Conclusions

In conclusion, the *Candida antarctica* lipase catalysed asymmetrisation of 4-substituted bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates has been developed. Although complete stereoselectivity of *Candida antarctica* lipase towards all substrates was not achieved in all cases, the results obtained have shown that a change of the reaction conditions can improve the stereoselectivity of the process. Our investigations of lipase-catalysed hydrolysis of 1,4-DHPs show the way for the synthesis of various chiral biologically active dihydropyridines containing different aryl and heterocyclic substituents in position 4.

2.4 Experimental

2.4.1 General

Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh or 70-230 mesh). ^{1}H NMR spectra were recorded on a Bruker WH 90/DC (90 MHz) or a Bruker AC-E 200 (200 MHz) or a Bruker Avance DPX 400 (400 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AC-E 200 (50 MHz) or a Bruker Avance DPX 400 (100 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to trimethylsilane (δ 0.00). Mass spectral data and accurate mass measurements were determined on a Finnigan MAT 95 mass spectrometer. Melting points were determined on a Boetius apparatus and are uncorrected. Optical rotation values were measured with a Perkin Elmer 241 digital polarimeter. Elemental analyses were determined on a Carlo-Erba elemental analyser. The reaction mixtures were analysed by HPLC on a 4.6x250 mm column packed with 5 μ Spherisorb ODS-2 (Phase Separations) using a Gynkotek 480 pump and Applied Biosystems 758A programmable absorbance detector at 254 nm. The solvent system acetonitrile/water/acetic acid (60:40:0.1) was used as mobile phase at a flow rate of 1.0 mL/min. Determination of enantiomeric excesses of the products **(+)-4a-f** was performed by direct analysis on a chiral column Chirex 3011, 4.6x250 mm, 5 μ (Phenomenex) using a Ginkotek 580A pump and

an Applied Biosystems 759A absorbance detector at 254 nm. Other Pirkle and cavity-type columns were useful for the analysis of e.e. of products of the reactions but this was the most universal one. The eluent was 0.05 M ammonium acetate in methanol at a flow rate of 1.0 mL/min for **(+)-4a-c,d,f** or dichloromethane/methanol/acetic acid (80:20:0.5) for compound **(+)-4e**. Peak areas were determined electronically with the Chromeleon chromatography data system, Dionex Softron GmbH (Germering, Germany). Enzymatic reactions were carried out in a New Brunswick Scientific Innova 4080 incubatory orbital shaker. Immobilised *Candida antarctica* lipase B, Novozym 435®, was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark).

2.4.2 Procedures and spectral data

General procedure of the synthesis of bis(ethoxycarbonylmethyl) 4-aryl -2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates 3a-e

Ethoxycarbonylmethyl acetoacetate **2 (**29.0 g, 0.15 mol), 0.077 mol of benzaldehyde **1a** or the corresponding substituted benzaldehyde **1b-e** and 5 mL (0.065 mol) of 25% aqueous ammonia were dissolved in 50 mL of ethanol and were heated under reflux. After 2 h of refluxing, 5 mL of 25% aqueous ammonia was added in two to three portions with 0.5 h intervals. The usual quantity of added ammonia was 10 mL (0.13 mol). The usual time of refluxing was 6 h. After cooling until -5°C the precipitated product was filtered and recrystallised from ethanol.

Bis(ethoxycarbonylmethyl) 2,6-dimethyl-4-phenyl-1,4-dihydro-3,5-pyridinedicarboxylate, 3a

Yield: 42%, mp 84-85°C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.23 (t, 6H, *J*=7.0 Hz, CH_2CH_3), 2.33 (s, 6H, 2xCH_3), 4.15 (q, 4H, *J*=7.0 Hz, CH_2CH_3), 4.55 (s, 4H, 2x$COOCH_2COO$), 5.09 (s, 1H, CH), 6.00 (br s, 1H, NH), 7.05-7.30 (m, 5H, C_6H_5). Anal. calcd for $C_{23}H_{27}NO_8$: C, 62.01; H, 6.11; N, 3.14; found: C, 62.06; H, 6.12; N, 3.14.

Bis(ethoxycarbonylmethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 3b

Yield: 55%, mp 101-102°C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.20 (t, 6H, *J*=7.0 Hz, CH_2CH_3), 2.30 (s, 6H, 2xCH_3), 4.10 (q, 4H, *J*=7.0 Hz, CH_2CH_3), 4.50 (s, 4H, 2x$COOCH_2COO$), 5.33 (s, 1H, CH), 6.25 (br s, 1H, NH), 6.45 (t, 1H, *J*=75 Hz, $OCHF_2$), 6.87-7.40 (m, 4H, C_6H_4). Anal. calcd for $C_{24}H_{27}F_2NO_9$: C, 56.36; H, 5.32; N, 2.74; found: C, 56.42; H, 5.33; N, 2.71.

Bis(ethoxycarbonylmethyl) 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, 3c

Yield: 67%, mp 157-159°C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.18 (t, 6H, *J*=7.0 Hz, CH_2CH_3), 2.33 (s, 6H, 2xCH_3), 4.09 (q, 4H, *J*=7.0 Hz, CH_2CH_3), 4.50 (s, 4H, 2x$COOCH_2COO$), 5.77 (s, 1H, CH), 6.57 (br s, 1H, NH), 7.10-7.67 (m, 4H, C_6H_4). Anal. calcd for $C_{23}H_{26}N_2O_{10}$: C, 56.32; H, 5.34; N, 5.71; found: C, 56.38; H, 5.32; N, 5.70.

Bis(ethoxycarbonylmethyl) 4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 3d

Yield: 69%, mp 130-132°C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.22 (t, 6H, *J*=7.0 Hz, CH_2CH_3), 2.33 (s, 6H, 2xCH_3), 4.15 (q, 4H, *J*=7.0 Hz, CH_2CH_3), 4.54 (s, 4H, $COOCH_2COO$), 5.05 (s, 1H, CH), 6.07 (br s, 1H, NH), 7.08 and 7.23 (two d, 4H, *J*=9 Hz, C_6H_4). Anal. calcd for $C_{23}H_{26}ClNO_8$: C, 57.56; H, 5.46; N, 2.92; found: C, 57.56; H, 5.47; N, 2.90.

Bis(ethoxycarbonylmethyl) 4-(2-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 3e

Yield: 38%, mp 115-117°C; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.16 (t, 6H, *J*=7.0 Hz, CH_2CH_3), 2.30 (s, 6H, 2xCH_3), 4.10 (q, 4H, *J*=7.0 Hz, CH_2CH_3), 4.50 (s, 4H, $COOCH_2COO$), 5.44 (s, 1H, CH), 6.41 (br s, 1H, NH), 6.88-7.41 (m, 4H, C_6H_4). Anal. calcd for $C_{23}H_{26}ClNO_8$: C, 57.56; H, 5.46; N, 2.92; found: C, 57.56; H, 5.45; N, 2.91.

Bis(ethoxycarbonylmethyl) 4-(3-pyridyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 3f

Ethoxycarbonylmethyl acetoacetate **2** (37.6 g, 90.2 mol), 9.4 mL (10.7g, 0.1 mol) of pyridine-3-carboxaldehyde **1f** and 10 mL (0.13 mol) of 25% aqueous ammonia solution were dissolved in 50 mL of ethanol and were heated under reflux for 3 h. After cooling until –5°C the precipitate was filtered off and recrystallised from ethanol to give 25.4 g (57%) of crystalline product, mp 143-145°C, ^{1}H NMR ($CDCl_3$, 90 MHz) δ 1.21 (t, 6H, *J*=7.0 Hz, CH_2CH_3), 2.34 (s, 6H, 2xCH_3), 4.17 (q, 4H, *J*=7.0 Hz, CH_2CH_3), 4.54 (s, 4H, 2x$COOCH_2COO$), 5.09 (s, 1H, CH), 7.06 (br s, 1H, NH), 7.17 (dd, 1H, $J_{5,4}$=8 Hz, $J_{5,6}$=4 Hz, H_5 Py), 7.65 (dt, 1H, $J_{4,5}$=8 Hz, $J_{4,2}$=$J_{4,6}$=2 Hz, H_4 Py), 8.32 (dd, 1H, $J_{6,5}$=4 Hz, $J_{6,4}$=2 Hz, H_6 Py), 8.50 (d, 1H, $J_{2,4}$=2 Hz, H_2 Py). Anal. calcd for $C_{22}H_{26}N_2O_8$: C, 59.19; H, 5.87; N, 6.27; found: C, 59.04; H, 5.86; N, 6.17.

General procedure for the enzymatic hydrolysis of 1,4-dihydropyridine-3,5-dicarboxylates 3a-e

A solution of 0.5 mmol of **3a,c,d** or 0.4 mmol of **3b,e** in 60 mL of acetonitrile was added to 340 mL of 20 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.5) and heated to 45°C, after which 600 mg of Novozym 435® was added. The resulting mixture was shaken at 350 rpm and heated at 45°C. Reactions were monitored by HPLC (see table Table 1 and 2 for further details about reaction times). After removal of the enzyme by filtration, the filtrate was adjusted to pH 5.0 by adding 1M HCl and extracted with chloroform (3x100mL). The combined organic layers were concentrated under reduced pressure. The residue was flash chromatographed on silica gel using the solvent system chloroform/isopropyl alcohol/acetic acid (100:20:0.1) to give the following monoacids.

(+)-3-Carboxymethyl 5-ethoxycarbonylmethyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate, (+)-4a

Yield: 181 mg (87%) as a precipitate from hexane, mp 169-170°C; $[\alpha]_D^{20}$ +49.9 (*c* 1.0, MeOH); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.17 (3H, t, *J*=7.1 Hz, CH_2CH_3), 2.31 (s, 6H, 2xCH_3), 4.10 (q, 2H, *J*=8.0 Hz, CH_2CH_3), 4.32 (ABq, 2H, $COOCH_2COO$), 4.60 (s, 2H, $COOCH_2COO$), 4.98 (s, 1H, CH), 7.12-7.21 (m, 5H, C_6H_5), 9.03 (s, 1H, NH); ^{13}C NMR (CD_3OD, 50 MHz) δ 14.41 (CH_3), 18.86 (CH_3), 18.91 (CH_3), 40.20 (CH), 60.41 (CH_2), 62.17 (CH_2), 63.26 (CH_2), 102.97, 104.10, 127.04 (CH), 128.69 (CH), 128.90 (CH), 147.51, 148.47, 148.92, 168.78, 169.48, 170.10, 176.51.

(+)-3-Carboxymethyl 5-ethoxycarbonylmethyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, (+)-4b

Yield: 85 mg (44%) as a precipitate from hexane, mp 151-153°C; $[\alpha]_D^{20}$ +44.7 (*c* 0.855, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.13 (t, 3H, *J*=7.1 Hz, CH_2CH_3), 2.26 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 4.08 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 4.24 (ABq, 2H, $COOCH_2COO$), 4.50 (ABq, 2H, $COOCH_2COO$), 5.25 (s, 1H, CH), 6.95-7.13 (m, 3H, C_6H_4), 7.00 (t, 1H, *J*=74.9 Hz, $OCHF_2$), 7.29 (dd, 1H, C_6H_4), 9.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 14.76 (CH_3), 19.10 (CH_3), 19.19 (CH_3), 34.91 (CH), 60.82 (CH_2), 61.33 (CH_2), 63.35 (CH_2), 100.73, 103.16, 117.94 (CH, t, *J*=252.9 Hz, $OCHF_2$), 118.29 (CH), 125.89 (CH), 128.26 (CH), 131.70 (CH), 139.81, 145.71, 148.10, 148.98, 167.27, 167.60, 169.00, 173.06.

(+)-3-Carboxymethyl 5-ethoxycarbonylmethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, (+)-4c

Yield: 153 mg (66%) as crystals from ether/petr. ether, mp 172-174°C; $[\alpha]_D^{20}$ +52.5 (*c* 1.0, MeOH); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.10 (t, 3H, *J*=7.0 Hz, CH_2CH_3), 2.32 (s, 6H, 2xCH_3), 4.04 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 4.25 (ABq, 2H, $COOCH_2COO$), 4.59 (s, 2H, $COOCH_2COO$), 5.07 (s, 1H, CH), 7.50 (t, 1H, C_6H_4), 7.73 (d, 1H, C_6H_4), 7.96 (d+s, 2H, C_6H_4), 9.33 (s, 1H, NH); ^{13}C NMR (CD_3OD, 50 MHz) δ 14.37 (CH_3), 18.85 (CH_3), 18.93 (CH_3), 40.55 (CH), 61.44 (CH_2), 62.20 (CH_2), 63.47 (CH_2), 102.21, 103.35, 122.06 (CH), 123.53 (CH), 130.08 (CH), 135.51 (CH), 148.36, 149.34, 149.38, 151.21, 168.18, 168.93, 169.89, 176.83.

(+)-3-Carboxymethyl 5-ethoxycarbonylmethyl 4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, (+)-4d

Yield: 70 mg (31%) as a viscous oil; $[\alpha]_D^{20}$ +5.5 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.24 (t, 3H, *J*=7.1 Hz, CH_2CH_3), 2.33 (s, 6H, 2xCH_3), 4.19 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 4.59 (ABq, 2H, $COOCH_2COO$), 4.60 (s, 2H, $COOCH_2COO$), 5.06 (s, 1H, CH), 6.45 (s, 1H, NH); 7.16 and 7.25 (two d, 4H, *J*=9 Hz, C_6H_4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.07 (CH_3), 19.10 (CH_3), 38.61 (CH), 60.06 (CH_2), 60.53 (CH_2), 61.49 (CH_2), 102.50, 102.59, 128.14 (CH), 129.24 (CH), 131.92, 145.58, 146.19, 146.39, 166.68, 168.70, 173.10.

(+)-3-Carboxymethyl 5-ethoxycarbonylmethyl 4-(2-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, (+)-4e

Yield: 53 mg (29%) as a precipitate from ether/petr. ether, mp 175-177°C; $[\alpha]_D^{20}$ +15.1 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.22 (t, 3H, *J*=7.2 Hz, CH_2CH_3), 2.34 (s, 3H, CH3), 2.36 (s, 3H, CH_3), 4.16 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 4.54 (ABq, 2H, $COOCH_2COO$), 4.60 (s, 2H, $COOCH_2COO$), 5.48 (s, 1H, CH), 6.28 (s, 1H, NH); 7.00-7.23 (m, 3H, C_6H_4), 7.39 (dd, 1H, 2-Cl-C_6H_4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.04 (CH_3), 19.20 (CH_3), 19.31 (CH_3), 37.05 (CH), 59.76 (CH_2), 60.12 (CH_2), 61.40 (CH_2), 102.60, 126.98 (CH), 127.41 (CH), 129.20 (CH), 131.60 (CH), 132.40, 145.66, 146.18, 146.32, 166.74, 166.78, 168.95, 172.97.

(+)-3-Carboxymethyl 5-ethoxycarbonylmethyl 2,6-dimethyl-4-(3-pyridyl)-1,4-dihydropyridine-3,5-dicarboxylate, (+)-4f

A solution of 223 mg (0.5 mmol) of **3f** in 20 mL of acetonitrile was added to 380 mL of 20 mM K_2HPO_4/KH_2PO_4 buffer solution and heated until 45°C after which 600 mg of Novozym 435® was

added. The resulting mixture was shaken at 350 rpm for 17 h at 45°C. After removal of enzyme by filtration, the filtrate was extracted with chloroform (2x100mL). The filtrate was acidified to pH 5.0 by adding 1M HCl and concentrated in vacuum. The residue was flash chromatographed on silica gel using chloroform/isopropyl alcohol/acetic acid (100:20:0.1) to (50:50:0.1) to give **(+)-4f**. Crystallisation from ethyl acetate gave 122 mg (58%) of **(+)-4f** as a powder, mp 187-192°C dec; $[\alpha]_D^{20}$ +23.7 (*c* 1.0, MeOH); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.13 (t, 3H, *J*=7.0 Hz, CH_2CH_3), 2.30 (s, 6H, 2xCH_3), 4.06 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 4.21 (ABq, 2H, $COOCH_2COO$), 4.59 (s, 2H, $COOCH_2COO$), 4.95 (s, 1H, CH), 7.23 (dd, 1H, Py), 7.57 (dt, 1H, Py), 8.29 (d, 1H, Py), 8.39 (br s, 1H, Py), 9.13 (s, 1H, NH).

(+)-3-Carboxymethyl 5-ethoxycarbonylmethyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (+)-4b by hydrolysis in water-saturated isopropyl ether

To a solution of 204 mg (0.40 mmol) of **3b** in 50 mL of water-saturated isopropyl ether, 600 mg of Novozym 435® was added and the resulting mixture was shaken at 350 rpm for 12 d (282 h) at 45°C. The enzyme was removed by filtration and washed additionally with methanol and the filtrate was concentrated under reduced pressure. The residue was flash chromatographed on silica gel with chloroform/isopropyl alcohol /acetic acid (100:20:0.1) to give 86 mg (45%) of **(+)-4b** as a solid and 95 mg (47%) of unreacted **3b**. Data for **(+)-4b:** $[\alpha]_D^{20}$ +72.6 (*c* 1.0, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) are identical to those for **(+)-4b** described above.

General procedure for the preparation of 6a-6f

All derivatives were prepared in the same manner as described below for compound **6c**.

3-Ethoxycarbonylmethyl 5-methoxycarbonylmethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, 6c

To a solution of 65 mg (0.14 mmol) of **(+)-4c** in 1 mL DMF, 29 mg (0.21 mmol) of K_2CO_3 was added at rt and the resulting mixture was stirred for 2 h after which 0.017 mL (0.28 mmol) of MeI was added. The reaction mixture was stirred for additional 2 h. The mixture was poured into water and extracted with $CHCl_3$. The extract was washed successively with water and brine, then dried over $MgSO_4$. After removal of solvent in vacuum the residue was flash chromatographed on silica gel with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (8:2:2) to give 46 mg (69%) of **6c**, mp 85-88°C (triturated with hexane-ether); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.24 (t, 3H, *J*=7.2 Hz, CH_2CH_3), 2.42 (s, 6H, 2xCH_3), 3.72 (s, 3H, CH_3), 4.19 (q, 2H, *J*=7.2 Hz, CH_2CH_3), 4.60 (s, 2H, $COOCH_2COO$), 4.61 (s, 2H, $COOCH_2COO$), 5.24 (s, 1H, CH), 6.03 (s, 1H, NH), 7.39 (t, 1H,

C_6H_4), 7.73 (dd, 1H, C_6H_4), 8.02 (dt, 1H, C_6H_4), 8.14 (t, 1H, C_6H_4); MS *m/z* (rel. abund.): 476 (M^+, 4), 387 (3), 373 (4), 356 (3), 355 (16), 354 (100), 345 (3), 326 (3), 296 (10), 268 (6); HRMS calcd for $C_{22}H_{24}N_2O_{10}$ 476.1431, found 476.1428.

3-Ethoxycarbonylmethyl 5-methoxycarbonylmethyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate, 6a

Yield: 65% as a powder triturated with hexane, mp 112-114°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.25 (t, 3H, *J*=7.1 Hz, CH_2CH_3), 2.38 (s, 6H, 2xCH_3), 3.72 (s, 3H, CH_3), 4.19 (q, 2H, *J*=7.2 Hz, CH_2CH_3), 4.59 (ABq, 2H, $COOCH_2COO$), 4.61 (s, 2H, $COOCH_2COO$), 5.14 (s, 1H, CH), 5.89 (s, 1H, NH), 7.10-7.36 (m, 5H, C_6H_5); MS *m/z* (rel. abund.): 431 (M^+, 4), 355 (11), 354 (100), 238 (3); HRMS calcd for $C_{22}H_{25}NO_8$ 431.1580, found 431.1578.

3-Ethoxycarbonylmethyl 5-methoxycarbonylmethyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 6b

Yield: 80% as a powder triturated with hexane, mp 83-85°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.22 (t, 3H, *J*=7.1 Hz, CH_2CH_3), 2.35 (s, 6H, 2xCH_3), 3.67 (s, 3H, CH_3), 4.15 (q, 2H, *J*=7.1 Hz, CH_2CH_3), 4.55 (ABq, 2H, $COOCH_2COO$), 4.57 (s, 2H, $COOCH_2COO$), 5.40 (s, 1H, CH), 5.96 (s, 1H, NH), 6.49 (t, 1H, *J*=75.3 Hz, $OCHF_2$), 6.97-7.42 (m, 4H, C_6H_4); MS *m/z* (rel. abund.): 497 (M^+, 6), 495 (4) 408 (3), 394 (4), 391 (4), 368 (9), 366 (5), 355 (16), 354 (100), 296 (5); HRMS calcd for $C_{23}H_{25}F_2NO_9$ 497.1497, found 497.1501.

3-Ethoxycarbonylmethyl 5-methoxycarbonylmethyl 4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 6d

Yield: 71% as a precipitate from hexane-ether, mp 131-132°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.25 (t, 3H, *J*=7.2 Hz, CH_2CH_3), 2.39 (s, 6H, 2xCH_3), 3.72 (s, 3H, CH_3), 4.20 (q, 2H, *J*=7.2 Hz, CH_2CH_3), 4.60 (s, 2H, $COOCH_2COO$), 4.61 (s, 2H, $COOCH_2COO$), 5.11 (s, 1H, CH), 5.83 (s, 1H, NH), 7.19 and 7.28 (two d, 4H, *J* =9 Hz, C_6H_4); MS *m/z* (rel. abund.): 465 (M^+, 4); 378 (3), 376 (3), 362(3), 356 (3), 355 (17), 354 (100), 348 (3), 334 (4), 268 (3); HRMS calcd for $C_{22}H_{24}ClNO_8$ 465.1190, found 465.1185.

3-Ethoxycarbonylmethyl 5-methoxycarbonylmethyl 4-(2-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 6e

Yield: 40% as a precipitate from hexane-ether, mp 91-93°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.21 (t, 3H, *J*=7.1 Hz, CH_2CH_3), 2.35 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 4.15 (q, 2H, *J*=7.2 Hz, CH_2CH_3), 4.57 (ABq, 2H, $COOCH_2COO$), 4.58 (s, 2H, $COOCH_2COO$), 5.50 (s, 1H, CH), 6.23 (s, 1H, NH); 7.00-7.24 (m, 3H, C_6H_4), 7.40 (dd, 1H, C_6H_4); MS *m/z* (rel. abund.): 465 (M^+, 3), 429 (3), 428 (13), 370 (4), 362 (3), 355 (14), 354 (100), 334 (3), 296 (5), 268 (4); HRMS calcd for $C_{22}H_{24}ClNO_8$ 465.1190, found 465.1186.

3-Ethoxycarbonylmethyl 5-methoxycarbonylmethyl 2,6-dimethyl-4-(3-pyridyl)-1,4-dihydropyridine-3,5-dicarboxylate, 6f

Yield: 60% as a powder triturated with hexane, mp 124-125°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.24 (t, 6H, *J*=7.2 Hz, CH_2CH_3), 2.39 (s, 6H, 2xCH_3), 3.71 (s, 3H, CH_3), 4.19 (q, 4H, *J*=7.2 Hz, CH_2CH_3), 4.59 (s, 2H, $COOCH_2COO$), 4.61 (s, 2H, $COOCH_2COO$), 5.14 (s, 1H, CH), 6.28 (br s, 1H, NH), 7.18 (dd, 1H, Py), 7.68 (dt, 1H, Py), 8.40 (br d, 1H, Py), 8.50 (br s, 1H, Py); MS *m/z* (rel. abund.): 432 (M^+, 5), 355 (18), 354 (100), 343 (5), 329 (6), 301 (5), 297 (3), 296 (23), 268 (8), 211 (3); HRMS calcd for $C_{21}H_{24}N_2O_8$ 432.1533, found 432.1532.

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Chapter 3

Effect of acyl chain length and branching on the enantioselectivity of *Candida rugosa* lipase in the kinetic resolution of 4-(2-difluoromethoxyphenyl)-substituted 1,4-dihydropyridine 3,5-diesters[*]

Abstract—Since 2,6-dimethyl-4-aryl-1,4-dihydropyridine 3,5-diesters themselves are not hydrolysed by commercially available hydrolases, derivatives with spacers containing a hydrolysable group were prepared. Seven acyloxymethyl esters of 5-methyl- and 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate were synthesised and subjected to *Candida rugosa* lipase (CRL) catalysed hydrolysis in wet diisopropyl ether. A methyl ester at the 5-position and a long or branched acyl chain at C(3) gave the highest enantiomeric ratio (*E* value). The most stereoselective reaction (*E*=21) was obtained with 3-[(isobutyryloxy)methyl] 5-methyl 4-(2-difluoromethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, and this compound was used to prepare both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate. The absolute configuration of the enzymatically produced carboxylic acid was established to be 4*R* by X-ray crystallographic analysis of its 1-(*R*)-phenylethyl amide.

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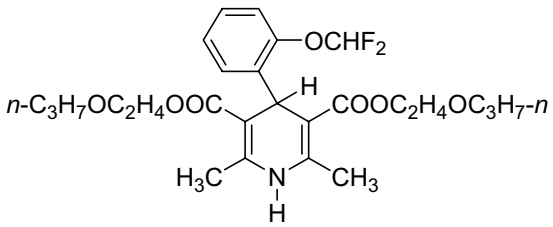
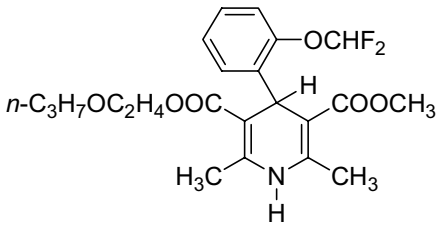
3.1 Introduction

The pharmacology of 1,4-dihydropyridine (1,4-DHP) derivatives is at the eve of a novel boom. After the synthesis, study and development of a set of antihypertensive and antianginal drugs,[1,2] the interest is growing towards pharmacological activities that are not connected with their calcium antagonist properties, like neurotropic (antiamnestic, anticonvulsant, neuroregulatory), antidiabetic, membrane protecting as well as anticancer and anti-inflammatory activities.[3-8]

Cerebrocrast (2,6-dimethyl-3,5-bis[2-(propoxy)ethoxycarbonyl]-4-[2-(difluoromethoxy)phenyl]-1,4-dihydropyridine) is a novel highly active compound with antidiabetic and antineurodegenerative activity, neuromodulatory, cognition enhancing and long-lasting memory improving properties at very low doses.[5,6] Anti-inflammatory effects of cerebrocrast have been reported recently.[8]



cerebrocrast



1

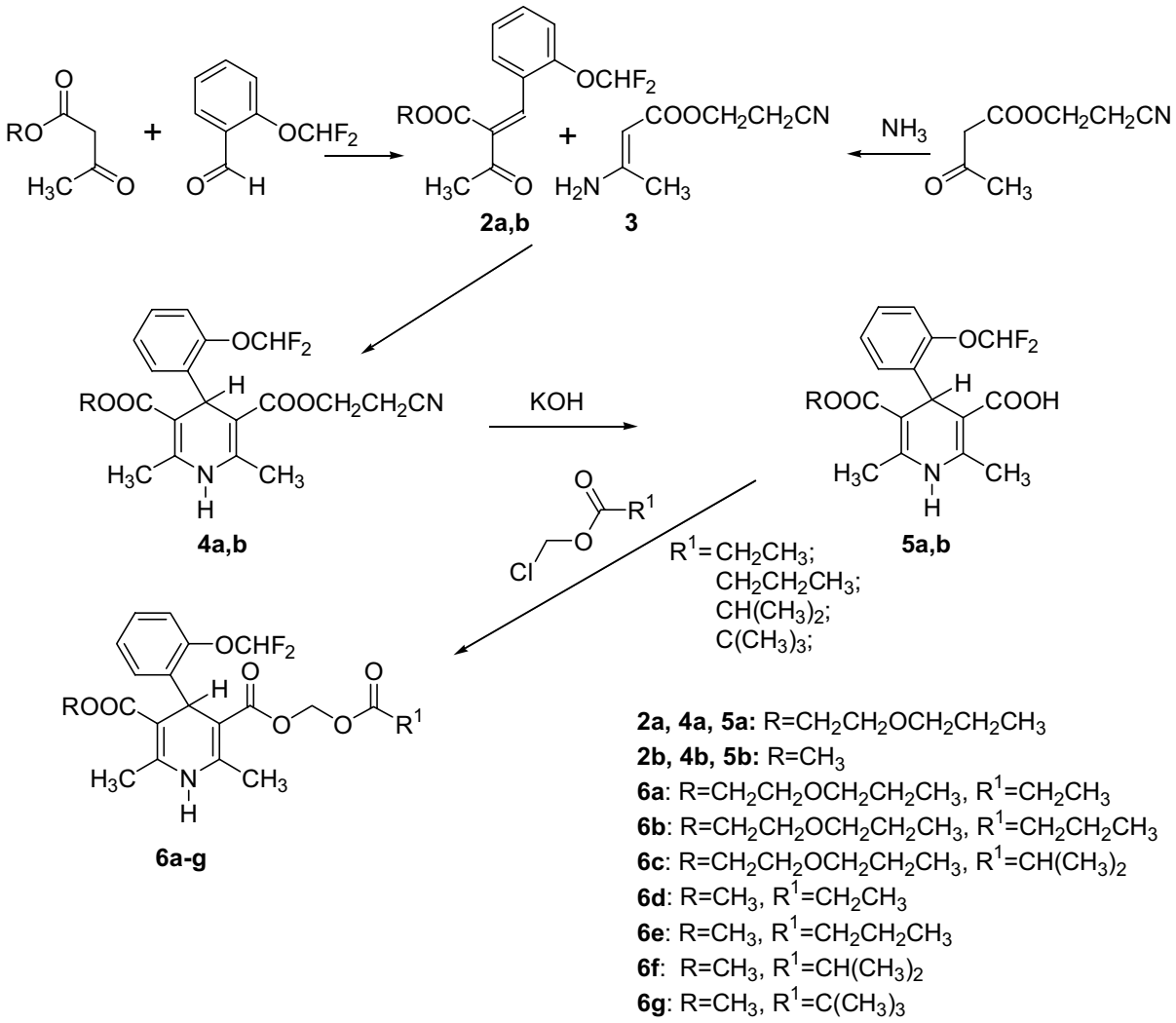
Pharmaceutical evaluations of chiral 1,4-DHPs revealed that their stereoisomers usually have different biological activities. Sometimes the undesired enantiomer caused serious side effects, while in other cases enantiomers were reported to even have the opposite action profile (calcium antagonist-calcium agonist; hypotensive activity-hypertensive activity).[9] Ever since the differences in biological action and toxicity for a number of unsymmetrical dihydropyridines were reported for the first time,[10] the demand for enantiopure dihydropyridines appeared, and in 1991, the first successful enantioselective chemoenzymatic transformation of a prochiral dihydropyridine-3,5-dicarboxylic diester was carried out.[11] The chemoenzymatic synthesis of some biological active DHPs, (e.g., Nicardipine, Nilvadipine) was recently achieved in several laboratories.[12,13]

Hydrolytic enzymes are not capable of hydrolysing alkyl esters at the position 3 and 5 of 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates, presumably because of steric and electronic factors.[11,14] To overcome this difficulty, spacers have been introduced at these positions. The spacer should contain a group that is easily hydrolysed by enzymes (*e.g.*, an ester group) in order to allow kinetic resolution or enzymatic asymmetrisation. The ethoxycarbonylmethyl spacer has been used to asymmetrise a number of 4-aryl-1,4-DHPs by us[15] and others.[16,17] Moderate to excellent e.e.

values were obtained, but this spacer group is not easy to remove. The acyloxymethyl moieties were introduced by the groups of Sih[18] and Achiwa[19] and have the advantage that, after enzymatic hydrolysis, a free carboxyl group is liberated due to the spontaneous loss of formaldehyde. This versatile spacer has also been applied for the kinetic resolution of sterically hindered tertiary alcohols.[20]

To investigate the differences in biological activities of both enantiomers of an asymmetric analogue of cerebrocrast, two chemoenzymatic approaches to the synthesis of both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate **1** using various acyloxymethyl spacers are described in this paper. The relationship between the structure of the substrates and the rate and selectivity of the enzymatic transformation is studied.

3.2 Results and discussion

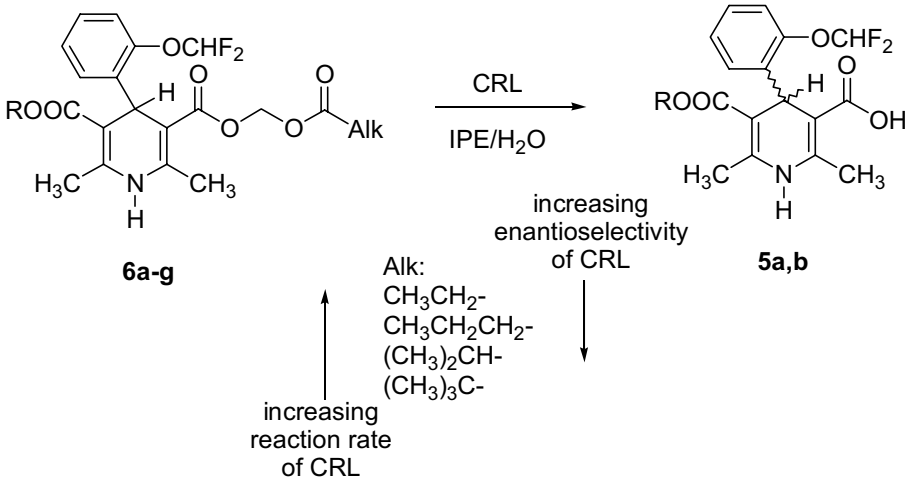


Scheme 1

The racemic substrates **6a-g** were synthesised as depicted in Scheme 1. These compounds have one of the desired ester groups at the 5-position and a variety of acyloxymethyl esters at the 3-position that are amenable to enzymatic hydrolysis. The first step is the reaction of the benzylidene acetoacetates **2a,b** and 2-cyanoethyl 3-aminocrotonate **3** by a modified Hantzsch cyclisation.

3,5-Bis(2-cyanoethyl) 1,4-dihydropyridinedicarboxylates have already been transformed by Seaprose S (*Aspergillus melleus*) with good enantioselectivity.[16,17] Unfortunately, this enzyme is not commercially available. Furthermore, in our hands, enzymes from the same species or genus such as protease P6 (*Aspergillus melleus*) and acylase 30,000 (*Aspergillus sp.*) hardly showed any activity toward compounds **4a,b**. However, the 2-cyanoethyl ester group can be easily removed under basic conditions[21] to give the monoacids **5a,b** in good yields. The substrates **6a-g** were synthesised from the isolated or *in situ* generated monoacids by treatment with the corresponding acyloxymethyl chloride prepared according to reported methods.[22,23] The alkylation occurred smoothly in the case of **6g**, but in the other cases, the products **6a-f** were isolated from mixtures of byproducts in 10-70% yield. The low yield of the propionyloxymethyl derivatives **6a** and **6d** is due to their instability. The set of derivatives **6a-g** differing in the bulkiness of the acyloxymethyl ester and alkyl ester at positions 3 and 5 allowed us to establish the relationship between the structure of the substrate and the reactivity and enantioselectivity of the enzyme.

Screening of the transformation of **6c** and **6f** with lipase AH, lipase PS, *Candida antarctica B* lipase (CAL-B), *Burkholderia cepacia* lipase and *Rhizomucor miehei* lipase showed that these enzymes can catalyse the hydrolysis of the substrates in wet diisopropyl ether. However, the enantiopreference was rather moderate and these enzymes were not investigated further. *Candida rugosa* lipase (CRL) was found to be the most active and enantioselective of all the tested enzymes, and this enzyme was used for all subsequent experiments (see Scheme 2).



Scheme 2

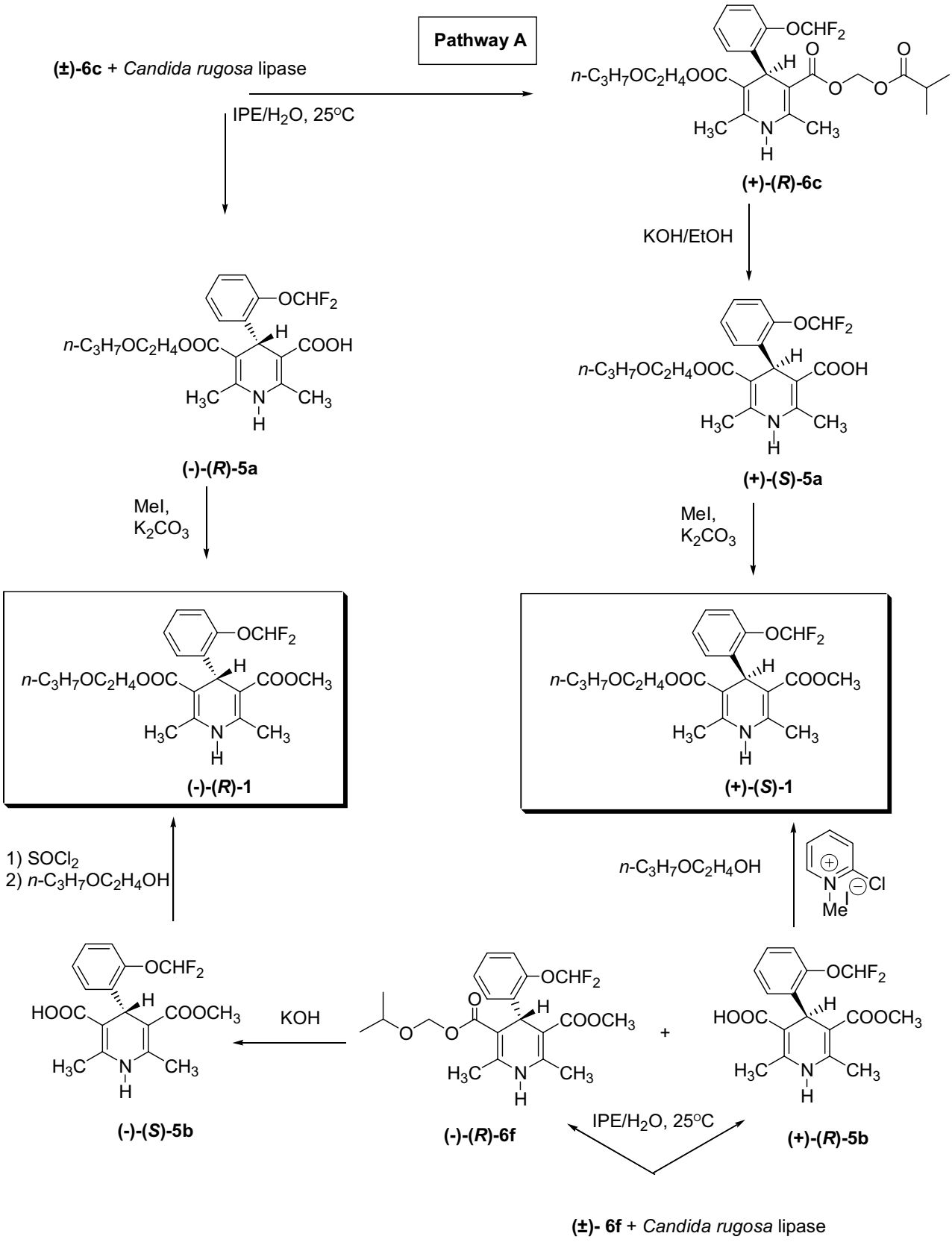
According to literature, CRL together with CAL-B are the most universal enzymes for different purposes and were already widely applied because of their unique stereoselectivity.[15,24,25]

Table 1. Enantiomeric ratio[26] (*E*) and reaction rate of the *Candida rugosa* lipase catalysed kinetic resolution of racemic 1,4-dihydropyridine substrates **6a-g** in water-saturated diisopropyl ether[a]

Compound	Enantiomeric ratio (E_p value)[b]	Time of 50% conversion, h
6a	1.7±0.07	4
6b	3.0 ±0.1	6
6c	11.0 ±0.6	6
6d	4.0 ±0.2	4.0
6e	6.0 ±0.1	4.5
6f	21.0 ±1.3	5
6g	-	no reaction

[a] For the details, see the Experimental Section. [b] The data were fitted using the computer program EIVFIT.[27]

The enantioselectivity of *Candida rugosa* lipase catalysed kinetic resolution of racemic substrates **6a-g** in water-saturated diisopropyl ether (IPE) was investigated, and the obtained data are summarised in Table 1. From this table it is clear that increasing the steric bulk of the acyl group in compounds **6** decreases the reaction rates.[28] The pivaloyloxymethyl derivative **6g** is not reactive toward CRL. Only CAL-B and *Rhizomucor miehei* lipase were able to hydrolyse **6g**: in 8 days of incubation with *Rhizomucor miehei* lipase, the conversion to monoacid **5b** was 35% with 13% e.e. Substrates having a methyl ester at C(5) give higher *E* values than those having 2-propoxyethyl esters. Furthermore, the enantioselectivity of CRL increased together with the steric hindrance of acyloxymethyl ester group. The highest enantiomeric ratio (*E*=21) was reached for the isobutyryloxymethyl ester **6f**. This rather high enantioselectivity is remarkable, since there are no less than 6 single bonds between the chiral centre and the carbonyl group where the serine residue of the enzyme attacks. This means that there is much conformational flexibility in the substrate, which increases the chance that both enantiomers will fit in the enzyme active site (in different conformations). Increasing the size and branching of the acyloxymethyl group may limit the available space for the 4-aryl-1,4-DHP moiety inside the enzyme and therefore enhance the steric recognition. Furthermore, it should be noted that the reaction is performed in an organic solvent and it is well-appreciated that reactions in nonaqueous solvents have increased enantioselectivity.[29]


Pathway A
(±)-6c + Candida rugosa lipase
IPE/H2O, 25°C
(+)-(R)-6c
KOH/EtOH
(-)-(R)-5a
(+)-(S)-5a
MeI, K2CO3
MeI, K2CO3
(-)-(R)-1
(+)-(S)-1
1) SOCl2
2) n-C3H7OC2H4OH
n-C3H7OC2H4OH
KOH
IPE/H2O, 25°C
(-)-(S)-5b
(-)-(R)-6f
(+)-(R)-5b
(±)- 6f + Candida rugosa lipase
Pathway B

Scheme 3

Table 2. Synthesis of both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylates, **(+)-(*S*)-1** and **(—)-(*R*)-1**

Pathway	Compound	e.e., %	$[\alpha]_D^{20}$
A	**(+)-(*R*)-6c**	62	+16.0 (MeOH)
A	**(—)-(*R*)-5a**	88[a]	–32.6 (MeOH)
A	**(+)-(*S*)-5a**	62	+29.8 (MeOH)
A	**(+)-(*S*)-1**	62	+13.0 ($CHCl_3$)
A	**(—)-(*R*)-1**	88	–17.0 ($CHCl_3$)
B	**(—)-(*R*)-6f**	79	–15.0 ($CHCl_3$)
B	**(+)-(*R*)-5b**	77	+42.7 ($CHCl_3$)
			–12.7 (MeOH)
B	**(—)-(*S*)-5b**	79	–46.9 ($CHCl_3$)
			+16.6 (MeOH)
B	**(+)-(*S*)-1**	71[b]	+16.8 ($CHCl_3$)
B	**(—)-(*R*)-1**	89[b]	–18.7 ($CHCl_3$)

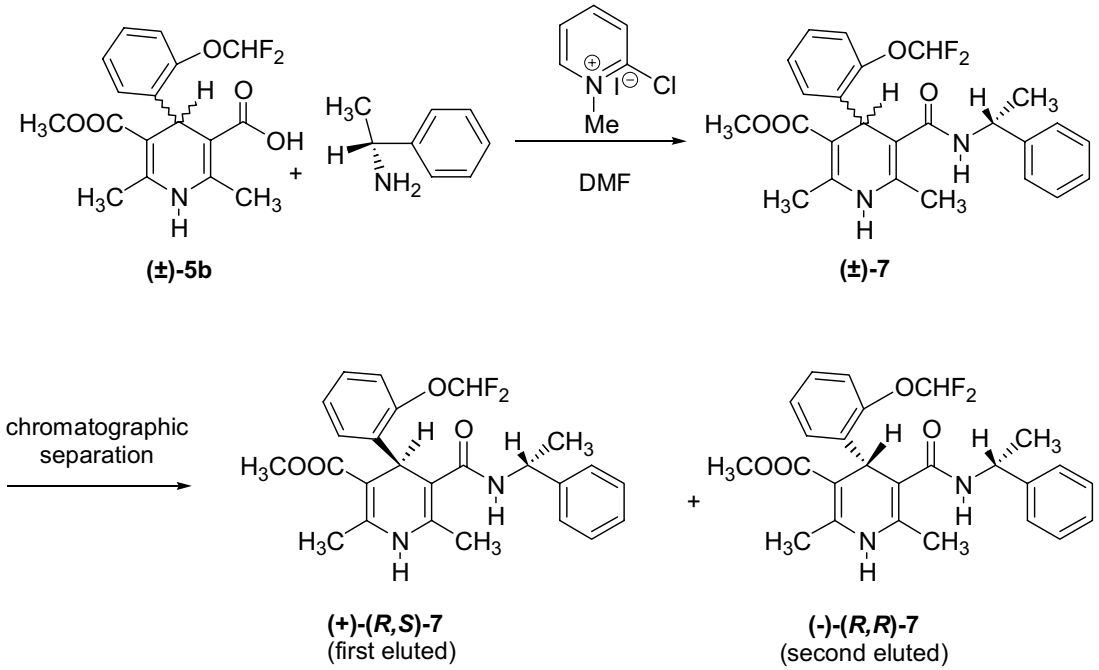
[a] Determined after crystallisation. [b] Obtained from another experiment, where the degree of conversion of the enzymatic reaction was higher than 50%.

Our goal was the preparative synthesis of both enantiomers of **1**. Several synthetic roads can be envisaged for this purpose. The two isobutyryloxymethyl derivatives **6c** and **6f** have been chosen as the starting materials, as in these cases the highest enantioselectivity of CRL was observed. The CRL-catalysed kinetic resolution was stopped halfway to give the optically active monoacid **5a,b** and the remaining optically active substrates **6c,f** (Scheme 3, Table 2). The remaining substrates **(+)-(*R*)-6c** and **(—)-(*R*)-6f** were hydrolysed chemically to the monoacids **(+)-(*S*)-5a** and **(—)-(*S*)-5b**. Pathway A is more preferable from a synthetic point of view because 2-propoxyethanol (propylcellosolve®) was already introduced to the dihydropyridine ring; however, pathway B gives higher e.e. values for the products.

The yield of esterification of the monoacid **(+)-(*R*)-5b** with 2-propoxyethanol (propylcellosolve®) using 2-chloromethylpyridinium iodide (Mukaiyama reagent)[30] was 22%, together with unconverted starting material. This forced us to look for a better method of esterification, which was found in treatment of **(—)-(*S*)-5b** with 4 equivalents of $SOCl_2$, and a subsequent reaction with propylcellosolve to give **(—)-(*R*)-6** in 45% yield. Using less $SOCl_2$ (1-2 equivalents) leads to decarboxylation instead of esterification.

Determination of e.e.'s of the products of the enzymatic reactions has been performed in several ways. The most convenient analysis using HPLC on chiral stationary phases was applied for compound **5a**, since the separation of enantiomers (R_s) was ~1.2. In other cases (**5b, 6c, 6f, 1**) the

optimal conditions of separation using different chiral HPLC columns were not found, and R_s was at best ~0.6-0.8, which was not sufficient for the determination of the e.e. of the enzymatic products. So, the data obtained by this method were only used to determine the approximate e.e. The determination of e.e. of **5b** has been done via coupling to (*R*)-(+)-α-methylbenzylamine;[31] analysis of the obtained diastereomers on a reversed phase column gave R_s >1.2. Enantiomeric excesses of the intermediates **6c,f** and target compound **1** derived from **6c** were assumed to be the same as for **5a** and **5b**. The enantiomeric excess of **1** obtained from **5b** has been determined by ^{1}H NMR using a chiral shift reagent.



Scheme 4

Initial attempts to synthesise derivatives suitable for the determination of the absolute configuration by X-ray diffraction analysis failed. A number of esters of **5b** and optically active or heavy atom-containing alcohols were synthesised, but none of them led to enantiopure crystalline material. Even when the racemic mixtures were crystalline, the enantiopure materials were found to be oils, until the derivative of **(±)-5b** and (*R*)-(+)-α-methylbenzylamine was synthesised. The epimers of **7** (Scheme 4) were separated on reversed phase silica gel, and both diastereomers appeared to be crystalline.

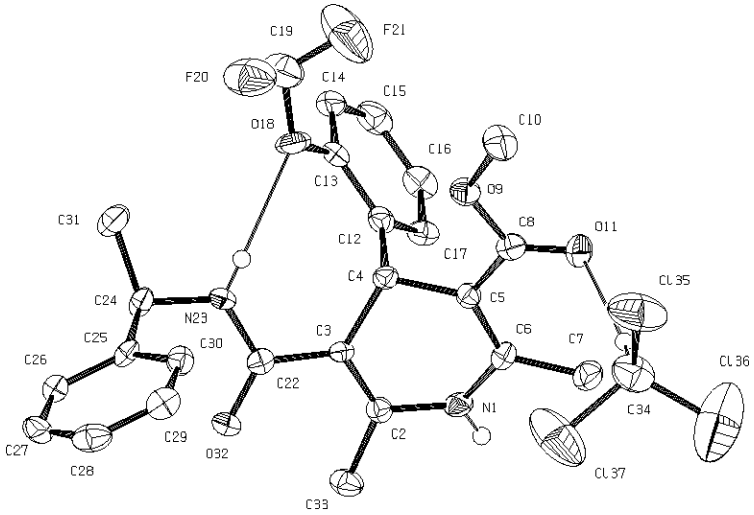


Figure 1

The crystals of the first eluted amide **(+)-(*R,S*)-7** were submitted to X-ray analysis. An ORTEP plot of **(+)-(*R,S*)-7** is given in Figure 1. Besides the indicated intramolecular hydrogen bond the structure also contains an intermolecular hydrogen bond, donated by N1—H to O32, creating an infinite one-dimensional chain of hydrogen-bonded molecules, running in the *a*-direction. The asymmetric unit contains one molecule of co-crystallised chloroform, involved in a short C-H···O interaction with O11. Further details of intra- and intermolecular geometry are given in Figure 2.

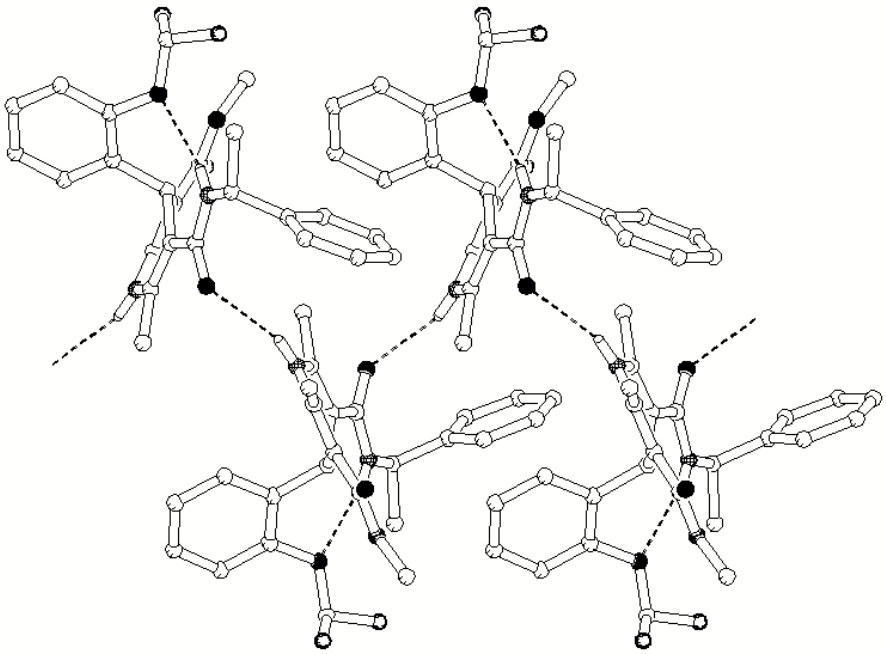


Figure 2

The absolute configuration was initially chosen to be in accordance with the known configuration of C24. The anomalous signal of the co-crystallised chloroform proved to be strong enough for the *ab initio* determination of the absolute configuration of an enantiopure sample.[32] The Flack *x*-parameter[33] amounted to -0.04(8); the *x* parameter for the inverted structure was 1.03(8), indicating a correct assignment of absolute structure.

The acid **(—)-(*S*)-5b,** derived from the remaining substrate **(—)-(*R*)-6f,** was converted to the corresponding amide **7** and appeared to have the same retention time as the **(+)-(*R,S*)**-isomer that was subjected to X-ray analysis. In the same way, the amide derived from the enzymatic product **(+)-(*R*)-5b** coeluted with **(—)-(*R,R*)-7**. So, in the hydrolysis of **6f**, CRL preferentially reacts with the ***S***-isomer. The same enantiopreference of CRL was observed for the hydrolysis of **6c** and other derivatives.

3.3 Conclusions

The chemoenzymatic synthesis of both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate has been performed in moderate to good optical yields, depending on the strategy of the synthesis. The reaction of *Candida rugosa* lipase was dependent on the structure of the substrates: increasing the steric bulk of the hydrolysed acyloxymethyl ester leads to lower reaction rates until the substrate becomes not reactive toward this enzyme at all. At the same time, the enantioselectivity of CRL increases until an enantiomeric ratio (*E*) of 21 is reached for the isobutyryloxymethyl ester. This fine-tuning of the structure of the acyl moiety on the spacer provides a useful method to perform kinetic resolution of hindered or unreactive carboxylic acids. Using this strategy, the CRL catalysed hydrolysis of the isobutyryloxymethyl derivative **6f** yielded a practical method for the synthesis of both enantiomers of a chiral 1,4-dihydropyridine. The absolute configuration of the enzymatic product was assigned by X-ray analysis of its (*R*)-(+)-1-phenylethylamine derivative.

3.4 Experimental Section

3.4.1 General

All reagents were purchased from Aldrich, Acros or Merck and used without further purification. HPLC grade solvents were from Labscan (Dublin, Ireland). Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh or 70-230 mesh) and Baker bond phase C18 for

flash from J.T.Baker (Deventer, The Netherlands). Preparative TLC was performed on 20x20 cm Silica gel TLC-PET F_{254} foils (Fluka). *Candida rugosa* lipase [lipase (EC 3.1.1.3) Type VII from *Candida rugosa*, 875 U/mg] was purchased from Sigma. Lipase AH, Lipase PS were gifts from Amano Pharmaceutical Co., Ltd. (Japan). Immobilised *Candida antarctica* lipase B (Novozym 435®) was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark). Lipase from *Burkholderia cepacia* (CHIRAZYME L-1, c.-f., lyo.) and *Rhizomucor miehei* lipase (CHIRAZYME L-9, c.-f, lyo.) were gifts from Boehringer-Mannheim (Mannheim, Germany). Enzymatic reactions were carried out in a New Brunswick Scientific Innova 4080 or G 24 incubatory orbital shaker at 25ºC. ^{1}H NMR spectra were recorded on a Bruker WH 90/DC (90 MHz) or a Varian Mercury 200BB (200 MHz) or a Bruker AC-E 200 (200 MHz) or a Bruker Avance DPX 400 (400 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AC-E 200 (50 MHz). Chemical shifts are reported in parts per million (ppm) relative to trimethylsilane (δ 0.00). Mass spectral data and accurate mass measurements were determined on a Finnigan MAT 95 mass spectrometer. Melting points were determined on a Boetius apparatus and are uncorrected. Optical rotation values were measured with a Perkin Elmer 241 digital polarimeter. Elemental analyses were determined on a Carlo-Erba elemental analyser. The conversions and *E* values of the enzymatic reactions were analysed by HPLC on a 4.6x250 mm column packed with 5 μm Spherisorb, ODS-2 (Phase Separations) with the solvent system acetonitrile/water/acetic acid (60:40:0.1) as the mobile phase at a flow rate of 1.0 mL/min using a Ginkotek 580A pump and an Applied Biosystems 759A absorbance detector at 254 nm. Alternatively, a 4.6x250 mm Alltima C18 5U (Alltech) column was used with the eluent methanol/water/acetic acid (68:38:0.01) equipped with a detector set at 254 nm. Determination of enantiomeric excesses of **5a** was performed by direct analysis on the chiral column Chirex 3011, 4.6x250 mm, 5 μm (Phenomenex) with detection at 254 nm. The eluent was methanol/dichloromethane (1:2) at a flow rate of 1.0 mL/min. Peak areas were determined electronically with the Chromeleon chromatography data system, Dionex Softron GmbH (Germering, Germany).

Enantiomeric excesses of **(—)-(*R*)-1** and **(+)-(*S*)-1** were determined by ^{1}H NMR (400 MHz) using $Eu(hfc)_3$ in $CDCl_3$ solution. The e.e. was calculated from the splitting of the resonance of one of the 2,6-CH_3 groups into two singlets.

3.4.2 Procedures and spectral data

2-[2-(Difluoromethoxy)benzylidene]methyl acetoacetate, 2b

2-(Difluoromethoxy)benzaldehyde (26.15 g, 0.15 mol) and methyl acetoacetate (21.37 g, 0.18 mol) were added to a mixture of 2-propanol (10 mL), piperidine (1 mL) and acetic acid (1 mL). After being stirred at rt for 6 h, the solvent was removed *in vacuo*. The residue was twice crystallised from diethyl ether-hexane mixture to give 21.80 g (53.2%) of **2b** as white crystals, mp 51-53°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.44 (3H, s, CH_3), 3.77 (3H, s, $COOCH_3$), 6.53 (1H, t, J_{H-F}=73.2 Hz, $OCHF_2$), 7.20-7.45 (4H, m, Ar-H), 7.82 (1H, s, CH). Anal. calcd for $C_{13}H_{12}F_2O_4$: C, 57.78; H, 4.47; found: C, 57.87; H, 4.28.

2-Cyanoethyl 3-aminocrotonate, 3[34]

This compound was prepared by a modified method: to 10.00 g (0.06 mol) of *2*-cyanoethyl acetoacetate, 15 mL (0.20 mol) of 25% aqueous ammonia and 0.3 mL of acetic acid were added with stirring. The reaction mixture was maintained at rt for 2 h, after which the white precipitate was filtered off. Crystallisation from methanol gave 6.50 g (71%) of **3** as white crystals: mp 88°C; (DMSO-d_6, 90 MHz) δ 1.85 (3H, s, CH_3), 2.60 (2H, t, *J*=6.5 Hz, CH_2CH_2CN), 4.00 (2H, t, *J*=6.5 Hz, CH_2CH_2CN), 4.45 (1H, s, CH), 7.20 (2H, br s, NH_2). Anal. calcd for $C_7H_{10}N_2O_2$: C, 54.53; H, 6.53; N, 18.17; found: C, 54.35; H, 6.51; N, 17.95.

3-(2-Cyanoethyl) 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 4a

2-(Difluoromethoxy)benzaldehyde (17.21 g, 0.10 mol) and propoxyethyl acetoacetate (18.82 g, 0.10 mol) were added to a mixture of 2-propanol (150 mL), a catalytic amount of piperidine, and acetic acid (0.5 mL). After being stirred at reflux for 5 h, the solvent was removed *in vacuo*. The residue was dissolved in ether and washed with water twice, dried over $MgSO_4$, and concentrated under reduced pressure to give 18.2 g (0.053 mol) of crude (E,Z)-2-[2-(difluoromethoxy)benzylidene]propoxyethyl acetoacetate **2a**, which was used without further purification: ^{1}H NMR ($CDCl_3$, 90 MHz) δ (major peaks) 0.80 and 0.88 (3H, t, *J*=7.0 Hz, $CH_2CH_2CH_3$), 1.46 (2H, sextet, *J*=7.0 Hz, $CH_2CH_2CH_3$), 2.25 and 2.38 (3H, s, CH_3), 3.30 (2H, t, *J*=7.0 Hz, $CH_2CH_2CH_3$), 3.40-3.70 (2H, m, $COOCH_2CH_2$), 4.15-4.35 (2H, m, $COOCH_2CH_2$), 6.48 and 6.50 (1H, t, *J*=72.5 Hz, $OCHF_2$), 6.95-7.50 (4H, m, Ar-H), 7.80 and 7.82 (1H, s, CH).

Crude **2a** (18.2 g, 0.053 mol) and 8.15 g (0.053 mol) of 2-cyanoethyl 3-aminocrotonate **3** were refluxed in 50 mL isopropyl alcohol for 6 h. After cooling until –5°C overnight, the precipitate

containing 90% of the main product was filtered off. The precipitate was flash chromatographed with chloroform/hexane/acetone (9:7:1) to give 17.5 g (69%) of **4a** as pale yellow crystals: mp 123-124°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.86 (3H, t, *J*=7.0 Hz, $CH_2CH_2CH_3$), 1.53 (2H, sextet, *J*=7.0 Hz, $CH_2CH_2CH_3$), 2.27 (3H, s, CH_3), 2.28 (3H, s, CH_3), 2.63 (2H, t, *J*=6.5 Hz, CH_2CH_2CN), 3.32 (CH_2, t, *J*=7.0 Hz, $CH_2CH_2CH_3$), 3.55 (2H, t, *J*=5.1 Hz, $COOCH_2CH_2O$), 4.12 (2H, t, *J*=5.3 Hz, $COOCH_2CH_2O$), 4.19 (2H, t, *J*=6.5 Hz, CH_2CH_2CN), 5.23 (1H, s, CH), 6.03 (1H, br s, NH), 6.58 (1H, dd, J_{H-F}=73.8, 76.8 Hz, $OCHF_2$), 6.96-7.18 (3H, m, Ar-H), 7.37 (1H, dd, *J*=2.0, 7.0 Hz, Ar-H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.50 (CH_3), 17.85 (CH_2), 19.55 (CH_3), 19.73 (CH_3), 22.78 (CH_2), 35.55 (CH), 58.18 (CH_2), 63.06 (CH_2), 68.56 (CH_2), 72.72 (CH_2), 101.57 (C), 103.10 (C), 116.91 (CN), 117.30 (CH, t, *J*=264.6 Hz, $OCHF_2$), 118.22 (CH), 125.18 (CH), 127.81 (CH), 131.71 (CH), 138.25 (C), 144.53 (C), 146.16 (C), 149.29 (C), 166.78 (C), 167.46 (C); IR (Nujol) 2260 cm^{-1} (CN); MS *m/z* (rel. abund.): 478 (M^+, 8), 391 (7), 368 (15), 347 (6), 336 (20), 335 (100), 249 (11), 196 (8), 43 (7), 32 (11); HRMS calcd for $C_{24}H_{28}F_2N_2O_6$ 478.1915, found: 478.1910. Anal. calcd for $C_{24}H_{28}F_2N_2O_6$: C, 60.24; H, 5.89; N, 5.85; found: C, 59.88; H, 5.90; N, 5.78;

3-(2-Cyanoethyl) 5-methyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 4b

A solution of 5.00 g (19 mmol) of **2b** and 2.86 g (19 mmol) 2-cyanoethyl 3-aminocrotonate was refluxed in 20 mL ethanol for 6 h. After cooling until –5°C, the precipitate was filtered off. Crystallisation from ethanol gave 5.20 g (69%) of **4b** as pale yellow crystals: mp 124-125°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.29 (3H, s, CH_3), 2.30 (3H, s, CH_3), 2.64 (2H, t, *J*=6.4 Hz, CH_2CH_2CN), 3.59 (3H, s, CH_3), 4.20 (2H, t, *J*=6.4 Hz, CH_2CH_2CN), 5.25 (1H, s, CH), 5.86 (1H, br s, NH), 6.52 (1H, dd, J_{H-F}=73.7, 76.6 Hz, $OCHF_2$), 6.96-7.19 (3H, m, Ar-H), 7.36 (1H, dd, *J*=2.0, 7.0 Hz, Ar-H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 17.81 (CH_2), 19.49 (CH_3), 19.80 (CH_3), 35.11 (CH), 50.92 (CH_3), 58.19 (CH_2), 101.71 (C), 103.32 (C), 116.91 (CN), 117.23 (CH, t, *J*=279.7 Hz, $OCHF_2$), 117.89 (CH), 125.23 (CH), 127.83 (CH), 131.47 (CH), 138.24 (C), 144.16 (C), 146.10 (C), 149.02 (C), 166.64 (C), 167.85 (C); IR (Nujol) 2260 cm^{-1} (CN); MS *m/z* (rel. abund.): 406 (M^+, 6), 264 (14), 263 (100), 210 (8); HRMS calcd for $C_{20}H_{20}F_2N_2O_5$ 406.1440, found 406.1333. Anal. calcd for $C_{20}H_{20}F_2N_2O_5$: C, 59.11; H, 4.96; N, 6.89; found: C, 59.00; H, 5.05; N, 6.72.

4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-[(2-propoxyethoxy)carbonyl]-1,4-dihydro-3-pyridinecarboxylic acid, 5a

Crushed KOH (0.73 g, 13 mmol) was added to a solution of 4.78 g (10 mmol) of **4a** in 40 mL of ethanol. The reaction mixture was stirred at 40°C for 3 h and then 6 h at rt. Then the reaction

mixture was evaporated and the residue was dissolved in water and washed twice with 30 mL chloroform. The ice cooled aqueous layer was acidified with HCl to pH 4.0-5.0. The precipitated product was filtered off, washed with water, and dried over $MgSO_4$ in desiccator to give 2.90 g (86%) of **5a** as a pale yellow powder: mp 138-140°C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 0.82 (3H, t, J=7.0 Hz, $CH_2CH_2CH_3$), 1.46 (2H, sextet, J=7.0 Hz, $CH_2CH_2CH_3$), 2.22 (6H, s, 2xCH_3), 3.30 (2H, t, J=7.0 Hz, $CH_2CH_2CH_3$), 3.45-3.51 (2H, m, $COOCH_2CH_2$), 3.95-4.06 (2H, m, $COOCH_2CH_2$), 5.12 (1H, s, CH), 6.93 (1H, t, J_{H-F}=76.6 Hz, $OCHF_2$), 6.92-7.30 (4H, m, Ar-H), 8.75 (1H, br s, NH), 11.63 (1H, br s, COOH); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 10.46 (CH_3), 18.28 (2xCH_3), 22.39 (CH_2), 34.85 (CH), 62.35 (CH_2), 67.95 (CH_2), 71.81 (CH_2), 100.70 (C), 101.80 (C), 117.10 (CH, t, J=254.6 Hz, $OCHF_2$), 117.66 (CH), 125.00 (CH), 127.39 (CH), 131.17 (CH), 139.17 (C), 145.04 (C), 145.94 (C), 148.43 (C), 167.04 (C), 168.74 (C). Anal. calcd for $C_{21}H_{25}F_2NO_6$: C, 59.29; H, 5.92; N, 3.29; found: C, 59.38; H, 6.00; N, 3.85.

4-[2-(Difluoromethoxy)phenyl]-5-(methoxycarbonyl)-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic acid, 5b

This compound was prepared via the same method used for compound **5a**, but beginning with 2.20 g (5.0 mmol) of **4b**, after addition of 0.30 g (5.3 mmol) of KOH, the reaction mixture was stirred for 6 h at rt, the washing step with chloroform was omitted. Compound **5b** was obtained in 86% (1.65 g), as a pale yellow powder: mp 153-154°C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.21 (3H, s, CH_3), 2.24 (3H, s, CH_3), 3.49 (3H, s, $COOCH_3$), 5.14 (1H, s, CH), 6.97 (1H, t, J_{H-F}=76.0 Hz, $OCHF_2$), 6.99-7.28 (4H, m, Ar-H), 8.76 (1H, br s, NH), 11.61 (1H, br s, COOH); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 18.12 (CH_3), 18.21 (CH_3), 34.23 (CH), 50.42 (CH_3), 100.96 (C), 101.85 (C), 117.07 (CH, t, J=254.6 Hz, $OCHF_2$), 117.43 (CH), 125.14 (CH), 127.42 (CH), 130.79 (CH), 139.30 (C), 145.14 (C), 145.63 (C), 148.05 (C), 167.51 (C), 168.68 (C). Anal. calcd for $C_{17}H_{17}NF_2O_5$: C, 57.79; H, 4.85; N, 3.96; found C, 57.65, H, 4.82, N, 3.85.

3-[(Propionyloxy)methyl] 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 6a

To a solution of 478 mg (2.0 mmol) of **4a** in 5 mL of ethanol was added a solution of 135 mg (2.4 mmol) of KOH in 1 mL of ethanol at rt. The resulting mixture was stirred for 1 h, after which the reaction mixture was evaporated until the solvent was completely removed. The residue was diluted with 2 mL of dry DMF, after which 139 mg (2.6 mmol) of propionyloxymethyl chloride was added. The reaction mixture was stirred overnight and then diluted with $CHCl_3$ and washed successively twice with water and twice with brine, dried over $MgSO_4$, and evaporated. The remaining residue

was flash chromatographed on silica gel with petroleum ether (bp 40-60°C)/ethyl acetate (7:3) to give 683 mg (67%) of 90% pure **6a** as a yellow oil, which can be purified on a preparative TLC silica gel plate with petroleum ether (bp 40-60°C)/acetone (5:2); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.89 (3H, t, *J*=7.5 Hz, $CH_2CH_2CH_3$), 1.05 (3H, t, *J*=7.5 Hz, CH_2CH_3), 1.60 (2H, sextet, *J*=7.5 Hz, $CH_2CH_2CH_3$), 2.15-2.30 (2H, m, CH_2CH_3), 2.24 (3H, s, CH_3), 2.27 (3H, s, CH_3), 3.36 (2H, t, *J*=7.4 Hz, $OCH_2CH_2CH_3$), 3.53-3.63 (2H, m, $COOCH_2CH_2O$), 4.11-4.17 (2H, m, $COOCH_2CH_2O$), 5.22 (1H, s, CH), 5.69 (2H, s, OCH_2O), 6.16 (1H, s, NH), 6.53 (1H, t, $J_{H\text{-}F}$=74.7 Hz, $OCHF_2$), 6.91-7.16 (3H, m, Ar-H), 7.35 (1H, dd, *J*=2.1, 7.4 Hz, Ar-H); ^{13}C ($CDCl_3$, 50 MHz) δ 8.65 (CH_3), 10.50 (CH_3), 19.38 (CH_3), 19.88 (CH_3), 22.80 (CH_2), 27.23 (CH_2), 36.09 (CH), 62.98 (CH_2), 68.47 (CH_2), 72.88 (CH_2), 78.41 (CH_2), 100.93 (C), 103.12 (C), 116.90 (CH, t, *J*=254.7 Hz, $OCHF_2$), 117.71 (CH), 124.70 (CH), 127.65 (CH), 132.15 (CH), 137.56 (C), 144.12 (C), 147.06 (C), 149.85 (C), 166.10 (C), 167.46 (C), 173.23 (C). Ms *m/z* (rel. abund.): 511 (M^+, 11), 424 (8), 408 (16), 369 (19), 368 (100), 294 (8), 282 (45), 196 (15), 43 (9), 31 (13); HRMS calcd for $C_{25}H_{31}F_2NO_8$ 511.2018, found 511.2022.

3-[(Isobutyryloxy)methyl] 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 6c

This compound was prepared via the same method used for compound **6a**. Beginning with 957 mg (2 mmol) of **4a**, 135 mg (2.4 mmol) of KOH, and 355 mg (2.6 mmol) of isobutyryloxymethyl chloride, **6c** was obtained in 72% (762 mg); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.89 (3H, t, *J*=7.4 Hz, $CH_2CH_2CH_3$), 1.01 (3H, d, *J*=6.8 Hz, $CHCH_3$), 1.05 (3H, d, *J*=6.8 Hz, $CHCH_3$), 1.56 (2H, sextet, *J*=7.4 Hz, $CH_2CH_2CH_3$), 2.25 (3H, s, CH_3), 2.29 (3H, s, CH_3), 2.42 (1H, septet, *J*=6.8 Hz, $CH(CH_3)_2$), 3.36 (CH_2, t, *J*=6.7 Hz, $CH_2CH_2CH_3$), 3.50-3.64 (2H, m, $COOCH_2CH_2O$), 4.04-4.20 (2H, m, $COOCH_2CH_2O$), 5.22 (1H, s, CH), 5.68 (2H, s, OCH_2O), 6.01 (1H, br s, NH), 6.55 (1H, dd, $J_{H\text{-}F}$=74.1, 76.2 Hz, $OCHF_2$), 6.93-7.15 (3H, m, Ar-H), 7.33 (1H, dd, *J*=2.1, 7.3 Hz, Ar-H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.50 (CH_3), 18.57 (2xCH_3), 19.34 (CH_3), 19.91 (CH_3), 22.79 (CH_2), 33.63 (CH), 36.00 (CH), 62.97 (CH_2), 68.45 (CH_2), 72.89 (CH_2), 78.51 (CH_2), 101.01 (C), 103.14 (C), 117.00 (CH, t, *J*=250.1 Hz, $OCHF_2$), 117.62 (CH), 124.75 (CH), 127.65 (CH), 132.08 (CH), 137.65 (C), 144.06 (C), 146.98 (C), 149.84 (C), 166.05 (C), 167.50 (C), 172.85 (C); Ms *m/z* (rel. abund.): 525 (M^+, 10), 408 (14), 383 (20), 382 (100), 320 (10), 294 (12), 282 (50), 226 (13), 196 (18), 43 (28); HRMS calcd for $C_{26}H_{33}F_2NO_8$ 525.2174, found 525.2175.

3-[(Isobutyryloxy)methyl] 5-methyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 6f

To a solution of 3.00 g (8.5 mmol) of **5b** in 6 mL of dry DMF, 1.41 g (10.2 mmol) of K_2CO_3 was added at rt and the reaction mixture was stirred for 2 h, after which 1.50 g (11.0 mmol) of isobutyryloxymethyl chloride was added. The mixture was stirred overnight, diluted with $CHCl_3$ and washed with water (three times) and brine, dried over $MgSO_4$, and evaporated. The remaining residue was flash chromatographed on silica gel with petroleum ether (bp 40-60°C)/ethyl acetate (2:1) followed by crystallisation from ethanol to give 2.67 g (71%) of **6f** as white crystals: mp 112-113°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (3H, d, *J*=6.7 Hz, CHC*H*$_3$), 1.06 (3H, d, *J*=6.7 Hz, CHC*H*$_3$), 2.27 (3H, s, CH_3), 2.31 (3H, s, CH_3), 2.44 (1H, septet, *J*=6.7 Hz, C*H*$(CH_3)_2$), 3.58 (1H, s, $COOCH_3$), 5.23 (1H, s, CH), 5.69 (2H, s, OCH_2O), 5.81 (1H, br s, NH), 6.53 (1H, dd, $J_{H\text{-}F}$=74.2, 76.1 Hz, $OCHF_2$), 6.93-7.16 (3H, m, Ar-H), 7.32 (1H, dd, *J*=2.1, 7.3 Hz, Ar-H); ^{13}C ($CDCl_3$, 50 MHz) δ 18.57 (2xCH_3), 19.28 (CH_3), 19.93 (CH_3), 35.65 (CH), 35.73 (CH), 50.90 (CH_3), 78.50 (CH_2), 101.11 (C), 103.31 (C), 116.84 (CH, t, *J*=256.7 Hz, $OCHF_2$), 117.57 (CH), 124.82 (CH), 127.70 (CH), 131.78 (CH), 137.69 (C), 143.98 (C), 147.01 (C), 149.70 (C), 166.05 (C), 167.99 (C), 175.88 (C); MS: m/z (rel. abund.) 453 (M^+, 10), 352 (7), 336 (20), 311 (14), 310 (80), 308 (8), 211(11), 210 (100); HRMS calcd for $C_{22}H_{25}NO_7F_2$ (M^+) m/z 453.1599, found 453.1597. Anal. calcd for $C_{22}H_{25}NO_7F_2$: C, 58.28; H, 5.56; N, 3.09; found: C, 58.12; H, 5.53; N, 3.03.

3-[(Butyryloxy)methyl] 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 6b

This compound was prepared via the same method used for compound **6f**, but beginning with 213 mg (0.5 mmol) of **5a**, 35 mg (0.25 mmol) of K_2CO_3, and 889 mg (0.65 mmol) of butyryloxymethyl chloride in 0.5 mL dry DMF. Flash chromatography on silica gel with petroleum ether (bp 40-60°C)/ethyl acetate (7:3) gave 137 mg (52%) of **6b** as a yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.86 (3H, t, *J*=7.6 Hz, $CH_2CH_2CH_3$), 0.88 (3H, t, *J*=7.6 Hz, $CH_2CH_2CH_3$), 1.53 (2H, sextet, *J*=7.6 Hz, $CH_2CH_2CH_3$), 1.55 (2H, sextet, *J*=7.6 Hz, $CH_2CH_2CH_3$), 2.18 (2H, t, *J*=7.6 Hz, $CH_2CH_2CH_3$), 2.27 (3H, s, CH_3), 2.29 (3H, s, CH_3), 3.35 (2H, t, *J*=7.6 Hz, $OCH_2CH_2CH_3$), 3.50-3.62 (2H, m, $COOCH_2CH_2O$), 4.05-4.19 (2H, m, $COOCH_2CH_2O$), 5.21 (1H, s, CH), 5.69 (2H, ABq, OCH_2O), 5.92 (1H, s, NH), 6.53 (1H, dd, $J_{H\text{-}F}$=74.3, 75.9 Hz, $OCHF_2$), 6.92-7.15 (3H, m, Ar-H), 7.33 (1H, dd, *J*=2.1, 7.4 Hz, Ar-H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.50 (CH_3), 13.51 (CH_3), 18.03 (CH_2), 19.45 (CH_3), 19.98 (CH_3), 22.80 (CH_2), 35.77 (CH_2), 36.11 (CH), 62.98 (CH_2), 68.46 (CH_2), 72.88 (CH_2), 78.39 (CH_2), 101.02 (C), 103.19 (C), 116.97 (CH, t, *J*=250.1 Hz, $OCHF_2$), 117.73 (CH), 124.72 (CH), 127.67 (CH), 132.14 (CH), 137.57 (C), 144.03 (C), 146.91 (C), 149.93 (C), 166.07

(C), 167.42 (C), 172.39 (C); Ms *m/z* (rel. abund.): 525 (M^+, 13), 408 (13), 383 (20), 382 (100), 294 (10), 283 (8), 282 (54), 196 (15), 43 (8); HRMS calcd for $C_{26}H_{33}F_2NO_8$ 525.2174, found 525.2182.

3-[(Propionyloxy)methyl] 5-methyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 6d

This compound was prepared via the same method used for compound **6f**, but beginning with 1.06 g (3.0 mmol) of **5b,** 0.62 g (4.5 mmol) of K_2CO_3, and 0.55 g (4.5 mmol) of propionyloxymethyl chloride in 2 mL of dry DMF. Flash chromatography on silica gel with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (10:10:1) followed by crystallisation from hexane-ethyl acetate and recrystallisation from methanol gave 0.13 g (10%) of **6d** as a pale yellow powder: mp 115-116°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.04 (3H, t, *J*=7.4 Hz, CH_2CH_3), 2.22 (2H, q, *J*=7.4 Hz, CH_2CH_3), 2.25 (3H, s, CH_3), 2.29 (3H, s, CH_3), 3.58 (3H, s, $COOCH_3$), 5.21 (1H, s, CH), 5.63 (2H, s, OCH_2O), 5.94 (1H, br s, NH), 6.54 (1H, t, J_{H-F}=75.0 Hz, $OCHF_2$), 6.88-7.32 (4H, m, Ar-H); ^{13}C ($CDCl_3$, 50 MHz) δ 8.66 (CH_3), 19.34 (CH_3), 19.42 (CH_3), 27.24 (CH_2), 35.78 (CH), 50.91 (CH_3), 78.40 (CH_2), 101.06 (C), 103.31 (C), 116.84 (CH, t, *J*=255.4 Hz, $OCHF_2$), 117.59 (CH), 124.79 (CH), 127.7 (CH), 131.85 (CH), 137.66 (C), 143.98 (C), 147.07 (C), 149.74 (C), 166.10 (C), 167.97 (C), 173.29 (C); Ms *m/z* (rel. abund.): 439 (M^+, 13), 352 (7), 336 (18), 308 (10), 294 (9), 211 (11), 210 (100), 57 (8); HRMS calcd for $C_{21}H_{23}F_2NO_7$ 439.1443, found 439.1437.

3-[(Butyryloxy)methyl] 5-methyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 6e

This compound was prepared via the same method used for compound **6f**, but beginning with 71 mg (0.20 mmol) of **5b,** 33 mg (0.24 mmol) of K_2CO_3, and 35 mg (0.26 mmol) of butyryloxymethyl chloride in 0.5 mL of dry DMF. Purification by flash chromatography on silica gel with petroleum ether (bp 40-60°C)/ethyl acetate (7:3) followed by crystallisation from ethanol gave 64 mg (70%) of **6e,** a pale yellow powder: mp 98-99°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.84 (3H, t, *J*=7.4 Hz, $CH_2CH_2CH_3$), 1.49 (2H, sextet, *J*=7.4 Hz, $CH_2CH_2CH_3$), 2.13 (2H, t, *J*=7.4 Hz, $CH_2CH_2CH_3$), 2.17 (3H, s, CH_3), 2.20 (3H, s, CH_3), 3.52 (1H, s, $COOCH_3$), 5.23 (1H, s, CH), 5.63 (2H, s, OCH_2O), 5.99 (1H, s, NH), 6.46 (1H, dd, J_{H-F}=74.4, 75.7 Hz, $OCHF_2$), 6.84-7.20 (3H, m, Ar-H), 7.26 (1H, dd, *J*=2.1, 7.3 Hz, Ar-H); ^{13}C ($CDCl_3$, 50 MHz) δ 13.50 (CH_3), 18.03 (CH_2), 19.28 (CH_3), 19.91 (CH_3), 35.72 (CH), 35.77 (CH_2), 50.90 (CH_3), 78.36 (CH_2), 101.04 (C), 103.30 (C), 116.84 (CH, t, *J*=255.4 Hz, $OCHF_2$), 117.60 (CH), 124.82 (CH), 127.70 (CH), 131.80 (CH), 137.71 (C), 144.04 (C), 147.13 (C), 149.00 (C), 166.10 (C), 167.99 (C), 172.47 (C); MS: m/z (rel. abund.) 453 (M^+,

14), 352 (8), 336 (20), 311 (15), 310 (90), 308 (9), 294 (8), 211 (11), 210 (100), 71 (4); HRMS calcd for $C_{22}H_{25}NO_7F_2$ 453.1599, found 453.1596.

3-Methyl 5-[(pivaloyloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 6g

This compound was prepared via the same method used for compound **6f**, but beginning with 0.35 g (1 mmol) of **5b**, 0.20 g (1.5 mmol) of K_2CO_3, and 0.30 mL (2 mmol) of pivaloyloxymethyl chloride in 3 mL of dry DMF. The mixture was poured into water, and the precipitate was filtered off and crystallised from methanol to give 0.32 g (69%) of **6f** as a white powder: mp 153-155°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.05 (9H, s, 3xCH_3), 2.25 (3H, s, CH_3), 2.29 (3H, s, CH_3), 3.58 (1H, s, $COOCH_3$), 5.22 (1H, s, CH), 5.69 (2H, ABq, OCH_2O), 5.92 (1H, br s, NH), 6.52 (1H, dd, $J_{H\text{-}F}$=74.0, 76.3 Hz, $OCHF_2$), 6.92-7.16 (3H, m, Ar-H), 7.31 (1H, dd, J=2.1, 7.4 Hz, Ar-H); ^{13}C ($CDCl_3$, 50 MHz) δ 19.33 (CH_3), 19.98 (CH_3), 26.71 (3xCH_3), 35.81 (CH), 38.60 (C), 50.87 (CH_3), 78.67 (CH_2), 101.23 (C), 103.31(C), 116.87 (CH, t, J=256.0 Hz, $OCHF_2$), 117.60 (CH), 124.84 (CH), 127.69 (CH), 131.78 (CH), 137.79 (C), 143.89 (C), 146.74 (C), 149.76 (C), 165.93 (C), 167.97 (C), 177.22 (C); MS: m/z (rel. abund.): 467 (M^+, 14), 336 (31), 334 (12), 325 (16), 324 (100), 294 (34), 224 (19), 210 (56), 166 (14), 57 (28); HRMS calcd for $C_{23}H_{27}F_2NO_7$ 467.1756, found 467.1748. Anal. calcd for $C_{23}H_{27}F_2NO_7$: C, 59.09; H, 5.82; N, 2.99; found: C, 58.95; H, 5.79; N, 3.11.

General procedure of *Candida rugosa* lipase catalysed kinetic resolution of racemic (±)-6c and (±)-6f

To a solution of 0.80 mmol of (±)-**6c,f** in 80 mL of water-saturated diisopropyl ether was added 200 mg of *Candida rugosa* lipase, and the resulting mixture was shaken for 6 h at 25°C and monitored by HPLC. When the conversion reached 50%, the enzyme was removed by filtration and washed additionally with chloroform. The filtrate was concentrated under reduced pressure. The residue was flash chromatographed on silica gel with chloroform/petroleum ether (bp 40-60°C)/isopropyl alcohol (10:20:1 ⇒ 5:5:1) to give the monoacids **(—)-(*R*)-5a** and **(+)-(*R*)-5b** and the remaining substrates **(+)-(*R*)-6c** and **(—)-(*R*)-6f**, respectively.

(4*R*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-[(2-propoxyethoxy)carbonyl]-1,4-dihydro-3-pyridinecarboxylic acid, (—)-(*R*)-5a

Yield: 139 mg (41%) as a white precipitate from ether, mp 62-65°C; 88% e.e. (determined after crystallisation), $[\alpha]_D^{20}$ –32.6 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90 (3H, t, J=7.4 Hz,

$CH_2CH_2CH_3$), 1.57 (2H, sextet, J=7.4 Hz, $CH_2CH_2CH_3$), 2.26 (3H, s, CH_3), 2.29 (3H, s, CH_3), 3.38 (CH_2, t, J=7.4 Hz, $CH_2CH_2CH_3$), 3.51-3.63 (CH_2, m, $COOCH_2CH_2$), 4.08-4.24 (CH_2, m, $COOCH_2CH_3$), 5.21 (1H, s, CH), 5.85 (1H, br s, NH), 6.39 (1H, dd, J_{H-F}=72.8, 77.8 Hz, , $OCHF_2$), 6.98-7.15 (3H, m, Ar-H), 7.35 (1H, dd, J=2.0, 7.6 Hz, Ar-H). The mass spectral data were identical to those described for **(±)-5a** and **(+)-(*S*)-5a**; HRMS calcd for $C_{21}H_{25}F_2N_1O_6$ 425.1650, found 425.1647.

(+)-3-[(Isobutyryloxy)methyl] 5-(2-propoxyethyl) (4*S*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, (+)-(*R*)-6c

Yield: 194 mg (46%) as a colorless viscous oil; 62% e.e., $[\alpha]_D^{20}$ +16.0 (*c* 1.0, MeOH); the ^{1}H NMR and mass spectral data were identical to those described for its racemic precursor **(±)-5c**; HRMS calcd for $C_{26}H_{33}F_2N_1O_8$ 525.2174, found 525.2171.

(+)-(4*R*)-4-[2-(Difluoromethoxy)phenyl]-5-(methoxycarbonyl)-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic acid, (+)-(*R*)-5b

Yield: 127 mg (45%) as a colorless viscous oil, which was triturated from the mixture of chloroform–hexane to give 90 mg (32%) of a white solid; mp 82-84°C; 77% e.e., $[\alpha]_D^{20}$ +42.7 (*c* 1.0, $CHCl_3$); $[\alpha]_D^{20}$ –12.7 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.28 (3H, s, CH_3), 2.29 (3H, s, CH_3), 3.58 (3H, s, $COOCH_3$), 5.21 (1H, s, CH), 5.71 (1H, br s, NH), 6.39 (1H, dd, J_{H-F}=73.1, 77.4 Hz, $OCHF_2$), 6.95-7.17 (3H, m, Ar-H), 7.33 (1H, dd, J=2.0, 7.2 Hz, Ar-H); mass spectral data were identical to those described for its racemic precursor (±)-**5b**; HRMS calcd for $C_{17}H_{17}NO_5F_2$ (M^+) m/z 353.1075, found 353.1074.

(—)-3-[(Isobutyryloxy)methyl] 5-methyl (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, (—)-(*R*)-6f

Yield: 164 mg (45%) as a colorless viscous oil; 79% e.e., $[\alpha]_D^{20}$ –15.0 (*c* 1.0, $CHCl_3$); the ^{1}H NMR and mass spectral data were identical to those described for its racemic precursor **(±)-6f**; HRMS calcd for $C_{22}H_{25}NO_7F_2$ (M^+) m/z 453.1599, found 453.1592.

(+)-(4*S*)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-5-[(2-propoxyethoxy)carbonyl]-1,4-dihydro-3-pyridinecarboxylic acid, (+)-(*S*)-5a

To a stirred solution of 158 mg (0.30 mmol) of **(+)-(*R*)-6c** in 3 mL of ethanol was added a solution of 19 mg (0.33 mmol) of KOH in 0.5 mL of ethanol. After being stirred at rt for 1.5 h the reaction

mixture was evaporated, diluted with water, acidified with diluted HCl until pH 5.0, and extracted with chloroform three times. The organic layer was washed with water and evaporated. The residue was chromatographed on silica gel with petroleum ether (bp 40-60°C)/dichloromethane/isopropyl alcohol (5:5:1) to give 87 mg (68%) of **(+)-(*S*)-5a** as a white precipitate from ether: mp 86-87°C; 62% e.e., $[\alpha]_D^{20}$ +29.8 (*c* 1.0, MeOH). The ^{1}H NMR ($CDCl_3$, 200 MHz) and mass spectral data were identical to those described for (±)-**5a** and **(—)-(*R*)-5a**; HRMS calcd for $C_{21}H_{25}F_2N_1O_6$ 425.1650, found 425.1645.

(—)-(4*S*)-4-[2-(Difluoromethoxy)phenyl]-5-(methoxycarbonyl)-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic acid, (—)-(*S*)-5b

This compound was prepared via the same method used for compound **(+)-(*S*)-5a**, without purification by flash chromatography. After pH adjustment to 5, the precipitated product was filtered off and thoroughly washed with water to give 84 mg (79%) of **(—)-(*S*)-5b** as a white precipitate: mp 87-89°C; 79% e.e., $[\alpha]_D^{20}$ –46.9 (*c* 1.0, $CHCl_3$); the ^{1}H NMR ($CDCl_3$, 200 MHz) and mass spectral data were identical to those described for (±)-**5b** and **(+)-(*R*)-5b**; HRMS calcd for $C_{17}H_{17}NO_5F_2$ (M^+) m/z 353.1075, found 353.1069.

General procedure for the determination of the enantiomeric ratio (*E* value) of the *Candida rugosa* lipase mediated kinetic resolution of racemic substrates 6a-f

To a solution of 0.04 mmol of (±)-**6a-d** in 4 mL of water-saturated diisopropyl ether was added 10 mg of *Candida rugosa* lipase and the resulting mixture was shaken at 25°C. During the investigated period several samples of 50 μL each were taken. The solvent was removed by passing a gentle stream of nitrogen. The samples taken from the reactions of **6a-c** were analysed for the conversion and enantiomeric excess on the chiral column Chirex 3011. The rate of the reactions of **6d-f** were analysed on a reversed phase HPLC column and enantiomeric excesses were determined after conversion to diastereomers of amide **7**: the sample was diluted with 50 μL of DMF and treated with an excess of (*R*)-(+)-α-methylbenzylamine, and kept for 0.5 h at rt after which a small excess of chloromethylpyridinium iodide was added. The reaction mixture was kept for another 2 h, evaporated, and diluted with mobile phase, and the pH of the sample was adjusted by adding acetic acid. The e.e. of the reactions of **6e,f** were analysed on a HPLC column packed with Spherisorb, ODS-2. The e.e. of the reaction of **6d** was analysed on an Alltima HPLC column.

General procedure for preparation of (—)-(*R*)-1 and (+)-(*S*)-1 from the monoacids (—)-(*R*)-5a *and* (+)-(*S*)-5a

To a stirred solution of 64 mg (0.15 mmol) of **(—)-(*R*)-5** or **(+)-(*S*)-5** in 0.4 mL of dry DMF was added 21 mg (0.15 mmol) of K_2CO_3 at rt, and resulting mixture was stirred for 2 h, after which 0.028 mL (0.45 mmol) of MeI was added. The reaction mixture was stirred for another 2 h, diluted with water and extracted with chloroform. The organic layer was washed with water and brine (twice) and dried over $MgSO_4$. After removal of the solvent *in vacuo*, the residue was chromatographed on a silica gel coated preparative TLC plate with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (10:1:1) to give the following.

(—)-3-Methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, (—)-(*R*)-1

Yield: 42 mg (63%) as a yellow oil; 88% e.e., $[\alpha]_D^{20}$ –17.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88 (3H, t, *J*=7.4 Hz, $CH_2CH_2CH_3$), 1.54 (2H, sextet, *J*=7.4 Hz, $CH_2CH_2CH_3$), 2.27 (3H, s, CH_3), 2.28 (3H, s, CH_3), 3.35 (CH_2, t, *J*=7.4 Hz, $CH_2CH_2CH_3$), 3.55 (2H, t, *J*=4.8 Hz, $COOCH_2CH_2O$), 3.58 (3H, s, $COOCH_3$), 4.13 (2H, t, *J*=5.1 Hz, $COOCH_2CH_2O$), 5.26 (1H, s, CH), 5.74 (1H, br s, NH), 6.88 (1H, t, $J_{H\text{-}F}$=75.3 Hz, $OCHF_2$), 6.98-7.16 (3H, m, Ar-H), 7.35 (1H, *J*=2.2, 7.2 Hz, Ar-H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 10.45 (CH_3), 19.43 (CH_3), 19.56 (CH_3), 22.76 (CH_2), 35.71 (CH), 50.76 (CH_3), 62.89 (CH_2), 68.47 (CH_2), 72.79 (CH_2), 102.58 (C), 102.70 (C), 116.97 (CH, t, *J*=255.0 Hz, $OCHF_2$), 118.04 (CH), 124.96 (CH), 127.57 (CH), 131.69 (CH), 138.31 (C), 144.37 (C), 144.68 (C), 149.43 (C), 167.50 (C), 168.05 (C); MS *m/z* (rel. abund.): 439 (M^+, 6), 352 (10), 351 (5), 336 (5), 308 (8), 297 (16), 296 (100), 252 (18), 210 (27), 131 (6), 119 (6), 85 (6), 83 (9), 69 (20); HRMS calcd for $C_{22}H_{27}NO_6F_2$ (M^+) m/z 439.1806, found 439.1800.

(+)-3-Methyl 5-(2-propoxyethyl) (4*S*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, (+)-(*S*)-1

Yield: 45 mg (68%) as a yellow oil; 62% e.e., $[\alpha]_D^{20}$ +13.0 (*c* 1.0, $CHCl_3$); the ^{1}H NMR ($CDCl_3$, 200 MHz) and mass spectral data were identical to those described for **(—)-(*R*)-1**; HRMS calcd for $C_{22}H_{27}NO_6F_2$ (M^+) m/z 439.1806, found 439.1801.

(+)-(*S*)-1 from (+)-(*R*)-5b

To a stirred solution of 91 mg (0.26 mmol) of **(+)-(*R*)-5b** in 0.2 mL of dry DMF was added 0.107 mL (0.77 mmol) of triethylamine at rt. After the solution was stirred for 30 min, 0.053 mL (0.46 mmol) of 2-propoxyethanol and 64 mg (0.28 mmol) of 2-chloromethylpyridinium iodide were

added, and the reaction mixture was stirred overnight. The reaction mixture was diluted with water and extracted with dichloromethane two times. The organic layer washed with water twice, dried over $MgSO_4$, and evaporated. The residue was flash chromatographed on silica gel with petroleum ether (bp 40-60°C)/dichloromethane/isopropyl alcohol (5:5:1) to give 53 mg (58%) of unreacted **(+)-(*R*)-5b** and 25 mg (22%) of **(+)-(*S*)-1** as a yellow oil: 71% e.e.; $[\alpha]_D^{20}$ +16.6 (*c* 1.0, $CHCl_3$). The ^1H NMR and mass spectral data were identical to those described for **(—)-(*R*)-1** above. Please note: **(+)-(*R*)-5b** was obtained from another CRL-catalysed hydrolysis of **6f** where the degree of conversion was higher than 50%.

(—)-(*R*)-1 from (—)-(*S*)-5b

To a stirred solution of 77 mg (0.22 mmol) of **(—)-(*R*)-5b** in 0.2 mL of dry DMF at 0°C 0.064 mL (0.88 mmol) of $SOCl_2$ was added after which the reaction mixture was allowed to come to rt. After being stirred for 2h, 0.05 mL (0.44 mmol) of 2-propoxyethanol was added and the reaction mixture was stirred for another 1h. The reaction mixture was diluted with water and extracted with chloroform. The organic layer was washed with water (twice) and brine, dried over $MgSO_4$ and evaporated. The residue was flash chromatographed on silica gel with ethyl acetate/petroleum ether (bp 40-60°C) (2:3) and purified again on a TLC silica plate with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (10:1:1) to give 43 mg (45%) of **(—)-(*R*)-1** as a yellow oil; 89% e.e., $[\alpha]_D^{20}$ –18.7 (*c* 1.0, $CHCl_3$). The ^1H NMR ($CDCl_3$, 200 MHz) and mass spectral data were identical to those described for **(—)-(*R*)-1**. HRMS calcd for $C_{22}H_{27}F_2NO_6$ 439.1806, found 439.1800. Please note: the starting material **(—)-(*R*)-5b** was obtained from another CRL catalysed hydrolysis of **6f** where the degree of conversion was higher than 50%.

Synthesis and chromatographic separation of epimers of methyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-5-({[(1*R*)-1-phenylethyl]amino}carbonyl)-1,4-dihydro-3-pyridinecarboxylate (*R,S*)-(+)-7 and **(*R,R*)-(—)-7**

To a solution of 141 mg (0.4 mmol) of **(±)-5b** in 0.2 mL of dry DMF was added 0.309 mL (2.4 mmol) of (*R*)-(+)-α-methylbenzylamine at rt, and the reaction mixture was stirred for 1 h, after which 180 mg (0.8 mmol) 2-chloromethylpyridinium iodide was added. The reaction mixture was stirred overnight, diluted with chloroform, washed with water (three times) and brine, dried over $MgSO_4$, and evaporated. The remaining residue was flash chromatographed on silica gel with chloroform/petroleum ether (bp 40-60°C)/isopropyl alcohol (9:7:1) to give 84 mg (46%) of **7** as a colorless viscous oil. The mixture of two diastereomers was separated by flash chromatography on Baker bond phase C18 with acetonitrile/water/acetic acid (60:40:0.1) to give the following.

Methyl (4*S*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-5-({[(1*R*)-1-phenylethyl]amino}-carbonyl)-1,4-dihydro-3-pyridinecarboxylate, *R,S*)-(+)-7

Yield: 29 mg (16%) as colorless needles from a mixture of EtOH, $CHCl_3$ and hexane, mp 101-103°C; $[\alpha]_D^{20}$ +63.2 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.29 (3H, d, *J*=7.0 Hz, CH_3), 2.19 (3H, s, CH_3), 2.22 (3H, s, CH_3), 3.51 (3H, s, $COOCH_3$), 4.96 (1H, quintet, *J*=7.0 Hz, CH), 5.03 (1H, s, CH), 5.65 (1H, br s, NH), 6.51 (1H, dd, J_{H-F}=69.8, 79.6 Hz, $OCHF_2$), 6.51 (1H, br d, *J*=7.0 Hz, NH), 6.00-7.37 (9H, m, C_6H_5+C_6H_4); ^{13}C NMR (50 MHz, $CDCl_3$+CD_3OD) δ 17.29 (CH_3), 18.38 (CH_3), 21.51 (CH_3), 33.69 (CH), 48.66 (CH), 50.31 (CH_3), 100.07 (C), 105.65 (C), 116.8 (CH, dd, *J*=254.4, 259.4 Hz, $OCHF_2$), 117.79 (CH), 125.46 (two CH from the phenyl), 126.16 (CH), 126.49 (CH), 127.67 (CH), 128.04 (two CH from the phenyl), 130.52 (CH), 138.85 (C), 139.73 (C), 143.40 (C), 146.53 (C), 147.03 (C), 167.67 (C), 168.34 (C). Ms *m/z* (rel. abund.): 456 (M^+, 30), 454 (20), 441 (27), 352 (14), 351 (76), 314 (17), 313 (100), 256 (11), 192 (13), 105 (26); HRMS calcd for $C_{25}H_{26}F_2N_2O_4$ 456.1861, found 456.1860. This isomer was the first to elute from the C18 column.

Methyl (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-5-({[(1*R*)-1-phenylethyl]amino}-carbonyl)-1,4-dihydro-3-pyridinecarboxylate, (*R,R*)-(—)-7

Yield: 29 mg (16%) as colorless needles from a mixture of $CHCl_3$ and hexane, mp 142-145°C; $[\alpha]_D^{20}$ –112.2 (*c* 1.0, MeOH) or $[\alpha]_D^{20}$ –146.0 (*c* 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.40 (3H, d, *J*=7.0 Hz, CH_3), 2.16 (3H, s, CH_3), 2.20 (3H, s, CH_3), 3.53 (3H, s, $COOCH_3$), 4.96 (1H, quintet, CH), 5.06 (1H, CH), 5.82 (1H, br s, NH), 6.42 (1H, dd, J_{H-F}=70.2, 79.2 Hz, $OCHF_2$), 6.52 (1H, br d, *J*=7.0 Hz, NH), 6.88-7.33 (9H, m, C_6H_5+C_6H_4); ^{13}C NMR ($CDCl_3$, 50 MHz) 19.36 (CH_3), 19.92 (CH_3), 22.17 (CH_3), 33.69 (CH), 49.07 (CH), 50.94 (CH_3), 101.87 (C), 105.74 (C), 116.8 (CH, dd, *J*=254.1; 260.2 Hz, $OCHF_2$), 118.05 (CH), 125.92 (two CH from the phenyl), 126.68 (two CH from the phenyl), 128.15 (CH), 128.30 (two CH from the phenyl), 131.04 (CH), 139.11 (C), 140.98 (C), 144.04 (C), 145.74 (C), 146.51 (C), 166.56 (C), 168.00 (C); Ms *m/z* (rel. abund.): 456 (M^+, 30), 454 (11), 441 (28), 352 (15), 351 (77), 314 (20), 313 (100), 308 (14), (11), 192 (13), 105 (21); HRMS calcd for $C_{25}H_{26}F_2N_2O_4$ 456.1861, found 456.1863. This isomer was the second to elute from the C18 column.

Crystal data for compound (*R,S*)-(+)-7

$C_{25}H_{26}F_2N_2O_4.CHCl_3$, M_r = 575.85, colorless crystal (0.05 × 0.10 × 0.30 mm), orthorhombic, space group $P2_12_12_1$ (no. 19) with *a* = 7.8831(10), *b* = 14.619(2), *c* = 23.101(3) Å, *V* = 2662.2(6) Å^3, *Z* = 4, D_x = 1.437 g cm^{-3}, 17412 reflections measured, 6034 independent, R_{int} = 0.0651, (1.5° < θ <

27.5°, T = 150 K, Mo Kα radiation, λ = 0.71073 Å) on a Nonius KappaCCD diffractometer on rotating anode. The structure was solved by automated direct methods (SHELXS86) and refined on F^2 (SHELXL-97) for 344 parameters. Refinement converged at a final $wR2$ value of 0.1415, $R1$ = 0.0578 (for 3626 reflections with $I > 2\sigma(I)$), S = 1.061. The N—H hydrogen atom co-ordinates were included as parameters in the refinement, all other hydrogen atoms were included on calculated positions, riding on their carrier atoms. A final difference Fourier showed no residual density outside -0.61 and 0.48 e $Å^{-3}$.

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Chapter 4

An efficient chemoenzymatic approach to enantiomerically pure 4-[2-(difluoromethoxy)phenyl] substituted 1,4-dihydropyridine-3,5-dicarboxylates*

Abstract—An efficient chemoenzymatic synthesis of (—)-3-methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate has been achieved. The key step is a highly stereoselective *Candida rugosa* lipase (CRL)–mediated asymmetrisation of the prochiral bis [(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate.

* Sobolev, A.; Franssen, M. C. R.; Vigante, B.; Cekavicus, B.; Makarova, N.; Duburs, G.; de Groot, Ae. *Tetrahedron: Asymmetry* **2001**, *12*, 3251-3256.

4.1 Introduction

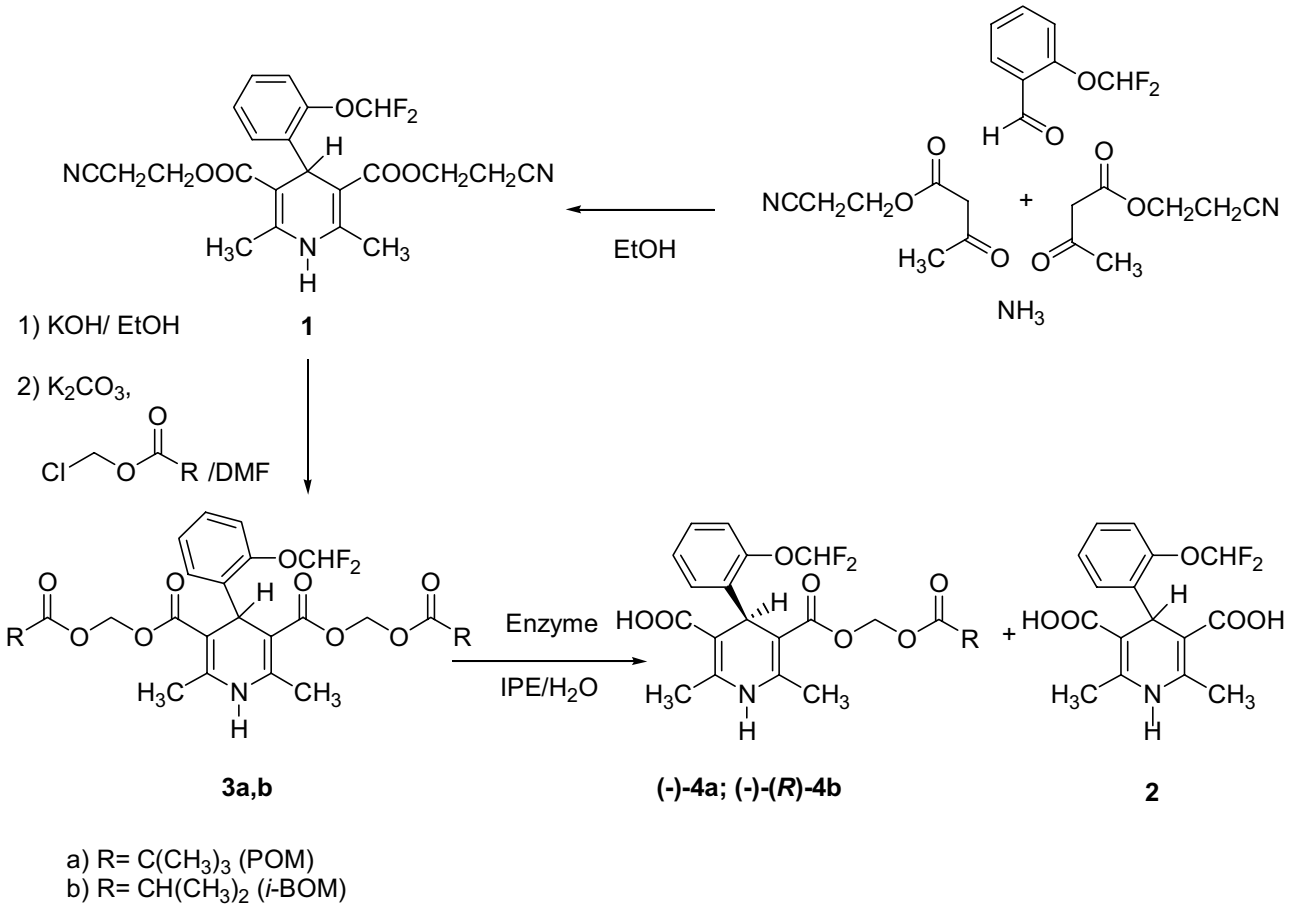
1,4-Dihydropyridines (1,4-DHPs) remain important because of their wide spectrum of biological activities, these include antidiabetic, nootropic, neuromodulatory, cognition and memory enhancing, and neuroprotective properties.[1-4] When substituents on the left side differ from those on the right side of a 1,4-DHP, the molecule is chiral, with C(4) as the stereogenic centre. The enantiomers of an unsymmetrical 1,4-DHP usually differ in their biological activities and could even have an exactly opposite activity profile. Chiral 4-aryl-1,4-DHPs have been the subject of extensive investigation as calcium antagonists for the last two decades[5-10] and the chemoenzymatic synthesis of chiral 1,4-DHPs has been reported by several research groups, including us.[11-16] In this chapter, the *Candida rugosa* lipase-catalysed enantioselective hydrolysis of bifunctional prochiral bis [(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridine-dicarboxylate **3**, which is a building block for the synthesis of potentially biologically active substances is presented. Compound **3** was used to prepare (—)-3-methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, which is an asymmetric analogue of cerebrocrast, a compound with cognition and memory enhancing, neuroprotective properties.[1] Antidiabetic and anti-inflammatory activities of cerebrocrast have been also reported recently.[2-4]

4.2 Results and discussion

The main task of our studies was to reach the best enantioselectivity for the enzyme-mediated hydrolysis of prochiral 4-[2-(difluoromethoxy)phenyl] substituted dihydropyridines. Since ester groups which are directly attached to the heterocyclic ring are not cleaved by most enzymes, the readily cleavable acyloxymethyl esters were introduced at the 3 and 5 positions of the 1,4-DHP. It has been observed previously[16,17] that the size of the transformed acyloxymethyl ester group of a 1,4-DHP exerts a significant influence on the enantioselectivity of the enzymatic hydrolysis. The enantioselectivity of CRL-mediated hydrolysis increased together with the steric hindrance of the acyloxymethyl ester group and the highest enantiomeric ratio (E=21) was reached for racemic 3-[(isobutyryloxy)methyl] 5-methyl 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate.[16] For this reason, the isobutyryloxymethyl and pivaloyloxymethyl derivatives **3a,b** were synthesised.

Substrates **3a,b** were prepared in a three-step sequence as depicted in Scheme 1. Bis cyanoethyl ester **1** was synthesised starting from 2-cyanoethyl acetoacetate, 2-(difluoromethoxy)benzaldehyde and ammonia by a Hantzsch cyclisation in 38% yield. The hydrolysis of the diester **1** with KOH

gave dicarboxylic acid **2** in 73% yield. The substrate **3a** was obtained by the treatment of **2** with pivaloyloxymethyl (POM) chloride in the presence of K_2CO_3 in 57% yield. Compound **3b** was obtained by the treatment of the *in situ* generated diacid **2** with isobutyryloxymethyl (*i*-BOM) chloride in 48% yield.



a) R= $C(CH_3)_3$ (POM)
b) R= $CH(CH_3)_2$ (*i*-BOM)

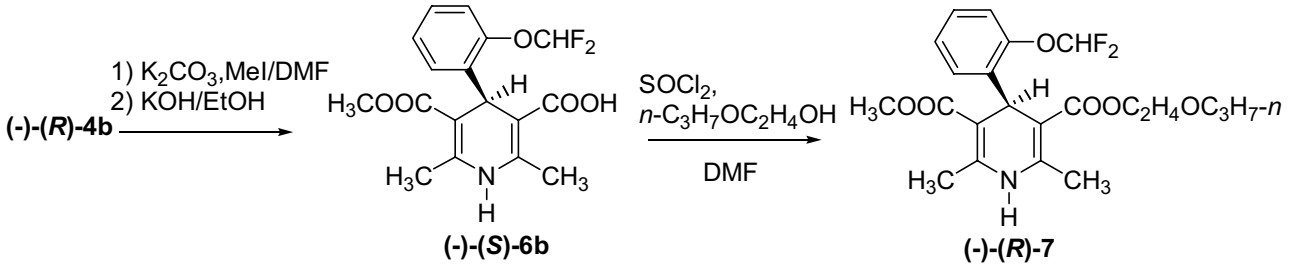
Scheme 1

The bis POM ester **3a** was first tested for hydrolysis with lipase AH, as this enzyme has been used for the asymmetrisation of its 4-(3-nitro)phenyl analogue.[18] The hydrolysis of **3a** by lipase AH in aqueous media at 45°C occurred with formation of **4a** in 25% e.e. When *Candida rugosa* lipase was applied for the hydrolysis of **3a** in water-saturated diisopropyl ether (IPE) at 45°C, the e.e. of monoacid **4a** was not constant and increased from 45% (25% of conversion in 1 day) until 84% of e.e. (34% of conversion in 9 days), together with concomitant hydrolysis to the diacid **2** (9% in 9 days). Apparently, the enantiomeric excess of **4a** was improved by the CRL-catalysed kinetic resolution of enantiomerically enriched **4a** to achiral diacid **2**, as the second hydrolytic step (see Chapter 1, section 1.6.1).[19,20] Longer reaction time led to higher enantiomeric purity but also to a low chemical yield of the product **4a** and therefore this method is not practical.

A better way to asymmetrise **3** involves hydrolysis of the bis *i*-BOM ester **3b**, which could be performed using *Candida rugosa* lipase as a catalyst in water-saturated IPE at rt, as shown in Scheme 1. It is necessary to monitor the reaction carefully by HPLC because of the subsequent hydrolysis to the diacid **2**. The reaction was interrupted when the ratio between the substrate, diacid **2** and **4b** was about 20-20-60. The enantioselectivity of the reaction was excellent (≥99%).

The enantiomeric excess of **4b** was determined by an enantioselective HPLC method using a mixture of **(+)-4b** and **(—)-4b** as a reference standard. This (almost racemic) reference standard was prepared by hydrolysis of **3b** with the lipases of *Rhizomucor miehei* and *Candida antarctica* B. The first enzyme gave **4b** with 11% e.e., whereas *Candida antarctica* lipase B gave **4b** in 13% e.e. with opposite stereopreference.

The monoacid **(—)-(*R*)-4b** was converted into the corresponding methyl ester **(—)-(*R*)-5b** by esterification with MeI in DMF with 87% yield (Scheme 2). The asymmetric analogue of cerebrocrast **(—)-(*R*)-7** was obtained after removal of the *i*-BOM group, treatment with $SOCl_2$, and a subsequent reaction with 2-propoxyethanol (propylcellosolve®) as described in Chapter 3.[16]



Scheme 2

The absolute configuration of **(—)-4b** was proven to be *R*. It was determined by the conversion of **(—)-4b** to **(—)-5b, (—)-6b** and **(—)-7** which showed the same sign of optical rotation as that of **(—)-(*R*)-5b**, **(—)-(*S*)-6b** and **(—)-(*R*)-7** (see Chapter 3, compounds **(—)-(*R*)-6f**, **(—)-(*S*)-5b**, **(—)-(*R*)-1**).[16] The absolute configuration was also proven by coupling of **(—)-(*S*)-6b** with (*R*)-(α)-methylbenzylamine and comparing their order of elution on a reversed phase HPLC column with the data described earlier (see Chapter 3, Scheme 4).[16]

CRL prefers to hydrolyse the *pro-R* ester group of substrate **3b** which is in agreement with the data described in Chapter 3, in which this enzyme reacts preferentially with the *S*-form of the racemic mixture of unsymmetrical analogues of compound **3,** producing *R*-monoacids.[16]

4.3 Conclusions

The present study demonstrates the usefulness of *Candida rugosa* lipase for the asymmetrisation of **3b** with an excellent enantiomeric purity. The enantioselectivity of the CRL-catalysed asymmetrisation of prochiral bifunctional substrates **3a** and **3b** to the monoacids **4a** and **4b** was enhanced by the second hydrolysis to achiral diacid **2** which is highly stereoselective. The synthesis of (—)-3-methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate **(—)-(*R*)-7** with ≥99% e.e. was achieved.

4.4 Experimental

4.4.1 General

All reagents were purchased from Aldrich, Acros or Merck and used without further purification. HPLC grade solvents were from Labscan (Dublin, Ireland). Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh or 70-230 mesh). Preparative TLC was performed on 20x20 cm Silica gel TLC-PET F_{254} foils (Fluka). *Candida rugosa* lipase (lipase (EC 3.1.1.3) Type VII from *Candida rugosa*, 875 U/mg) was purchased from Sigma. Lipase AH was gift from Amano Pharmaceutical Co., Ltd. (Japan). Immobilised *Candida antarctica* lipase B (Novozym 435®) was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark). *Rhizomucor miehei* lipase (CHIRAZYME L-9, c.-f, lyo.) was a gift from Boehringer-Mannheim (Mannheim, Germany). Enzymatic reactions were carried out in a New Brunswick Scientific Innova 4080 incubatory orbital shaker at 25ºC. ^{1}H NMR spectra were recorded on a Varian Mercury 200BB (200 MHz) or a Bruker AC-E 200 (200 MHz) or a Bruker Avance DPX 400 (400 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AC-E 200 (50 MHz). Chemical shifts are reported in parts per million (ppm) relative to trimethylsilane (δ 0.00). Mass spectral data and accurate mass measurements were determined on a Finnigan MAT 95 mass spectrometer. Melting points were determined on a Boetius apparatus and are uncorrected. Optical rotation values were measured with a Perkin Elmer 241 digital polarimeter. Elemental analyses were determined on a Carlo-Erba elemental analyser. The reaction mixtures were analysed by HPLC on a 4.6x250 mm column packed with 5 μm Spherisorb ODS-2 (Phase Separations) with solvent system acetonitrile/water/acetic acid (60:40:0.1) as mobile phase at a flow rate of 1.0 mL/min using a Gynkotek 480 pump and Applied Biosystems 758A programmable absorbance detector at 254 nm. Determination of enantiomeric excesses of **4a** and **4b** was performed by analysis on an enantioselective column Chirex 3011, 4.6x250 mm, 5 μm (Phenomenex) using a Ginkotek 580A

pump and an Applied Biosystems 759A absorbance detector at 254 nm. The eluent was methanol/dichloromethane (1:2) at a flow rate of 1.0 mL/min. Peak areas were determined electronically with the Chromeleon chromatography data system, Dionex Softron GmbH (Germering, Germany). The enantiomeric purity of **(—)-(*R*)-6b** was measured after coupling to (*R*)-(+)-α-methylbenzylamine on a reversed phase ODS-2 column.[16] The enantiomeric excess of **(—)-(*R*)-7** has been determined by ^{1}H NMR using a chiral shift reagent.[16] The enantiomeric excess of the intermediate **(—)-(*R*)-5b** was assumed to be the same as for **(—)-(*R*)-4b**, **(—)-(*S*)-6b** and **(—)-(*R*)-7**.

4.4.2 Procedures and spectral data

Bis(2-cyanoethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridine-dicarboxylate, 1

A solution of 10 g (58 mmol) 2-(difluoromethoxy)benzaldehyde, 18.2 g (117 mmol) 2-cyanoethyl acetoacetate and 10 mL (130 mmol) of 25% aqueous ammonium solution in 50 mL of ethanol was stirred under reflux for 6 h. After cooling the mixture, the precipitate was filtered off and crystallised from ethanol to give 10 g (38%) of **1** as yellow crystals: mp 138-139°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.31 (s, 6H, 2,6-CH_3), 2.65 (t, 4H, *J*=6.8 Hz, 3,5-CH_2CN), 4.22 (t, 4H, *J*=6.8 Hz, 3,5-OCH_2-), 5.25 (s, 1H, 4-CH), 5.95 (br s, 1H, NH), 6.60 (t, 1H, J_{H-F}=78.0 Hz, $OCHF_2$), 7.00-7.19 (m, 3H, Ar-H), 7.37 (dd, 1H, *J*=2.2, 7.3 Hz, Ar-H); ^{13}C NMR ($CHCl_3$, 50 MHz) δ 17.95 (2xCH_2); 19.66 (2xCH_3); 35.33 (CH); 58.27 (2xCH_2); 101.94 (2xC); 116.97, (t, CH, *J*=274.2 Hz, $OCHF_2$); 117.34 (2xCN); 117.99 (CH), 125.28 (CH), 128.04 (CH), 131.57 (CH), 137.91 (C), 146.06 (2xC), 149.15 (C), 166.66 (2xC); MS *m/z* (rel. abund.): 445 (M+, 6); 375 (6), 347 (11), 303 (17), 302 (100), 278 (6), 277 (33), 249 (8), 205 (13), 196 (8); HRMS calcd for $C_{22}H_{21}F_2N_3O_5$ 445.1449, found 445.1442; Anal. calcd for $C_{22}H_{21}F_2N_3O_5$: C, 59.32; H, 4.75; N, 9.43; found: C, 59.33; H, 4.73; N, 9.40.

4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylic acid, 2

To a stirred solution of 2 g (4.5 mmol) of **1** in 50 mL of ethanol was added a solution of 0.55 g (10 mmol) of KOH in 5 mL ethanol. After stirring the mixture at rt for 2 h the solvent was evaporated and the residue was dissolved in water. After cooling down the solution was adjusted to pH 4-5 by adding dilute aqueous HCl. The precipitate was filtered off and washed thoroughly with water to give 1.11 g (73%) of **2**, mp 138-140°C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.19 (s, 6H, 2,6-CH_3), 5.08 (s, H, 4-CH), 6.78 (t, 1H, *J*=75.4 Hz, $OCHF_2$), 6.9-7.3 (m, 4H, Ar-H), 8.52 (br s, H, NH), 11.38 (br s, 2H, 3,5-COOH); ^{13}C NMR (DMSO-d_6, 200 MHz) δ 18.29 (2xCH_3); 34.90 (CH),

101.71 (2xC); 117.26 (t, CH, *J*=246.5 Hz, $OCHF_2$), 117.96 (CH), 125.23 (CH), 127.31 (CH), 131.15 (CH), 139.89 (C), 145.05 (2xC), 148.28 (C), 168.87 (2xC); Anal. calcd for $C_{16}H_{15}F_2NO_5$: C, 56.64; H, 4.46; N, 4.13; found: C, 56.32; H, 4.45; N, 4.20.

Bis [(pivaloyloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, 3a

Compound **2** (3.66 g, 10.8 mmol) was dissolved in 10 mL of DMF, after which 3.70 g (26.9 mmol) of K_2CO_3 was added. The mixture was stirred for 2 h at rt and 3.74 mL (25.92 mmol) of pivaloyloxymethylchloride was added and the resulting mixture was stirred for an additional 24 h. The reaction mixture was diluted with 100 mL of chloroform, washed successively twice with water and twice with brine, dried and evaporated. The residue was triturated with methanol and crystallised from diluted methanol to give 3.5 g (57%) of **3a**, mp 105-107°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.06 (s, 18H, 3,5-C(CH_3)$_3$), 2.26 (s, 6H, 2,6-CH_3), 5.18 (s, H, 4-CH), 5.68 (s, 4H, 3,5-$COOCH_2O$), 6.04 (br s, H, NH), 6.51 (t, 1H, *J*=75.0 Hz, $OCHF_2$), 6.88-7.11 (m, 3H, Ar-H), 7.31 (dd, 1H; *J*=2.0, 7.3 Hz, Ar-H); ^{13}C ($CDCl_3$, 50 MHz) δ 19.79 (2xCH_3), 26.73 (6xCH_3), 36.33 (CH), 38.61 (2xC), 78.90 (2xCH_2), 101.62 (2xC), 116.79 (t, CH, *J*=255.5 Hz, $OCHF_2$), 117.54 (CH), 124.73 (CH), 127.78 (CH), 132.25 (CH), 136.94 (C), 146.13 (2xC), 150.22 (C), 162.84 (2xC), 177.14 (2xC). MS *m/z* (rel. abund.): 567 (M+, 11); 437 (8), 436 (33), 425 (22), 424 (100), 394 (11), 364 (7), 294 (9), 196 (17), 57 (14); HRMS calcd for $C_{28}H_{35}F_2NO_9$ 567.2288, found 567.2273; Anal. calcd for $C_{28}H_{35}F_2NO_9$: C, 59.25; H, 6.22; N, 2.47; found: C, 58.96; H, 6.22; N, 2.41.

Bis [(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, 3b

To a stirred solution of 2.00 g (4.5 mmol) of **1** in 50 mL of ethanol was added a solution of 0.55 g (10 mmol) of KOH in 5 mL ethanol. After being stirred at rt for 2 h the solvent was evaporated. The residue was diluted with 5 mL of dry DMF, after which 1.41 g (10.35 mmol) of isobutyryloxymethyl chloride[21] was added. The reaction mixture was stirred for 18 h, then diluted with water and extracted with chloroform. The organic layer was washed successively twice with water and twice with brine, dried and evaporated. The resulting product was flash chromatographed on silica gel [3:1 petroleum ether (bp 40-60°C)/EtOAc] to give 1.17 g (48%) of **3b** as a yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.03 (d, 6H, *J*=6.8 Hz, 2xCH_3), 1.06 (d, 6H, *J*=6.8 Hz, 2xCH_3), 2.27 (s, 6H, 2,6-CH_3), 2.44 (septet, 2H, *J*=6.8 Hz, 3,5-C*H*(CH_3)$_2$), 5.18 (s, 1H, 4-CH), 5.69 (s, 4H, 3,5-$COOCH_2O$), 6.24 (br s, 1H, NH), 6.76 (t, 1H, J_{H-F}= 74.0 Hz, $OCHF_2$), 6.92-7.12 (m, 3H, Ar-H); 7.32 (dd, 1H, *J*=2.0, 7.3 Hz, Ar-H); ^{13}C ($CDCl_3$, 50 MHz): δ 18.53 (4xCH_3), 19.83 (2xCH_3), 33.61 (2xCH), 36.20 (CH), 78.70 (2xCH_2), 101.59 (2xC), 116.74 (t, CH, *J*=255.29 Hz, $OCHF_2$), 117.41

(CH), 124.60 (CH), 127.76 (CH), 132.21 (CH), 136.82 (C), 146.20 (2xC) 150.18 (C), 165.86 (2xC), 175.74 (2xC); MS *m/z* (rel. abund.): 539 (M+, 11), 422 (30), 397 (20), 396 (100), 322 (7), 297 (8), 296 (54), 294 (18), 196 (43), 71 (8), 43 (11); HRMS calcd for $C_{26}H_{31}NO_9F_2$ (M+) m/z 539.1967, found 539.1963.

(—)-4-[2-(Difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-5-{[(pivaloyloxy)methoxy]-carbonyl}-3-pyridinecarboxylic acid, 4a

A solution of 200 mg (0.35 mmol) of **3a** in 30 mL of acetonitrile was added to 500 mL of 20 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.5) and heated to 45°C, after which 600 mg of Lipase AH was added. The resulting mixture was shaken at 350 rpm and 45°C for 5 days, then the pH of the solution was adjusted to 5.0 by adding 1 M aqueous HCl and extracted three times with ethyl acetate. The organic layers were dried and concentrated under reduced pressure. The residue was crystallised from methanol to give: 140 mg (87%) of **4a**, mp 175-177°C, $[\alpha]_D^{20}$ +1.3 (*c* 1.0, MeOH), e.e.=25%; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.02 (s, 9H, $C(CH_3)_3$), 2.21 (s, 6H, 2,6-CH_3), 5.09 (s, H, 4-CH), 5.62 (s, 2H, $COOCH_2O$), 6.93 (t, 1H, *J*=75.0 Hz, $OCHF_2$), 6.93-7.25 (m, 4H, Ar-H), 8.92 (br s, H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz): 18.24 (CH_3), 18.65 (CH_3); 26.56 (3xCH_3), 35.12 (CH), 38.09 (C), 78.86 (CH_2), 99.21 (C), 102.43 (C), 117.11 (t, CH, *J*=254.74 Hz, $OCHF_2$), 117.61 (CH), 124.98 (CH), 127.62 (CH), 131.32 (CH), 138.55 (C), 144.68 (C), 148.20 (C), 148.84 (C), 165.66 (C), 168.75 (C), 176.40 (C); MS *m/z* (rel. abund.): 453 (M+, 2), 409 (23), 310 (18), 294 (25), 278 (55), 267 (16), 266 (100), 250 (14), 236 (35), 152 (71), 57 (20); HRMS calcd for $C_{22}H_{25}NO_7F_2$ (M+) m/z 453.1599, found 453.1592. Anal. calcd for $C_{22}H_{25}NO_7F_2$: C, 58.27; H, 5.56; N, 3.09; found: C, 57.95; H, 5.54; N, 3.00.

(—)-(4*R*)-4-[2-(Difluoromethoxy)phenyl]-5-{[(isobutyryloxy)methoxy]carbonyl}-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic acid, (—)-*R*-4b

To a solution of 0.539 g (1 mmol) of **3b** in 50 mL of water-saturated IPE was added 0.200 g of *Candida rugosa* lipase and the resulting mixture was shaken for 3.5 h at rt. After removal of the enzyme by filtration, the filtrate was concentrated under reduced pressure. The residue was flash chromatographed on silica gel with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (100:40:5 ⇒ 100:100:20) to give 0.125 g (23 %) of unreacted **3b** and 0.240 g (55%) of **(—)-*R*-4b,** mp 149-151°C or 129-130°C (dec.), from ether-hexane; $[\alpha]_D^{20}$ +26.5 (*c* 1.0, $CHCl_3$), –24.0 (*c* 1.0 MeOH); e.e.≥99 %; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.08 (d, 3H, *J*=6.8 Hz, CH_3), 1.11 (d, 3H, *J*=6.8 Hz, CH_3), 2.31 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.48 (septet, 1H, *J*=6.8 Hz C*H*$(CH_3)_2$), 5.21 (s, 1H, 4-CH), 5.73 (ABq, 2H, $COOCH_2O$), 6.45 (dd, 1H, $J_{H\text{-}F}$=74.4, 76.0 Hz, $OCHF_2$), 7.00-7.16 (m, 3H,

Ar-H), 7.37 (dd, 1H, *J*=2.0, 7.6 Hz, Ar-H); ^{13}C ($CDCl_3$+CD_3OD, 50 MHz) δ 18.45 (2xCH_3), 18.69 (CH_3), 19.03 (CH_3), 33.73 (CH), 35.72 (CH), 78.77 (CH_2), 100.66 (C), 102.71 (C), 117.10 (t, CH, *J*=254.95 Hz, $OCHF_2$), 118.07 (CH), 124.98 (CH), 127.67 (CH), 131.88 (CH), 138.46 (C), 145.87 (C), 148.26 (C), 149.58 (C), 166.62 (C), 170.45 (C), 176.28 (C); MS *m/z* (rel. abund.): 439 (M^+, 2) 395 (17), 296 (12), 294 (19), 278 (28), 253 (9), 252 (64), 250 (11), 196 (13), 152 (100), 44 (18); HRMS calcd for $C_{21}H_{23}F_2NO_7$ 439.1443, found 439.1438. Anal. calcd for $C_{21}H_{23}NO_7F_2$: C, 57.40; H, 5.28; N, 3.19; found: C, 57.69; H, 5.12; N, 3.00.

(—) 3-[(Isobutyryloxy)methyl] 5-methyl (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, (—)-*R*-5b

To a solution of 0.77 g (1.75 mmol) of **(—)-*R*-4b** in 1 mL DMF was added 0.12g (1.75 mmol) of K_2CO_3. The reaction mixture was stirred for 2 h at rt, after which 0.218 mL (3.5 mmol) of MeI was added and the mixture was stirred for another 2 h. The reaction mixture was diluted with water and extracted with chloroform. The organic layers were washed twice with water and brine, dried, and evaporated. The remaining residue was flash chromatographed on silica gel with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (10:1:1) to give 0.69 g of **5b** (1.52 mmol, 87%) as a yellow oil: $[\alpha]_D^{20}$ –17.6 (*c* 1.0, $CHCl_3$); e.e.≥99 %; (see Chapter 3, compound **(—)-(*R*)-6f**[16] 79% e.e., $[\alpha]_D^{20}$ –15.0 (*c* 1.0, $CHCl_3$)). The ^{1}H NMR spectrum was in accordance with the one described in Chapter 3 for compound (±)-**6f**.[16] MS: m/z (rel. abund.) 453 (M^+, 9), 352 (7), 336 (18), 311 (11), 310 (80), 308 (8), 294 (7), 211(11), 210 (100), 69 (11), 43 (7). HRMS calcd for $C_{22}H_{25}NO_7F_2$ 453.1599, found 453.1592.

(—)-(4*S*)-4-[2-(Difluoromethoxy)phenyl]-5-(methoxycarbonyl)-2,6-dimethyl-1,4-dihydro-3-pyridinecarboxylic acid, (—)-(*S*)-6b

This compound was prepared by the same method used in Chapter 3.[16] The product was characterised giving: mp 78-79°C triturated from ether–hexane, e.e.≥99% (Chapter 3, compound **(—)-(*S*)-5b**[16] mp 87-89°C, 79% e.e.); $[\alpha]_D^{20}$ –51.7 (*c* 1.0, $CHCl_3$) (Chapter 3, compound **(—)-(*S*)-5b**[16] $[\alpha]_D^{20}$ –46.9 (*c* 1.0, $CHCl_3$); the ^{1}H NMR was in accordance with the one described in Chapter 3 for compound **(—)-(*S*)-5b**.[16]

(—)-3-Methyl 5-(2-propoxyethyl) (4*R*)-4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate, (—)-(*R*)-7

This compound was prepared by the same method used in Chapter 3.[16] The oily product was characterised giving: e.e.≥99%, $[\alpha]_D^{20}$ –19.7 (*c* 1.0, $CHCl_3$) (Chapter 3, compound **(—)-(*R*)-1**[16] 88%

e.e., $[\alpha]_D^{20}$ –17.0 (*c* 1.0, $CHCl_3$)). The ^1H NMR and mass spectral data were identical with those described in Chapter 3.[16]

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Chapter 5

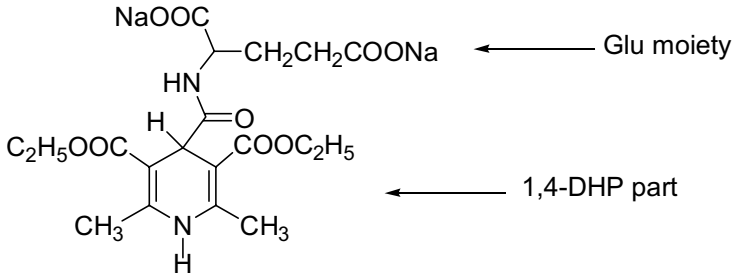
Enantioselective lipase-catalysed kinetic resolution of acyloxymethyl and ethoxycarbonylmethyl esters of 1,4-dihydroisonicotinic acid derivatives [*]

Abstract—The lipase-catalysed kinetic resolution of four derivatives of 4-[(acyloxy)methyl] and 4-ethoxycarbonylmethyl 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridinetricarboxylates has been investigated. Whereas the enantioselectivity of lipases towards the acyloxymethyl derivatives was rather low, the *Candida antarctica* lipase B (Novozym 435®, CAL-B) catalysed hydrolysis of the ethoxycarbonylmethyl ester of 1,4-dihydroisonicotinic acid was enantioselective. In water-saturated diisopropyl ether at 45°C the enantioselectivity of CAL-B toward the ethoxycarbonylmethyl ester was rather moderate (E=13.8), but it was enhanced at rt and +4°C (E=21.5 and E=28.9, respectively). A high enantiomeric ratio (E=45.3) was reached at subzero temperatures, although at the expense of the reaction rate.

[*] Sobolev, A.; Franssen, M. C. R.; Poikans, J.; Duburs, G.; de Groot, Ae. *Tetrahedron:Asymmetry* **2002**, 13, 2389-2397

5.1 Introduction

1,4-Dihydroisonicotinic acid derivatives are important precursors for the synthesis of a novel class of amino acid-containing biologically active 1,4-dihydropyridines (1,4-DHP). The disodium salt of 2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine-4-carboxamido)-glutaric acid (glutapyrone; fig. 1), unlike classical 1,4-DHPs, lacks calcium antagonistic activity and possesses an unusually broad spectrum of biological activities at low concentrations such as neuromodulatory and neuroregulatory action. It is an anticonvulsant, stress-protective, antiarrhythmic, cognition and memory enhancing drug of long-term activity.[1-4]



Glutapyrone

The synthesis of a number of asymmetric structural analogues of nifedipine led to the development of new generations of highly selective calcium antagonist preparations.[5] Enantiomers of unsymmetrical 1,4-DHP usually differ in their biological activities[6-8] and could even have an opposite action profile.[9] Yamanouchi (Japan) was the first to introduce the most potent diastereomer of barnidipine[10,11] in Japan in 1992 and in the Netherlands in 2000, however other calcium antagonists are marketed as racemic mixtures to this day.

Whereas the synthesis and pharmacology of enantiomerically pure 4-aryl-1,4-DHPs as novel calcium antagonists have been widely studied,[12,13] 1,4-dihydroisonicotinic acid derivatives were not synthesised until quite recently and even less information is available on their enantiomerically pure derivatives. The synthesis of unsymmetrical 1,4-dihydroisonicotinic acid derivatives in enantiopure form is highly necessary for the elucidation of their mechanism of action which should result in more efficient and selective preparations *via* rational drug design.

The stereoselective syntheses of 4-alkyl-, aryl- or pyridyl-1,4-DHP-3,5-dicarboxylates *via* enzyme-catalysed transformations of activated esters of 1,4-dihydro-3/5-carboxylic acids has been performed by several research groups.[12-16] Disregarding the fact that the reaction centre (the place of enzyme attack) was quite remote from the stereogenic carbon atom at position 4, in many cases it was possible to obtain excellent e.e.'s of the reaction products.[12,13,17] For acyloxymethyl esters of

1,4-DHP-3,5-dicarboxylates, the enantioselectivity of *Candida rugosa* lipase (CRL) and lipase AH increased together with the length or branching of the acyl chain, whereas the reversed effect was described for lipase PS in water-saturated diisopropyl ether (IPE).[16,18] Generally, CRL and *Candida antarctica* lipase B (CAL-B) are very efficient catalysts and are widely used in practice. Initially, CAL-B has been designed as an additive to detergents due to its extreme resistance against deactivation by oxidising agents and temperature.[19] The usefulness of CAL-B was reported at subzero temperatures[20] as well as at 90°C[21].

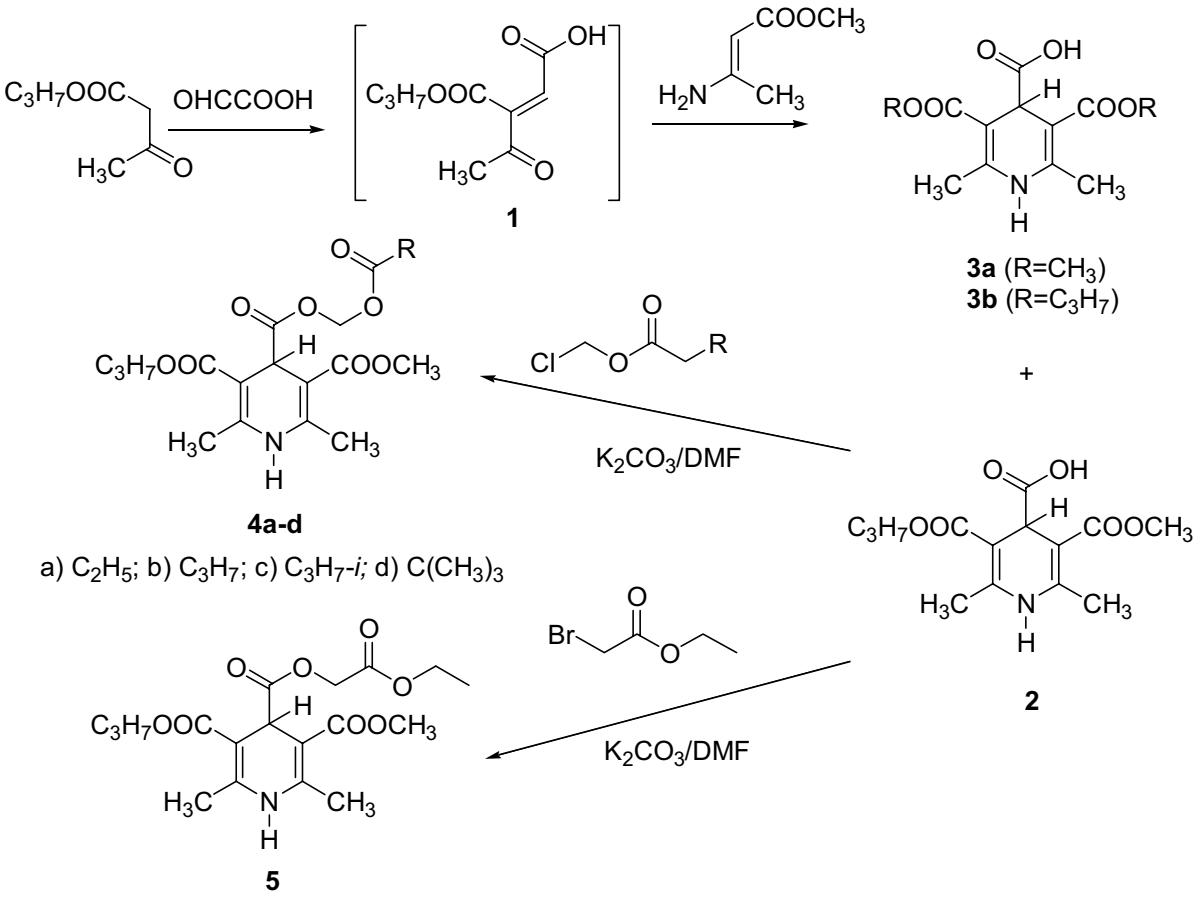
In this chapter, the synthesis of enantiomerically enriched derivatives of 1,4-dihydroisonicotinic acid as key intermediates for the synthesis of unsymmetrical potentially biologically active compounds, *via* lipase-catalysed kinetic resolution of acyloxymethyl and ethoxycarbonylmethyl esters of 1,4-dihydroisonicotinic acid is described.

5.2 Results and discussion

5.2.1 Synthesis of 1,4-dihydroisonicotinic acid 2 and the enzymatically labile esters 4a-d and 5

The synthesis of unsymmetrical derivatives of 1,4-dihydroisonicotinic acid **2** is not very efficient. Glyoxylic acid in reaction with acetoacetates readily forms bis-1,3-dicarbonyl derivatives leading to symmetric 1,4-dihydropyridines **3**.[22] In case of the three component synthesis (glyoxylic acid, alkyl acetoacetate, alkyl β-aminocrotonate) a complicated mixture is formed, wherein symmetric products prevail. Separation of mixtures of symmetrical and unsymmetrical derivatives of 1,4-dihydroisonicotinic acid (**2** and **3a,b**) by chromatography turned out to be problematic. As a result of extensive studies, a method for the synthesis of unsymmetrical 1,4-dihydroisonicotinic acid **2** was elaborated (Scheme 1).

The formation of the undesired symmetrical by-product **3a** is due to coupling of 3-aminocrotonate to unreacted glyoxylic acid. Therefore, to prevent formation of **3a**, propyl acetoacetate was first coupled to glyoxylic acid in a ratio of 3:1, forming *in situ* intermediate **1**. The treatment of the reaction mixture with hydrochloric acid and the subsequent work-up with acetic anhydride markedly reduces the formation of the second by-product (**3b**). The condensation of intermediate **1** with a limited amount of methyl 3-aminocrotonate gives 1,4-dihydroisonicotinic acid **2** as the major reaction product in 13% overall yield.



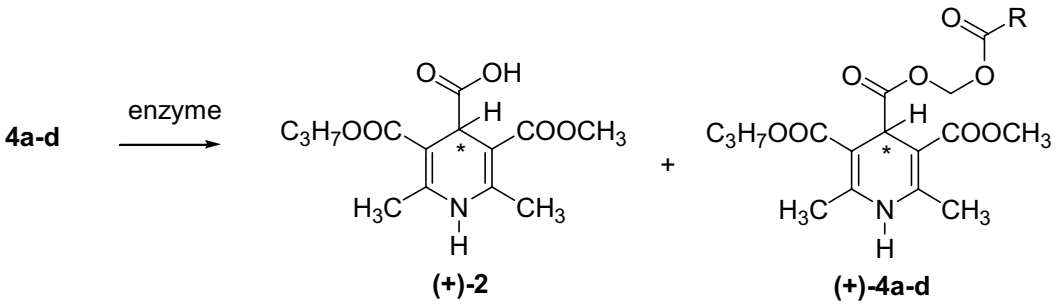
Scheme 1

A chemoenzymatic approach is used for the resolution of 1,4-dihydroisonicotinic acids in the current studies. Similarly to 3,5-dialkyl esters of 1,4-DHPs,[23] methyl and ethyl esters of 1,4-dihydroisonicotinic acid were stable towards treatment with a number of commercially available hydrolases. 1,4-Dihydroisonicotinic acid **2** was turned into a substrate for hydrolases by the attachment of a suitable spacer group. Since there is no literature on enzymatic transformations of these compounds, a primary screening of enzymes and substrates had to be performed. For this purpose, a set of acyloxymethyl esters **4a-d** was prepared by coupling of 1,4-dihydroisonicotinic acid **2** with the corresponding acyloxymethyl chloride by reported methods[16] in 20-72% yields (Scheme 1). The corresponding ethoxycarbonylmethyl ester **5** of 1,4-dihydroisonicotinic acid **2** was synthesised in 83% yield using ethyl bromoacetate (Scheme 1).

5.2.2 Kinetic resolution of acyloxymethyl esters of 1,4-dihydroisonicotinic acid, 4a-d

The lipase-catalysed hydrolysis of compounds **4a-d**, which differ in size and branching of acyloxymethyl ester group, has been investigated. Several commercially available lipases exhibited hydrolytic activity toward these substrates (Scheme 2). The lipases AH, PS and Chirazym L-2 gave

low *E* values (<2) for all derivatives of **4**. Higher selectivities were obtained for CRL and CAL-B (*i.e.*, its immobilised form Novozym 435®). The results of the screening in organic solvents and aqueous media are listed in Table 1. CAL-B possessed an opposite stereopreference toward substrates **4a-d** compared to other tested enzymes.



Scheme 2

Since the enantioselectivity of the enzymes was still too low for practical application, we decided to investigate the temperature dependence of the reaction. Thus, the CRL and Novozym 435® catalysed conversions of **4b** in water saturated IPE in a temperature range from –18°C to +45°C were investigated. The enantioselectivities of both enzymes were rather low and slightly dependent on temperature. The enantioselectivity of CRL increased with the raise of temperature (entries 7-9), whereas the enantioselectivity of Novozym 435® dropped with increase of temperature from *E*=2.3 until *E*=1.7.

CRL showed better enantioselectivity than the other tested enzymes toward acyloxymethyl esters **4a-c** in aqueous medium, however, the *E* value did not exceed 9 (entries 5,6,10,11). Increasing the content of acetonitrile from 10% to 15% in phosphate buffer in order to reach better solubility of the substrates **4b**,**d** also gave better enantioselectivity of CRL (entries 4,5 and 10,11). The use of other co-solvents, such as *t*-BuOH and DMSO, to the phosphate buffer was not successful, as CRL was absolutely not selective towards substrate **4c** under the given reaction conditions. The influence of the bulkiness of the acyloxymethyl group on the enantioselectivity of CRL was not so clear in the examples **4a-d** and in all cases the enantioselectivity was moderate. However, the bulkiness of the acyloxymethyl group did affect the reactivity of the enzyme. The bulky *t*-butyl substituted acyloxymethyl ester **4b** was not reactive at rt and became a substrate for enzymes only at higher temperatures (entries 12-14; Table 1), similarly to the corresponding bis [(pivaloyloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate.[16]

Isobutyryloxymethyl derivative **4c** was used for the preparative synthesis of both enantiomers of 1,4-dihydroisonicotinic acid **2** using CRL in phosphate buffer pH 7.5, modified with 15% of acetonitrile. The CRL-mediated kinetic resolution of **4c** was carried out for 6.5 h with HPLC

control until 50% conversion was reached. The enantiomeric excess of the reaction product (+)-**2** was 61% and for the remaining (+)-**4c** the e.e. was 65%, the *E* value of the conversion was 8.0.

Table 1. Lipase-catalysed hydrolysis of acyloxymethyl esters of 1,4-dihydroisonicotinic acid **4a-d**[a]

Entry	Substrate	Lipase	Reaction medium[a]	Enzyme[d] mg	T, °C	Conversion			*E* value[f]	Major Enantiomer of **2**[e]
						Time, h	%	e.e.$_p$, %		
1	**4a**	Novozym-435®	IPE/H_2O	2	rt	3	55	52	5.0±0.4	1 (—)
2	**4a**	Novozym-435®	*t*-BuOMe/H_2O	2	rt	3	35	57	5.8±0.7	1 (—)
3	**4a**	*Candida rugosa*	IPE/H_2O	2	rt	3	19	41	1.6±0.05	2 (+)
4	**4a**	*Candida rugosa*	Buffer A	1	rt	3.2	40	61	5.0±0.4	2 (+)
5	**4a**	*Candida rugosa*	Buffer B[c]	0.2	rt	17	38	68	7.9±0.2	2 (+)
6	**4b**	*Candida rugosa*	Buffer B	0.2	rt	18	48	66	9.0±0.8	2 (+)
7	**4b**	*Candida rugosa*	IPE/H_2O	4	45	5	58	35	3.2±0.3	2 (+)
8	**4b**	*Candida rugosa*	IPE/H_2O	4	4	22	46	35	2.7±0.4	2 (+)
9	**4b**	*Candida rugosa*	IPE/H_2O	4	-18	23	7	38	2.3±0.1	2 (+)
10	**4c**	*Candida rugosa*	Buffer A	1	rt	3	40	63	7.0±0.7	2 (+)
11	**4c**	*Candida rugosa*	Buffer B	0.5	rt	6.5	52	61	8.0±0.7 E_{tot}=7.9	2 (+)
12	**4d**	*Candida rugosa*	Buffer B	1	rt	12	0	-	-	-
13	**4d**	*Candida rugosa*	Buffer B	1	45	120	12	9	1.2±0.03	2 (+)
14	**4d**	*Candida rugosa*	IPE/H_2O	4	45	104	21	51	3.7±0.2	2 (+)

[a] Typical reaction conditions: 0.01 mmol of substrate **4a-d** was dissolved in 8 mL of phosphate buffer A or phosphate buffer B or 0.8 mL of water-saturated IPE or *t*-BuOMe. [b] Buffer A: 10% solution of acetonitrile in 20 mM K-phosphate buffer, pH 7.5. [c] Buffer B: 15% solution of acetonitrile in 20 mM K-phosphate buffer, pH 7.5. [d] Amount enzyme means mg solid. [e] The number means the order of elution of enantiomers of **2** on the β-cyclodextrin chiral column, for details see the experimental section. [f] $E=E_p=\{\ln[1-c(1+e.e._p)]\}/[\ln(1-c(1-e.e._p)]$; $E_{tot}=\{\ln[e.e._p(1-e.e._s)]/[e.e._p+e.e._s]\}/\{\ln[e.e._p(1+e.e._s)]/[e.e._p+e.e._s]\}$.

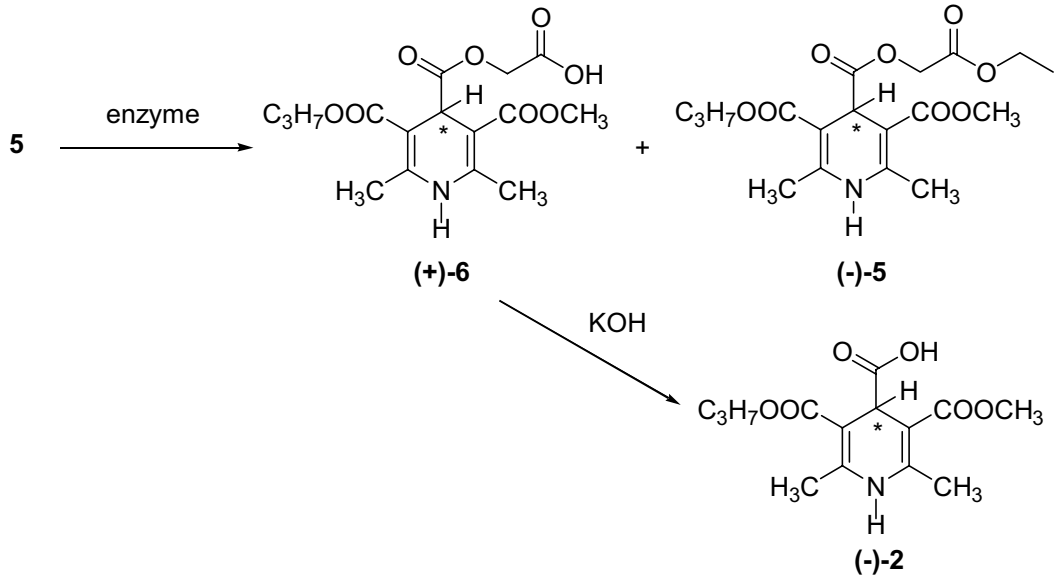
5.2.3 Kinetic resolution of ethoxycarbonylmethyl ester of 1,4-dihydroisonicotinic acid, 5

Preliminary screening tests revealed that lipases CRL, CAL-B, AH, *Rhizomucor miehei*, protease P6 and acylase 30,000 were capable of hydrolysing the ethoxycarbonylmethyl ester of 1,4-dihydroisonicotinic acid **5** (Scheme 3, for details see Table 2). Similarly to the lipase-catalysed hydrolysis of bis(ethoxycarbonylmethyl) substituted 4-substituted 1,4-dihydropyridine-3,5-dicarboxylates,[24] only CAL-B catalysed the hydrolysis of **5** with reasonable enantioselectivity (*E*=8.0 at 45°C and *E*=13.7 at rt) at the 'outer' ester group of the ethoxycarbonylmethyl ester at the 4-position. However, it is worth to note that a trace of **2** was detected. The transition from

phosphate buffer pH 7.5 modified with acetonitrile to water-saturated IPE inclines CAL-B to hydrolyse **5** more stereoselectively (entries 4,5 and 7,8; Table 2). The effect of temperature on the CAL-B catalysed enantioselective hydrolysis was studied in detail (see Table 2). At 45°C the enantioselectivity of the relatively fast reaction was moderate (*E*=13.8), whereas acceptable enantioselectivity of enzyme was reached at rt (*E*=21.5), albeit at the expense of the reaction rate. The highest enantiomeric ratio (*E*=45.3) was reached at –12°C, however, after 2 months of incubation only 35% of the substrate was converted.

During the crystallisation of **(+)-6** with an e.e. of 75% it was found that the e.e. of the crystals was much lower than the e.e. of the mother liquor.

It is possible to obtain both enantiomers of **2** from (+)-**6** and (—)-**5** by chemical hydrolysis in low yields. The reaction was not studied in detail, but the possible cause of the low yields is decarboxylation during the hydrolysis and work-up.[25] The complexity of the reaction mixture obtained also hampers the isolation of the product. The acid **(+)-6** with 85% e.e. gave **(—)-2** with 67% e.e.; the loss of the e.e. occurred during the crystallisation.



Scheme 3

Table 2. Enzyme-catalysed hydrolysis of ethoxycarbonylmethyl esters of 1,4-dihydroisonicotinic acid **5**[a]

Entry	Lipase	Reaction medium	Enzyme[b] mg	T, °C	Conversion			*E* value[e]	Major Enantiomer of **6**[d]
					Time	%	e.e.$_p$, %		
1	Protease P6	Buffer[a]	2	rt	24 h	8	10	1.3±0.04	2 (—)
2	Acylase 30,000	Buffer[a]	2	rt	24 h	5	0	1	-
3	CRL	Buffer[a]	2	rt	24 h	5	0	1	-
4	Novozym 435®	Buffer[a]	7.5	45	28 h	49	62	8.0±1.1	1 (+)
5	Novozym 435®	Buffer[a]	7.5	rt	28 h	33	79	13.7±2.1	1 (+)
6	CRL	IPE/H_2O	3.5	rt	15 d	7	0	1	-
7	Novozym 435®	IPE/H_2O	7.5	45	46 h	47 calcd	75 (67[c])	14.0±2.0 E_{tot}=13.9	1 (+)
8	Novozym 435®	IPE/H_2O	7.5	rt	27 d	51 calcd	79 (81[c])	23±5.8 E_{tot}=21.1	1 (+)
9	Novozym 435®	IPE/H_2O	4.5	4	15 d	46	87	28.9±4.1	1 (+)
10	Novozym 435®	IPE/H_2O	7.5	-12	63 d	35 calcd	93 (50[c])	E_{tot}=45.3	1 (+)

[a] Typical reaction conditions: 0.01 mmol of **5** was dissolved in 8 mL of 15% solution of acetonitrile in 20 mM K-phosphate buffer, pH 7.5 or in 0.5 mL of water-saturated IPE (entries 8 and 10: 0.75 mL). [b] Amount enzyme means mg solid. [c] The e.e. of remaining substrate **5.** [d] The number of enantiomer means the order of elution of enantiomers of **6** on the β-cyclodextrin chiral column, for details see the experimental section. [e] $E=E_p=\{\ln[1-c(1+e.e._p)]\}/\{\ln[1-c(1-e.e._p)]\}$; $E_{tot}=\{\ln[e.e._p(1-e.e._s)]/[e.e._p+e.e._s]\}/\{\ln[e.e._p(1+e.e._s)]/[e.e._p+e.e._s]\}$.

To estimate the enantiomeric ratio (*E* value)[26] of the enzymatic kinetic resolutions, the e.e.'s of the products of reactions **2** and **6** were measured during the reaction. Enantiomeric ratios were calculated using the Chen equations[26,27] or the computer programs ''EIVFIT''[28] and ''Selectivity-Win-1.0''[19]. Monitoring the stereochemical course of the reactions in IPE was difficult, as the available normal-phase chiral columns were found to be not enantioselective toward the 1,4-dihydroisonicotinic acid derivatives **2** and **6.** Only a reversed-phase β-cyclodextrin chiral column was suitable for the analysis of 1,4-dihydroisonicotinic acids **2** and **6**, however, a trace of IPE or *t*-BuOMe dramatically decreased the separations on β-cyclodextrin CSP. On the other hand, preparation of samples for analysis without IPE, declined the accuracy of the measurement of the conversion, and curve deviations were significant. Reliable conclusions about the conversion and enantioselectivity of the enzymes in IPE should be based on comparison of the e.e.'s of both the reaction product **2** or **6** and the remaining substrate **5**.

5.3 Conclusions

Two different strategies of lipase-catalysed resolution of unsymmetrical 1,4-dihydroisonicotinic acid derivatives have been developed. The first method consists of resolution of acyloxymethyl esters of 1,4-dihydroisonicotinic acid **4a-d**, the second offers the resolution of the ethoxycarbonylmethyl ester of 1,4-dihydroisonicotinic acid **5**. The lipase-catalysed kinetic resolutions of four acyloxymethyl derivatives **4a-d** have been studied with variation of the enzyme, the structure of the acyloxymethyl group and the reaction conditions. *Candida rugosa* lipase showed better enantioselectivity towards acyloxymethyl esters **4a-c** than the other tested enzymes, however, the *E* value did not exceed 9 in aqueous medium. The influence of steric bulk of the acyl group of the acyloxymethyl ester on the enantioselectivity of the enzymes was not so clear. *Candida antarctica* lipase B catalysed hydrolysis of the ethoxycarbonylmethyl ester of 1,4-dihydroisonicotinic acid **5** at the 'outer' ester group of the ethoxycarbonylmethyl substituent was enantioselective. The transition from aqueous medium to water-saturated IPE led to a better stereoselectivity of CAL-B in the hydrolysis of **5**. The reaction time is longer and the enantioselectivity is better at lower reaction temperature. In water-saturated diisopropyl ether at 45°C the enantioselectivity of CAL-B toward the ethoxycarbonylmethyl ester was moderate (E=13.8), but was enhanced at rt and +4°C (E=21.5 and E=28.9, respectively). A high enantiomeric ratio (E=45.3) was reached at subzero temperatures, although at the expense of the reaction rate.

5.4 Experimental

5.4.1 General

All reagents were purchased from Aldrich, Acros or Merck and used without further purification. HPLC grade solvents were from Labscan. Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh or 70-230 mesh). Preparative TLC was performed on 20x20 cm Silica gel TLC-PET F_{254} foils (Fluka). *Candida rugosa* lipase (lipase (EC 3.1.1.3) Type VII from *Candida rugosa,* activity 875 U/mg, contains lactose as an extender) was purchased from Sigma. Acylase 30,000 (*Aspergillus sp*., activity ≥ 30,000 U/g), Protease P6 (*Aspergillus melleus*, activity ≥ 60,000 U/g), Lipase AH (*Pseudomonas sp.*, activity unknown), Lipase PS (*Pseudomonas cepacia*, activity ≥ 30,000 U/g) and Lipase PS 800 (activity 839,000 U/g) were gifts from Amano Pharmaceutical Co., Ltd. (Japan). Immobilised *Candida antarctica* lipase B (Novozym 435®, activity 5600 or 7200 P.L.U. (propyl laureate units)/g) was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark). *Candida antarctica* lipase fraction B (CHIRAZYME L-2, lyo., activity > 173,000 U/g),

Rhizomucor miehei lipase (CHIRAZYME L-9, c.-f, lyo., activity ≥ 15.2 U/g) were gifts from Boehringer-Mannheim (Mannheim, Germany). Enzymatic reactions were carried out in a New Brunswick Scientific Innova 4080 incubatory orbital shaker (250 rpm). ^{1}H NMR spectra were recorded on a Bruker AC-E 200 (200 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AC-E 200 (50 MHz). Chemical shifts are reported in parts per million (ppm) relative to trimethylsilane (δ 0.00). Mass spectral data and accurate mass measurements were determined on a Finnigan MAT 95 mass spectrometer. Melting points were determined on a Boetius apparatus and are uncorrected. Optical rotation values were measured with a Perkin Elmer 141 or 241 digital polarimeter. Elemental analyses were determined on a Carlo-Erba elemental analyser.

The analysis of the reaction mixtures and the determination of enantiomeric excesses of **2** and **6** were performed by analysis on an enantioselective column LiChroCART 250-4 ChiraDex, 5 μm (Merck) using a Ginkotek 580A pump and an Applied Biosystems 759A absorbance detector at 254 nm. The e.e. of **6** was determined using a 15% solution of acetonitrile in 0.01 M (NaH_2PO_4/Na_2HPO_4) phosphate buffer, pH 4.1, as eluent at a flow rate of 0.8 mL/min. The solvent system acetonitrile/water/acetic acid (10/90/0.1) at a flow rate of 0.8 mL/min was used for the determination of e.e. of **2**. Peak areas were determined electronically with the Chromeleon chromatography data system, Dionex Softron GmbH (Germering, Germany).

5.4.2 Procedures and spectral data

3-(Methoxycarbonyl)-2,6-dimethyl-5-(propoxycarbonyl)-1,4-dihydro-4-pyridinecarboxylic acid, 2

A mixture of propyl acetoacetate (18 mL, 0.12 mol), 50% aqueous glyoxylic acid (4.5 mL, 0.04 mol) and morpholine (0.2 mL, 0.002 mol) in ethanol (5 mL) was left at room temperature for 16 h. Then concentrated HCl (0.5 mL) was added and the reaction mixture was evaporated under reduced pressure. The residue was treated with acetic anhydride (4 mL, 0.042 mol) and after standing for 30 min, the acetic anhydride was removed in vacuum. The residue was cooled and methyl 3-aminocrotonate (1 g, 0.0087 mol) was added under stirring. The reaction mixture was dissolved in ethyl acetate (5 mL) and stored in a refrigerator for 16 h. The precipitate was filtered, washed with ethyl acetate and dried to give 2 (1.5 g, 12.6%) as a white powder, mp 187-190°C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.88 (t, 3H, *J*=7.5 Hz, CH_3), 1.58 (sextet, 2H, *J*=7.5 Hz, CH_2), 2.21 (s, 3H, CH_3), 2,22 (s, 3H, CH_3), 3.60 (s, 3H, CH_3), 3.90-4.10 (m, 2H, CH_2), 4.59 (s, 1H, CH), 8.89 (br s, 1H, NH), 11.92 (br s, 1H, COOH); ^{13}C (DMSO-d_6, 50 MHz): δ 10.47 (CH_3), 17.91 (2xCH_3), 21.75 (CH_2), 38.24-40.75 (CH, overlap with DMSO-d_6), 50.81 (CH_3), 64.74 (CH_2), 96.97 (C),

97.31 (C), 145.81 (C), 146.15 (C), 166.85 (C), 167.32 (C), 174.48 (C); MS: *m/z* (rel. abund.) 297 (M^+, 0.7), 253 (19), 252 (100), 238 (10), 211 (8), 210 (73), 209 (8), 206 (12), 178 (11), 165 (8), 150 (8); HRMS calcd for $C_{14}H_{19}NO_6$ 297.1212, found 297.1210. Anal. calcd for $C_{14}H_{19}NO_6$: C, 56.56; H, 6.44, N, 4.71; found: C, 56.51; H, 6.29; N, 4.62.

3-Methyl 4-[(propionyloxy)methyl] 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridine-tricarboxylate, 4a

To a solution of **2** (1.188 g, 4.0 mmol) in dry DMF (2 mL), K_2CO_3 (0.828 g, 6 mmol) was added at rt and the reaction mixture was stirred for 2 h, after which propionyloxymethyl chloride (0.735 g, 6 mmol) was added. The mixture was stirred overnight, diluted with $CHCl_3$ and washed with water (three times) and brine, dried over $MgSO_4$ and evaporated. The remaining residue was flash chromatographed on silica gel with chloroform/petroleum ether (bp 40-60°C)/isopropyl alcohol (10:3:1 ⇒ 10:10:1) to give **4a** (0.31 g, 20.2%) as a white precipitate from hexane/ethyl acetate, mp 70-72°C and unreacted **2 (**0.39 g, 32.8%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.94 (t, 3H, *J*=7.5 Hz, CH_3), 1.10 (t, 3H, *J*=7.5 Hz, CH_3), 1.66 (sextet, 2H, *J*=7.5 Hz, CH_2), 2.28 (s, 6H, 2xCH_3), 2.31 (q, 2H, *J*=7.5 Hz, CH_2), 3.70 (s, 3H, CH_3), 3.91-4.17 (m, 2H, CH_2), 4.86 (s, 1H, CH), 5.68 (s, 2H, CH_2) 6.19 (br s, 1H, NH); ^{13}C ($CDCl_3$, 50 MHz): δ 8.75 (CH_3), 10.58 (CH_3), 19.16 (2xCH_3), 22.08 (CH_2), 27.28 (CH_2), 40.50 (CH), 51.35 (CH_3), 65.85 (CH_2), 79.37 (CH_2), 97.61 (C), 97.96 (C), 146.11 (C), 146.39 (C), 166.95 (C), 167.39 (C), 172.61 (C), 172.95 (C); MS: *m/z* (rel. abund.) 383 (M^+, 0.06), 253 (14), 252 (100), 238 (2), 211 (3), 210 (25), 204 (3), 178 (3), 165 (4), 150 (2), 57 (5); HRMS calcd for $C_{18}H_{25}NO_8$ 383.1580, found 383.1575. Anal. calcd for $C_{18}H_{25}NO_8$: C, 56.39; H, 6.57; N, 3.65; found: C, 56.40; H, 6.61; N, 3.67.

4-[(Butyryloxy)methyl] 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridine-tricarboxylate, 4b

This compound was prepared *via* the same method as used for compound **4a**, starting from 0.297 g (1 mmol) of **2**, 1.5 mL of DMF, 0.242 g (1.75 mmol) of K_2CO_3 and 0.273 g (2 mmol) of butyryloxymethyl chloride. Flash chromatography on silica gel with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (10:3:1) followed by crystallisation from ether/hexane gave **4b** (0.285 g, 72%) as a white precipitate, mp 71-73°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, 3H, *J*=7.4 Hz, CH_3), 0.89 (t, 3H, *J*=7.4 Hz, CH_3), 1.56 (sextet, 2H, *J*=7.4 Hz, CH_2), 1.62 (sextet, 2H, *J*=7.4 Hz, CH_2), 2.22 (t, 2H, *J*=7.4 Hz, CH_2), 2.23 (s, 6H, 2xCH_3), 3.65 (s, 3H, CH_3), 3.92-4.13 (m, 2H, CH_2), 4.82 (s, 1H, CH), 5.64 (s, 2H, CH_2) 6.14 (br s, 1H, NH); ^{13}C ($CDCl_3$, 50 MHz): δ 10.57 (CH_3), 13.55 (CH_3), 18.11 (CH_2), 19.16 (2xCH_3), 22.09 (CH_2), 35.79 (CH_2), 40.46 (CH), 51.33 (CH_3), 65.85 (CH_2), 79.35 (CH_2), 97.64 (C), 98.00 (C), 146.08 (C), 146.37 (C), 166.93 (C), 167.37

(C), 172.09 (C), 172.53 (C); MS: *m/z* (rel. abund.) 397 (M^+, 0.06), 253 (14), 252 (100), 211 (2), 210 (22), 204 (2), 178 (2), 165 (3), 150 (3), 71 (3), 43 (3); HRMS calcd for $C_{19}H_{27}NO_8$ 397.1737, found 397.1736.

4-[(Isobutyryloxy)methyl] 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridine-tricarboxylate, 4c

This compound was prepared *via* the same method as used for compound **4a**, starting from 0.346 g (1.16 mmol) of **2**, 1.5 mL of DMF, 0.242 g (1.75 mmol) of K_2CO_3 and 0.318 g (2.3 mmol) of isobutyryloxymethyl chloride. Flash chromatography on silica gel with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (10:3:1) followed by crystallisation from ether/hexane gave **4c** (0.286 g, 62%) as a white precipitate, mp 64-65°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.89 (t, 3H, *J*=7.4 Hz, CH_3), 1.08 (d, 6H, *J*=7.0 Hz, 2xCH_3), 1.62 (sextet, 2H, *J*=7.4 Hz, CH_2), 2.24 (s, 6H, 2xCH_3), 2.51 (septet, 1H, *J*=7.0 Hz, CH), 3.66 (s, 3H, CH_3), 3.92-4.13 (m, 2H, CH_2), 4.82 (s, 1H, CH), 5.64 (s, 2H, CH_2), 5.95 (br s, 1H, NH); ^{13}C ($CDCl_3$, 50 MHz): δ 10.51 (CH_3), 18.55 (2xCH_3), 19.05 (2xCH_3), 22.04 (CH_2), 33.76 (CH), 40.41 (CH), 51.28 (CH_3), 65.80 (CH_2), 79.29 (CH_2), 97.52 (C), 97.89 (C), 146.15 (C), 146.45 (C), 166.87 (C), 167.29 (C), 172.48 (C), 175.48 (C); MS: *m/z* (rel. abund.): 338 ((M-$COOCH_3$)$^+$, 8), 280 (59), 254 (32), 253 (100), 252 (85), 238 (28), 211 (52), 210 (95), 178 (47), 165 (50), 150 (45), 71 (71), 43 (50). Anal. calcd for $C_{19}H_{27}NO_8$: C, 57.42; H, 6.85; N, 3.52; found: C, 57.42; H, 6.84; N, 3.42.

3-Methyl 4-[(pivaloyloxy)methyl] 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridine-tricarboxylate, 4d

This compound was prepared *via* the same method as used for compound **4a**, starting from 0.297 g (1.0 mmol) of **2**, 0.5 mL of DMF, 0.138 g (1.0 mmol) of K_2CO_3 and 0.301 g (2 mmol) of pivaloyloxymethyl chloride. Flash chromatography on silica gel with petroleum ether (bp 40-60°C)/ethyl acetate (1:1) gave **4d** (0.249 g, 60.6%) as a white powder from hexane-ether, mp 52-54°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.94 (t, 3H, *J*=7.4 Hz, CH_3), 1.15 (s, 9H, 3xCH_3), 1.67 (sextet, 2H, *J*=7.4 Hz, CH_2), 2.28 (s, 6H, 2xCH_3), 3.70 (s, 3H, CH_3), 3.97-4.17 (m, 2H, CH_2), 4.87 (s, 1H, CH), 5.68 (s, 2H, CH_2), 6.11 (br s, 1H, NH); ^{13}C ($CDCl_3$, 50 MHz): δ 10.56 (CH_3), 19.22 (2xCH_3), 22.09 (CH_2), 26.79 (3xCH_3), 38.65 (C), 40.32 (CH), 51.35 (CH_3), 65.84 (CH_2), 79.44 (CH_2), 97.73 (C), 98.09 (C), 146.01 (C), 146.34 (C), 166.86 (C), 167.28 (C), 172.17 (C), 175.89 (C); MS: *m/z* (rel. abund.) 411 (M^+, 0.07), 294 (1) 280 (2) 254 (1) 253 (11), 252 (100), 211 (1), 210 (12), 178 (1), 165 (2), 57 (2); HRMS calcd for $C_{20}H_{29}NO_8$ 411.1893, found 411.1888. Anal. calcd for $C_{20}H_{29}NO_8$: C, 58.38; H, 7.11; N, 3.40; found: C, 58.38; H, 7.07; N, 3.41.

4-Ethoxycarbonylmethyl 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridine-tricarboxylate, 5

This compound was prepared *via* the same method as used for compound **4a**, starting from 0.595 g (2 mmol) of **2**, 1.0 mL of dry DMF, 0.276 g (2 mmol) of K_2CO_3 and 0.334 mL (3 mmol) of ethyl bromoacetate. The reaction time after addition of ethyl bromoacetate was 2 h. Flash chromatography on silica gel with petroleum ether (bp 40-60°C)/ethyl acetate (1:1) gave **5 (**0.67 g, 83%) as a white precipitate from ether/hexane, mp 70-72°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.94 (t, 3H, *J*=7.4 Hz, CH_3), 1.22 (t, 3H, *J*=7.2 Hz, CH_3), 1.69 (sextet, 2H, *J*=7.4 Hz, CH_2), 2.28 (s, 6H, 2xCH_3), 3.73 (s, 3H, CH_3), 3.99-4.15 (m, 2H, CH_2), 4.14 (q, 2H, *J*=7.2 Hz, CH_2), 4.53 (s, 2H, CH_2), 4.97 (s, 1H, CH), 6.43 (br s, 1H, NH); ^{13}C ($CDCl_3$, 50 MHz): δ 10.58 (CH_3), 14.08 (CH_3), 19.07 (2xCH_3), 22.12 (CH_2), 40.08 (CH), 51.34 (CH_3), 61.06 (CH_2), 61.29 (CH_2), 65.80 (CH_2), 97.71 (C), 98.06 (C), 146.21 (C), 146.52 (C), 167.11 (C), 167.53 (C), 167.62 (C), 173.56 (C); MS: *m/z* (rel. abund.) 382 ($(M-H)^+$, 0.05), 338 (2), 324 (2), 296 (3), 280 (2), 254 (2), 253 (14), 252 (100), 211 (2), 210 (16), 165 (3), 150 (2); Anal. calcd for $C_{18}H_{25}NO_8$: C, 56.39; H, 6.57; N, 3.65; found: C, 56.47; H, 6.63; N, 3.61.

***Candida rugosa* lipase-catalysed kinetic resolution of racemic 4c**

A solution of **4c** (135 mg, 0.34 mmol) in acetonitrile (40 mL) was added to K_2HPO_4/KH_2PO_4 buffer (20 mM, pH 7.5, 230 mL) after which *Candida rugosa* lipase (17 mg) was added. The resulting mixture was shaken at 250 rpm for 7.5 h at 25°C until the conversion reached 50% according to HPLC. The remaining **(+)-4c** was extracted from the reaction mixture with CH_2Cl_2 (3x100 mL), washed with water, dried over $MgSO_4$ and concentrated under reduced pressure to give **(+)-4c** (66 mg, 49%) as an amorphous triturate from ether-hexane; $[\alpha]_D^{20}$ +2.8 (*c* 1, $CHCl_3$) or $[\alpha]_D^{20}$ +4.4 (*c* 1, MeOH); 65% e.e. The water layer was adjusted to pH 5.0 by adding dilute aqueous HCl and extracted with ethyl acetate (5x200 mL). The extract was concentrated under reduced pressure. The residue was triturated from ether-hexane to give **(+)-2 (**42.0 mg, 42%) of as a white powder from hexane-ether, mp 161-163°C; $[\alpha]_D^{20}$ +2.0 (*c* 2, MeOH); 61% e.e. The ^{1}H NMR spectra were identical to those described for the racemic precursors **2** and **4c**. Anal. calcd for **(+)-4c** ($C_{19}H_{27}NO_8$): C, 57.42; H, 6.85; N, 3.52; found: C, 57.29; H, 6.85; N, 3.55. Anal. calcd for (+)**-2 (**$C_{14}H_{19}NO_6$): C, 56.56; H, 6.44, N, 4.71; found: C, 56.80; H, 6.37; N, 4.81.

Novozym 435®-catalysed kinetic resolution of racemic 5 at 45°C

To a solution of **5** (0.383 g, 1 mmol) in water-saturated IPE (50 mL) was added Novozym 435® (0.750 g) and the resulting mixture was shaken at 250 rpm for 48 h at 45°C until the conversion

reached 50% according to HPLC. After removal of the enzyme by filtration, the filtrate was concentrated under reduced pressure. The residue was flash chromatographed on silica gel with petroleum ether (bp 40-60°C)/ethyl acetate (1:1) to give unreacted **(—)-5 (**0.195 g, 51%). After changing of the mobile phase to dichloromethane/isopropyl alcohol/acetic acid (7:3:0.1) **(+)-6 (**0.170 g, 48%) was obtained.

(+) 4-Carboxymethyl 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridine-tricarboxylate, (+)-6

(+)-6 Was obtained as a viscous oil, e.e. 75%, $[\alpha]_D^{20}$ +5.3 (*c* 1, MeOH) or $[\alpha]_D^{20}$ +5.0 (*c* 1, acetone), and was crystallised from ether to give **(+)-6** (11.2 mg), mp 82-84°C, e.e. 46%. Repeated crystallisation of mother liquor containing **(+)-6** from ethyl acetate gave **(+)-6** (4.1 mg), e.e. 35%, mp 109-111°C, the e.e. of the mother liquor was 85%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (t, 3H, *J*=7.4 Hz, CH_3), 1.58 (sextet, 2H, *J*=7.4 Hz, CH_2), 2.23 (s, 6H, 2xCH_3), 3.60 (s, 3H, CH_3), 3.87-4.10 (m, 2H, CH_2), 4.42 (s, 1H, CH), 4.78 (s, 2H, CH_2), 9.01 (br s, 1H, NH); ^{13}C (DMSO-d_6, 50 MHz): δ 10.46 (CH_3), 18.70 (2xCH_3), 21.70 (CH_2), 38.20-40.75 (CH, overlap with DMSO-d_6), 50.91 (CH_3), 60.82 (CH_2), 64.84 (CH_2), 96.06 (C), 96.33 (C), 146.86 (C), 147.05 (C), 166.51 (C), 166.95 (C), 168.83 (C), 172.55 (C); MS: *m/z* (rel. abund.): 355 (M^+, 0.04), 324 (0.06), 296 (3), 268 (5), 253 (14), 252 (100), 211 (4), 210 (33), 192 (2), 178 (4), 165 (4), 150 (4); HRMS calcd for (M-OCH_3)$^+$, 324.1447, found 324.1443.

(—) 4-Ethoxycarbonylmethyl 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridine-tricarboxylate, (—)-5

(—)-**5** Was obtained as a viscous oil, e.e. 67% (determined after conversion to **(—)-6**); $[\alpha]_D^{20}$ –4.4 (*c* 1, $CHCl_3$) or $[\alpha]_D^{20}$ –4.1 (*c* 1, MeOH). The ^{1}H NMR spectrum was identical to that described for its racemic precursor **5**. MS: *m/z* (rel. abund.): 382 ((M-H)$^+$, 0.09), 338 (1), 324 (3), 296 (4), 253 (14), 252 (100), 211 (2), 210 (23), 185 (3), 165 (3), 150 (3); HRMS calcd for (M-H)$^+$ 382.1502 and for (M-C_2H_5O)$^+$ 338.1240, found for (M-H)$^+$ 382.1500 and for (M-C_2H_5O)$^+$ 338.1239.

Novozym 435®-catalysed kinetic resolution of racemic 5 at 25°C

The kinetic resolution of racemic **5** was performed *via* the same procedure as used for the resolution of **5** at 45°C, but in 75 mL of water-saturated IPE. The incubation with Novozym 435® for 27 days gave **(+)-6** (0.167 g, 47%) as a viscous oil, 79% e.e., $[\alpha]_D^{20}$ +6.4 (*c* 1, MeOH) and **(—)-5** (0.191 g, 49.8%) as a viscous oil, 81% e.e. (determined after conversion to **(—)-6**); $[\alpha]_D^{20}$ –5.3 (*c* 1, $CHCl_3$). The ^{1}H NMR spectra were identical to those described for the racemic precursors **5** and **(+)-6**.

Novozym 435®-catalysed kinetic resolution of racemic 5 at –12°C

The kinetic resolution of racemic **5** was performed *via* the same procedure as used for the resolution of **5** at 45°C, but in 75 mL of water-saturated IPE. The incubation with Novozym 435® for 63 days in the refrigerator at –12°C gave **(+)-6** (0.120 g, 34%) as a viscous oil, 93% e.e., $[\alpha]_D^{20}$ +7.0 (*c* 1, MeOH) and **(—)-5** (0.225 g, 59%) as a viscous oil, 50 % e.e. (determined after conversion to **(—)-6**); $[\alpha]_D^{20}$ –3.2 (*c* 2, $CHCl_3$). The ^{1}H NMR spectra were identical to those described for the racemic precursors **5** and **(+)-6**.

(—) 3-(Methoxycarbonyl)-2,6-dimethyl-5-(propoxycarbonyl)-1,4-dihydro-4-pyridine-carboxylic acid, (—)-2

To a solution of **(+)-6** (0.105 g, 0.294 mmol) with an e.e. of 85% in ethanol (3 mL) was added a solution of KOH (0.042 g, 0.75 mmol) in ethanol (1 mL). After being stirred under reflux for 1.5 h, the reaction mixture was evaporated, diluted with water, acidified with dilute aqueous HCl until pH 5.0, and extracted four times with ethyl acetate. The organic layer was evaporated and chromatographed on silica gel with petroleum ether (bp 40-60°C)/ethyl acetate (1:1) to give a product (0.037 g) that was purified again on TLC-PET foil with petroleum ether (bp 40-60°C)/chloroform/isopropyl alcohol (10:10:1) as eluent to give **(—)-2 (**0.007 g, 8%) as a white precipitate from ether: mp 162-164°C; 67% e.e., $[\alpha]_D^{20}$ –4.77 (*c* 0.524, MeOH). The ^{1}H NMR spectrum was identical to that described for its racemic precursor **2.**

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Chapter 6

Candida rugosa lipase-catalysed kinetic resolution of 3-(isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno[3,2-*b*]pyridine-3-carboxylate*

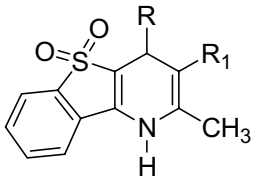
Abstract—The lipase-catalysed kinetic resolution of 3-(isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno[3,2-*b*]pyridine-3-carboxylate has been performed. The most enantioselective reaction (*E*=28) was a transesterification with *n*-butanol in water-saturated toluene, at 45°C.

* Sobolev, A.; Zhalubovskis, R.; Franssen, M. C. R.; Vigante, B.; Cekavicus, B.; Duburs, G.; de Groot, Ae. *manuscript in preparation.*

6.1 Introduction

Pharmacological activities as well as metabolism of drugs depend on their interaction with biological matrices (so-called drug targets). The drug targets, such as proteins (receptors, enzymes), nucleic acids and biomembranes (phospholipids and glycolipids) have complex three-dimensional structures, which are capable to recognise and bind specifically the ligand (drug) molecule in only one of the many possible arrangements in three-dimensional space.[1,2] As a result of this direct correlation between drug stereochemistry and biological activity, the governing bodies that regulate the approval of new medicines in the USA[3,4] and Europe[5] have issued specific rules pertaining to the development of stereoisomeric drugs.[2,6,7]

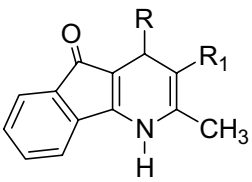
Chirality plays an important role in determining the activity of 1,4-dihydropyridines (1,4-DHPs) and both quantitative and qualitative differences have been reported.[8,9] The standard resolution technique of monocyclic 1,4-DHPs, such as incorporation of an enzymatically labile group has been pioneered by group of Sih[10] and Achiwa[11] and has been successfully used also by our research group.[12,13] Polycyclic 1,4-DHPs in enantiopure form are desired for extended pharmacological studies, since racemic 1,4-dihydrobenzothieno[3,2-*b*]pyridine-5,5-dioxides[14] **1** and 5-oxo-4,5-dihydro-1,4-indeno[1,2-*b*]pyridines[15] **2** have exhibited various biological activities. Many representatives of both classes of compounds (*e.g.* **1** [R = phenyl, 4-bromophenyl, 4-nitrophenyl] and **2c**) show coronary dilating activities.[16-18] 1,4-DHP **2a**[19] is an active glutathione S-transferase inhibitor; compounds **2b**[19] and **2d**[20] have exhibited anticancer activities.



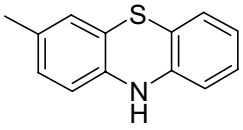
1

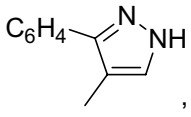
R=H, CH_3, C_6H_5, 4-Cl-C_6H_4, 4-CH_3O-C_6H_4, 4-OH-C_6H_4, 4-Br-C_6H_4, 2,3-$(CH_3O)_2C_6H_3$, 4-NO_2-C_6H_4, CH=CH-C_6H_5

R_1=COOAlk, $COCH_3$, CN, $C(O)SC_2H_5$, $C(S)OC_2H_5$, $C(S)SC_2H_5$

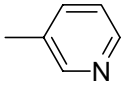
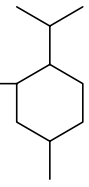


2

2a R=  , R_1= C(O)SAlk

2b R=  , R_1=$COOCH_3$

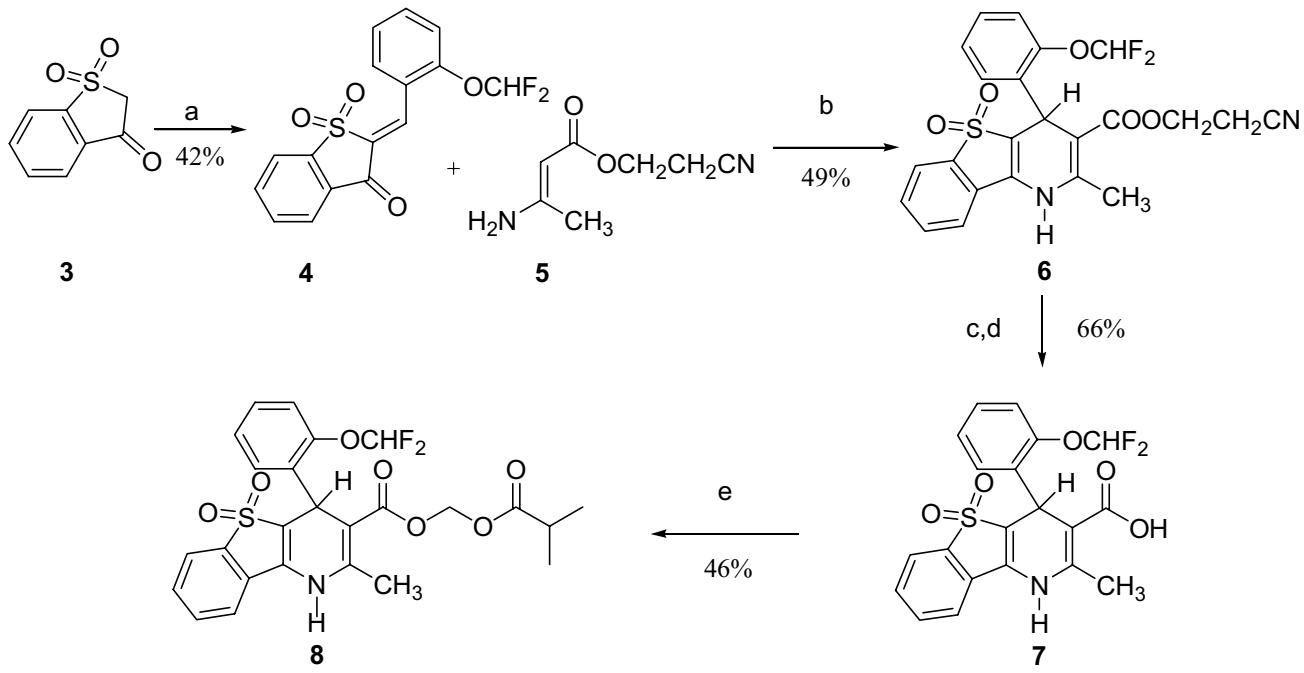
2c R= C_6H_4, 2-OH-C_6H_4, 2- and 4-NO_2-C_6H_4;
R_1=$C(O)SC_2H_5$, $C(O)SCH_2C_6H_5$

2d R=  , R_1=COO 

Herein, we report the *Candida rugosa* lipase-catalysed kinetic resolution of 3-(isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzo-thieno[3,2-*b*]pyridine-3-carboxylate.

6.2 Results and discussion

The synthesis of enantiopure polycyclic 1,4-DHPs has been performed *via* lipase-catalysed kinetic resolution of the corresponding acyloxymethyl derivative **8**. The racemic acyloxymethyl ester **8** has been prepared in a four-step sequence as depicted in scheme 1. Thus, condensation of benzo[*b*]thiophen-3(2H)-one 1,1-dioxide[21] **3** with 2-(difluoromethoxy)benzaldehyde, followed by Hantzsch cyclisation of the intermediate **4** with 2-cyanoethyl 3-aminocrotonate **5** furnished the polycyclic 1,4-DHP **6** framework according to the earlier reported method.[14] The hydrolysis of cyanoethyl ester **6** with KOH gave carboxylic acid **7**. The last step consists of esterification of **7** with isobutyryloxymethyl chloride.[13]



[a] Reagents and conditions: (a) 2-(difluoromethoxy)benzaldehyde, AcOH, piperidine, Δ, 3h; (b) EtOH:AcOH (20:1), Δ, 3h; (c) EtOH, KOH, rt, 3h; (d) H_2O, HCl; (e) $ClCH_2OC(O)CH(CH_3)_2$, K_2CO_3, DMF, rt, 3h.

Scheme 1

The choice of enzymes for primary screening was based on previous work of our group[12,13,22] and literature data.[11,23] The primary screening was carried out in water-saturated diisopropyl ether (IPE) at 45°C. From the tested hydrolases (Lipase PS and AH, *Candida antarctica* lipase B [Novozym

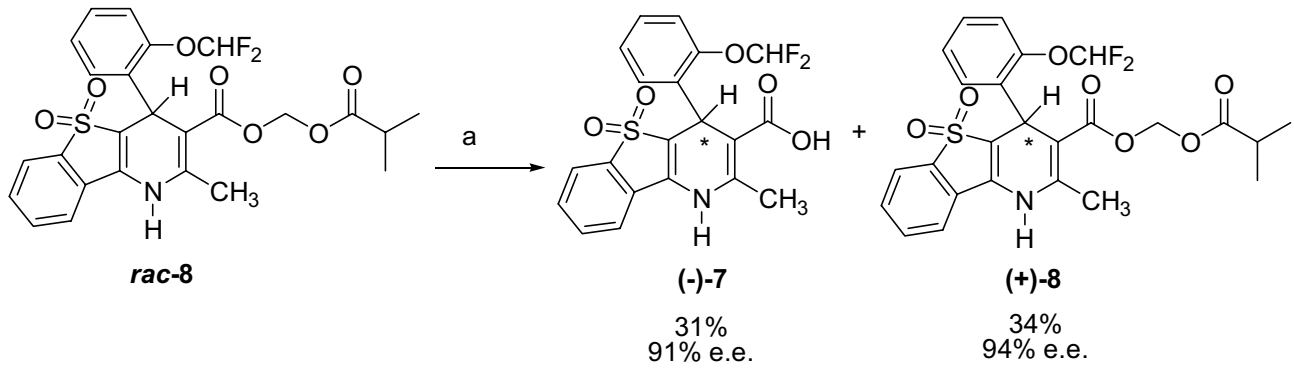
435® and Chirazyme L-2, c.-f., C3, lyo., (CAL-B)], *Rhizomucor miehei* lipase, *Burkholderia cepacia* lipase and *Candida rugosa* lipase [CRL]), only CRL showed significant hydrolytic activity towards substrate **8**. CRL-mediated hydrolysis of **8** in water-saturated IPE at 45°C (Table 1, entry 1) occurred with moderate enantiosectivity (*E* value[24]=12). The very low solubility of the substrate in IPE and the moderate enantioselectivity of CRL led us to investigate other reaction conditions. The influence of the solvent on the enantioselectivity of CRL was studied in more detail and some examples are given in Table 1. It was found that the use of toluene as the solvent increased the solubility of substrate **8**. CRL-catalysed hydrolysis in toluene that was saturated with water at the reaction temperature occurred with moderate selectivity (Table 1, entry 2). CRL was found not active when toluene was used with *n*-butanol as nucleophile in the absence of water (Table 1, entry 3). Better results were obtained when 5-50 mM *n*-butanol in water-saturated toluene was used as the reaction medium (Table 1, entries 4-7). Using less *n*-butanol at higher temperatures appeared to give the most enantioselective reaction (*E*=28) (Table 1, entry 6). The amount of water in the reaction mixture seems to be important for the enantioselectivity of CRL, as the *E* value is 22 for the reaction where the toluene was water-saturated at rt, whereas an *E* value of 28 was obtained in the case where the toluene was water-saturated at 45°C (Table 1, entry 6). The experimentally determined water content in toluene was around 0.045% and 0.10% at 25°C and 45°C, respectively.[25]

Table 1. CRL-catalysed kinetic resolution of **8**

Entry	T, °C	Reaction medium	Conversion			*E* value[a]
			Time, h	%	e.e.$_p$, %	
1	45	IPE/H_2O[b]	6	49	69	12.0±1.0
2	25	toluene/H_2O[c]	20	25	82	14.0±0.8
3	45	50 mM *n*-butanol in toluene[d]	4	-	-	-
4	45	50 mM *n*-butanol in toluene/H_2O[e]	5.5	42	77	16.0±1.4
5	25	5 mM *n*-butanol in toluene/H_2O[e]	6.0	41	83	23.0±1.6
6	45	5 mM *n*-butanol in toluene/H_2O[e]	2.0	31	90	28.0±2.8
7	45	5 mM *n*-butanol in toluene/H_2O[f]	4.5	25	89	22.0±2.7

[a] The enantiomeric ratio (*E* value) was calculated using the computer program EIVFIT[26] [b] A solution of 5 mg of **8** in 20 ml of water-saturated at room temperature IPE with 5 mg of CRL was shaken at 250 rpm. [c] A solution of 5 mg of **8** in 5 ml of toluene that was water-saturated at the reaction temperature with 5 mg of CRL was shaken at 250 rpm. [d] A solution of 5 mg of **8** in 5 ml of 50 mM *n*-butanol in toluene with 5 mg of CRL was shaken at 250 rpm. [e] A solution of 5 mg of **8** in 5 ml of 5-50 mM *n*-butanol in toluene that was water-saturated at the reaction temperature with 5 mg of CRL was shaken at 250 rpm. [f] A solution of 5 mg of **8** in 5 ml of 5 mM *n*-butanol in toluene that was water-saturated at room temperature with 5 mg of CRL was shaken at 250 rpm.

The monoacid **7** and the remaining ester **8** were isolated after 2 h of reaction with CRL when the conversion reached ~30%, in order to have a good enantiomeric excess of the reaction product. The enantiomerically pure monoacid **7** appeared to have an optical rotation of –61.4° (*c* 0.5, acetone) whereas the enantiomerically enriched remaining ester had an optical rotation of +29.1° (c 1.0, acetone). The remaining substrate **(+)-8** preferentially crystallises as a single enantiomer. After purification of **(+)-8** *via* crystallisation from diluted methanol, the e.e. of the product was much higher than expected (94%).



[a] Reagents and conditions: (a) *Candida rugosa* lipase, 5 mM *n*-butanol solution in toluene that was water-saturated at the reaction temperature, 2 h at 45°C.

Scheme 2

6.3 Conclusions

The *Candida rugosa* lipase-catalysed kinetic resolution of 3-(isobutyryloxy)methyl 4-[2-(difluoro-methoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno[3,2-*b*]pyridine-3-carboxylate has been developed. The enantioselectivity of *Candida rugosa* lipase can be improved by changing the reaction medium and the temperature. The change of the reaction medium from water-saturated diisopropyl ether to 5 mM solution of *n*-butanol in toluene resulted in a higher enantiomeric ratio (*E*=28).

6.4 Experimental

6.4.1 General

All reagents were purchased from Aldrich, Acros or Merck and used without further purification. HPLC grade solvents were from Labscan (Dublin, Ireland). Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh or 70-230 mesh). Preparative TLC was performed on 20x20 cm Silica gel TLC-PET F_{254} foils (Fluka). *Candida rugosa* lipase, (lipase (EC 3.1.1.3) Type VII from *Candida rugosa*, 875 U/mg) was purchased from Sigma. Lipase AH and Lipase PS were gifts from Amano Pharmaceutical Co., Ltd. (Japan). Immobilised *Candida antarctica* lipase B (Novozym 435®) was a gift from Novo Nordisk A/S (Bagsvaerd, Denmark). *Rhizomucor miehei* lipase (Chirazyme L-9, c.-f, lyo.), *Candida antarctica* lipase B (Boehringer Mannheim, Chirazyme L-2, c.-f., C3, lyo.) and *Burkholderia cepacia* lipase (Chirazyme L-1, c.-f., lyo.) were gifts from Boehringer-Mannheim (Mannheim, Germany). Enzymatic reactions were carried out in a New Brunswick Scientific Innova 4080 incubatory orbital shaker. ^{1}H NMR spectra were recorded on a Bruker WH 90/DC (90 MHz) or a Bruker AC-E 200 (200 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AC-E 200 (50 MHz). Chemical shifts are reported in parts per million (ppm) relative to trimethylsilane (δ 0.00). Mass spectral data were determined on a AEI MS-905 mass spectrometer. Melting points were determined on a Boetius apparatus and are uncorrected. Optical rotation values were measured with a Perkin Elmer 241 digital polarimeter. Elemental analyses were determined on a Carlo-Erba elemental analyser. The conversions and enantiomeric excesses of all enzymatic reactions were analysed by HPLC on an enantioselective column Chirex 3011, 4.6x250 mm, 5 μm (Phenomenex) using a Ginkotek 580A pump (Germering, Germany) and an Applied Biosystems 759A absorbance detector at 254 nm or a LC-1110 pump and a LC-1200 UV/Vis detector at 254 nm, GBC (Dandenong, Australia). The eluent was 0.05 M ammonium acetate in MeOH at a flow rate of 1 mL/min. Peak areas were determined electronically with the Chromeleon chromatography data system, Dionex Softron GmbH (Germering, Germany) or DP-800, GBC (Dandenong, Australia).

6.4.2 Procedures and spectral data

2-[2-(Difluoromethoxy)benzylidene]-benzo[*b*]thiophen-3(2H)-one 1,1-dioxide, 4

Benzo[*b*]thiophen-3(2H)-one 1,1-dioxide **3** (1.64 g, 9 mmol), 2-(difluoromethoxy)benzaldehyde (1.55 g, 9 mmol) and piperidine (0.06 mL) in acetic acid (20 ml) was stirred under reflux for 3 h. After storing in the refrigerator the precipitated product was filtered to give 1.38 g of crude **4**. The

precipitate was crystallised from methanol to give white crystals of **4** (1.27 g, 42%), mp 195-198°C; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 7.10-8.40 (m, 9H, Ar-H and =CH), 7.40 (1H, t, J_{H-F}=74.0 Hz, $OCHF_2$). MS: 336 (M+). Anal. calcd for $C_{16}H_{10}F_2O_4S$: C 57.14; H 3.00; S 9.53; found: C 56.20; H 2.94; S 10.66.

3-(2-Cyanoethyl) 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno-[3,2-*b*]pyridine-3-carboxylate, 6

To a solution of **4** (1.34 g, 4 mmol) in a mixture of ethanol (20 mL) and acetic acid (4 mL), 2-cyanoethyl 3-aminocrotonate **5** (0.62 g, 4 mmol) was added. The reaction mixture was stirred under reflux for 3 h. After refrigeration of the mixture, the orange precipitate was filtered off. The crude product was crystallised from methanol-acetic acid to give a yellow powder of **6** (0.92 g, 49%); mp 230-234°C; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 2.40 (s, 3H, CH_3, overlap with DMSO-d_6), 2.70 (t, 2H, *J*=6.5 Hz, CH_2CH_2CN), 4.05 (t, 2H, *J*=6.5 Hz, CH_2CH_2CN), 5.30 (s, 1H, CH), 6.90-8.10 (m, 8H, Ar-H), 6.97 (1H, t, J_{H-F}=76.0 Hz, $OCHF_2$), 9.80 (s, 1H, NH). MS: 472 (M^+). Anal. calcd for $C_{23}H_{18}F_2N_2O_5S$: C 58.47; H 3.84; N 5.93; S 6.79; found: C 57.79; H 3.74; N 5.59; S 6.57.

4-[2-(Difluoromethoxy)phenyl]-5,5-dioxo-2-methyl-1,4-dihydrobenzothieno[3,2-*b*]pyridine-3-carboxylic acid, 7

1.89 g (4 mmol) of **6** in ethanol (20 ml) was heated under reflux until dissolution was complete, after which it was cooled down. Crushed KOH (0.28 g, 5 mmol) was added to the reaction mixture at rt and this mixture was then stirred for 3 h at the same temperature before being evaporated. The residue was diluted with water. The ice-cooled solution was acidified with diluted aqueous HCl to pH 4.0-5.0. The precipitated product was filtered off, washed with water and crystallised from methanol to give a yellow powder of **7** (1.11 g, 66%), mp 223-225°C; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 2.40 (s, 3H, CH_3, overlap with DMSO-d_6), 5.30 (s, 1H, CH), 6.98 (1H, t, J_{H-F}=76.0 Hz, $OCHF_2$), 7.04-8.10 (m, 8H, Ar-H), 9.55 (br s, 1H, NH), 10.90 (br s, 1H, COOH). Anal. calcd for $C_{20}H_{15}F_2NO_5S$: C 57.28; H 3.60; N 3.34; S 7.65; found C 56.95; H 3.52; N 3.39; S 7.71.

3-(Isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzo-thieno[3,2-*b*]pyridine-3-carboxylate, 8

To a solution of 0.53 g (1.3 mmol) of **7** in 7 mL of dry DMF, 0.21 g (1.5 mmol) of K_2CO_3 was added at rt and the reaction mixture was stirred for 2 h, after which 0.22 g (1.6 mmol) of isobutyryloxymethyl chloride was added. The mixture was stirred for 3 h, poured into ice cold water and extracted with $CHCl_3$. The organic layer was washed with water (three times) and brine, dried over $MgSO_4$, and evaporated. The remaining residue was crystallised from methanol to give

0.30 g (46%) of **8** as a yellow powder; mp 185-187°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.05 (d, 3H, *J*=6.9 Hz, CHC*H*$_3$); 1.07 (d, 3H, *J*=6.9 Hz, CHC*H*$_3$); 2.40 (septet, 1H, *J*=6.9 Hz, C*H*$(CH_3)_2$); 2.44 (s, 3H, CH_3); 5.43 (s, 1H, CH); 5.69 (ABq, 2H, OCH_2O); 6.54 (t, 1H, $J_{H\text{-}F}$=74.4 Hz, $OCHF_2$); 6.59 (br s, 1H, NH); 7.00-7.64 (m, 8H, Ar-H). MS: 519 (M^+). Anal. calcd for $C_{25}H_{23}F_2NO_7S$: C, 57.80; H, 4.46; N, 2.70; S, 6.17; found: C, 57.64; H, 4.35; N, 2.64; S, 6.26.

***Candida rugosa* lipase-catalysed kinetic resolution of racemic 8**

To a solution of 85 mg (0.16 mmol) of **8** in 85 mL of 5mM *n*-butanol in toluene that was water-saturated at 45°C was added 85 mg of *Candida rugosa* lipase and the resulting mixture was shaken for 2 h at 45°C. The reaction mixture was diluted with 200 ml of acetonitrile, evaporated and directly flash chromatographed on silica gel with chloroform/petroleum ether (bp 40-60°C)/acetone/ethanol (9:7:2:2) to give **(—)-7** and **(+)-8**. The e.e.'s of both compounds were determined after crystallisation.

(—)-4-[2-(difluoromethoxy)phenyl]-5,5-dioxo-2-methyl-1,4-dihydrobenzothieno[3,2-*b*]-pyridine-3-carboxylic acid, (—)-7

Yield: 21 mg (31%) as a yellow powder from methanol-water; mp 166-167°C; 91% e.e.; $[\alpha]_D^{20}$ –61.4, (*c* 0.5, acetone); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.46 (s, 3H, CH_3); 5.36 (s, 1H, CH); 6.98 (t, 1H, $J_{H\text{-}F}$=74.3 Hz, $OCHF_2$); 7.03-7.34 (m, 4H, Ar-H); 7.58-8.08 (m, 4H, Ar-H); 9.71 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz) δ 18.35 (CH_3); 30.12 (CH); 102.07 (C); 110.16 (C); 116.82 (CH, t, $J_{C\text{-}F}$=250.0 Hz, $OCHF_2$); 116.83 (CH); 120.27 (CH); 121.20 (CH); 125.19 (CH); 126.04 (C); 128.07 (CH); 130.00 (CH); 130.65 (CH); 132.93 (CH); 135.36 (C); 135.75 (C); 137.86 (C); 146.22 (C); 148.31 (C); 167.75 (C). Anal. calcd for $C_{20}H_{15}F_2NO_5S$: C, 57.28; H, 3.60; N, 3.34; S, 7.65; found: C, 56.72; H, 3.40; N, 3.37; S, 7.75.

(+)-3-(Isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydro-benzothieno[3,2-*b*]pyridine-3-carboxylate, (+)-8

Yield: 29 mg (34%) as a yellow powder from methanol-water; mp 85-86°C; 94% e.e.; $[\alpha]_D^{20}$ +29.1 (*c* 1.0, acetone); ^{1}H NMR (DMSO-d_6, 200 MHz) δ 0.92 (d, 3H, *J*=6.9 Hz, CHC*H*$_3$); 0.97 (d, 3H, *J*=6.9 Hz, CHC*H*$_3$); 2.39 (septet, 1H, *J*=6.9 Hz, C*H*$(CH_3)_2$); 2.48 (s, 3H, CH_3); 5.34 (s, 1H, CH); 5.62 (ABq, 2H, *J*=5.8 Hz, OCH_2O); 7.02 (t, 1H, $J_{H\text{-}F}$=74.2 Hz, $OCHF_2$); 7.07-7.32 (m, 4H, Ar-H); 7.60-8.09 (m, 4H, Ar-H); 9.99 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 50 MHz) δ 18.00 (CH_3); 18.04 (CH_3); 18.69 (CH_3); 29.90 (CH); 32.61(CH); 78.26 (CH_2); 99.63 (C); 110.94 (C); 116.56 (CH); 116.75 (CH, t, $J_{C\text{-}F}$=255.1 Hz, $OCHF_2$); 120.38 (CH); 121.27 (CH); 125.02 (CH); 125.76 (C);

128.25 (CH); 129.87 (CH); 130.77 (CH); 133.03 (CH); 134.88 (C); 135.02 (C); 137.71 (C); 148.47 (C); 149.61(C); 164.50 (C); 174.58 (C). MS: 519 (M^+). Anal. calcd for $C_{25}H_{23}F_2NO_7S$: C, 57.80; H, 4.46; N, 2.70; S, 6.17; found: C, 57.50; H, 4.31; N, 2.62; S, 6.29.

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Chapter 7

Discussion and conclusions

7.1 Introduction

1,4-Dihydropyridines (1,4-DHPs) are widely used for the treatment of arterial hypertension and ischemic heart disease.[1] Besides the well-known ability of 1,4-DHPs to control the influx of calcium into cells, many other activities have been described recently.[2,3] 1,4-DHPs with novel pharmacological activities, such as neuroprotective, antineurodegenerative, cognition and memory enhancing, antidiabetic, anti-inflammatory and antiviral are the main subjects of study.[4-8]

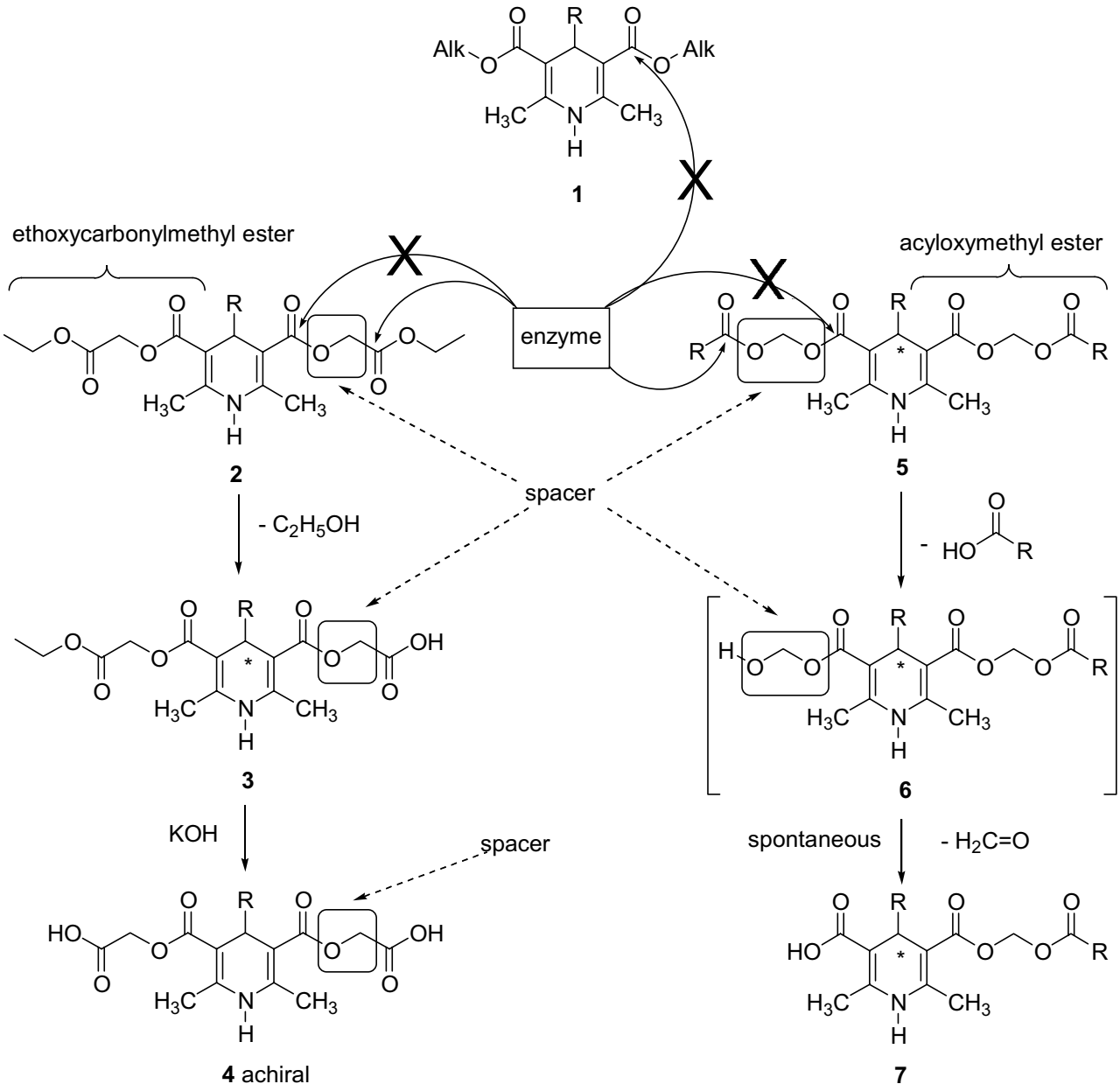
Chiral 1,4-DHPs show advantages compared to the corresponding achiral or racemic analogues.[9] Unequal and even opposite activities of enantiomers and diastereomers of 1,4-DHPs have been described.[9-11] Often enantiopure compounds show many additional advantages, such as better activity, fewer side effects and less toxicity.[12] Chemical, toxicological and pharmacological data have to be obtained for individual enantiomers and the racemate before registration will become possible.[13,14] Therefore methodologies for the synthesis of enantiopure 1,4-DHP's have to be developed. However, the classical Hantzsch synthesis of 1,4-DHPs does not lead to enantiopure compounds, so other methodologies have to be developed.

Synthetic approach. One method for the preparation of enantiopure compounds is chemo-enzymatic synthesis. This method provides a number of distinct advantages compared to other methods, such as mild reaction conditions, high selectivity, application in water and in organic solvents, and other (see Chapter 1).

4-Aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates **1** having alkyl esters at the positions 3 and 5 are exceptionally stable towards enzymatic hydrolysis, as well as chemical hydrolysis because of electronic and steric reasons. The conversion of this inert ester into to an ester that can be attacked successfully by enzymes may circumvent this problem. Such a situation can be achieved by replacing the alkyl group of the first 'inner' ester by an enzymatically labile ester on a spacer.[15,16] The groups that have been investigated for this purpose, are the ethoxycarbonylmethyl and the acyloxymethyl moieties. After enzymatic hydrolysis of the labile 'outer' ester, and separation of the products, removal of the spacer will lead to the desired chiral 1,4-DHP (Scheme 1).

Ethoxycarbonylmethyl esters. Lipases are capable to cleave the 'outer' ester group of the ethoxycarbonylmethyl esters **2** (Scheme 1). The resulting 'inner' carboxymethyl ester **3** is inert to enzymatic hydrolysis and only under strong alkaline or acidic conditions hydrolysis to the corresponding carboxylic acid can be achieved. If the 1,4-DHP contains other hydrolysable groups (*e.g.* another ethoxycarbonylmethyl ester) the selective hydrolysis of the 'inner' carboxymethyl ester turns out to be a problem. Thus, chemical hydrolysis of carboxymethyl ester **3** will take place at both sides of the molecule to give the achiral bis(carboxymethyl) derivative **4**.

A drawback of this approach is that the carbonyl group of the reacting ester in **2** is shifted by the spacer three bonds further away from the stereogenic centre at C(4), which will affect the enantioselectivity of the enzymes.



Scheme 1

Acyloxymethyl esters. The problem of selective hydrolysis of the ethoxycarbonylmethyl esters mentioned above can be circumvented by the use of acyloxymethyl esters, which can be hydrolysed completely by lipases (Scheme 1). The lipase-catalysed hydrolysis of acyloxymethyl esters **5** to the corresponding carboxylic acid **7** is in fact a two-step process. After the enantioselective enzymatic hydrolysis of the 'outer' ester, the unstable hydroxymethyl group in ester **6** splits off spontaneously with loss of formaldehyde, thus liberating the 'inner' ester. The drawback of this approach is that

the carbonyl group of the reacting ester (*i.e.*, the place where the enzyme attacks) is shifted by the spacer four bonds further away from the stereogenic centre at C(4).

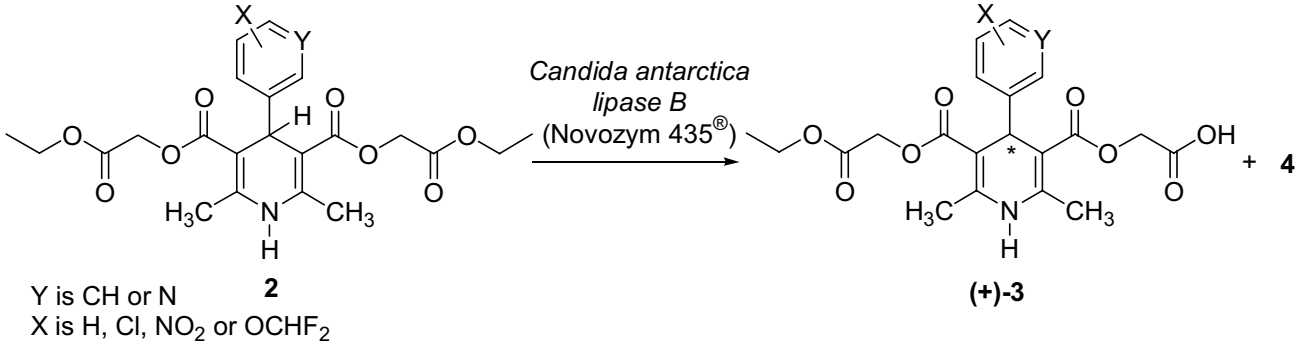
Comparison of both methodologies. Both the ethoxycarbonylmethyl esters **2** and the acyloxymethyl esters **5** of the 1,4-DHP's are suitable substrates for lipases. The reactive centre of the ethoxycarbonylmethyl esters **2** is one bond closer to the stereogenic carbon at C(4) of the 1,4-DHP in comparison to the corresponding acyloxymethyl esters **5**, what generally could result in a better stereoselectivity of the enzyme. On the other hand, a free carboxyl group is obtained directly after hydrolysis of the acyloxymethyl ester **5**, while the product of the hydrolysis of the ethoxycarbonylmethyl ester is the carboxymethyl ester **3**, which is not easy to hydrolyse further. Only when the 1,4-DHP contains no other reactive groups (*e.g.* another ethoxycarbonylmethyl ester), the carboxymethyl ester can be selectively removed by chemical hydrolysis to give the corresponding carboxylic acid.

For ethoxycarbonylmethyl esters **2** a direct enantioselective ester exchange *via* a transesterification reaction is possible *via* enzymatic asymmetrisation or kinetic resolution. The direct enantioselective synthesis of other esters from acyloxymethyl esters **5** *via* transesterification is not possible and the reaction product in this case will be the corresponding carboxylic acid **7**.

7.2 4-Aryl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates

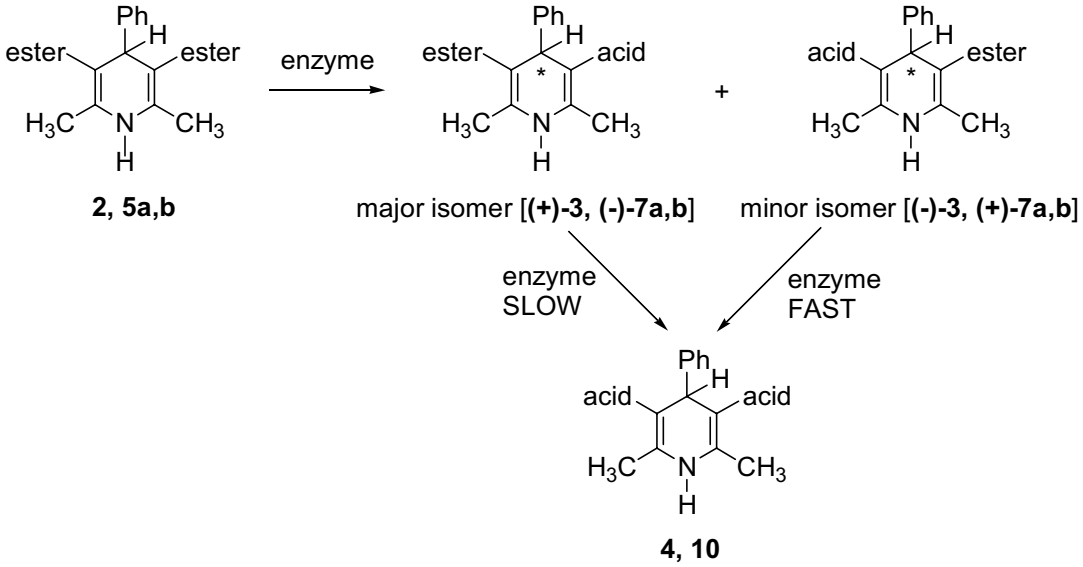
7.2.1 Prochiral 4-pyridyl or 4-aryl bis(ethoxycarbonylmethyl) 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates

The enantioselective enzyme-catalysed asymmetrisation of 4-pyridyl or 4-aryl substituted bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates **2** has been developed (Chapter 2; Scheme 2). Extensive screening yielded *Candida antarctica* lipase B (CAL-B, Novozym 435®) as the preferred biocatalyst. The hydrolysis is still enantioselective although the place of enzyme attack is five bonds away from the stereogenic centre at C(4).



Scheme 2

The asymmetrisation of the prochiral bifunctional 1,4-DHPs **2** is accompanied by further hydrolysis of the monoester (+)-**3** to the symmetrical diester **4**. This reaction decreases the chemical yield of (+)-**3**, but it increases the e.e. of (+)-**3** because the second hydrolysis is in fact a kinetic resolution (see Scheme 3 below). This increase of e.e. is indeed observed in our case.



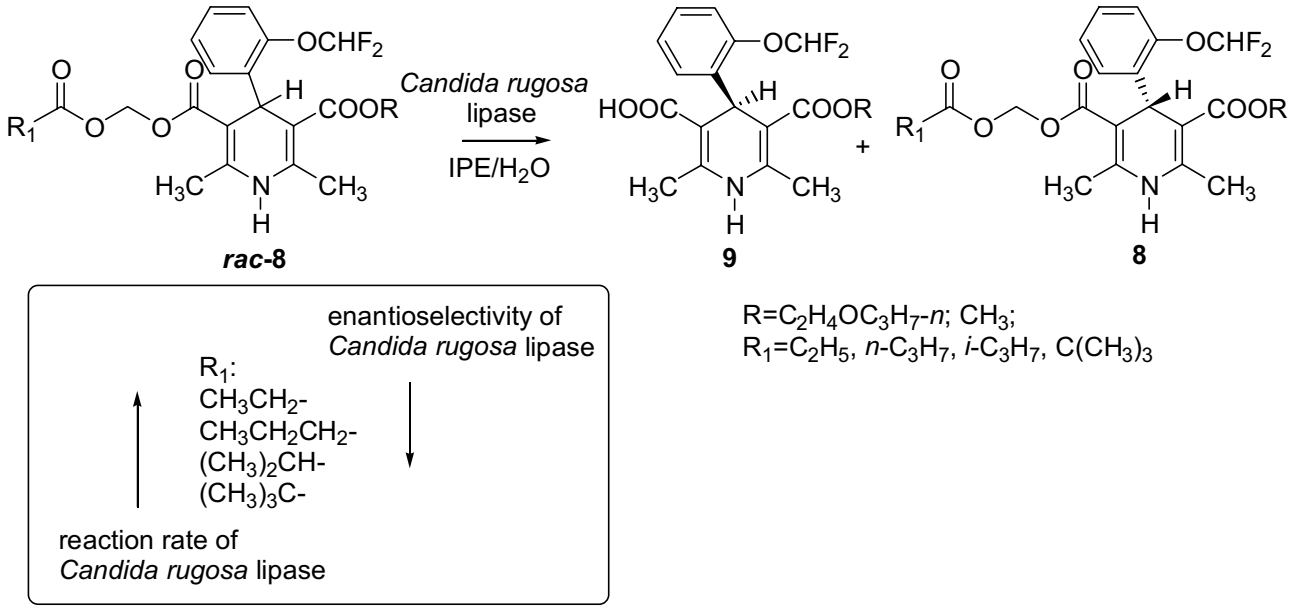
Scheme 3

The enantiomeric excesses of monoacids (+)-**3** varies from 68 to 97%, depending on the substituent at position 4. Although complete stereoselectivity of CAL-B towards the substrates was not achieved in all cases, the obtained results have shown that high e.e. values can be obtained by optimisation of the reaction medium. Another advantage of the method is that the synthesis of substrates **2** consists of one step *via* Hantzsch cyclisation. A disadvantage of the method is that only the 'outer' ester group of the ethoxycarbonylmethyl ester can be cleaved by CAL-B and the resulting carboxymethyl ester is difficult to remove.

7.2.2 Acyloxymethyl esters of 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylates

The use of acyloxymethyl derivatives for the synthesis of enantiopure 1,4-DHPs is advantageous, since acyloxymethyl esters split off completely during enzymatic hydrolysis. The synthesis of acyloxymethyl esters is rather complicated and consists of Hantzsch cyclisation and modification of the ester moieties in the 3- and 5-positions. The fact that the chiral centre is further away from the reaction centre (6 bonds) is not detrimental for enantioselective *Candida rugosa* lipase-catalysed hydrolysis of 4-aryl-1,4-DHPs having an acyloxymethyl group (Chapters 3 and 4; Schemes 4 and 5).

Kinetic resolution of racemic acyloxymethyl esters. Kinetic resolution of acyloxymethyl esters of 1,4-DHPs ***rac*-8** offers two enantiopure products (**9** and **8**) with opposite stereochemistry in up to 50% chemical yields (Scheme 4).

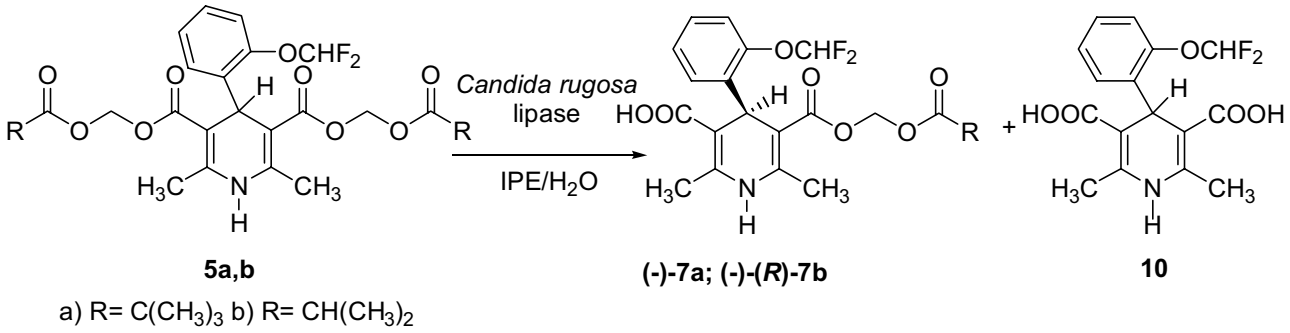


Scheme 4

The reaction of *Candida rugosa* lipase (CRL) with racemic acyloxymethyl esters of 5-methyl- and 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate ***rac*-8** in water-saturated IPE is dependent on the structure of the substrates. When the steric bulk of the acyloxymethyl ester ***rac*-8** is increased, lower reaction rates are observed, until the substrate becomes not reactive towards this enzyme at all (Chapter 3; Scheme 4). At the same time, the enantioselectivity of CRL increases together with the steric bulk of the acyloxymethyl ester group. The most stereoselective reaction (E[17]=21) was obtained for the derivative bearing an (isobutyryloxy)methyl ester at C(3) and a methyl ester at C(5). CRL lipase reacts preferentially with the ***S***-form of the racemic substrates ***rac*-8**, producing ***R***-monoacids **9**. The absolute configuration of the enzymatically produced carboxylic acids **9** was established to be 4*R* by X-ray crystallographic analysis of its (*R*)-(+)-1-phenylethylamine derivative.

Asymmetrisation of prochiral acyloxymethyl esters. An efficient CRL-catalysed asymmetrisation of bis acyloxymethyl derivatives **5a,b** has been developed (Chapter 4, Scheme 5). The enantioselectivity of the CRL-catalysed asymmetrisation of the prochiral bifunctional substrates bis[(pivaloyloxy)methyl] and bis[(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate **5a,b** to the corresponding monoacids **7a,b** was enhanced by the second hydrolysis to the achiral 1,4-dihydro-3,5-pyridinedicarboxylic acid **10**, which was apparently very stereoselective (Scheme 3). The second hydrolytic step decreases the

chemical yield of the desired monoacid **7a,b**. Slow reaction rates, incomplete enantioselectivity of CRL towards bis [(pivaloyloxy)methyl] derivative **5a** as well as a low chemical yield of the product **7a** made the use of this substrate not very practical. The enantiomeric excess of the *R*-monoacid **7b** obtained *via* the CRL–mediated asymmetrisation of the prochiral bis[(isobutyryloxy)methyl] derivative **5b** in water-saturated IPE at rt was ≥99%. It appears from the high enantioselectivity of CRL-catalysed hydrolysis of **5b** and the high enantiomeric ratio of CRL-catalysed kinetic resolution of ***rac*-8** bearing an isobutyryloxymethyl ester at C(3) that the isobutyryl moiety has the best fit in the active site of CRL. Because of its very high enantioselectivity, the CRL-mediated asymmetrisation of **5b** can serve as a useful method for the synthesis of enantiopure 2-(difluoromethoxy)phenyl substituted 1,4-DHPs.



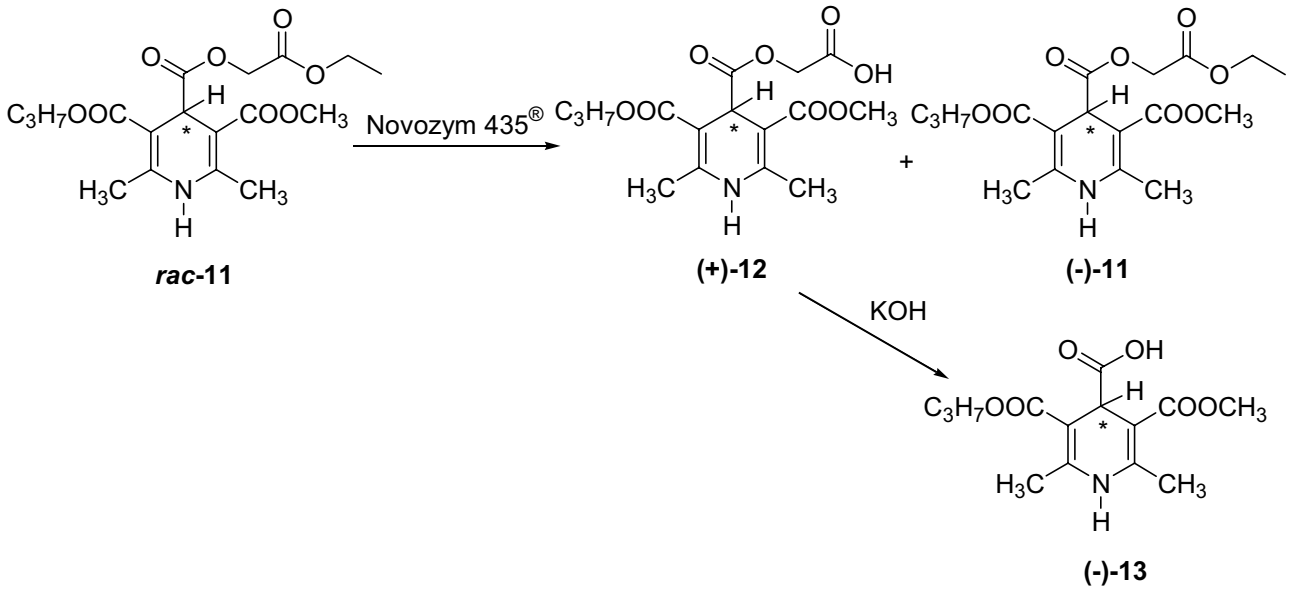
Scheme 5

7.3 Kinetic resolution of 1,4-dihydro-3,4,5-pyridinetricarboxylates

7.3.1 4-Ethoxycarbonylmethyl ester of 1,4-dihydro-3,4,5-pyridinetricarboxylate

The enzyme-catalysed kinetic resolution of the ethoxycarbonylmethyl ester of 1,4-dihydro-3,4,5-pyridinetricarboxylate ***rac*-11** has been investigated (Scheme 6; Chapter 5). In these compounds the ester that has to be hydrolysed is located at C(4) and again only the 'outer' ester group of the ethoxycarbonylmethyl ester can be cleaved by lipases. CAL-B was found as the most enantioselective enzyme. CAL-B still controls the stereochemistry at this reaction site, which is at a four-bond distance from the stereogenic centre at C(4). The transition from aqueous medium to water-saturated diisopropyl ether (IPE) led to a better stereoselectivity of CAL-B in the hydrolysis reaction. The reaction time is longer and the enantioselectivity is better at lower reaction temperature. In water-saturated IPE the enantioselectivity of CAL-B at 45°C toward the ethoxycarbonylmethyl ester was moderate (E=13.8), but was enhanced at rt and +4°C (E=21.5 and E=28.9, respectively). A high enantiomeric ratio (E=45.3) was reached at subzero temperatures, although at the expense of the reaction rate.

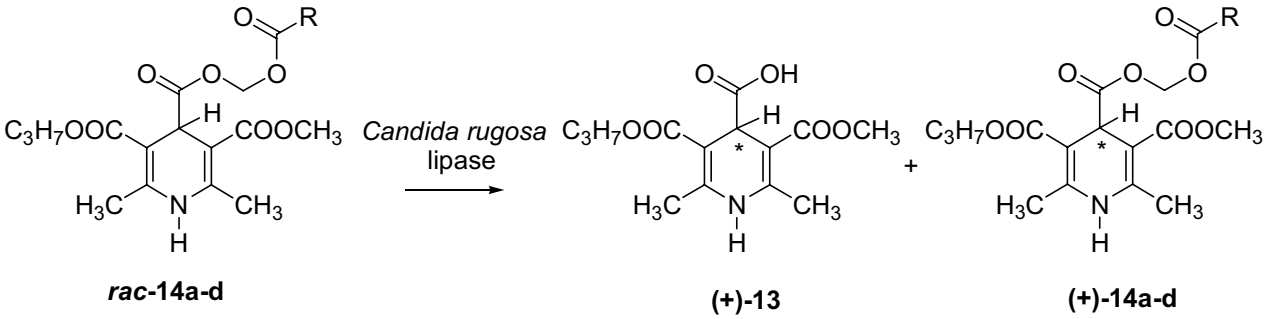
It is possible to cleave the carboxymethyl ester **12** by chemical hydrolysis in low yield, which is probably caused by decarboxylation during the hydrolysis and work-up. The stability of 1,4-dihydroisonicotinic acid **13** has not been specially studied by us; however, photochemical oxidation or isomerisation together with decarboxylation of a symmetrical analogue of **13** has been reported.[18]



Scheme 6

7.3.2 4-[(Acyloxy)methyl] esters of 2,6-dimethyl-1,4-dihydro-3,4,5-pyridinetricarboxylates

The acyloxymethyl esters of 1,4-dihydro-3,4,5-pyridinetricarboxylates ***rac*-14** have also been subjected to enzymatic hydrolysis. The use of this group is more advantageous, since it can be cleaved completely during the enzymatic hydrolysis, thus avoiding the decarboxylation problem. The lipase-catalysed kinetic resolution of four 4-[(acyloxy)methyl] 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridinetricarboxylates ***rac*-14** has been studied with variation of the enzyme, the structure of acyloxymethyl group and the reaction conditions (Chapter 5; Scheme 7). The distance of five bonds between the reacting carbonyl group and the stereogenic centre at C(4) is longer than in the ethoxycarbonylmethyl esters ***rac*-11** mentioned in Scheme 6, but one bond shorter than in the 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates **5** and ***rac*-8**. However, the screening has shown only low or moderate enantioselectivity of enzymes toward the acyloxymethyl esters in ***rac*-14**. The influence of steric bulk of the acyl group of the acyloxymethyl ester on the enantioselectivity of the enzymes was not so clear as in the case of **5** and ***rac*-8**. The most enantioselective enzyme was CRL, for which enantiomeric ratio's *E*=8 and *E*=9 were reached for the 4-isobutyryloxymethyl ***rac*-14c** and 4-butyryloxymethyl ***rac*-14b** derivatives, respectively.

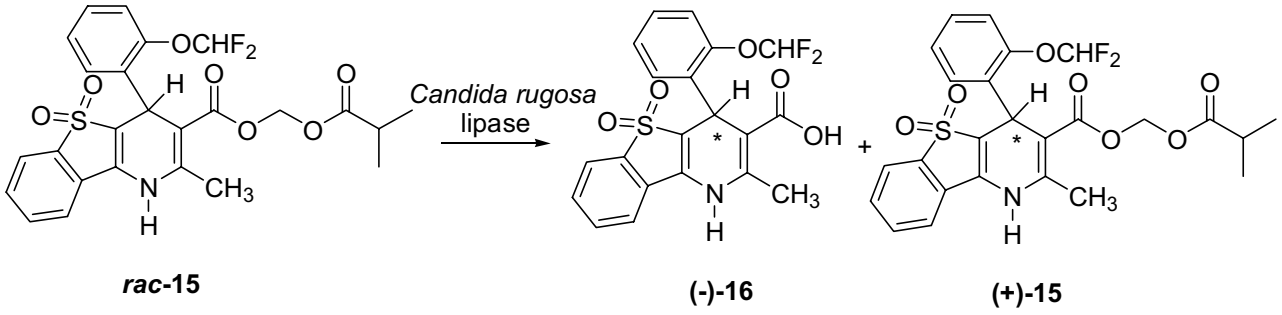


a) C_2H_5; b) C_3H_7; c) C_3H_7-*i;* d) $C(CH_3)_3$

Scheme 7

7.4 1,4-Dihydrobenzothieno[3,2-*b*]pyridine-5,5-dioxide

The CRL-catalysed kinetic resolution of 3-(isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno[3,2-*b*]pyridine-3-carboxylate ***rac*-15** has been developed (Chapter 6; Scheme 8). The 1,4-dihydrobenzothieno[3,2-*b*]pyridine-5,5-dioxides are possessing coronary dilating and anticancer activities. As the highest enantioselectivity of CRL-catalysed hydrolysis of the acyloxymethyl esters **5** and ***rac*-8** was found for the (isobutyryloxy)methyl derivatives **5b** and ***rac*-8**, the corresponding (isobutyryloxy)methyl derivative of 1,4-dihydrobenzothieno[3,2-*b*]pyridine-5,5-dioxide ***rac*-15** has been synthesised. A remarkably good *E* value was obtained for the CRL-catalysed resolution of this big 1,4-DHP derivative. The enantioselectivity of CRL can be improved by changing the reaction medium and the temperature. The transition from water-saturated IPE to a solution of *n*-butanol in toluene that was water-saturated at 45°C resulted in the increase of enantiomeric ratio from *E*=12 to *E*=28. More derivatives of the polycyclic 1,4-DHP ***rac*-15** would be worth to study.



Scheme 8

7.5 General conclusions

Chemoenzymatic methods for the enantioselective synthesis of 4-aryl-1,4-dihydropyridines, 1,4-dihydroisonicotinic acid derivatives and 1,4-dihydrobenzothieno[3,2-*b*]pyridine-5,5-dioxides have been developed. In this way, versatile enantiopure building blocks for unsymmetrical potentially pharmacologically active compounds have become accessible.

Lipases are not capable to cleave the alkyl esters of 4-aryl-1,4-dihydropyridine-3,5-dicarboxylates and 1,4-dihydro-3,4,5-pyridinetricarboxylates. Lipase-catalysed synthesis of enantiopure 1,4-dihydropyridinecarboxylates can be performed well, when the ester moieties contain enzymatically labile groups on spacers, such as ethoxycarbonylmethyl and acyloxymethyl.

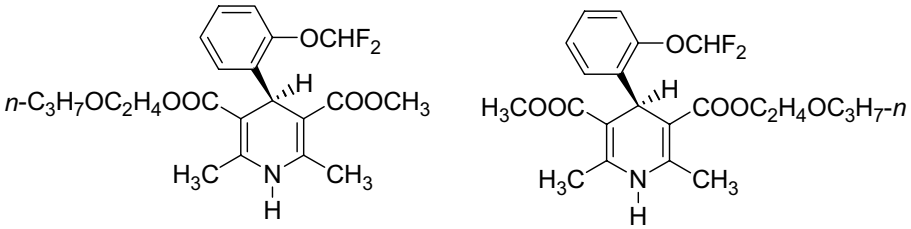
The reacting carbonyl group of the ethoxycarbonylmethyl ester or acyloxymethyl ester is shifted, respectively, for three or four bonds away from the stereogenic centre at C(4). Nevertheless, a good degree of selectivity of *Candida antarctica* lipase B and *Candida rugosa* lipase were achieved in many cases. Asymmetrisations of 4-substituted bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates **2** occurred with e.e.'s of 68-97%. An *E* value of 21 was obtained for kinetic resolutions of racemic acyloxymethyl esters of 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate ***rac*-8**. The e.e. of the asymmetrisation product of bis[(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridine-dicarboxylate **5b** was ≥99%. A high enantiomeric ratio (*E*=45.3) was reached for the ethoxycarbonylmethyl ester of 1,4-dihydro-3,4,5-pyridinetricarboxylate ***rac*-11**. Enantiomeric ratio's of 8-9 were reached in the kinetic resolution of acyloxymethyl derivatives of 1,4-dihydro-3,4,5-pyridinetricarboxylate ***rac*-14**. *E*=28 was reached in kinetic resolution of the isobutyryloxymethyl ester of 1,4-dihydrobenzothieno[3,2-*b*]pyridine-5,5-dioxide ***rac*-15**. These results prove that the generally accepted idea that remote chiral centres cannot be recognised well by enzymes is not true for the cases studied.

The products of the hydrolysis of acyloxymethyl esters of 1,4-DHPs **5**, ***rac*-8**, ***rac*-14** and ***rac*-15** are the corresponding carboxylic acids. The products of the hydrolysis of ethoxycarbonylmethyl esters **2** and ***rac*-11** are the carboxymethyl esters, which are not easy to remove selectively.

The obtained results show the different catalytic properties of CRL and CAL-B. CRL is more enantioselective toward acyloxymethyl esters of 1,4-DHPs, while CAL-B is well suited for the hydrolysis of ethoxycarbonylmethyl esters of 1,4-DHPs. Increasing the size and branching of the acyloxymethyl ester of 1,4-DHP is a tool for the enhancement of enantioselectivity of CRL. The enantioselectivity of CRL and CAL-B can be altered by the change of the reaction medium and reaction temperature.

7.6 Outlook

These studies show that our investigations of lipase-catalysed hydrolyses of acyloxymethyl and ethoxycarbonylmethyl esters of 1,4-DHPs provide useful methods for the enantioselective synthesis of several novel 1,4-dihydropyridine derivatives (Schemes 4-9). The synthesis of the (+)-(*S*)- and the (—)-(*R*)-isomer of the asymmetric cerebrocrast analogue 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate **17** has been performed in moderate to good enantiomeric excesses, depending on the strategy of the synthesis. A wide variety of asymmetric derivatives of cerebrocrast for pharmacological tests can be synthesised from enantiopure compounds **7-9**.



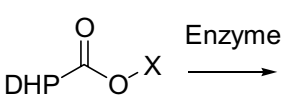
(+)-(*S*)-17 **(-)-(*R*)-17**

Future trends of applications of hydrolytic enzymes in the synthesis of enantiopure dihydropyridinecarboxylates as new pharmacologically important compounds are outlined in Scheme 9. The skeletons of dihydropyridines depicted in Scheme 9 represent series of compounds, synthesised in Laboratory of Membrane Active Compounds and β-diketones of Latvian Institute of Organic Synthesis (Riga, Latvia). Many of these compounds have shown different biological activities and therefore show perspective for further biological investigations. The presented methodology can find applications in the enantioselective synthesis of a wide range of potentially pharmacologically active dihydropyridines, such as Hantzsch-type 1,4-DHPs, 1,4-dihydroisonicotinic acid derivatives, polycyclic 1,4-DHP derivatives, polycyclic and monocyclic 1,2-DHPs and others.

The absolute configurations of several synthesised compounds still have to be established. Since the absolute configurations of **3**, **13** and **16** cannot be determined by the comparison of the signs of specific optical rotations with reference compounds, 3-D structures can be established only by X-ray diffraction analyses. Due to a low quality or lack of crystals of diastereomeric derivatives of 1,4-DHPs **3** and **13** with optically active alcohols and amines, it was not possible to determine their absolute configurations. The problems with crystallisation of single stereoisomers may even occur if the corresponding diastereomeric mixture is a crystalline substance.[19] This problem has to be solved in future research. A possible way to obtain crystalline materials for X-ray analysis is the synthesis of an extended set of enantiopure 1,4-DHP and 1,2-DHP derivatives with optically active alcohols and amines. This approach is outlined in the Scheme 10.

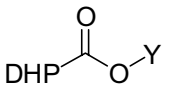
methodology

substrate



Enzyme

chiral key intermediate

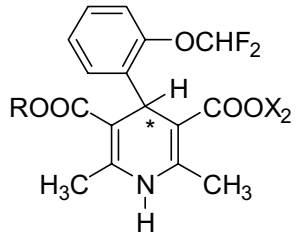


enantiopure compounds for pharmacological tests

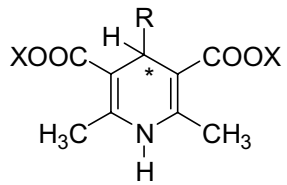
X_1=$CH_2COOC_2H_5$;
X_2=$CH_2OC(O)Alk$

Y_1=CH_2COOH
Y_2=H

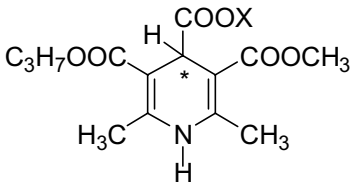
resolved compounds



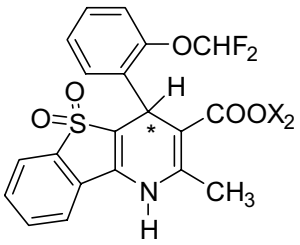
R=$C_2H_4OC_3H_7$; CH_3



when X=X_1, R=Ar; 3-pyridyl
when X=X_2, R=2-($OCHF_2$)Ph



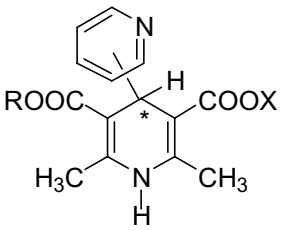
X=X_1; X_2

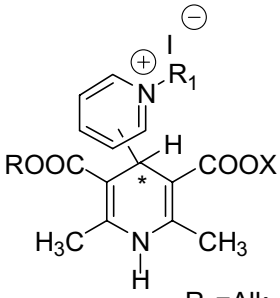


X_1=$CH_2COOC_2H_5$;
X_2=$CH_2OC(O)Alk$

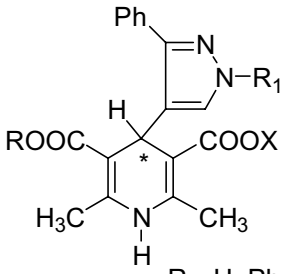
future trends

4-aryl or 4-heteryl-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylates:



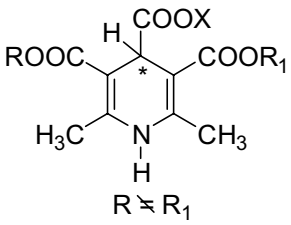


R_1=Alk



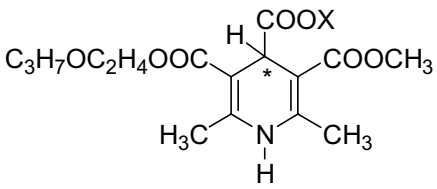
R_1=H; Ph

1,4-dihydroisonicotinic acid derivatives:

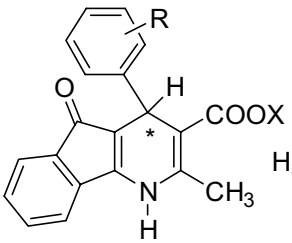


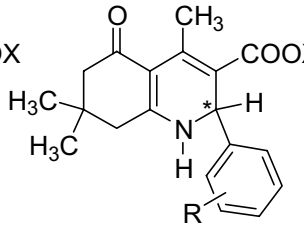
R ≠ R_1

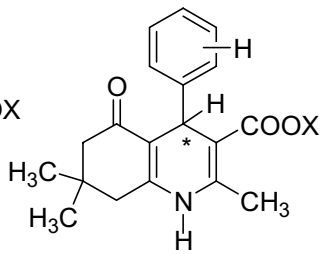
e.g.:



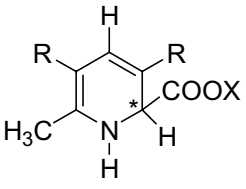
polycyclic 1,4- and 1,2-dihydropyridines:

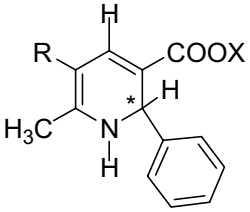




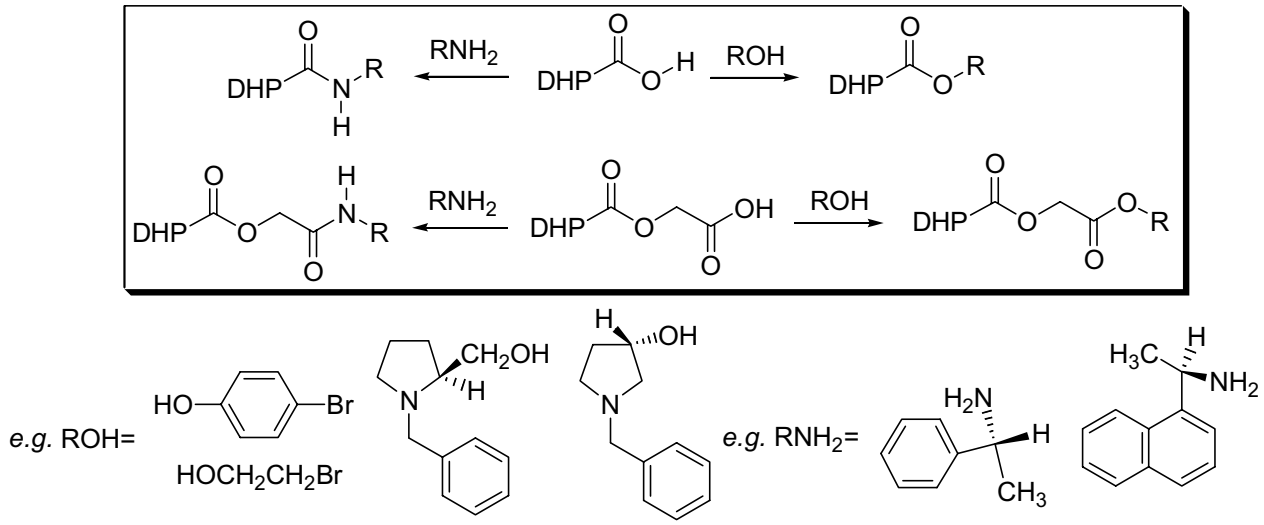


1,2-dihydropyridines:





Scheme 9



Scheme 10

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Summary

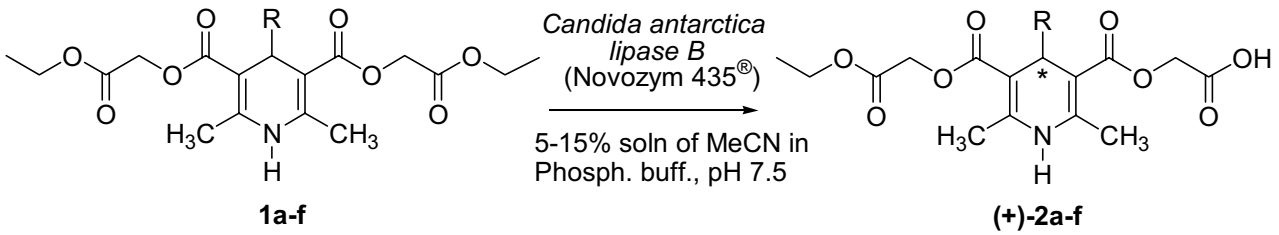
Chirality is important for the activity of many biologically active compounds, since differences in biological properties of stereoisomers occur frequently. The exact stereochemical composition of each new chiral compound as well as toxicological and pharmacological data for racemic and enantiomerically pure compounds are required for their approval as new chiral medicines in the EU.

The research described in this thesis deals with the chemoenzymatic synthesis of 1,4-dihydropyridine derivatives (1,4-DHPs) in enantiopure form as the key intermediates for chiral analogues of symmetrical biologically active compounds. The use of enzymes is an advantageous alternative to classical chemical methods, as enzymes are efficient catalysts with high chemo-, regio- and stereoselectivity under mild conditions.

In Chapter 1, a literature review is given about the synthesis of 1,4-dihydropyridine derivatives, and their biological activities. Synthesis of 1,4-DHPs by cyclocondensation reactions and reduction of pyridines are described whereby special attention is paid to stereoselective chemical and biotechnological methods for the synthesis of enantiopure 1,4-DHPs.

Derivatives of bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates have shown antimetastatic activities as well as activities against the Herpes simplex virus. The first objective of the current research is the enzyme-catalysed hydrolysis of these compounds as described in Chapter 2.

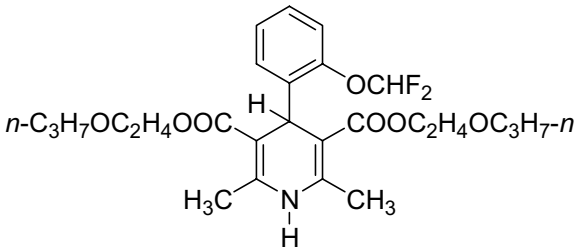
Prochiral bis(ethoxycarbonylmethyl) substituted 4-aryl-1,4-dihydropyridine-3,5-dicarboxylates **1a-f** are hydrolysed enantioselectively by *Candida antarctica* lipase B (Novozym 435®) (Scheme 1). The enantiomeric excesses range from 68 to 97%, depending on the substituent at position 4. In some cases, the e.e. can be significantly increased by changing the solvent system.



a: R=C_6H_5 (e.e.=93%); b: R=2-$OCHF_2$-C_6H_4 (e.e.=79%; *e.e.=97% in H_2O-satd IPE); c: R=3-NO_2-C_6H_4 (e.e.=77%); d: R=4-Cl-C_6H_4 (e.e.=68%) e: R=2-Cl-C_6H_4 (e.e.=72%); f: R=3-pyridyl (e.e.=82%)

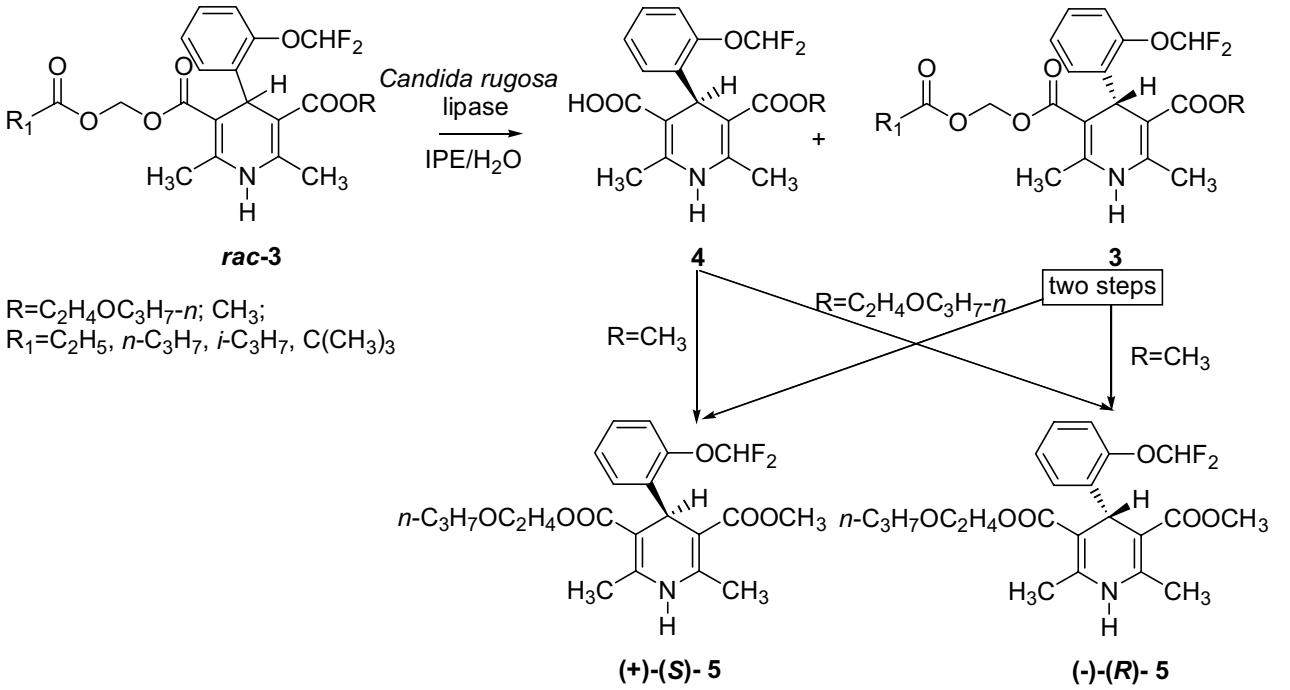
Scheme 1

Cerebrocrast (2,6-dimethyl-3,5-bis[2-(propoxy)ethoxycarbonyl]-4-[2-(difluoromethoxy)phenyl]-1,4-dihydropyridine) is a highly active neuroprotector. This compound has been found active in the treatment of diabetes and various inflammatory disorders. Chapter 3 is devoted to the synthesis of chiral analogues of cerebrocrast in enantiopure form *via* enzyme-catalysed kinetic resolution of 2,6-dimethyl-4-[2-(difluoromethoxy)phenyl]-1,4-dihydropyridine 3,5-diesters.



Cerebrocrast

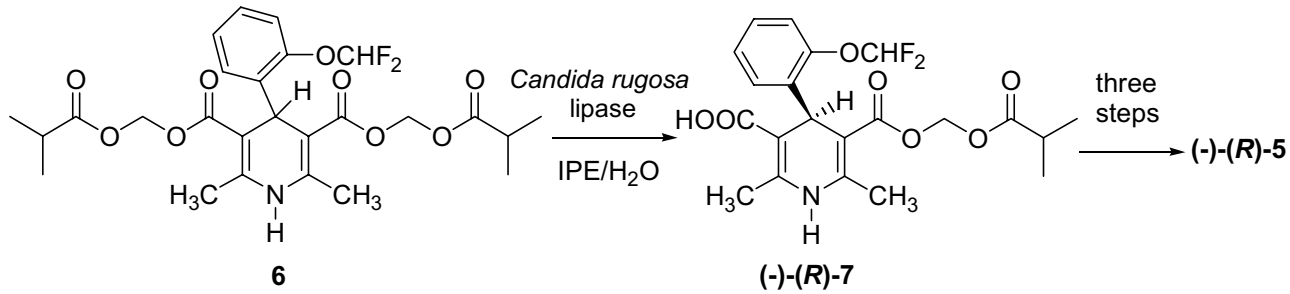
Alkyl esters at the 3- and 5-positions of 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates are not hydrolysed by commercially available hydrolases, and 4-substituted bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylates can be cleaved by lipases only at the 'outer' ester group. Therefore, derivatives have been prepared which contain a spacer that spontaneously detaches after enzymatic hydrolysis of the 'outer' ester group (Chapter 3). Seven acyloxymethyl esters of 5-methyl- and 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate **3** have been synthesised and subjected to *Candida rugosa* lipase (CRL) catalysed hydrolysis in wet diisopropyl ether (Scheme 2). A methyl ester at the 5-position and a long or branched acyl chain at C(3) give the highest enantiomeric ratio (*E* value). The most stereoselective reaction (*E*=21) is obtained with 3-[(isobutyryloxy)methyl] 5-methyl 4-(2-difluoromethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate **3**, and this compound is used to prepare both enantiomers of 3-methyl 5-(2-propoxyethyl) 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylate **5**.



Scheme 2

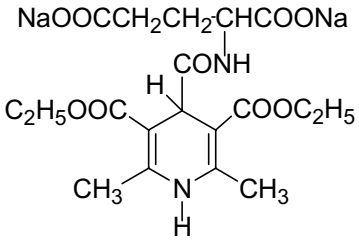
The absolute configuration of the enzymatically-produced carboxylic acid has been established to be 4*R* by X-ray crystallographic analysis of its 1-(*R*)-phenylethyl amide.

In Chapter 4, an efficient chemoenzymatic synthesis of (—)-(*R*)-**5** is described (Scheme 3). The key step in this approach is the asymmetrisation of a symmetrical bifunctional substrate. The enantioselectivity of *Candida rugosa* lipase–mediated asymmetrisation of the prochiral bis[(isobutyryloxy)methyl] 4-[2-(difluoromethoxy)phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate is excellent (≥99%).



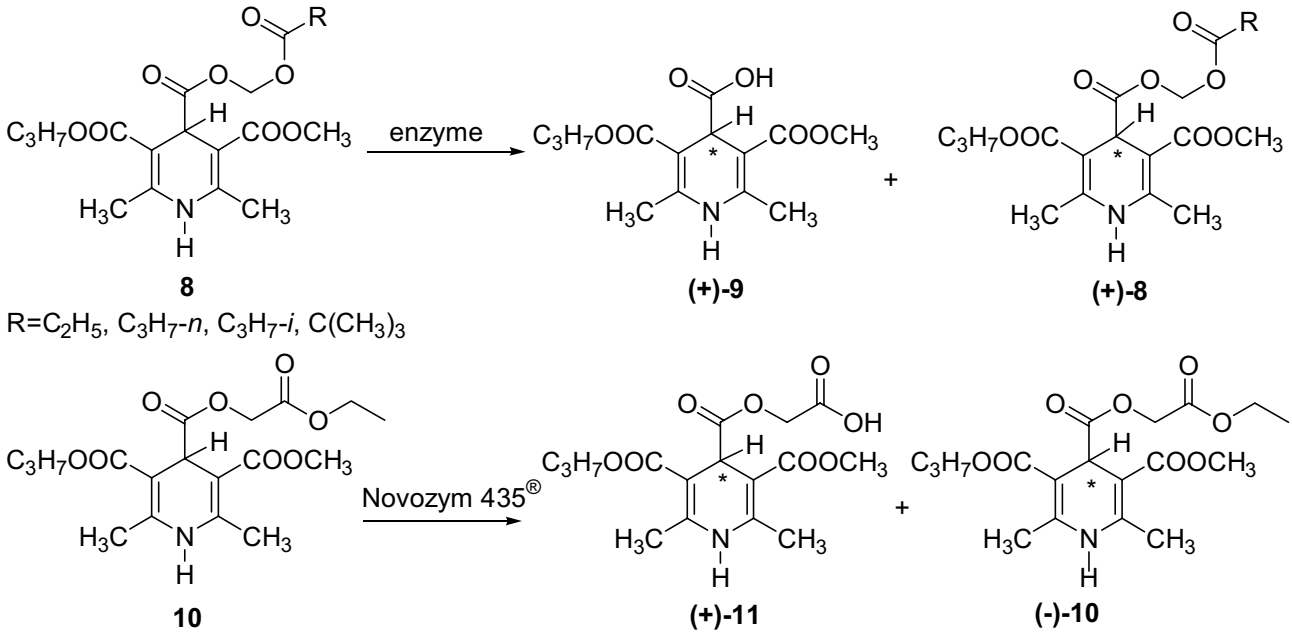
Scheme 3

The disodium salt of 2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine-4-carboxamido)-glutaric acid (glutapyrone) possesses an unusually broad spectrum of biological activities at low concentrations such as neuromodulatory and neuroregulatory action. It is an anticonvulsant, stress-protective, antiarrhythmic, cognition and memory enhancing compound.



Glutapyrone

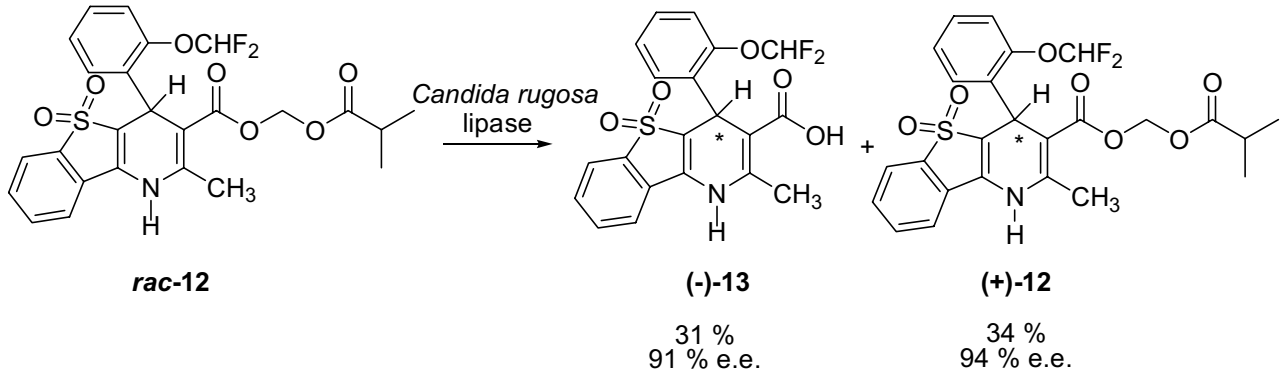
3,4,5-Trialkyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridinetricarboxylates like glutapyrone are inert to the attack of hydrolytic enzymes. The exchange of at least one alkyl group to an enzymatically labile moiety (*e.g.* acyloxymethyl or ethoxycarbonylmethyl) turns these 1,4-dihydroisonicotinic acid derivatives into substrates for hydrolytic enzymes. Since acyloxymethyl and ethoxycarbonylmethyl derivatives of 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates have been recognised as being susceptible to lipases (see Chapters 2-4), the corresponding derivatives of 1,4-dihydroisonicotinic acid **8** and **10** have been prepared (see Scheme 4). The lipase-catalysed kinetic resolution of five derivatives of 4-[(acyloxy)methyl] and 4-ethoxycarbonylmethyl 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridinetricarboxylates **8** and **10** has been investigated, and the results are described in Chapter 5. Whereas the enantioselectivity of lipases towards the acyloxymethyl derivatives **8** is rather low, the *Candida antarctica* lipase B (Novozym 435®) catalysed hydrolysis of the ethoxycarbonylmethyl ester of 1,4-dihydroisonicotinic acid **10** is enantioselective. In water-saturated diisopropyl ether at 45°C the enantioselectivity of Novozym 435® toward the ethoxycarbonylmethyl ester **10** is rather moderate (E=13.8), but it is enhanced at rt and +4°C (E=21.5 and E=28.9, respectively). A high enantiomeric ratio (E=45.3) is reached at subzero temperatures, although at the expense of the reaction rate.



Scheme 4

Polycyclic 1,4-DHPs in enantiopure form are desired for extended pharmacological studies, since racemic 1,4-dihydrobenzothieno[3,2-*b*]pyridine-5,5-dioxides and 5-oxo-4,5-dihydro-1,4-indeno[1,2-*b*]pyridines possess various biological activities such as coronary dilating and anticancer; they have also been found active as glutathione S-transferase inhibitors.

The lipase-catalysed kinetic resolution of enzymatically labile 3-(isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno[3,2-*b*]pyridine-3-carboxylate ***rac*-12** is described in Chapter 6 (Scheme 5). The most enantioselective reaction (*E*=28) is a CRL-mediated transesterification with *n*-butanol in water-saturated toluene, at 45°C.



***rac*-12** **(-)-13** **(+)-12**

31 %
91 % e.e.

34 %
94 % e.e.

Scheme 5

The main results of this study are evaluated in Chapter 7. The approaches to overcome inactivity of hydrolytic enzymes toward simple esters of 1,4-DHPs, with their advantages, disadvantages and limitations, are discussed. The perspectives of the applications of chemoenzymatic approaches to the synthesis of enantiopure pharmacologically important novel dihydropyridine derivatives are also outlined.

It can be concluded that the use of hydrolytic enzymes, acting on hydrolysable groups on spacers, is a useful and widely applicable method for the enantioselective synthesis of hydrogenated pyridines.

Samenvatting

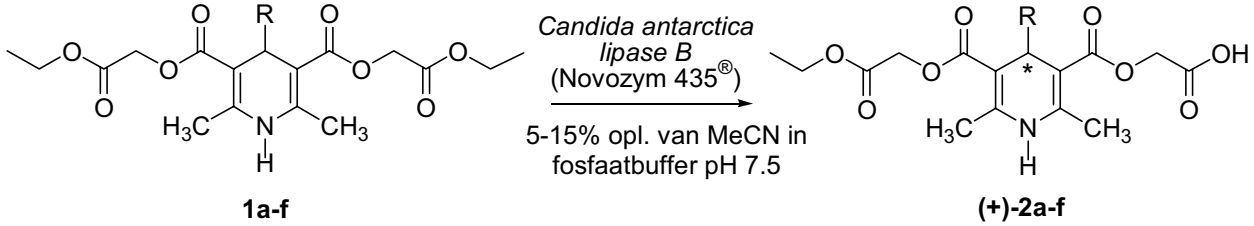
Chiraliteit is belangrijk voor de activiteit van veel biologisch actieve verbindingen, gezien het feit dat stereo-isomeren vaak verschillende biologische activiteiten hebben. Voor de goedkeuring van nieuwe chirale medicijnen in de EU is het noodzakelijk om informatie te verstrekken over de exacte stereochemische samenstelling van de stof, alsmede over de toxicologie en farmacologie van de zuivere enantiomeren en van het racemaat.

Het onderzoek beschreven in dit proefschrift betreft de chemo-enzymatische synthese van 1,4-dihydropyridine derivaten (1,4-DHPs) in enantiomeer zuivere vorm, als essentiële bouwstenen voor chirale analoga van symmetrische biologisch actieve verbindingen. Het gebruik van enzymen hierbij heeft voordelen ten opzichte van klassieke chemische methoden omdat enzymen reacties efficiënt katalyseren onder milde condities en met hoge chemo-, regio- en stereoselectiviteit.

In Hoofdstuk 1 wordt een literatuuroverzicht gegeven van de synthese van 1,4-dihydropyridine derivaten en hun biologische activiteiten. De synthese van 1,4-DHPs door middel van cyclo-condensatie reacties en via reductie van pyridines wordt beschreven waarbij speciale aandacht wordt gegeven aan stereoselectieve chemische en biotechnologische methoden voor de synthese van enantiozuivere 1,4-DHPs.

Van enkele 4-gesubstitueerde bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylaat derivaten is aangetoond dat ze metastase remmende activiteit hebben en dat ze actief zijn tegen het Herpes simplex virus. Het eerste doel van het onderhavige onderzoek betreft de enzym-gekatalyseerde hydrolyse van deze verbindingen. De resultaten zijn beschreven in Hoofdstuk 2.

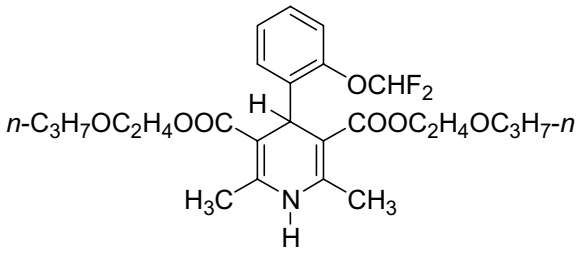
Prochirale bis(ethoxycarbonylmethyl) gesubstitueerde 4-aryl-1,4-dihydropyridine-3,5-dicarboxylaten **1a-f** worden enantioselectief gehydrolyseerd door *Candida antarctica* lipase B (Novozym 435®) (Schema 1). De enantiomere overmaat variëert van 68 tot 93%, afhankelijk van de substituent op de 4-positie. In een aantal gevallen kan de e.e. aanzienlijk verhoogd worden door optimalisatie van het oplosmiddel.



a: R=C_6H_5 (e.e.=93%); b: R=2-$OCHF_2$-C_6H_4 (e.e.=79%; * 97% in H_2O-verz. IPE); c: R=3-NO_2-C_6H_4 (e.e.=77%); d: R=4-Cl-C_6H_4 (e.e.=68%) e: R=2-Cl-C_6H_4 (e.e.=72%); f: R=3-pyridyl (e.e.=82%)

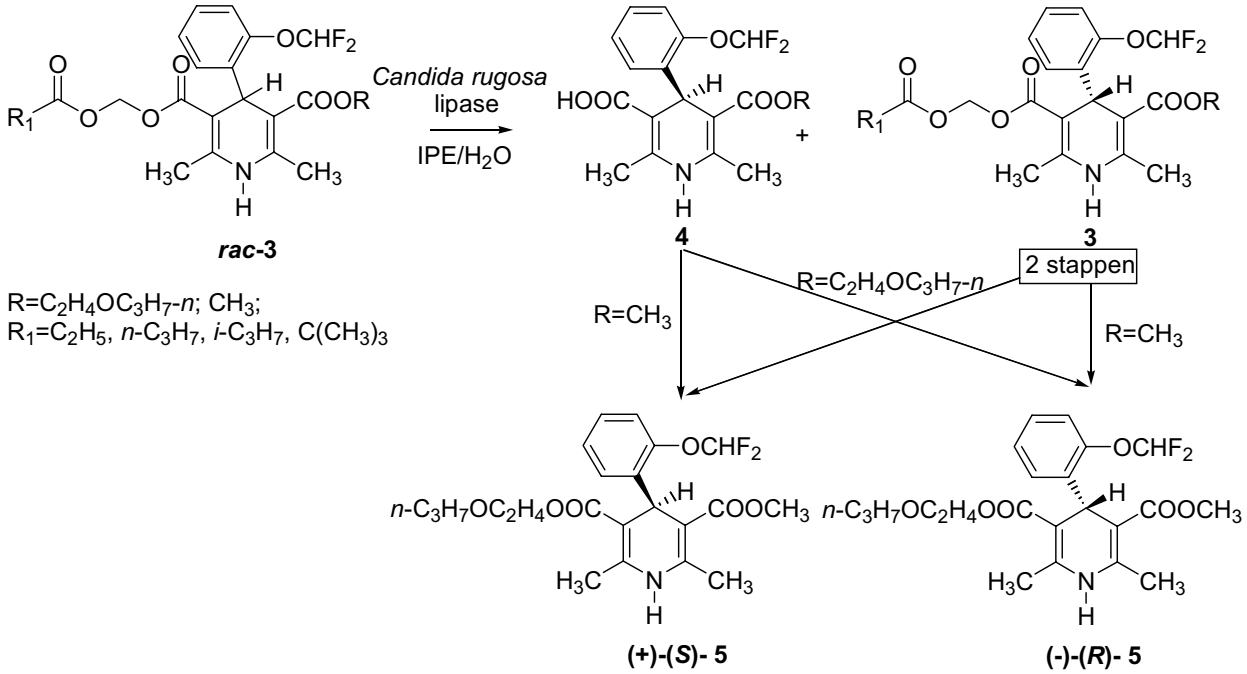
Schema 1

Cerebrocrast (2,6-dimethyl-3,5-bis[2-(propoxy)-ethoxycarbonyl]-4-[2-(difluormethoxy)fenyl]-1,4-dihydropyridine) is een zeer actieve neuronenbeschermende stof. Voorts is cerebrocrast effectief in de behandeling van diabetes en als ontstekingsremmer. Hoofdstuk 3 is gewijd aan de synthese van chirale analoga van cerebrocrast in enantiozuivere vorm door middel van enzymgekatalyseerde kinetische resolutie van 2,6-dimethyl-4-[2-(difluormethoxy)fenyl]-1,4-dihydropyridine 3,5-di-esters.



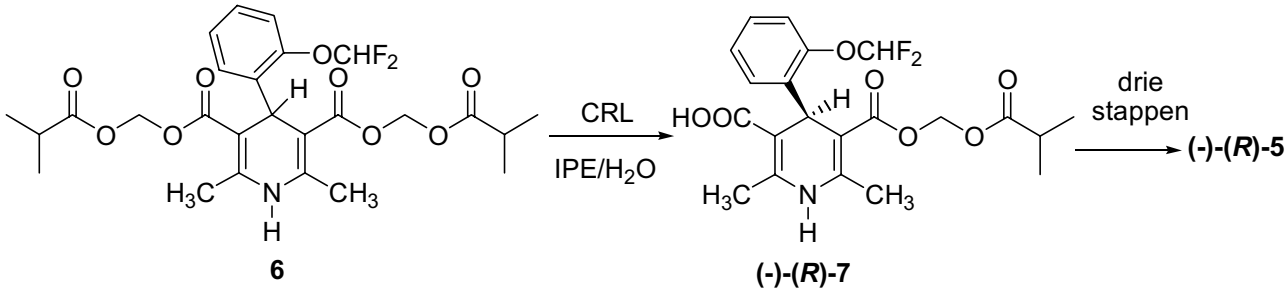
Cerebrocrast

Alkylesters op de 3- en 5-posities van 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylaten worden niet gehydrolyseerd door commercieel verkrijgbare hydrolasen, en 4-gesubstitueerde bis(ethoxycarbonylmethyl) 1,4-dihydropyridine-3,5-dicarboxylaten worden alleen op de buitenste estergroep gesplitst door lipasen. Om deze reden zijn derivaten gemaakt die een spacer bevatten die spontaan afsplitst na enzymatische hydrolyse van de 'buitenste' ester groep (Hoofdstuk 3). Zeven acyloxymethylesters van 5-methyl- and 5-(2-propoxyethyl) 4-[2-(difluormethoxy)fenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylaat **3** zijn gesynthetiseerd en onderworpen aan een *Candida rugosa* lipase (CRL) gekatalyseerde hydrolyse in natte di-isopropylether (Schema 2). Een methylester op de 5-positie en een lange of vertakte acylketen op C(3) geeft de hoogste enantiomere ratio (*E*-waarde). De meest stereoselectieve reactie (*E*=21) is verkregen met 3-[(isobutyryloxy)methyl] 5-methyl 4-(2-difluormethoxyfenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylaat **3** en deze verbinding is gebruikt om beide enantiomeren te bereiden van 3-methyl 5-(2-propoxyethyl) 4-[2-(difluormethoxy)fenyl]-2,6-dimethyl-1,4-dihydro-3,5-pyridinedicarboxylaat **5**. De absolute configuratie van het enzymatisch gevormde carbonzuur is 4*R*, hetgeen vastgesteld is door een kristalstructuurbepaling van het overeenkomstige 1-(*R*)-fenylethylamide.



Schema 2

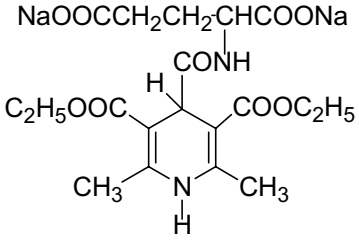
In Hoofdstuk 4 is een efficiënte synthese beschreven van (—)-(*R*)-**5** (Schema 3). De sleutelreactie in deze benadering is de asymmetrisering van een symmetrisch bifunctioneel substraat. De enantioselectiviteit van de *Candida rugosa* lipase–gekatalyseerde asymmetrisering van het prochirale bis[(isobutyryloxy)methyl] 4-[2-(difluormethoxy)fenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylaat is uitstekend (≥99%).



Schema 3

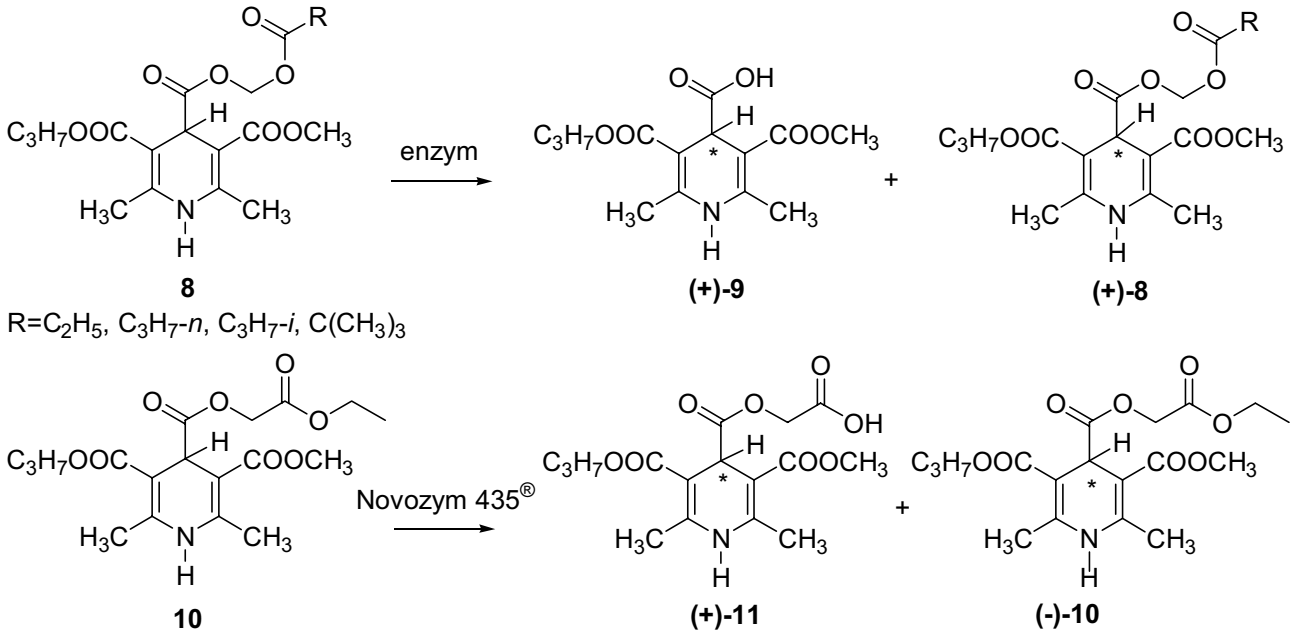
Het dinatriumzout van 2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine-4-carboxamido)-glutaarzuur (glutapyron) bezit een ongebruikelijk groot spectrum van biologische activiteiten bij lage concentraties, zoals het reguleren van pré- en postsynaptische afgifte en opname van neurotransmitters. Andere tot nu gevonden werkingen zijn: remming van convulsies (ongecontroleerde spiersamentrekkingen), beschermend tegen chemische stressfactoren

(oxidatoren, calcium e.d.), regulatie van het hartritme, verbeteren van het leervermogen en het geheugen.



Glutapyrone

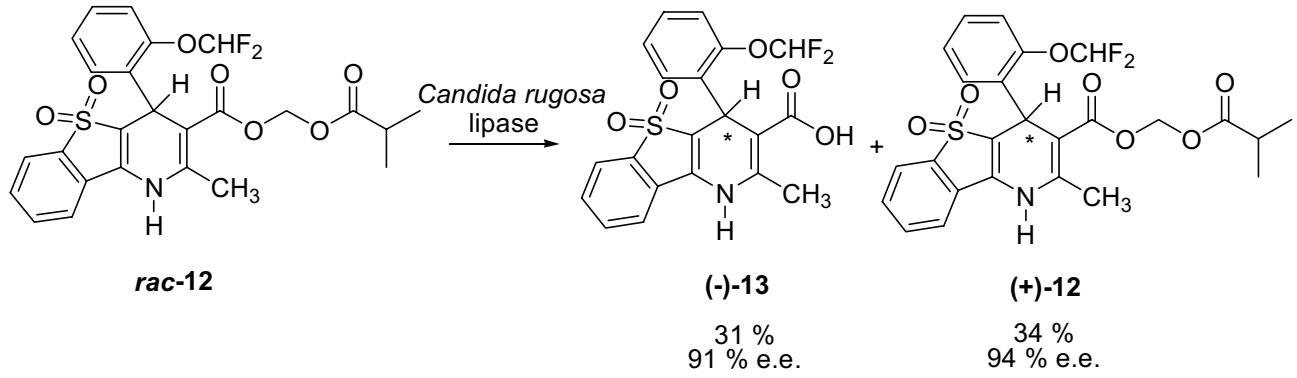
3,4,5-Trialkyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridinetricarboxylaten zoals glutapyron zijn inert ten opzichte van hydrolytische enzymen. Het vervangen van minstens één van de alkylgroepen door een spacer met een estergroep (bijv. acyloxymethyl of ethoxycarbonylmethyl) verandert 1,4-dihydroisonicotinezuurderivaten in substraten voor hydrolytische enzymen. Omdat eerder onderzoek aan acyloxymethyl en ethoxycarbonylmethylderivaten van 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylaten heeft uitgewezen dat deze stoffen goed herkend worden door lipasen (zie Hoofdstuk 2-4), zijn de overeenkomstige derivaten van 1,4-dihydroisonicotinezuur (**8** en **10**) gesynthetiseerd. De lipase-gekatalyseerde kinetische resolutie van vijf verschillende derivaten van 4-[(acyloxy)methyl] en 4-ethoxycarbonylmethyl 3-methyl 5-propyl 2,6-dimethyl-1,4-dihydro-3,4,5-pyridinetricarboxylaten **8** en **10** is onderzocht (Schema 4) en de resultaten zijn beschreven in Hoofdstuk 5. De enantioselectiviteit van lipasen ten opzichte van de acyloxymethyl derivaten **8** is vrij laag, maar de *Candida antarctica* lipase B (Novozym 435®) gekatalyseerde hydrolyse van de ethoxycarbonylmethylester van 1,4-dihydroisonicotinezuur **10** is enantioselectief. De enantioselectiviteit van Novozym 435® ten opzichte van de ethoxycarbonylmethylester **10** is tamelijk laag in di-isopropylether, verzadigd met water, bij 45°C (E=13,8), maar wordt verhoogd indien de reactie wordt uitgevoerd bij kamertemperatuur of +4°C (E=21,5 en E=28,9, respectievelijk). Een hoge enantiomere ratio (E=45,3) wordt verkregen bij temperaturen onder het vriespunt maar dit gaat ten koste van de reactiesnelheid.



Schema 4

Enantiozuivere polycyclische 1,4-DHPs zijn benodigd voor uitgebreide farmacologische studies, omdat is gebleken dat racemische 1,4-dihydrobenzothieno[3,2-*b*]pyridine-5,5-dioxides en 5-oxo-4,5-dihydro-1,4-indeno[1,2-*b*]pyridines diverse biologische activiteiten bezitten zoals vaatverwijdend en antikanker; ook is gevonden dat ze actieve glutathion S-transferase remmers zijn.

De lipase-gekatalyseerde kinetische resolutie van het enzymatisch hydrolyseerbare 3-(isobutyryloxy)methyl 4-[2-(difluormethoxy)fenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno-[3,2-*b*]pyridine-3-carboxylaat **12** is beschreven in Hoofdstuk 6 (Schema 5). De meest enantioselectieve reactie (*E*=28) is een CRL-gekatalyseerde transesterificatie met *n*-butanol in water-verzadigde tolueen bij 45°C.



Schema 5

De belangrijkste resultaten van dit onderzoek worden geëvalueerd in Hoofdstuk 7. De gekozen benaderingen om de lage activiteit van hydrolytische enzymen ten opzichte van eenvoudige esters van 1,4-DHPs te omzeilen wordt besproken, met alle voordelen, nadelen en beperkingen. Voorts worden de perspectieven geschetst van de toepassing van chemo-enzymatische benaderingen voor de synthese van enantiozuivere, farmacologisch interessante, nieuwe 1,4-dihydropyridine derivaten. Samenvattend kan gezegd worden dat het gebruik van hydrolytische enzymen die reageren met hydrolyseerbare groepen op spacers een goede en algemeen toepasbare methode is voor de enantioselectieve synthese van gehydrogeneerde pyridines.

Kopsavilkums

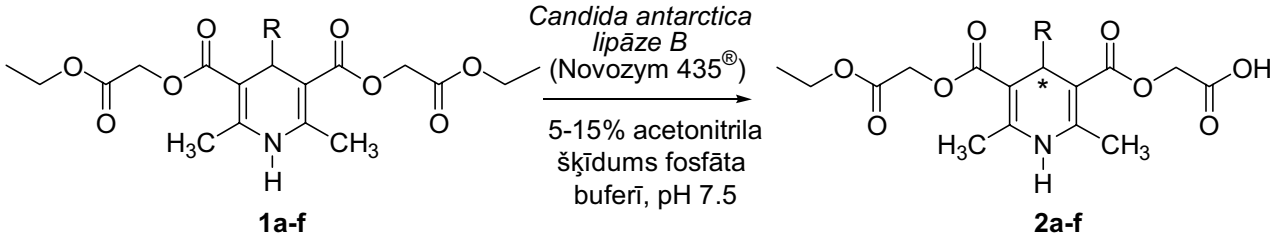
Hiralitāte ietekmē daudzu bioloģiski aktīvo savienojumu iedarbību, jo stereoizomēru bioloģiskās īpašības ļoti bieži savstarpēji krasi atšķiras un var būt pat diametrāli pretējas. Eiropas Savienībā un ASV jauno hirālu ārstniecības preparātu ieviešanai klīnikā ir nepieciešams noskaidrot savstarpējās enantiomēru attiecības preparātā, izpētīt racemiskā maisījuma un atsevišķu enantiomēru toksicitāti un farmakoloģisko iedarbību.

Šī zinātniski pētnieciskā darba galvenais mērķis ir enantiotīru 1,4-dihidropiridīna (1,4-DHP) atvasinājumu ķīmioenzimātisko sintēzes metožu izstrādāšana jaunu nesimetrisku bioloģiski aktīvu savienojumu iegūšanai. Enzīmu lietošanai organiskajā sintēzē, salīdzinot ar klasiskām ķīmiskām metodēm, ir virkne priekšrocību, jo enzīmi ir augsti efektīvi regio- un stereoselektīvi katalizatori, turklāt enzimātiski katalizējamās reakcijas var veikt maigos apstākļos.

1. nodaļā ir literatūras apskats par 1,4-dihidropiridīna atvasinājumu sintēzi un to bioloģiskām īpašībām, un apkopoti 1,4-dihidropiridīnu sintēzes ceļi ciklokondensācijas un piridīnu reducēšanas reakcijās. Īpaša uzmanība pievērsta stereoselektīvām ķīmiskām un biotehnoloģiskām enantiotīru 1,4-DHP sintēzes metodēm.

Bis(etoksikarbonilmetil) 1,4-dihidropiridīn-3,5-dikarboksilātu atvasinājumiem piemīt anti-metastatiskā aktivitāte, kā arī antivirālā aktivitāte pret Herpes simplex vīrusu. Darba pamatuzdevums bija enzimātiski katalizējama 4-aizvietotu bis(etoksikarbonilmetil) 1,4-dihidropiridīn-3,5-dikarboksilātu hidrolīzes izpēte. Rezultāti ir atspoguļoti 2. nodaļā.

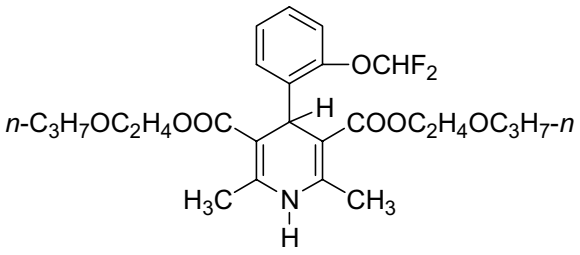
Prohirālu bis(etoksikarbonilmetil)aizvietotu 4-aril-1,4-dihidropiridīn-3,5-dikarboksilātu **1a-f** enzimātiskā hidrolīze veikta enantioselektīvi ar *Candida antarctica* lipāzi B (Novozym 435®) (1. shēma). Atkarībā no aizvietotāja 4. vietā, enantiomēra pārākums (e.e.) svārstījās no 68 līdz 97%. Dažos gadījumos e.e. varētu ievērojami uzlabot, mainot šķīdinātāju sistēmu.



a: R=C_6H_5 (e.e.=93%); b: R=2-$OCHF_2$-C_6H_4 (e.e.=79%; *e.e.=97% diizopropilēterī, kas piesātināts ar ūdeni);
c: R=3-NO_2-C_6H_4 (e.e.=77%); d: R=4-Cl-C_6H_4 (e.e.=68%) e: R=2-Cl-C_6H_4 (e.e.=72%); f: R=3-piridils (e.e.=82%)

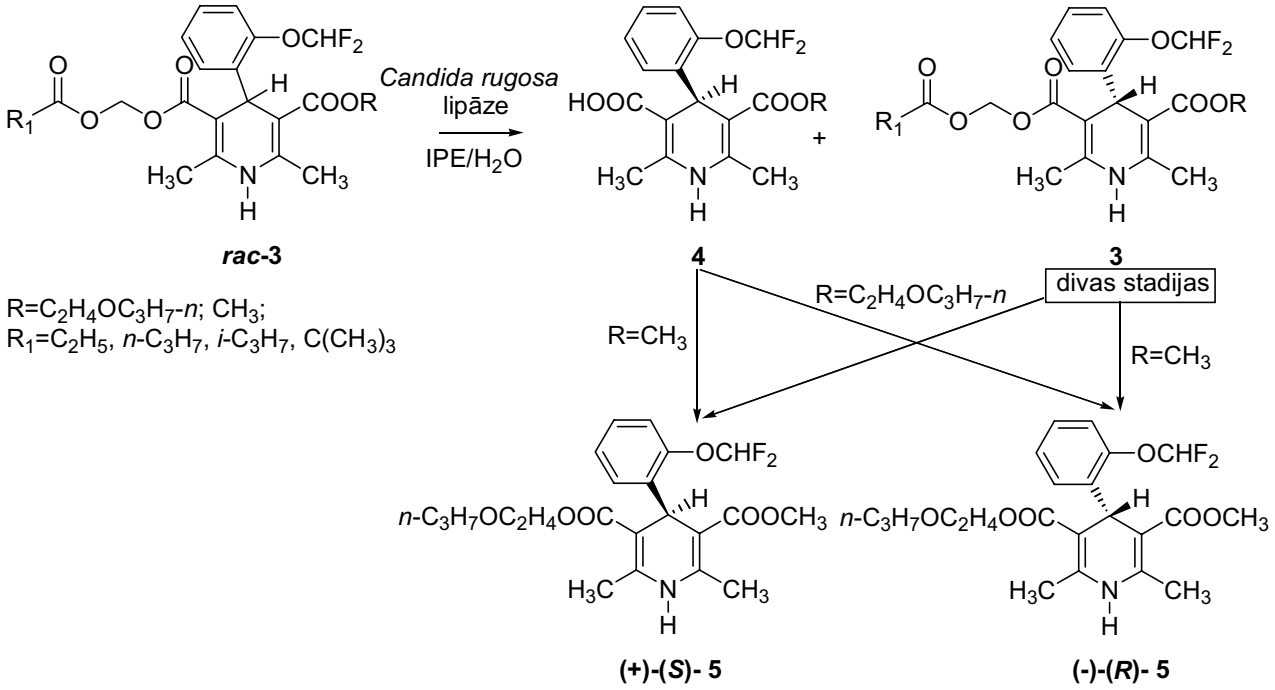
1. shēma

Cerebrokrasts (2,6-dimetil-3,5-bis[2-(propoksi)etoksikarbonil]-4-[2-(difluormetoksi)fenil]-1,4-dihidropiridīns) ir aktīvs neiroprotektors. Nesen veiktie pētījumi parādīja, ka cerebrokrastam piemīt arī antidiabētiskās un pretiekaisuma īpašības. Cerebrokrasta enantiotīru nesimetrisko analogu iegūšana ir aprakstīta 2. nodaļā. Kā visatbilstošākā metode šī mērķa sasniegšanai bija izvelēta 2,6-dimetil-4-[2-(difluormetoksi)fenil]-1,4-dihidropiridin 3,5-diesteru kinētiska sadalīšana.



Cerebrokrasts

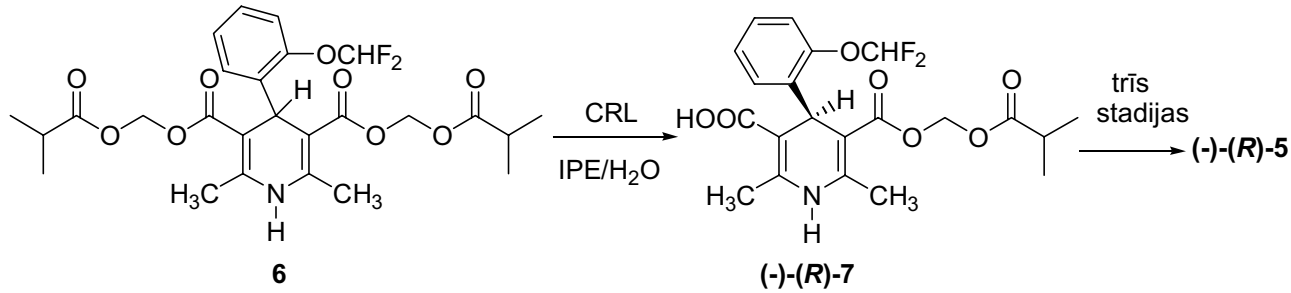
Enzimātiskās hidrolīzes procesa pētījumos, izmantojot vairākas komerciāli pieejamas hidrolāzes, noskaidrots, ka 2,6-dimetil-4-aril-1,4-dihidropiridīnkarbonskābju-3,5-dialkilesteri, kas tieši piesaistīti pie 1,4-DHP cikla, nepakļaujas enzimātiskai hidrolīzei stērisko un elektronisko faktoru dēļ. Tā kā lipāzes šķeļ bis(etoksikarbonilmetil) 1,4-dihidropiridīn-3,5-dikarboksilātus pie attālinātā estera grupējuma, tika sintezēti aciloksimetilatvasinājumi **3** (3. nodaļa). Enzīmi hidrolizē aciloksimetilesterus pie attālinātā estera grupējuma, bet reakcijas produkts ir nestabils un spontāni nošķeļas līdz brīvai karboksilgrupai (2. shēma). Izpētītas septiņas 5-metil- un 5-(2-propoksietil)-4-[2-(difluormetoksi)fenil]-2,6-dimetil-1,4-dihidro-3,5-piridīndikarboksilātu aciloksimetil atvasinājumu **3** hidrolīzes reakcijas, pielietojot *Candida rugosa* lipāzi (CRL) ar ūdeni piesātinātā diizopropilēterī (2. shēma). Visaugstākā procesa enantioselektivitāte ir sasniegta tad, kad 1,4-DHP molekula satur metilesteri 5. vietā un garu vai sazarotu acilķēdi pie C(3) atoma. Visaugstākā enantioselektivitāte (E=21; E vienība ir enantiomēru attiecība) ir sasniegta 3-[(izobutiriloksi)metil] 5-metil 4-(2-difluormetoksifenil)-2,6-dimetil-1,4-dihidropiridīn-3,5-dikarboksilāta **3** gadījumā, kuru izmantoja 3-metil 5-(2-propoksietil) 4-[2-(difluormetoksi)fenil]-2,6-dimetil-1,4-dihidro-3,5-piridīndikarboksilāta **5** abu enantiomēru iegūšanai.



2. shēma

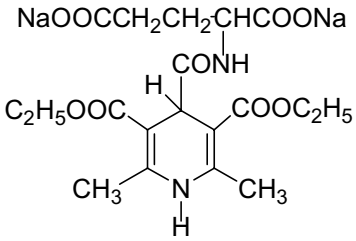
Enzimātiskā ceļā iegūto karbonskābju absolūtā konfigurācija 4*R* noskaidrota rentgenstruktūras analīzē ar diastereomēru metodi, analizējot 1-(*R*)-feniletilamīda un attiecīgo 1,4-DHP-3-karbonskābes atvasinājumu.

4. nodaļā ir aprakstīta efektīva daudzpakāpju sintēze (—)-(*R*)-**5** iegūšanai, kuras pamatā ir simetriskā bifunkcionālā substrāta enzimātiskā asimetrizācija (3. shēma). *Candida rugosa* lipāzes katalizējamās prohirālā bis[(izobutiriloksi)metil] 4-[2-(difluormetoksi)fenil]-1,4-dihidro-2,6-dimetil-3,5-piridīndikarboksilāta asimetrizācijas enantioselektivitāte ir ≥99%.



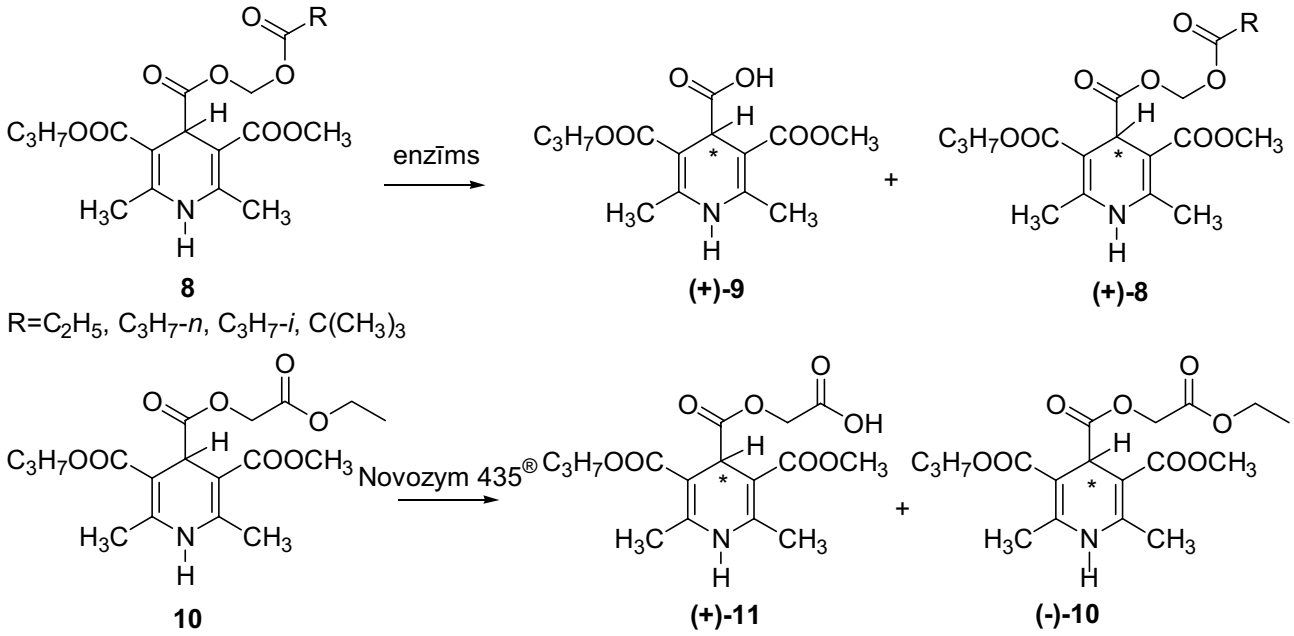
3. shēma

Glutapironam (2-(2,6-dimetil-3,5-dietoksikarbonil-1,4-dihidropiridīn-4-karboksamido)-glutārskābes dinātrija sāls) piemīt īpaši plašs bioloģisko aktivitāšu spektrs. Jau zemās koncentrācijās tam konstatēta antikonvulsanta, stress-protektīva, antiaritmiska, kognīcijas spēju un atmiņu uzlabojoša aktivitātes, kā arī neiromodulējoša un neiroregulējoša darbība.



Glutapirons

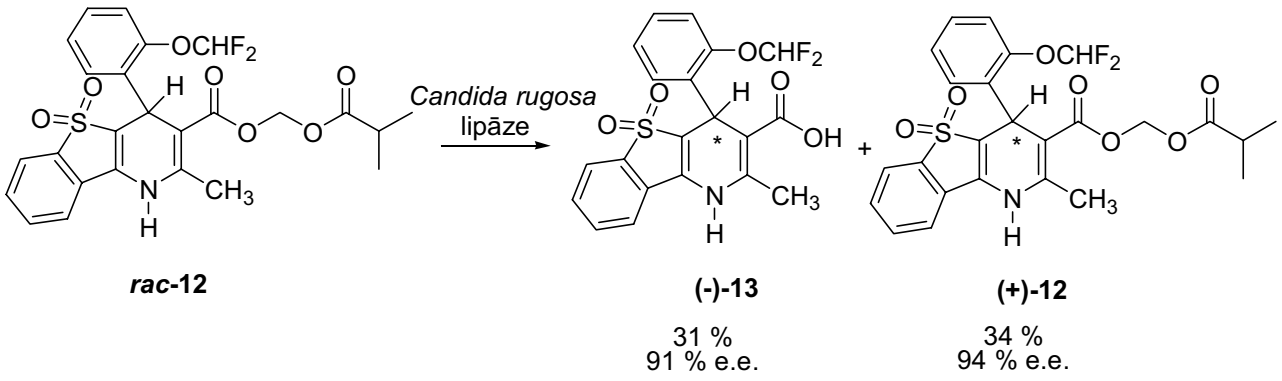
3,4,5-Trialkil-2,6-dimetil-1,4-dihidro-3,4,5-piridīntrikarboksilāti ir inerti pret hidrolītisko enzīmu iedarbību. Noskaidrots, ka 1,4-DHP molekulai jāsatur vismaz viens enzimātiski labils aizvietotājs, piemēram, aciloksimetil vai etoksikarbonilmetil. Tā kā iepriekš (skatīt 2.-4. nodaļas) tika pierādīts, ka 2,6-dimetil-4-aril-1,4-dihidropiridīn-3,5-dikarboksilātu aciloksimetil un etoksikarbonilmetil atvasinājumi ir viegli stereoselektīvi hidrolizējami ar lipāzēm, tad tika sintezēti atbilstoši 1,4-dihidroizonikotīnskābes atvasinājumi **8** un **10**. Izpētīta piecu racēmisku 4-[(aciloksi)metil] un 4-etoksikarbonilmetil 3-metil 5-propil 2,6-dimetil-1,4-dihidro-3,4,5-piridīntrikarboksilātu **8** un **10** atvasinājumu ar lipāzem katalizējamā kinētiskā sadalīšana. Rezultāti ir atspoguļoti darba 5. nodaļā. Pretēji diezgan zemai lipāžu enantioselektivitātei, hidrolizējot aciloksimetil atvasinājumus **8**, *Candida antarctica* lipāzes B (Novozym 435®) pielietošana ievērojami uzlaboja 1,4-dihidroizonikotīnskābes etoksikarbonilmetilestera **10** hidrolīzes enantioselektivitāti. Novozym 435® katalizējamās etoksikarbonilmetilestera **10** hidrolīzes 45°C ar ūdeni piesātinātā diizopropilēterī enantioselektivitāte ir vidēja (E=13,8). Pierādīts, ka reakcijas temperatūras pazemināšana uzlabo reakcijas enantioselektivitāti: tā E=21,5 iegūta istabas temperatūrā un E=28,9 sasniegta +4°C. Visai augsta procesa enantioselektivitāte (E vienība) (E=45,3) ir sasniegta temperatūrās zem 0°C, bet arī šaja gadījumā reakcija norit ļoti lēni.



4. shēma

Farmakoloģiskie pētījumi parādīja, ka racēmiskiem dihidrobenzotieno[3,2-*b*]piridīn-5,5-dioksīdiem un 5-okso-4,5-dihidro-1,4-indeno[1,2-*b*]piridīniem piemīt dažādas bioloģiskās aktivitātes, tādas kā koronāri dilatējošā un pretvēža; savienojumi ir arī aktīvi glutationa S-transferāzes inhibitori. Tādējādi, enantiotīriem policikliskiem 1,4-DHP ir nepieciešamas paplašinātas farmakoloģiskās pārbaudes.

Lipāžu katalizējamā enzimātiski labilā 3-(izobutiriloksi)metil-4-[2-(difluormetoksi)fenil]-2-metil-5,5-diokso-1,4-dihidrobenzotieno[3,2-*b*]piridīn-3-karboksilāta **12** kinētiskā sadalīšana ir aprakstīta 6. nodaļā (5. shēma). Visaugstākā enantioselektivitāte (*E*=28) ir sasniegta CRL katalizējamā *rac*-**12** pāresterifikācijas reakcijā ar *n*-butanolu toluolā, kas piesātināts ar ūdeni 45°C.



***rac*-12** **(-)-13** **(+)-12**

31 %
91 % e.e.

34 %
94 % e.e.

5. shēma

Galvenie zinātniski pētnieciskā darba rezultāti ir izvērtēti 7. nodaļā. Ir izstrādātas stēriski un elektroniski apgrūtinātu 1,4-DHP enzimātiskās hidrolīzes metodes. Noskaidrotas ķīmioenzimātiskās enantioselektīvas 1,4-DHP hidrolīzes priekšrocības, trūkumi un ierobežojumi. Atrastās ķīmioenzimātiskās, enantioselektīvas 1,4-DHP sintēzes metodes ir perspektīvas jaunu farmakoloģiski aktīvu enantiotīru dihidropiridīna atvasinājumu iegūšanai.

Parādīta enzimātisko enantioselektīvo funkcionālo grupu transformācijas metožu lielā praktiskā nozīme un pielietošanas iespējas hidrogenēto piridīnu enantioselektīvajā sintezē.

Curriculum Vitae

Arkadij Sobolev (Arkādijs Soboļevs, Аркадий Владимирович Соболев) was born on the 27th of September, 1970 in Riga, Latvia. In 1987 he left from Secondary School of Riga № 78. From 1987 he studied at the Faculty of Chemistry of the University of Latvia (formerly Latvian State University) in Riga, Latvia, where graduated in 1993. In 1990 as a third year student he started his professional career in the Latvian Institute of Organic Synthesis (IOS, Riga, Latvia) in the Laboratory of Membrane Active Compounds and β-diketones guided by Dr. Chem. Hab. Prof. Gunars Duburs under supervision of Dr. Chem. Brigita Vigante. From 1993 to 1995 he participated in the International Science Foundation long-term research grants programme ”Investigation of formation mechanisms of derivatives of pyridine and its hydrogenated forms”. In 1994, as a Tempus programme student he participated in the research project carried out at the Department of Organic Chemistry of Wageningen Agricultural University in Wageningen, The Netherlands (in the meantime Laboratory of Organic Chemistry of Wageningen University). After completion of a one-year study programme at the Faculty of Chemistry of the University of Latvia, he obtained his MSc degree in 1995. From 1995 to 1998 he participated in the Programme for Co-operation in Science and Technology with Central and Eastern Europe (Copernicus programme) devoted to the investigation and elaboration of novel chiral drugs prepared by biotechnological procedures. From 1996 to 2001 he was PhD student of the co-operative PhD programme between Wageningen University (WU) and IOS guided by Dr. M. C. R. Franssen, Prof. Ae. de Groot and Prof. G. Duburs. From 1999 to 2001 he was also involved in collaboration between IOS and WU by means of the NATO Linkage grant “Elaboration of enzyme-catalysed enantioselective synthesis of optically active dihydropyridines’’. He is currently an assistant in the Laboratory of Membrane Active Compounds and β-diketones of IOS. The research performed during his PhD studies is described in this thesis.

List of publications relevant to this thesis:

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6. Sobolev, A.; Zhalubovskis, R.; Franssen, M. C. R.; Cekavicus, B.; Vigante, B.; Duburs G.; de Groot, Ae. *Candida rugosa* lipase-catalysed kinetic resolution of 3-(isobutyryloxy)methyl 4-[2-(difluoromethoxy)phenyl]-2-methyl-5,5-dioxo-1,4-dihydrobenzothieno[3,2-*b*]pyridine-3-carboxylate. – in preparation.

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