Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

Kristoffer Klungseth

Chemo-enzymatic synthesis of enantiopure penbutolol and penbutolol derivative 1-*tert*-butylamino)-3-(4-cyclopentylphenoxy)propan-2-ol

Master's thesis in TKJ4900 - Organic Chemistry
Supervisor: Associate professor Elisabeth Egholm Jacobsen
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Preface

This master's thesis was carried out in the spring of 2021, as the final part of a master's degree at the Department of Chemistry, Faculty of Natural Sciences, Norwegian University of Science and Technology (NTNU).

I would like to express my grattitude to my supervisor, Associate professor Elisabeth Egholm Jacobsen for guidance through this thesis, and for putting together weekly meetings with this incredible research group: Lukas Bocquin and Susanne Troøyen which held lectures in biocatalysis, (Lukas even brought croissants). Mari Rødseth, Anna Tennfjord and Raymond Trohjell which worked in the same lab as me. I also have other friends, I promise! Even though I haven't seen any of them for over a year because of this Covid-19 pandemic. One of my friends, who is a very wise man, once told me: "NTNU is the 5 worst, or 6 best years of your life!". Through my 6 years at NTNU, I have always lived by that motto.

The pandemic has made this last year and a half hard for all of us. Also, a paragliding accident caused me to postpone the writing of this thesis for over a month. Throughout all of this, I would not have been able to cope as well as I did without my girlfriend: Amalie Mello. My deepest gratitude goes out to her.

Now, it looks like the pandemic is finally coming to an end. Also, I just got a PlayStation 5 (PS5), so things are looking brighter.

That's it for now, I hope everyone who reads this thesis enjoys it.

Sincerely
Kristoffer Klungseth
- Author of this awesome thesis.

Abstract

In this thesis, two enantiopure compounds were synthesized using *Candida antarctica* lipase B (CALB) as enantioselective biocatalyst in kinetic resolution. (*S*)-1-(*tert*-butylamino)-3-(4-cyclo-pentylphenoxy)propan-2-ol was synthesized in 96% enantiomeric excess and 29% yield. This was done by CALB-catalyzed kinetic resolution of 1-chloro-3-(4-cyclopentylphenoxy)propan-2-ol, followed by amination with *tert*-butylamine. This synthesis was used as a model for the synthesis of the β-blocker penbutolol.

1-Chloro-3-(2-cyclopentylphenoxy)propan-2-ol, a building block for penbutolol, was synthesized in 58% yield. This was done by a substitution reaction of 2-cyclopentyl phenol with epichlorohydrin, followed by epoxide ring opening with lithium chloride and acetic acid. These reactions have been investigated, and the results are discussed in this thesis.

(*S*)-Penbutolol was also synthesized in 96% enantiomeric excess. This was done by CALB-catalyzed kinetic resolution of the building block 1-Chloro-3-(2-cyclopentylphenoxy)propan-2-ol, followed by amination with *tert*-butylamine. This resulted in 68% purity, but suggestions are made in this thesis of how to purify it further. The enantioselectivity and catalytic ability of the biocatalyst CALB towards the different substrates are also investigated and discussed.

Sammendrag

I denne oppgaven ble to enantiomert rene stoffer syntetisert ved å bruke *Candida antarctica* lipase B (CALB) som enantioselektiv biokatalysator i kinetisk resolusjon. (*S*)-1-(*tert*-butylamin)-3-(4-syklopentylfenoksy)propan-2-ol ble syntetisert i 96% enantiomerisk eksess og 29% utbytte. Dette ble utført ved CALB-katalysert kinetisk resolusjon av 1-klor-3-(4-syklopentylphenoksy)propan-2-ol, etterfulgt av aminering med *tert*-butylamin. Denne syntesen ble brukt som en modell for syntese av β-blokkeren penbutolol.

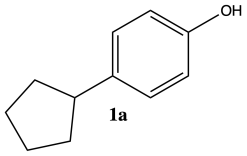
1-Klor-3-(2-syklopentylfenoxy)propan-2-ol, en byggestein for penbutolol, ble syntetisert i 58% utbytte. Dette ble utført ved en substitusjonsreaksjon av 2-syklopentylfenol med epiklorhydrin, etterfulgt av epoksid-ringåpning med litiumklorid og eddiksyre. Disse reaksjonene har blitt undersøkt, og resultatene er diskutert i denne oppgaven.

(*S*)-Penbutolol ble også syntetisert i 96% enantiomerisk eksess. Dette ble utført ved CALB-katalysert kinetisk resolusjon av byggesteinen 1-klor-3-(2-syklopentylphenoksy)propan-2-ol, etterfulgt av aminering med *tert*-butylamin. Dette ga 68% renhet, men det er diskutert hvordan det kan rengjøres bedre. Enantioselektiviteten og den katalytiske aktiviteten til CALB mot forskjellige substrater er også undersøkt og diskutert.

Symbols and abbrieviations

δ	Chemical shift in an NMR spectrum
API	Active pharmaceutical ingredient
c	Conversion
CALB	*Candida antarctica* lipase B
COSY	Correlation spectroscopy
E	Enantiomeric ratio
ee	Enantiomeric excess
ee_s	Enantiomeric excess of substrate
ee_p	Enantiomeric excess of product
$eq.$	Molar equivalents used in a reaction
et al.	And others
FDA	United States Food and Drug Administration
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single-quantum correlation spectroscopy
in vacuo	Under reduced pressure
Mol%	Molar percentage
N	Theoretical plate number over an HPLC column
NME	New molecular entity
NMR	Nuclear magnetic resonance
R_f	Retention factor on a TLC plate
R_s	Resolution between two peaks on a chromatogram
THF	Tetrahydrofuran
TLC	Thin layer chromatography
t_R	Retention time in minutes on HPLC
w	Width of a peak in HPLC at the baseline
$w1/2$	Width of a peak in HPLC at half its height

Mentioned compounds



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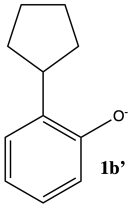

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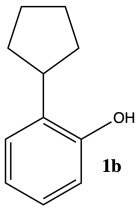

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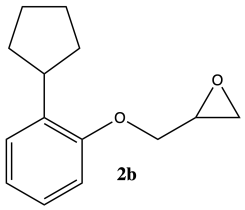

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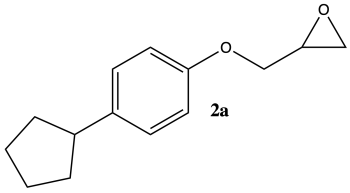

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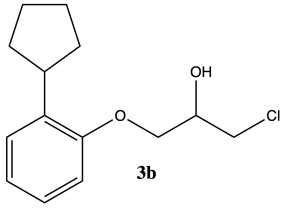

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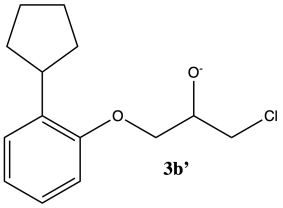

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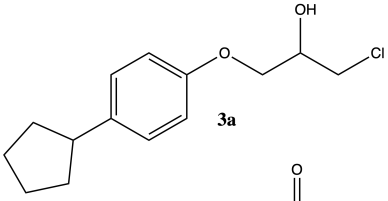

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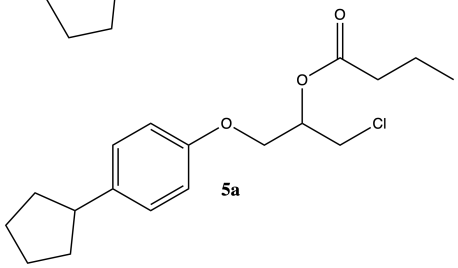

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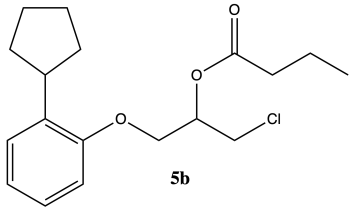

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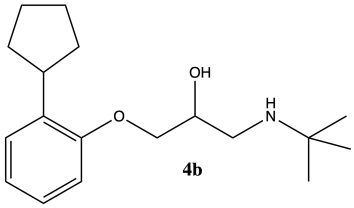

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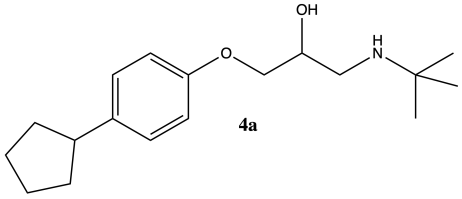

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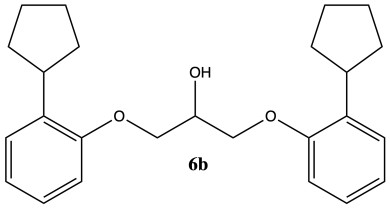

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Contents

1 Introduction

1.1 Chirality

A chiral compound is a compound that is not identical to its mirrored compound. A pair of unidentical mirrored molecules are called enantiomers. Enantiomers have identical chemical properties except for interaction with other chiral compounds.[1] Hence, enantiomers can have very different biological properties. All proteins/enzymes, sugars and receptors in the human body are chiral.[2] Thus, different enantiomers will have different effects on the body. A stereocenter is an atom or a region in a molecule that, if two substituents switch places, the mulecule will have a different stereoisomery. If there is only one stereocenter in a molecule, switching two substituents will cause mirroring, thus causing enantiomers. In the case of more than one stereocenter, and switching substituents causes unidentical, but not mirrored compounds, the molecule is classified by which way it rotates plane-polarized light. It can be to the right which is called dextrorotary (+) or to the left which is called levorotary (−).[3]

Figure 1.1 shows the *S*- and *R*-enantiomer of 1-phenylethanol. The stereocenter of 1-phenylethanol is a carbon atom with four different substituents: Ph, CH_3, OH and H. If any of the substituents switches place with another, the compound will be mirrored, thus creating a pair of enantiomers. A chiral center (stereocenter in a single tetraedrical atom) can have either *R*- or *S*-configuration. A stereocenter can be either a tetraedrical atom, a part of a molecule, or a stereogenic axis or plane. A molecule with n stereocenters, can have up to n^2 different stereoisomers.

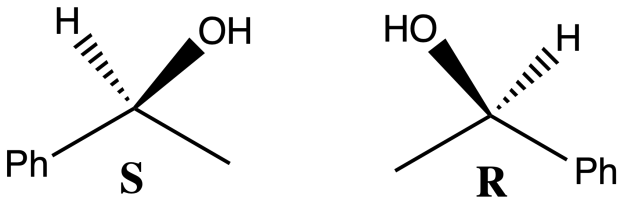


Figure 1.1: The structure of 1-phenylethanol in its *S*-enantiomer to the left and its *R*-enantiomer to the right.

1.2 Chirality in pharmaceuticals

A finished pharmaceutical product (FPP) contains several components. The substance in an FPP that furnishes the intended pharmacological effect is called active pharmaceutical ingredient (API).[4] In chiral APIs, the enantiomer that provides the wanted pharmacological effect is called the automer, while the enantiomer that provides small to no effect, or possibly side effects, is called the distomer.[5]

A horrifying example of side effects caused by a distomer is thalidomide. Thalidomide was developed in the early 1950s, and was used by pregnant women in treatment of nausea.[6] However, it turned out this drug could cause severe malformations on the fetus. This teratogenic effect was attributed to the *S*-enantiomer.[7] However, administration of the *R*-enantiomer could not prevent the side effects because thalidomide is racemized in the body.

Today, the United States Food and Drug Administeation (FDA) requires all manufacturers to investigate the chemical and pharmacological properties of all enantiomers/diastereomers of chiral drugs to determine their individual safety and efficacy.[8] The development of enantiomerically pure pharmaceuticals has become increasingly important over the last decades, and remains a main target for many academic and industrial researchers. In the years between 2001-2010, 63% of all newly approved drugs worldwide, so called New Molecular Entities (NMEs), were single enantiomers.[9] Only 9% were racemates and the rest were achiral compounds. Although it has been well established that the two enantiomers of the same compound can have different pharmacological effects, several chiral drugs are still being manufactured and sold as racemates.[10]

1.3 Synthesis of enantiomerically pure compounds

There are three ways to synthesize enantiomeracally pure compounds: Using a compound from "The chiral pool", asymmetric synthesis or by resolution.[11]

The chiral pool refers to the domain of chiral, non-racemic compounds from nature.[12] This method involves using enantiopure compounds as starting materials that is provided by nature. Using the chiral pool is often unbeatable in terms of cost efficiency beacause the

chiral coumpounds are provided from natural sources in great abundance.[13] However, as long syntheses are needed for more complex target compounds, the chiral pool is not always an option.

Asymmetric synthesis is when a prochiral compound is converted into an enantiopure compound by utilizing an enantiopure reagent or co-reagent, or an enantiomerically selective catalyst. Some of the most known and used asymmetric syntheses are the epoxidation and dihydroxylation developed by Sharpless *et al.*[14,15] Asymmetric synthesis have been largely developed through the last decades, but often still requires expensive reagents, catalysts and/or extreme reaction conditions.[16]

Resolution means separating a racemic mixture into enantiomers. Resolution was first performed by Louis Pasteur in 1848, when he discovered that crystals of sodium ammonium tartrate consisted of mirrored geometrical shapes.[7] He manually sorted the crystals into two piles and discovered that they rotated polarized light in opposite directions. Tartaric acid was then known as racemic acid, which is were the word racemate comes from.[17] In modern day, many different methods to perform resolution have been developed: Classical,[18] spontaneous,[17] by chromatography,[17] by extraction,[19] by electrophoresis,[20] by membrane,[21] or kinetically.[18] Resolution makes it possible to synthesize compounds in great enantiomeric purity with cheap reagents and/or catalysts. However, a big drawback with resolution is that the theoretical maximum yield is only 50% from the racemic mixture, which makes it much less desireable for the industry unless both enantiomers can be utilized, or the unwanted enantiomer can be easily racemized.

1.3.1 Kinetic resolution

Kinetic resolution is a prosess where one enantiomer of a racemic mixture is selectively transformed into a product, while the other enantiomer is left unreacted.[22] This can be done with either a chiral reagent or a chiral catalyst. This will lower the activation barrier for one of the enantiomers, causing different reaction rates. While performing kinetic resolution, it is important to stop the reaction at the right time to get the best possible enantiomeric purity for both substrate and product in as high yield as possible. Enantiomeric excess (*ee*),

Conversion (c), and Enantiomeric ratio (E) are parameters that helps the understanding of kinetic resolution. This is illustrated in Figure 1.2.

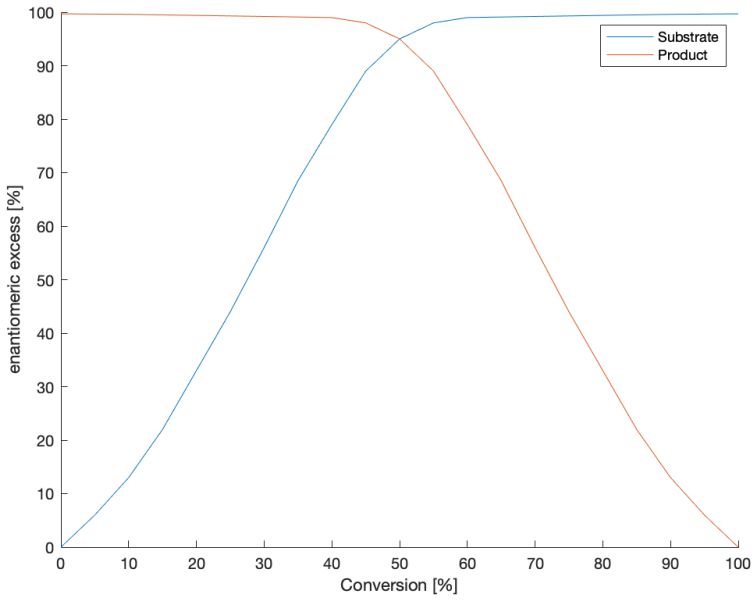


Figure 1.2: A theoretical plot showing the enantiomeric excess of both the substrate and the product against the conversion during a reaction in kinetic resolution.

1.3.2 Enantiomeric excess

Enantiomeric purity is decided by enantiomeric excess (*ee*). Enantiomeric excess is a measurement of the difference in the amount of one enantiomer compared to the other in a mixture. A racemate will have an *ee* of 0%, while a pure enantiomer will have 100% *ee*. Enantiomeric excess is calculated with equation 1.1, where R is the amount of one enantiomer and S is the amount of the other enantiomer.[23]

$$ee = \frac{R - S}{R + S} \tag{1.1}$$

1.3.3 Conversion

A 100% enantioselective kinetic resolution will never reach a higher conversion than 50%. If the conversion exceeds 50%, the *ee* will decrease as both enantiomers are converting. That

makes it very important to monitor the conversion while performing kinetic resolution. The conversion, c, in a kinetic resolution is calculated with equation 1.2, where ee_s is the *ee* of the substrate, while ee_p is the *ee* of the product.[23]

$$c = \frac{ee_s}{ee_s + ee_p} \tag{1.2}$$

1.3.4 Enantiomeric ratio

Enantiomeric ratio (E) is used to quantify reactions, and correlates *ee* with the conversion. E-values describes the relation between the rate constants of the enantiomers in a reaction, and gives information about the enantioselectivity of the reaction. E can be calculated when c and *ee* of either substrate or product are known (equation 1.3), or when *ee* of both are known (equation 1.4).[23]

$$E = \frac{\ln((1-c)(1-ee_x))}{\ln((1-c)(1+ee_x))} \tag{1.3}$$

$$E = \frac{\ln(\frac{1-ee_s}{1+ee_s/ee_p})}{\ln(\frac{1+ee_s}{1+ee_s/ee_p})} \tag{1.4}$$

1.4 Chemical analysis methods

1.4.1 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy (NMR) is the most common method for analyzing the structure of organic compounds. NMR will only work on compounds that have at least one nucleus with spin, which means it has an odd number of protons and neutrons. Examples of this are the proton (^{1}H), and the 13- and 15- isotopes of carbon and nitrogen (^{13}C and ^{15}N).

NMR is performed by introducing a sample to a magnetic field. The magnetic moment of all nuclei with spin will orient either with or against the magnetic field. The nuclei that are oriented with the magnetic field have lower energy levels than the nuclei oriented against the

magnetic field.[24] Changing the orientation of these nuclei demands energy that comes from electromagnetic radiation that is sent through the sample. When this energy is absorbed by a nucleus, it will be in resonance with the electromagnetic radiation. The amount of energy absorbed is dependent on the electron density and movement around the respective nucleus (molecular shielding). The molecular shielding effects will induce a magnetic field around the nucleus. This causes the nucleus to experience a net field with a different intensity than the magnetic field from the NMR instrument.[25] Thus, different electronic environments around nuclei causes electromagnetic radiation of different wavelengths to be absorbed, which gives rise to its chemical shift (δ).

An NMR experiment yields a spectrum where each peak corresponds to nuclei with a certain chemical shift. Integration of these peaks can be used to determine how many nuclei correnspponds to that particular chemical shift, and thus how many that have identical electronic environment in a molecule. One-dimensional (1D) NMR gives information about one particular type of nucleus in a sample, while two-dimensional (2D) NMR can give information about the bonding between neighbouring nuclei.[24] The most common 1D NMR experiments are ^{1}H NMR and ^{13}C NMR, while the most common 2D NMR experiments are Correlation Spectroscopy (COSY), Heteronuclear Single-Quantum Correlation Spectroscopy (HSQC) and Heteronuclear Multiple-Bond Correlation Spectroscopy (HMBC).

1.4.2 High performance liquid chromatography

Chromatography is a series of separation techniques commonly used in analytical and synthetic chemistry. The principle of chromatography is to separate compounds based on differences in affinity towards a mobile phase and a stationary phase.[26] This is done by moving a mixture of compounds through the stationary phase, using the mobile phase. Compounds that have higher affinity towards the stationary phase will interact with it more and thus be more *retained*; meaning it will take longer to travel through the stationary phase. Compounds that have higher affinity towards the mobile phase will not interact as much with the stationary phase, and thus travel through it quicker; it will *eluate* quicker. Many differences in chemical properties can be used to separate compounds with chromatography, like

molecular size, electrical properties and polarity. Separating compounds based on differences in polarity is the most common chromatographic technique. When the stationary phase is more polar than the mobile phase, it is called normal phase, and when the mobile phase is more polar than the stationary phase, it is called reverse phase.[27,28]

When the stationary phase is solid and the mobile phase is liquid, it is called liquid-solid chromatography (LSC), which is usually referred to as simply liquid chromatography (LC). Modern LC-instruments uses high pressure pumps to keep a stable flow through columns packed with small particles, typically 3-5 μm . This is called high performance liquid chromatography (HPLC).[26] HPLC is a very common analysis technique both in research and industry, and can be used for both qualitative and quantitative analysis.

Normal phase HPLC columns usually consists of tightly packed particles of silica-derivatives. Because of the tighly packed stationary phase and high pressure, HPLC gives many possible interactions between the stationary phase and applied compounds. This gives extremely effective separation, and high number of theoretical plates. The number of theoretical plates, N, can be calculated from equation 1.5. In this equation, t_R is the retention time, which means the time it takes for a compound to travel through the column with the applied eluent, w is the width of the peak at the baseline, and $w_{1/2}$ is the width of the peak at half its height.[29]

$$N = 16(\frac{t_R}{w})^2 = 5.56(\frac{t_R}{w_{1/2}})^2 \tag{1.5}$$

If the theoretical plate number decreases drastically between analyses, it might mean the column is damaged or broken.

To ensure that peaks on the chromatogram is properly separated, resolution, R_s is used. R_s is calculated from equation 1.6.[29] Here, t_{R1} and t_{R2} are the retention times of the first and second peak, w_1 and w_2 are the width of those peaks at the baseline, and $w_{1/2_1}$ and $w_{1/2_2}$ are the width of the first and second peak at half their heights.

$$R_s = 2\frac{t_{R2} - t_{R1}}{w_1 + w_2} = 1.18\frac{t_{R2} - t_{R1}}{w_{1/2_1} + w_{1/2_2}} \tag{1.6}$$

For proper separation that can be used for quantitative analysis, a minimum R_s-value of 1.5 is required. This is called baseline separation.[30] Most modern day HPLC software will calculate plate numbers and resolution automatically.

1.4.3 Chiral HPLC

Enantiomers have the same physical and chemical properties, and can only be separated by interactions with other chiral substances. This means that in order to separate enantiomers by chiral chromatographic methods, a chiral selector needs to be added. The enantioselectivity of a chomatographic system is determined by the interactions between the chiral selector and the enantiomers.[26] A chiral complex is formed between the chiral selector and the enantiomers. In order to achieve enantiomeric separation, the chiral complex needs to be more stable for one of the enantiomers. This is illustrated in Figure 1.3. Enantiomer 1 bonds with the chiral selector in three interactions, while enantiomer 2 only bonds in 2 interactions. Enantiomer 1 then forms a more stable complex with the chiral selector. If the chiral selector is added to the stationary phase, this will cause enantiomer 1 to be more retained. In Figure 1.3, there are three bonding interactions for enantiomer 1, and two bonding interactions for enantiomer 2. There can also be more than three interactions, or less, and interactions can be either bonding or repulsive.

In HPLC, chiral selectors are usually added to the stationary phase. In the last few decades, a large number of chiral stationary phases have been developed using both small chiral molecules and polymers with chiral recognition abilities.[7] Examples of this are polysaccharides, cyclic saccharides, crown ethers, amino acids and proteins. In Figure 1.4, the stationary phase of a Chiralcel® OD-H column is shown.[31] The stationary phase is made up of silica gel which is a common stationary phase for HPLC columns. The chiral selector is cellulose tris-(3,5-dimethylphenyl-carbamate), which is coated on the silica particles.

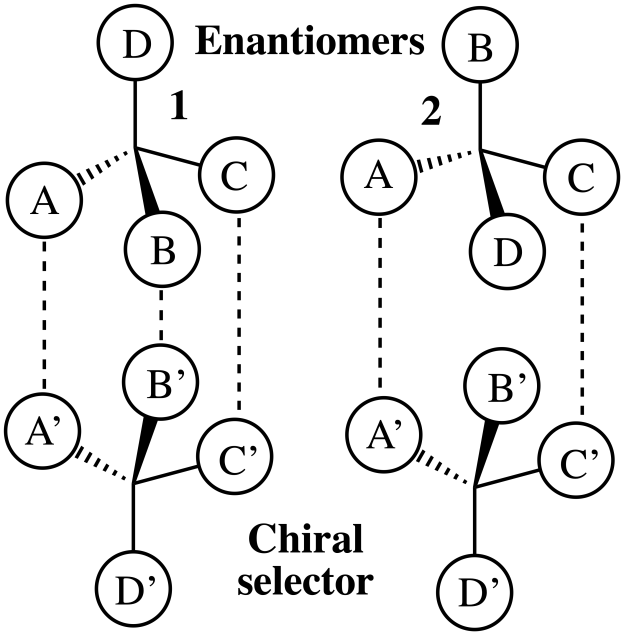


Figure 1.3: Formation of chiral complexes between two enantiomers and a chiral selector. Enantiomer 1 forms a more stable complex and is retaned more by the chiral selector.

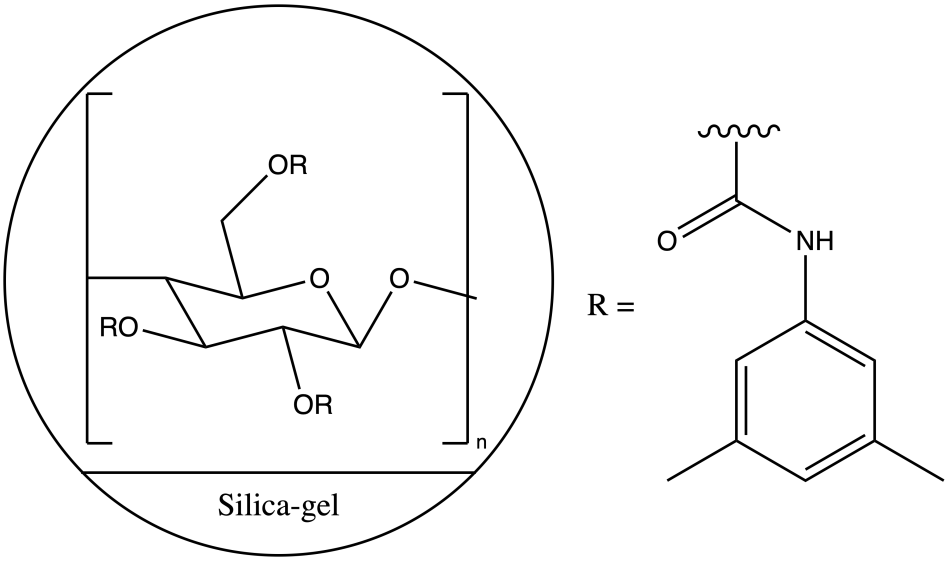


Figure 1.4: The stationary phase of a Chiralcel® OD-H column. It consists of cellulose tris-(3,5-dimethylphenyl-carbamate) coated on 5 μm silica gel particles.[31]

1.5 Green chemistry

In 1998, Paul Anastas and John Warner developed a set of principles for making chemical reactions and processes more environmentally friendly.[32] The idea of green chemistry is to develop new reactions and reaction conditions that can provide better chemical syntheses in terms of resource efficiency, energy efficiency, product selectivity, operational simplicity and health and environmental safety. Biocatalysis of organic reactions can follow all these principles and are a good example of green chemistry.

1.6 Biocatalysis in organic chemistry

All biochemical reactions in the human body are catalyzed by enzymes.[33] Enzymes can catalyze organic reactions like any other catalyst by binding to the substrate and reducing the activation energy of the reaction. Enzymes can be extremely powerful catalysts, typically enhancing reactions rates by a factor of 10^8 to 10^{10} , and sometimes even up to 10^{19}.[34] Compared to other catalysts used in organic synthesis that often include heavy metals, biocatalysts are much more environmentally friendly. They can be effective in mild reaction conditions, low temperatures and in water as well as organic solvents. Enzymes are also completely renewable and degradable, and can be recycled to be used for several organic syntheses. They can also be enantioselective towards a wide range of substrates, and are therefore suitable catalysts for kinetic resolution.

Enzymes are generally categorized into six main groups based on what kind of reactions they catalyze.[35] The different enzyme classes and what kind of reactions they catalyze, are shown in Table 1.1.

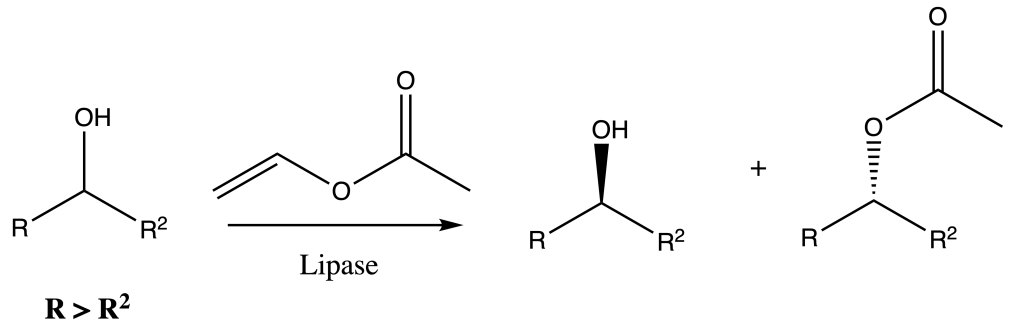
Table 1.1: Classification of enzymes and the reactions they catalyze.

Enzyme class	Reaction type
Oxidoreductases	Oxido-reduction reactions
Transferases	Transferring groups like methyl, ketone acyl *etc.*
Hydrolases	Hydrolytic cleavage/formation of esters, amides, lactones *etc.*
Lyases	Addition and elimination reactions
Isomerases	Structural or geometric changes within one molecule
Ligases	Formation/cleavage of C-O, C-S, C-N and C-C bonds

1.6.1 Lipases

Lipases are a group of enzymes that help the digestion of fat. They belong to the hydrolases-class of enzymes, and can be found in plants and fungi, as well as animals and humans. In living organisms, lipases work by catalysing the hydrolysis of triglycerides, and the reverse reaction. Because of their ability to break down triglycerides, lipases are widely used in the processing of fats and oil, detergent and degreasing formulations, but are also used for synthetic purposes in industries like food processing, paper manufacture, cosmetics, pharmaceuticals and fine organic chemistry.[36]

In synthetic chemistry, lipases are the most important group of enzymes used for biocatalysis of organic reactions.[37] Lipases are regio- and stereoselective, and can accept a wide range of substrates. They are also active in both water and organic solvents. Lipases are used for selective kinetic resolution by esterification of secondary alcohols or hydrolysis of esters (reverse reaction). Esterification is catalyzed in organic solvents, and the hydrolysis is favoured in aqueous solutions. In 2008, Q. Jing and R. Kazlauskas discovered an empiric rule that can be used to predict the stereoselectivity based on the relative sizes of the substituents.[38] This is shown in Scheme 1.1.



Scheme 1.1: Kinetic resolution of a secondary alcohol with a lipase catalyst, where R is bigger than R^2.

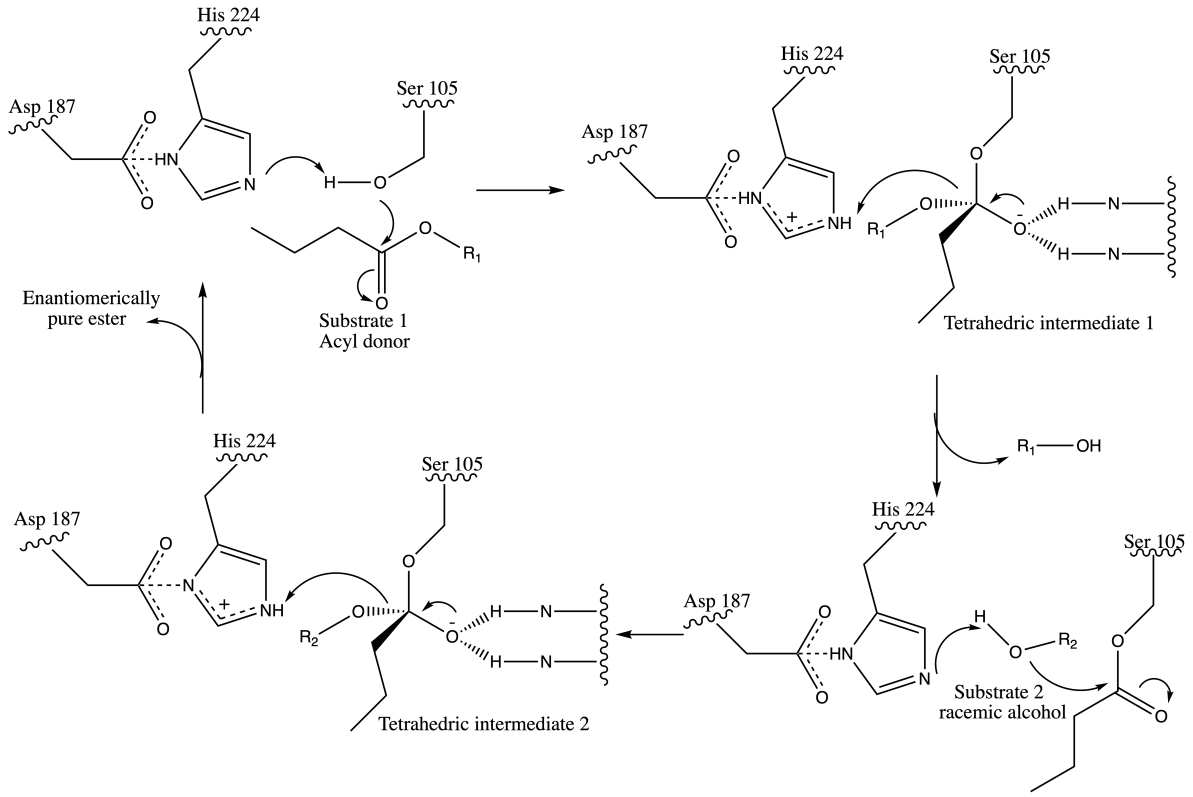
Access to the active site of a lipase occurs through interfacial activation at the lipid-water interface.[39] Lipases contain a helical oligopeptide that shields the active site. Upon interaction with a hydrophobic interface, this oligopeptide unit is folded to provide free access to the active site. The active site of a lipase generally consists of a triad of serine, histidine and aspartate.[39]

1.6.2 *Candida antarctica* lipase B

Candida antarctica lipase B (CALB) is a robust enzyme that is derived from the yeast species *Candida antarctica*. CALB is characterized as a serine hydrolase, and is known for being highly enantioselective with strong catalytic abilities. The enzyme consists of 317 amino acid residues, and the active site, like other lipases, is composed of a catalytic triade of serine, histidine and aspartate.[40] The catalytic cycle with CALB follows a serin-hydrolase mechanism.[41] It is a two-step mechanism with acylation, followed by an acyl-enzyme intermediate, and then deacylation. The mechanism is illustrated in Scheme 1.2.

Studies and simulations have shown that the structure of CALB is very stable in organic solvents.[40,42] However, the stability of the active site is considerably lower in polar organic solvents than unpolar. This causes the catalytic activity to be higher in unpolar solvents, which causes higher reaction rates. This is not always desired, as more polar solvents and lower activity have previously caused higher enantioselectivity.[43] Also, water contents in the solvents can have an impact on the activity. Water causes the enzyme to become more

unstable and flexible, which lowers the activity.[42] This does not necessarily decrease the enantioselectivity, but it will cause catalytic effect to favour hydrolysis over esterification.



Scheme 1.2: The serin-hydrolase mechanism.[41]

Structural studies of CALB have revealed a small α-helix lid close to the active site.[44] Because of the small lid and narrow entrance, CALB have long been considered an atypical lipase with no interfacial activation.[45] Instead, the narrow entrance is what causes the high selectivity towards substrates. However, in 2015, Zisis *et al.* discovered with experiments that CALB displayed an enhanced catalytic rate for large, bulky substrates when adsorbed to a hydrophobic interface.[46] Computer simulations also showed a high mobility for the small α-helix lid, which suggests a more open entrance to the active site.

CALB have shown to be extremely effective in kinetic resolution of secondary alcohols, and have even shown better results than other lipases for a wide array of substrates.[47] CALB also has the same stereoselectivity towards secondary alcohols as other lipases shown in Scheme 1.1. In 2000, Jacobsen *et al.* investigated the substrate requirements to obtain

high enantioselectivity of kinetic resolution catalyzed by CALB.[48] They discovered that the enantioselectivity decreased with increasing length of the carbon chain on the small group. The highest selectivity was obtained with a chain of two carbon atoms.

1.7 β -Antagonists

β-Adrenergic antagonists (β-blockers) are widely used drugs in treatment of hypertension, angina pectoris, glaucoma, anxiety and obesity.[49] They work by antagonizing β-Adrenergic receptors in the sympathic nervous system, and thereby blocking the effects of endogenous catecholamines like epinephrine (adrenaline) and norepinephrine (noradrenaline).[50] This will cause the heart rate to slow down, and the blood pressure to decrease. β-blockers can also help open up arteries and veins to increase blood flow.

There are three types of β-adrenergic receptors. β_1- β_2- and β_3-receptors. β_1-receptors are located mainly in the heart and kidneys, β_2-receptors are located mainly in the lungs, and β_3-receptors are located in fat cells.[5,51] β-blockers can be either selective or non-selective. Non-selective β-blockers antagonize both β_1- and β_2-receptors, which gives a widespread effect through the whole body.[52] Some β-blockers are selective towards β_1-receptors which are mainly located in the heart region. These are called cardioselective β-blockers.[5].

The distribution and delivery of β-blockers in the body are controlled by a cascade of processes, each with specific stereochemical requirements.[53] These involve multiple enzymes, transport proteins and receptors. Because of all these stereoselective processes, all available β-blockers have at least one chiral center.[54] The *S*-enantiomers of β-blockers are known to have much higher affinity towards β-receptors, and can have more than 100 times the potency of the *R*-enantiomer.[55] Also, the *R*-enantiomer of some β-blockers have displayed undesireable side effects.[17] This means that synthesis of enantiopure β-blockers is extremely important as it can increase the pharmaceutical effect as well as getting rid of unwanted side effects.

1.8 Penbutolol

Penbutolol is a non-selective β-blocker used in treatment of hypertension.[56] Penbutolol inhibits β_1-adrenergic receptors in both the heart and kidneys. This causes the heart rate to slow down which lowers blood pressure. Penbutolol also prevents release of renin in the kidneys, which is a hormone that causes constriction of blood vessels. Penbutolol was approved as a hypertension drug by the FDA in 1987.[57] It was withdrawn from sale in the USA in 2015, but not due to reasons of safety or effectiveness.[58] Penbutolol is available as penbutolol sulphate in its levorotary isomer.[59] This will give the S-enantiomer of penbutolol when dissolved in the body. The structure of (−)-penbutolol sulphate is shown in Figure 1.5.

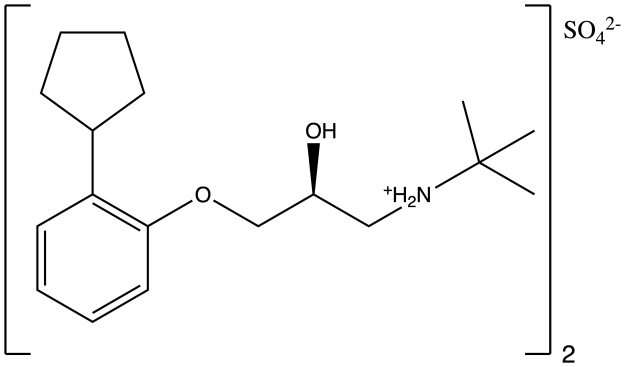


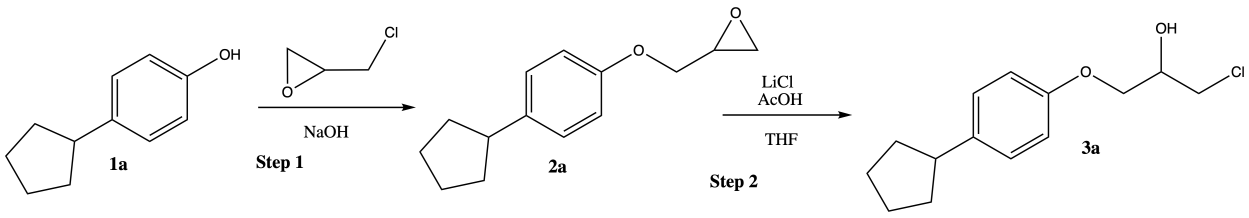
Figure 1.5: The structure of (−)-penbutolol sulphate.

The syntheses of penbutolol, however, usually requires harsh reaction conditions and hazardous transition metal catalysts, which is not in line with the principles of green chemistry. Like the one performed by Phukan and Sudalai, where they synthesized (S)-penbutolol in 95% *ee* by employing Sharpless asymmetric dihydroxylation.[49] Later, Hamaguchi *et al* were able to synthesize (S)-penbutolol in 100 % ee by by utilizing enzyme catalyzed kinetic resolution of 3-(tert-butyl)-5-(hydroxymethyl)oxazolidin-2-one with lipoprotein lipase amano 3.[60,61]

1.9 Synthetic route to Penbutolol

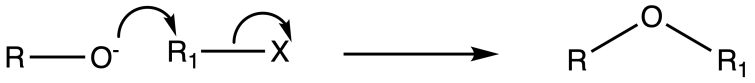
1.9.1 Previously performed synthesis

In the authors previous project, 1-chloro-3-(4-cyclopentylphenoxy)propan-2-ol (**3a**) was synthesized as a building block, laying grounds for this thesis.[62] This synthesis is shown in Scheme 1.3.



Scheme 1.3: The synthesis performed in the authors previous project.[62]

Step 1 in the synthesis is a Williamson ether synthesis between 4-cyclopentyl phenol (**1a**) and 2-(chloromethyl)oxirane (epichlorohydrin). The Williamson ether synthesis is an S_N2 type reaction that forms an ether from an organohalide and an alkoxide. The mechanism for a general Williamson ether synthesis is shown in Scheme 1.4.

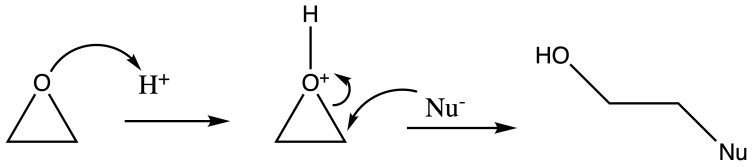


Scheme 1.4: The mechanism for a Williamson ether synthesis.[63] X is a halide and R and R_1 are organic substituents.

Employing Williamson ether synthesis with epichlorohyrdin is very useful in pharmaceutical industry, and epichlorohydrin is an especially useful reactant in production of many β-blockers.[64]

Step 2 in the previously performed synthesis[62], is an epoxide ring opening of 2-((4-cyclopentylphenoxy)methyl)oxirane (**2a**) to form 1-chloro-3-(4-cyclopentylphenoxy)propan-2-ol (**3a**). The highly strained three-membered ring of an epoxide makes it more susceptible to nucleophilic attacks. In acidic environment, the epoxide will be protonated which enhances this effect and allows for weak nucleophiles. The mechanism for epoxide ring opening in acidic

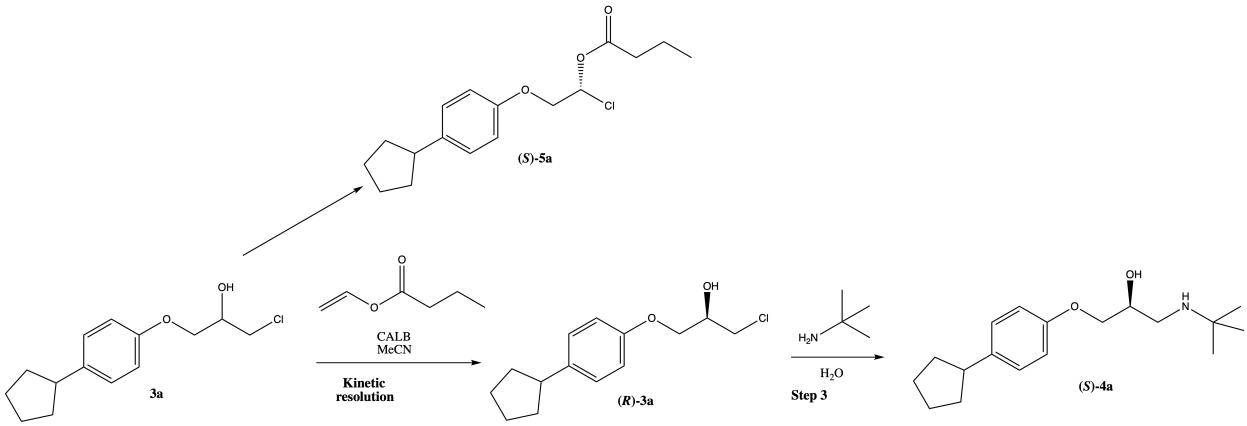
environment is illustrated in Scheme 1.5.[63] This kind of β-hydroxylation of nucleophiles is one of the most valuable and generally applied methods for the synthesis of β-substituted alcohols.[65] Nucleophilic attack generally occurs at the least substituted carbon in high regioselectivity.



Scheme 1.5: The mechanism for a nucleophilic epoxide ring opening in acidic environment.[63]

1.9.2 Intended synthetic approaches in this thesis

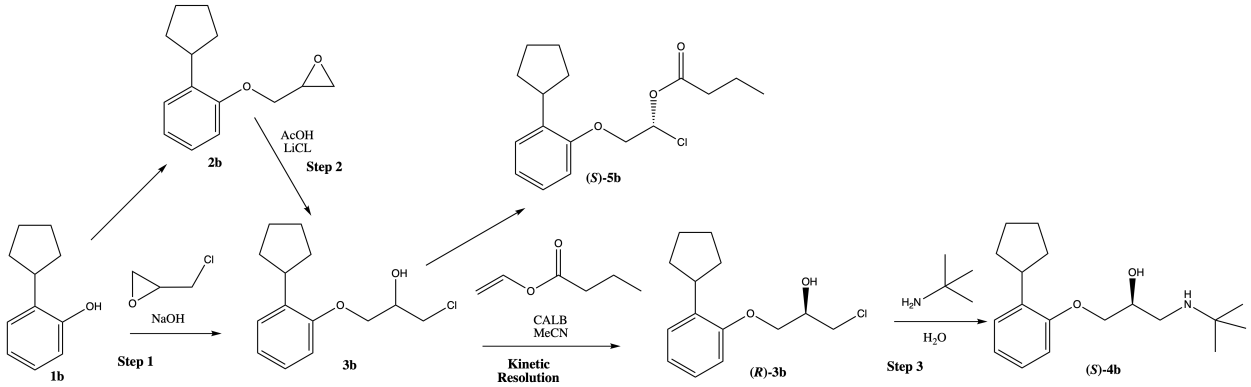
Scheme 1.6 shows the intended synthetic pathway to (*S*)-1-(*tert*-butylamino)-3-(4-cyclopentylphenoxy)propan-2-ol ((*S*)-**4a**). Kinetic resolution is performed on the previously synthesized **3a** with vinyl butyrate and CALB as the stereoselective catalyst. This will form (*S*)-1-chloro-3-(4-cyclopentylphenoxy)propan-2-yl butyrate ((*S*)-**5a**), and leave (*R*)-**3a** unreacted. Step 3 is then a substitution reaction with *tert*-butylamine to form (*S*)-**4a**. The synthesis of (*S*)-**4a** is performed as a model for the synthesis of (*S*)-penbutolol ((*S*)-**4b**), as the starting material **1a** is a lot cheaper than 2-cyclopentyl phenol (**1b**).



Scheme 1.6: Planned synthetic route to enantiopure (*S*)-1-(*tert*-butylamino)-3-(4-cyclopentylphenoxy)propan-2-ol ((*S*)-**4a**).

Scheme 1.7 shows the intended synthetic pathway to (*S*)-**4b**. The synthesis is similar

to the synthesis of **4a** described in Schemes 1.3 and 1.6, with the only difference being the cyclopentyl ring in ortho-position in the starting material **1b**, compared to the para-positioning in **1a**. Step 1 and 2 are carried out based on the results of the previous synthesis of **3a**,[62] to form 2-((2-cyclopentylphenoxy)methyl)oxirane (**2b**) and then 1-chloro-3-(2-cyclopentylphenoxy)propan-2-ol (**3b**). That is followed by CALB-catalyzed kinetic resolution with vinyl butyrate to form (*S*)-1-chloro-3-(2-cyclopentylphenoxy)propan-2-yl butyrate ((*S*)-**5b**), leaving (*R*)-**3b** unreacted. Amination with *tert*-butylamine (Step 3) is the last step to form enantiopure β-blocker (*S*)-penbutolol ((*S*)**4b**).



Scheme 1.7: Planned synthetic route to enantiopure (*S*)-penbutolol ((*S*)-**4b**).

2 Results and discussion

2.1 Synthesis of 1-chloro-3-(2-cyclopentylphenoxy)propan-2-ol (3b)

2.1.1 Step 1: Substitution reaction with 2-cyclopentyl phenol (1b) and epichlorohydrin

Step 1 reactions with **1b** and epichorohydrin were carried out based on the similar reactions performed previously with **1a**.[62] **1b**, epichorohydrin and aqueous sodium hydroxide (NaOH) were mixed which gave a dark green solution, that turned to a pale yellow after stirring over night. This reaction yielded a mixture of epoxide **2b** and alcohol **3b**. To find optimal reaction conditions, several parallels were carried out using different amounts of NaOH, different reaction times and solvent. The resulted amounts of **1b**, **2b** and **3b** in the reaction

mixtures after these experiments are shown in Table 2.1. For more details about every experiment, see Section A in the Appendix.

Table 2.1: Resulted amounts of **2b** and **3b**, together with amount of unconverted **1b**, in the step 1 reactions performed in different reaction conditions. In experiment F, additional 0.5 equivalents of NaOH were added after 20 hours.

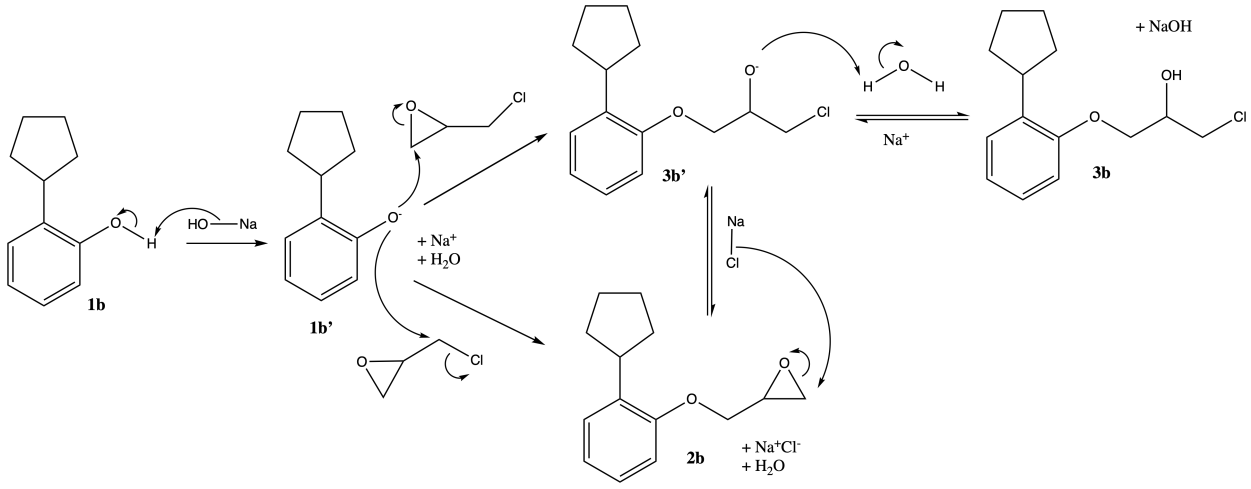
Experiment [-]	Time [h]	Epichloro-hydrin [$eq.$]	NaOH [$eq.$]	THF [mL]	Amount **1b** [%]	Amount **2b** [%]	Amount **3b** [%]
A	20	2	1	2	30	55	15
B	20	2	2	2	27	63	10
C	20	2	2	0	13	52	35
D	20	2	1.5	0	14	33	53
E	44	2	1.5	0	10	36	54
F	20+8	2	1.5+0.5	0	8	53	39
G	96	2	0.5	0	25	9	66

The first discovery shown in these results is from the use of tetrahydrofuran (THF) as solvent. The first two experiments (A and B) that were run with THF as solvent, resulted in 70% and 73% conversion of starting compound **1b**, while running the same reaction without any added organic solvent resulted in 87% conversion (Experiment C). Also, more of the desired product **3b** compared to **2b** were formed without THF as solvent. These results show that the aqueous mixture of NaOH and epichlorohydrin was sufficient as solvent in this reaction.

As for most chemical reactions, increased reaction time did cause increased conversion. However, letting the reaction stir for 44 hours (Experiment E) compared to 20 hours (Experiment D), only caused a 4% increase in conversion. This suggests that the reaction would have to stir for very long in order to achieve full conversion. This is also seen in Experiment G, where stirring for four days resulted in 75% conversion, which shows that other parameters are more important.

The amount of NaOH added to the reaction turned out to be an impactful factor, but also the

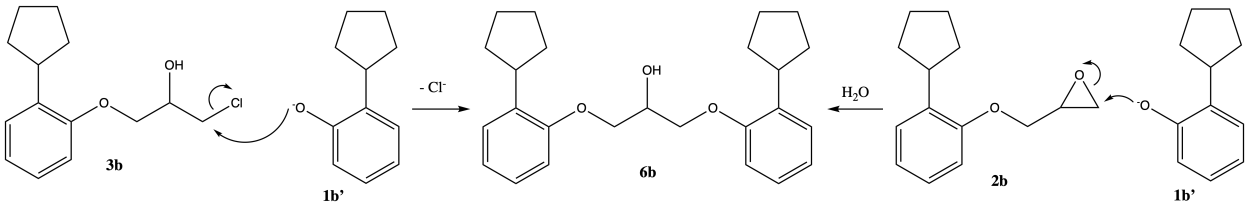
most difficult to optimize. An increased amount of NaOH resulted in increased conversion, but also caused the formation of epoxide **2b** to be more prioritized compared to **3b**. This could be explained by the suggested reaction mechanism, which is shown in Scheme 2.1. **1b** is first deprotonated by NaOH to form 2-cyclopentylphenolate (**1b'**), which can then attack epichlorohydrin on two different carbons. Either on the chloro-carbon forming **2b**, or on the epoxide ring to form 1-chloro-3-(2-cyclopentylphenoxy)propan-2-olate (**3b'**). **2b** and **3b'** creates an equilibrium with ring opening/closing of the epoxide with/knocking out Cl^-. **3b'** can also be protonated in the aqueous solution to form alcohol **3b**, which will recycle the NaOH. Because NaOH is recycled in the formation of **3b**, adding a large abundance of NaOH will, by Le Chatelier's principle,[66] cause more **3b'** to remain deprotonated. This will in turn cause the equilibrium to shift more towards formation of **2b**. In experiment G, a catalytic amount of NaOH (0.5 equivalents) were added. The conversion was still larger than 50%, which proves that NaOH is recycled and works as a catalyst in formation of alcohol **3b**.



Scheme 2.1: Suggested mechanism for step 1 in the synthesis of penbutolol.

The highest conversion of **1b** achieved was by addition of extra NaOH after the reaction was stirred for 20 hours, and letting it stir for another 8 hours. This resulted in 92% conversion (Experiment F). However, when more than 1.5 equivalents of NaOH were used, trace amounts of dimer 1,3-bis(2-cyclopentylphenoxy)propan-2-ol (**6b**) could be observed. This was not seen in reactions where 1.5 equivalents of NaOH or less were used. Dimerization can happen in

two ways, by epoxide ring opening of **2b**, or by nucleophilic attack on the chloro-carbon on **3b**. Mechanisms for the two possible dimerizations are shown in Scheme 2.2.



Scheme 2.2: Mechanism for the possible dimerization in Step 1.

Another factor that made the reactions difficult, is the similarities in polarity of the starting compound (**1b**), and the intermediate product **2b**. Attempts were made to monitor the carried out reactions over time, using thin layer chromatography (TLC). However, **1b** and **2b** had the same retention factor (R_f), which caused difficulties distinguishing them during the reaction. Also, product **3b** had the same R_f-value as the dimer **6b**. This means that high observed conversion to **3b** on TLC is not necessarily positive. The R_f-values for all relevant compounds, and the eluents used, are shown in Table 2.2.

Table 2.2: R_f-values of all relevant compunds in their applied eluent compositions.

Compound	Eluent [*pentane:EtOAc*]	R_f-value
3a	90:10	0.27
5a	90:10	0.65
1b	90:10	0.39
2b	90:10	0.39
3b	90:10	0.20
6b	90:10	0.20
3b	80:20	0.39
5b	80:20	0.76

The crude products after workup from all the step 1 reactions, were all yellow colored oils. The yellow color disappeared after flash chromatography, leaving a blank oil. The oil was

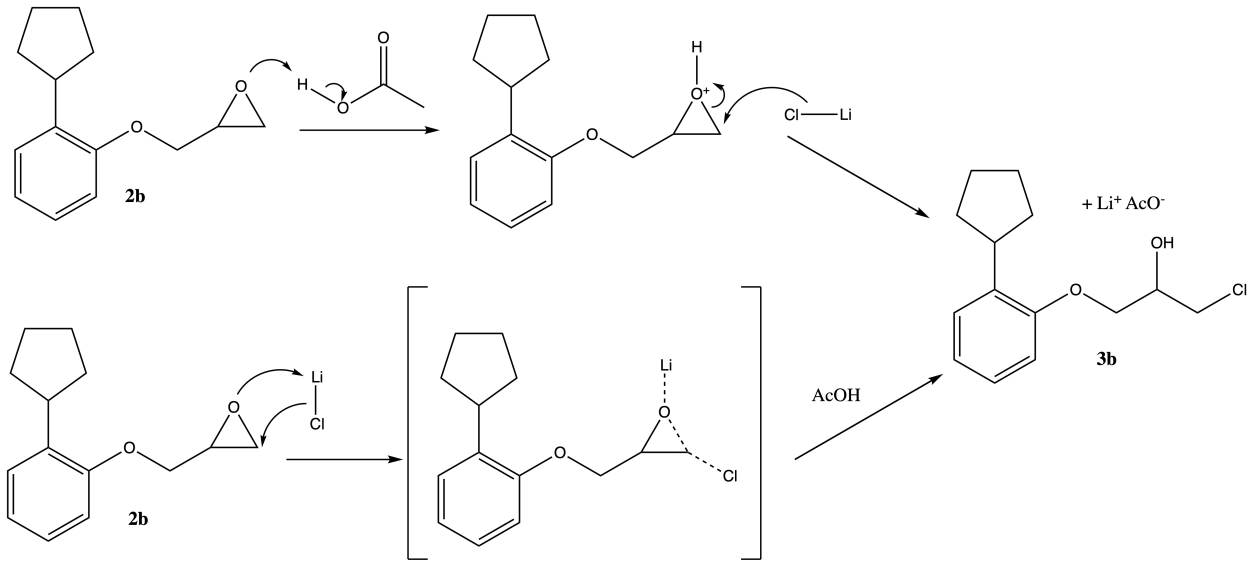
more viscous with larger amount of **3b** compared to **2b**. The yellow color is believed to come from oligomers of epichlorohydrin, as they are reported to be yellow-brown in color.[67]

By interpreting the results from all performed step 1 reactions, a suggestion to achieve better results would be to use less NaOH at the start of the reaction to shift it more towards formation of **3b**. This should be combined with several additions of small amounts of NaOH over time and longer reaction time to achieve higher conversion. Also, to avoid dimerization, combined NaOH additions should not exceed 1.5 equivalents.

2.1.2 Step 2: epoxide ring opening of 2-((2-cyclopentylphenoxy)-methyl)oxirane (2b)

In step 2, the goal was to open the epoxide ring on the remaining **2b**, and convert it to the desired product **3b**. To do this, lithium chloride (LiCl) and acetic acid (AcOH) were added to the reaction mixture from step 1. Two mechanisms are suggested for this reaction, which is shown in Scheme 2.3. The first shows a mechanism similar to the one illustrated in Scheme 1.5, with the epoxide being protonated by AcOH, followed by nucleophilic attack from the weak nucleophile Cl^-. The second is a direct attack by Cl^-, which is made possible because the free electrons at the oxygen is coordinated by Li^+ as a Lewis acid. Experiments to determine the correct mechanism were not conducted, so it is unknown which is the main mechanism or if it is a combination of the two.

The Step 2 reaction were performed both with and without THF as solvent, which both resulted in 98% conversion. More details about these reactions are presented in Table 2.3. Also, no by-products could be observed. The reason full conversion was not achieved could be because of the mixture of compounds in the reaction mixture from step 1. Because of the similar R_f-values, epoxide **2b** could not be fully isolated by flash chromatography before step 2. When there is only 2% of **2b** left in the reaction mixture, and other compounds are present as well, random encounters with the right reactants become more rare.



Scheme 2.3: Two suggested mechanisms for the epoxide ring opening of **2b** to form **3b**.

The highest achieved yield of **3b** from step 1 and 2 combined, was 58%. Most of the yield loss is thought to come from the complex mechanism and equilibria in step 1.

Step 2 were performed both with and without separating out the formed **3b** from step 1, by flash chromatography. Although some yield were lost over the flash column, having the formed **3b** in the reaction mixture caused greater loss. This is because more yield was lost during workup and extraction of the product.

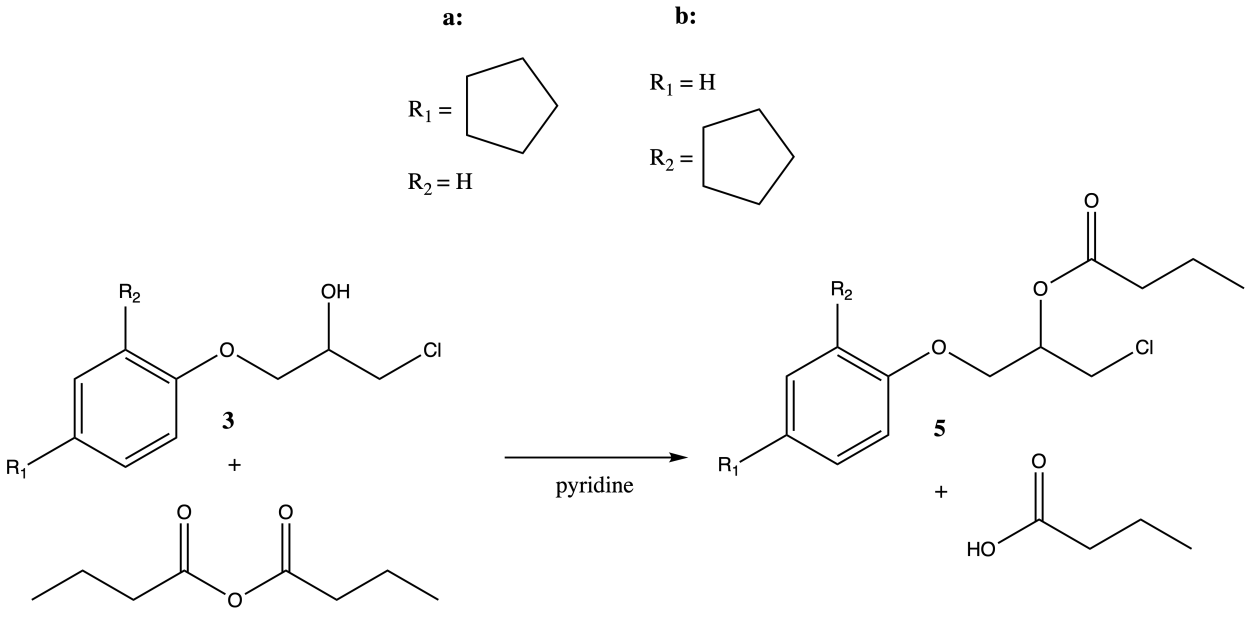
Table 2.3: Conversion of epoxide ring opening reaction of **2b** showing reactants with and without THF as solvent.

Time [*h*]	AcOH [*eq.*]	LiCl [*eq.*]	Solvent	Conversion [%]
20	3	3	THF	98
20	3.5	3	none	98

2.2 Derivatization and chiral analysis

Before kinetic resolutions could be performed, chiral analyses of compounds **3** and **5** had to be performed in order to achieve separation of the enantiomers. To synthesize the esters

5, derivatization was performed by mixing **3**, butyric anhydride and pyridine and heating it at 60 °C for 1 hour. Derivatization of both **3a** and **3b** resulted in full conversion. The derivatization reaction is shown in Scheme 2.4.



Scheme 2.4: Derivatization of **3a** and **3b** with butyric anhydride to form **5a** and **5b**.

Chiral analyses were performed on HPLC. Separation of **3a** with an R_s-value of 1.85, and separation of **3b** with an R_s-value of 2.19 was achieved. Methods for this is shown in Table 2.4.

Table 2.4: Table that shows the achieved enantiomeric separations with retention times of the *S*- and *R*-enantiomers, and resolution in the applied HPLC methods.

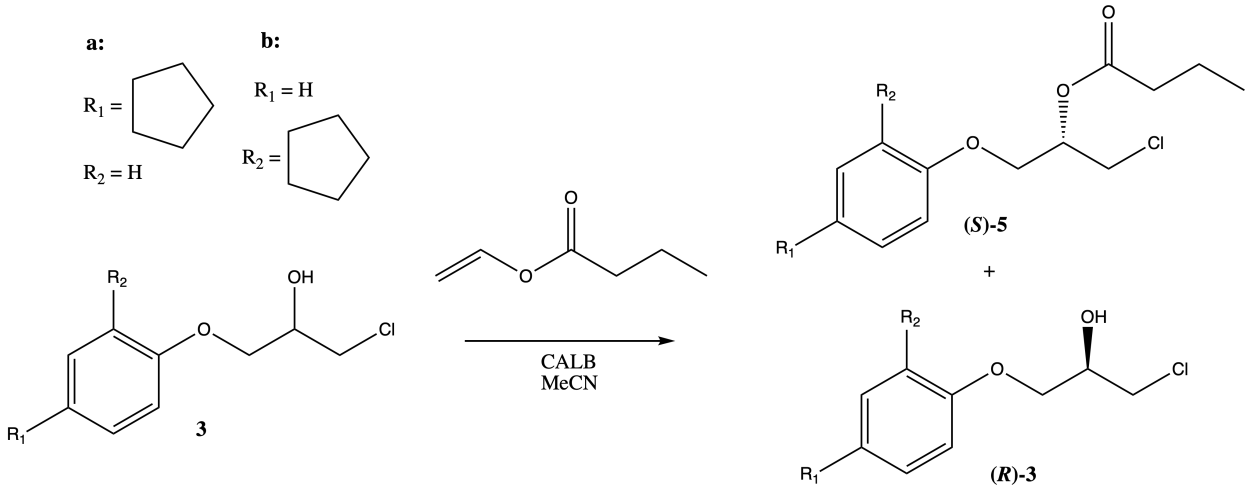
Compound	Column	Eluent [hexane:iPrOH]	t_R (S) [min]	t_R (R) [min]	R_s
3a	Chiralcel® OD-H	90:10	7.7	8.8	1.85
3b	Chiralcel® OD-H	95:5	12.0	13.7	2.19

Enantiomeric separation of the esters (**5**) was not achieved. Many different eluents were attempted, as well as a normal phase (Chiralcel® OD-H) and a reverse phase Chiralcel® OD-RH) column, but none resulted in sufficient separation. The highest R_s-value achieved

for **5a** was 0.67, on the normal phase column with 99:1 hexane:iPrOH. For **5b**, the peaks could not be distinguished at all.

2.3 Kinetic resolutions of 3a and 3b

Kinetic resolutions were performed by dissolving alcohol **3** in dry acetonitrile (MeCN), and mixing in vinyl butyrate and CALB. The mixture was shaken at 30 °C and the reaction was monitored on chiral HPLC over 48 hours. The kinetic resolution is illustrated in Scheme 2.5.



Scheme 2.5: Kinetic resolution of **3a** and **3b**.

Two parallels of the kinetic resolution reactions were performed with **3a**, and one with **3b**. Becuase enantiomeric separation of the esters (**5**) were not achieved, the ee_p could not be determined. This means that no plot like the one shown in Figure 1.2 could be made. The *ee* for alcohol **3** was still calculated, and plots were made showing ee_s over time. Figures 2.1 and 2.2 show the ee_s over time for the kinetic resolutions of **3a**, and Figure 2.3 shows the ee_s over time for the kinetic resolution of **3b**.

The first kinetic resolution of **3a** started off successfully, with ee_s gradually increasing up to 83% after 10 hours. However, over night the ee_s started decreasing, and after 48 hours it went all the way down to 11%. This should not have happened, because even if the reaction had reached more than 50% conversion, the ee_s should still be high as the ee_p would go down. This means the reaction must have reversed itself. This was thought to be because

of water contamination, causing the ester (**5a**) to be hydrolysed. Because of this, another kinetic resolution was carried out, where extra care was taken to keep the reactants and solvent dry. This time the ee_s went all the way up to 100% after 14 hours. However, over night it still went down to 71% at 22 hours. Then it increased up to 100%, before decreasing to 92% after 48 hours.

These results were difficult to interpret. One theory is that the water content in the reaction mixture was lower than the first reaction, but still enough to affect the reaction. It has been proven by Jacobsen and Anthonsen that water content in CALB can affect the selectivity of CALB-catalyzed kinetic resolutions of secondary alcohols.[68] Also, as mentioned in Section 1.6.2, water can change the overall structure and stability of the enzyme. CALB may catalyze the esterification first, but when contact with water occurs, structural changes to the enzyme and/or its active site happens to catalyze the hydrolysis instead. Now, the hydrolysis is catalyzed until the structure is stabilized and changed back to catalyze esterification. This could explain why the reaction seems to go back and forth without reaching an equilibrium. In the first kinetic resolution, the water content was too high for the enzyme to stabilize, causing hydrolysis to continue once it had started. These results show how extremely complex enzymes can be, and understanding all the mechanisms of an enzyme demands a lot of research and experiments.

The kinetic resolution of **3b** was more successful, as seen in Figure 2.3. The ee_s increased gradually, and reached 97% after 14 hours. It reached 100% over night, and stayed there. This shows the incredible stereoselectivity of CALB. Before this experiment, it was hypothesized that the kinetic resolution with **3b** might not work as well as with **3a**. The ortho-substituted cyclopentyl group could act as a "hook", preventing the substrate from entering the narrow entrance of CALB. However, the results showed that this was not the case. This could be because the entrance to the active site is opened a little more by some kind of interfacial activation, like described in Section 1.6.2. Building on the theory from the kinetic resolution of **3a**, this could be the reason the resolution with **3b** only moves in one direction. The entrance to the active site is only opened upon interaction with a hydrophobic substance, and not with water, which prevents hydrolysis.

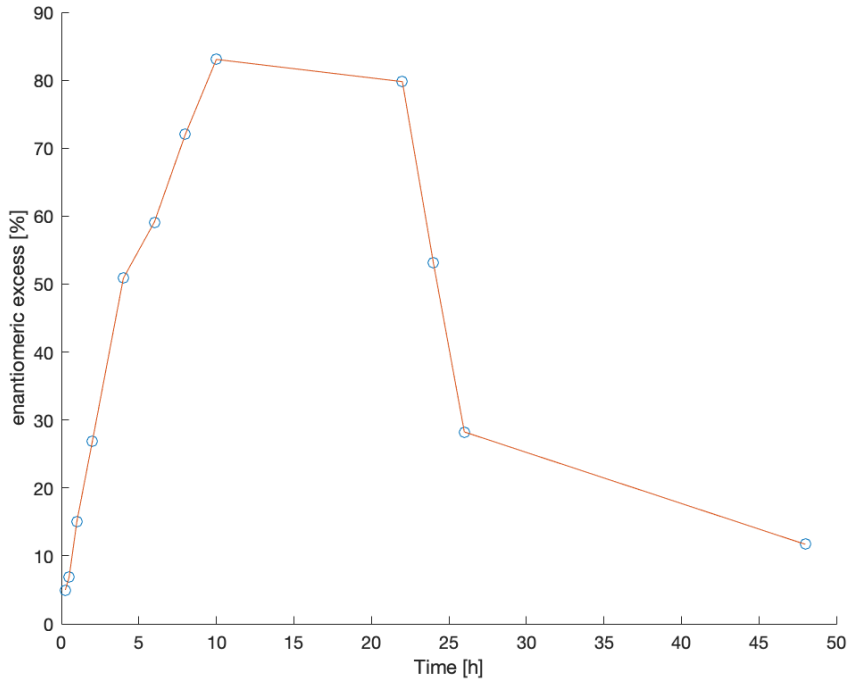


Figure 2.1: Plot showing ee_s over time in the first kinetic resolution performed on alcohol **3a**.

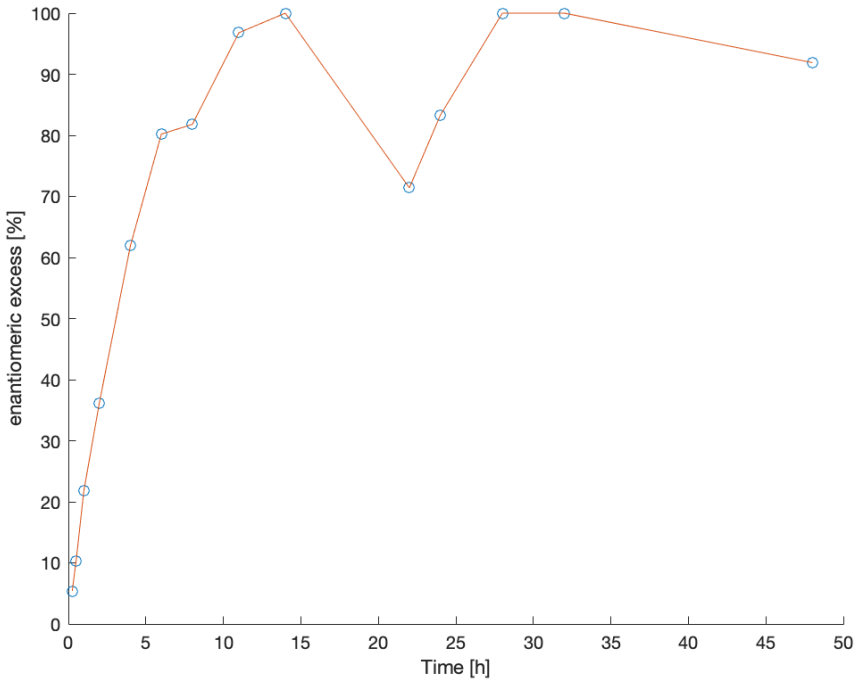


Figure 2.2: Plot showing ee_s over time in the second kinetic resolution performed on alcohol **3a**.

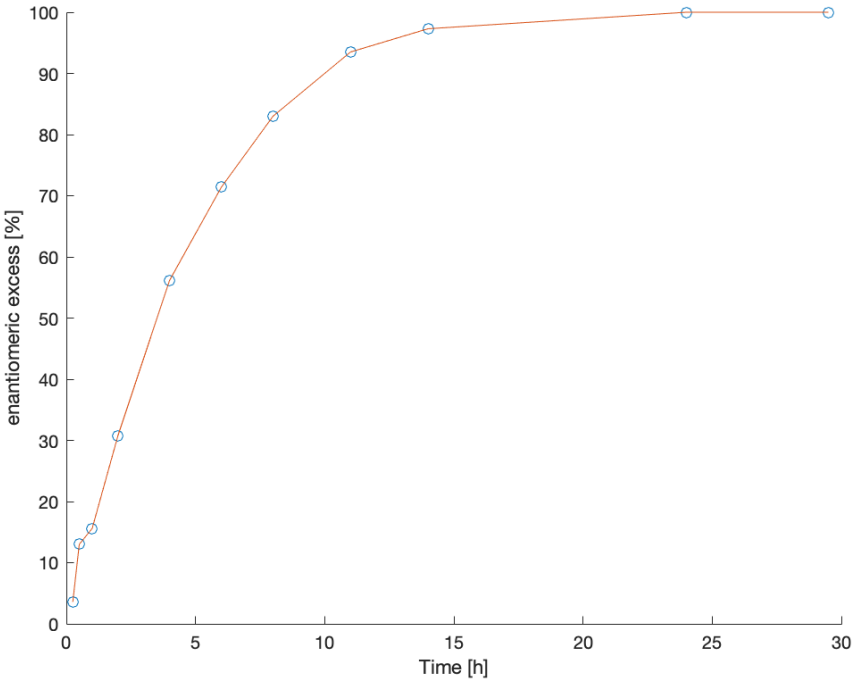


Figure 2.3: Plot showing ee_s over time in the kinetic resolution performed on alcohol **3b**.

After interpreting the results from the monitored kinetic resolutions, larger scale resolutions were conducted with both **3a** and **3b**. The resolution of **3a** was stopped after 14 hours, and the resolution of **3b** was stopped after 16 hours. After workup and separation of **3** and **5** by flash chromatography, both resolutions yielded alcohol (*R*)-**3** in 96% *ee*. The resolution of **3a** gave 34% yield.

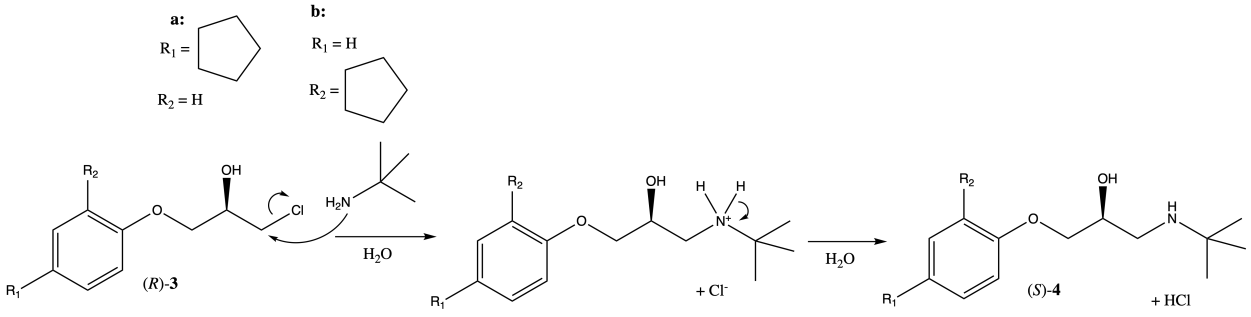
As for **3b**, the product was only in 81% purity as it still contained 19 mol% of butyric acid, which was formed in the kinetic resolution reaction. Because hydrolysis of ester **5b** did not occur, at least not to a large degree, the butyric acid must have been formed in a side reaction. Over five equivalents of vinyl butyrate were used, so this could have happened without affecting the resolution. The butyric acid was also difficult to get rid of, because it came out in the same fraction as **3b** in flash chromatography. The butyric acid could not be seen on TLC, which made it too difficult for method development on TLC. Because of this, the butyric acid was kept in the product mixture for step 3.

In an attempt to get calculated enantiomeric ratios for the large scale kinetic resolutions,

hydrolysis of the formed esters **5** were carried out. However, no conversion could be seen in any of the reactions attempted. Hydrolyses were attempted with both NaOH and HCl, and in heating up to 95 °C. Nevertheless, the only peaks that could be seen on HPLC, were the esters **5**. For more details about the reaction conditions of all these hydrolysis attempts, see Section A.5 in the Appendix. Even though the hydrolysis attempts were unsuccessful, some results can still be drawn from these experiments. In order to keep the enantiomeric excess of the esters when hydrolyzed, CALB was not used as catalyst in any of these attempts. Because nothing happened without the enzyme, this proves that the hydrolysis that occurred in the kinetic resolution of **3a** must have been catalyzed by CALB. This also could strengthen the theory discussed above.

2.4 Step 3: Synthesis of 1-(tert-butylamino)-3-(4-cyclopentylphen-oxy)propan-2-ol (4a) and penbutolol (4b)

Step 3 in the synthesis of **4a** was performed by mixing the enantiopure (*R*)-**3a** in *tert*-butylamine and water, and stirring in room temperature. A suggested mechanism for this reaction is shown in Scheme 2.6. This is an S_N2 type reaction, with the amine as nucleophile.



Scheme 2.6: Suggested mechanism for step 3 of the synthesis. Amination with *tert*-butylamine through an S_N2-reaction.

The reaction with (*R*)-**3a** resulted in 95% conversion. After trituration with *n*-pentane, this gave pure (*S*)-**4a** as white crystals in 86% yield, and still 96% *ee*. This gives a combined yield of 29% from racemic **3a**. The synthesis of (*S*)-**4a** was originally used as a model for synthesis of the enantiopure β-blocker (*S*)-penbutolol. However, because of the similarity

of the reactive sites, **4a** might possess β-antagonizing properties too, although no literature were found for it. Most nonselective β-blockers, like penbutolol, have an ortho-substituted group on the aromatic ring.[69] As for the cardioselective β-blockers, most of them have a para-substituted group on the aromatic ring instead.[70–72] This could suggest that **4a**, with its para-substituted cyclopentyl group, does possess more cardioselective β-antagonizing properties. However, because the synthesis was simpler, and the starting compound cheaper, this have likely been tested before. Therefore it could be that no literature were found because of publication bias, as negative results are not as likely to be published in pharmaceutical science.[73]

The amination of (*R*)-**3b** was performed in the same way as the reaction with (*R*)-**3a**, but with the product mixture containing 81 mol% (*R*)-**3b** and 19 mol% butyric acid. This resulted in 80% conversion from (*R*)-**3b** to (*S*)-**4b**. Because of the increased amount of impurities in this reaction mixture, a full recrystallization in *n*-pentane was performed instead of trituration. This gave a white powder, that after NMR analysis was revealed to be hydroxylamine (NH_2OH). The filtrate was the recovered as a mixture of 68 mol% (*S*)-**4b** in 96% *ee*, 17 mol% (*R*)-**3b** and 15 mol% butyric acid. It is unclear why **4b** was dissolved in pentane while **4a** was not, but it could be some kind of interaction with the acidic environment created by butyric acid. Because of time restrictions, no further attempts were made to purify this product mixture. However, this would most likely be possible to separate with flash chromatography. Since it is known from the purification attempt after the kinetic resolution that **3b** and butyric acid have the same R_f-value, method development on TLC would now be possible.

2.5 Characterization of compounds

NMR was used for characterization of all compounds in this thesis. ^{1}H NMR was utilized for analysis of crude products and intermediates, and measurements of conversion and yield. For the isolated products and intermediates, full characterizations were performed by the use of ^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC. For synthesis route **a** (synthesis of (*S*)-**4a**), The starting compound **3a** had already been fully characterized with all ^{1}H- and

^{13}C-shifts assigned in the authors previous project.[62] The assigned chemical shifts for all relevant compounds in this synthesis are shown in Figure 2.4. All these structures were confirmed with both 1D- and 2D NMR. For synthesis route **b** (synthesis of (*S*)-**4b**), only **3b** and **5b** were fully isolated and could be fully characterized. For **2b** and **4b**, ^{1}H shifts were assigned through careful analysis of the spectra using coupling constants, and comparing to the similar assigned shifts for **3b** and **5b**. All assigned chemical shifts for these compounds, as well as the starting compound **1b** are shown in Figure 2.5.

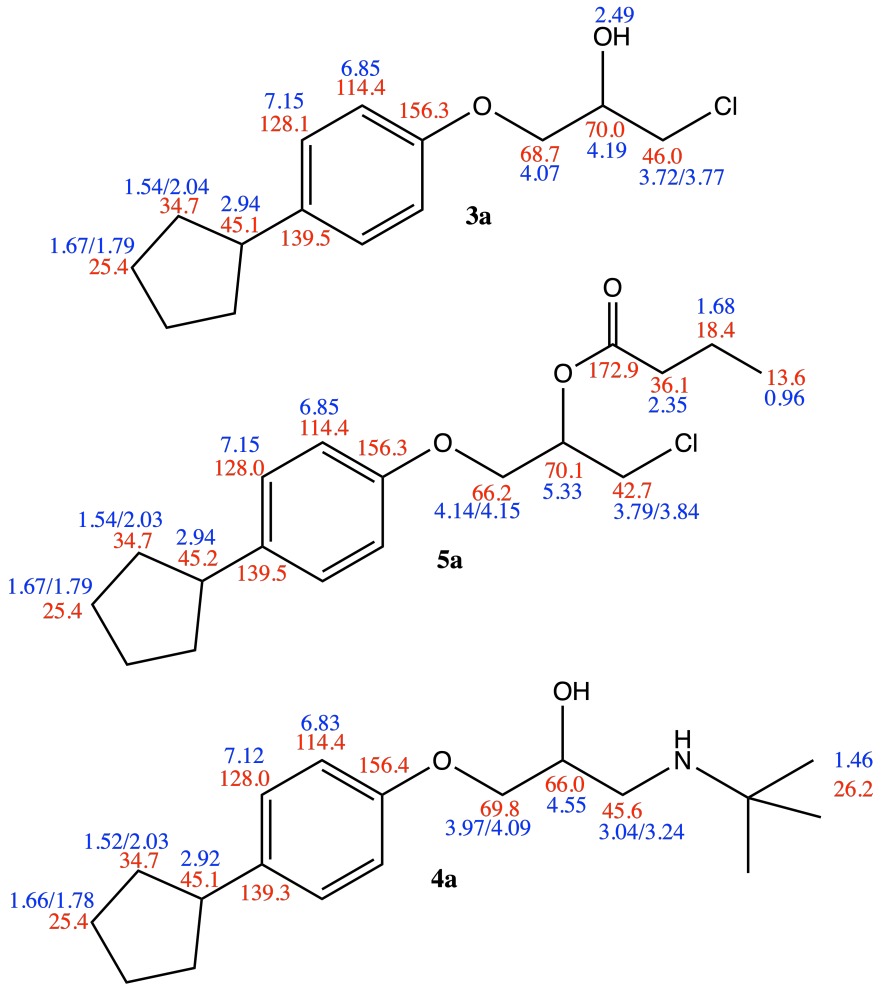


Figure 2.4: Assigned chemical shifts for all relevant compounds in the synthetic route to **4a**. ^{1}H shifts are written in blue, and ^{13}C shifts are written in red. **3a** was characterized in the authors previous project.[62]

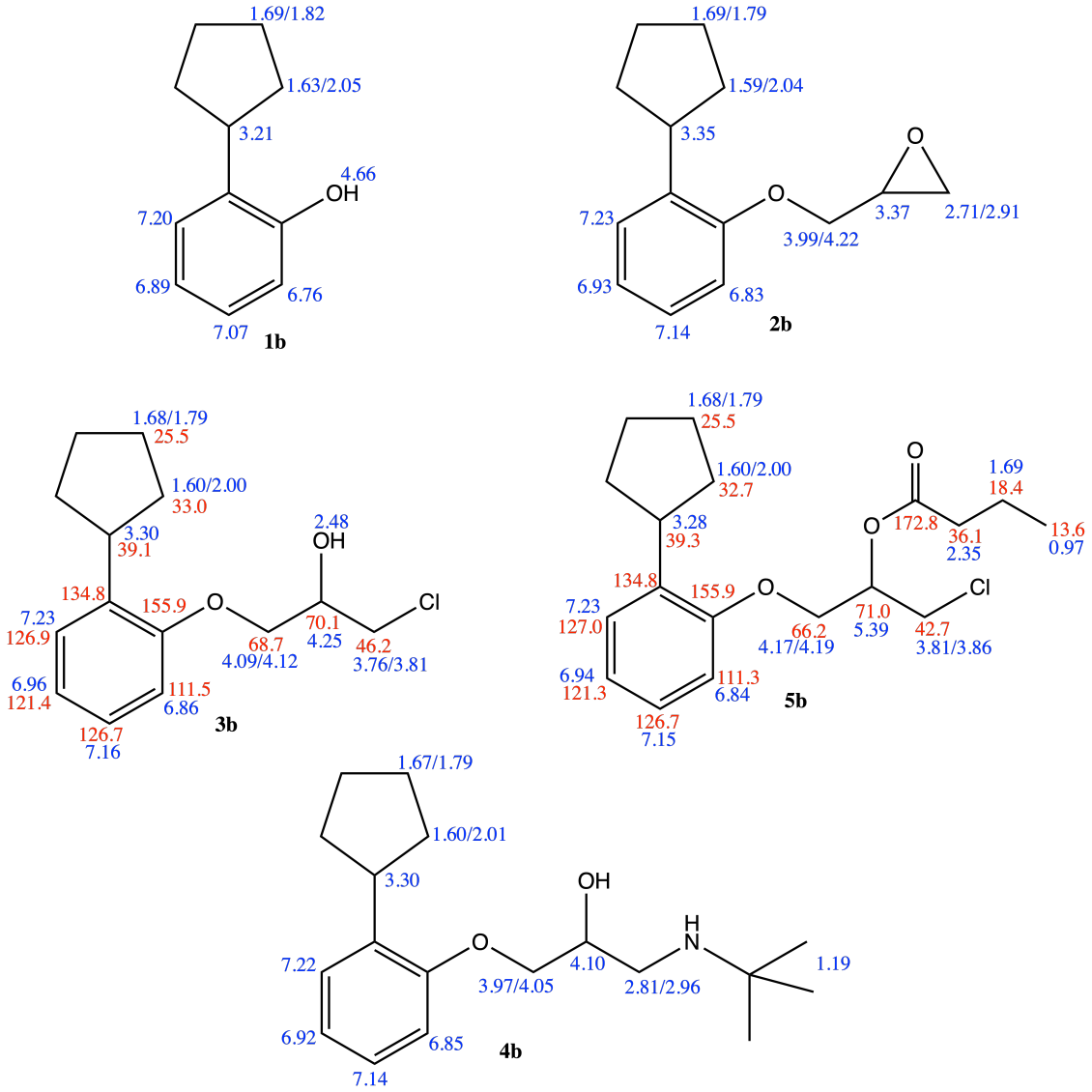


Figure 2.5: Assigned chemical shifts for all relevant compounds in the synthetic route to **4b**. ^{1}H shifts are written in blue, and ^{13}C shifts are written in red.

3 Future work

Organic syntheses can always be improved, and there is a lot that can be done to improve the results from this thesis. To improve yield, the complex equilibria and mechanisms in step 1 should be investigated further. Because of the difficulties monitoring the reaction on TLC, the only way to to this is to run more reactions. Longer reaction times and gradual addition of NaOH are suggested improvements to start with. The reaction should also be

tested with other bases than NaOH, and with different amounts of epichlorohydrin. Another thing that could help understanding the reaction, is to run a full timelapse of it. This should be done by taking out small samples of the reaction mixture over a long time, while stirring, for analysis on NMR.

Step 2 did work well in this thesis, with 98% conversion. This step should be run again to see if it could be more environmentally- and cost efficient by adding less of the reactants, or if 100% conversion could be reached with more. Also, experiments should be conducted to confirm which of the mechanisms suggested in Scheme 2.3 is happening. Two experiments are suggested in order to confirm the right mechanism: The reaction could be performed with only addition of HCl. If **3b** is formed, it will confirm the first mechanism in Scheme 2.3, because the lithium is not present to coordinate the oxygen and the epoxide has to be protonated for a weak nucleophile like Cl^- to attack. The reaction could also be performed with only addition of LiCl in aqueous solution. If **3b** is formed, it will confirm the second mechanism in Scheme 2.3, because with no acid present, the oxygen has to be coordinated by a lewis acid like Li^+ for the weak nucleophile Cl^- to attack. With an abundance of water in the solution, this should be enough to protonate at least some of the lithium-coordinated intermediate, which would prove this mechanism.

For the kinetic resolution, there are a lot of things that could be further studied. First, more method development should be tried on chiral HPLC, in order to achieve enantiomeric separation of the esters **5a** and **5b**. The Chiralcel® OD-H column did not seem to work with any eluent composition, but it was also the only normal phase column that was used. Other columns should be tested, as their chiral selectors might have more suitable interactions with the esters (**5**). Achieving enantiomeric separation of the esters **5**, will allow for much more information to study the kinetic resolution. Many more experiments could be conducted in order to investigate the kinetic resolution. It could be attempted with different solvents, different acyl donors or other enzymes.

Step 3 in this thesis seemed to work extremely well in formation of **4a**, but not for **4b**. This reaction should be performed on racemic **3b** first, to see if the final product is really that different, or if recrystallization with *n*-pentane will work without the presence of butyric

acid. Also, the reaction should be performed without the addition of water. If the reaction still works, this will prevent formation of NH_2OH. Purification by flash chromatography could also work to purify the product.

4 Conlusion

In this thesis, the final product (*S*)-**4a** was synthesized in 29% yield and 96% *ee*. This was done by enzyme-catalyzed kinetic resolution of alcohol **3a** using CALB, followed by amination with *tert*-butylamine. This was used as a model for the synthesis of β-blocker (*S*)-penbutolol ((*S*)-**4b**).

The alcohol **3b** was also synthesized in 58% yield. This was synthesized as a building block for **4b**. Kinetic resolution of **3b** gave (*R*)-**3b** in 96% *ee*, but only in 81% purity, as butyric acid was not removed by purification. Amination with *tert*-butylamine gave (*S*)-**4b** in 96% *ee* and 68% purity, with 17% **3b** and 15% butyric acid. Purification attempts of this were not successful.

5 Experimental

5.1 General

All experiments and analyses in this project were performed at the Department of Chemistry, Faculty of Natural Sciences, Norwegian University of Science and Technology, Trondheim, Norway.

All chemicals used are of analytical grade, commercially available and were purchased from Sigma Aldrich Norway. Solvents used for HPLC are of HPLC grade.

Enzyme used was *Candida Antarctica* Lipase B (CALB) (Novozym 435 from Novozymes AS, 1-2% water content, activity 10000 PLU/g; LC200204) immobilized on macroporous acrylresin.

All HPLC analyses were performed on an Agilent HPLC 1100 instrument with manual injec-

tor (Rheodyne 77245i/Agilent, 10 μL loop), with a Chiralcel® OD-H column (Daicel, Chiral Technologies Europe, 250 · 4.6 mm ID). Isocratic eluation with 90:10 n-hexane:isopropanol for compounds **3a** and **5a**, and 95:5 n-hexane:isopropanol for compounds **3b** and **5b**. Flow: 1 mL/min, injection volume: 10 μL, detection at 254 nm.

All NMR analyses were performed on a Bruker 600 MHz Avance III HD instrument equipped with 5-mm cryogenic CP-TCI z-gradient probe and SampleCase. $CDCl_3$ was used as solvent.

TLC analyses were performed on Merck silica $F_2$54 and detected by UV at λ=254 nm.

Flash chromatography were performed using silica gel from VWR Chemicals with pore size 60 Å, 230-400 mesh and 40-63 mm particle size.

For some of the reactions described below, several parallels were carried out at different reaction conditions. For detailed descriptions about every experiment, see section A in the Appendix.

5.2 Synthesis of 1-chloro-3-(2-cyclopentylphenoxy)propan-2-ol (3b)

5.2.1 Step 1: Substitution reaction with 2-cyclopentyl phenol (1b) and epichlorohydrin

2-Cyclopentyl phenol (**1b**) (0.2161 g, 1.33 mmol) and epichlorohydrin (0.2539 g, 2.74 mmol) were mixed in NaOH (1 M, 2.00 mL), to a dark green solution. The solution was stirred for 44 hours, to a cloudy yellow solution. Products were extracted with EtOAc (4·8 mL) and washed with water (6 mL) and brine (2·6 mL). The solution was dried over $MgSO_4$ and solvents were removed *in vacuo* to a yellow oil (0.2521 g) containing **1b** (10 mol%), 2-((2-cyclopentylphenoxy)methyl)oxirane (**2b**) (36 mol%) and 1-chloro-3-(2-cyclopentylphenoxy)-propan-2-ol (**3b**) (54 mol%). NMR (**2b**): ^{1}H, δ (ppm): 1.59 (m, 2H), 1.69 (m, 2H), 1.79 (m, 2H), 2.04 (m, 2H), 2.71 (dd, 1H) 2.91 (dd, 1H), 3.35 (m, 1H) 3.37 (m, 1H), 3.99 (dd, 1H) 4.22 (dd, 1H), 6.83 (dd, 1H), 6.93 (td, 1H), 7.14 (td, 1H), 7.23 (dd, 1H).

5.2.2 Step 2: Epoxide ring opening of 2-((2-cyclopentylphenoxy)methyl)oxirane (2b)

A reaction mixture from Step 1 (0.6278 g) containing 23 mol% **1b**, 0.57 mol% **2b** and 20 mol% **3b** was separated by flash chromatography (90:10 *n*-pentane/-EtOAc). Fraction 1 (blank oil, R_f=0.39) contained a mixture (0.4235 g) of **1b** (30 mol%) and **2b** (70 mol%). Fraction 2 (blank oil, R_f=0.20) contained isolated **3b** (0.0998 g, 0.39 mmol). To fraction 1, LiCl (3 *eq.*), AcOH (3 *eq.*) and THF (3 mL) were added and the solution was stirred for 20 hours. The crude product was extracted with EtOAc (4·10 mL) and washed with water (2·8 mL) and brine (2·8 mL). The solution was dried over $MgSO_4$ and solvents were removed *in vacuo* to a blank oil. Flash chromatography (90:10 *n*-pentane/EtOAc, R_f=0.20) gave **3b** (0.2692 g, 1.06 mmol) as a blank oil that was mixed with the **3b** from the previous flash chromatography to give **3b** (0.3690 g, 1.45 mmol) in a combined yield of 58%.
NMR (**3b**): ^{1}H, δ (ppm): 1.60 (m, 2H), 1.68 (m, 2H), 1.79 (m, 2H), 2.00 (m, 2H), 2.48 (d, 1H), 3.30 (m, 1H), 3,76 (dd, 1H), 3.81 (dd, 1H), 4.09 (dd, 1H), 4.12 (dd, 1H), 4.25 (sxt, 1H), 6.86 (dd, 1H), 6.96 (td, 1H), 7.16 (td, 1H), 7.23 (dd, 1H). ^{13}C, δ (ppm): 25.5 (2C), 33.0 (2C), 39.1 (1C), 46,2 (1C), 68.7 (1C), 70.1 (1C), 111.5 (1C), 121.4 (1C), 126.7 (1C), 126.9 (1C), 134.8 (1C), 155.9 (1C).

5.3 Derivatization

5.3.1 Formation of 1-chloro-3-(4-cyclopentylphenoxy)propan-2-yl butyrate (5a)

1-Chloro-3-(4-cyclopentylphenoxy)propan-2-ol (**3a**) (1 drop), pyridine (1 drop) and butyric anhydrie were mixed and heated at 60 °C for 1 hour. Full conversion to 1-chloro-3-(4-cyclopentylphenoxy)propan-2-yl butyrate (**5a**) was achieved. Samples of **3a** and **5a** were analyzed by chiral HPLC (90:10, hexane:iPrOH), t_R (min): 4.7 (**5a**), 7.7 ((*S*)-**3a**), 8.8 ((*R*)-**3a**). Enantiomeric separation for **5a** was not achieved.

5.3.2 Formation of 1-chloro-3-(2-cyclopentylphenoxy)propan-2-yl butyrate (5b)

(**3a**) (1 drop), pyridine (1 drop) and butyric anhydrie were mixed and heated at 60 °C for 1 hour. Full conversion to 1-chloro-3-(2-cyclopentylphenoxy)propan-2-yl butyrate (**5b**) was

achieved. Samples of **3b** and **5b** were analyzed by chiral HPLC (95:5, hexane:iPrOH), t_R (min): 5.2 (**5b**), 12.0 ((*S*)-**3b**), 13.7 ((*R*)-**3b**). Enantiomeric separation for **5b** was not achieved.

5.4 Kinetic resolutions

5.4.1 Small scale kinetic resolution of 3a

3a (0.0226 g, 0.089 mmol), vinyl butyrate (64 μL, 0.057 g, 0.50 mmol), CALB (0.0445 g) and MeCN (3.0 mL) were mixed and molecular sieves were added. The mixture was placed in an incubator shaker (30 °C, 200 rpm.). Samples of 150 μL were taken out regularly over 48 hours for chiral HPLC analysis.

5.4.2 Small scale kinetic resolution of 3b

3b (0.0250 g, 0.097 mmol), vinyl butyrate (70 μL, 0.062 g, 0.55 mmol), CALB (0.0419 g) and MeCN (3.0 mL) were mixed and molecular sieves were added. The mixture was placed in an incubator shaker (30 °C, 200 rpm.). Samples of 150 μL were taken out regularly over 30 hours for chiral HPLC analysis.

5.4.3 Large scale kinetic resolution of 3a

3a (0.1216 g, 0.48 mmol), vinyl butyrate (341 μL, 0.304 g, 2.66 mmol), CALB (0.2263 g) and MeCN (15.0 mL) were mixed and molecular sieves were added. The mixture was placed in an incubator shaker (30 °C, 200 rpm.) for 14 hours. Enzymes and molecular sieves were filtered off and solvents were removed *in vacuo*. The reaction mixture was separated flash chromatography (90:10, n-pentane:EtOAc). Fraction 1 (R_f=0.65) gave isolated **5a** (0.0516 g, 0.159 mmol). NMR: ^{1}H, δ (ppm): 0.96 (t, 3H), 1.54 (m, 2H), 1.67 (m, 2H), 1.68 (q, 2H) 1.79 (m, 2H), 2.03 (m, 2H), 2.35 (m, 2H), 2.94 (m, 1H), 3.79 (dd, 1H), 3.84 (dd, 1H), 4.14 (dd, 1H), 4.15 (dd, 1H), 5.33 (p, 1H), 6.85 (dt, 2H), 7.15 (dt, 2H). ^{13}C, δ (ppm): 13.6 (1C), 18.4 (1C), 25.4 (2C), 34.7 (2C), 36.1 (1C), 42.7 (1C), 45.2 (1C), 66.2 (1C), 70.1 (1C), 114.4 (2C), 128.0 (2C), 139.5 (1C), 156.3 (1C), 172,9 (1C).

Fraction 2 (R_f=0.27) gave isolated (*R*)-**3a** (0.0412 g, 0.162 mmol, *ee*=96%) in 34% yield.

5.4.4 Big scale kinetic resolution of 3b

3b (0.2141 g, 0.84 mmol), vinyl butyrate (600 μL, 0.534 g, 4.69 mmol), CALB (0.3874 g) and MeCN (25.0 mL) were mixed and molecular sieves were added. The mixture was placed in an incubator shaker (30 °C, 200 rpm.) for 16 hours. Enzymes and molecular sieves were filtered off and solvents were removed *in vacuo*. The reaction mixture was separated by flash chromatography (80:20, n-pentane:EtOAc). Fraction 1 (R_f=0.76) gave isolated **5b** (0.1148 g, 0.35 mmol). NMR: ^{1}H, δ (ppm): 0.97 (t, 3H), 1.60 (m, 2H), 1.68 (m, 2H), 1.69 (q, 2H) 1.79 (m, 2H), 2.00 (m, 2H), 2.35 (m, 2H), 3.28 (m, 1H), 3.81 (dd, 1H), 3.86 (dd, 1H), 4.17 (dd, 1H), 4.19 (dd, 1H), 5.39 (p, 1H), 6.84 (dd, 1H), 6.94 (td, 1H), 7.15 (td, 1H), 7.23 (dd, 1H). ^{13}C, δ (ppm): 13.6 (1C), 18.4 (1C), 25.5 (2C), 32.7 (2C), 36.1 (1C), 39.3 (1C), 42.7 (1C), 66.2 (1C), 71.0 (1C), 111.3 (1C), 121.3 (1C), 126.7 (1C) 127.0 (1C), 134.8 (1C), 155.9 (1C), 172,8 (1C).

Fraction 2 (R_f=0.27) gave 0.1438 g of a mixture of 65 mol% (*R*)-**3b** (*ee*=96%) and 35 mol% butyric acid.

5.5 Hydrolysis reactions

5.5.1 Hydrolysis of 5a

5a (0.010 g, 0.030 mmol) was mixed in NaOH (1 M, 1.5 mL), and refluxed at 95 °C for 8 hours. Product was extracted with EtOAc (2 mL), and solvents were removed by flushing with nitrogen. No conversion was observed (HPLC).

5.5.2 Hydrolysis of 5b

5b (0.009 g, 0.028 mmol) was mixed in NaOH (1 M, 1.5 mL), and refluxed at 95 °C for 8 hours. Product was extracted with EtOAc (2 mL), and solvents were removed by flushing with nitrogen. No conversion was observed (HPLC).

5.6 Step 3: Amination reactions

5.6.1 Synthesis of (*S*)-1-(*tert*-butylamino)-3-(4-cyclopentylphenoxy)propan-2-ol ((*S*)-4a)

(*R*)-3a (0.0283 g, 0.111 mmol, 96% *ee*), *t*−$BuNH_2$ (1.5 mL, 15.1 mmol) and H_2O (0.45 mL) were mixed and stirred at room temperature for 12 hours. Remaining *t*−$BuNH_2$ and water were removed *in vacuo* which gave crude product as white crystals (0.0437 g). The crude was triturated with n-pentane (4·0.5 mL) to form isolated (*S*)-1-(*tert*-butylamino)-3-(4-cyclopentylphenoxy)propan-2-ol ((*S*)-**4a**) (0.0278 g, 0.095 mmol, 96% *ee*), as a white powder in 86% yield. NMR: ^{1}H, δ (ppm): 1.46 (s, 9H), 1.52 (m, 2H), 1.67 (m, 2H), 1.78 (m, 2H), 2.03 (m, 2H), 2.92 (m, 1H), 3.04 (dd, 1H), 3.24 (dd, 1H), 3.97 (dd, 1H), 4.09 (dd, 1H), 4.55 (s, 1H), 6.83 (d, 2H), 7.12 (d, 2H). ^{13}C, δ (ppm): 25.4 (2C), 26.2 (3C), 34.7 (2C), 45.1 (1C), 45.6 (1C), 66.0 (1C), 69.8 (1C), 114.4 (2C), 128,0 (2C), 139.3 (1C), 156.4 (1C).

5.6.2 Synthesis of (*S*)-penbutolol ((*S*)-4a)

A (0.0559 g) mixture of (*R*)-3b (81 mol%) and butyric acid (19 mol%) were dissolved in *t*−$BuNH_2$ (3 mL, 30.2 mmol) and H_2O (0.90 mL), and stirred at room temperature for 13 hours. The solvents were removed *in vacuo*, and the crude product was recrystallized in *n*-pentane (2.5 mL) to give white crystals (0.0175 g), which were characterized as hydroxylamine (NH_2OH). NMR: ^{1}H, δ (ppm): 1.55 (s, 2H), 8.31 (s, 1H).

The filtrate was recovered to give (*S*)-penbutolol ((*S*)-**4b**) (0.0520 g, 96% *ee*) in 68 % purity, (17% (*R*)-**3b** and 15% butyric acid). NMR **4b**: ^{1}H, δ (ppm): 1.19 (s, 9H), 1.60 (m, 2H), 1.67 (m, 2H), 1.79 (m, 2H), 2.01 (m, 2H), 2.81 (dd, 1H), 2.96 (dd, 1H), 3.30 (m, 1H) 3.97 (dd, 1H), 4.05 (dd, 1H), 4.10 (s, 1H), 6.85 (dd, 1H), 6.92 (td, 1H), 7.14 (td, 1H), 7.22 (dd, 1H).

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A List of experiments

A.1 Step 1

Experiment A:

1b: 0.2335 g, epichlorohydrin: 0.2789 g (2 eq.), NaOH (1 M): 1.475 mL (1 eq.), THF: 2 mL. Stirred 20 hours.

Experiment B:

1b: 0.2259 g, epichlorohydrin: 0.2712 g (2 eq.), NaOH (2 M): 1.395 mL (2 eq.), THF: 2 mL. Stirred 20 hours.

Experiment C:

1b: 0.2117 g, epichlorohydrin: 0.2454 g (2 eq.), NaOH (2 M): 1.305 mL (2 eq.). Stirred 20 hours.

Experiment D:

1b: 0.2420 g, epichlorohydrin: 0.2807 g (2 eq.), NaOH (1 M): 2.240 mL (1.5 eq.). Stirred 20 hours.

Experiment E:

1b: 0.2161 g, epichlorohydrin: 0.2539 g (2 eq.), NaOH (1 M): 2.000 mL (1.5 eq.). Stirred 44 hours.

Experiment F:

1b: 0.1971 g, epichlorohydrin: 0.2290 g (2 eq.), NaOH (2 M): 0.915 mL (1.5 eq.). Stirred 20 hours. Then more NaOH (2 M) were added: 0.305 mL (0.5 eq.). Then stirred another 8 hours.

Experiment G:

1b: 0.2057 g, epichlorohydrin: 0.2540 g (2 eq.), NaOH (0.5 M): 1.300 mL (0.5 eq.). Stirred 96 hours.

A.2 Step 2

Experiment ABC:

Reaction mixtures from experiment A, B and C were combined to a mix of 0.6278 g, containing **1b** (0.7 mmol), **2b** (1.68 mmol) and **3b** (0.59 mmol). This mixture was separated by flash chromatography (90:10) n-pentane/EtOAc. Fraction 1 contained 0.4235 g of a mix with **1b** (30 mol%) and **2b** (70 mol%). Fraction 2 contained **3b** (0.0998 g).

To fraction 1: AcOH (0.2501 g, 3 eq.), LiCL (0.1750 g, 3 eq.) and THF (3 mL) were added. Stirred 20 hours. Purification by flash chromatography (90:10) n-pentane/EtOAc gave **3b** (0.2692 g).

Experiment DEF:

Reaction mixtures from experiment D, E and F were combined to a mix of 0.7219 g, containing **1b** (0.34 mmol), **2b** (1.25 mmol) and **3b** (1.57 mmol). AcOH (0.2673 g, 3.5 eq.) and LiCl (0.1679 g, 3 eq.) were added. Stirred 20 hours. Purification by flash chromatography gave **3b** (0.3891 g).

A.3 Derivatization

Experiment Deriv$_a$:

3a: 1 drop, pyridine: 1 drop, butyric anhydride: 5 drops. 60 °C, 1 hour.

Experiment: Deriv$_b$:

3b: 1 drop, pyridine: 1 drop, butyric anhydride: 5 drops. 60 °C, 1 hour.

A.4 Enzyme reactions

A.4.1 Small scale

Experiment Enz$_{a1}$

3a: 0.0228 g, CALB: 0.0384 g, Vinyl butyrate: 64 μL, MeCN: 3 mL. Incubator shaker at 30 °C. Samples taken at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 22, 24, 26 and 48 hours.

Experiment Enz$_{a2}$

3a: 0.0226 g, CALB: 0.0445 g, Vinyl butyrate: 64 μL, MeCN: 3 mL. Incubator shaker at 30 °C. Samples taken at 0.25, 0.5, 1, 2, 4, 6, 8, 11, 14, 22, 24, 28, 32 and 48 hours.

Experiment Enz$_b$

3b: 0.0250 g, CALB: 0.0419 g, Vinyl butyrate: 70 μL, MeCN: 3 mL. Incubator shaker at 30 °C. Samples taken at 0.25, 0.5, 1, 2, 4, 6, 8, 11, 14, 24, 24, and 29.5 hours.

A.4.2 Big scale

Experiment EnzBig$_a$

3a: 0.1216 g, CALB: 0.2263 g, vinyl butyrate: 341 μL, MeCN: 15 mL. Incubator shaker at 30 °C, 14 hours. Purified by flash chromatography (80:20) n-pentane/EtOAc.

Experiment EnzBig$_b$

3b: 0.2141 g, CALB: 0.3874 g, vinyl butyrate: 600 μL, MeCN: 25 mL. Incubator shaker at 30 °C, 16 hours. Purified by flash chromatography (80:20) n-pentane/EtOAc.

A.5 Hydrolysis

Experiment H$_1$

5a: 1 drop, NaOH (1 M): 5 drops. 60 °C, 1 hour.

Experiment H$_2$

5a: 1 drop, NaOH (0.2 M): 5 drops. Incubator shaker at 30 °C, 20 hours.

Experiment H$_3$

5a: 0.010 g, NaOH (1 M): 1.5 mL. Refluxed at 95 °C, 8 hours.

Experiment H$_4$

5b: 0.009 g, NaOH (1 M): 1.5 mL. Refluxed at 95 °C, 8 hours.

Experiment H$_5$

5a: 1 drop, HCl (0.2 M): 2 mL. Refluxed at 95 °C, 4 hours.

A.6 Last step

Experiment Amin$_a$

(*R*)-3a: 0.0283 g, *t*−BuNH$_2$: 1.5 mL, H$_2$O: 450 μL. Stirred 12 hours.

Experiment Amin$_b$

(*R*)-3b: 0.0559 g, *t*−BuNH$_2$: 3 mL, H$_2$O: 900 μL. Stirred 13 hours.

B NMR spectra

B.1 Full characterization of 3b

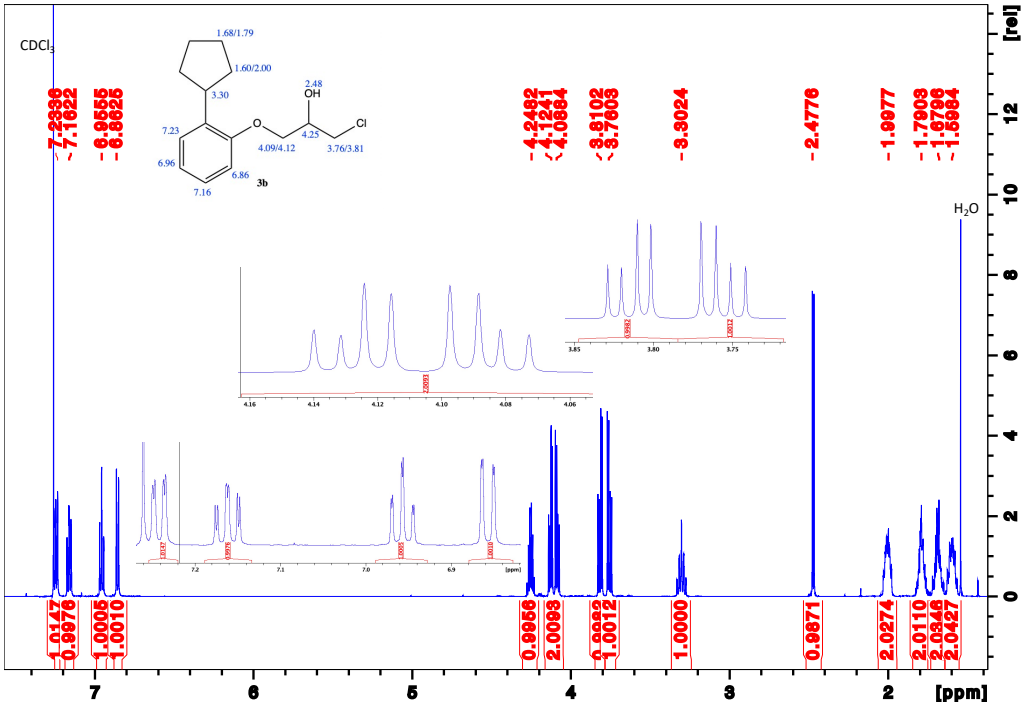

Figure B.1: ^{1}H NMR spectrum of the isolated alcohol **3b**.

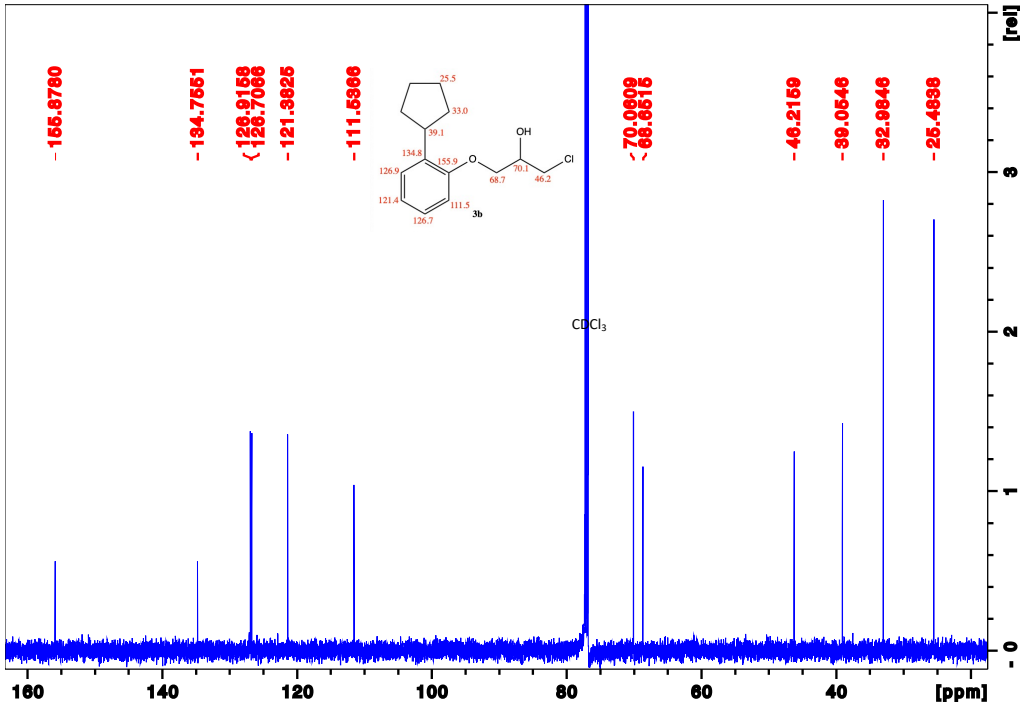

Figure B.2: ^{13}C NMR spectrum of the isolated alcohol **3b**.

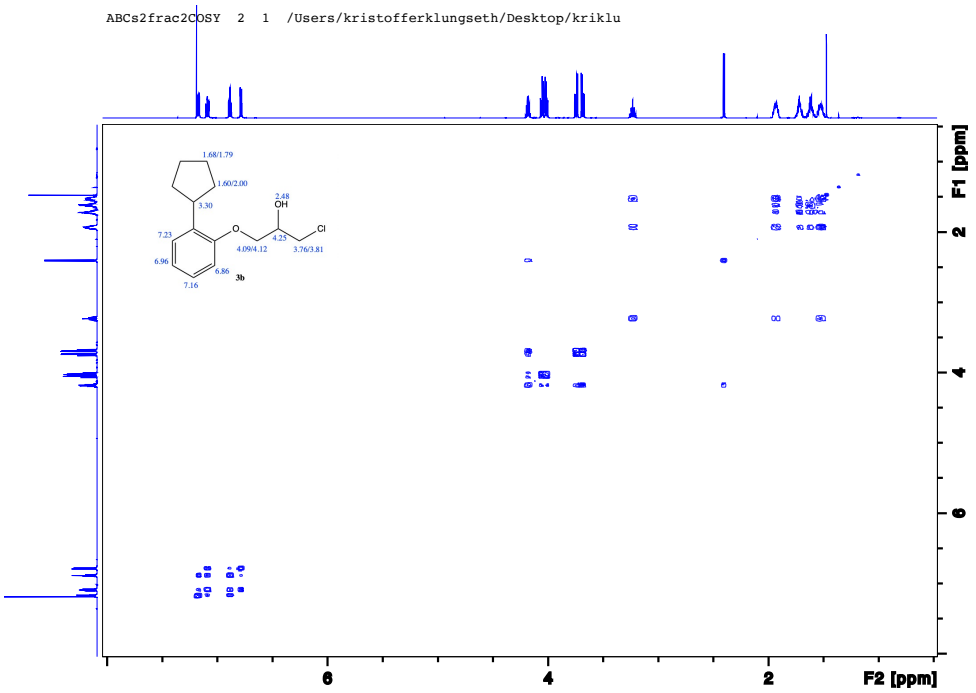

Figure B.3: COSY spectrum of the isolated alcohol **3b**.

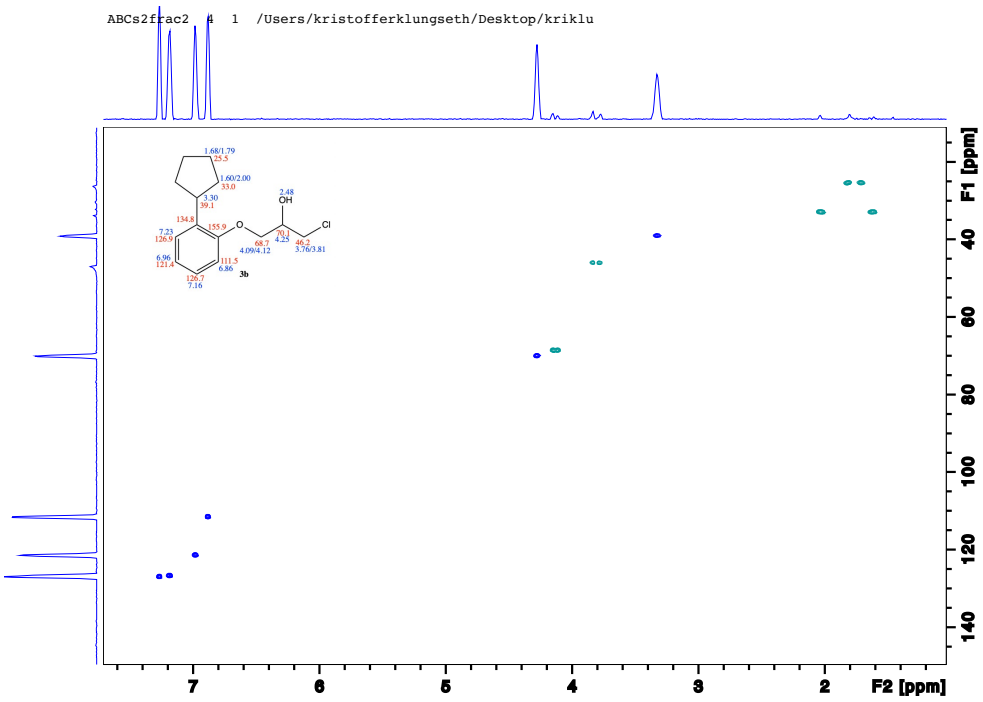

Figure B.4: HSQC spectrum of the isolated alcohol **3b**.

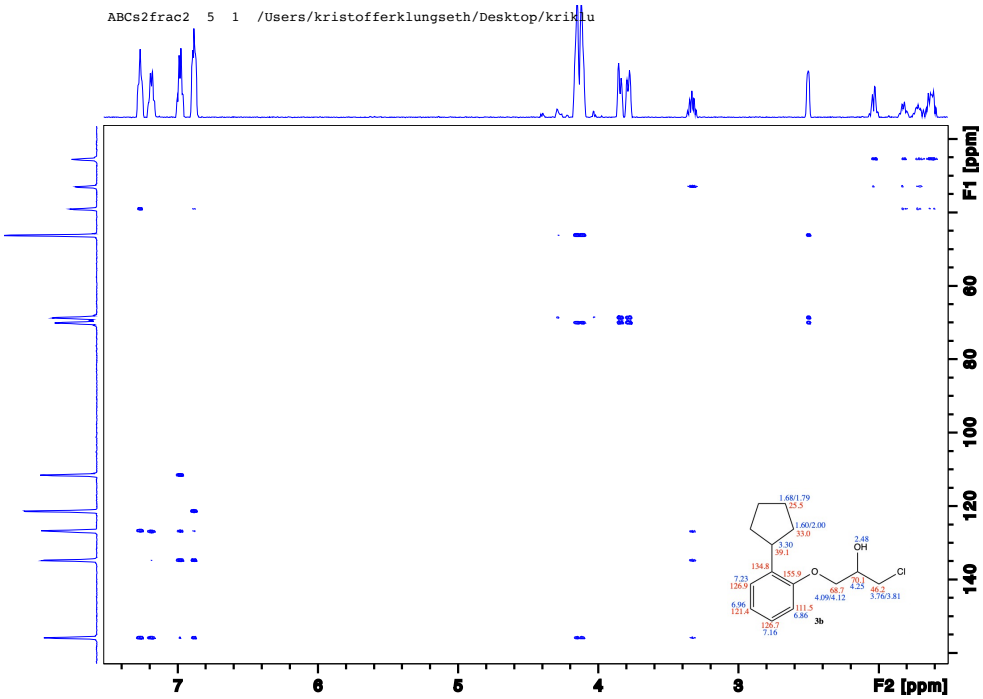

Figure B.5: HMBC spectrum of the isolated alcohol **3b**.

B.2 Full characterization of 5b

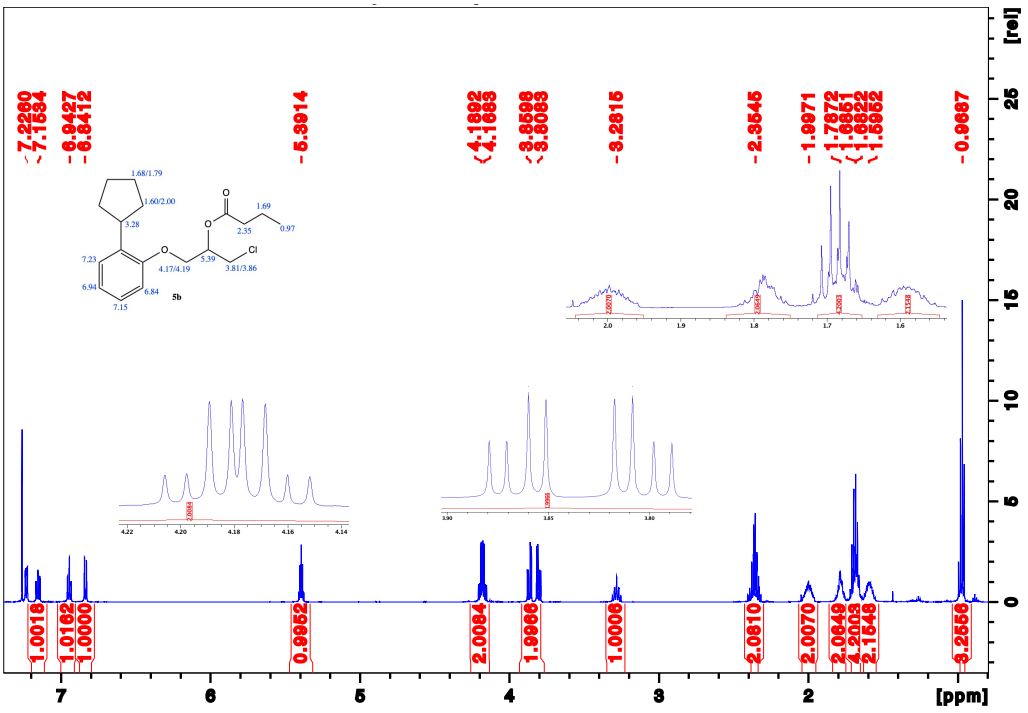

Figure B.6: ^{1}H NMR spectrum of the ester **5b**.

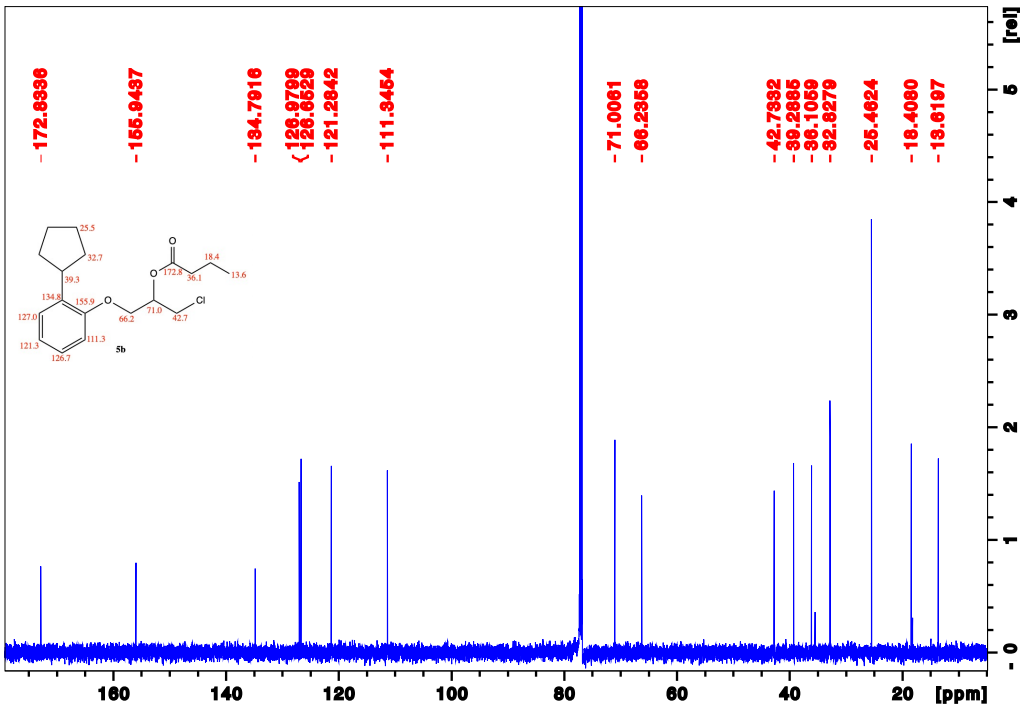

Figure B.7: ^{13}C NMR spectrum of the ester **5b**.

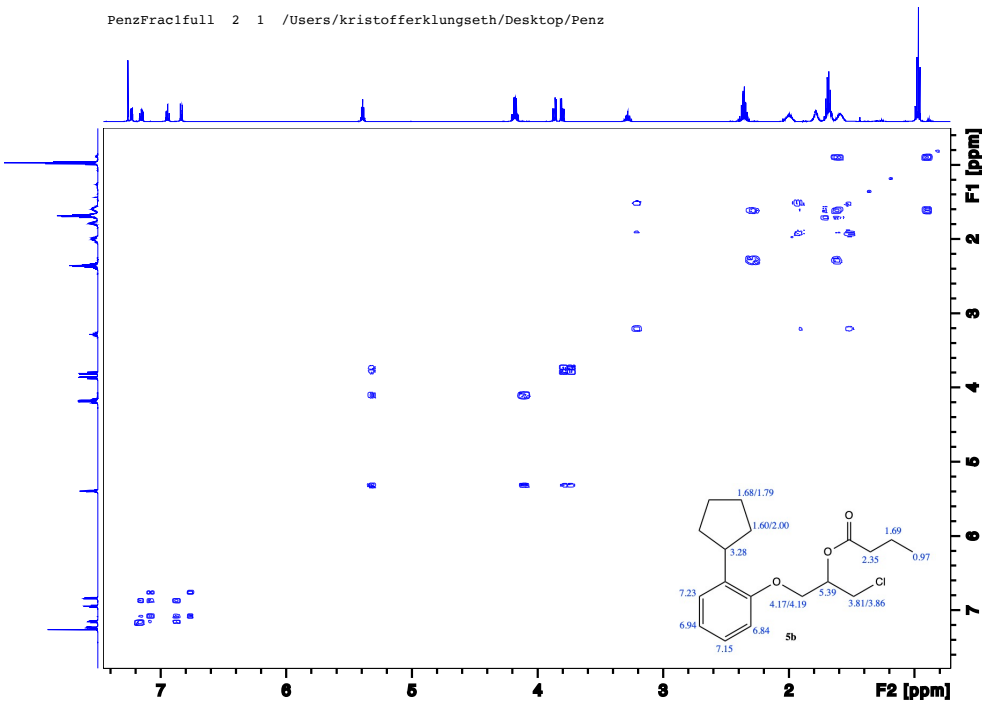

Figure B.8: COSY spectrum of the ester **5b**.

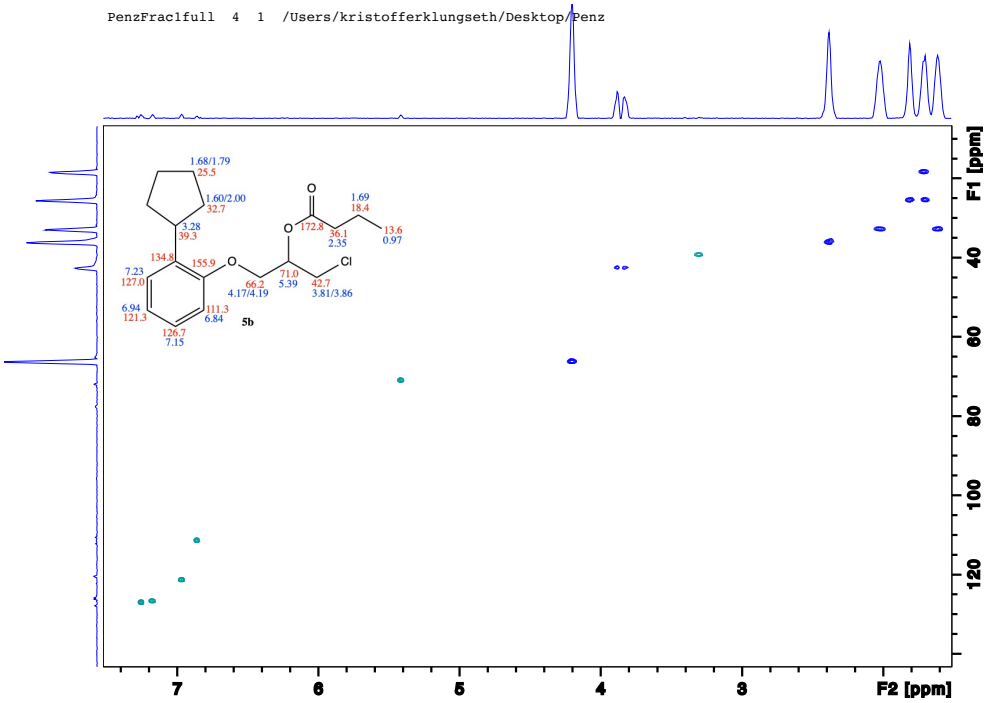

Figure B.9: HSQC spectrum of the ester **5b**.

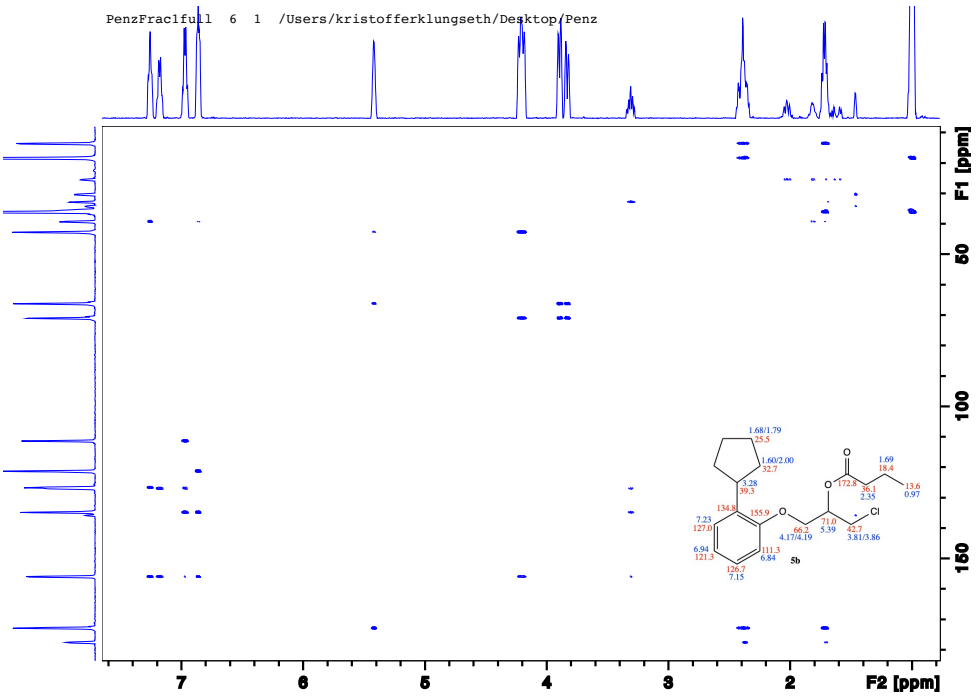

Figure B.10: HMBC spectrum of the ester **5b**.

B.3 Full characterization of 5a

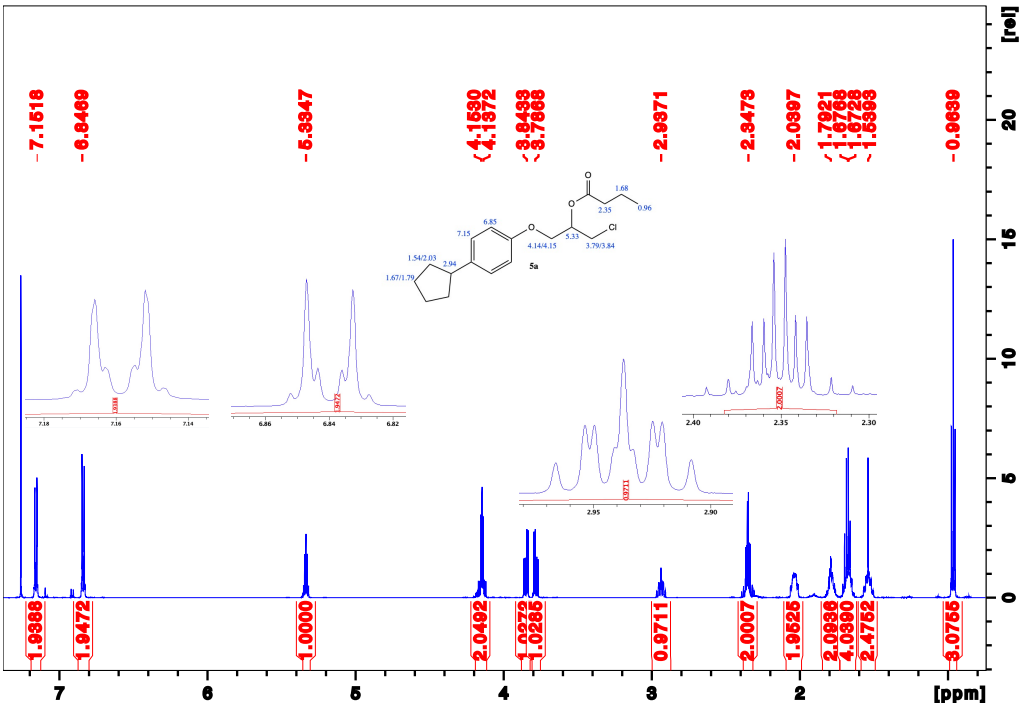

Figure B.11: ^{1}H NMR spectrum of the ester **5a**.

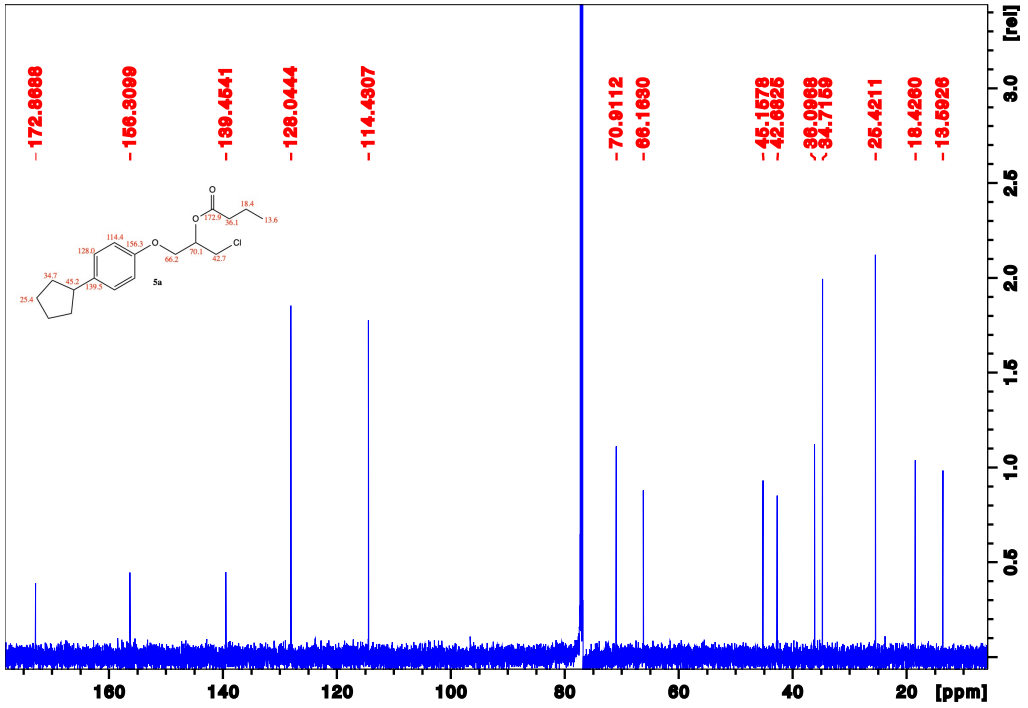

Figure B.12: ^{13}C NMR spectrum of the ester **5a**.

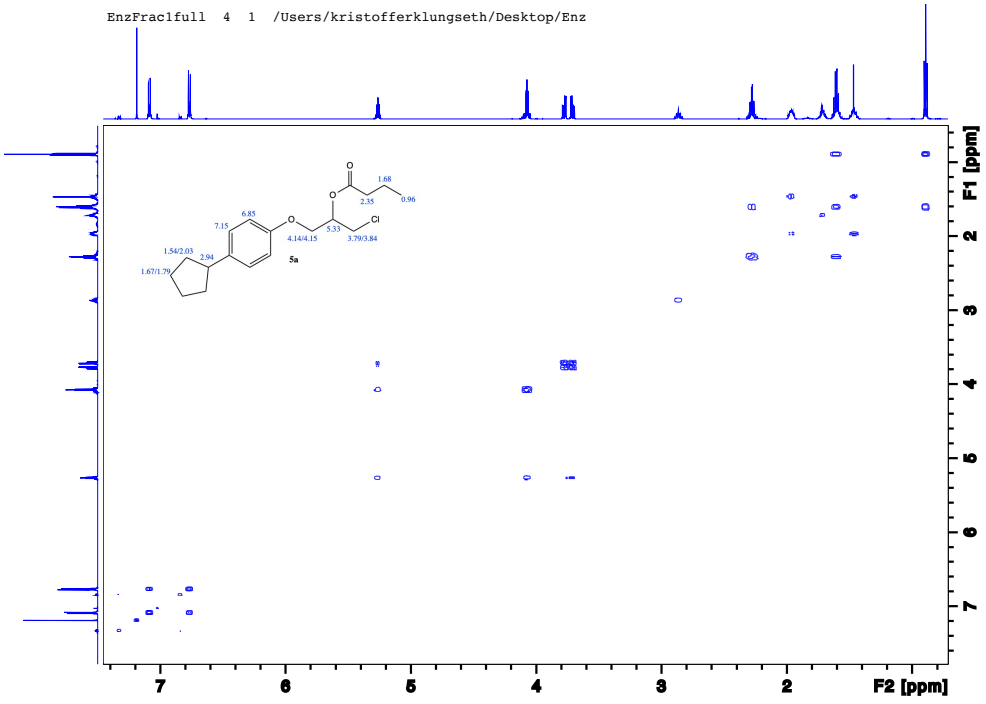

Figure B.13: COSY spectrum of the ester **5a**.

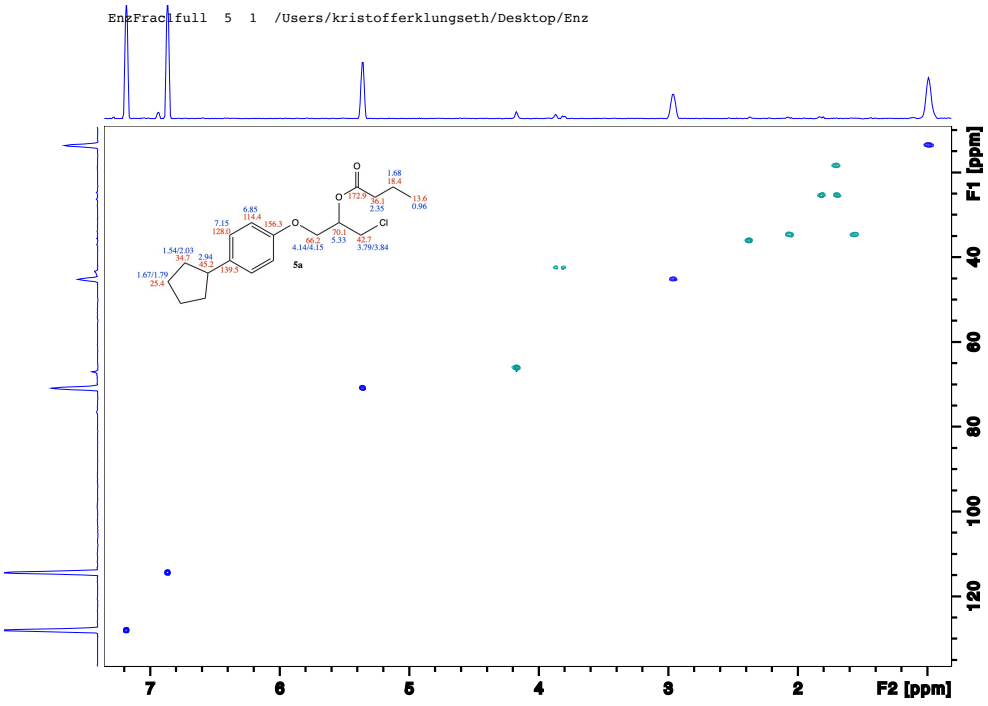

Figure B.14: HSQC spectrum of the ester **5a**.

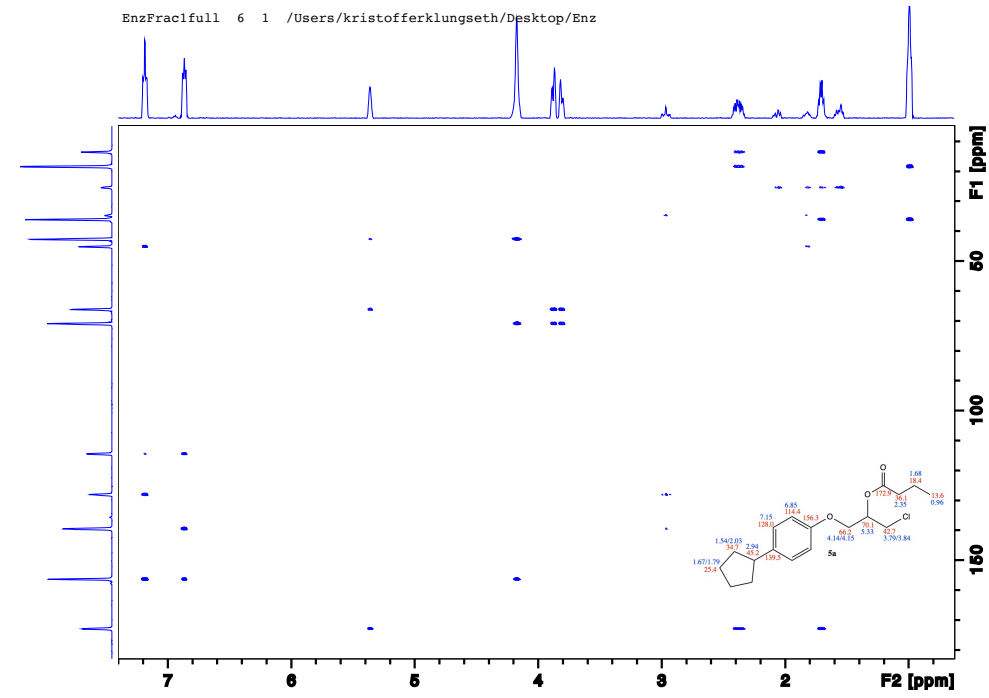

Figure B.15: HMBC spectrum of the ester **5a**.

B.4 Full characterization of 4a

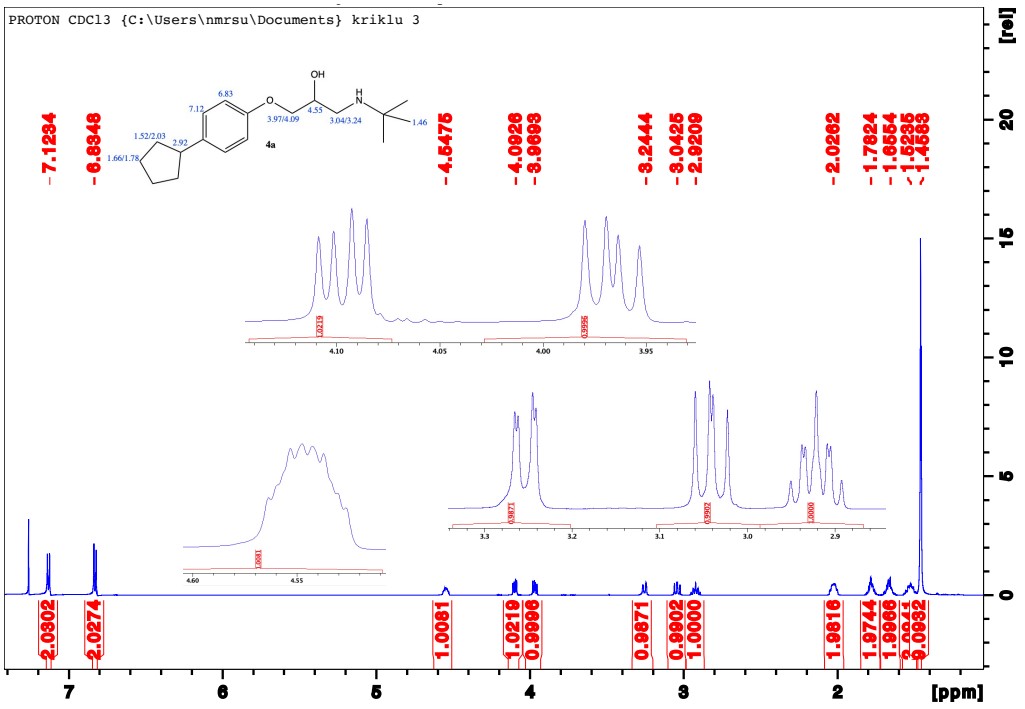

Figure B.16: ^{1}H NMR spectrum of the final product **4a**.

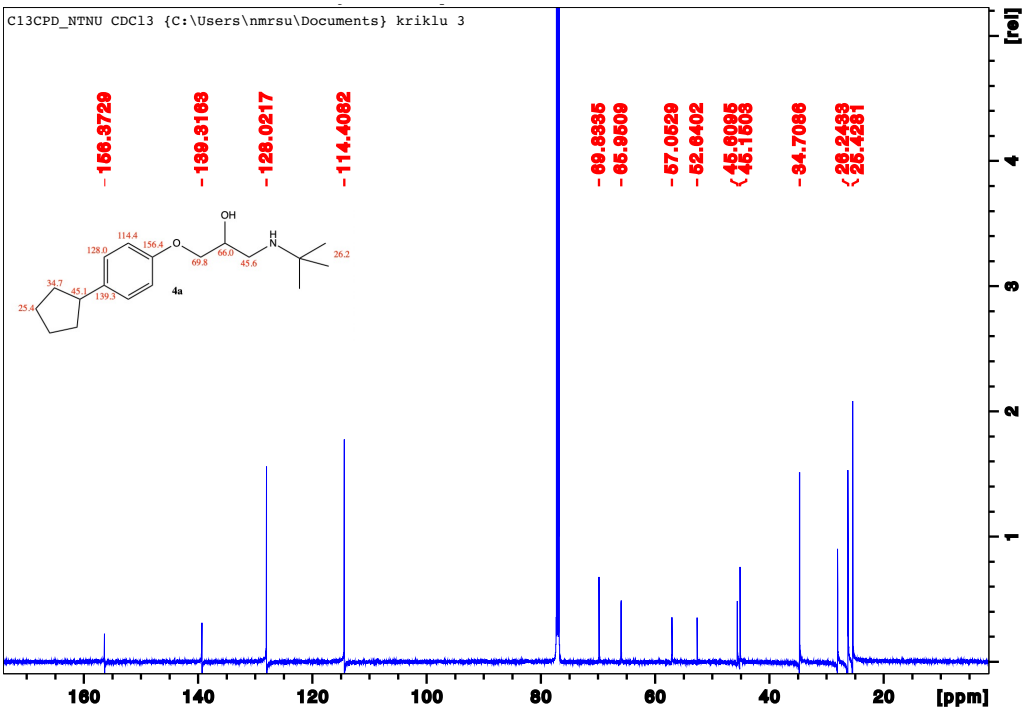

Figure B.17: ^{13}C NMR spectrum of the final product **4a**.

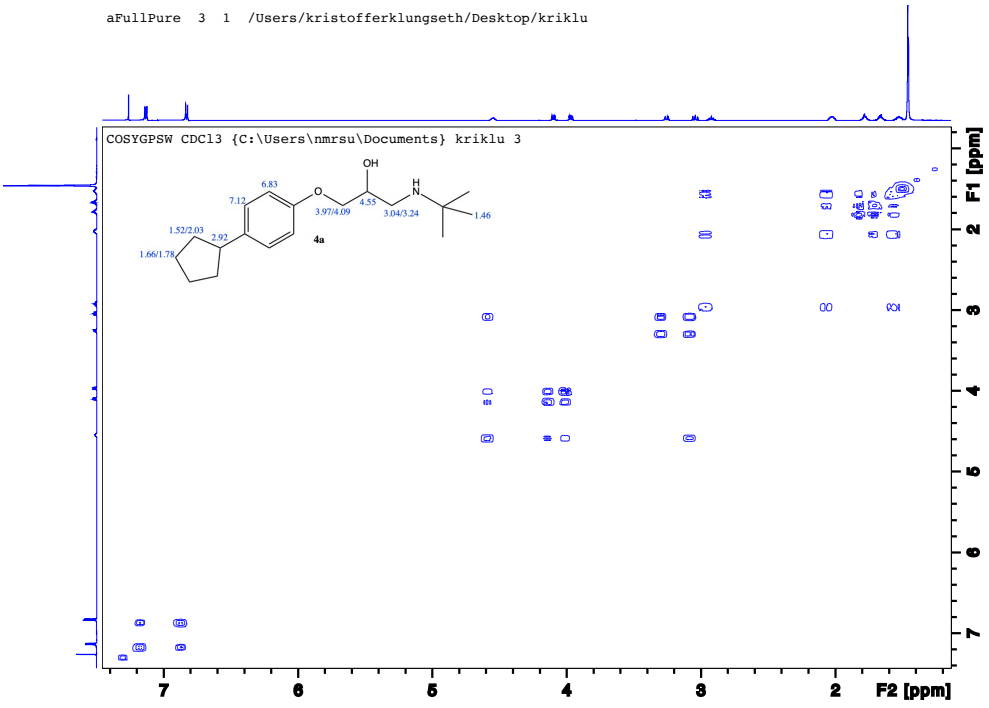

Figure B.18: COSY spectrum of the final product **4a**.

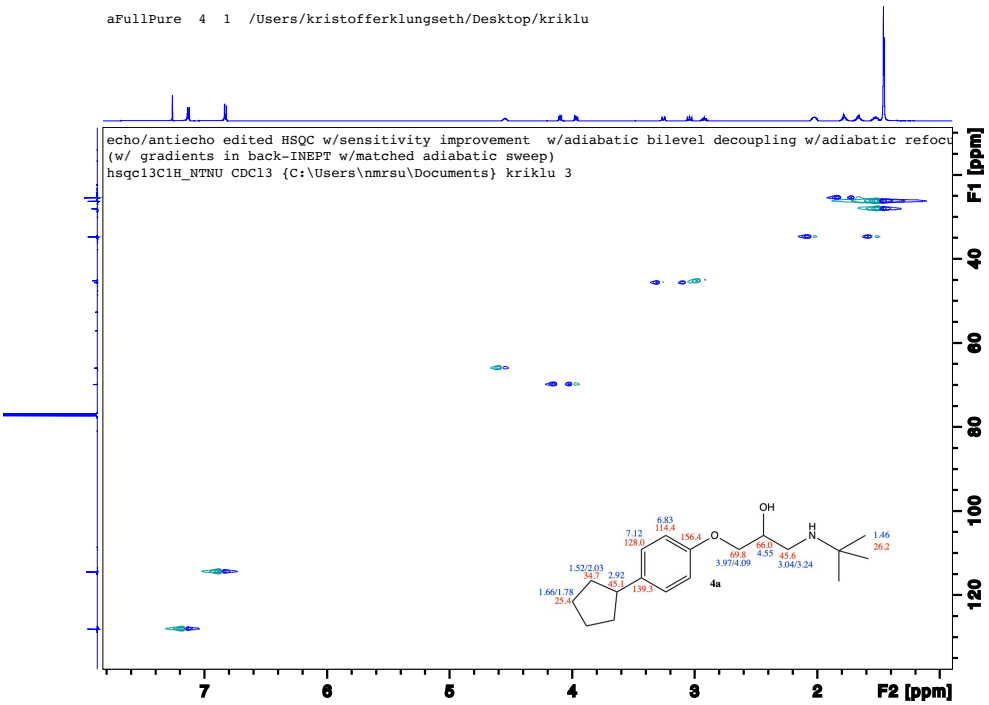

Figure B.19: HSQC spectrum of the final product **4a**.

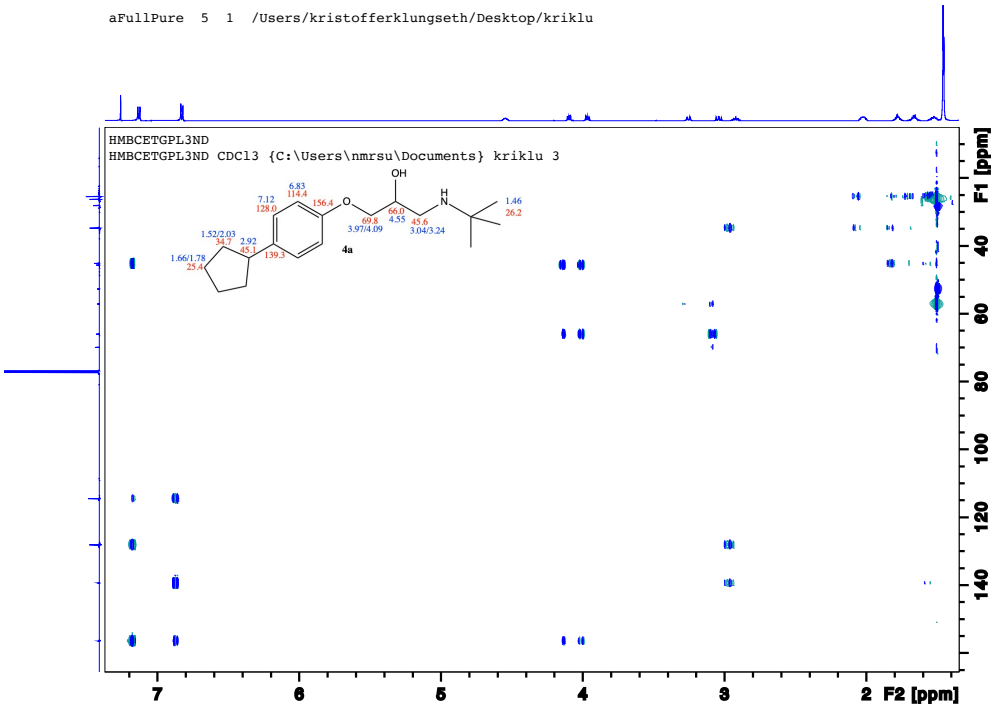

Figure B.20: HMBC spectrum of the final product **4a**.

B.5 ^{1}H NMR spectra

Some of the ^{1}H NMR spectra obtained throughout the syntheses are shown in this section.

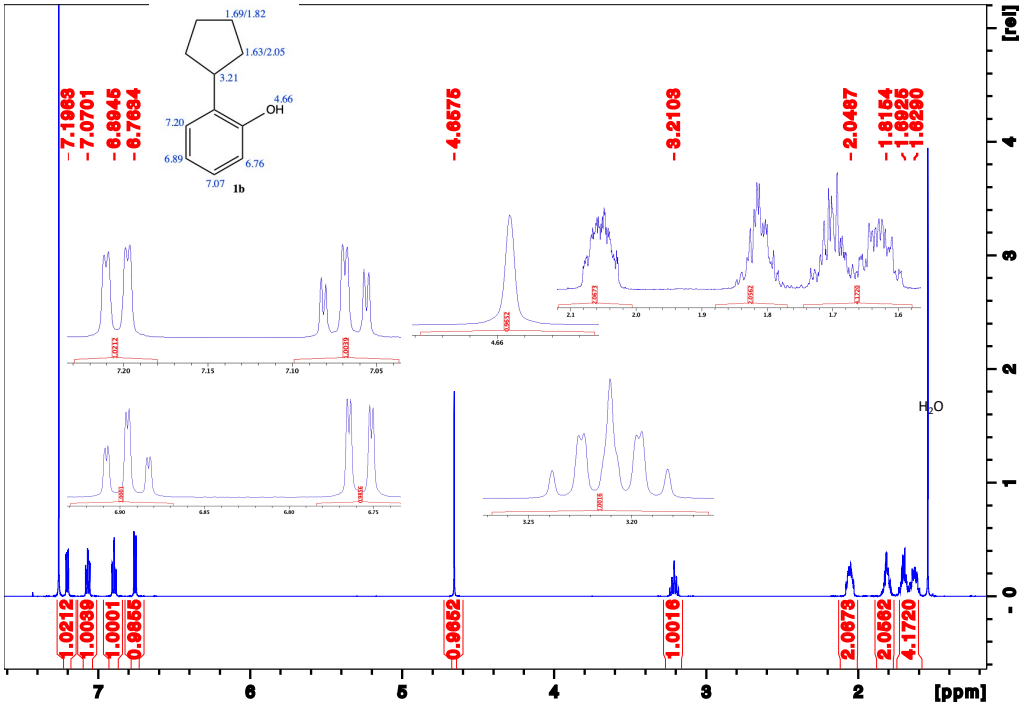

Figure B.21: ^{1}H NMR spectrum of the starting compound **1b**.

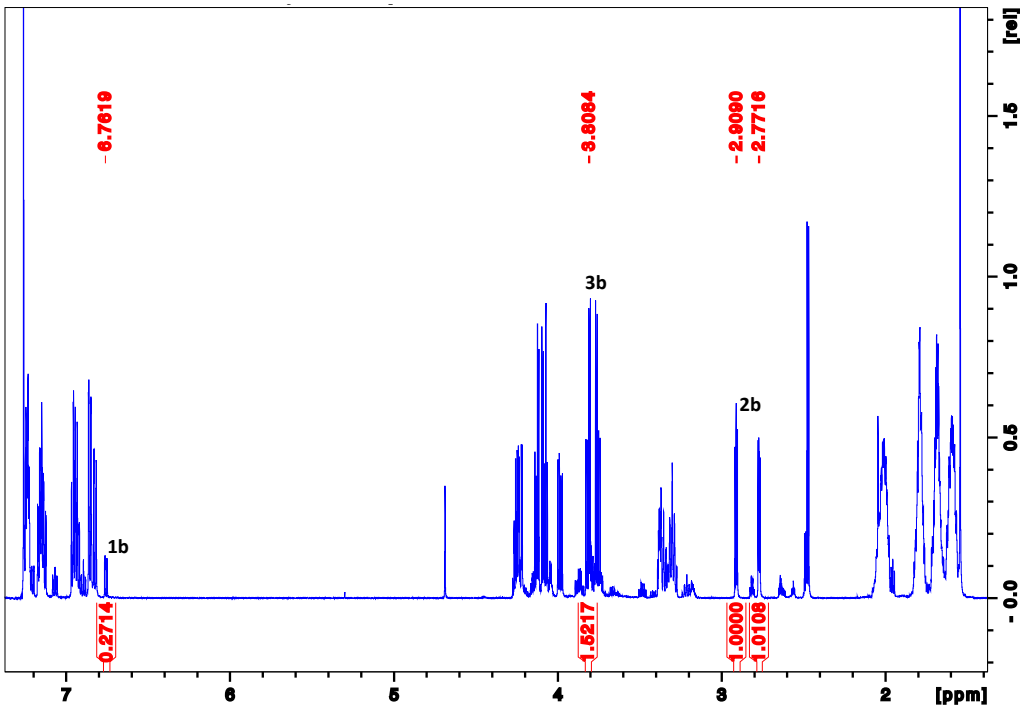

Figure B.22: ^{1}H NMR spectrum of the reaction mixture of the most successful step 1 reaction (Experiment E). The mixture contains 54 mol% **3b**, 36 mol% **2b** and 10 mol% **1b**.

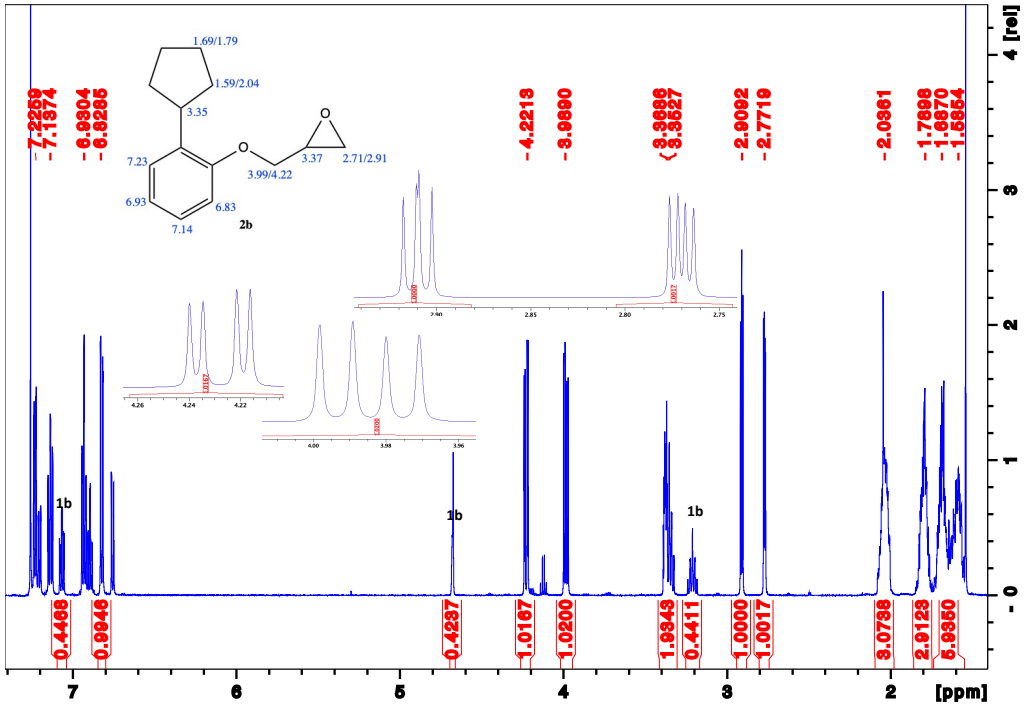

Figure B.23: ^{1}H NMR spectrum of the first fraction in flash chromatography of the reaction mixture used in Experiment ABC. The mixture contains 70 mol% **2b** and 30 mol% **1b**.

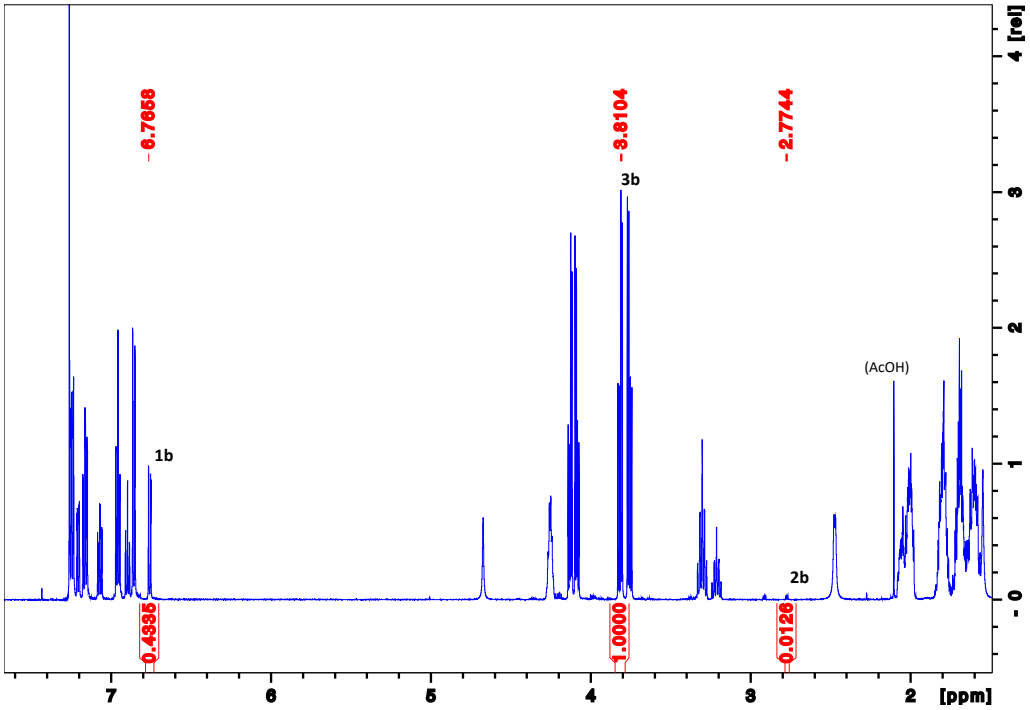

Figure B.24: ^{1}H NMR spectrum of the reaction mixture after step 2 in Experiment ABC.

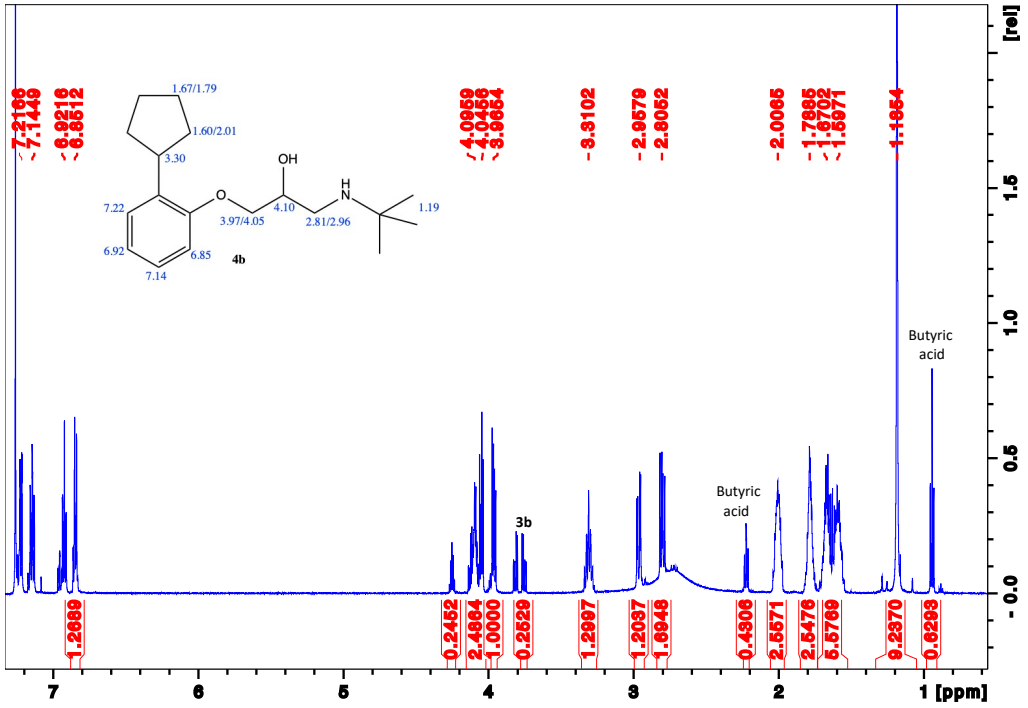

Figure B.25: ^{1}H NMR spectrum of the final product (*S*)-penbutolol ((*S*)-**4b**). The product is in 68% purity, with 17 mol% **3b** and 15mol% butyric acid.

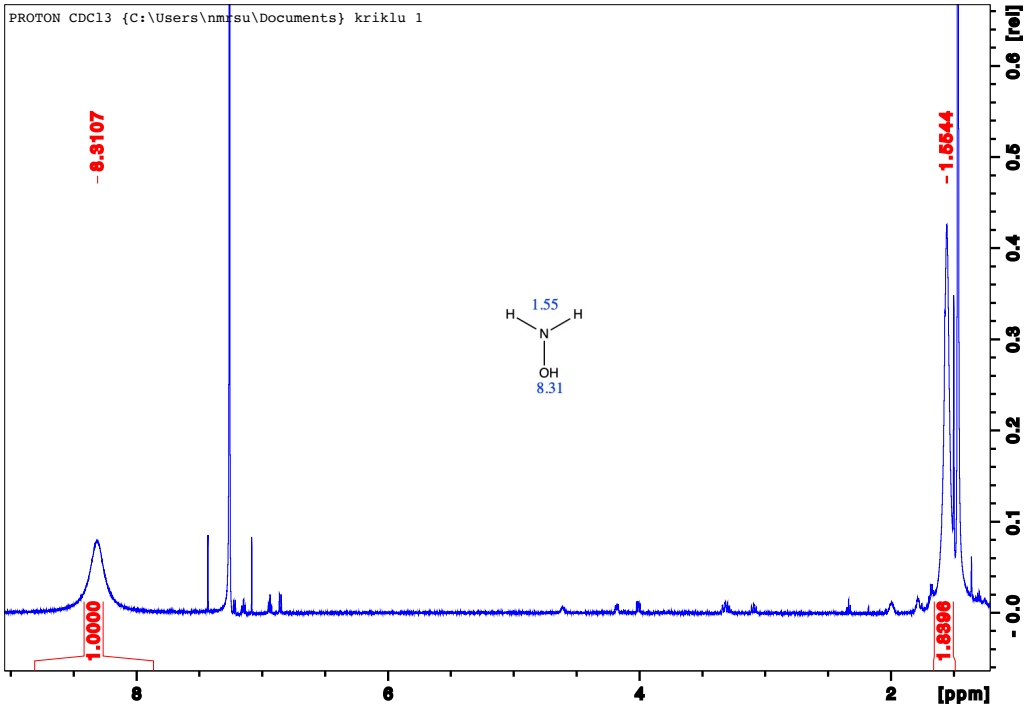

Figure B.26: ^{1}H NMR spectrum of the isolated by-product hydroxylamine.

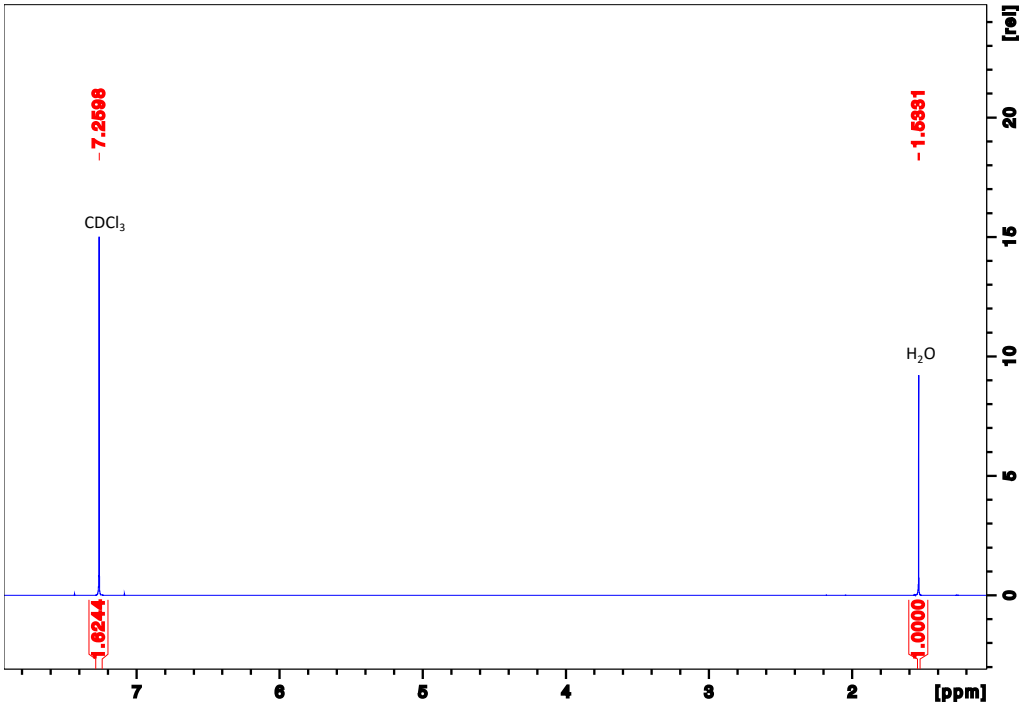

Figure B.27: ^{1}H NMR spectrum of a blank sample of the $CDCl_3$ used for all NMR alasyses reveals that it contains water.

C Chromatograms

C.1 Kinetic resolution of 3a

Figure C.1 shows the HPLC-chromatogram of the enantioenriched alcohol (*R*)-**3a**, after the large scale kinetic resolution. Figures C.2 to C.27 shows chromatograms of all samples taken during small scale kinetic resolutions of **3a**. All these analyses were performed with 90:10 *n*-hexane:isopropanol.

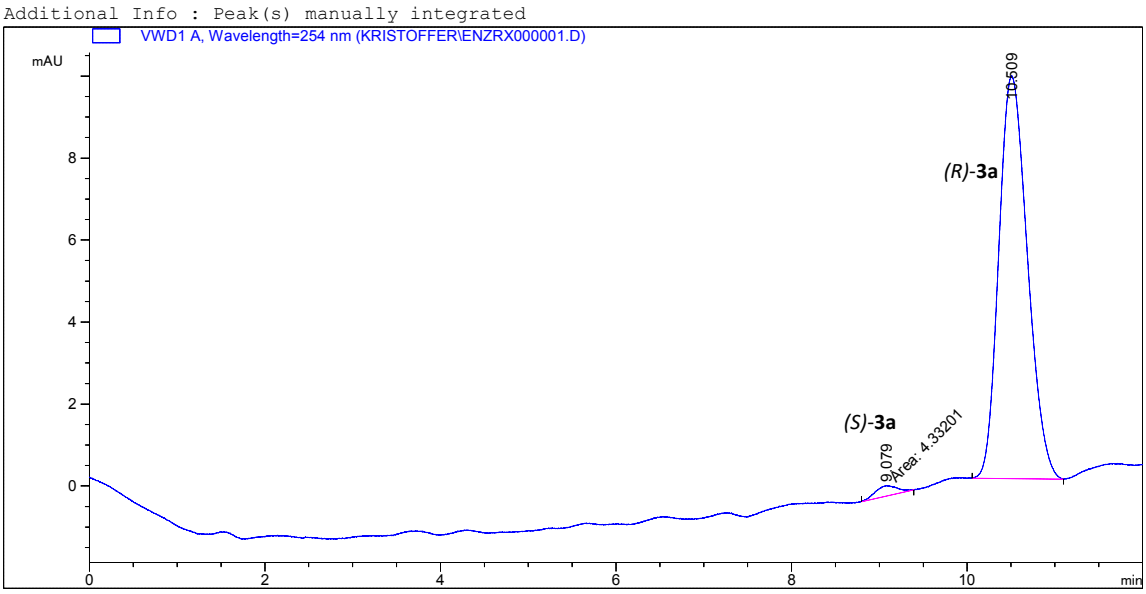

Figure C.1: HPLC-chromatogram of the isolated alcohol (*R*)-**3a** after large scale kinetic resolution catalyzed by CALB. The alcohol is in 96% *ee*.

C.1.1 First parallel

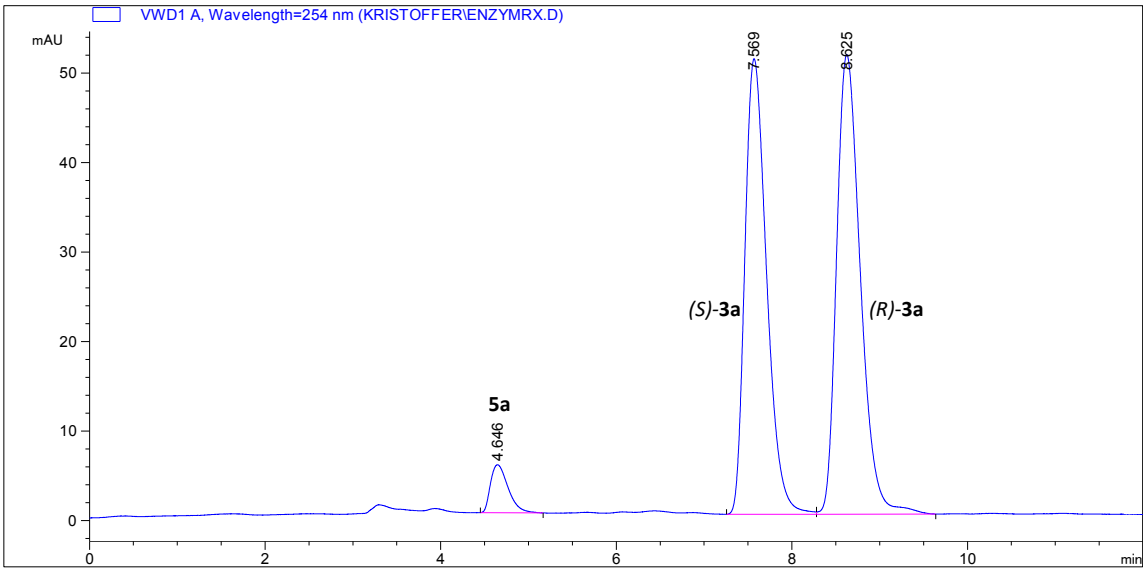

Figure C.2: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 15 minutes reaction time. $ee_s = 5\%$.

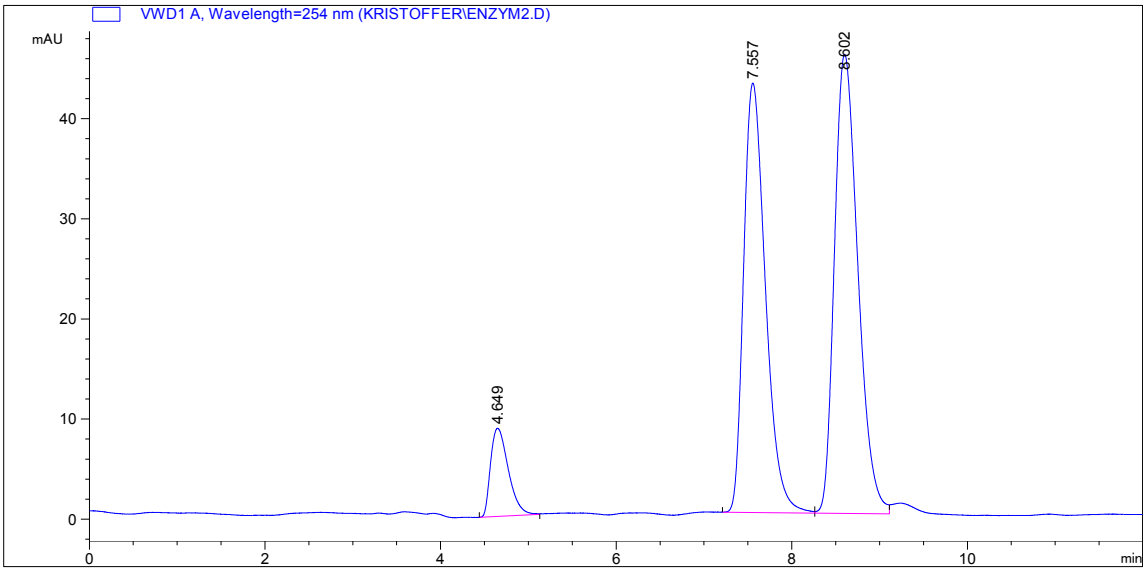

Figure C.3: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 30 minutes reaction time. $ee_s = 7\%$.

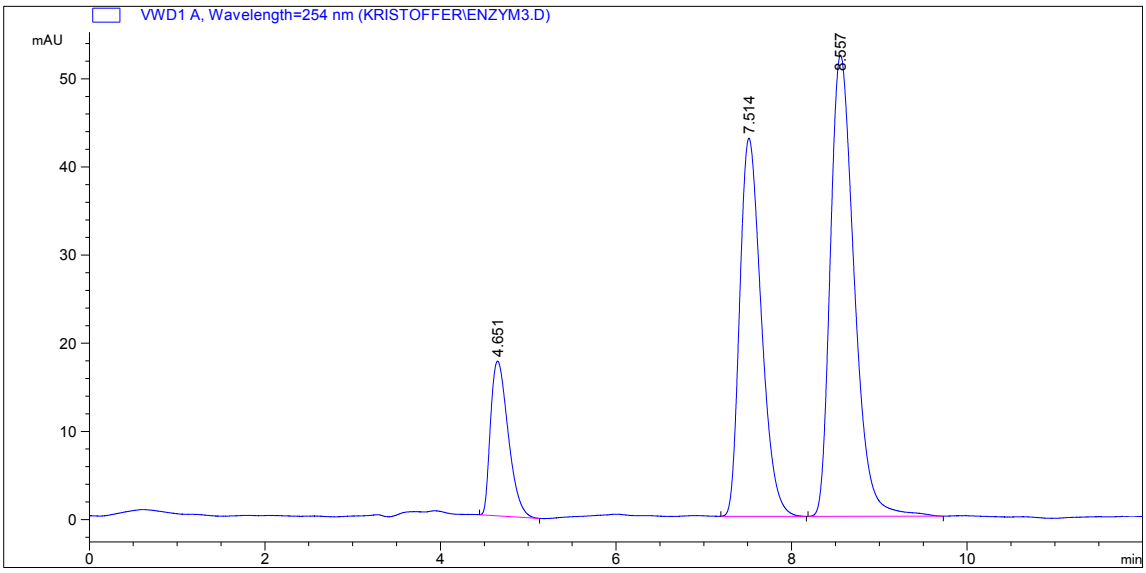

Figure C.4: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 1 hour reaction time. $ee_s = 15\%$.

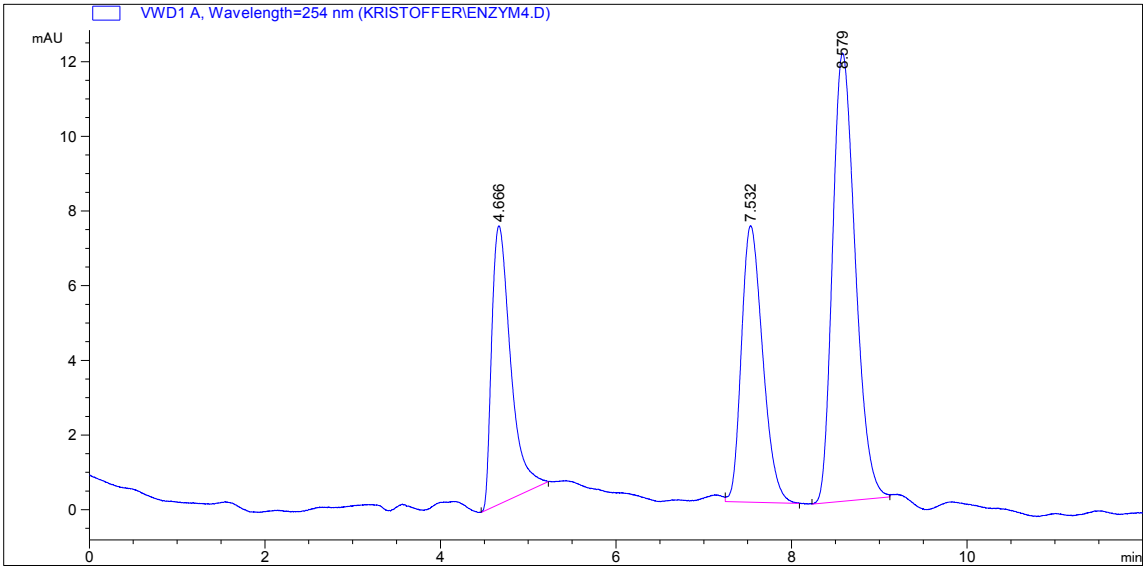

Figure C.5: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 2 hours reaction time. $ee_s = 27\%$.

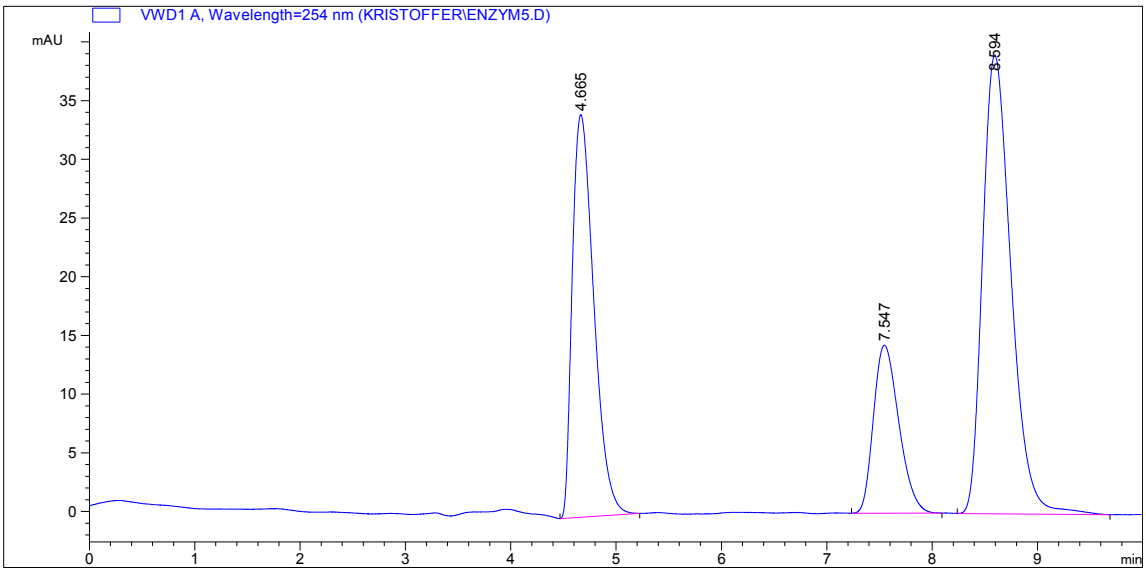

Figure C.6: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 4 hours reaction time. $ee_s = 51\%$.

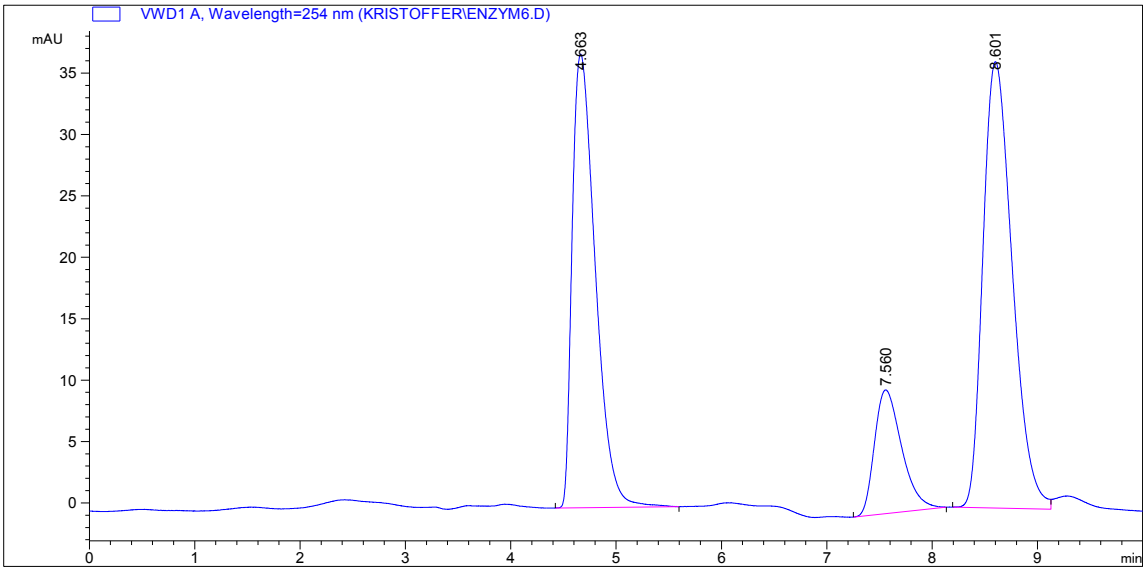

Figure C.7: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 6 hours reaction time. $ee_s = 59\%$.

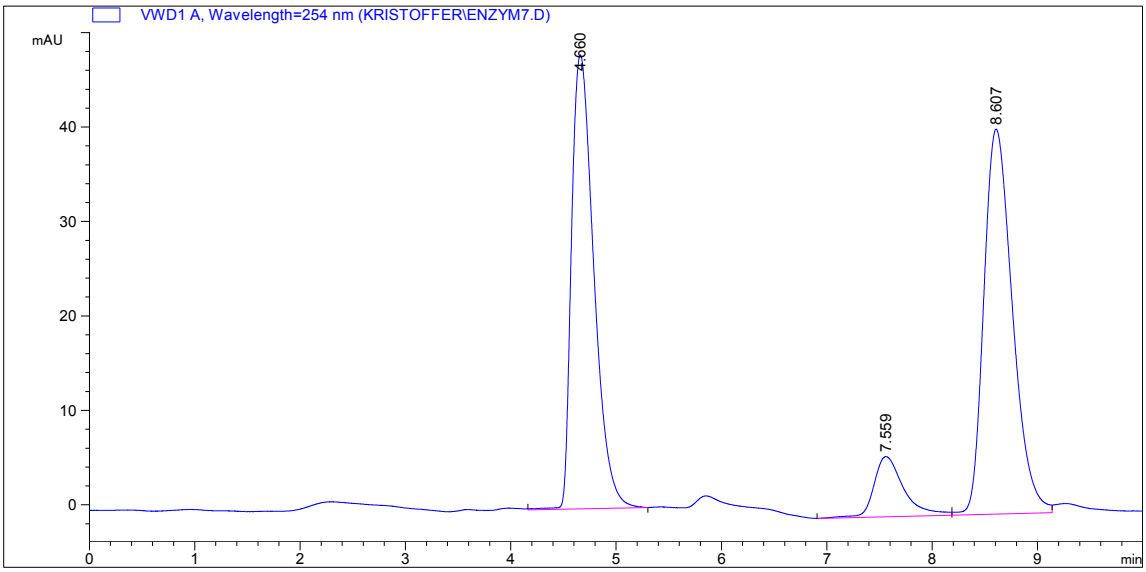

Figure C.8: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 8 hours reaction time. $ee_s = 72\%$.

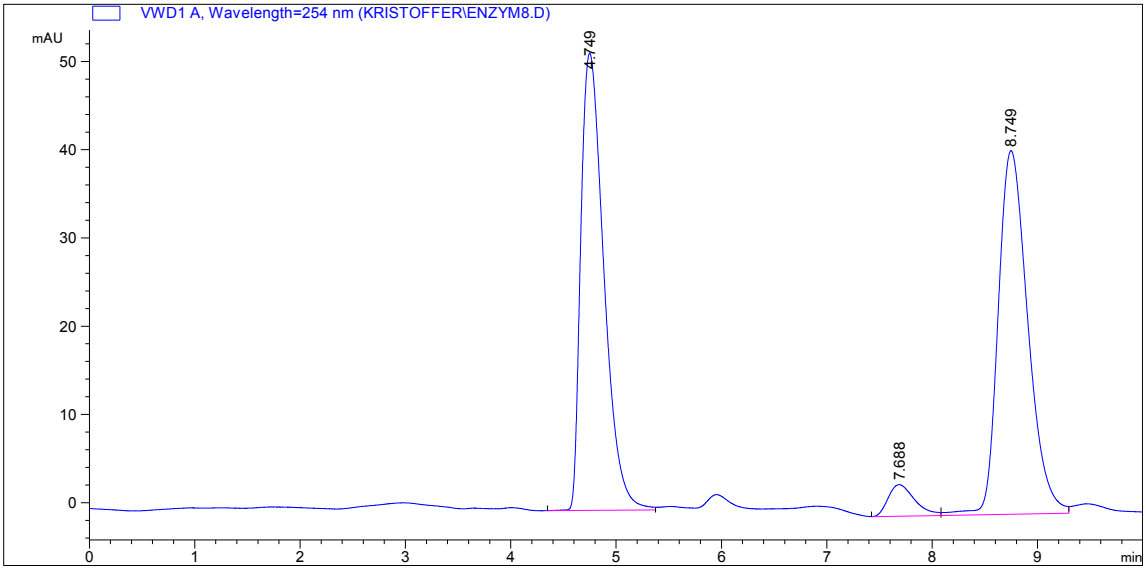

Figure C.9: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 10 hours reaction time. $ee_s = 83\%$.

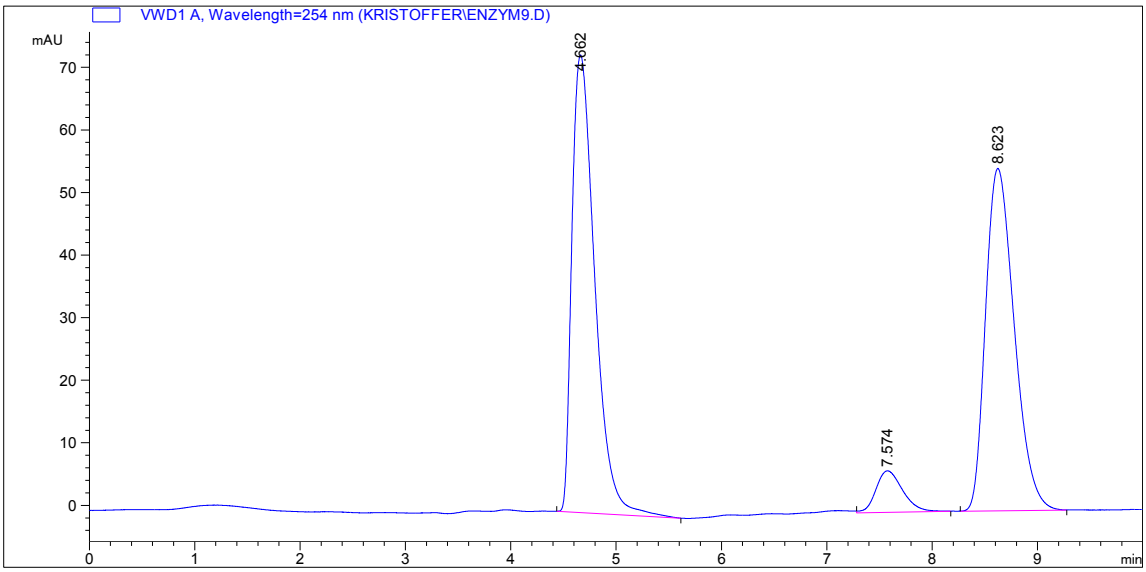

Figure C.10: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 22 hours reaction time. $ee_s = 80\%$.

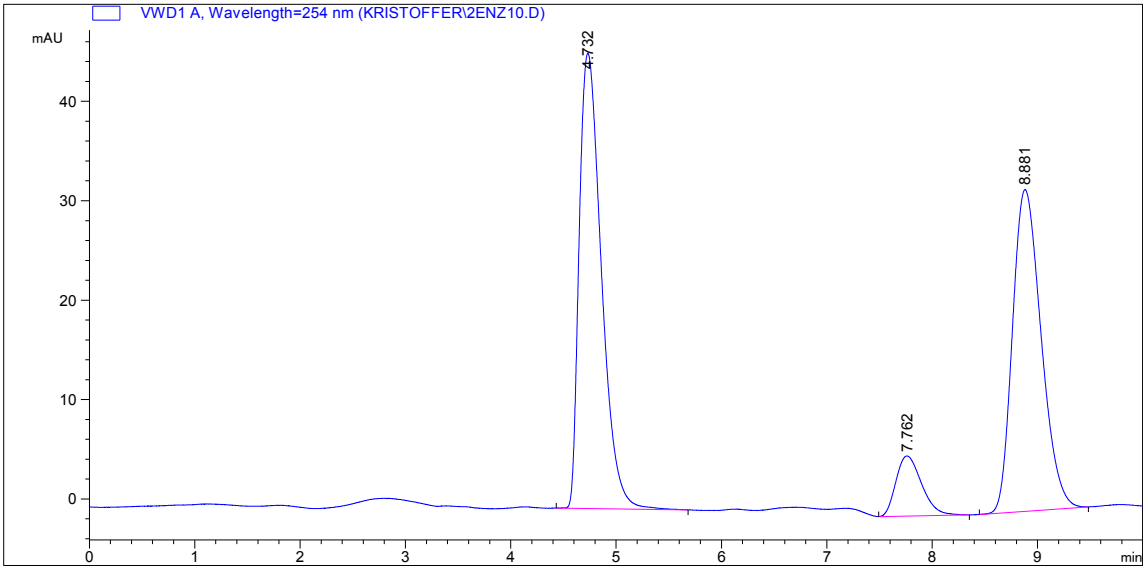

Figure C.11: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 24 hours reaction time. $ee_s = 53\%$.

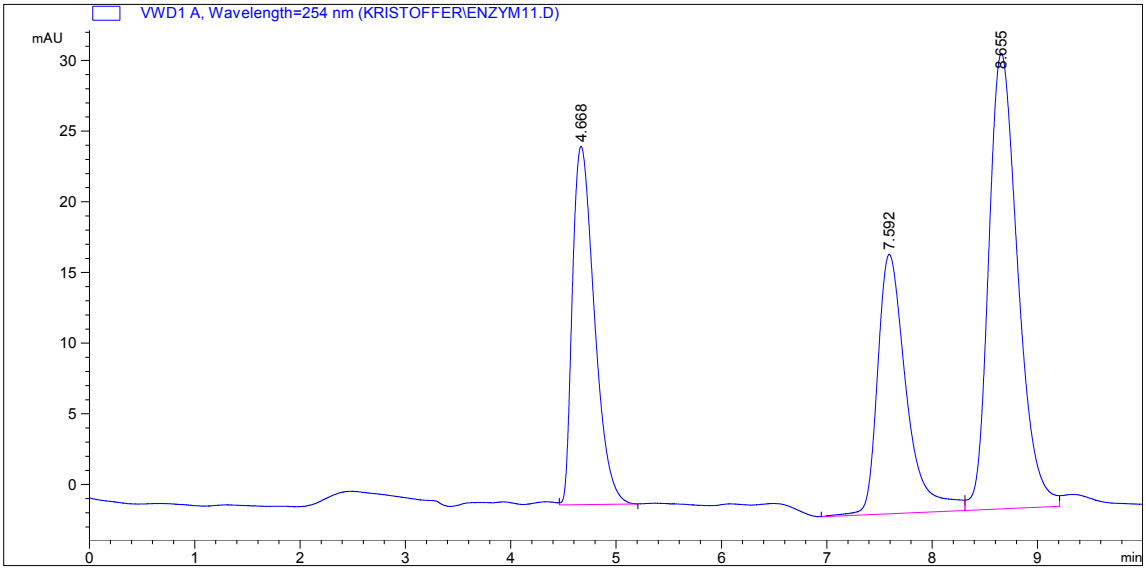

Figure C.12: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 26 hours reaction time. $ee_s = 28\%$.

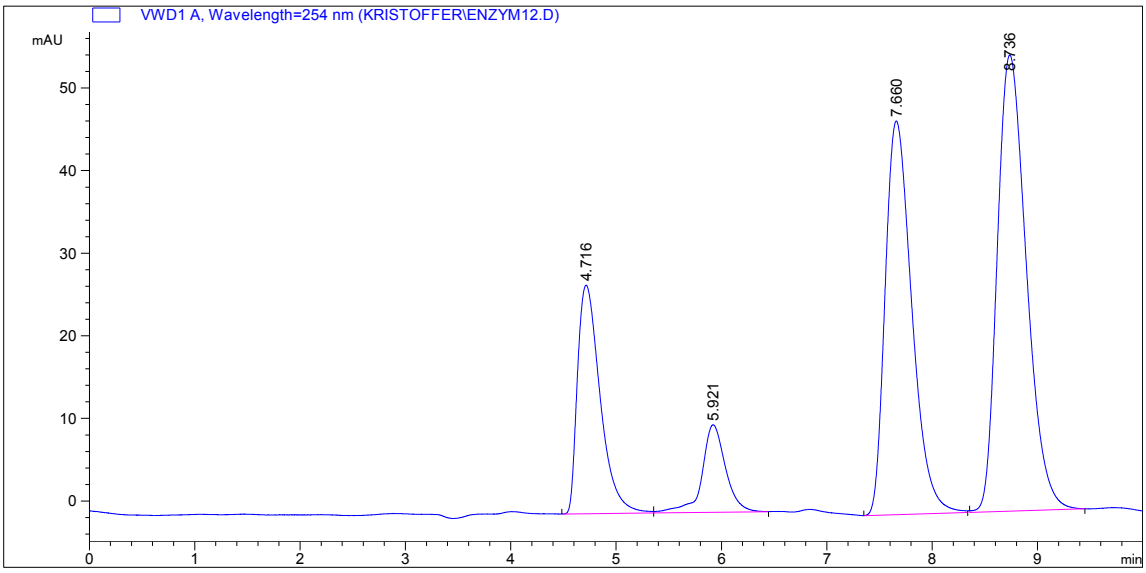

Figure C.13: HPLC-chromatogram of the first CALB-catalyzed kinetic resolution performed on **3a**, after 48 hours reaction time. $ee_s = 12\%$.

C.1.2 Second parallel

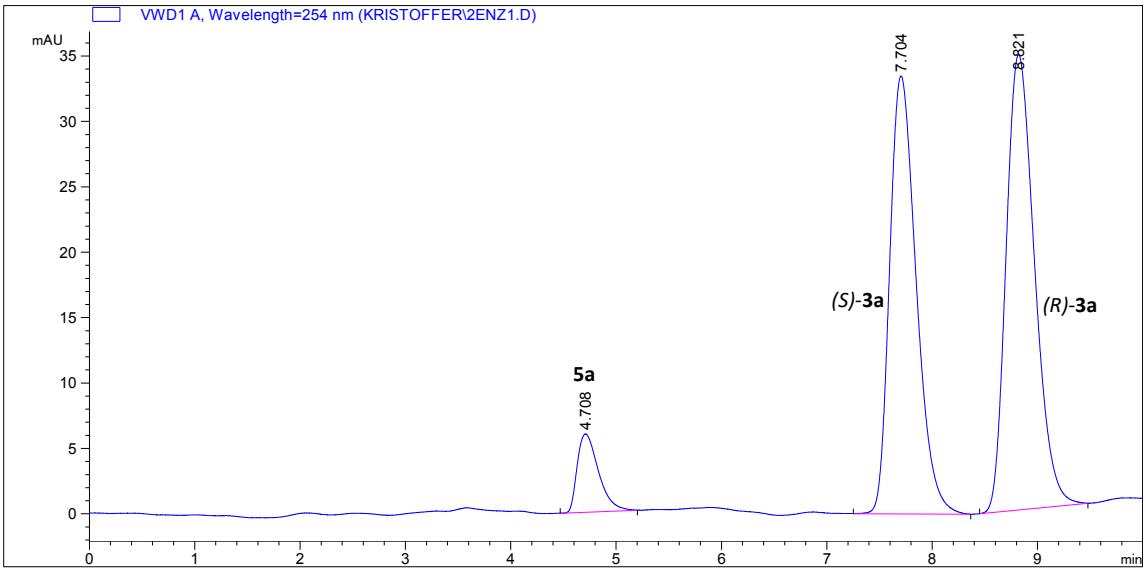

Figure C.14: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 15 minutes reaction time. $ee_s = 5\%$.

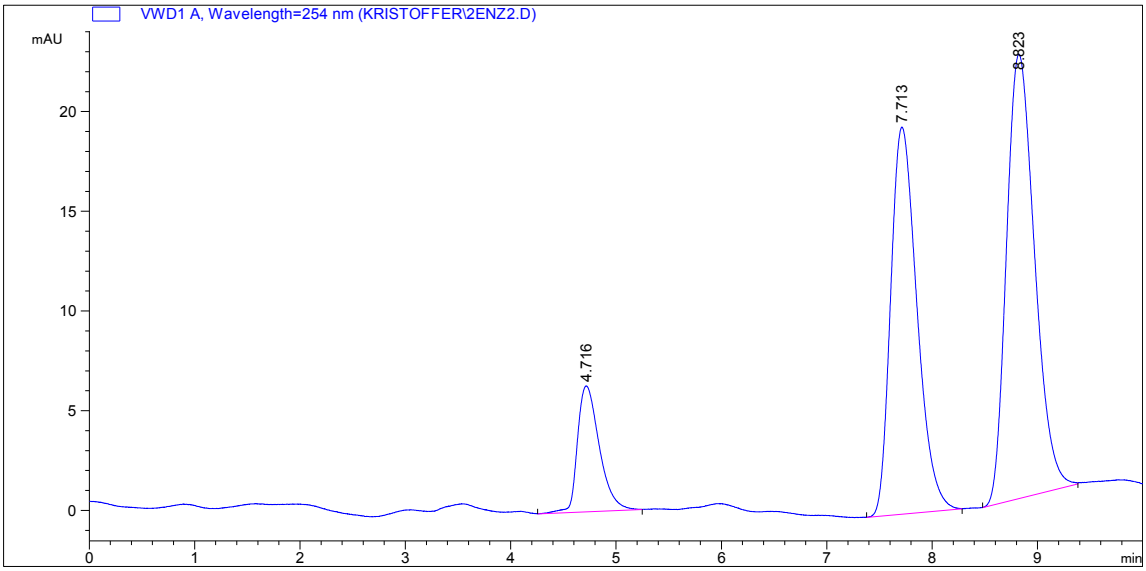

Figure C.15: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 30 minutes reaction time. $ee_s = 10\%$.

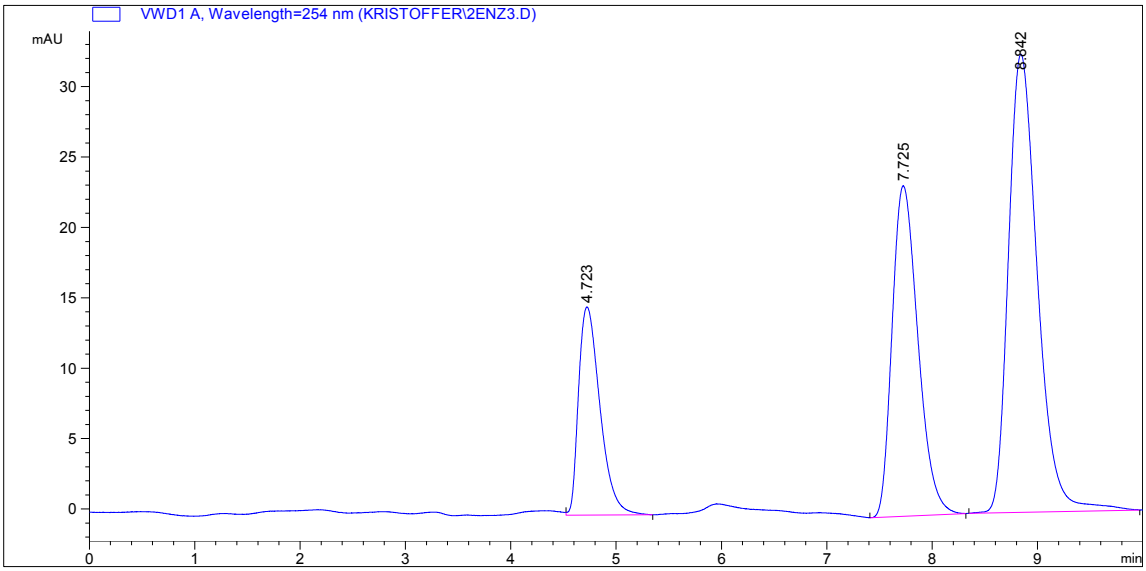

Figure C.16: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 1 hour reaction time. $ee_s = 22\%$.

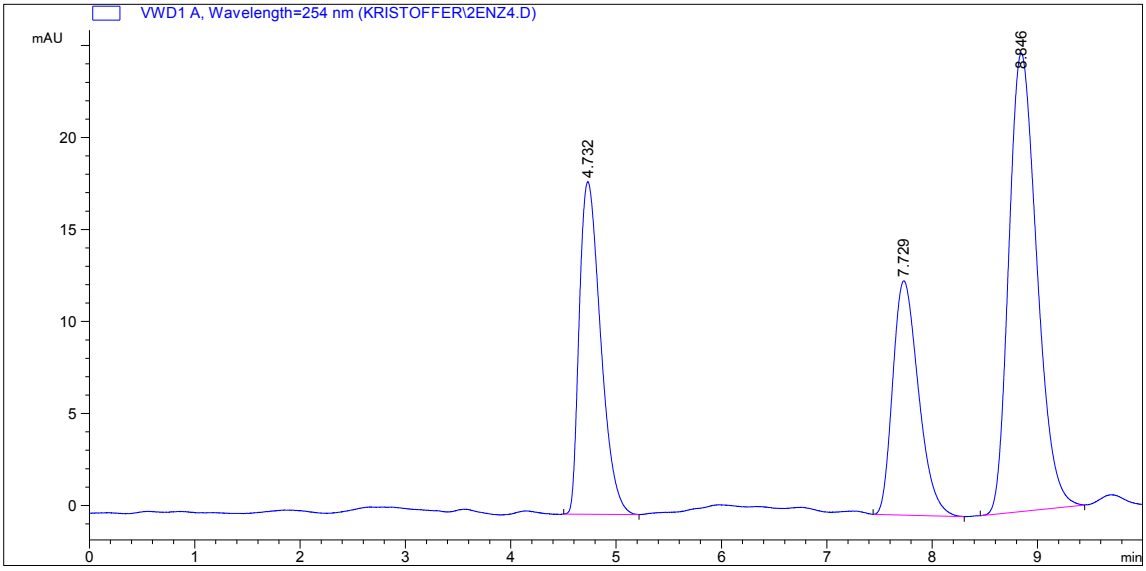

Figure C.17: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 2 hours reaction time. $ee_s = 36\%$.

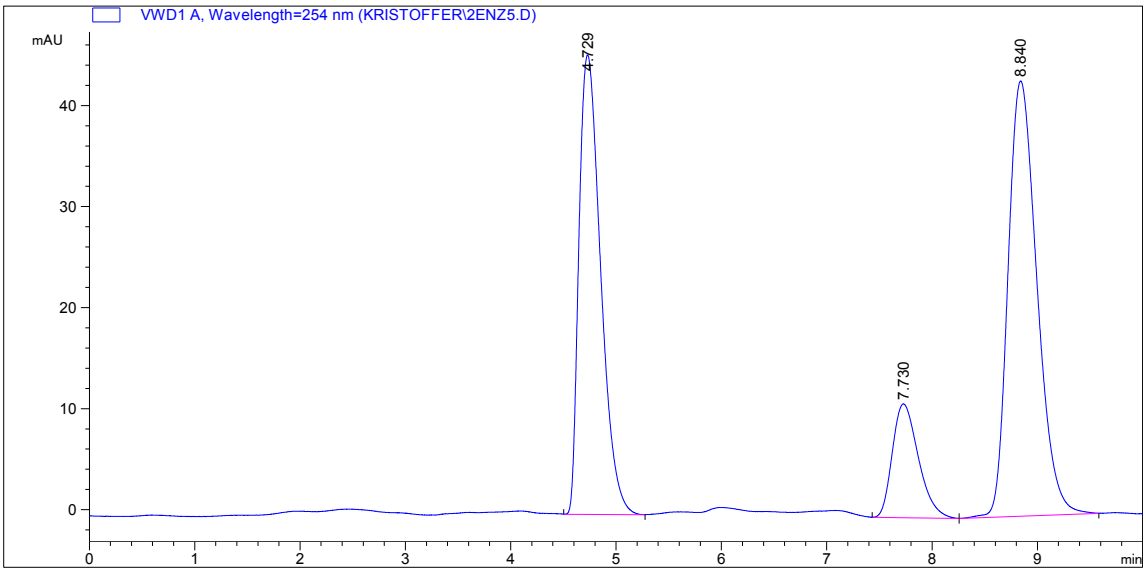

Figure C.18: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 4 hours reaction time. $ee_s = 62\%$.

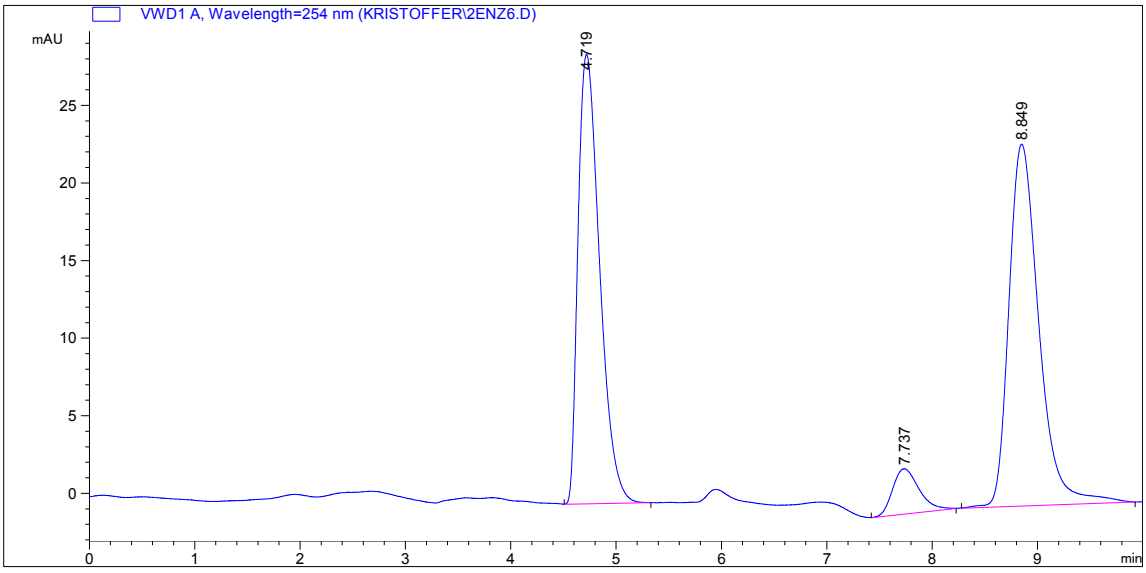

Figure C.19: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 6 hours reaction time. $ee_s = 80\%$.

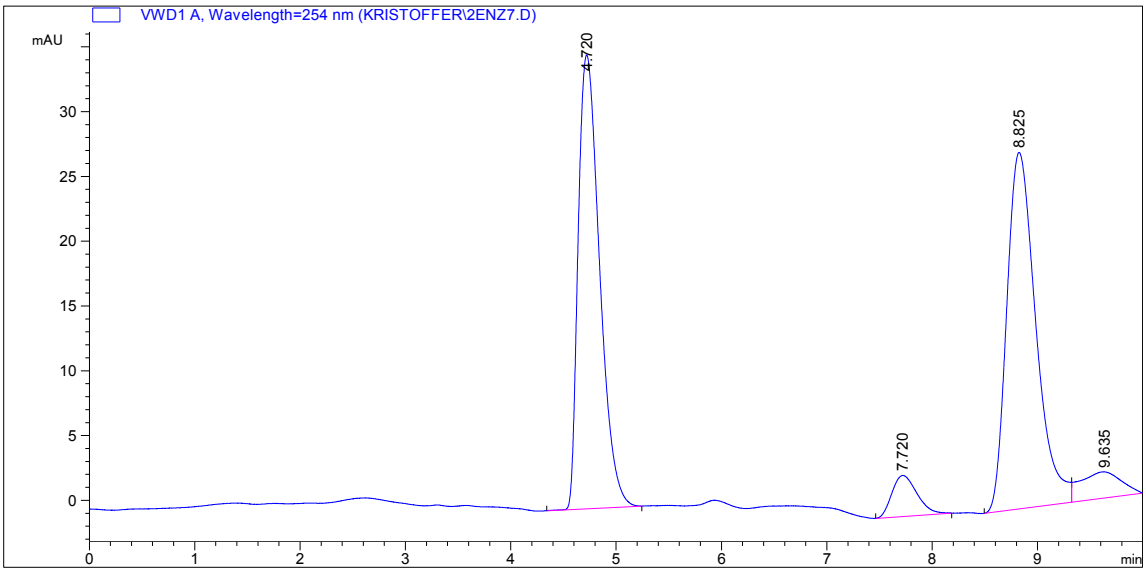

Figure C.20: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 8 hours reaction time. $ee_s = 82\%$.

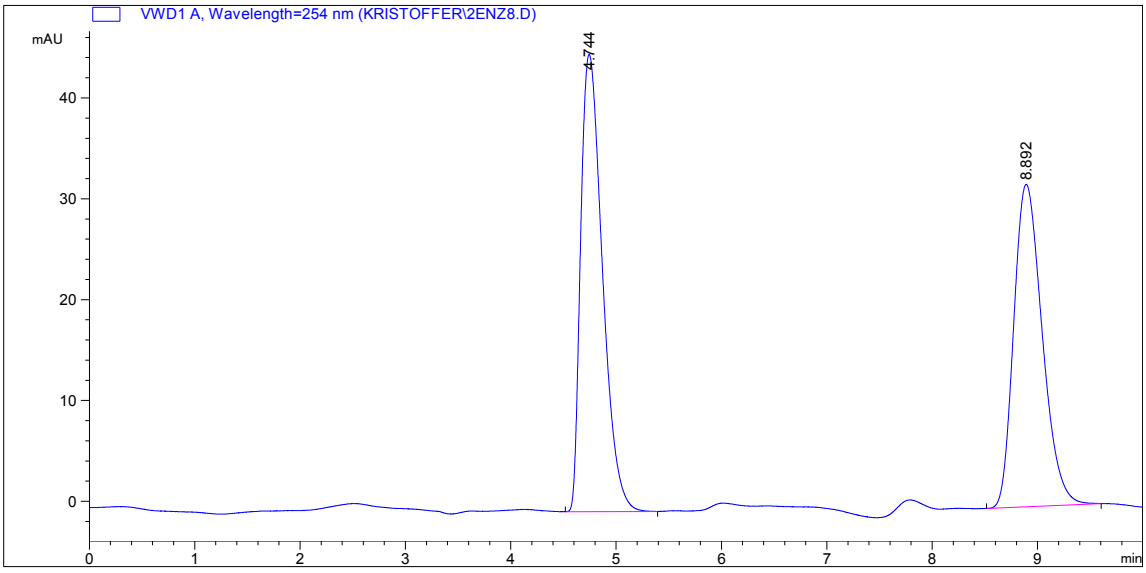

Figure C.21: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 11 hours reaction time. $ee_s = 97\%$.

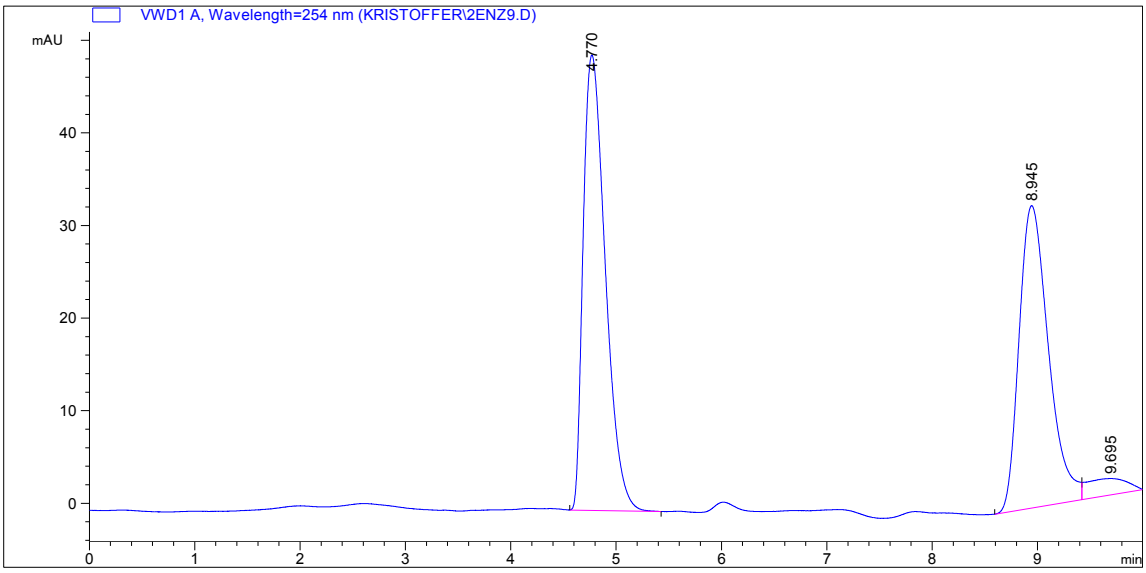

Figure C.22: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 14 hours reaction time. $ee_s = 100\%$.

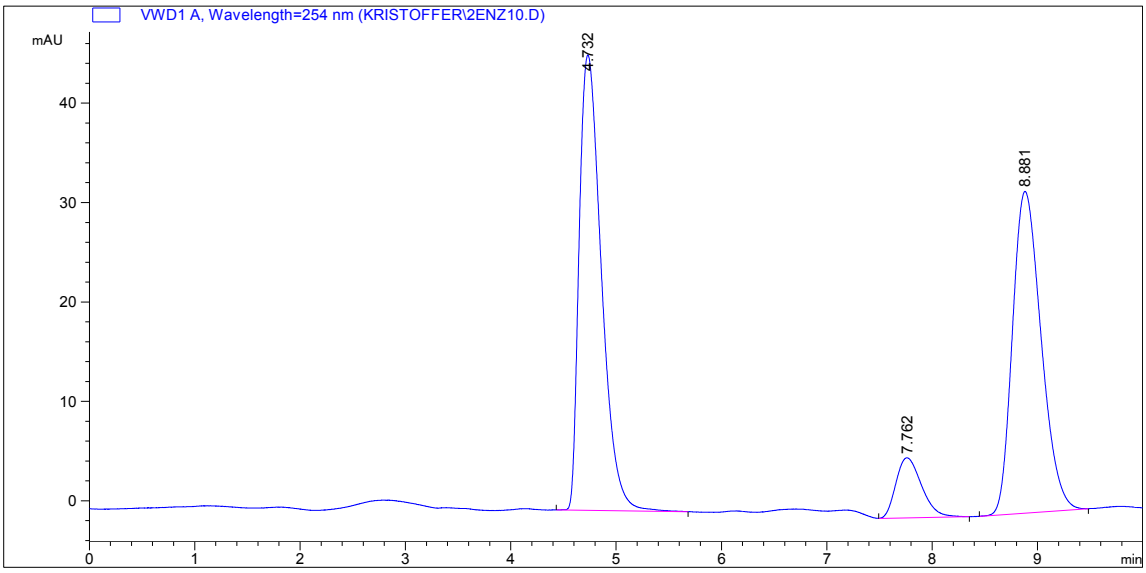

Figure C.23: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 22 hours reaction time. $ee_s = 71\%$.

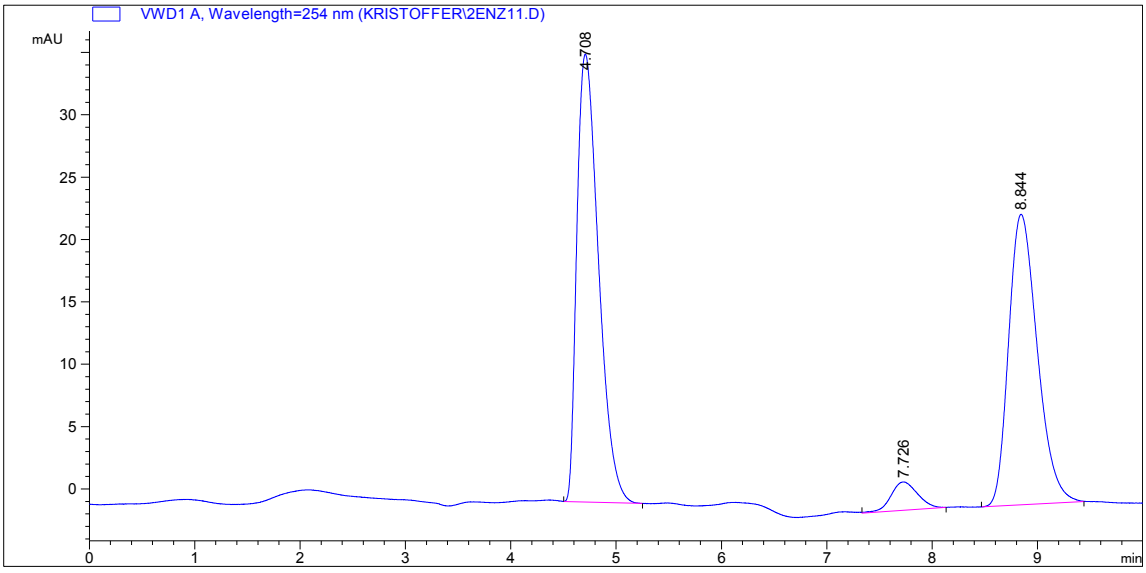

Figure C.24: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 24 hours reaction time. $ee_s = 83\%$.

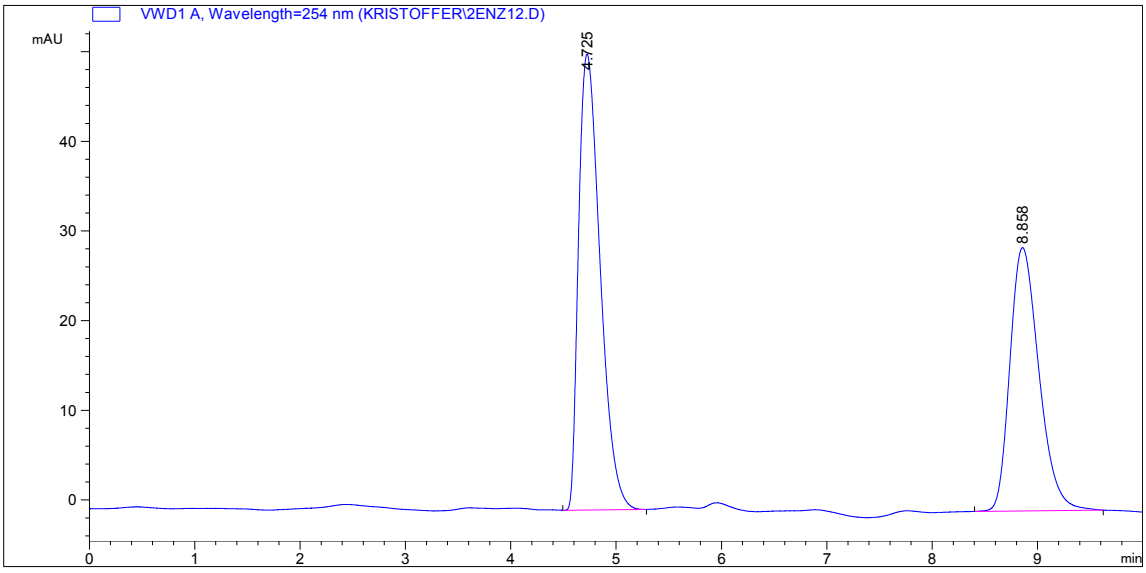

Figure C.25: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 28 hours reaction time. $ee_s = 100\%$.

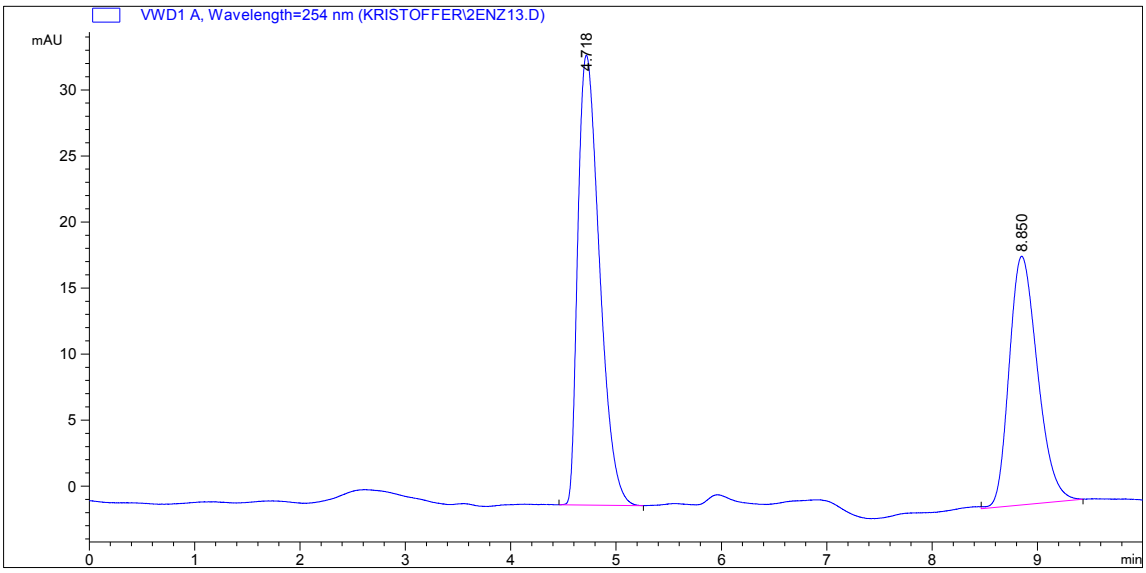

Figure C.26: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 32 hours reaction time. $ee_s = 100\%$.

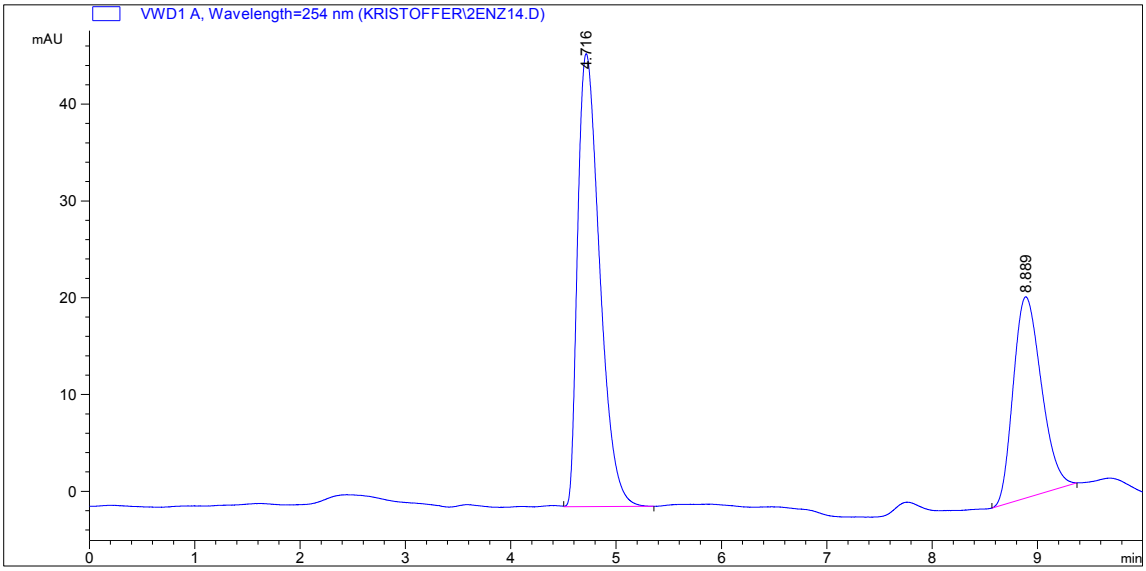

Figure C.27: HPLC-chromatogram of the second CALB-catalyzed kinetic resolution performed on **3a**, after 48 hours reaction time. $ee_s = 92\%$.

C.2 Kinetic resolution of 3b

Figure C.28 shows the HPLC-chromatogram of the enantioenriched alcohol (*R*)-**3b**, after the large scale kinetic resolution. Figures C.29 to C.39 shows chromatograms of all samples taken during the small scale kinetic resolution of **3b**. All these analyses were performed with 95:5 *n*-hexane:isopropanol.

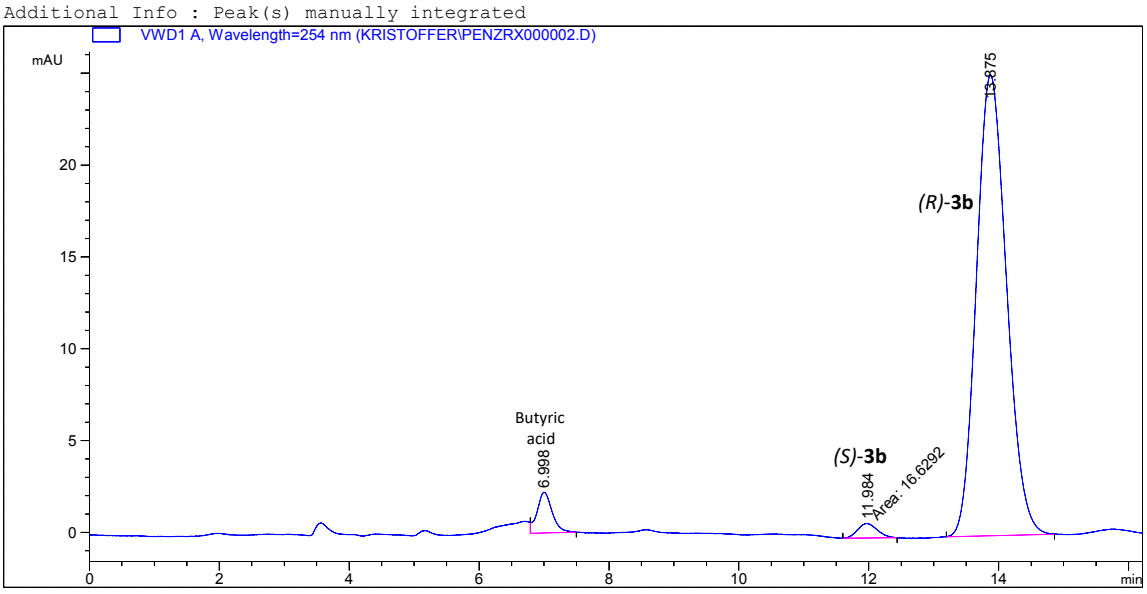

Figure C.28: HPLC-chromatogram of the alcohol (*R*)-**3b** after large scale kinetic resolution catalyzed by CALB. The alcohol is in 96% *ee*.

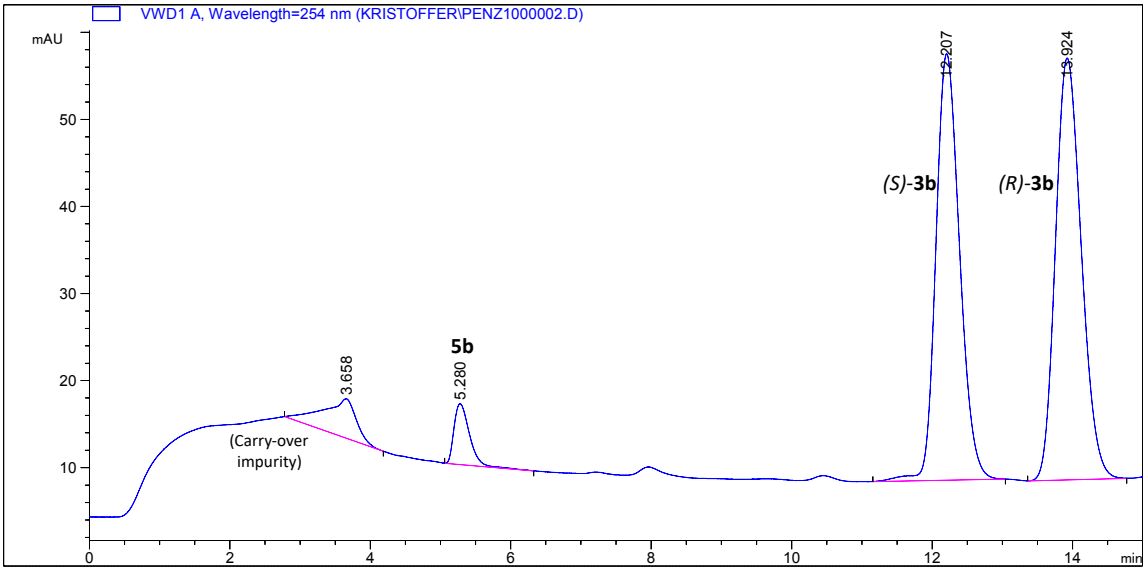

Figure C.29: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 15 minutes reaction time. $ee_s = 4\%$.

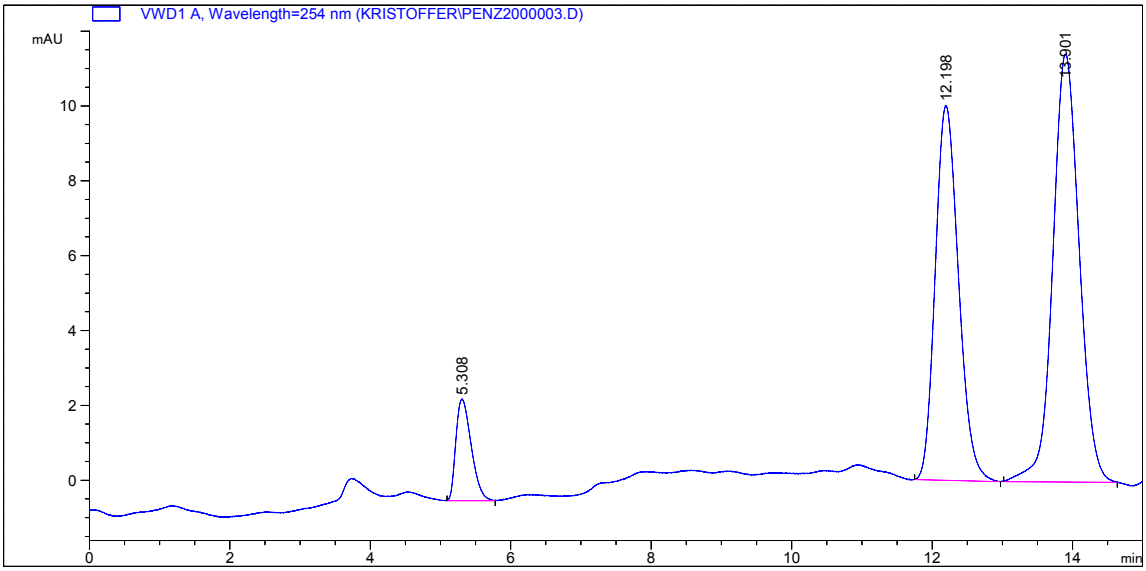

Figure C.30: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 30 minutes reaction time. $ee_s = 13\%$.

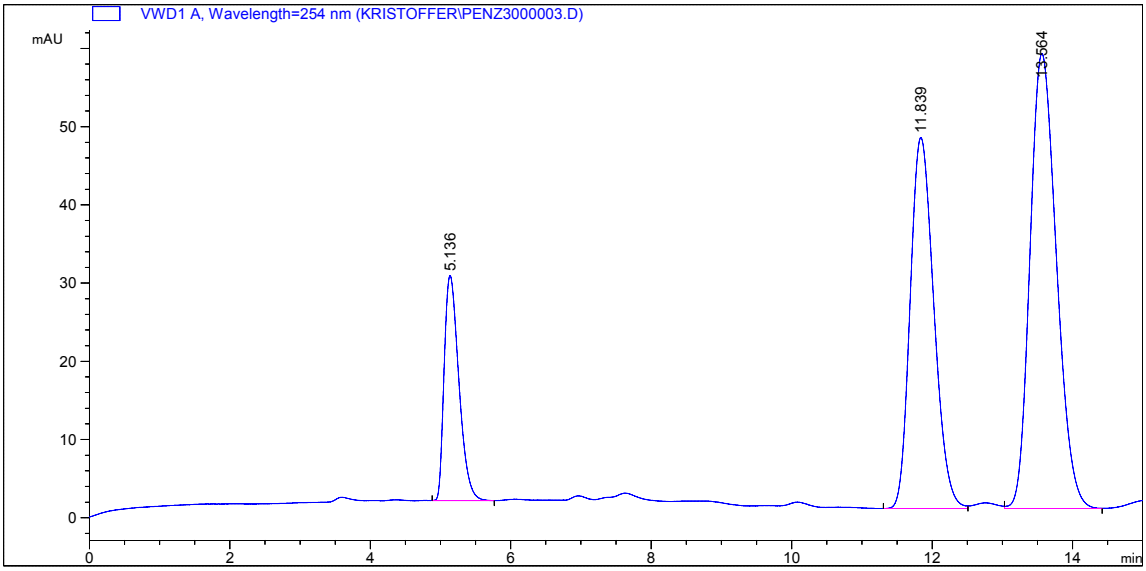

Figure C.31: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 1 hour reaction time. $ee_s = 16\%$.

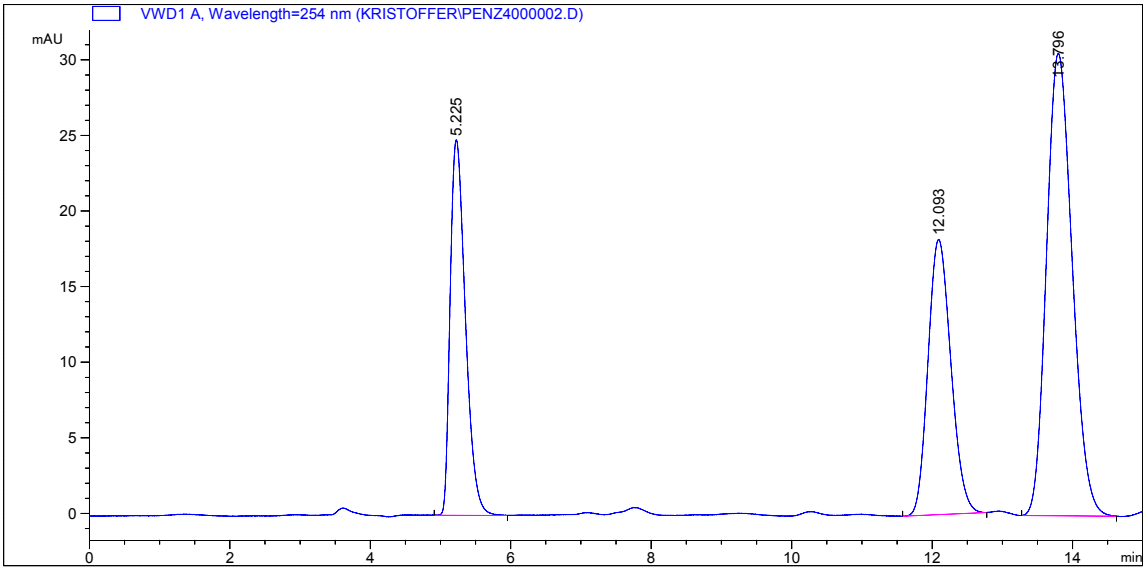

Figure C.32: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 2 hours reaction time. $ee_s = 31\%$.

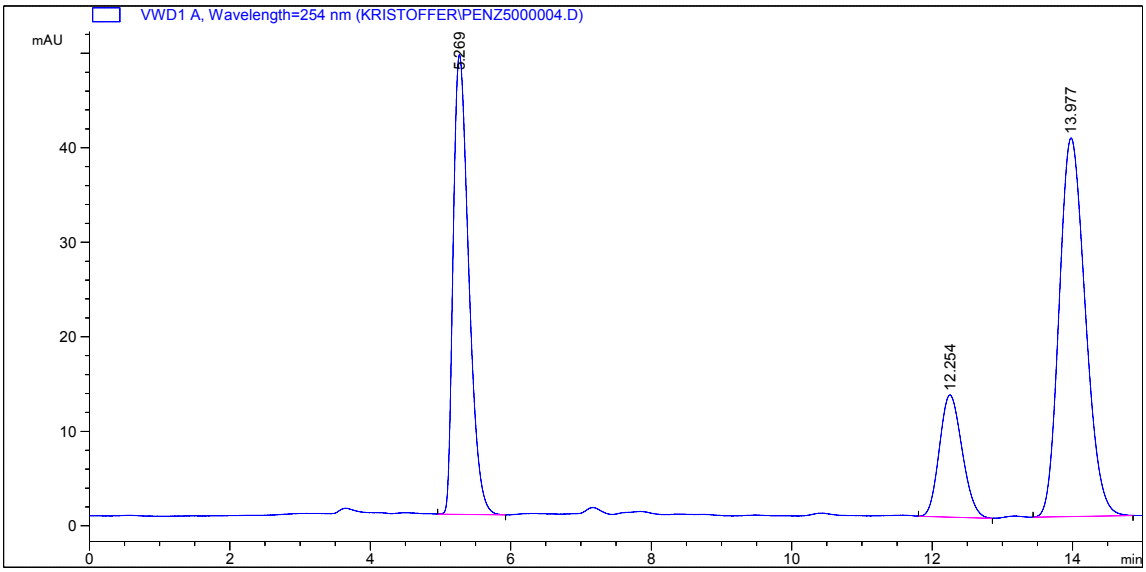

Figure C.33: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 4 hours reaction time. $ee_s = 56\%$.

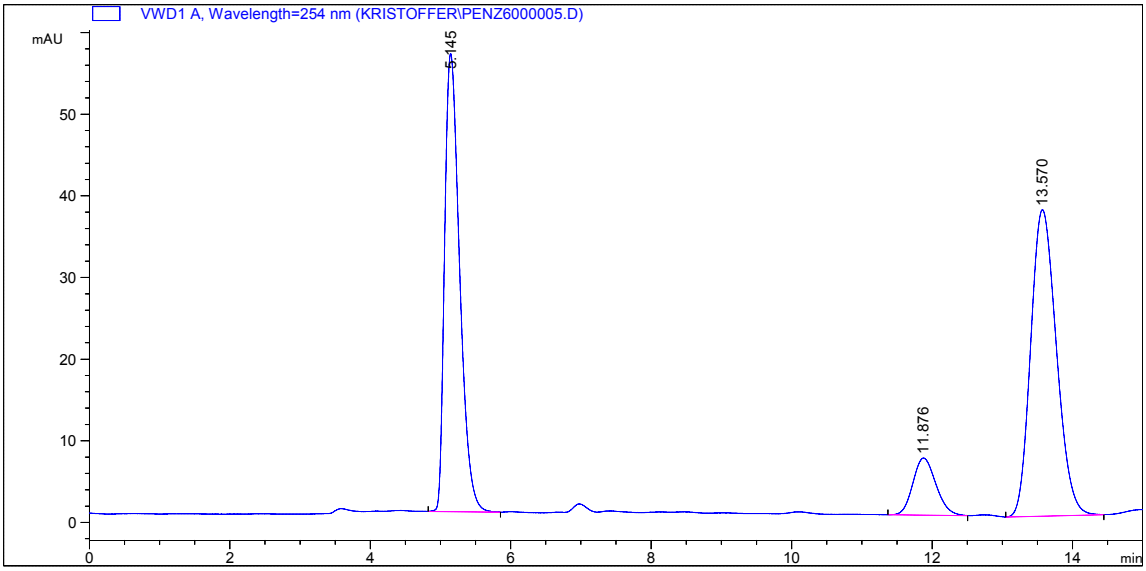

Figure C.34: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 6 hours reaction time. $ee_s = 71\%$.

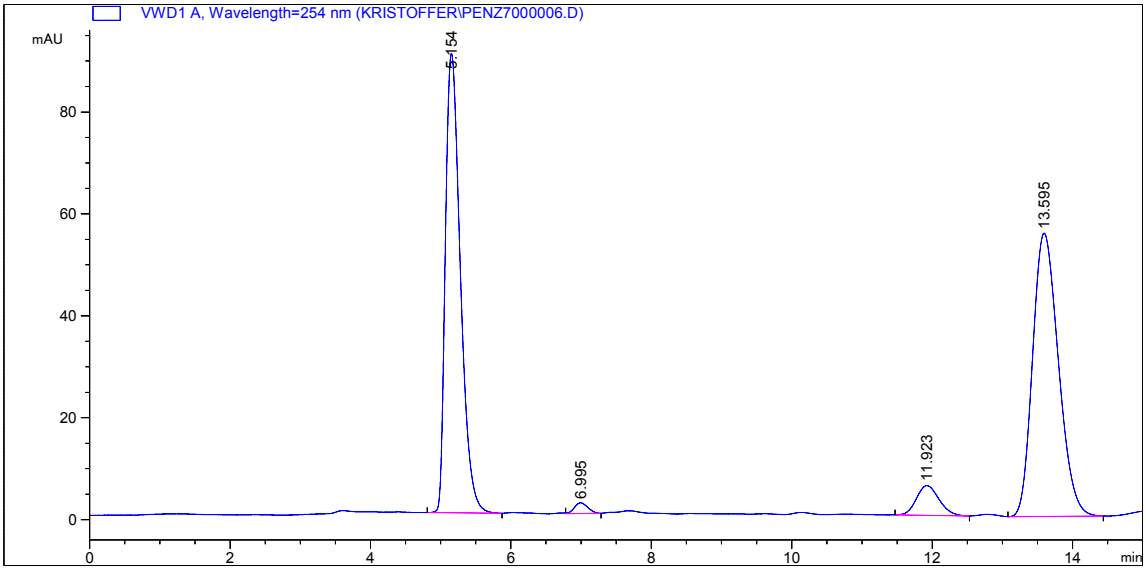

Figure C.35: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 8 hours reaction time. $ee_s = 83\%$.

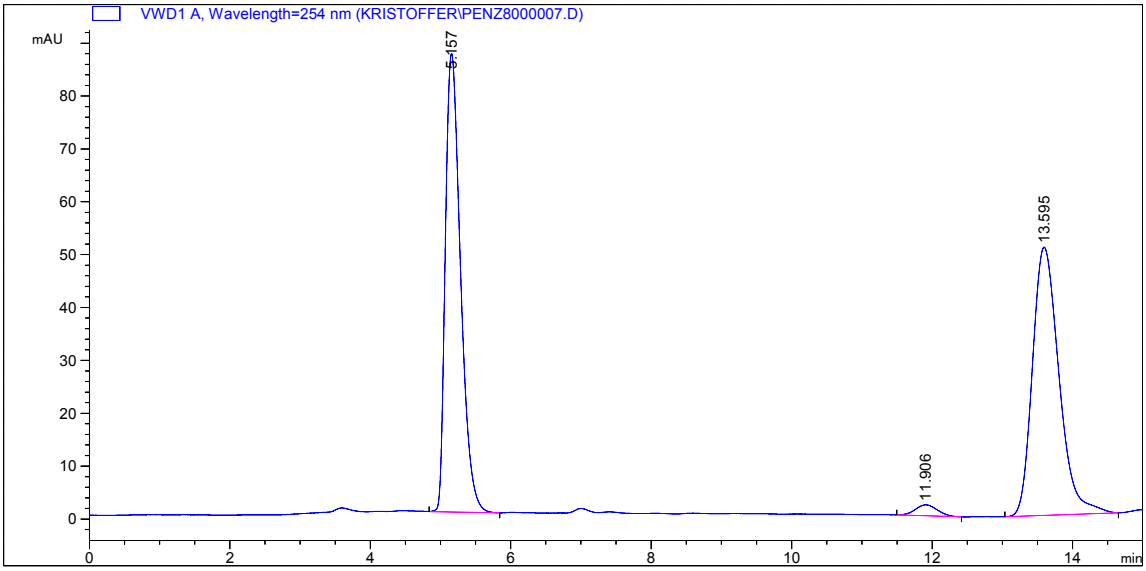

Figure C.36: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 11 hours reaction time. $ee_s = 94\%$.

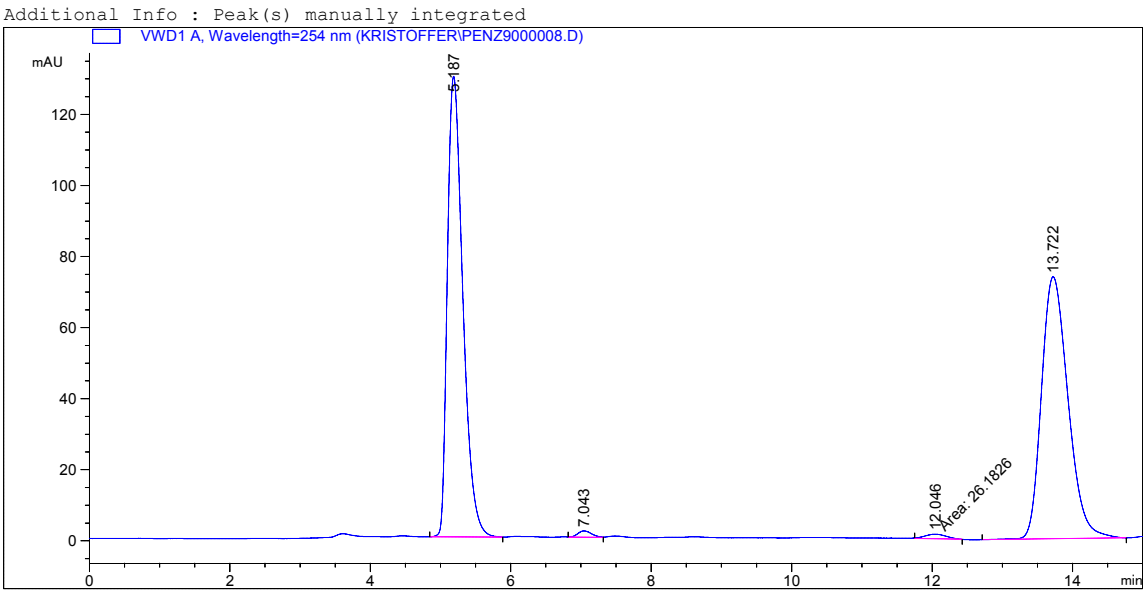

Figure C.37: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 14 hours reaction time. $ee_s = 97\%$.

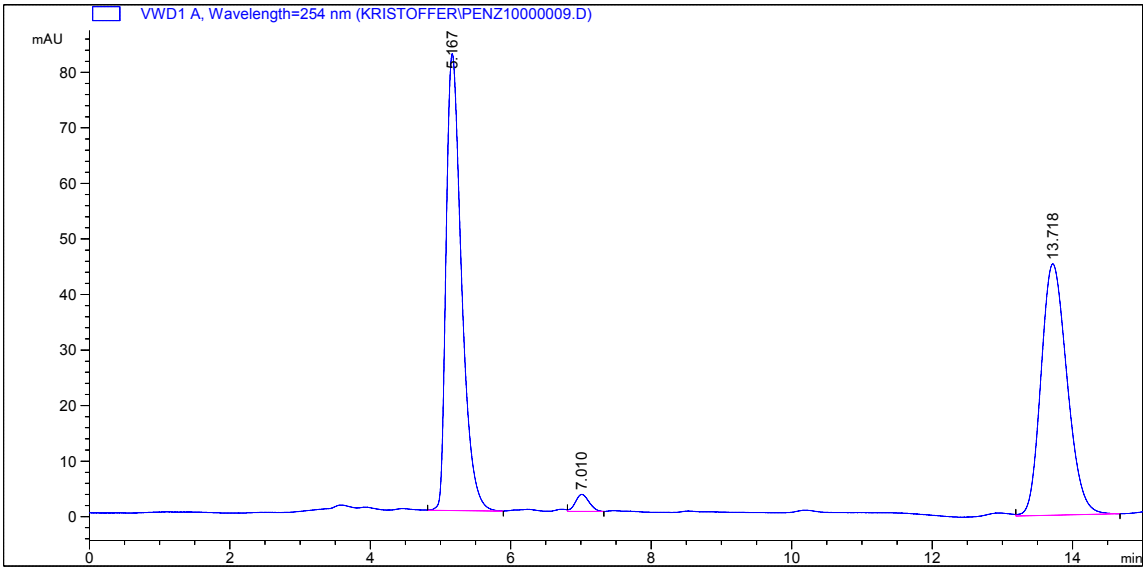

Figure C.38: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 24 hours reaction time. $ee_s = 100\%$.

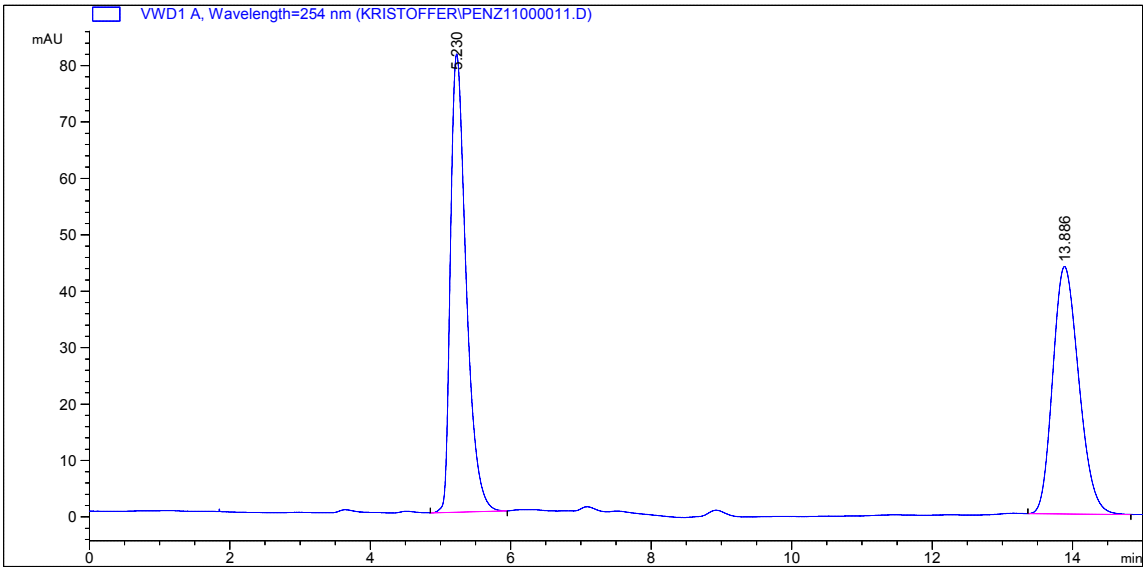

Figure C.39: HPLC-chromatogram of the CALB-catalyzed kinetic resolution performed on **3b**, after 4 hours reaction time. $ee_s = 100\%$.

Kristoffer Klungseth

Synthesis and studies of penbutolol

NTNU
Norwegian University of
Science and Technology