Supplementary material for

Resolution of a chiral amine and recovery of unwanted enantiomer by racemization: towards a greener industrial process

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The main purpose of this experiment is to point out that a classical enantiomer resolution process, frequently regarded as a low tech process, can effectively compete with an asymmetric synthetic process, particularly at industrial scale processes.

Resolution of racemic mixtures is often applied for the production of enantiomeric pure compounds in fine chemical industrial chemistry. One of the main drawbacks of this well-established technology is that half of the molecules produced in the synthetic process are waste, as they will not be incorporated in the final product. However, if the unwanted isomer can be recycled by racemization or by converting it into a non-chiral intermediate of the process, the wasted material can be reduced to a minimum. A well design industrial process can, in theory, recover all the “waste” material and reintroduce it in the synthetic process.

When an acidic proton is present at the chiral centre, as for example in the α-carbonyl carbon atom, subjecting the unwanted isomer to basic conditions is a very effective way to produce a racemic mixture.

In the present experiment, an enantiomer of 1-phenyl-1,2,3,4-tetrahydroisoquinoline cannot be directly racemized. However, using the *N*-chlorination and elimination sequence a non-chiral imine is formed from the amine, giving the opportunity to recycle the unwanted material.

Additional notes on the experiment and on the reactions

This experiment can be divided in two main parts. In the first one (lab sessions A), the racemic phenyl-1,2,3,4-tetrahydroisoquinoline is prepared. This can be done by the instructor to provide availability of the starting material to the resolution/racemization experiment to be performed by the students. The second part of the present experiment (lab sessions B) includes the enantiomeric resolution and the recycling process of the unwanted isomer by an imine formation.

The experimental procedure was tested four times in the laboratory by an undergraduate student and the experiment was executed following the experimental written procedure included in the book, without any direct intervention of the authors. The yields and quality of the obtained products are included below.

Based on the experimental procedure included above, one of us developed an industrial scalable process. Due to confidentiality of the data, the exact conditions of the adapted conditions are not disclosed in this document but several multi-kilogram batches were executed with success, the quality and the yields exceeded those reported in the present experiment.

N-(2-phenylethyl)benzamide

At 20-25 ºC the reaction is quick and a slow addition of the acyl chloride can control the heat released in the transformation. Using 0-5 ºC during the addition will result in the accumulation of reactants and additional stirring time at ambient temperature is required to get the reaction complete. Yields are consistently higher than 90%.

1-phenyl-3,4-dihydroisoquinoline

Hydrochloric acid is released during the cyclization and the reaction is complete when the gas release is no longer noticeable. Yields usually in the range 80-90%.

Racemic 1-phenyl-1,2,3,4-tetrahydroisoquinoline

Although it is not required, anhydrous methanol (or pre-dried methanol) can be used. Yields usually in the range 80-90%.

Resolution of racemic 1-phenyl-1,2,3,4-tetrahydroisoquinoline

The resolution process is relatively robust but at least 30 minutes of stirring before filtering is required. Stirring for 1.5 hours before filtering will benefit the efficiency of the enantiomeric separation without yield loss but this seems not to be crucial for the experiment. Enantiomeric excess is routinely higher than 85%. (*S*)-1-phenyl-1,2,3,4-tetrahydroisoquinoline optical rotatory power +13.5 ° (1.15 g/100 mL, chloroform; wavelength: 589.3 nm, 25 °C)[11], (*S*)-1-phenyl-1,2,3,4-tetrahydroisoquinoline optical rotatory power -10.9 ° (1.1 g/100 mL, chloroform; wavelength: 589.3 nm; 25 °C)[12].

Isolated enantiomers of 1-phenyl-1,2,3,4-tetrahydroisoquinoline (or its tartareate salt) can be purified by further crystallization (as described in the experiment or as in Bolchi *et al.*, 2013[10]).

Oxidation of (S)-1-phenyl-1,2,3,4-tetrahydroisoquinoline to 1-phenyl-3,4-dihydroisoquinoline

Robust reaction with typical yield higher than 85%.

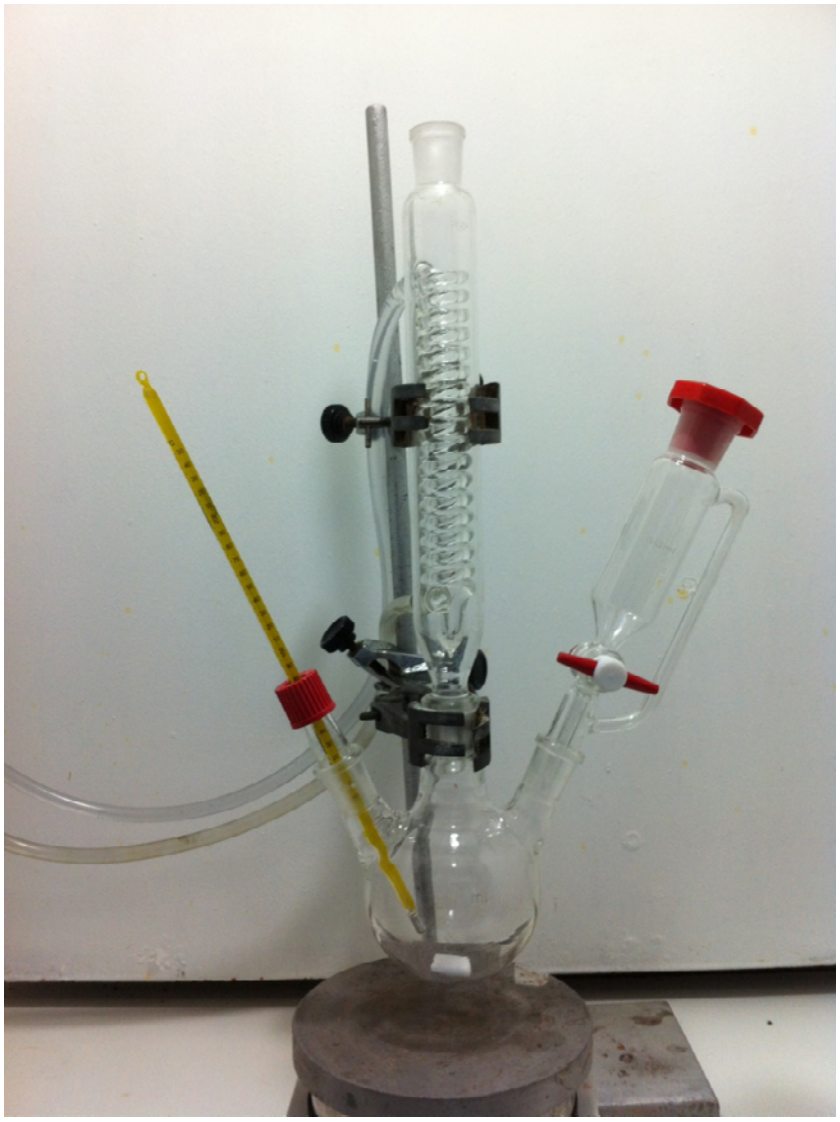


Figure SM 18.1.1 – Amide formation apparatus.

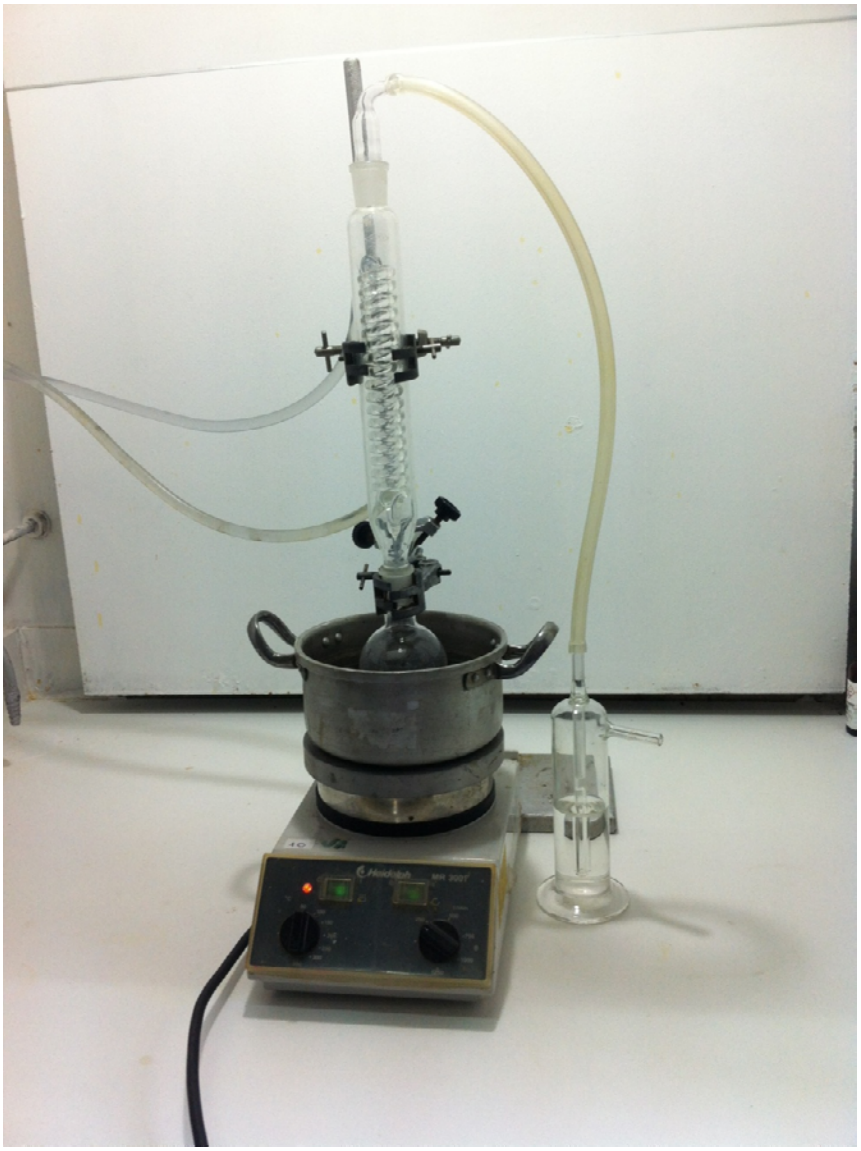


Figure SM 18.1.2 - Bischler−Napieralski cyclization apparatus.

NMR spectra

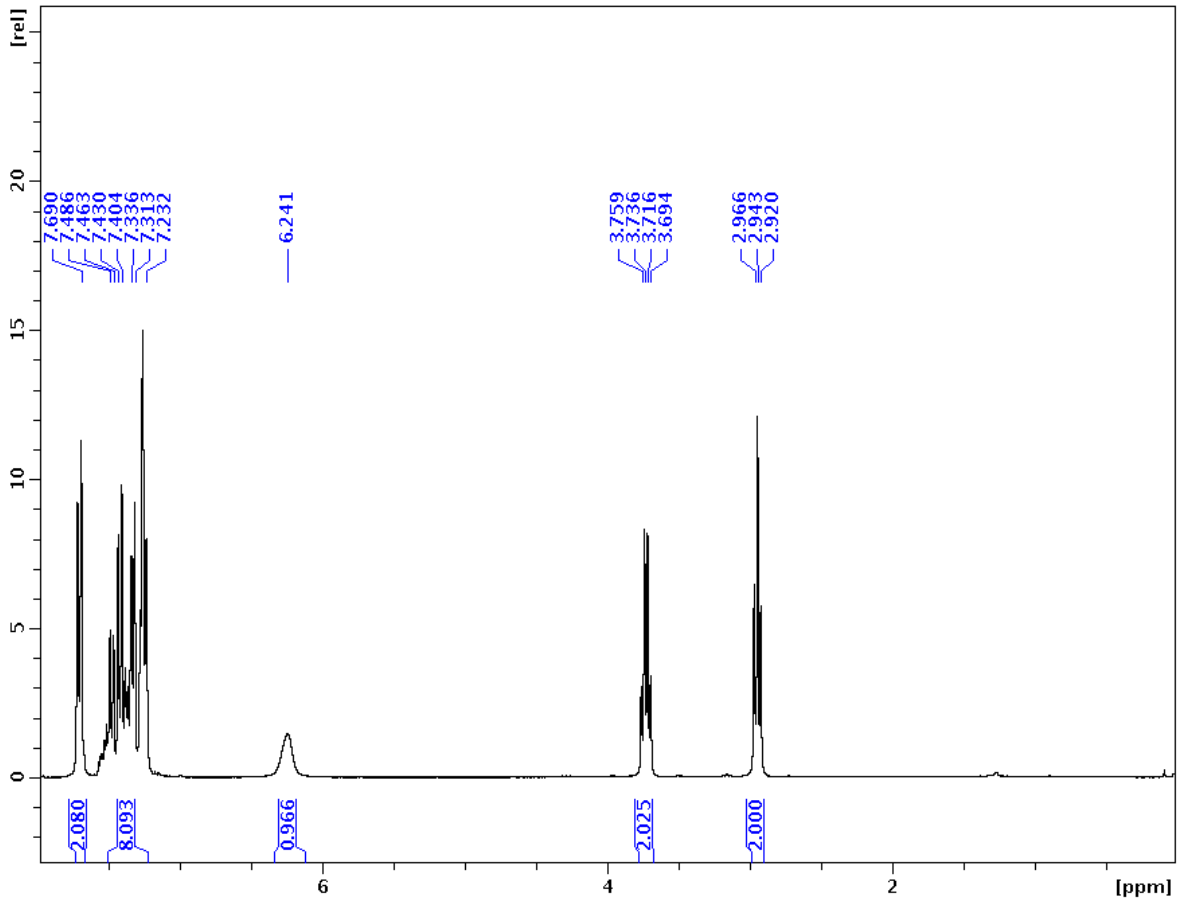
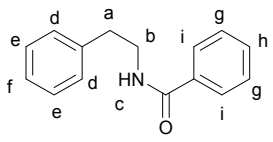


Figure SM 18.1.3 - ^{1}H NMR spectrum of *N*-(2-phenylethyl)benzamide (300 MHz, $CDCl_3$).

^{1}H NMR ($CDCl_3$): δ 2.94 (2H, t), 3.72 (2H, m), 6.24 (1H, br), 7.23-7.49 (m, 8H), 7.60-7.69 (m, 2H).



a 2.94 (2H, t)
b 3.72 (2H, m)
c 6.24 (1H, br)
d,e,f,g,h 7.23 - 7.69 (m, 8H).
i 7.23 - 7.69 (m, 2H).

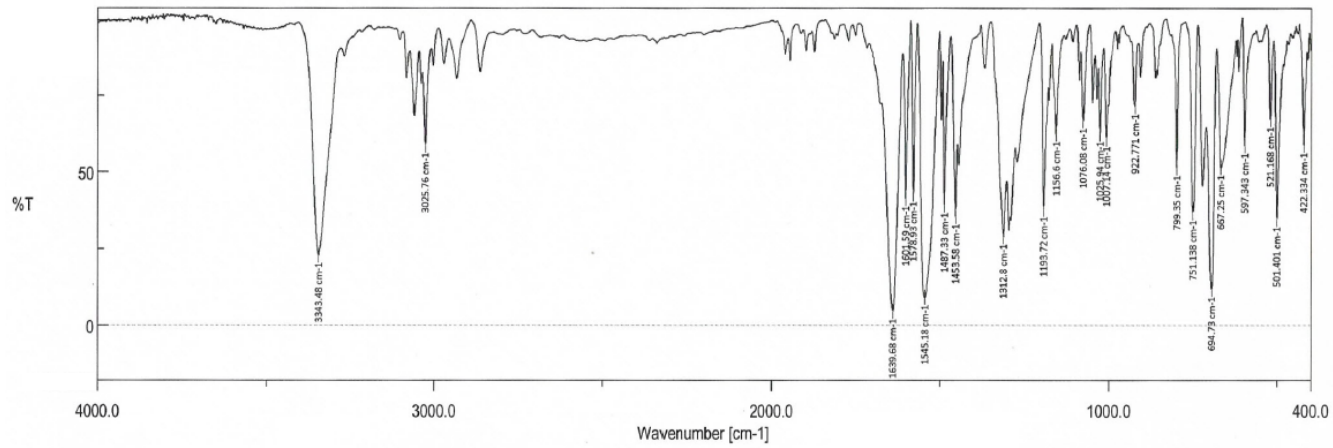


Figure SM 18.1.4– FT-IR (KBr disk) spectrum of *N*-(2-phenylethyl)benzamide.

 (cm^{-1}) 3343 (N-H stretch), 1639 (C=O stretch), 1545 (N-H bend).

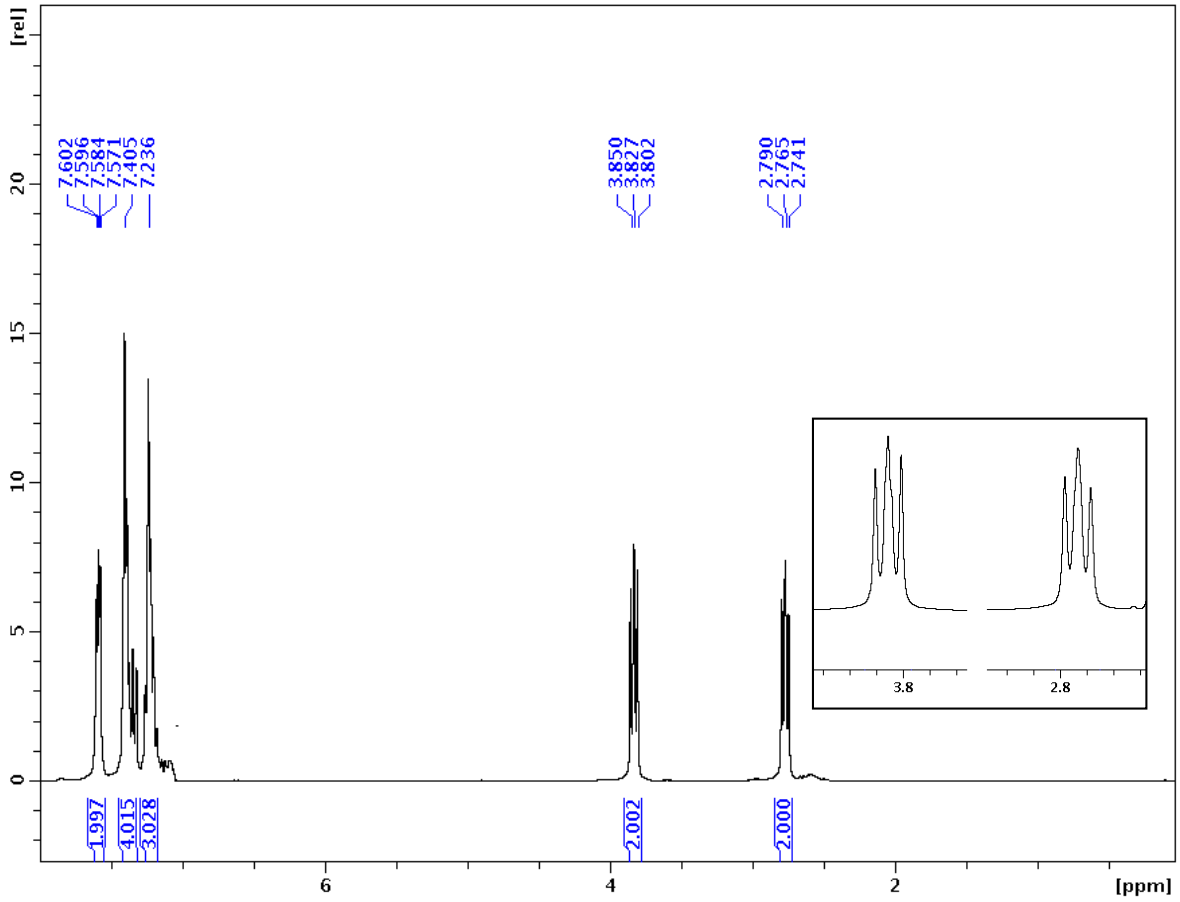
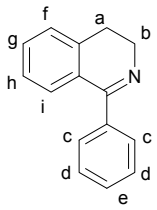


Figure SM 18.1.5 - ^{1}H NMR spectrum of 1-phenyl-3,4-dihydroisoquinoline (300 MHz, $CDCl_3$).

1H NMR ($CDCl_3$): δ 2.77 (2H, t), 3.83 (2H, t), 7.24-7.60 (m, 9H).



a 2.77 (2H, t)
b 3.83 (2H, t)
c,d,e,f,g,h,i 7.24-7.60 (m, 9H).

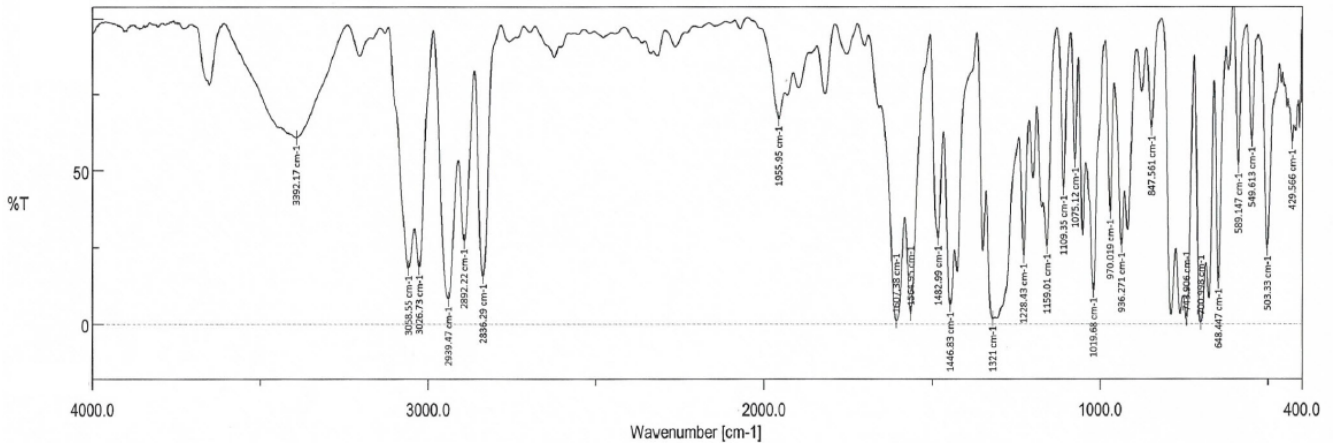


Figure SM 18.1.6– FT-IR spectrum (KBr disk) of 1-phenyl-3,4-dihydroisoquinoline.

 (cm^{-1}) 3058, 3026 (sp^3 C-H stretch), 1607 (C=N stretch), 1564 (C=C aromatic stretch).

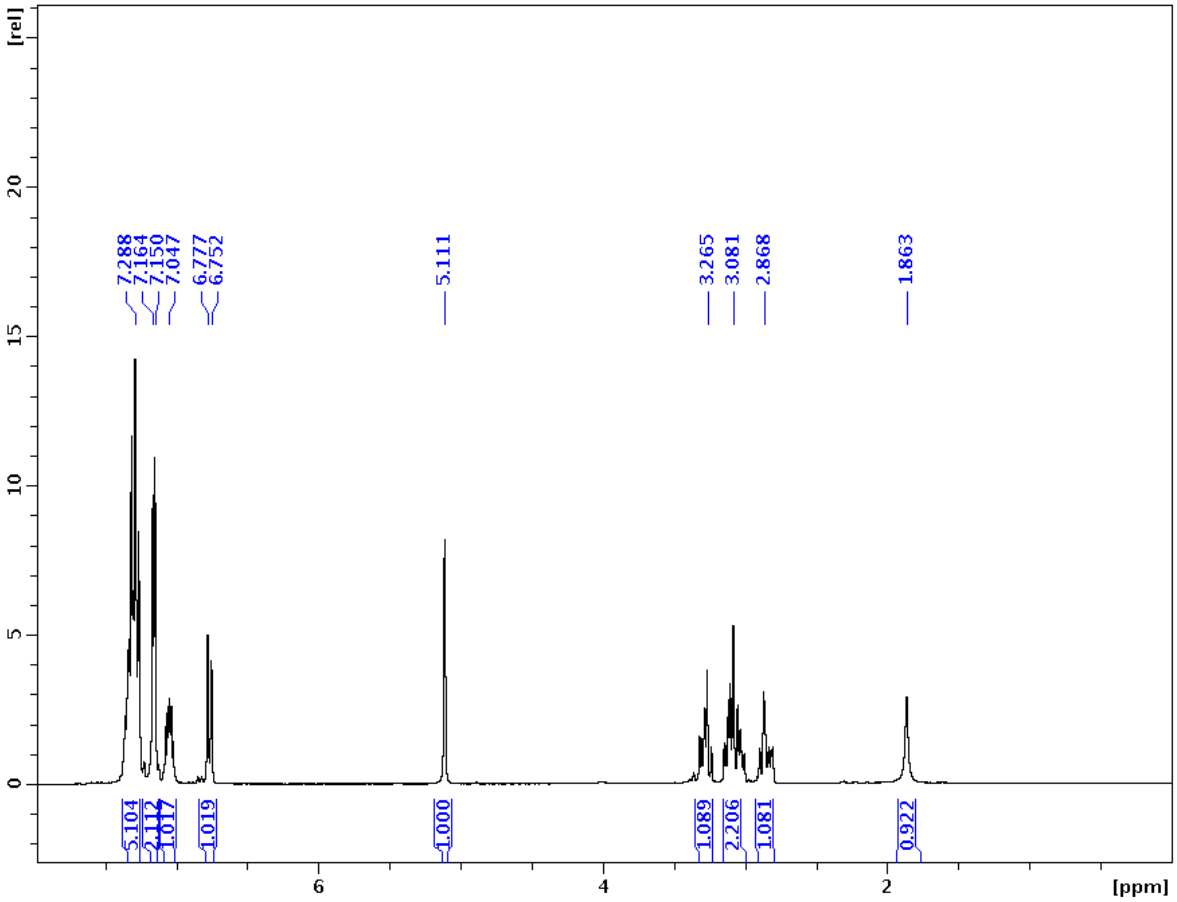
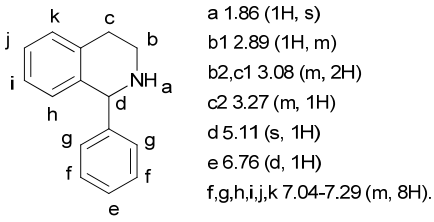


Figure SM 18.1.7- ^{1}H NMR spectrum of *rac*-1-phenyl-1,2,3,4-tetrahydroisoquinoline (300 MHz, $CDCl_3$).

^{1}H NMR ($CDCl_3$): δ 1.86 (1H, s), 2.89 (1H, m), 3.08 (m, 2H), 3.27 (m, 1H), 5.11 (s, 1H), 6.76 (d, 1H), 7.04-7.29 (m, 8H).



a 1.86 (1H, s)
b1 2.89 (1H, m)
b2,c1 3.08 (m, 2H)
c2 3.27 (m, 1H)
d 5.11 (s, 1H)
e 6.76 (d, 1H)
f,g,h,i,j,k 7.04-7.29 (m, 8H).

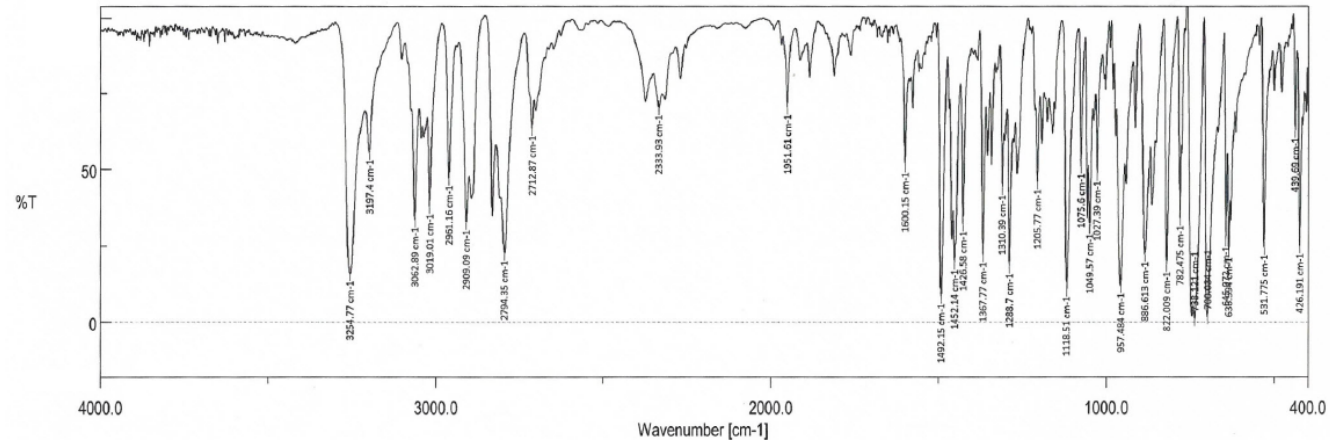


Figure SM 18.1.8- FT-IR spectrum (KBr disk) of *rac*-1-phenyl-1,2,3,4-tetrahydroisoquinoline.

 (cm^{-1}) 3254 (N-H stretch), 3026, 3019 (sp^3 C-H stretch).

Hints for questions

1. Determine the reaction yields.

Yields should be calculated with the dry weight of the products. Yield for the 1-phenyl-3,4-dihydroisoquinoline synthesis process can be determined using the product volume and its density (1.07 g/mL).

2. Calculate the enantiomeric excess of (*R*) and (*S*)-1-phenyl-1,2,3,4-tetrahydroisoquinoline obtained during session B1.

Percent enantiomeric excess is determined by the ratio of observed specific rotation and the specific rotation of the enantiopure compound.

$$\%e.e. = \frac{[\alpha]_{observed}}{[\alpha]_{enantiopure}} . 100$$

3. What is the structural relation between the two tartaric acid salts formed during session B1? Is this fact important or not to the separation process by crystallization?

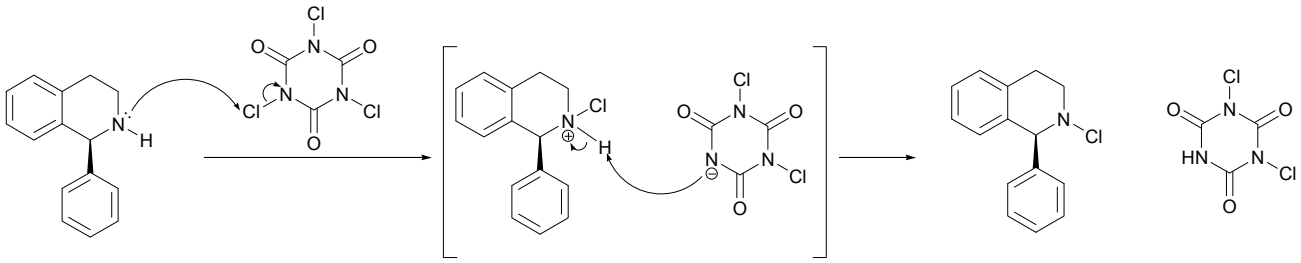
Using (+)-tartaric acid, two different salts with the basic amine (1-phenyl-1,2,3,4-tetrahydroisoquinoline) will be formed. The two salts are diastereomeric and have different solubility profiles, one of the them crystallizes while the other remains in solution. This behavior is crucial to the separation process. Filtration separates efficiently the two diasteromeric salts.

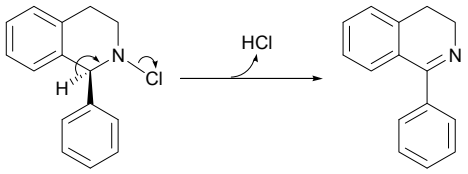
4. Why can the sequence *N*-chlorination, imine formation and reduction can be considered as a recycling process for the unwanted enantiomer of 1-phenyl-1,2,3,4-tetrahydroisoquinoline?

N-chlorination followed by spontaneous elimination of HCl of an enantiometer of 1-phenyl-1,2,3,4-tetrahydroisoquinoline occurs with the oxidation of the C-N bond to form an imine. The resulting product is achiral and can be reintroduced into the process in the reduction step.

5. Write the mechanisms for the chlorination end elimination reactions to form the 1-phenyl-3,4-dihydroisoquinoline from 1-phenyl-1,2,3,4-tetrahydroisoquinoline.

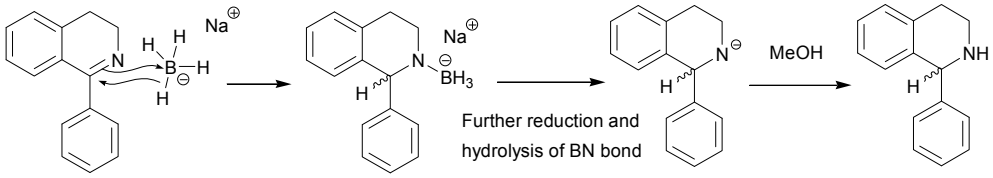
A simplified mechanism is shown below:





6. Write the mechanism of the sodium borohydride reduction.

An outline of the reaction mechanism is shown below:



Synthesis of racemic phenylalanine methyl ester and its kinetic resolution catalyzed by α-chymotrypsin

Supplementary Material

Additional background/discussion to be optionally delivered to the student

Session 1

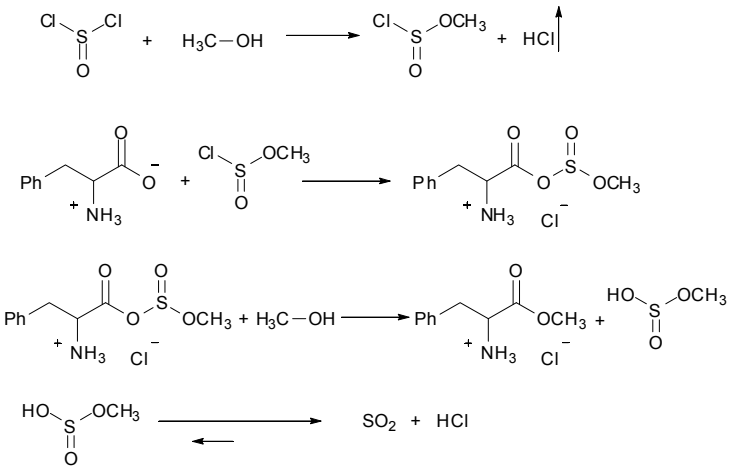
The desired methyl ester could be obtained in principle in two ways:

- By Fischer esterification under acid catalysis (HCl) using methanol as solvent. This is a very general method to synthesize esters.
- Using thionyl chloride in methanol. This is not a general method, being peculiar for aminoacids, for the reasons detailed below.

The second approach was chosen in this experiment for various reasons:

- Reaction is faster.
- Conditions are milder.
- The reaction is irreversible (Fischer esterification is an equilibrium reaction).
- Since we start from an aminoacid in zwitterionic form the acid used in Fischer esterification cannot be just catalytic. More than 1 equivalent would be needed.
- The work-up is very easy, whereas in Fischer esterification it is necessary to neutralize the excess acid before solvent evaporation.

The reaction likely proceeds through the mechanism shown in Scheme SM 18.2.1



Scheme SM 18.2.1. Mechanism of ester formation.

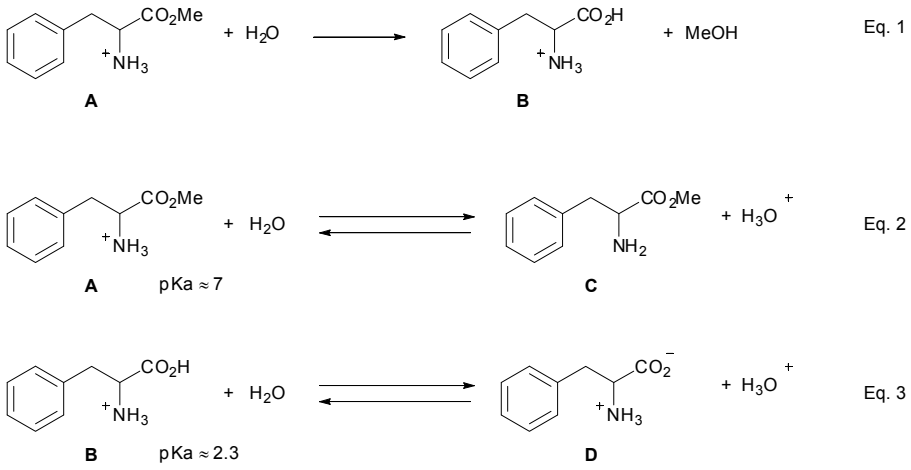
From this mechanism some important points can be deduced:

- There are no side products, except for HCl and SO_2 that, being gases at room temperature, mostly exit from the reaction vessel during the reaction (and are captured by soda lime). The remaining traces are co-evaporated with methanol, leaving the nearly pure crystalline product.
- The reaction works well with α-aminoacids, thanks to their low pKa (around 2.5), that makes the concentration of the zwitterion form sufficient to undergo the reaction shown in the second row of Scheme SM 18.2.1. With standard carboxylic acids (pKa around 5) the concentration of the carboxylate would be too low.
- It is important that thionyl chloride reacts with just one equivalent of methanol, because dimethyl sulphite is unable to promote the esterification. Therefore the temperature control during mixing of this two reagents is very important. At higher temperatures, dimethyl sulphite may be formed.
- Methyl chlorosulphite prefers to react with the carboxylic group to form a mixed anhydride than with the amino group. The reason is that the amino group is completely protonated under these acidic conditions.
- Since two toxic gases evolve from the reaction, it is important to trap them (working in a fume hood is not enough!). Soda lime is used for this goal. Soda lime is a mixture of NaOH and CaO. NaOH alone would not be well suited since, due to the exothermic reaction with HCl and SO_2, would melt down, obstructing the trap. In addition NaOH is not equally efficient for trapping water from the air, inhibiting entry of moisture into the reaction apparatus. On the other hand, soda lime maintains its initial shape and the indicator allows seeing when it becomes exhausted.

Session 2

α-Chymotrypsin is a serine protease, which is extracted from mammalian pancreas. In this experiment we use an enzyme of bovine origin. α-Chymotrypsin is one of the most studied enzymes, thanks to the possibility to obtain it in crystalline form. Although proteases are enzymes whose natural function is to hydrolyse proteins (and therefore amidic bonds), they are able to hydrolyse esters as well, with a similar mechanism. Often proteases are selective for the so called "scissile" aminoacid, that is the aminoacid whose acyl group is involved in the reaction. This is because the α-substituents occupy a pocket in the active site named the "specificity pocket". For chymotrypsin this pocket is hydrophobic and relatively large and thus prefers aromatic aminoacids such as phenylalanine, tyrosine and tryptophan. The same selectivity applies for esters. Moreover, since the active site is chiral, only when the aminoacid is of the L series, the benzyl residue is able to occupy the specificity (hydrophobic) pocket. Therefore reaction of the D derivatives is very slow, and kinetic resolution is quite efficient. α-Chymotrypsin works best at slightly acidic pH, because at higher pH it tends to self digest.

If one looks at the stoichiometry of hydrolysis reaction (Eq. 1), it is not obvious why the pH decreases. However, one should keep in mind the two acid-base equilibria depicted in Eq. 2 and 3, remembering that the reaction is carried out at pH = 5.15. At this pH, the equilibrium of Eq. 2 is shifted to the left. Actually, only a very small amount of NaOH solution is needed, before adding the enzyme, to adjust the pH to 5.15, proving that the substrate essentially remains in the ammonium form **A**. In contrast, after the hydrolysis, the ammonium ion **B**, which is much more acidic, is completely converted into the zwitterionic form **D**, since at pH 5.15 the equilibrium of eq. 3 in shifted to the right. Therefore one equivalent of H_3O^+ is formed and a decrease of pH is observed. In order to maintain it at the starting value, an equimolar amount of NaOH must be added.



Scheme SM 18.2.2. Stoichiometric hydrolysis equation and acid-base equilibria.

At the end of the reaction, addition of NaOH brings the pH to 9.5 and shifts the equilibrium of Eq. 2 to the right, converting the unreacted D-methyl ester into **C**, which is rather hydrophobic and can be extracted by an organic solvent. Meanwhile the hydrolysis product (phenylalanine) is converted to the anionic form, which is very polar and thus remains in the aqueous phase. In this experiment L-phenylalanine (the product of hydrolysis reaction) is not isolated, but if one wants to perform this task, the aqueous phase (after extraction) should be acidified to the isoelectric point (about 6). At this pH, the concentration of zwitterion **D** is maximum and **D** can be precipitated by concentrating the solution.
A practical problem associated with the extraction of **C** is the likely formation of emulsions due to the emulsifying power of the enzyme. Addition of some crystal of solid NaCl can help in breaking emulsions.

Note to the instructor

General

This experiment has been given for several years to students of the second year of bachelor course in Chemistry. The second part has also been given to students of the first year of the bachelor course in Biotechnology. Typically classes of 25-35 students were hosted at a time in the laboratory.
The second year bachelor students in Chemistry had already attended a complete course of Organic Chemistry at the first year and have just followed a course that describes classes of biologically important organic compounds, including aminoacids.
For this experiments, students typically work in pair. This is particularly useful for the kinetic resolution (session II) where one of the student handles the burette, while the other one takes note of the added quantity at time intervals.

Session 1

It is important to use a reaction flask with a broad joint (29/32) because it facilitates both addition of phenylalanine and product filtration.
In our lab we use flat-bottomed flasks because we heat them directly with a stirring hot plate. However, if other heating means are used (e.g. sand baths, oil baths), round-bottomed flasks are equally suitable.
Apart from the safety precautions necessary to handle thionyl chloride, the most critical part of the experiment is the evaporation of the solvent. It is important to stop it at the right moment (see picture below), since excessive evaporation leads to the precipitation of a glassy solid that may be nearly impossible to convert into a crystalline powder upon addition of diethyl ether. In all cases it is better to add a few mL of methanol (not more than 5), grinding the thick oily residue with a spatula. Only when the precipitation has started, one can add diethyl ether.
Typical yields are between 60 and 75% and the melting point is 159-160°C

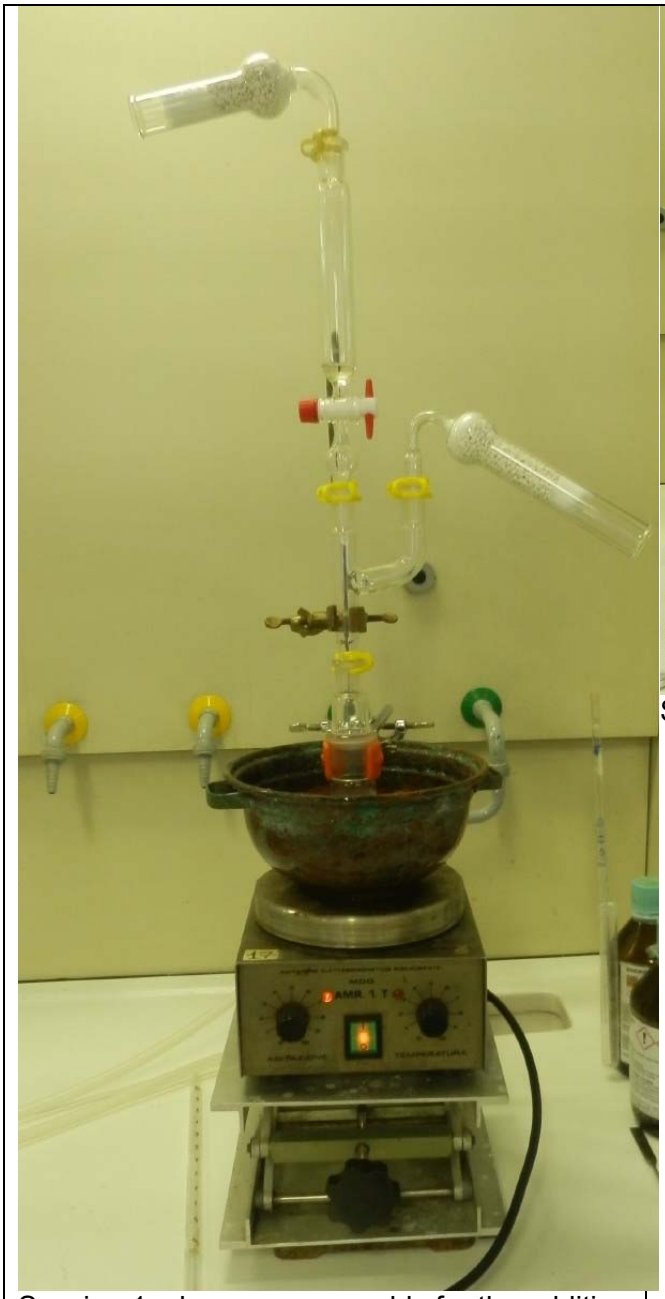
Session 2

The rate of enzymatic hydrolysis depends on the activity of the enzyme. We typically use α-Chymotrypsin type II purchased from Sigma-Aldrich (code C4129) (> 40 units/mg). With this enzyme the reaction is usually complete in 1 h.
A typical error made by students is to use aqueous HCl instead of ethereal HCl for the precipitation of the hydrochloride. This obviously leads to a two phase system. The solution is to bring back the pH to 9.5 and repeat the extraction.
Typical yields of 35-40% are obtained. Optical purities are always > 95%.
Although the optical power measurment takes only about 15-30 minutes for each student pair, if there is only one polarimeter available it is clearly impossible to perform the polarimetric measure during the same session for everybody. This experiment can thus be split in three parts. The last part will be

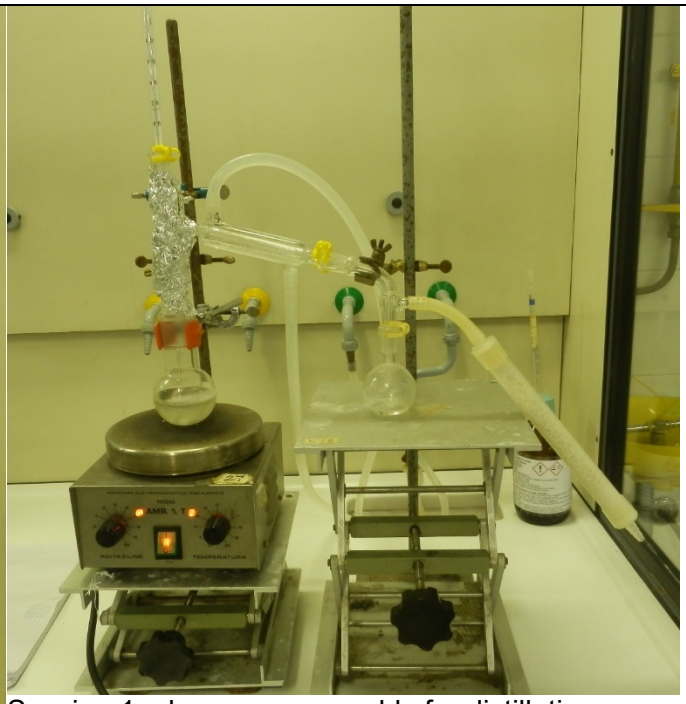
carried out by the students during another morning or afternoon, by appointment and under the supervision of a technician.

The reported $[\alpha]_D$ of pure L-phenylalanine methyl ester hydrochloride is = +38.0 (c 2, ethanol).

PICTURES



Session 1: glassware assembly for the addition of $SOCl_2$ to methanol.



Session 1: glassware assembly for distillation

Session 1: the flask containing crude product after methanol distillation

Session 1: the product after crystallization (addition of methanol and then diethyl ether)

Lipase Catalyzed Kinetic Resolution of Racemic 1-Phenylethanol

Supplementary Material

Background

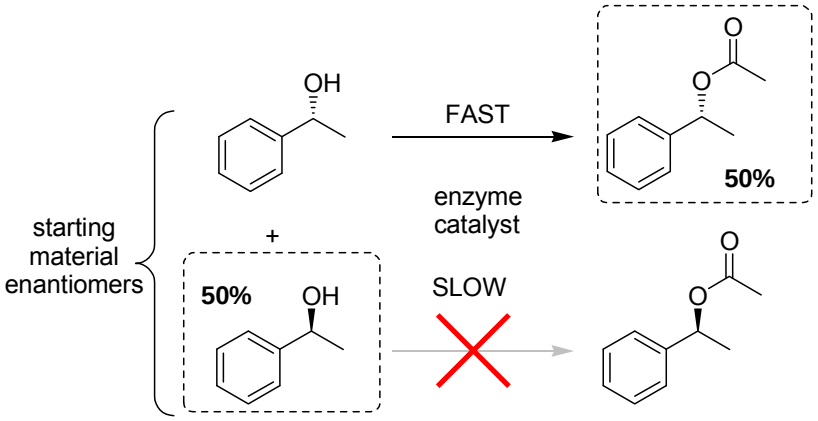
Stereochemistry as the topic can be motivated and introduced to the students by different means. One well inspiring approach is to demonstrate the different effects that enantiomers may have in various biological systems due to the chiral nature of the receptors. In fact, the increasing demand for enantiopure compounds for various modern applications, such as pharmaceuticals and agrochemicals, was our initial source of inspiration when developing the kinetic resolution laboratory experiment for undergraduate students.

In general, the main methods used for producing optically pure and isolated enantiomers or diastereomers, both in industry and academia, are based on:

1. asymmetric synthesis
2. classical racemate resolution by forming pairs of diastereomers which can then be isolated by conventional techniques
3. kinetic resolution

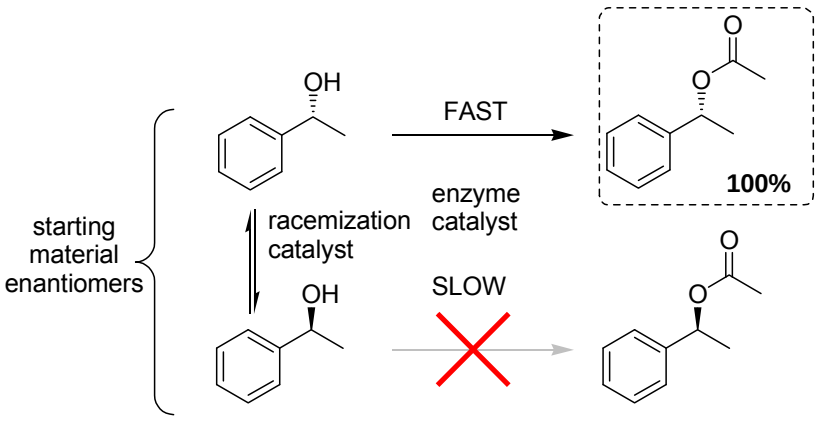
This laboratory experiment demonstrates the enzyme catalyzed kinetic resolution of two enantiomers using 1-phenylethanol as a model substrate. The starting material is extensively studied and the reaction is well documented by several research groups worldwide.

The scope of the laboratory work can be expanded by including the comparison between kinetic resolution (KR) and dynamic kinetic resolution (DKR) on discussion level. The enzyme catalyzed kinetic resolution of a racemic starting material is a useful tool for obtaining both enantiomers as optically pure products (Scheme SM **18.3.**1). The method is, however, limited to a theoretical yield of 50% for one enantiomer, as also mentioned in the experiment description. Notably, this characteristic feature of KR is not necessarily a drawback if both enantiomers are useful. The students can be reminded that the commercially available pure enantiomers of 1-phenylethanol, have possibly been produced utilizing a kinetic resolution of *rac*-1-phenylethanol. Notably, the separation of enantiomers increases the prize of the 1-phenylethanol substantially, which can easily be demonstrated by comparing the prices of the commercially available racemic 1-phenylethanol and the pure enantiomers.



Scheme SM 18.3.1 The concept of enzyme catalyzed kinetic resolution.

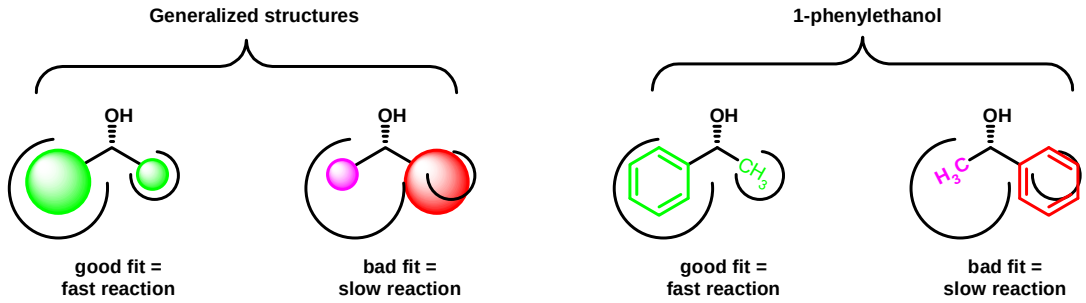
In order to overcome the drawback of 50% yield, various types of dynamic kinetic resolution (DKR) processes have been developed. In a DKR process, the slower reacting enantiomer is racemized *in situ* to the faster reacting enantiomer by a catalytic process (Scheme SM **18.3.**2). In a DKR process, the yield of 100% can be achieved, at least in theory. There is a range of modern catalysts, both homogeneous and heterogeneous, by which kinetic resolutions can efficiently be shifted into a DKR.[1,2,3] For example, the chemoenzymatic DKR of *sec*-alcohols, such as 1-phenylethanol, catalyzed by enzymes, such as CALB, combined with homogeneous half-sandwich type ruthenium based racemization catalysts are shown to be a very powerful tool for the synthesis of enantiomerically pure esters and, after hydrolysis, the corresponding chiral parent alcohol.[4,5]



Scheme SM 18.3.2 The concept of dynamic kinetic resolution utilizing both an enzyme and a racemization catalyst.

In order to determine which of the starting material enantiomers will be the faster reacting in a lipase catalyzed kinetic resolution, the so called *Kazlauskas rule* can be applied.[6] This rule is based on the size or bulkiness of the substrate and how it fits in specific pockets in the active site of the enzyme. Interestingly, most of the wild-type lipases, as also CALB, prefer to catalyze the

same enantiomer structure in the starting material (see Scheme SM **18.3.**3). In agreement with the *Kazlauskas rule,* CALB catalyze the esterification of (*R*)-1-phenylethanol.



Scheme SM 18.3.3 General demonstration of the Kazlauskas rule. Green color indicates good fit, in contrast to purple and red color which indicates bad fit.

Similar CALB catalyzed reactions with focus on education have previously been published in the literature.[7] Nevertheless, the objective of the experiment described here is to illustrate the easiest possible method to separate enantiomers and to follow the kinetic resolution by chiral chromatography. The experiment is aimed to strengthen the undergraduate student's knowledge and understanding in stereochemistry.

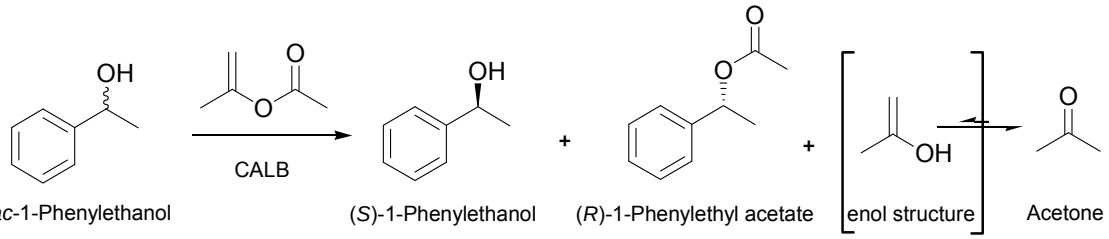
Study Level of the Laboratory Work

The requirement for the students performing the laboratory work has been that they have participated and completed the basic level studies in chemistry including the introductory and basic level course in organic chemistry. The laboratory work is planned to be as easy as possible to operate in order to be feasible even for students who have not been intensely trained in laboratory practice. Not surprisingly, we have found the laboratory experiment to be more easily conducted by students having practical laboratory background, including the handling of small laboratory apparatus as micropipettes. For the more trained students the modified version, where the two sessions are merged, is applicable (see Modifications of the experimental procedure).

Contextualization of the Laboratory Work

The laboratory experiment described here supports and practices the bachelor level students' knowledge in optical isomerism, exemplified by the enantiomers of 1-phenylethanol. The laboratory work gives a good practical example on how the separation of enantiomers can be achieved, even in large scale. Furthermore, answering the questions encourages the students to practice how to draw and identify enantiomers.

The explanation of how the reaction by-product, acetone, is formed can be a challenging and somewhat unexpected task for the students. The students can have some difficulties to identify the keto-enol tautomerism and some help can be needed (Scheme SM **18.3.**4). Anyhow, this theoretical exercise is a good supplement to the practical operation and teaches the students to think in chemical terms. The discussion with the students can be further developed to include the concept of chemical equilibrium and how the formation of acetone shifts or affects the balance between the esterification and reverse reaction, i.e. the hydrolysis. One further subject that can be addressed is the catalyst's ability to increase the reaction rate but not the thermodynamic equilibrium.



Scheme SM 18.3.4 Reaction scheme of the kinetic resolution with CALB and isopropenyl acetate displaying the keto-enol tautomerism.

Modifications of the Experimental Procedure

1. Optimization of the experiment procedure can be done by merging the two sessions. This modification has been found useful at our laboratory and works also well. The shorter time for conducting the experiment includes, though, more focused work and is recommended for smaller groups (fewer students) due to the requirement of a more intense guidance. The shorter laboratory work has found to be especially feasible for students who are more experienced in the laboratory practice.
2. The utilization of mild stirring speed is to prevent the undesirable grinding of the catalyst beads. The experiment can also be carried out using a horizontal linear shaker instead of a magnetic stirrer, when using the 4 mL vials as reaction vessels. In this case, the linear shaker speed is set to approximately 170 rpm.
3. One further modification is obviously to scale up the reaction. If the reaction is performed at larger scale (e.g. 1 g scale), some modifications of the catalyst and substrate concentrations are preferred. Additionally, for preparative scale reactions, the utilization of a mechanical stirrer should be considered. When the resolution is performed in larger scale, the isolation of the reaction products becomes more feasible. The isolation of the reaction product can be done by column chromatography or distillation.

4. GC peaks of underivatized alcohols are seldom very sharp and symmetric. The Varian CP7502 GC column applied, together with analytical details described below, separates the 1-phenylethanol enantiomers very well and the peak width is not an issue. Nevertheless, the reaction samples can be derivatized using for example propionic anhydride. In this case, the GC run time or oven temperature should be increased.
5. It is also possible to use (*S*)-1-phenylethyl acetate as a reference compound. The acetylation of (*S*)-1-phenylethanol is then carried out as described in the experimental procedure.

Experimental details

All chemicals were purchased from commercial sources and used without further purification except for the MTBE that was dried for at least 24 hours before use over 4 Å molecular sieves.

The GC instrumentation used was Agilent Technologies 6850 GC. The GC was equipped with a Varian CP7502 CP-Chirasil-Dex CB. Column dimensions; length: 24.8 m; diameter: 250 µm; film thickness: 0.25 µm. The analysis method details; inlet mode: split (1/50); inlet temperature: 250 °C; oven mode: isothermic temperature profile at 120 °C; run time: 18.00 min; post run temperature: 190 °C, post run time: 3.00 min.

Results

The enzymatic kinetic resolution described here has proved to be a reliable experiment giving results that are easy to reproduce. Representative chiral GC chromatograms are attached to this document (Schemes SM **18.3.**12 - SM **18.3.**17) and the calculated results collected in Tables SM **18.3.**1 and SM **18.3.**2.

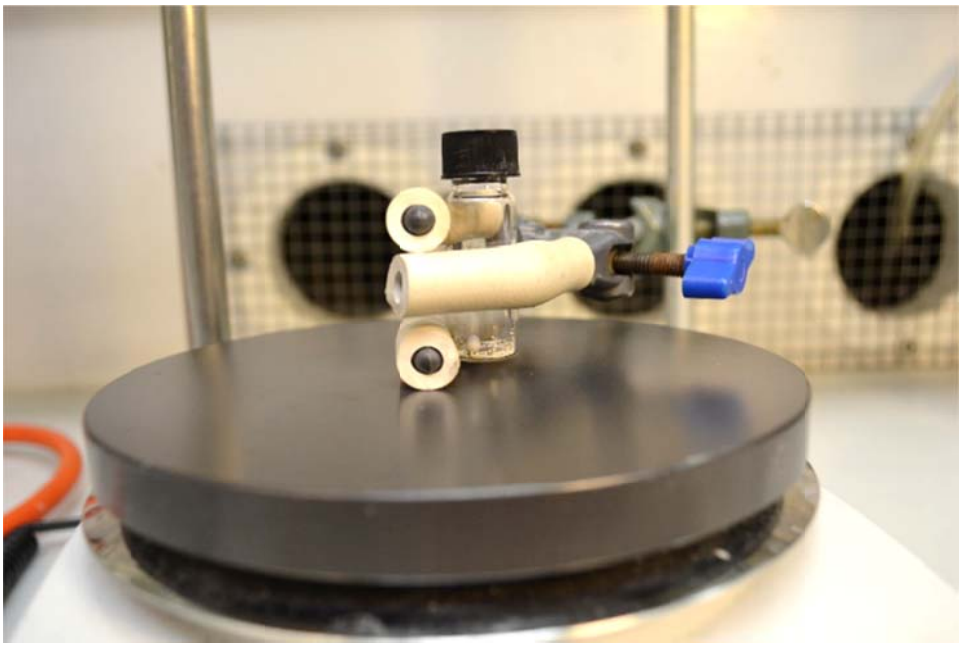
Table SM 18.3.1 Retention times for the compounds involved in the experiment.

Compound	**Retention time [min]**
(*S*)-1-phenylethyl acetate	8.64
(*R*)-1-phenylethyl acetate	9.74 ± 0.01
(*R*)-1-phenylethanol	13.16 ± 0.01
(*S*)-1-phenylethanol	14.12 ± 0.05

Table SM 18.3.2 The obtained *ee*-values, *E*-values and conversions at 0 min, 30 min, 60 min and 180 min.

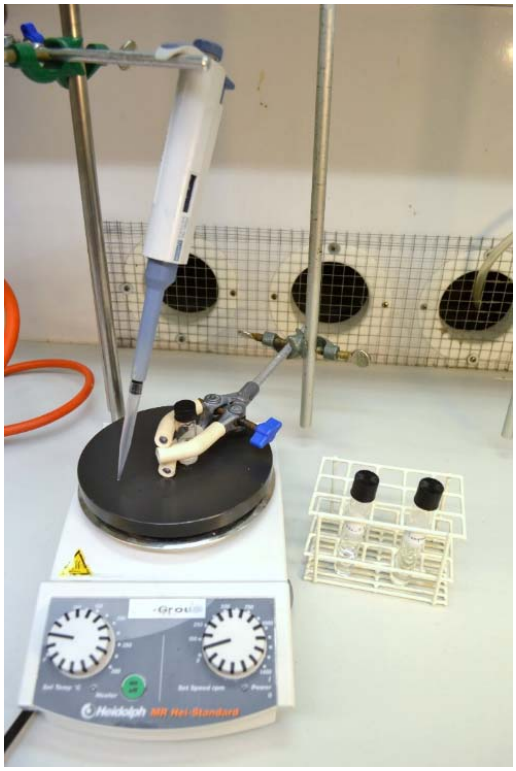
Reaction time [min]	$ee_{1\text{-phenylethanol}}$ **[%]**	$ee_{1\text{-phenylethyl acetate}}$ **[%]**	***E*-value**	**conversion [%]**
0	0	0	-	-
30	49.5	> 99.9	> 200 (≈ 3300)	33
60	73.5	> 99.9	> 200 (≈ 4400)	42
180	> 99.9	> 99.9	> 200 (≈ 15200)	50

Photos of experiment



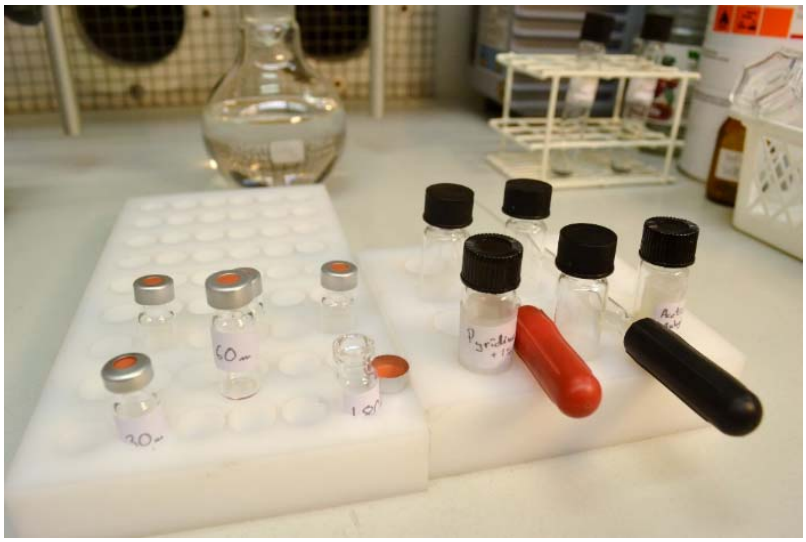
Scheme SM 18.3.7 The reaction vessel, a 4 mL vial, loaded with a small magnet stirring bar and cap containing 10 mg enzyme beads.

Scheme SM 18.3.8 Addition of 1.7 mL dry MTBE (4 Å mol. sieves) using a plastic syringe.



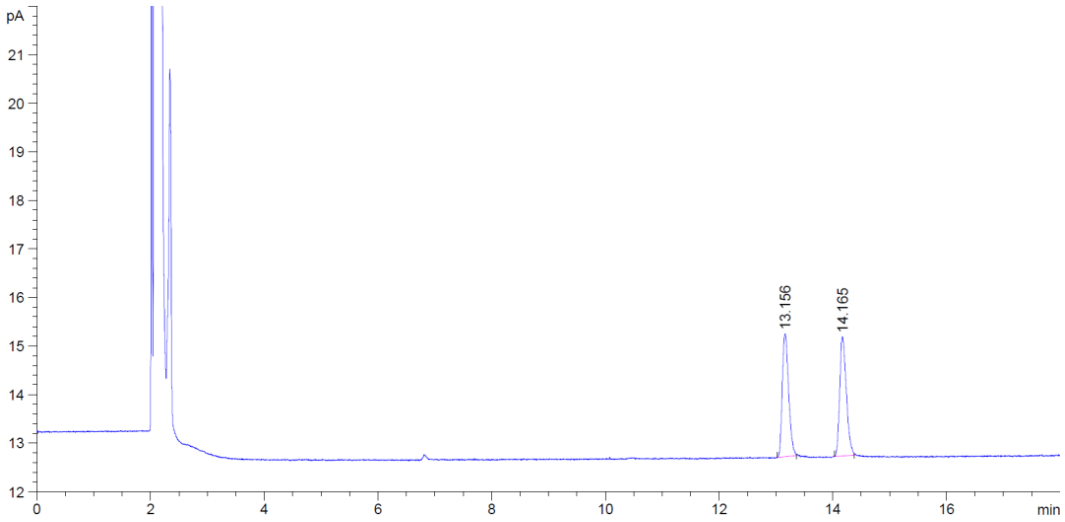
Scheme SM 18.3.9 Addition of *rac*-1-phenylethanol and isopropenyl acetate using a micropipette.

Scheme SM 18.3.10 Samples taken from the reaction are filtered with a syringe filter.

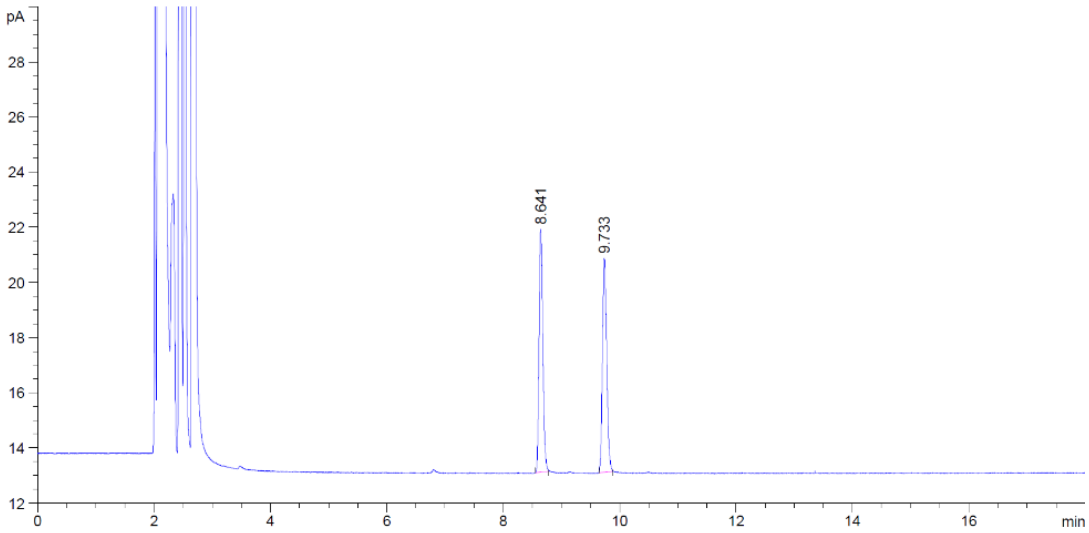


Scheme SM 18.3.11 30, 60 and 180 min reaction samples to the left and to the right GC sample derivatization reagents pyridine (containing <1% DMAP) and acetic anhydride.

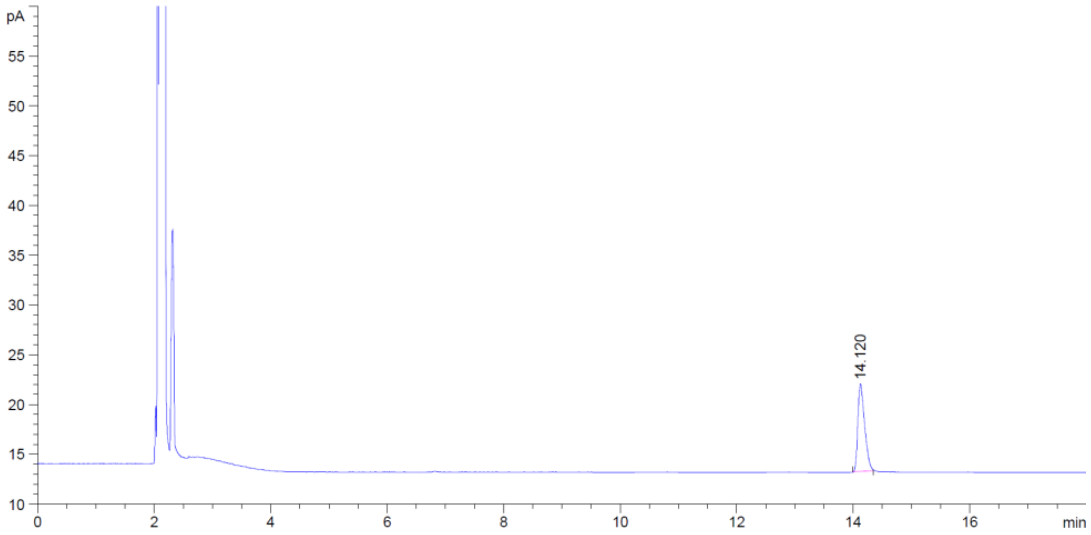
GC chromatograms



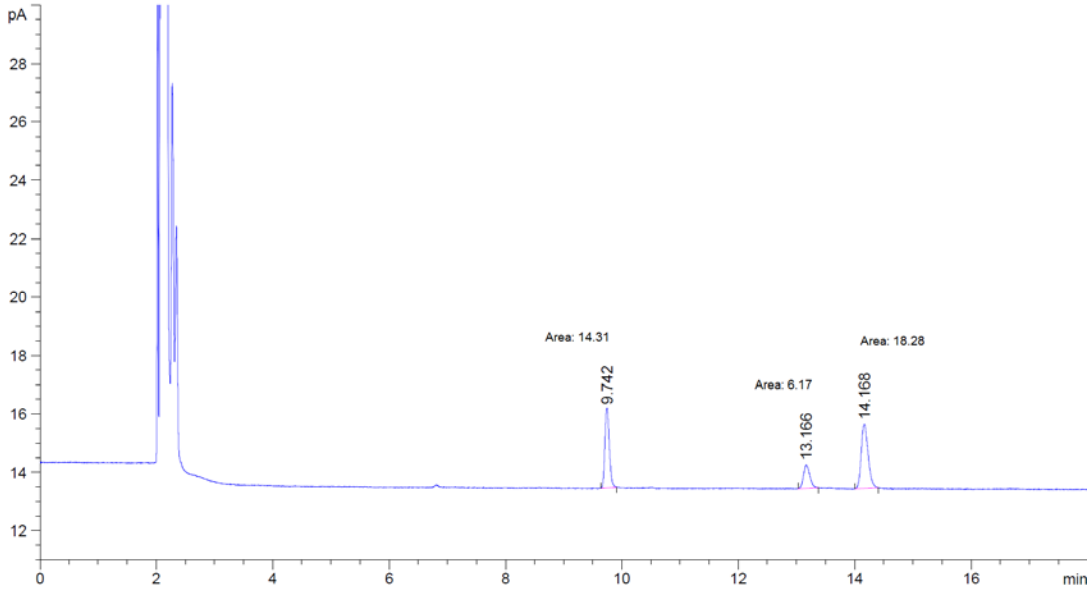
Scheme **SM 18.3.12** Chiral GC chromatogram of *rac*-1-phenylethanol.



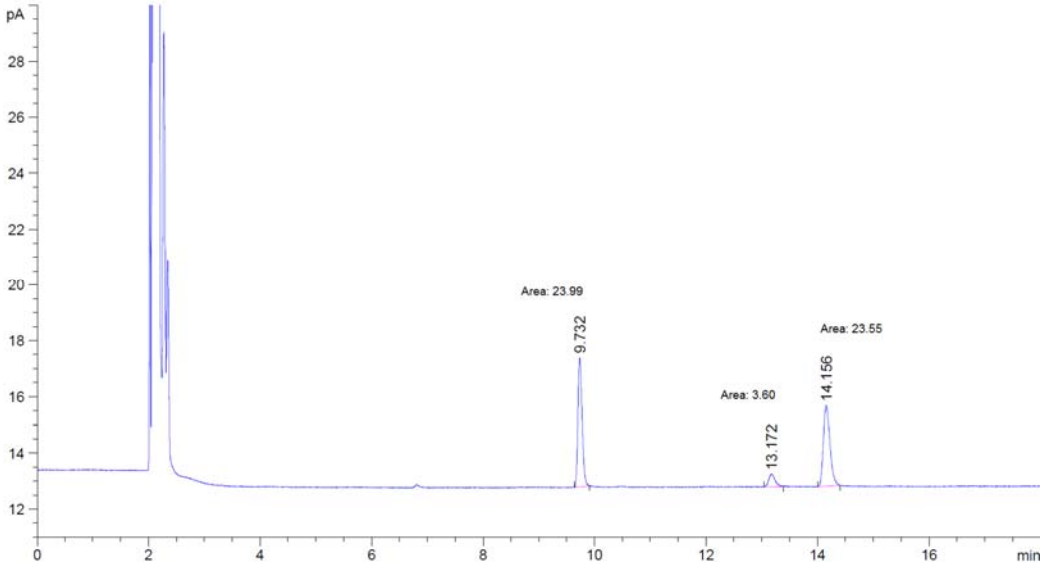
Scheme SM 18.3.13 Chiral GC chromatogram of *rac*-1-phenylethyl acetate.



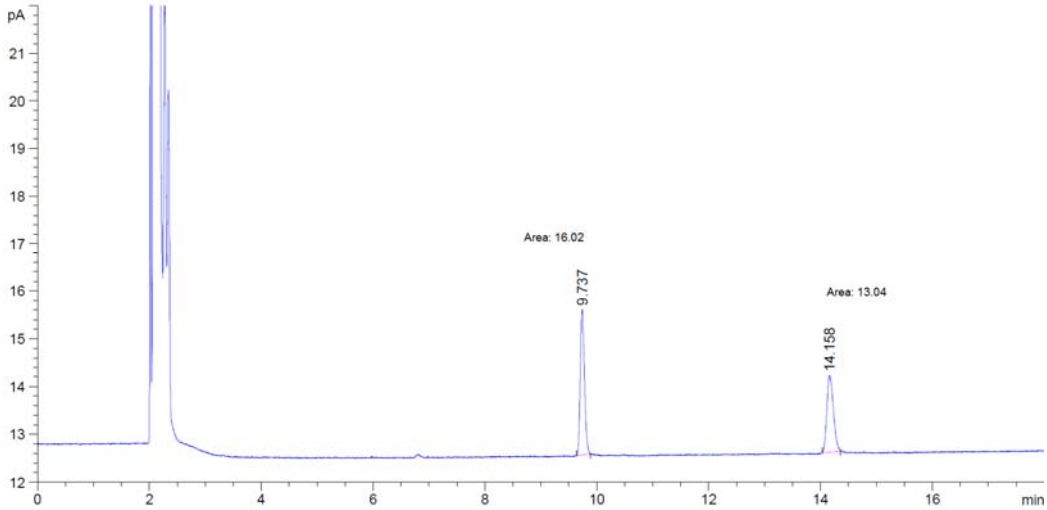
Scheme SM 18.3.14 Chiral GC chromatogram of (*S*)-1-phenylethanol.



Scheme SM 18.3.15 Chiral GC Chromatogram of the 30 min reaction sample.



Scheme SM 18.3.16 Chiral GC Chromatogram of the 60 min reaction sample.



Scheme SM 18.3.17 Chiral GC Chromatogram of the 180 min reaction sample.

[1] B. Martín-Matute, J.-E. Bäckvall, *Curr. Opin. Chem. Biol.,* 2007, **11**, 226.
[2] A. N. Parvulescu, J. Janssens, J. Vanderleyden, D. De Vos, *Top. Catal.,* 2010, **53**, 931.
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[5] D. Mavrynsky, L. T. Kanerva, R. Sillanpää, R. Leino, *Pure Appl. Chem.,* 2011, **83**, 479.
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[7] (a) J. Drouin, J. Costante, E. Guibé-Jampel, *J. Chem. Ed.,* 1997, **74**, 992. (b) D. Stetca, I. W. C. E. Arends, U. Hanefeld, *J. Chem. Ed.,* 2002, **79**, 1351. (c) F. Rebolledo, R. Liz, *J. Chem. Ed.,* 2005, **82**, 930. (d) C. M. Monteiro, C. A. M. Afonso, N. M. T. Lourenço, *J. Chem. Ed.,* 2010, **87**, 423. (e) J. A. Faraldos, J.-L. Giner, D. H. Smith, M. Wilson, K. Ronhovde, E. Wilson, D. Clevette, A. E. Holmes, K. Rouhier, *J. Chem. Ed.,* 2011, **88**, 334.

Enzymatic kinetic resolution and separation of sec-alcohols methodology based on fatty esters

Supplementary Material

Experiment Notes

General Remarks

All reagents used are commercially in Aldrich and Fluka. The enantiomer excesses (ee) were determined by GC analysis, performed using Trace Focus Unicam, FID detection, using capillary column astec chiraldex^{TM} G-TA (30m × 0.25mm × 0.12µm, ref.73033AST); Injector: 250ºC; detector: 250ºC; split ratio = 6, column flow (H_2): 60kPas (1.2 mL/min); oven:100ºC for 15 minutes and ramp 8ºC/min to 155ºC). NMR spectra were recorded at room temperature in a Bruker AMX 300 $CDCl_3$ as solvent and $(CH_3)_4Si$ (^{1}H) as internal standard. All coupling constants are expressed in Hz. For the procedures under vacuum it was used a diaphragm pump (1-760 mmHg).

Instructor notes for the experiment

As mentioned before, all chemicals are commercial available, but if necessary, the acylating agent (ethyl myristate) can be prepared by the undergraduate students. Nevertheless, students should be alerted that some of long fatty acids commercially available (used as starting material for the ester synthesis) are a mixture of several different ones. In this case, if the acylating agent is not properly purified by fractional distillation several difficulties can come out during isolation step of the 1-phenylethanol enantiomers resolution (please s*ee* Note 1.).

Enzymatic transesterification

- Some product may be lost during the distillation (2-3%), so it is advised to control the temperature and pressure carefully and adapt a trap to the apparatus.
- The reaction can also be well performed in a smaller scale (10 mmol) with good yields and enantiomer excesses and in higher scale (82 mmol, see details in Note 2). The recycle of the enzyme and ethyl ester is also possible without substantial loss of the enzyme activity.
- The distillation should be complete. The (*S*)-enantiomer that remains in the solution will deteriorate the *ee* of the second step.

Note 1: As part of the organic chemistry laboratory course, the enzymatic resolution of 1-phenylethanol was performed by one group undergraduate students.

The enzymatic resolution of 1-phenylethanol was performed following the general procedure; however a higher reaction scale was used because of being more appropriate for undergraduate classes. In this case it was used 4.52 g, 37.0 mmol of racemic 1-phenylethanol; 1.75g of CAL B (Novozym 435®) and 9.49g, 37.0 mmol of ethyl myristate. The reaction mixture was filtrated and the enzyme washed with hexane (3 x 45 mL). The enriched *(S)*-1-phenylethanol was isolated in 3.16g, 70% yield and 61% *ee* by distillation under reduced pressure (1mmHg, 60ºC, 2h or 20 mmHg, 84ºC, 2h30). For the isolation of bound enantiomer by enzymatic transesterification was used ethanol (1-2.5 eq.). The reaction mixture was filtrated again and the enzyme washed with hexane (3 x 45 mL). The enriched *(R)*-1-phenylethanol was isolated in 1.75g, 39% yield and 76% *ee* by distillation under reduced pressure (1mmHg, 60ºC, 2h or 20 mmHg, 84ºC, 2.5h).

After isolating both enantiomers, the overall yield of both isolated enantiomers was higher than 100%. After analysing the reaction, we noticed that this result is due to the occurrence of distillation of several smaller fatty esters as impurities during the isolation step of each enantiomer. When the acylating agent was previously purified by distillation no problems occur during isolation of 1-phenylethanol enantiomers. Using the same conditions and with a more efficient distillation, the students were able to isolate (*S*)-1-phenylethanol in 41% yield and 80% ee, from the first enzymatic reaction. From the second enzymatic reaction, (*R*)-1-phenylethanol was also isolated in 56% yield and 63% ee.

Note 2: As part of the organic chemistry laboratory course, the enzymatic resolution of 1-phenylethanol was performed by one group of undergraduate students.

Another experiment of enzymatic resolution of 1-phenylethanol was also performed following the general procedure using a higher reaction scale and more common laboratory tools in order to become more feasible in undergraduate classes (Figure SI 2). In this case it was used 10.0 g, 81.7 mmol of racemic 1-phenylethanol; 3.0 g of CAL B (Novozym 435®) and 23.28 g, 81.7 mmol of ethyl myristate. The reaction mixture was filtrated and the enzyme washed with hexane (3 x 100 mL). The enriched *(S)*-1-phenylethanol was isolated in 5.8 g, 58% yield and 40% *ee* by distillation under reduced pressure (20 mmHg, 84ºC, 3h, Figure SI 4). For isolation of the bound enantiomer by enzymatic transesterification enantiomer was used ethanol (2.5 eq.). The reaction mixture was filtrated again and the enzyme washed with hexane (3 x 100 mL). The enriched *(R)*-1-phenylethanol was isolated in 3.8 g, 38% yield and 62% *ee* by distillation under reduced pressure (20 mmHg, 84ºC, 2.5 h).

Figures

Experiment Photos

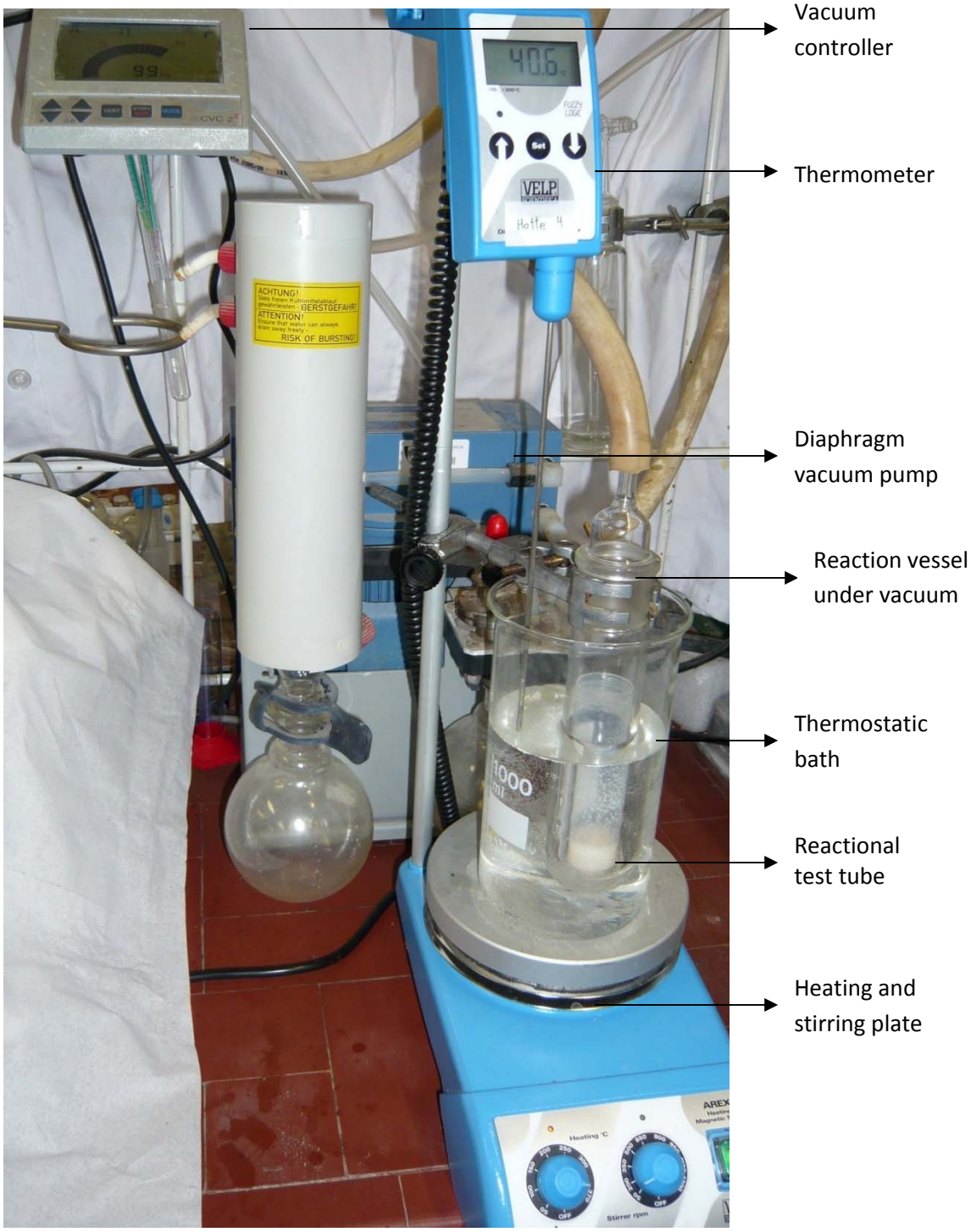


Figure SI 1- Apparatus used for the kinetic enzymatic resolution.

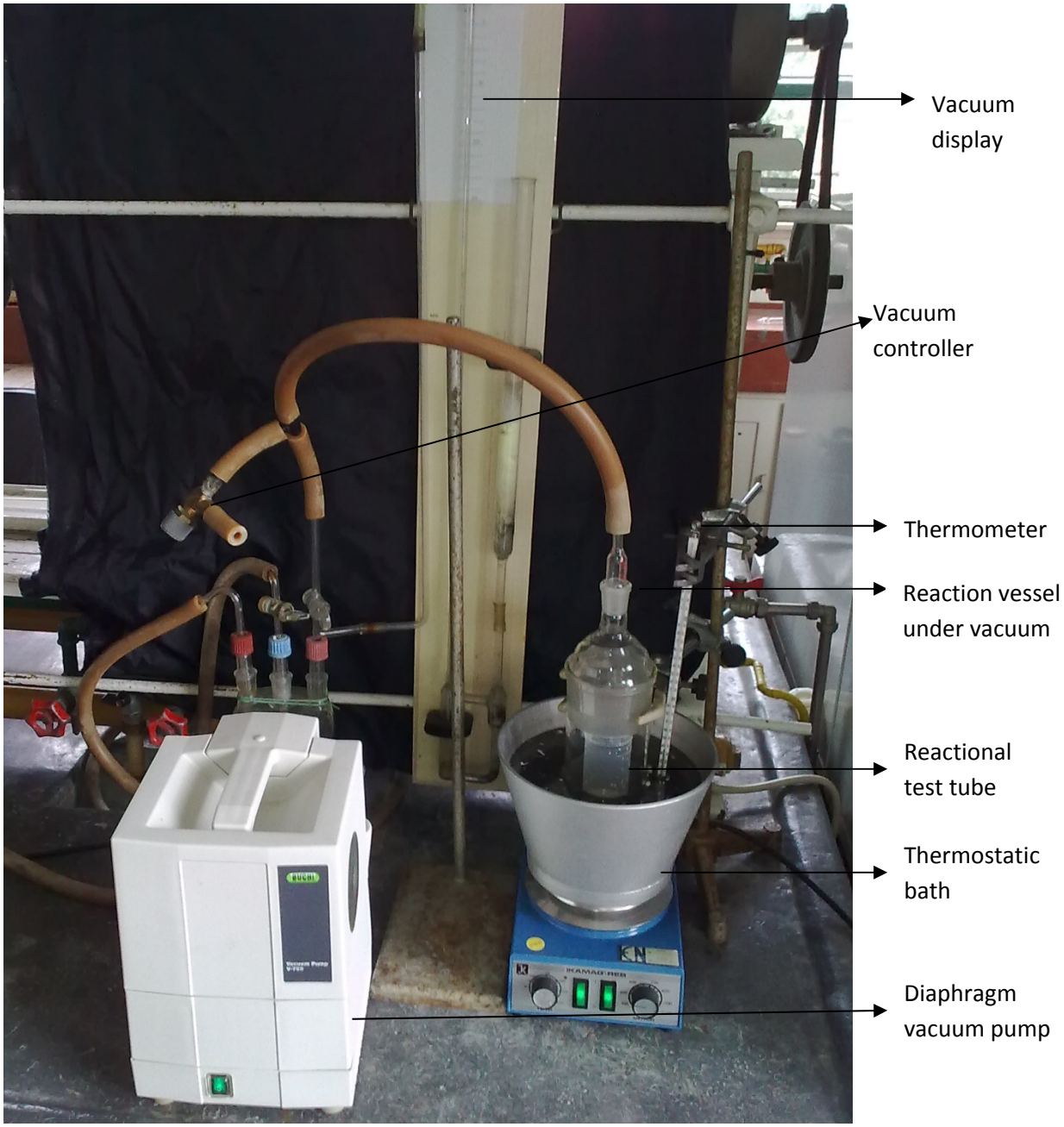


Figure SI 2 - Apparatus used for the kinetic enzymatic resolution in higher scale (10 g of racemic 1-phenylethanol).

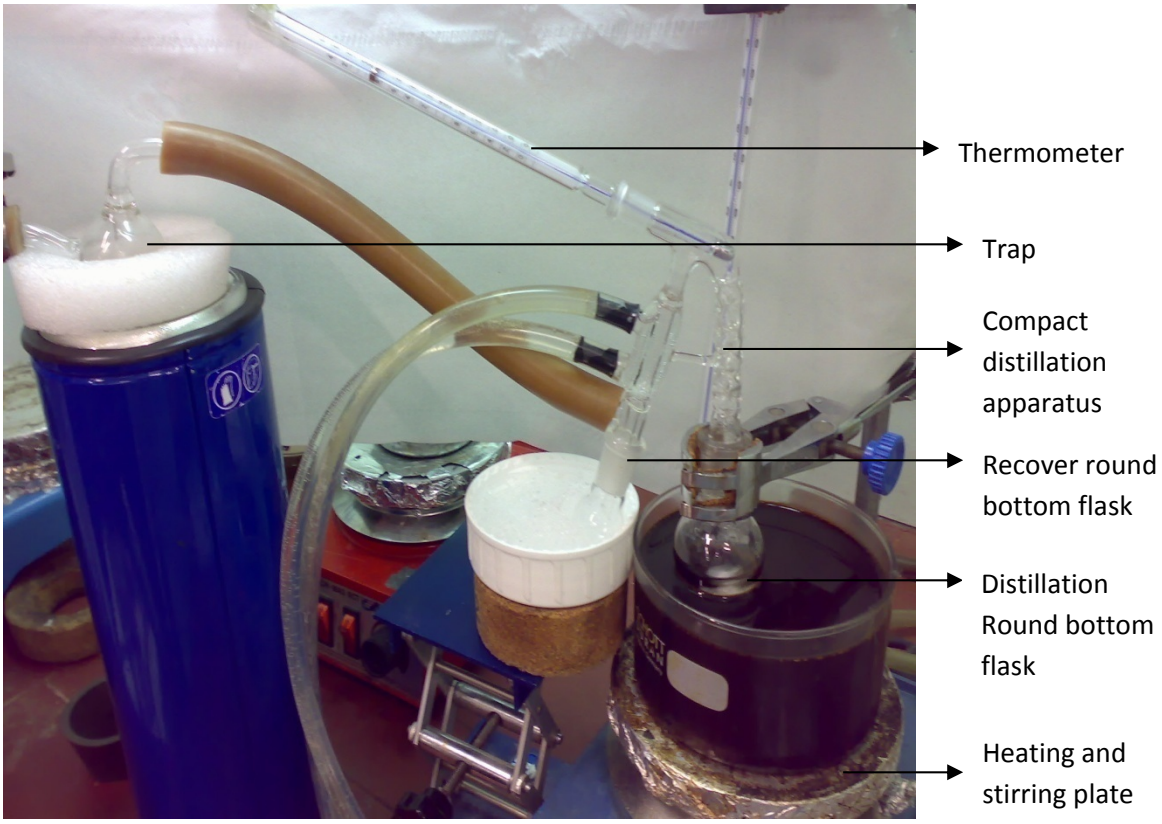


Figure SI 3 – Apparatus for the distillation under vacuum of the 1-phenylethanol.

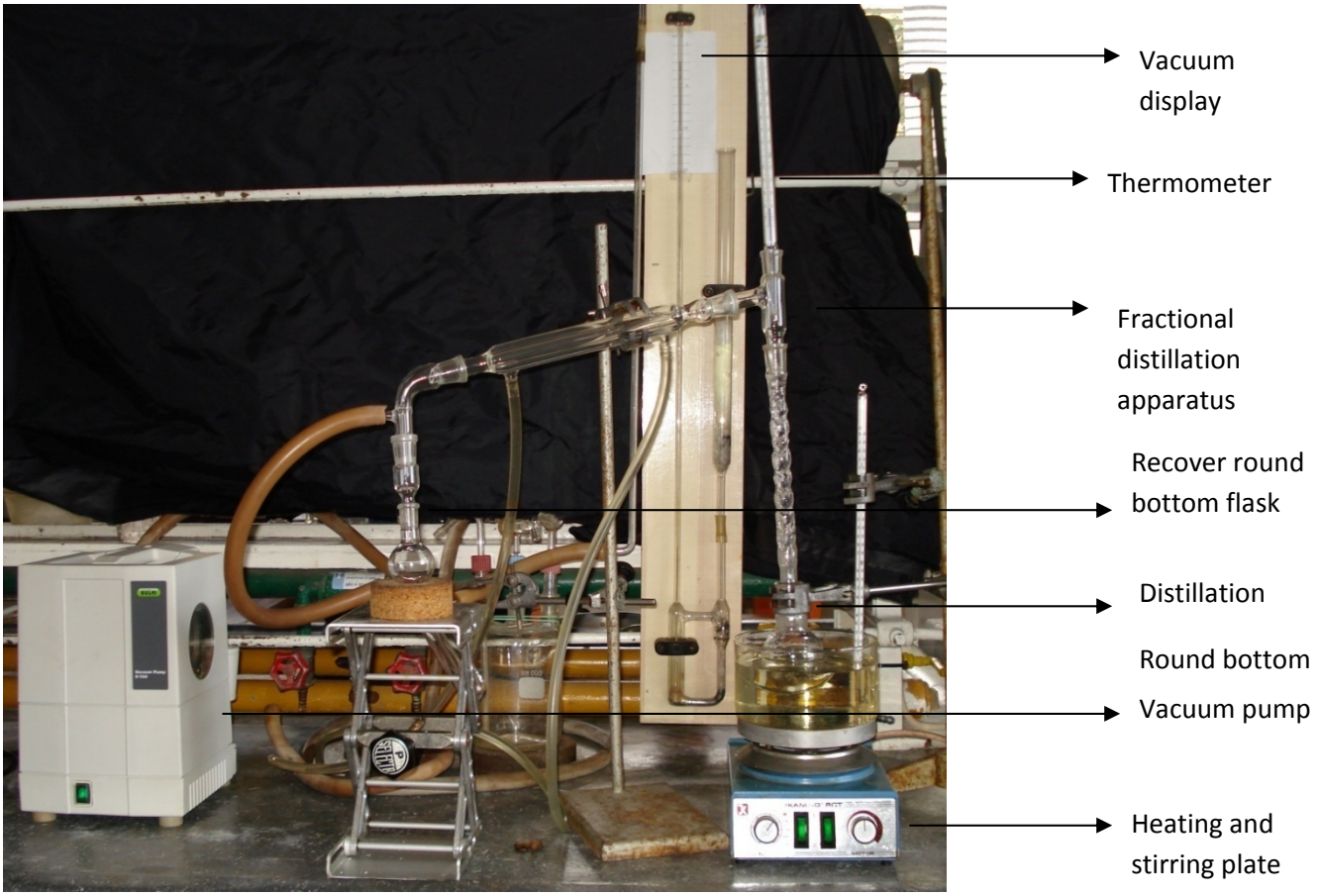


Figure SI 4 – Apparatus for the distillation under vacuum of the 1-phenylethanol in a higher scale (10 g of racemic 1-phenylethanol).

Products characterisation by ^{1}H and ^{13}C NMR and GC chromatograms

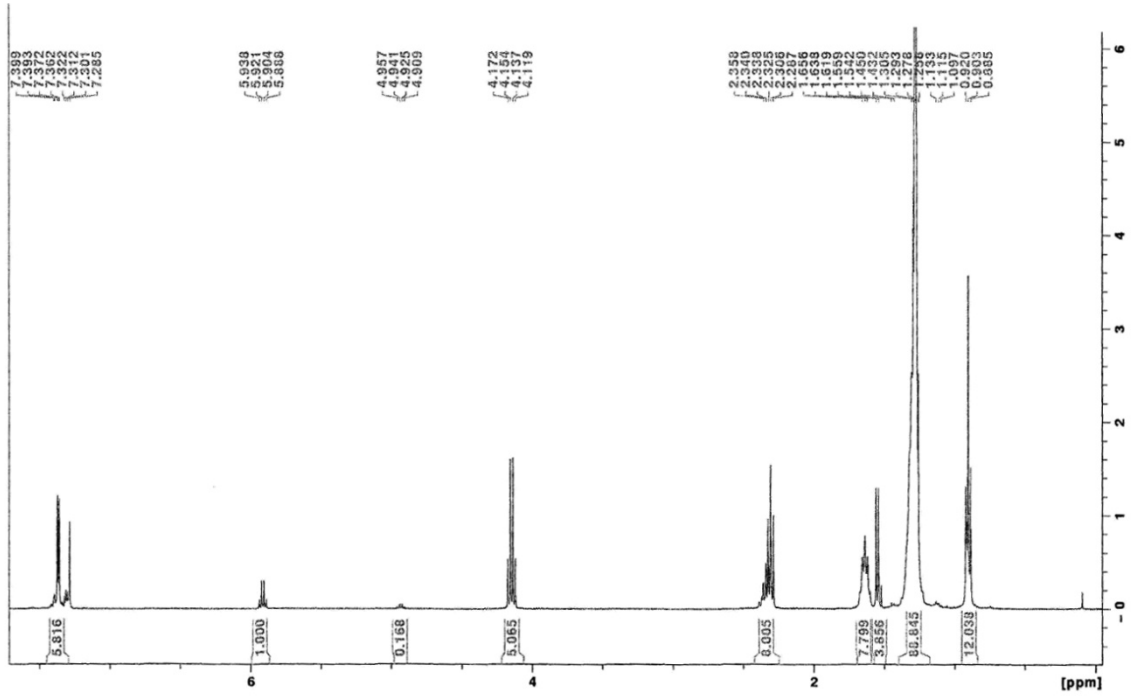


Figure SI 5. ^{1}H NMR spectrum (in $CDCl_3$) of the reaction medium after the first step containing ethyl myristate and (*R*)-1-phenylethyl myristate.

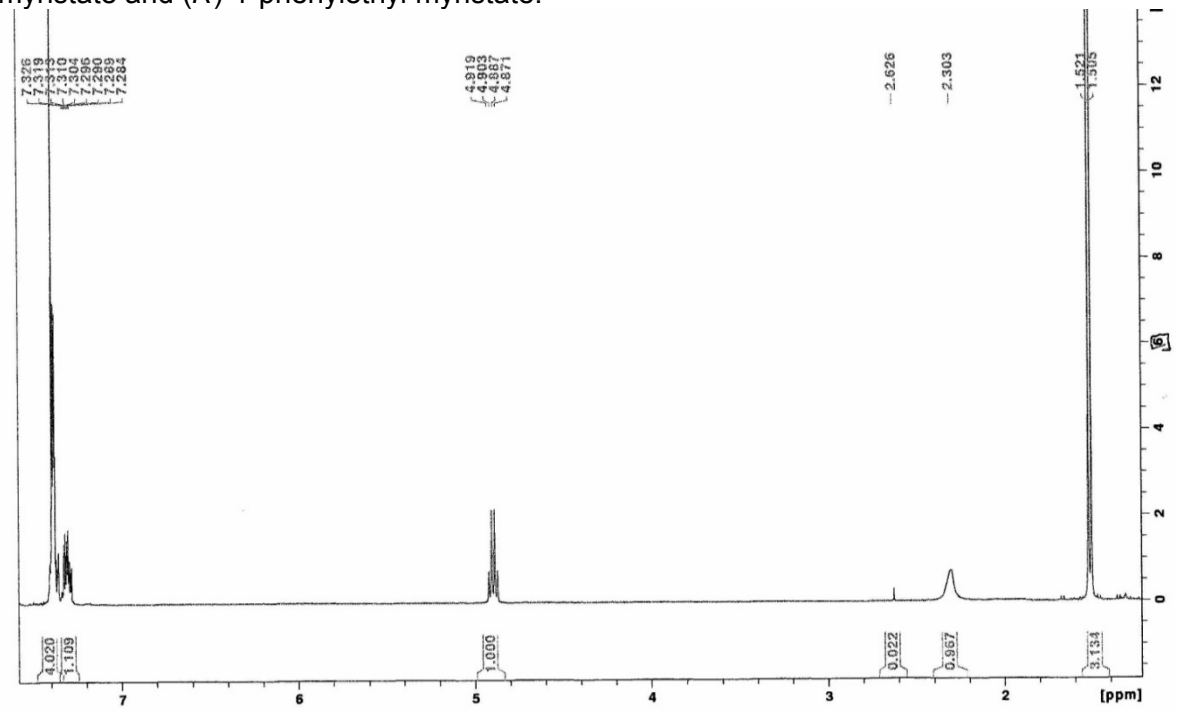


Figure SI 6. ^{1}H NMR spectrum (in $CDCl_3$) of the distilled (S)-1-phenylethanol after the first step.

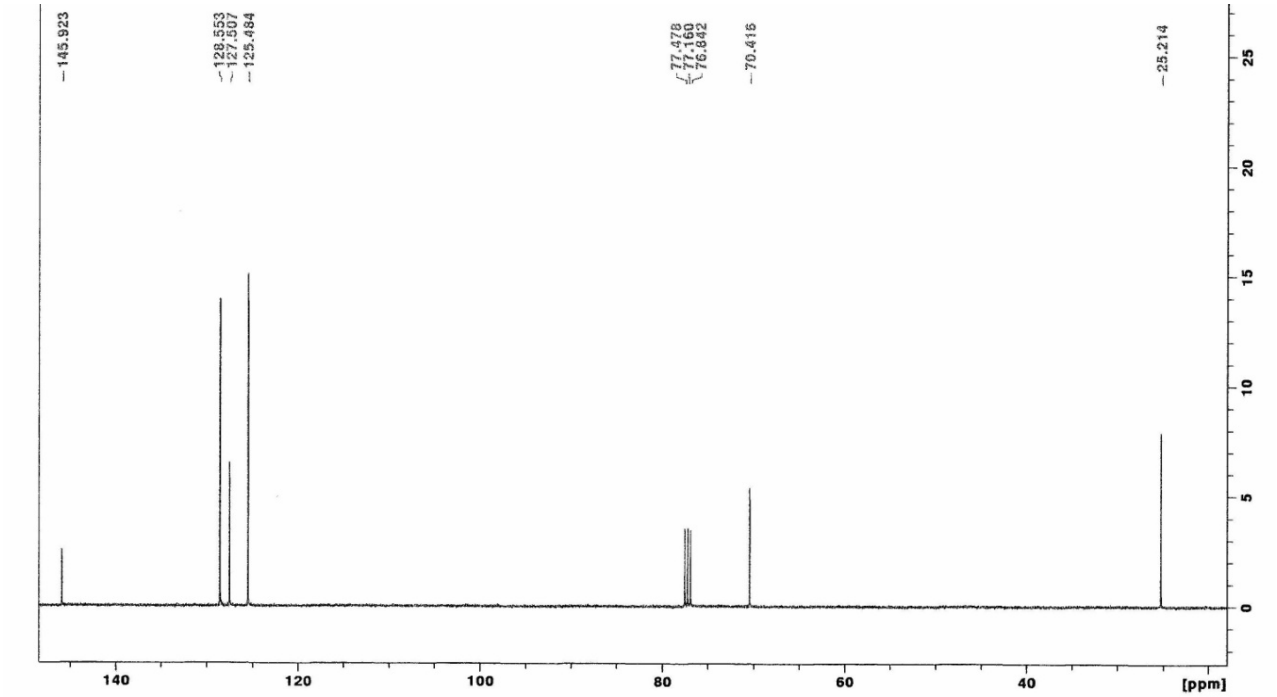


Figure SI 7. ^{13}C NMR spectrum (in $CDCl_3$) of the distilled (S)-1-phenylethanol after the first step.

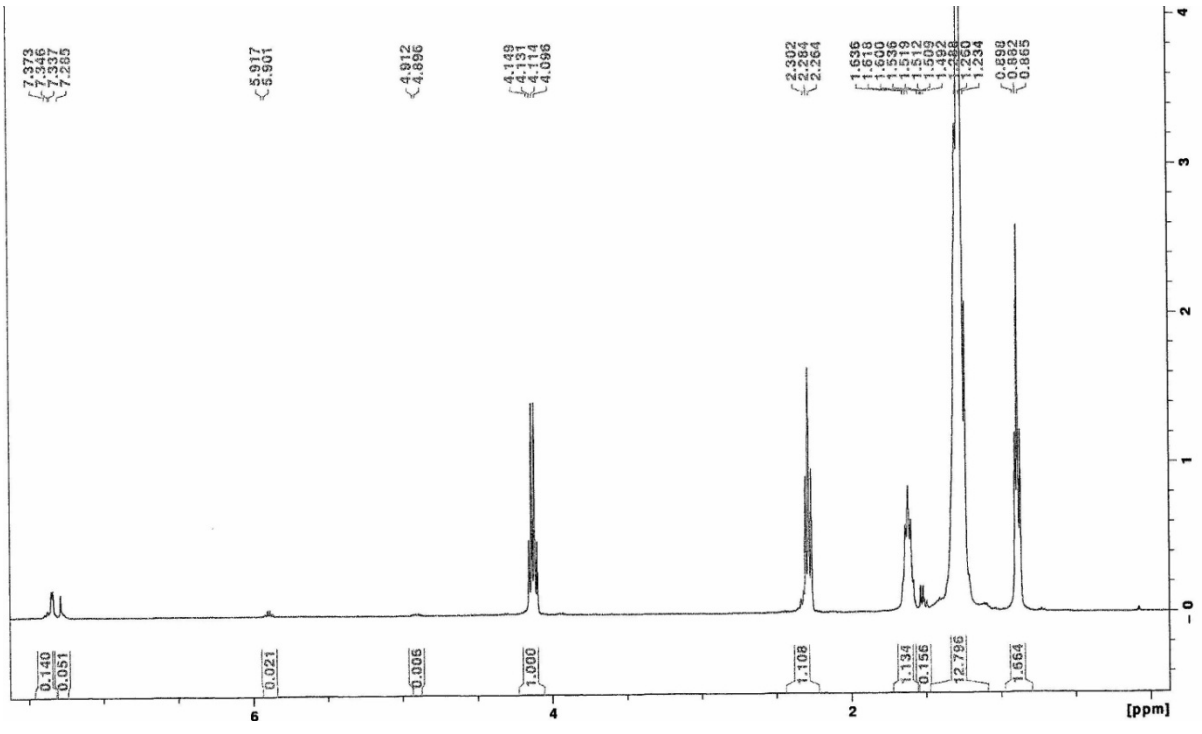


Figure SI 8. ^{1}H NMR spectrum (in $CDCl_3$) of the ethyl myristate after the second step.

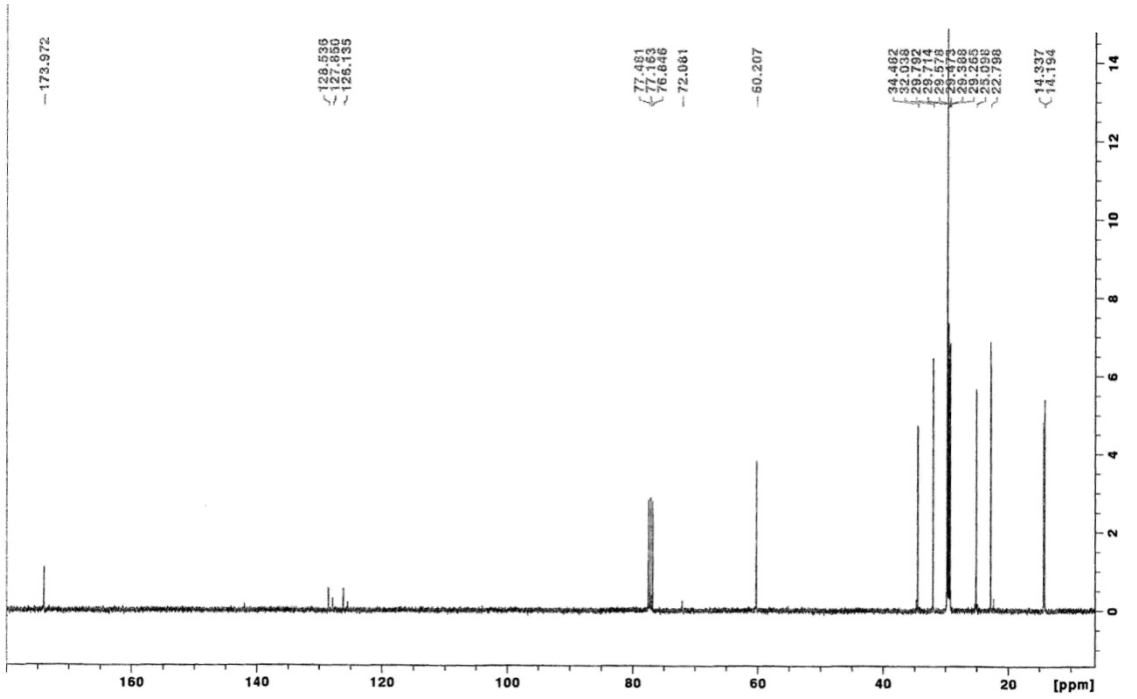


Figure SI 9. ^{13}C NMR spectrum (in $CDCl_3$) of the ethyl myristate after the second step.

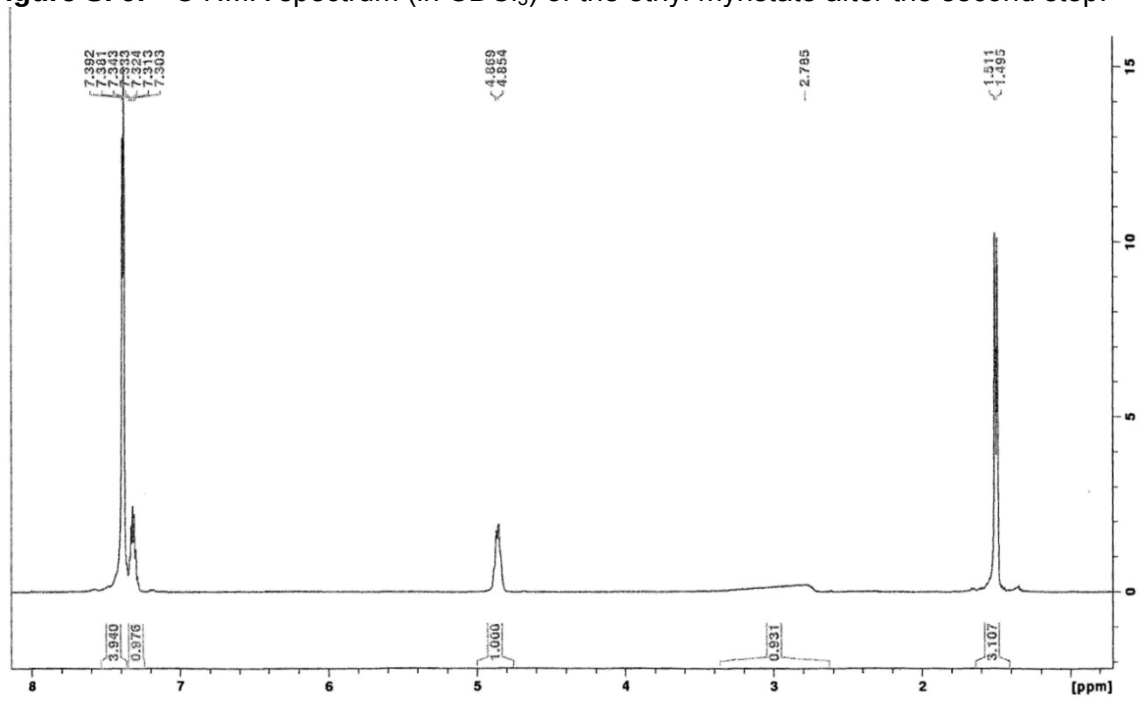


Figure SI 10. ^{1}H NMR spectrum (in $CDCl_3$) of the (*R*)-1-phenylethanol after the second step.

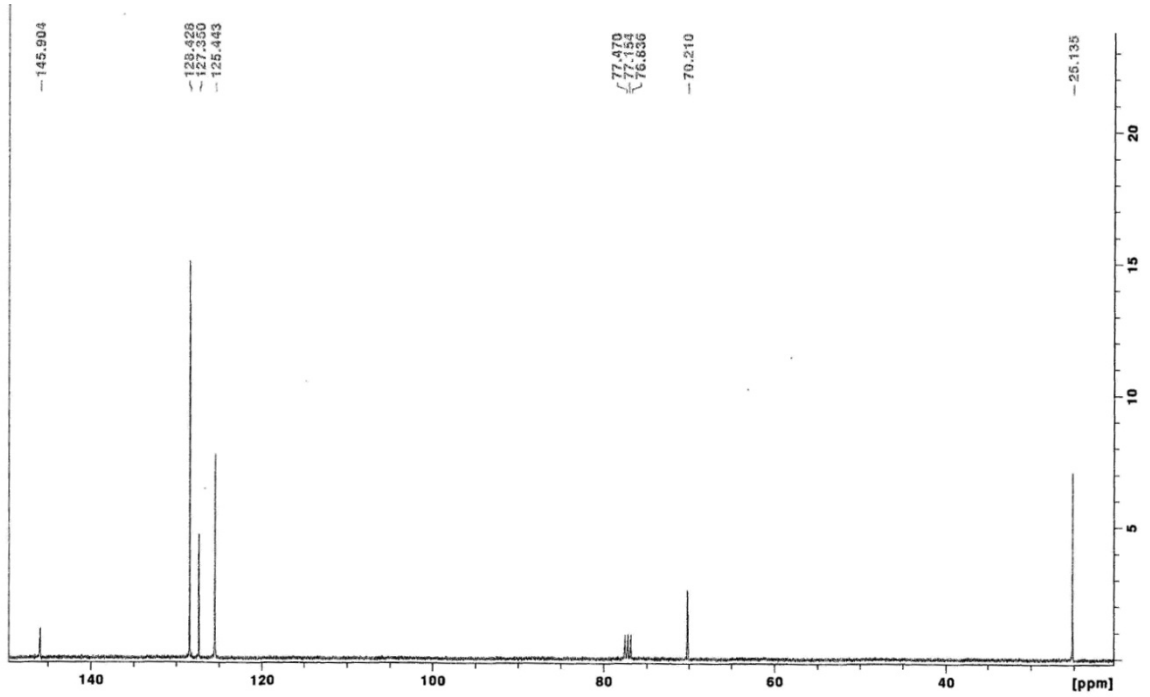


Figure SI 11. ^{13}C NMR spectrum (in $CDCl_3$) of the (*R*)-1-phenylethanol after the second step.

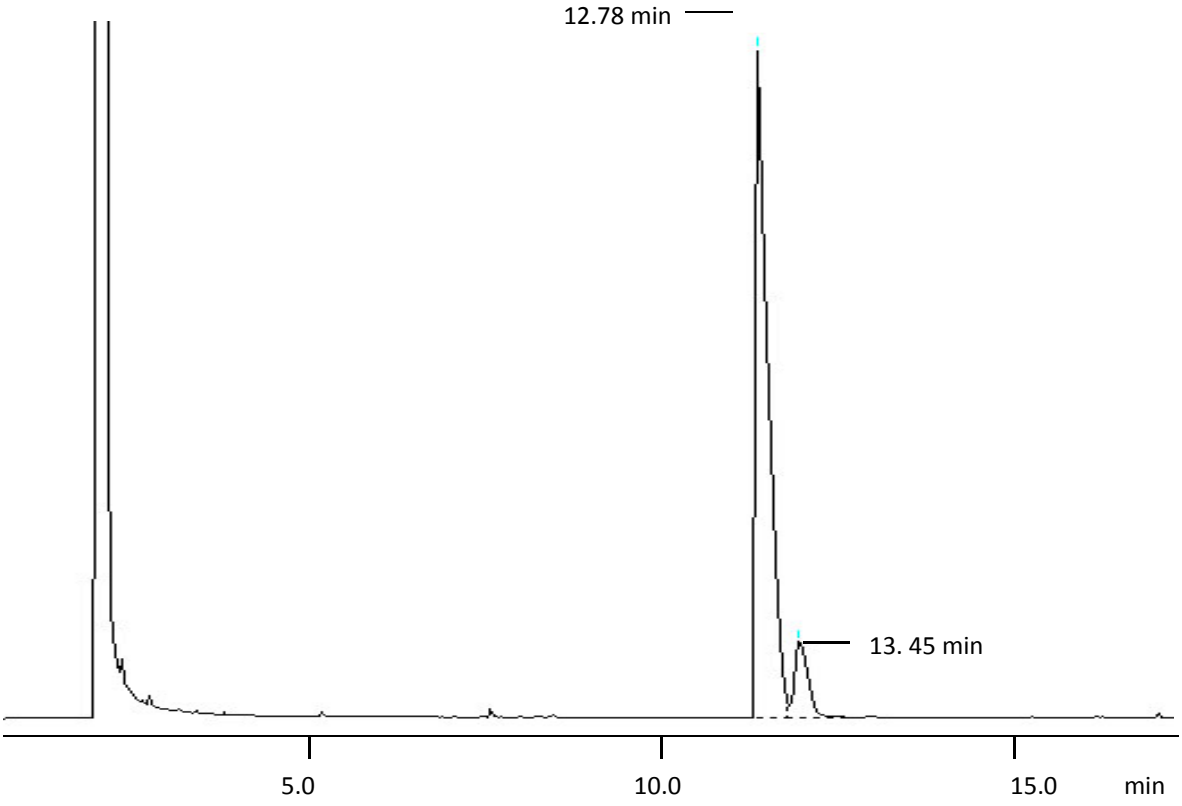


Figure SI 12. GC chromatogram of the distilled (*S*)-1-phenylethanol (*ee* =80%).

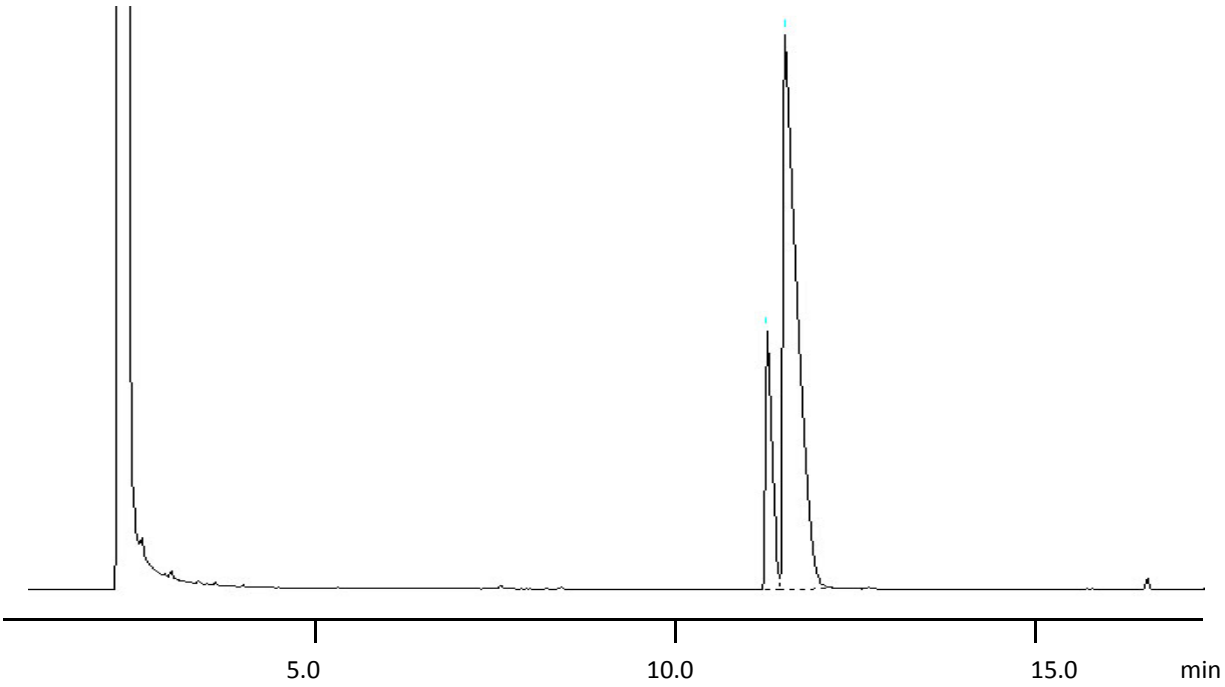


Figure SI 13 GC chromatogram of the distilled (*R*)-1-phenylethanol (*ee*=63%).

Enzymatic kinetic resolution and preparative separation of secondary alcohols

Supplementary Material

Experiment Notes

Instructor notes for the experiment

This experiment was developed by this research team and afterwards performed by one group of two undergraduated Chemistry students in Instituto Superior Tecnico, University of Lisbon inserted in the course of Laboratories II.

Note 1 – Preparation of the Ionic Liquid [Aliquat][dca]

The preparation of the ionic liquid is extremely reproducible. However, sometimes it is necessary to dry the ionic liquid under reduce pressure and stirring (if necessary heat it up until 60ºC) for 18h, in order to remove the residual water. Avoid to heat the liquid ionic over 60ºC in order to circumvent the degradation of the dicyanamide anion. If possible, quantify the amount of water present in the IL by Karl-Fischer titration. The amount of water in the reaction medium is crucial because it can react with the vinyl butyrate and form the butanoic acid. Especially in this experiment, where it is not used a significant excess of vinyl butyrate, all the carefulness is necessary.

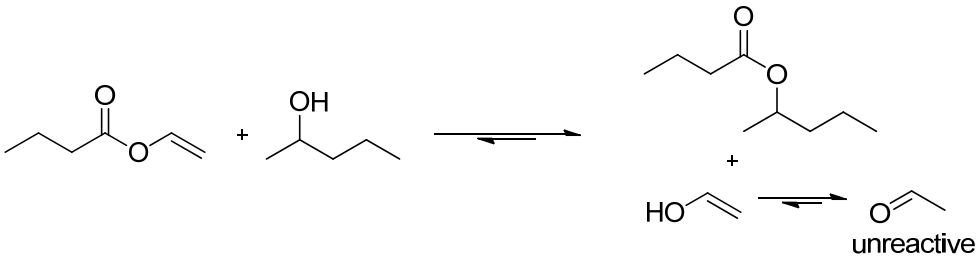
Note 2 - Enzymatic resolution

The enzymatic reaction was carry out by the students in smaller scale (4.0 g). They obtained in the first step (distillation at 20 mmHg, water bath at 50ºC) 31% yield and 72% *ee* (measured by Chiral GC) of (*S*)-(+)-2-pentanol. The figures presented show the spectral data obtained and described for the IL, 2-pentanol and pentyl *n*-butyrate.

Note 3

This work demonstrates the potential of enzymatic transformations and, simultaneously, the advantage of the usage of non-volatile reaction medium for the isolation of the volatile product. The distillation under vacuum allows to teach the effect of the pressure in the boiling point of volatile products. In this way, by using the temperature-pressure diagram (Figure 10), the students can predict the boiling point of the volatile product at different pressures and decide the best temperature to perform the distillation.

The use of vinyl butyrate as acyl donor allows to discuss the importance of irreversible resolution. The reversibility of the reaction may cause erosion of the *ee* in the first step and the yield in the second step.



Also, the usage of IL may be discussed, taking especial attention to their properties, applications and the possibility of reutilization. Another point of discussion is the atom efficiency and E-factor and the sustainability of this approach. Alternatively, there is another approach, developed by this team, for

resolution of secondary alcohols using an ionic acylating agent and the same enzyme in both enzymatic steps.[1]

Figures - *Products characterisation by ^{1}H and ^{13}C NMR, IR spectra*

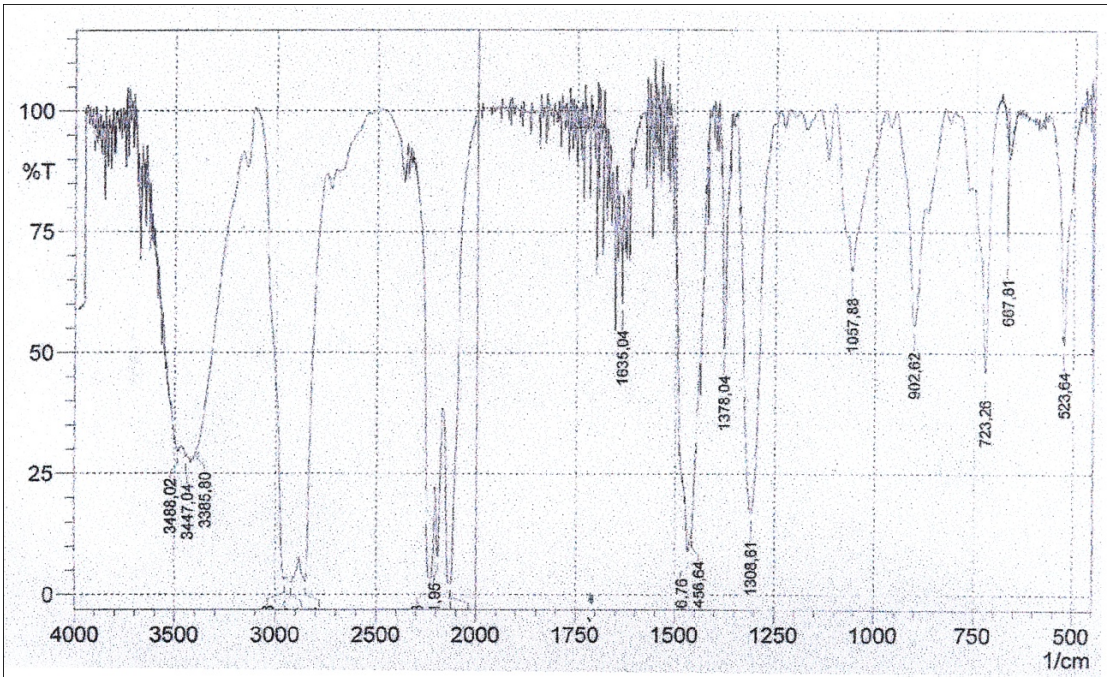


Figure 1. Infrared spectrum (film) of the IL [Aliquat][dca].

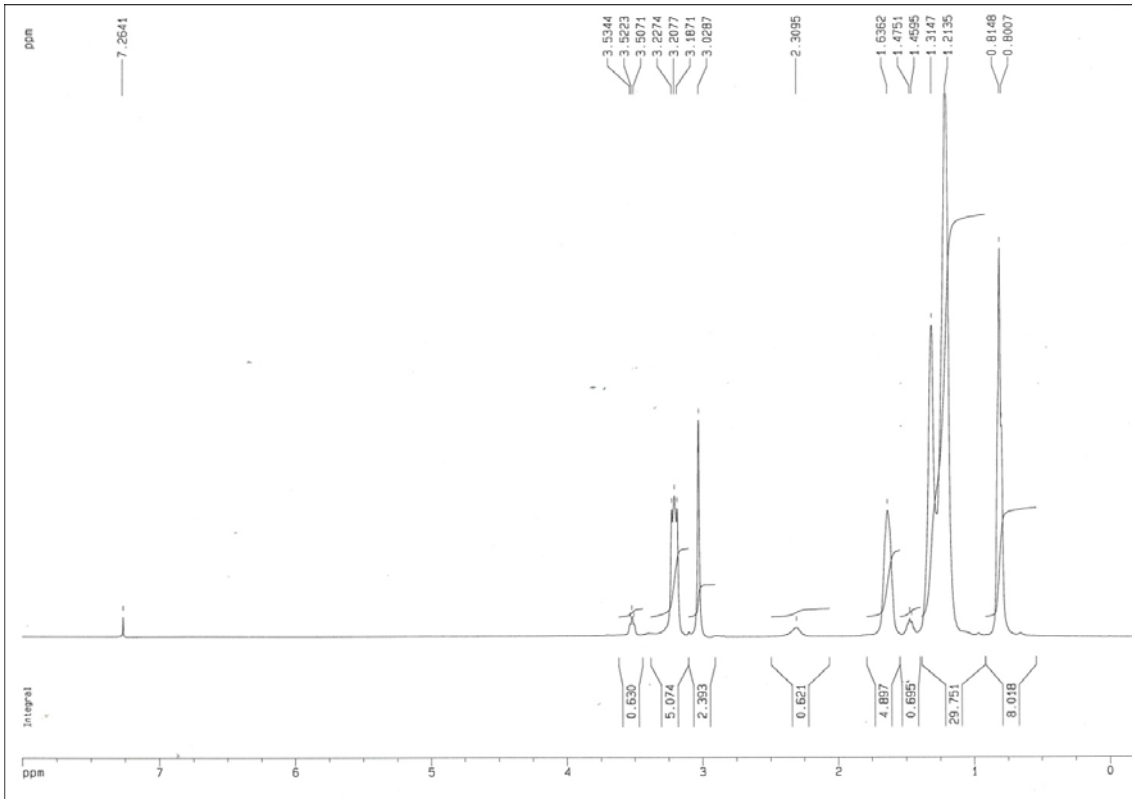


Figure 2. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of the IL [Aliquat][dca].

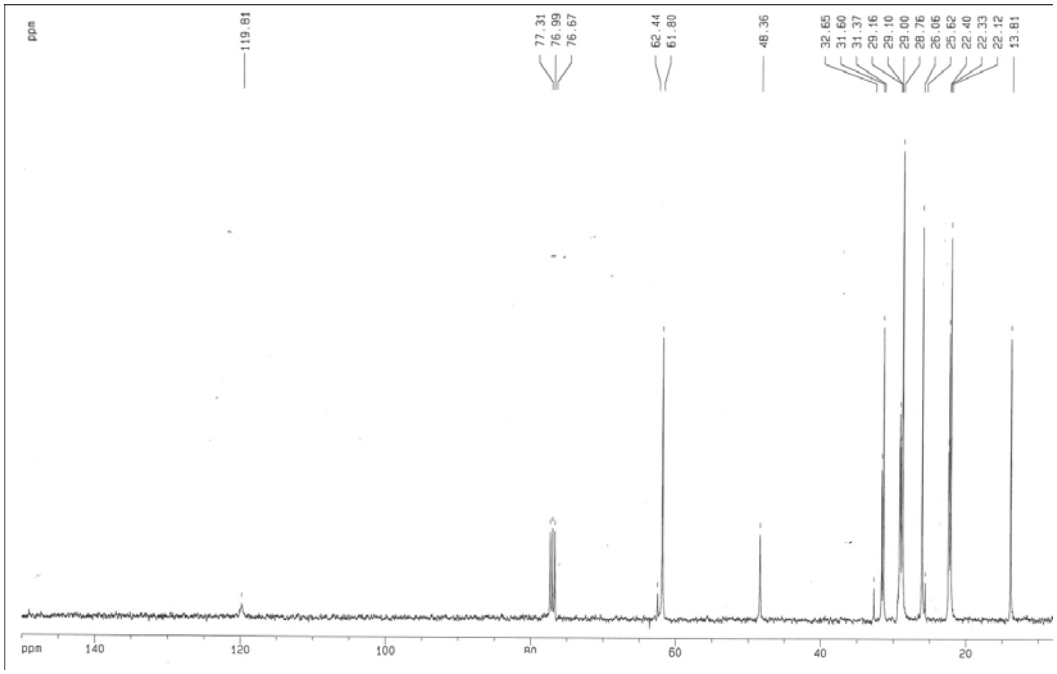


Figure 3. ^{13}C NMR (400 MHz, $CDCl_3$) spectrum of the IL [Aliquat][dca].

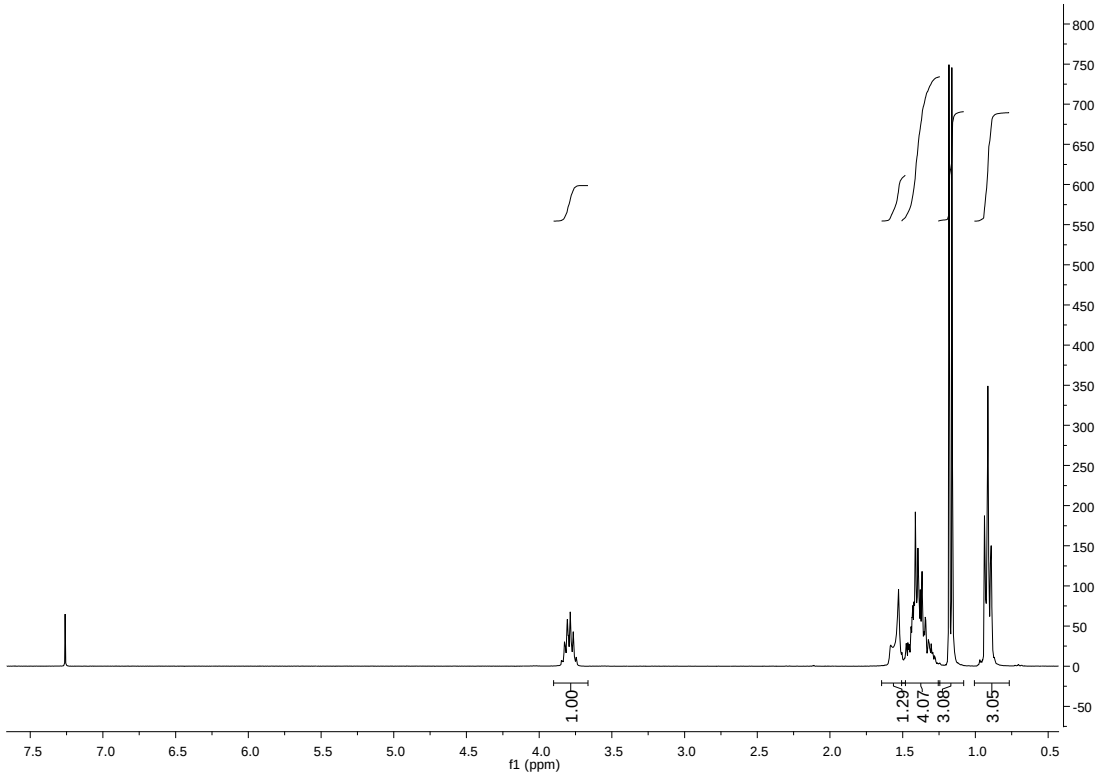


Figure 4. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of the commercial 2-pentanol 98% (Riedel-de Haen®)

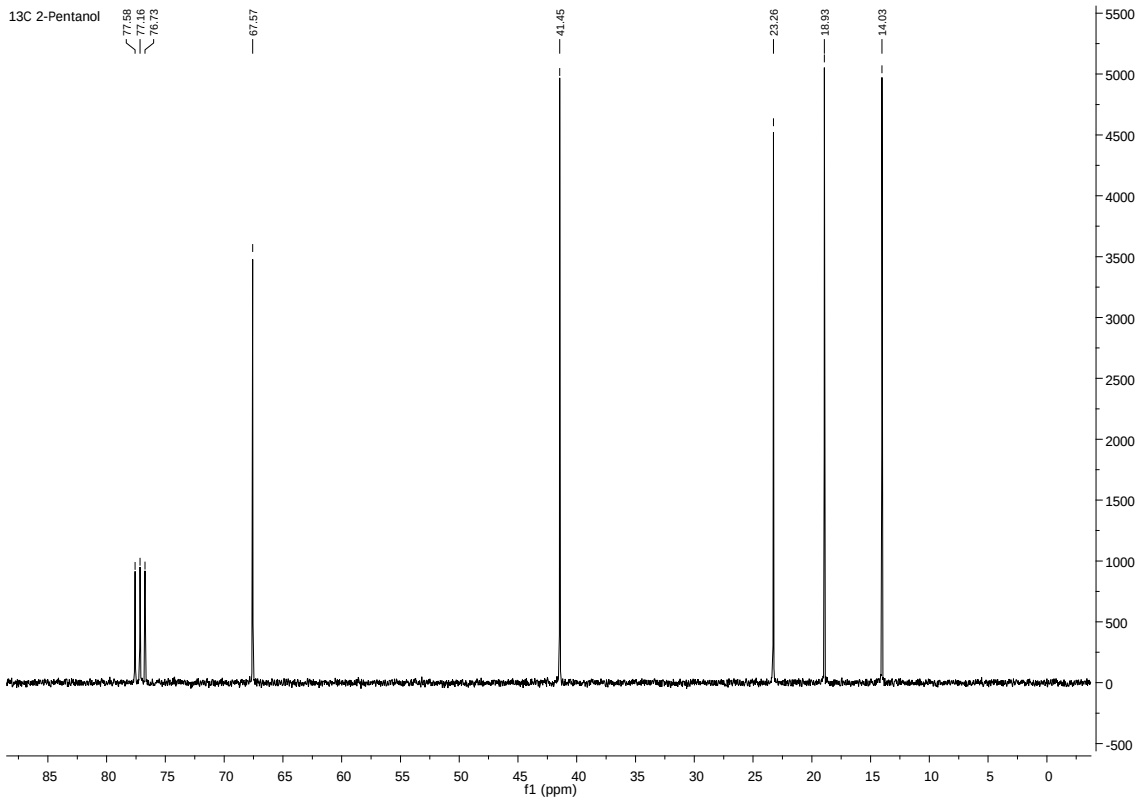
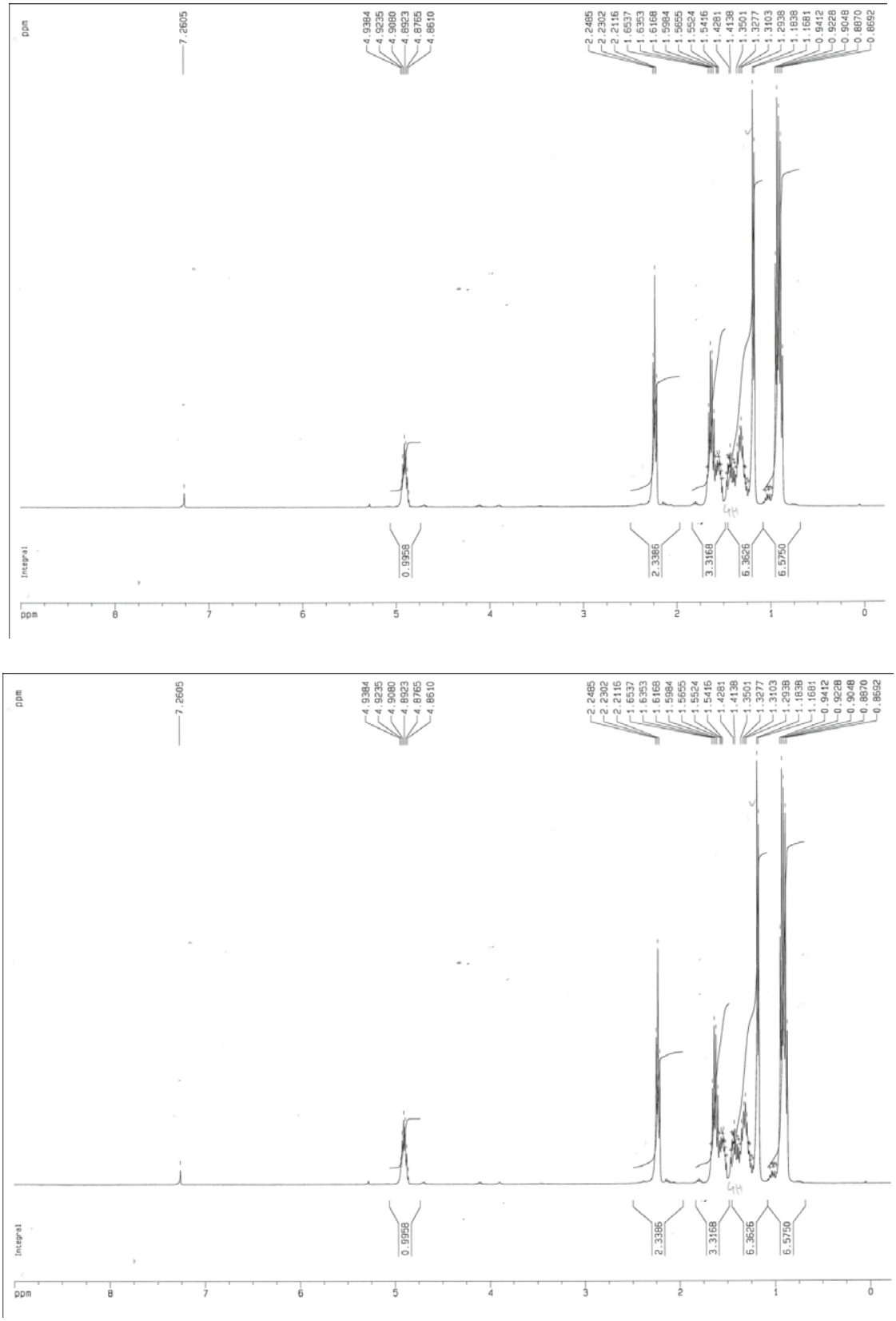


Figure 5. ^{13}C NMR (300 MHz, $CDCl_3$) spectrum of the commercial 2-pentanol 98% (Riedel-de Haen®)



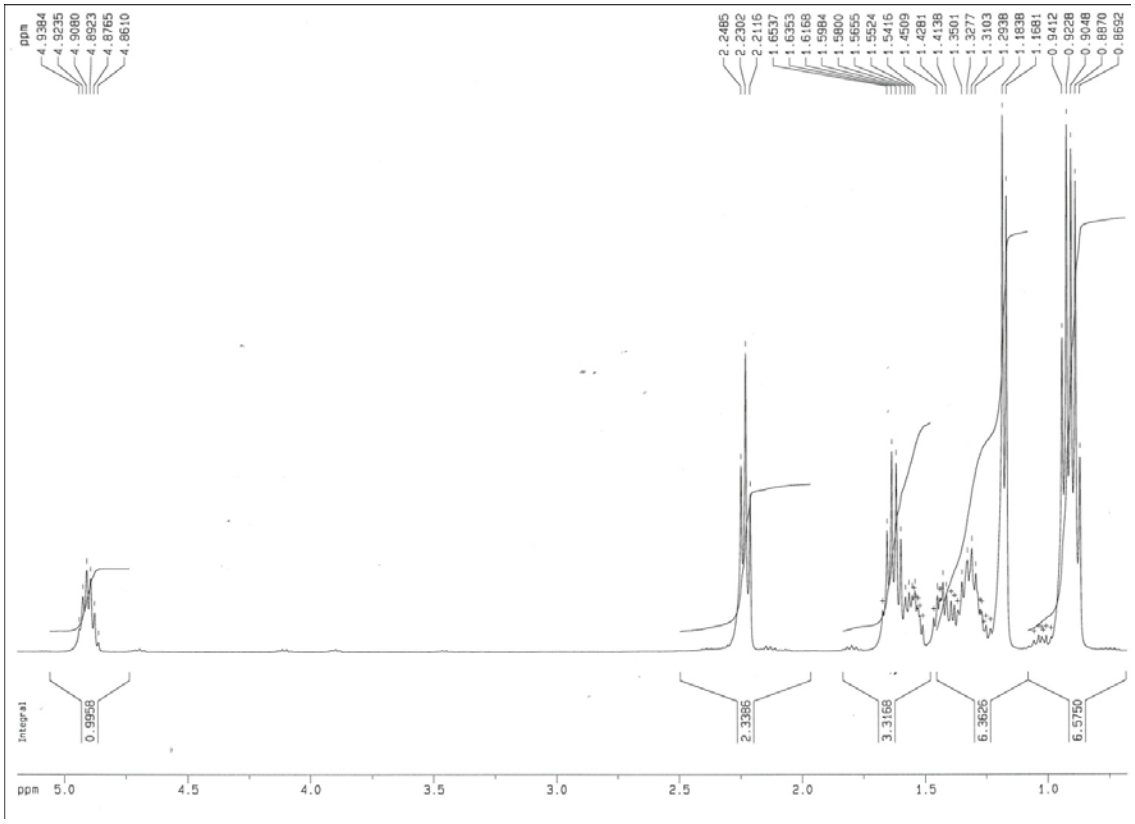
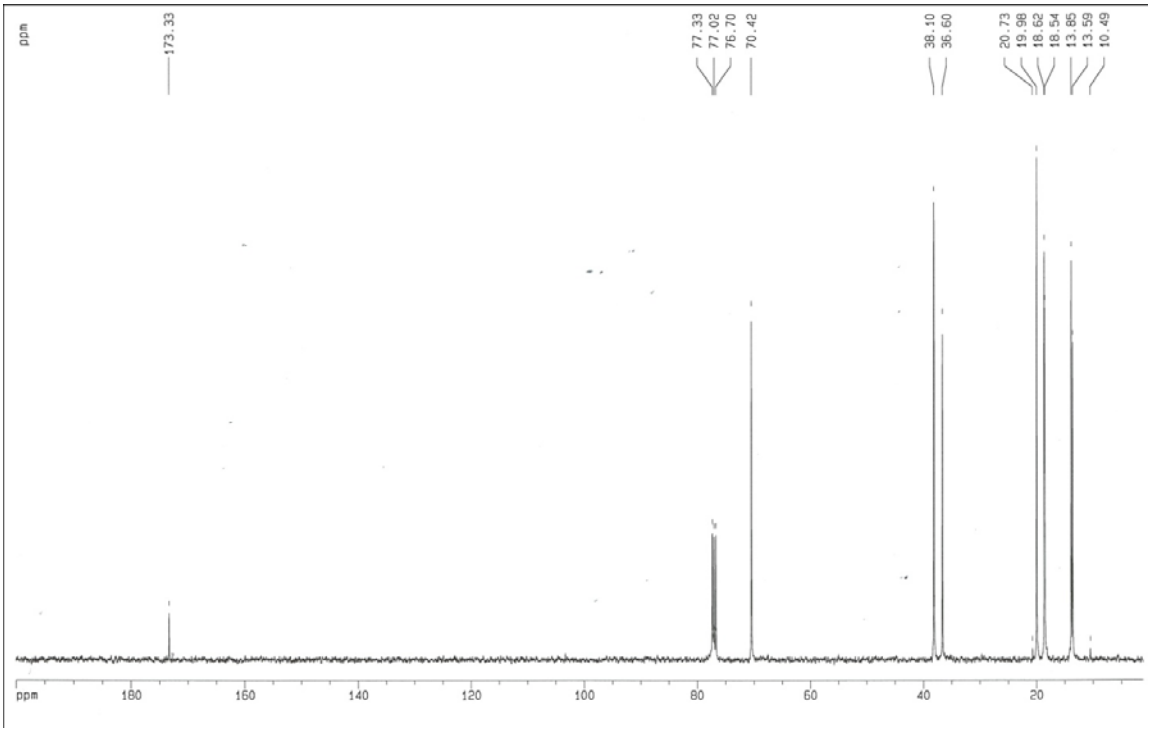


Figure 6. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of 2-pentyl butyrate obtained.[2]



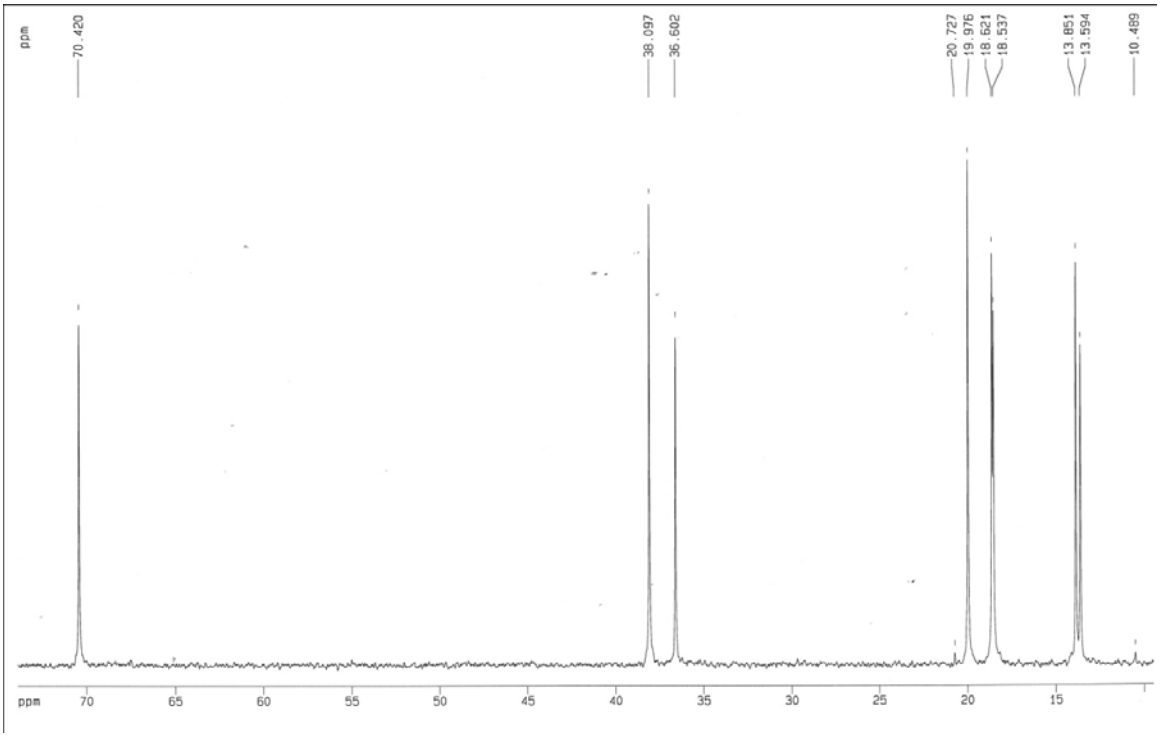


Figure 7. ^{13}C NMR ($CDCl_3$) spectrum of 2-pentyl butyrate obtained.[2]

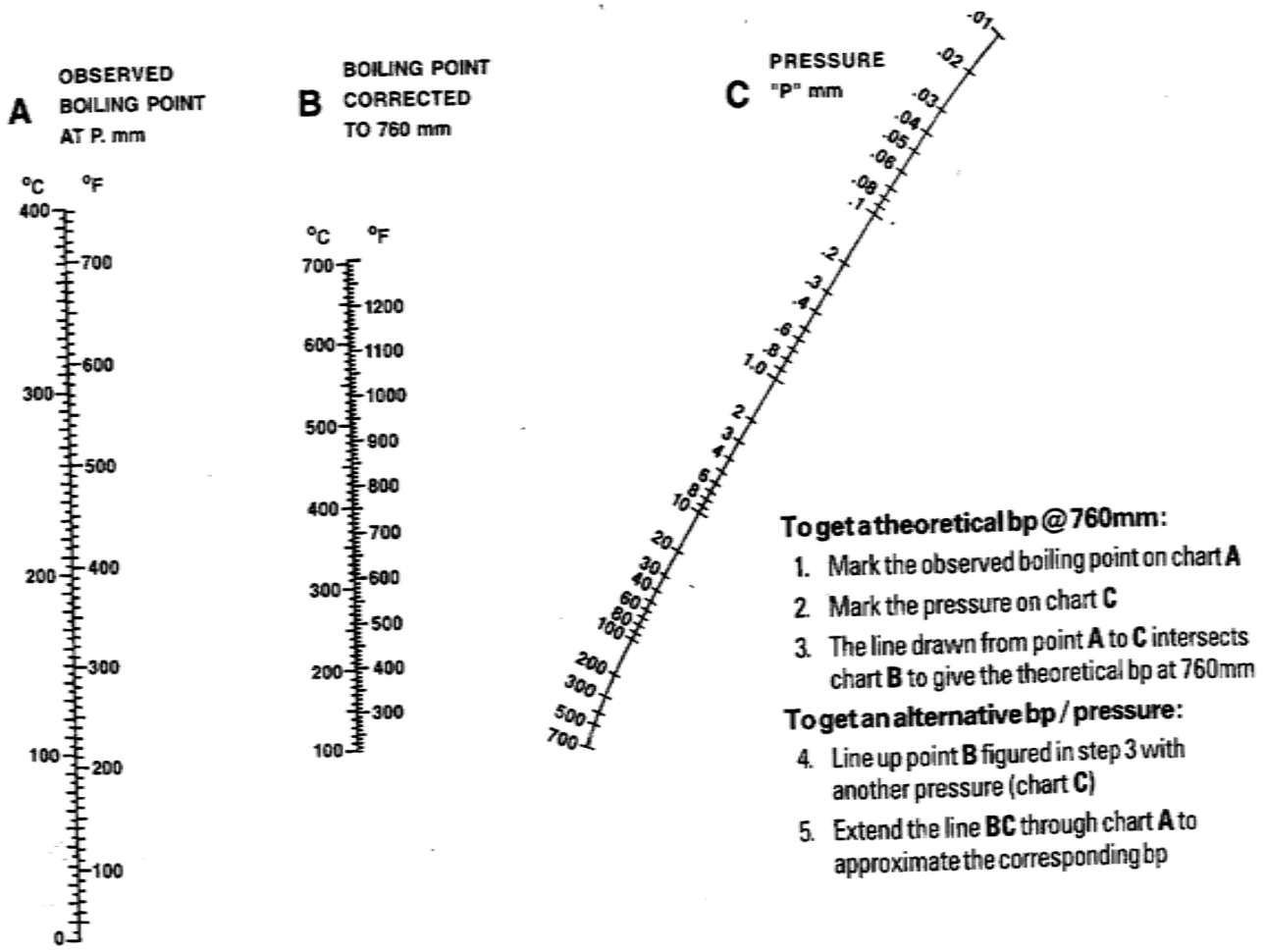


Figure 8. Temperature-pressure diagram for prediction of boiling point (Aldrich catalogue).

[1] *One-Pot Enzymatic Resolution and Separation of sec-Alcohols Based on Ionic Acylating Agents;* N. M. T. Lourenço, C. A. M. Afonso; *Angew. Chem. Int. Ed.*, **2007,** *46,* 8178.

[2] Spectra consistent with SDBS database accessed on 28th February 2015 (http://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_disp.cgi?sdbsno=17072)

Catalyzed Resolution and Simultaneous Selective Crystallization

Supplementary Material

Notes for the instructor for a successful experiment:

1. Performing TLC

TLC was performed using DC-Alufolien Kieselgel 60 F_{254} (Merck) silica gel plates and analyzed by staining upon heating with anisaldehyde solution (3 ml anisaldehyde, 10 ml conc. H_2SO_4 in 90 ml cold EtOH).

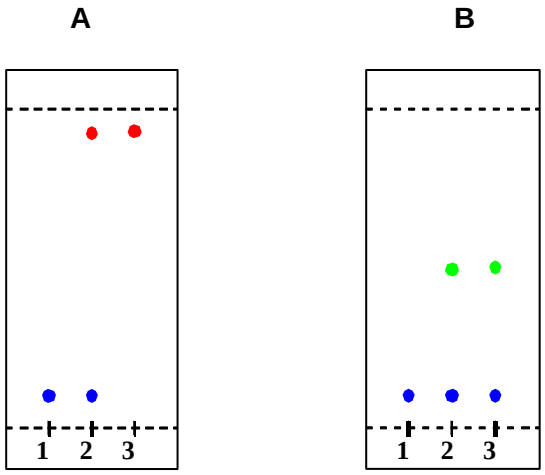
1,2-dodecanediol:

TLC: Rf=0.14 (6/10 EtOAc/petroleum ether).

1,2-dodecanediol bisbutyrate:

TLC: Rf = 0.39 (0.4/10 EtOAc/petroleum ether).

TLC analysis performed in section 1.4. should look like the TLC **A** and the one performed in section 2.1. should be like TLC **B**:



A: 1 – 1,2-dodecanediol

2 – 1,2-dodecanediol and reaction mixture put together

3 – sample from the reaction mixture (containing diester – red dot)

B: 1 – 1,2-dodecanediol

2 – 1,2-dodecanediol and reaction mixture put together

3 – sample from the reaction mixture (containing 2-monoester – green dot and (S)-1,2-dodecanediol – blue dot)

2. Parameters that can be changed

The duration of the enzymatic methanolysis can be prolonged from 48 hours up to 240 hours. Varying the reaction time does not influence the enantiomeric purity of the product because the reaction is quite stereospecific and, in addition, the crystallization process has proven to be enantio- and chemoselective.

3. Melting points:

(*S*)-1,2-dodecanediol m_p=68-70°C

rac-1,2-dodecanediol m_p=58-60°C

4. Optical rotation

(*S*)-1,2-dodecanediol $[\alpha]_D^{20}$ -14 (c 1.0; EtOH).

This value of specific rotation has been measured for the (*S*)-1,2-dodecanediol sample which was analyzed by HPLC over a chiral stationary phase ((*S*)-1,2-dodecanediol was analyzed in the form of 1-tosylate). The *er* determined was >99.9/0.1.

5. Results obtained previously

This experiment has been previously performed by second year master's students (31 persons). With 31 students the average yield was 10%, and the ee obtained >99%.

Normally, the students have continued the work evaporating their residual crude methanolysis product (mother liquor) and dissolving it in 2 mL of chloroform on slight heating. The following step has been stirring the solution magnetically for a couple of minutes, cooling it simultaneously on an ice-bath. As a result, an additional portion of (*S*)-1,2-dodecanediol has been crystallized out and filtered off. The quality of the additional portion of the diol has been practically identical to that of the former one. The amount of the additional product has been 70-190 mg (the overall yield: 17-29%).