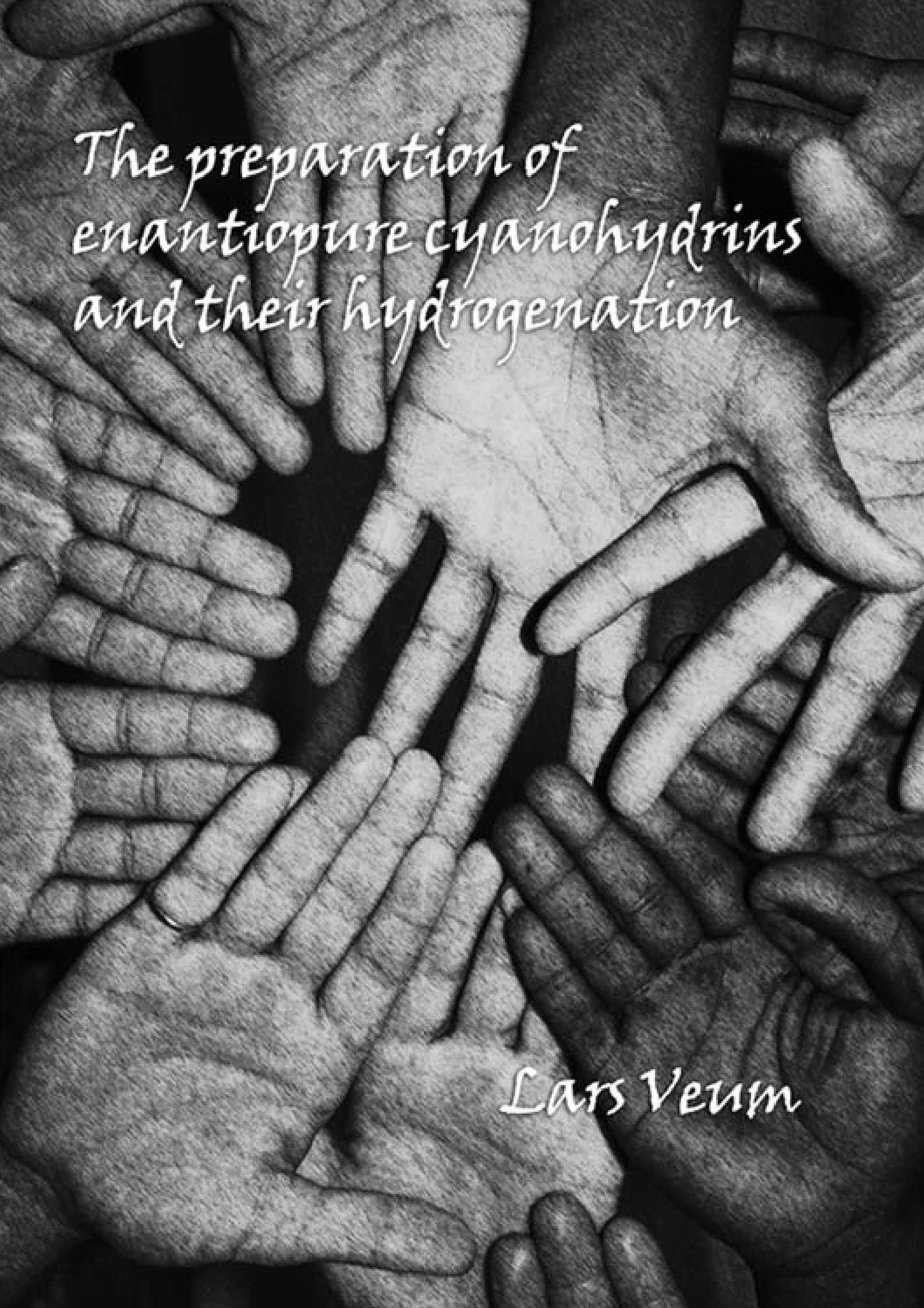
The preparation of enantiopure cyanohydrins and their hydrogenation
Lars Veum

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The preparation of enantiopure cyanohydrins and their hydrogenation

Proefschrift

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus Professor dr. ir. J. T. Fokkema,
voorzitter van het College voor Promoties,
in het openbaar te verdedigen
op 20 maart 2006 om 15.30 uur

door

Lars VEUM

Sivilingeniør i kjemi, Norges teknisk-naturvitskaplege universitet, Trondheim (Noreg)
geboren te Voss, Noorwegen

Dit Proefschrift is goedgekeurd door de promotor:

Prof. dr. R. A. Sheldon

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Dr. U. Hanefeld	Technische Universiteit Delft
Dr. R.V. A. Orru	Vrije Universiteit Amsterdam

Dr. U. Hanefeld heeft als begeleider in belangrijke mate aan de totstandkoming van het proefschrift bijgedragen.

Contents

Chapter 1

Introduction: Carrier enabled catalytic reaction cascades

Summary: This chapter summarizes the work described in this thesis. The focus is put on the cascade reactions which has been performed and on the importance of immobilising, either the enzyme or the chemical catalyst for these cascades to be successful.

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Introduction

When organic chemists set out to develop their first syntheses their curiosity drove them to try many daring combinations of chemicals. This led to the successful synthesis of numerous heterocyclic, aromatic ring systems.[1] The growing understanding of the underlying reaction mechanisms enabled Robinson already in 1917 to perform a cascade of reactions to obtain tropinone starting from succindialdehyde, methylamine and the calcium salt of acetone dicarboxylic acid.[2] However, after this milestone in the development of chemistry, most reactions were performed in a step-by-step approach for a long time. Efficient cascades of reactions, multi-component reactions and domino reactions only became fashionable again during the last decade or two. Similarly catalysis and more specifically biocatalysis were introduced into organic chemistry already in the nineteenth century,[3] but their application in total synthesis is still not such that their full potential has been realised.[4]

The combination of different types of catalysis, chemo-catalysis and biocatalysis, opens the way to many attractive reaction cascades. The two types of catalysts complement each other: transition metals are particularly versatile for (enantioselective) oxidations and reductions, tasks often difficult to perform with enzymes, due to problems with co-factor regeneration. On the other hand hydrolytic reactions and their reversal are readily performed with the aid of enzymes, while chemically they often require drastic reaction conditions and generate large amounts of salts as waste. Another interesting opportunity arises in the area of the enantioselective synthesis of C-C bonds.[4] A common point of criticism is that there are not enough enzymes for this purpose. Dynamic kinetic resolutions offer versatile solutions for this shortcoming. By combining a chemical catalyst that forms a C-C bond racemically with a hydrolytic enzyme that catalyses the enantioselective formation of an ester, hydrolases can be utilised in a chemo-enzymatic cascade of reactions to synthesise new C-C bonds.[5]

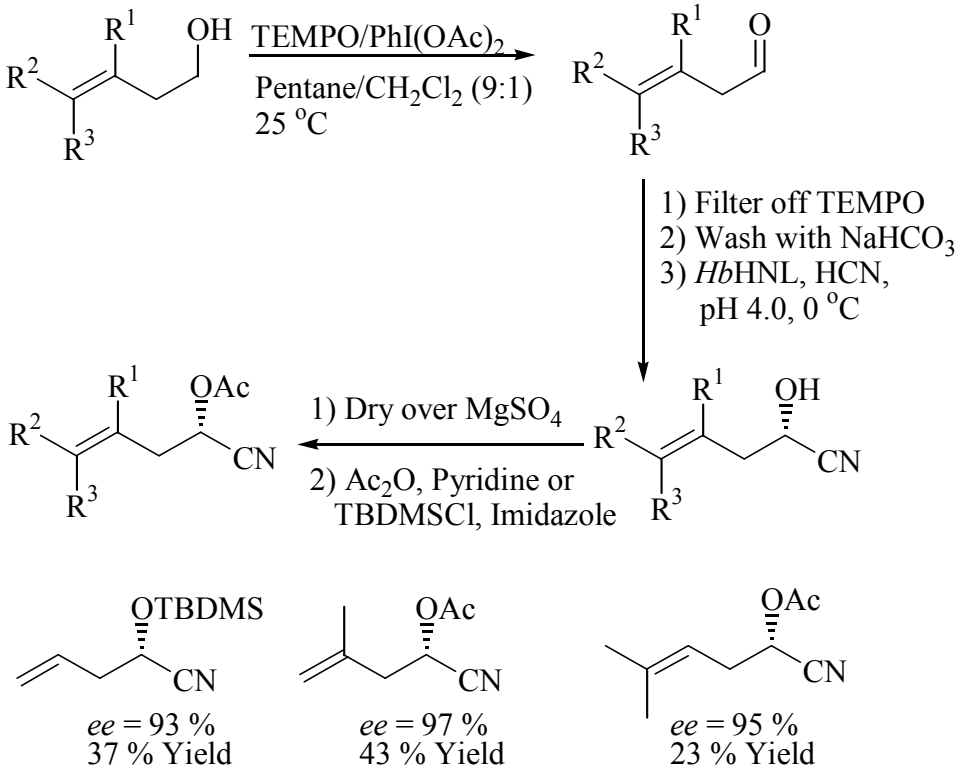
In order to reap the full potential of these catalytic reaction cascades, special care has to be taken that the catalysts do not inhibit or deactivate each other. We would here like to discuss a few examples out of our recent research that demonstrate the importance of immobilizing either of the catalysts for their successful application in catalytic reaction cascades.

Chemical oxidation and enzymatic C-C bond formation

During a collaborative study[6] with Dr. Orru (Vrije Universiteit Amsterdam) towards a chemo-enzymatic *de novo* synthesis of non-natural nucleosides containing a 3'-deoxyribose moiety, the optically pure (*S*)-γ,δ-unsaturated cyanohydrins in Scheme 1 were identified as key intermediates. A straightforward approach towards such intermediates would be the oxidation of γ,δ-unsaturated alcohols to their corresponding aldehydes followed by a C-C bond formation catalysed by the highly (*S*)-selective hydroxynitrile lyase from *Hevea brasiliensis* (*Hb*HNL).[4,7,8] However, the manipulation of the β,γ-unsaturated aldehydes is not uncomplicated. Their preparation is hampered by isomerisation into α,β-unsaturated aldehydes during work up, in particular under basic conditions. It was therefore chosen to perform the reaction sequence as a cascade using the reaction mixture from the oxidation reaction directly for the hydrocyanation reaction without isolating the aldehyde. Although *Hb*HNL works well under optimised conditions, it is very sensitive when applied outside this optimum. For a successful cascade, it was therefore essential that the oxidation proceeded not only without isomerisation of the product but also with reactants and waste products, which were harmless for the enzyme reaction.

As discussed above the oxidation reaction was performed with a chemical catalyst, since enzymatic oxidations are often cumbersome. The reagents of choice for the oxidation were catalytic amounts of the relatively stable organic nitroxyl radical 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) with $PhI(OAc)_2$ as the stoichiometric oxidant.[9] However, in order to couple it to the *Hb*HNL-reaction, some modifications had to be made. First of all, the reaction produces acetic acid, which is an efficient inhibitor of the enzyme. This was removed at the end of the oxidation, without isomerisation of the product, by simply washing with a saturated solution of $NaHCO_3$ at 0 °C. Since TEMPO was suspected to have a negative effect on the enzyme, it was immobilised ensuring an easy removal prior to the *Hb*HNL-catalysed step.[10] This immobilisation also allowed the TEMPO to be recycled at least once without any loss of activity. By exchanging the solvent from the normally used CH_2Cl_2, to a CH_2Cl_2 : pentane (1:9) mixture, not only did the solvent become more suitable for the enzyme reaction,[11] but both the conversion and the reaction time of the oxidation were improved dramatically. A complete conversion of the alcohol could be achieved within one hour.

When the oxidation and the enzyme reaction were coupled together, the whole cascade proceeded smoothly to the desired cyanohydrins, with excellent optical purities (Scheme 1). As the enzyme reaction was performed under mildly acidic conditions to prevent any chemical background reaction, nearly no isomerisation of the aldehyde was observed. When the reaction cascade was performed with homogeneous TEMPO the *ee*'s of the products were significantly lower (5 – 26 % lower). This demonstrates the great importance of using immobilised TEMPO for this reaction and confirmed our earlier concerns about the compatibility of the oxidation catalyst and the enzyme. While conversions in the separate steps were excellent, the isolated yields over all three steps, performed within one day, were moderate. As the reactions were performed on a 100 mg scale and the compounds involved in this reaction cascade are relatively polar and/or volatile, some loss/low yields could not be avoided.



Scheme 1 The three step "one pot" cascade reaction from γ,δ-unsaturated alcohols to their corresponding protected (*S*)-γ,δ-unsaturated cyanohydrins.

The *Hb*HNL was also immobilised in a sol-gel (similar to the carrier of TEMPO).[12] Although the *Hb*HNL immobilised through encapsulation was active, significant leaching was observed and therefore this enzyme preparation was not applied in the reaction cascade.

(*S*)-γ,δ-unsaturated cyanohydrins could be obtained with excellent optical purity in good yields by a chemo-enzymatic reaction cascade starting from γ,δ-unsaturated alcohols with virtually no isomerisation of the intermediate β,γ-unsaturated aldehyde.[6] For this cascade to proceed with excellent *ee*'s, it was essential to immobilise the chemical catalyst and to remove it prior to the enzymatic step.

Encapsulation of *Hb*HNL in a sol-gel matrix

With increased stability, recyclability and a facile separation of the enzyme from the reaction mixture as the main motive, the immobilisation of enzymes has attracted much attention over the last years. One of the many promising techniques is the sol-gel technique which allows for the synthesis of chemically inert glasses encapsulating enzymes.[13] These glasses can in theory be moulded into any desirable shape with a high porosity (up to 98 % pore volume) and a relatively high mechanical and thermal resistance.

In the case of *Hb*HNL this technique is particularly interesting. It has previously been shown that *Hb*HNL immobilised on celite is dependent on being surrounded by a discrete water layer in order to be active in an organic solvent.[14] This can be assured by filling the pores of the gel with a suitable buffer, and, since the water inside the pores has a reduced mobility and its propensity to partition into the organic phase is negligible, there will be no separate water layer in the reaction.

Although the sol-gel technique has been successfully applied to a wide range of lipases, the *Hb*HNL offered a more difficult challenge as it is particularly sensitive to conditions outside its optimum. Even the substrates and the natural product of the enzyme, HCN, deactivate the enzyme. Indeed, when a standard encapsulation procedure was followed, the enzyme was completely deactivated due to methanol formed during the hydrolysis of the sol-gel precursors. A new immobilisation method was therefore developed. By replacing most of the methanol with water prior to the addition of the enzyme, the conditions became milder and as much as 65 % of the initial activity of the enzyme could be recovered after the immobilisation.[12]

The aqua gels (the pores of the glass were filled with the selected buffer) containing the enzyme were not dried but used directly in an organic solvent for the synthesis of enantiopure

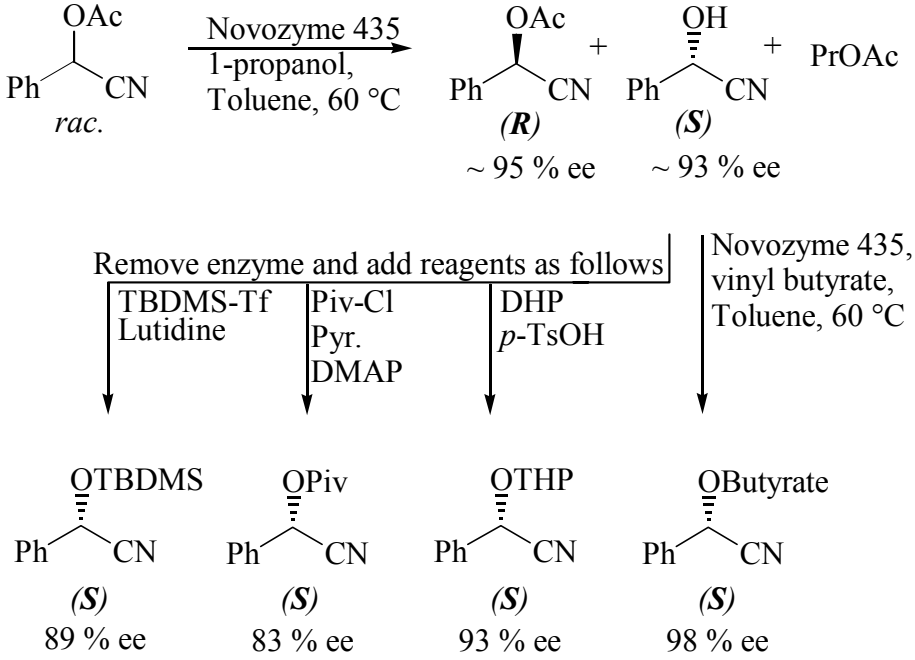
cyanohydrins. Both the yield and the optical purity of the products were comparable to what has been reported for the free enzyme under similar conditions. Filtration tests also showed that there was no leakage of the enzyme from the gels into the organic layer.

When the gels were recycled the activity of the biocatalyst dropped by ~50 % in each cycle. Since the enzyme is encapsulated in the pores of the gel, the enzyme will also readily leach during the washing procedure if the "capsules" are broken during the reaction. As the enzyme is relatively sensitive, a drop in activity is also to be expected due to the reaction conditions (HCN, substrate).

Hence, *Hb*HNL, an enzyme very sensitive to organic solvents and requiring a near neutral pH, was successfully immobilized in a sol-gel matrix.[12] Although the recyclability was relatively poor, the developed method has proved valuable by its successful application in the encapsulation of the methanol sensitive lipase from *Candida rugosa*.

Kinetic resolution and reprotection

In addition to HNL's, lipases have also successfully been used for the synthesis of enantiopure cyanohydrins. One attractive application is the kinetic resolution (KR) starting from acylated cyanohydrins (Scheme 2).[7,8,15]



Scheme 2 Kinetic resolution of mandelonitrile acetate and the protection of the formed (*S*)-cyanohydrin.

The starting material can easily be prepared in a one-pot procedure from the corresponding aldehyde without use of the highly toxic HCN. Furthermore, the remaining enantiomer can, if desired, readily be racemised and resubmitted to the kinetic resolution, allowing for nearly quantitative yields of the product. Still, the resulting (*S*)-cyanohydrins are relatively unstable and racemise easily. The kinetic resolution should therefore ideally be coupled with a protection reaction, directly and without any elaborate workup. Moreover, an enzyme should be found that is not only enantioselective but also recyclable. To achieve this, a range of immobilised lipases were screened for this reaction, and the widely available (*S*)-selective lipase B from *Candida antarctica* (CAL-B), immobilised by adsorption on a macroporous acrylic resin ("Novozyme 435") was found to be particularly enantioselective for the aromatic substrates, with E > 100.[16,17]

In order to recycle the enzyme at the end of this cascade, it is crucial that the enzyme is removed prior to any chemical protection reaction. Due to their size, the enzyme beads could easily be "filtered off" by simply transferring the liquid through a cannula to another reaction flask. Recycling experiments showed that the enzyme could be reused at least four times without any loss of enantioselectivity.[15]

After the straightforward removal of the enzyme, the (*S*)-cyanohydrins could readily be protected under basic conditions as TBDMS-ethers and pivalates, and under acidic conditions as THP-ethers. The THP-ether had a slightly higher *ee* than the pivalates and the TBDMS-ethers, probably due to the tendency of the cyanohydrins to racemise under basic conditions.

Protection under neutral conditions was realised by simply adding vinyl butyrate to the reaction mixture at the end of the KR, without prior removal of the enzyme. The enzyme then protected the (*S*)-cyanohydrin as its butyrate (Scheme 2). The optical purity of the resulting (*S*)-butyrates had increased compared to the (*S*)-cyanohydrins, due to the enantioselectivity of the enzyme.

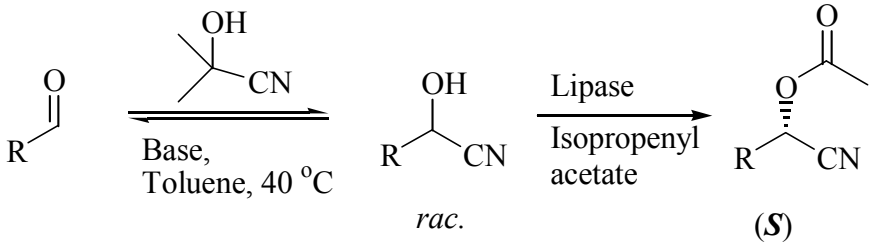
All the protected (*S*)-cyanohydrins and the (*R*)-cyanohydrin acetates could then easily be separated by column chromatography in both excellent yields and optical purities. The isolated yields of the (*R*)-acetates were generally > 90 % while the yields of the protected (*S*)-cyanohydrins were slightly lower (> 80 %), due to some decomposition of the intermediate cyanohydrin to its aldehyde.

Thus the enantiopure, protected cyanohydrins were made accessible via a cascade consisting of an immobilised lipase catalysing the kinetic resolution, followed by the re-protection of the resulting (S)-cyanohydrins.[15]

Dynamic kinetic resolutions enable hydrolase-catalysed C-C bond formations

As shown above, HNL's are excellent catalysts for the addition of HCN to aldehydes, yielding enantiopure cyanohydrins. Although the HNL's accept a relatively broad substrate range it is limited and in particular their application in dry organic solvents can be troublesome.

Since lipases in general have a wide substrate range and can be used in pure organic solvents, the lipase-catalysed dynamic kinetic resolution (DKR) of cyanohydrins is particularly interesting (Scheme 3). This catalytic cascade reaction was already described in 1991, and is the first example where a lipase is used in an enantioselective C-C bond formation.[5] However, in spite of its elegance, there are only few examples of its successful application.[18] This is mainly due to long reaction times, moderate enantioselectivities and the failure of this DKR when applied to aliphatic substrates.[5,19] A more active, enantioselective and readily available enzyme could therefore help increasing the attractiveness of this DKR. Encouraged by the results obtained with the immobilised CAL-B (Novozyme 435, see chapter 3.1), we set out to explore its potential for the DKR.



Scheme 3 The enantioselective synthesis of cyanohydrin esters *via* a dynamic kinetic resolution.

This cascade of reactions combines a base-catalysed equilibrium between an aldehyde, acetone cyanohydrin and the resulting cyanohydrin of the aldehyde, with a lipase-catalysed acylation of one enantiomer of the racemic cyanohydrin. As the remaining enantiomer of the

cyanohydrin is racemised by the base, theoretically 100 % yield of the corresponding cyanohydrin acetate can be obtained.

There are also other advantages of this approach than the luring prospects of quantitative yields and high optical purity. While the traditional HNL approach is based on an equilibrium reaction where an excess of HCN is crucial for obtaining good yields, the DKR makes use of the cheap and relatively safe acetone cyanohydrin, which generates the HCN *in situ*. Furthermore, the last step of the DKR is a practically irreversible reaction, reducing the required amount of acetone cyanohydrin to merely 2 equivalents.

Relatively early in this work, it was found that the base-catalysed formation of the cyanohydrin, its racemisation and acylation all proceeded smoothly when performed separately. When the reactions were combined, however, the process hardly gave more than a meagre 16 % yield.[20] The problem was ascribed to the water bound to the carrier of the enzyme. During the reaction this water is readily released to the reaction media and used by the enzyme to hydrolyse the acylating agent, generating acetic acid. The acetic acid in turn neutralised the base, which completely suppressed the reaction. For each molecule of acetic acid that is neutralised, one molecule of water is liberated, which again can be used to produce more acetic acid until there is either no acylating agent left or until the entire base is neutralised. Thus the choice of carrier is essential to the success of the overall DKR.

Yet another difficulty could be identified when aliphatic substrates were used for the reaction. The yields were in line with what can be obtained from a kinetic resolution, indicating that the standard base for this reaction (OH^- conditioned Amberlite) is not strong enough to efficiently racemise the remaining enantiomer of the cyanohydrin.

Various approaches were explored to meet these two challenges. Obvious solutions to the water induced acidification such as adding more base or molecular sieves to the reaction did not yield the desired results. In contrast, an increased amount of base led to a base-catalysed polymerisation of HCN. This polymer turned out to be a highly efficient inhibitor of the lipase, causing a full stop of the reaction.

The possibility of replacing the traditionally used base with solid buffers (CAPSO pK_a 9.6,CAPS pK_a 10.4) was also investigated (For the structures of these bases see Table 1,

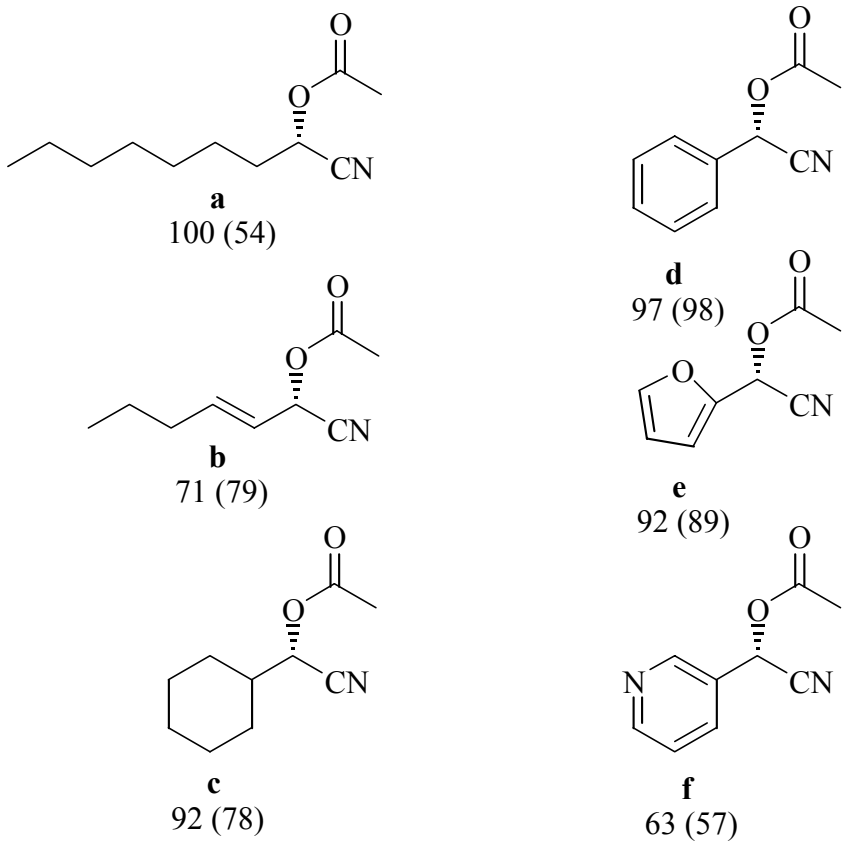
chapter 5). It had previously been shown that solid buffers can be efficiently used to adjust the ionisation state of enzymes in organic media and to maintain it.[21] It was found that the buffers did improve the results dramatically, both in respect to the water issue and the racemisation of the aliphatic cyanohydrin. However, since a relatively large amount of solid buffers (1-2 grams of buffer/g product) would be necessary for the reaction to proceed smoothly, other solutions were pursued.

To use NaCN as a base in the reaction turned out to be a straightforward solution for the aliphatic substrates. Not only was NaCN a strong enough base to efficiently racemise the aliphatic cyanohydrin but the reaction also proceeded to 100 % conversion with a high *ee* for cyclohexanecarbaldehyde, implying that water caused no problem in this case. The advantage of the cyanide salt is that it neutralises any acetic acid, yielding HCN and the sodium acetate. In contrast to the water formed during the neutralisation of the Amberlite, HCN does not take part in a destructive cycle but conveniently adds to the aldehyde yielding the racemic cyanohydrin.[22] When used in combination with an aromatic substrate, the yields were also excellent; however, the products were nearly racemic. The reason for this must be that the NaCN is strong enough as a base to catalyse the chemical acylation. Other cyanide salts were also probed ($Zn(CN)_2$ and CuCN) but they were not sufficiently basic to catalyse the reaction. Another solution was therefore necessary for the aromatic substrates.

The only difference between the reaction performed with Novozyme 435 and the few existing successful DKRs of cyanohydrins, except for the solvent and the enzyme itself, is the carrier of the enzyme. Previously, the various lipases have always been immobilised on a celite, probably due to the effective and simple procedure. By simply immobilising the CAL-B on Celite R-633, both the yield and *ee* of mandelonitrile actetate had significantly increased and a nearly quantitative (97 % yield) and enantioselective (98 % *ee*) reaction was achieved.[23]

The reason for this difference is that Novozyme 435 is immobilised on a relatively hydrophobic carrier, and any water attached to this carrier will be liberally released to the reaction medium. In contrast to this, celite is capable of binding water relatively tightly via hydrogen bridges, and is therefore not releasing any water into the reaction mixture. Indeed celite has even been used to control water activities. The celite immobilised CAL-B was also used in combination with an aliphatic substrate and NaCN, but in this case water is less disturbing and thus Novozyme still proved to be the better solution.

A wide range of substrates was tested for the reaction, and in most cases both excellent yields and enantioselectivities could be obtained (Scheme 4). Compared to the first described DKR of cyanohydrins, the reaction time has been significantly shortened, and both the yields and enantioselectivities have been improved. The *ee* of aliphatic cyanohydrins was also improved in spite of the known poor selectivity of CAL-B towards straight chain aliphatic substrates.



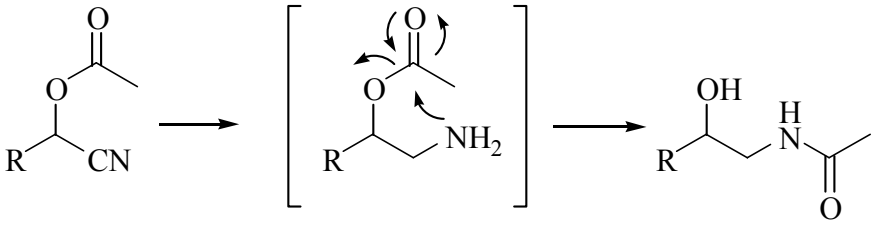
Scheme 4 Starting from the corresponding aldehydes, compounds **a** and **c** were prepared using Novozyme 438 in combination with NaCN, while the remaining and compounds **b**, **d**-f were prepared using CAL-B adsorbded on Celite in combination with Amberlite (according to scheme 3). The yields (*ee*) [%] were obtained after four days (two days for **a** and **b**). The yield of **c**, **d** and **e** are isolated yields.

Hence, the challenges presented by water and an insufficient racemisation of the intermediate cyanohydrin (in the case of aliphatic substrates) were solved based on the understanding of the influence of the carrier on the reaction. Using the readily available CAL-B with straightforward modifications of either the chemical catalyst or the carrier of the enzyme, excellent yields and enantioselectivities in the DKR of cyanohydrins starting from both aliphatic and aromatic aldehydes were achieved.[22,23,24]

Reductions and migrations

Due to its known pharmacological activity, *N*-acyl-β-amino alcohols are a highly interesting structural motif. Their facile conversion into β-secondary aminoalcohols, an important class of compounds in the pharmaceutical and agrochemical industry makes them a valuable building block too. Their preparation starting from free cyanohydrins, normally consists of two steps, a reduction ($LiAlH_4$, BH_3 or catalytic hydrogenation under strongly acidic conditions)[8,25] followed by an acylation. As this procedure generates a relatively large amount of waste, and, an intermediate work-up is necessary, a novel strategy was developed.

It was found that the hydrogenation of cyanohydrin esters was followed by a rapid intramolecular acyl migration to yield the *N*-acyl-β-aminoalcohols directly (Scheme 5). This one-pot two-step cascade reaction proceeds with a high atom efficiency under neutral conditions from readily available starting materials and is a significant improvement compared to the previous two-step procedure. Since the newly generated primary amines are rapidly acylated, the formation of dimeric secondary amines, a side reaction normally observed in the catalytic hydrogenation of nitriles under neutral conditions was also conveniently suppressed.

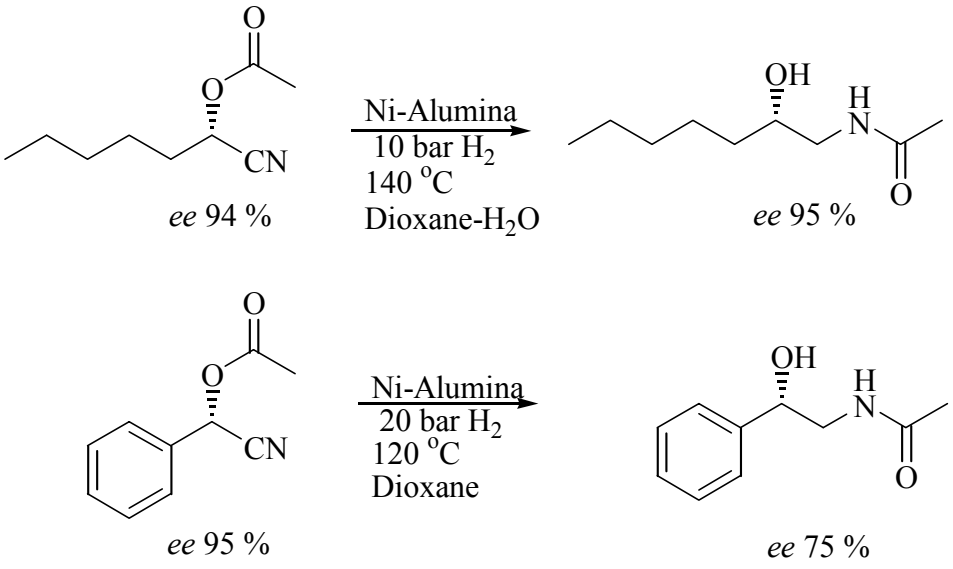


Scheme 5: Catalytic hydrogenation and subsequent rearrangement of acylated cyanohydrins.

Since benzylic C-O bonds are readily cleaved by hydrogenation, the reaction was optimised separately for substrates containing a benzylic and a non-benzylic C-O bond. For the optimisation strategy, a multivariable statistical experimental design was chosen.[26] This consisted of an initial small design (only around 7 % of all possible combinations) where the results were used to drastically narrow down the parameter space for the second design. The conditions were further fine-tuned in a third design. By using this strategy only 70 reactions in total for each substrate type, from around 2000 possible combinations of the tested parameters (metal, support, solvent, temperature, reaction time, additives and pressure) were needed to find the optimum. As a result both time and chemicals could be saved.

For substrates with a non-benzylic C-O bond, the hydrogenation and the subsequent acyl migration proceeded smoothly using nickel on alumina in dioxane at 140 °C and 10 bar H_2 with water as an additive. Yields of up to 90 % could be obtained, also when the acyl group was varied. In spite of the weak benzylic C-O bond, yields up to 50 % could be obtained for this type of substrates using nickel on alumina in dioxane at 120 °C and 20 bar H_2.

Chiral *N*-acyl-β-aminoalcohols are a highly import group of compounds in organic chemistry. The applications vary from protected chiral building blocks to intermediates in the synthesis in pharmaceuticals such as denopamine.[27] With this objective, the reaction was also performed with chiral substrates (Scheme 6).



Scheme 6: Catalytic hydrogenation of enantiopure acylated cyanohydrins.

We were very pleased to see that the optical purity of the substrate without a benzylic C-O bond could be retained in the product after the cascade reaction. However for the substrate with a benzylic C-O bond, there was a small decrease in the *ee* during the reaction. This is probably due to racemisation of the substrate caused by basic side products formed in the reaction.

In short, a novel one step cascade reaction for preparing chiral *N*-acyl β amino alcohols from acylated cyanohydrins was developed. A design of experiment strategy proved to be highly efficient in identifying the optimal reaction conditions. Since only the first reaction is catalysed, it is strictly speaking no cascade of catalytic reactions. However, the carrier of the

catalyst does play a crucial role since many of the screened supports did not allow the desired reaction to take place.[28]

Conclusions

When we set out to develop the chemistry described above, we focused on products and catalysts. However, during the course of our investigations it became more and more obvious that a parameter that organic chemists tend to neglect was the unifying factor between the very different reactions we were studying. Carriers and supports of catalysts are normally not taken into consideration when planning synthetic strategies.[29] Often they are barely mentioned in synthesis.

When developing catalytic cascades, seemingly unimportant parameters become important. Carriers immobilise catalysts and thus allow keeping them separated (Schemes 1 and 2), avoiding inhibition and deactivation. Moreover, they enable efficient recycling of the often expensive and toxic catalysts.

For the DKR the carrier of CAL-B has a very different additional function. The Celite R-633 obviously maintains low water activities in the reaction mixture. Only at such low water activities are efficient lipase-catalysed DKR's possible (Scheme 3 and 4).

Next to these rather unexpected features of the carriers, the carriers can influence the catalyst that is immobilised on them. This is the case for the Ni-catalyst (Schemes 5 and 6). It performs best and most selectively when supported by alumina.

To summarise, carriers enable catalytic cascades, by ensuring compartmentalisation, by controlling the reaction conditions and by fine-tuning the catalyst. They often play a larger role than expected and should be taken into consideration when planning reaction cascades, chemo-enzymatic, enzymatic or purely chemical.

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Chapter 2

A mild chemo-enzymatic oxidation-hydrocyanation cascade

Summary: Oxidation-hydrocyanation of γ,δ-unsaturated alcohols using (immobilised) TEMPO/PhI(OAc)$_2$ in combination with *Hb*HNL proceeds smoothly. After (*in situ*) protection, the resulting cyanohydrin derivatives were obtained in good overall yields and high *ee*'s. A mild TEMPO-catalysed oxidation protocol is described that yields β,γ-unsaturated aldehydes without isomerisation of the double bond and that is compatible with a subsequent *Hb*HNL-catalysed hydrocyanation performed in the same pot.

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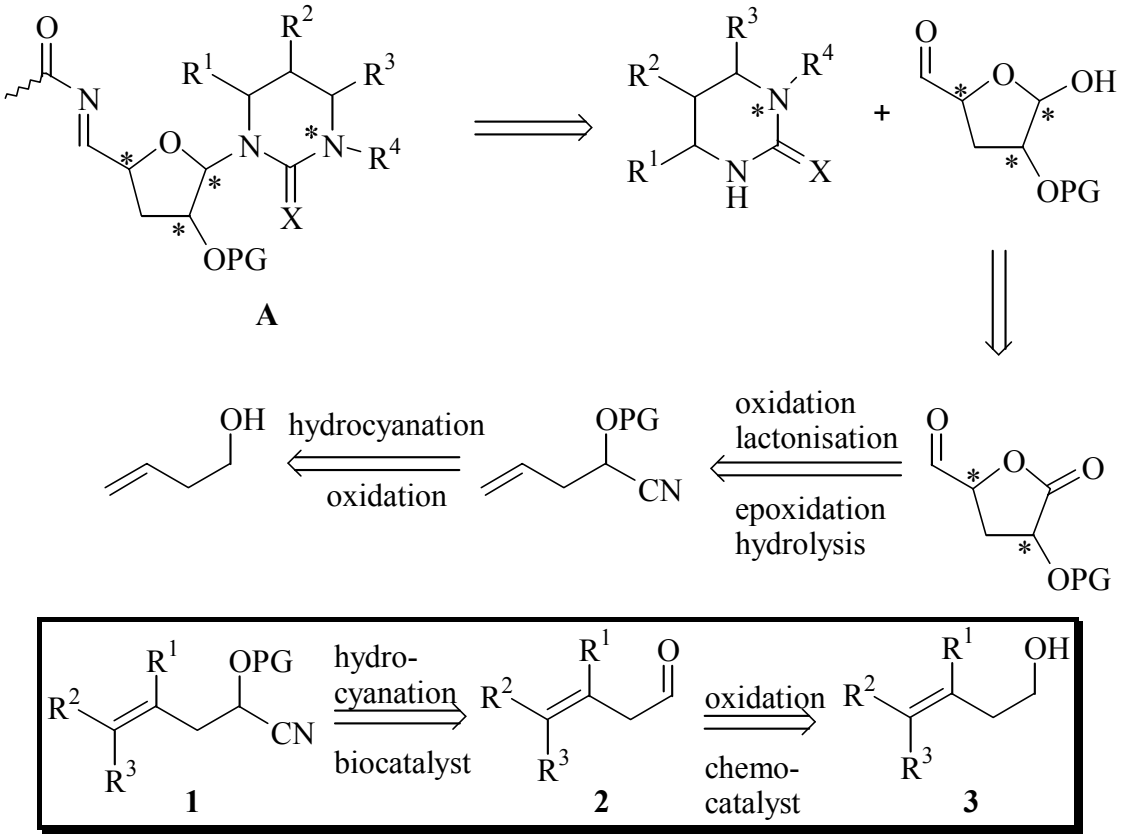
Introduction

Cyanohydrins are versatile chiral building blocks,[1,2,3] which can be transformed into α-aminonitriles,[4,5,6] α-hydroxyesters,[7] aminoalcohols,[8] 2,3-disubstituted piperidines,[9] and 3-hydroxytetrahydropyridines.[10] The cyanohydrins are conveniently accessed by hydroxynitrile lyase (HNL) catalysed hydrocyanation of the corresponding aldehydes.[11] These biocatalysts are well established and both the (*R*)- and (*S*)-enantiomer of the cyanohydrins can be prepared in high yields and *ee*'s.[12,13,14] During our studies towards a chemo-enzymatic *de novo* synthesis of non-natural nucleosides (**A**) containing a 3'-deoxy ribose moiety we required a series of differently substituted, optically pure, γ,δ-unsaturated cyanohydrins **1**. For this, a biocatalytic hydrocyanation of β,γ-unsaturated aldehydes **2** was identified as a key-step. See Scheme 1 for a retro-synthesis.



Scheme 1: Retro synthesis of non-natural nucleosides containing a 3'-deoxyribose moiety

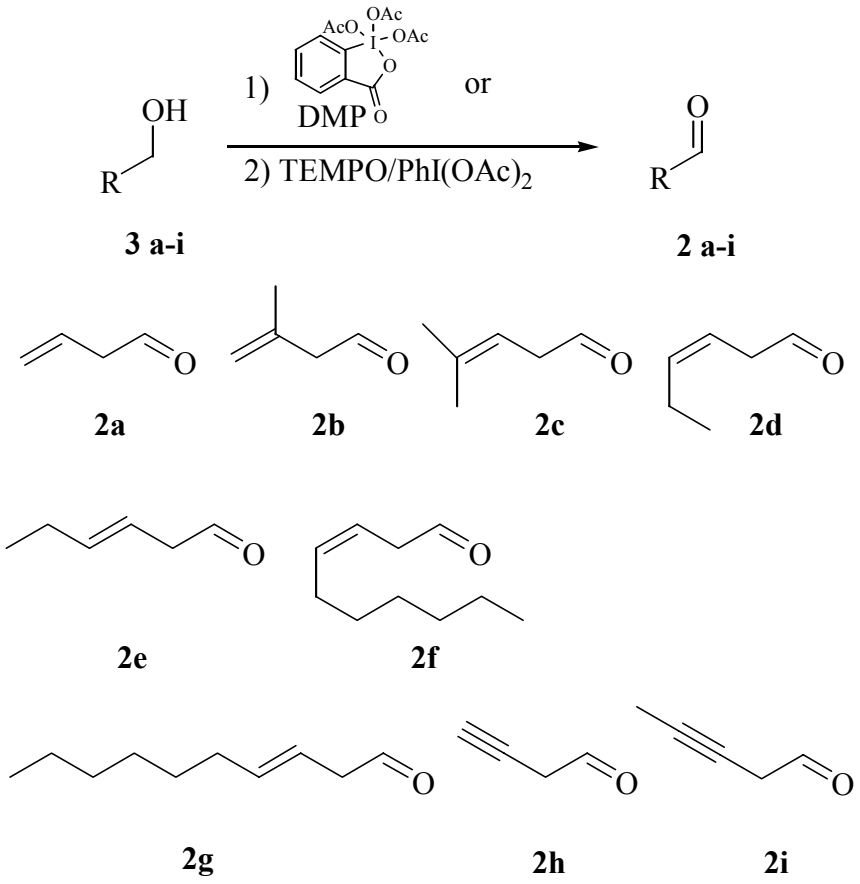
Oxidation of γ,δ-unsaturated primary alcohols **3** to the corresponding aldehydes **2** is the most direct way to prepare the required substrates for the HNL-catalysed hydrocyanation. However, such an oxidation is often hampered by isomerisation of the double bond resulting in mixtures of β,γ- and α,β-unsaturated isomers, which are difficult to separate.[15,16,17,18,19]

The work described in this chapter aims at a mild oxidation-hydrocyanation cascade to produce optically pure γ,δ-unsaturated (*S*)-cyanohydrins **1** starting from readily available primary alcohols **3**. Since enzymatic redox reactions tend to be difficult due to problems with co-factor regeneration, a chemical reagent is envisaged for the oxidation step. The enantioselective C-C bond formation under close to neutral conditions, however, is difficult to perform with a chemical catalyst, therefore the efficient and highly enantioselective *Hb*HNL will be employed.[20]

(*R*)-Cyanohydrins are accessible in good yields and *ee*'s using the hydroxynitrile lyase *Prunus amygdalus* (*Pa*HNL) from almonds, which catalyses the hydrocyanation efficiently.[12] In general, (*S*)-cyanohydrins are readily available using the hydroxynitrile lyase from the rubber tree *Hevea brasiliensis* (*Hb*HNL).[13,14,21,22,23,24,25,26,27] However, reports on the enantioselective synthesis of γ,δ-unsaturated cyanohydrins **1** from β,γ-unsaturated aldehydes **2** are scarce.[28,29] Only two examples are known of (*R*)-**1** produced by *Pa*HNL, while no examples exist for the preparation of (*S*)-**1** using (*S*)-selective enzymes such as *Hb*HNL.

Results and discussion

Many of the most widely employed oxidations like, *e.g.,* Swern-oxidation or chromium(VI)-based reagents (PDC, PCC, etc) require relatively alkaline conditions. Such conditions are not suitable for the transformation of primary alcohols **3** to yield the desired β,γ-unsaturated aldehydes **2** because they promote isomerisation of the double bond to give the α,β-unsaturated isomers.



Scheme 2: Oxidation of γ,δ-unsaturated alcohols

Application of the very mild Dess-Martin periodinane (DMP) reagent to oxidise **3** (Scheme 2) without isomerisation seemed feasible but a couple of practical disadvantages were encountered.[30,31] Commercial DMP is relatively expensive while at the same time its preparation is not entirely trivial and its storage can lead to degradation.[32] When oxidising primary alcohols of relatively low molecular weight, a large amount of DMP is needed to convert the alcohol to the aldehyde. For example, 1 g (13.9 mmol) of alcohol **3a** is converted to the corresponding aldehyde **2a** almost quantitatively, but this can only be achieved by using 12 g (28.3 mmol) of DMP. Thus the Dess-Martin oxidation suffers from poor atom efficiency and the reaction work-up is troublesome. Consequently, the use of DMP in larger scale production of β,γ-unsaturated aldehydes becomes less convenient. Furthermore, we found that the filtration/evaporation procedure, which is necessary after the Dess-Martin oxidation, was accompanied by some isomerisation of the product aldehyde. Therefore we turned to another method to oxidise alcohols **3** to the corresponding aldehydes **2** (Scheme 2).

The use of relatively stable organic nitroxyl radicals, like 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO), as catalysts for the mild oxidation of alcohols has found widespread application.[33,34] Among the numerous described variants, the oxidation of sensitive primary alcohols that employs a catalytic amount of TEMPO together with $PhI(OAc)_2$ as the primary oxidant is particularly interesting.[35,36] In this reaction an equilibrium between the alcohol (ROH) and $PhI(OAc)_2$ is responsible for the exchange of one or both acetate ligands around the iodine atom (for OR). The acetic acid that is formed catalyses dismutation of TEMPO to hydroxylamine and the corresponding oxoammonium salt. The latter species is responsible for the selective oxidation of the primary alcohol to the aldehyde while being reduced to hydroxylamine. Then $PhI(OAc)_2$ completes the catalytic cycle via regeneration of TEMPO.[35]

Table 1. Oxidation of **3a** with TEMPO/$PhI(OAc)_2$.

Entry	$PhI(OAc)_2$ [eq]	TEMPO [eq]	Solvent	Conversion[a] [%]
1	1.1	0.1	CH_2Cl_2	15
2	1.1	0.2	CH_2Cl_2	38
3	1.2	0.2	CH_2Cl_2	50
4	1.1	0.1	Pentane:CH_2Cl_2 = 9:1	100

[a] conversion of **3a** after 2.5h at room temperature determined by ^{1}H-NMR.

Initially the reaction was performed following the literature procedure[35] by using a 0.2 M solution of **3a** in CH_2Cl_2, 1.1 equiv. of $PhI(OAc)_2$, and 0.1 equiv. of TEMPO (entry 1, table 1). After 2.5 h only 15 % of **3a** had been converted to the desired β,γ-unsaturated aldehyde **2a**. Although more oxidising agent improved the conversion, still only an unsatisfactory 38 or 50 % conversion (entries 2 and 3, table 1) was detected after 2.5 h. Prolonged reaction times did not result in a better conversion but instead showed isomerisation to the undesired α,β-unsaturated aldehyde to occur. As CH_2Cl_2 would be an unsuitable solvent for *Hb*HNL catalysed hydrocyanation, an attempt was made to change to a solvent mixture of pentane and CH_2Cl_2.[37] To our satisfaction **3a,** dissolved in a 9:1 mixture of pentane and CH_2Cl_2, respectively, with 1.1 equiv. of $PhI(OAc)_2$, and 0.1 equiv. of TEMPO, gave complete and selective conversion to **2a** after 2.5 h (entry 4, table 1).

A number of γ,δ-unsaturated primary alcohols **3** were treated under similar conditions and in table 2 the results are summarised. Conditions were optimised towards a minimum degree of

isomerisation and optimum conversion. As can be seen, homo-allylic primary alcohols **3a-3g** were readily converted to the corresponding β,γ-olefinic aldehydes **2a-2g**. Also homo-propargylic primary alcohols can be oxidised to yield the corresponding aldehydes, however, 1-butynol **3h** proved to react sluggishly.

Table 2: Optimised TEMPO/$PhI(OAC)_2$-oxidation of γ,δ-unsaturated alcohols **3**

substrate	mmol **3a-3i**	Conversion[a] [%]	$PhI(OAc)_2$ [eq]	TEMPO [eq]	Pentane: CH_2Cl_2	Conc. [mM]	React. time [min]
3a	27.5	quant.	1.1	0.1	9:1	0.20	150
3b	5	quant.	1.1	0.1	9:2	0.36	120
3c	2	quant.	1.1	0.1	9:2	0.36	40
3d	2	quant.	1.1	0.1	9:2	0.36	100
3e	2	quant.	1.1	0.1	9:2	0.36	100
3f	4	quant.	1.2	0.2	2:1	0.66	30
3g	4	quant.	1.2	0.2	2:1	0.66	30
3h	0.25	0	1.2	0.2	1:1	1	--
3i	0.5	41	1.8	0.2	3:2	1	70

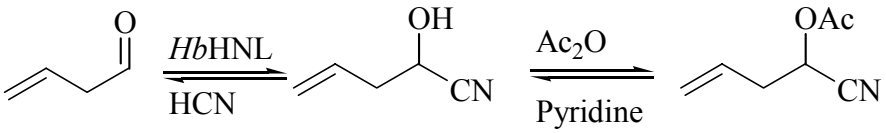
[a] conversion of **3** according to ^{1}H-NMR

It should be noted that the above TEMPO/$PhI(OAc)_2$ oxidation protocol encounters some problems during reaction work-up. Even when the aldehyde was carefully co-distilled with dethylether under reduced pressure, a significant isomeration of the aldehyde was observed. This provided an additional incentive to develop the planned reaction cascade. However, during the oxidation two equivalents of acetic acid are formed. As this acid would cause an immediate deactivation of the *Hb*HNL it had to be removed prior to the hydrocyanation reaction.[38] By washing the reaction mixture at the end of the TEMPO/$PhI(OAC)_2$ oxidation of **3a** with a saturated solution of $NaHCO_3$, all the acetic acid was removed completely. To our satisfaction no isomerisation was detected.

Since both the formation of cyanohydrins and the isomerisation of the aldehyde 2a is base-catalysed, the enzyme reaction should be performed in a mildly acidic buffer. A direct transfer of the conditions that were used in the *Pa*HNL-catalysed synthesis of the (*R*)-

enantiomer of **1a** is not possible. *Pa*HNL and *Hb*HNL are structurally not related and, their optimum reaction conditions are different.[39] Initially two different pH's (4.0 and 5.0) and two different temperatures (0° and 25° C) were investigated for the *Hb*HNL catalysed addition of HCN to the *in-situ* generated aldehyde **2a** following a general procedure.[40] The results are summarised in table 3.

Table 3. Selectivity at complete conversion of the *Hb*HNL catalysed hydrocyanation of **2a** at various pH and temperatures.[a]



2a **1a** **4a**

Entry	pH	T [°C]	*ee* of **4a** [%]
1	4.0	0	82
2	5.0	0	71
3	5.0	25	66

[a] Initially, *Hb*HNL and 3 equiv of HCN were used but after 30 min the reaction stopped. Complete conversion was only achieved by the addition of extra *Hb*HNL and 1.5 equiv HCN (see Experimental).

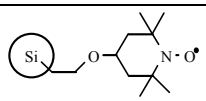
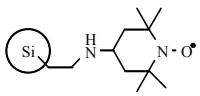
When the enzymatic hydrocyanation was performed at 0 °C and pH 4.0 the desired γ,δ-unsaturated cyanohydrin **1a** was formed with reasonable enantioselectivity (*ee* = 82 %, entry 1, Table 3). Even though this result is in line with *ee*'s obtained previously with other short-chain aldehydes[1] it is still insufficient for synthetic use. The relatively low *ee* is not caused by chemical background reaction, as this is virtually absent under these conditions. Therefore, other reasons were investigated.

The *Hb*HNL catalysed hydrocyanation of **2a** as described above stopped after 30 min (see Table 3). Apparently the enzyme was deactivated and additional *Hb*HNL and HCN were needed to allow the reaction to complete. After $NaHCO_3$ neutralisation of the crude reaction mixture that results from the oxidation part of the highly reactive TEMPO is still present. To minimise a potentially harmful effect of TEMPO on the selectivity of the *Hb*HNL we investigated the influence of different immobilised variants of TEMPO in the oxidation-

hydrocyanation cascade. This should allow an efficient removal of TEMPO from the reaction mixture just before addition of the *Hb*HNL. Furthermore, such a procedure opens the way to a more atom-efficient protocol that enables recycling of the TEMPO-catalyst.

TEMPO was immobilised on colloidal silica according to a known procedure[41] and compared to commercial TEMPO immobilised on silica gel. The TEMPO immobilisates were screened in the oxidation of **3a** for optimal conversion and minimal isomerisation of the aldehyde product **2a** (Table 4). The data in Table 4 (entry 1) show that oxidation of **3a** using TEMPO immobilised on colloidal silica proceeds about three times faster than the oxidation of **3a** using soluble TEMPO (Table 2). After 40 min at RT the reaction was complete and no isomerisation was observed. The TEMPO on colloidal silica could easily be recovered (by filtration) and re-used at least once without any loss of activity. The commercial TEMPO on silica (entry 2, Table 4) gave, under similar reaction conditions, only a few percent of 3-butenal **2a**. In the ^{1}H-NMR of the reaction mixture, the un-reacted alcohol **3a** was clearly identified together with signals that could only be attributed to the undesired isomer of 3-butenal, crotonaldehyde.

Table 4: Oxidation of **3a** using TEMPO immobilised on colloidal silica and commercial TEMPO immobilised on silica.[a]

Entry	Structure	Loading [mmol/g]	Equiv.	**2a** [%][b]	Reaction time
1[c]		0.27	0.16	>98	40 min
2[d]		0.61	0.35	0[e]	3.5 h

[a] 1.2 equiv of $PhI(OAc)_2$ in a 0.2 M solution of **3a** in a 1:9 CH_2Cl_2:pentane mixture at room temperature.

[b] Conversion determined by ^{1}H-NMR.

[c] TEMPO immobilised on colloidal silica.

[d] Commercialy TEMPO immobilised on silica, purchased from Sigma-Aldrich.

[e] The reaction yielded the unwanted isomer of **2a**, crotonaldehyde.

Finally, the oxidation of **3a** catalysed by immobilised TEMPO was combined with the *Hb*HNL catalysed hydrocyanation to arrive at the desired cyanohydrin **1a**.[42] After the

oxidation reaction, the immobilised TEMPO was removed by filtration and the resulting mixture neutralised with a saturated $NaHCO_3$ solution. The resulting solution is directly used in the *Hb*HNL catalysed formation of γ,δ-unsaturated **1a**. Almost optically pure **1a** (*ee* = 93 %; entry 1, Table 5) could be isolated as its TBDMS-ether **5a** in 37 % overall yield[43] starting from the γ,δ-unsaturated primary alcohol **3a**. Although, under these optimised conditions, the isolated yield of **1a** is 5-10 % lower compared to the yield obtained after the oxidation-hydrocyanation sequence using soluble TEMPO, the optical purity of **1a** is considerably higher (93 % *versus* 82 %; entry 1, table 5 *versus* entry 1, Table 3) when performing this cascade using immobilised TEMPO.

The above procedures were used to prepare γ,δ-unsaturated cyanohydrins **1b-1e**, starting from the corresponding primary alcohols **3b-3e**. The resulting cyanohydrins were directly protected as acetates. The results are summarised in Table 5. A similar trend with regard to isolated yield and optical purity as observed for the synthesis of **5a** was also found for the preparation of **4b-4e**. In general, the *ee*'s of **4b-4e** (entries 2-5, Table 5) are significantly higher whereas their isolated yields (calculated from **3b-3e**) are somewhat lower if they are prepared by the immobilised TEMPO/*Hb*HNL cascade compared to the *ee*'s and yields obtained by applying the same cascade by using soluble TEMPO. As the reactions were performed on a 100 mg scale and the compounds involved in this reaction cascade are relatively polar and/or volatile, some loss/low yields could not be avoided.

Table 5. The three-step cascade oxidation-hydrocyanation and protection reaction starting from **3a-3e** using both homogeneous and heterogeneous TEMPO in combination with *Hb*HNL.

Entry	Substrate	Product	pH	Homogeneous	Heterogeneous
				Yield[a] (ee) [b] [%]	Yield[a] (ee) [b] [%]
1	**3a**	**5a**	4.0	nd. (82)	37 (93)
2	**3b**	**4b**	4.0	54 (92)	43 (97)
3	**3c**	**4c**	5.0	48 (78)	23 (95)
4	**3d**	**4d**	5.0	51 (72)	7 (91)
5	**3e**	**4e**	5.0	52 (61)	26 (87)

[a] isolated yields over 3 steps.

[b] *ee* determined by chiral GC.

When testing both pH 4.0 and 5.0 for aldehydes **2c**-**2e,** pH 5.0 proved to be favourable and was therefore utilized. Finally, the γ,δ-unsaturated primary alcohols **3f** and **3g** were readily oxidised by the TEMPO/PhI(OAC)$_2$ procedure to the corresponding aldehydes **2f** and **2g**. However, both aldehydes proved unreactive towards *Hb*HNL catalysed hydrocyanation. This can be attributed to the length of the alkyl chain, which is known to be crucial for *Hb*HNL activity.[11,12,13,14]

Conclusion

In summary, we have developed an efficient oxidation-hydrocyanation cascade protocol that produces optically enriched γ,δ-unsaturated cyanohydrins in good yields starting from the corresponding primary alcohols. The oxidation of the alcohols with TEMPO and PhI(OAc)$_2$ is a mild and selective method to prepare β,γ-unsaturated aldehydes, which are otherwise difficult to access as they readily undergo isomerisation to the α,β-unsaturated analogues. The thus generated aldehydes can be used directly in the subsequent *Hb*HNL catalysed hydrocyanation to give the desired optically enriched γ,δ-unsaturated cyanohydrins. Moreover, when the *Hb*HNL catalyst was used in cascade with the TEMPO catalyst immobilised on colloidal silica *ee*'s up to 97 % and overall yields up to 43 % of the final cyanohydrin derivatives were obtained. The TEMPO-catalyst could be re-used at least once without any loss of catalytic activity.

Experimental

General Remarks: 1H and 13C nuclear magnetic resonance (NMR) spectra are recorded in CDCl3 on a Bruker Avance 250 (250. 13 MHz and 62.90 MHz respectively) or Bruker Avance 400 (400.13 MHz and 100.61 MHz respectively) with chemical shifts (δ) reported in ppm downfield from tetramethylsilane. MS and HRMS data were measured at 70 eV with a Finnigan MAT900 spectrometer. To follow the course of the reactions, samples were taken directly from the reaction mixtures, dissolved in $CDCl_3$ and analysed by 1H-NMR. For the oxidation and enzyme reaction, the conversion was determined by monitoring the disappearance of the H_2C-O (δ 3.59 - 3.76) and the *H*C=O (δ 9.66 - 9.74) signal respectively. Flash column chromatography was performed with Baker 7024-02 silica gel (40 μ, 60 Å) solvents were petroleum ether (PE) with a boiling range between 40 °C and 60 °C, and ethyl acetate (EA). Thin layer chromatography (TLC) was performed using silica plates from Merck (Kieselgel 60 F_{254} on aluminium with fluorescence indicator). Compounds on TLC

were visualised by UV-detection or 5 % (w/v) aqueous $KMnO_4$. The racemic cyanohydrin acetates were prepared from the corresponding aldehydes according to literature.[44] The enantiomeric excess of the acylated (*S*)-cyanohydrins **4** was determined on a Shimadzu GC-17A, equipped with a β-cyclodextrin column (CP-Chirasil-Dex CB 25m × 0.32 mm ID), a FID detector, and a Shimadzu Auto-injector AOC-20i. The carrier gas was He with a linear gas velocity of 75 cm/s at 155kPa. The GC-retention times are summarised in Table 6. Optical rotations were measured on an AA-10 automatic polarimeter from Optical Activity Ltd. Pro analysis grade γ,δ-unsaturated primary alcohols **3a-3e**, **3h** and **3i** were all commercially available and used without purification except for 3-butenol **3a**, which was distilled and stored under nitrogen and over mol sieves 4A. Primary alcohols **3f** and **3g** were prepared by hydrogenation and LAH reduction, respectively, starting from 3-decynol following literature procedures.[45,46] TEMPO immobilised on silica gel (70-120 mesh) was purchased from Sigma-Aldrich. The loading of the TEMPO on colloidal silica was found by elemental analysis on a Elementar Vario EL III analyzer. The hydroxynitrile lyase from *Hevea brasiliensis* (*Hb*HNL) was a generous gift from DSM (M. Wubbolts, NL). The activity of the *Hb*HNL (13.1 U/mg protein solution) was determined according to standard procedures.[47,48]

Table 6. Temperature program and retention times for the GC analysis of acetates **4a-4e**.

Compound	Temperature (°C)	R_t **(*R*) 4a-e** (min)	R_t **(*S*) 4a-e** (min)
4a	100	3.13	4.16
4b	135	1.13	1.20
4c	135	1.61	1.81
4d	135	1.67	1.91
4e	135	1.63	1.83

Colloidal silica: A mixture of tetramethoxysilane (25 ml) and acidic water (50 ml, pH adjusted to 2.8 by addition of concentrated HCl) was stirred until a homogeneous mixture was formed. After ageing at room temperature for 18 h, the gel was crushed to a fine powder and the water was removed by azeotropic distillation with toluene. The solid was collected by filtration and dried at 120 ºC over-night and further crushed in a mortar to a fine white glass like powder. The particle size was not determined. The TEMPO was immobilised on the colloidal silica according to literature.[41]

General Oxidation-hydrocyanation procedure A, using homogeneous TEMPO and *Hb*HNL: To a solution of γ,δ-unsaturated primary alcohol **3a-e** in a pentane/CH_2Cl_2 mixture $PhI(OAc)_2$ and TEMPO were added, all quantities are according to Table 2. The reaction mixture was stirred at room temperature until full conversion of the alcohol was reached (according to ^{1}H-NMR). Then, saturated $NaHCO_3$ solution was added at 0 °C to the reaction mixture until the CO_2 development ended. The organic layer was isolated and hydroxynitrile lyase from *Hevea Brasiliensis* (1.45 KU/mmol **3a-e**) dissolved in an equivolume of 0.1 M citrate buffer (pH 4.0 or pH 5.0) at 0 ºC to generate a 1:1 mixture (v/v) of organic phase to buffer was added. The mixture was stirred vigorously until a stable emulsion was obtained, after which HCN dissolved in MTBE was added. [The HCN-solution was prepared by dissolving sodium cyanide (3.0 equivalents) in water (10 ml) and adjusting the pH of the solution to 4.8 by addition of citric acid. This aqueous solution was extracted with MTBE (3 × 8 ml) at 0 ºC]. After the hydrocyanation was complete (according to ^{1}H-NMR) the organic layer was isolated and dried over $MgSO_4$. In the cases where the emulsion was too stable, it was extracted with CH_2Cl_2 (5-10 ml). After evaporation of the solvents, the resulting oil was dissolved in CH_2Cl_2 (2 ml/mmol substrate) and acetic anhydride (3 eq.), pyridine (2 eq.) and 4-DMAP were added to the solutions of (*S*)-cyanohydrins **1b-e**. The reaction mixture was stirred overnight, washed with 1 % HCl (2 × 10 ml), water (2 × 10 ml), followed by washing with saturated $NaHCO_3$ (2 × 10 ml) and water (2 × 10 ml). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography. In the case of **1a**, starting from 6 mmol, only an analytical sample was derivatised in the same manner. This showed an ee of 82 %.

(2*S*)-2-acetoxy-4-methyl- 4-pentenenitrile (4b): The title compound was prepared from **3b** (430 mg, 5 mmol) according to general procedure A, using pH 4.0. **(*S*)-4b** was obtained as a clear oil (414 mg, 54 % yield, 92 % ee). For characterisation, see **(*S*)-4b** obtained from general procedure B.

(2*S*)-2-acetoxy-5-methyl-4-hexenenitrile (4c): The title compound was prepared from **3c** (200 mg, 2 mmol) according to general procedure A, using pH 5.0. **(*S*)-4c** was obtained as a clear oil (161 mg, 48 % yield, 78 % ee). For characterisation, see **(*S*)-4c** obtained from general procedure B.

(2*S*,4*Z*)-2-acetoxy-4-heptenenitrile (4d): The title compound was prepared from **3d** (200 mg, 2 mmol) according to general procedure A, using pH 5.0. **(*S*)-4d** was obtained as a clear oil (171 mg, 51 % yield, 72 % ee). For characterisation, see **(*S*)-4d** obtained from general procedure B.

(2*S*,4*E*)-2-acetoxy-4-heptenenitrile(4e): The title compound was prepared from **3e** (200 mg, 2 mmol) according to general procedure A, using pH 5.0. **(*S*)-4e** was obtained as a clear oil (174 mg, 52 % yield, 61 % ee). For characterisation, see **(*S*)-4e** obtained from general procedure B.

General Oxidation-hydrocyanation procedure B using TEMPO immobilisates and *Hb*HNL: A 0.2 M solution of γ,δ-unsaturated primary alcohols **3** (1 equiv) in a 9:1 pentane/CH_2Cl_2 mixture, $PhI(OAc)_2$ (1.1 equiv) was mixed with immobilised TEMPO (TEMPO on colloidal silica: 0.27 mmol/g, 0.16 equiv or commercial TEMPO on silica gel: 0.61 mmol/g, 0.35 equiv) and stirred at room temperature until completion (according to ^{1}H-NMR). After filtration of immobilised TEMPO, a saturated $NaHCO_3$ solution was added to the reaction mixture at 0 °C until the CO_2 development ended. The organic layer was isolated and to this was added hydroxynitrile lyase from *Hevea Brasiliensis* (1.45 KU/mmol **3a-e**) dissolved in an equivolume of 0.1 M citrate buffer (pH 4.0 or pH 5.0) at 0 ºC to generate a 1:1 mixture (v/v) of organic phase to buffer. The mixture was stirred vigorously until a stable emulsion was obtained, after which HCN (3 equiv) dissolved in MTBE was added. [The HCN-solution was prepared by dissolving sodium cyanide (3.0 eq) in water (10 ml) and adjusting the pH of the solution to 4.8 by addition of citric acid. This aqueous solution was extracted with MTBE (3 × 8 ml) at 0 ºC]. After the hydrocyanation was complete (according to ^{1}H-NMR) the organic layer was separated and dried over $MgSO_4$. In the cases where the emulsion were too stable, it was extracted with CH_2Cl_2 (5-10 ml). After evaporation of the solvents, the resulting oil was dissolved in CH_2Cl_2 (2 ml/mmol substrate) and acetic anhydride (3 eq.), pyridine (2 eq.) and 4-DMAP were added to the solutions of (*S*)-cyanohydrins **1b-e**. The reaction mixture was stirred overnight, washed with 1 % HCl (2 × 10 ml), water (2 × 10 ml), followed by washing with saturated $NaHCO_3$ (2 × 10 ml) and water (2 × 10 ml). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography. In the case of **1a**, only an analytical

sample was derivatised in the same manner to give the acetate with an ee of 95 %. The remaining solution was derivatised as the corresponding TBS-ether **5a** (see below).

(2*S*)-2-acetoxy-4-methyl- 4-pentenenitrile(4b): The title compound was prepared from **3b** (431 mg, 5 mmol) according to general procedure B, using pH 4.0. **(*S*)-4b** was obtained as a clear oil (330 mg, 43 % yield, 97 % ee); R_f (PE/EA, 95 : 5) = 0.27; $[\alpha]^{22}_D$ = -74 (*c* = 1, $CHCl_3$); ^{1}H-NMR (250.13 MHz): δ = 1.83 (s, 3H, C*H*$_3$C=C), 2.16 (s, 3H, CH_3C=O), 2.64 (d, *J* = 7.1 Hz, 2H, C*H*$_2$CHCN), 4.98 (d, *J* = 18.9 Hz, 2H, *H*$_2$C=CCH$_3$), 5.50 (t, *J* = 7.1 Hz, 1H, CH-CN); ^{13}C-NMR (62.90 MHz): δ = 20.3 (*C*H$_3$C=O), 22.3 (*C*H$_3$C=C), 40.4 (CH_2), 59.7 (CH-O), 116.10 (CH=*C*H$_2$), 116.7 (CN), 137.6 (*C*H=CH$_2$), 168.9 (C=O). MS ($C_8H_{11}O_2N$, m/z, relative intensity) 153 (M^+, 2), 111 (4), 93 (100), 66 (68), 55 (32).

(2*S*)-2-acetoxy-5-methyl-4-hexenenitrile (4c): The title compound was prepared from **3c** (75 mg, 0.75 mmol) according to general procedure B, using pH 5.0. **(*S*)-4c** was obtained as a clear oil (29 mg, 23 % yield, 95 % ee); R_f (PE/EA, 95 : 5) = 0.27; $[\alpha]^{22}_D$ = - 46 (*c* = 1, $CHCl_3$); ^{1}H-NMR (250.13 MHz): δ = 1.71 (s, 3H, C*H*$_3$C=C), 1.79 (s, 3H, C*H*$_3$C=C), 2,18 (s, 3H, C*H*$_3$C=O), 2.61-2.67 (m, 2H, C*H*$_2$), 5.19 (t, *J* = 7.2 Hz, 1H, C=C*H*), 5.30 (t, *J* = 6.9 Hz, 1H, C*H*CN); ^{13}C-NMR (100.61 MHz): δ=17.9 (*cis*-*C*H$_3$), 20.3 (*C*H$_3$C=O), 25.7 (*trans*-*C*H$_3$), 31.1 (CH_2), 60.9 (CH-O), 114.9 (C=*C*H$_2$), 116.7 (CN), 138.5 (*C*=CH$_2$), 168.9 (CO); MS ($C_9H_{13}O_2N$, m/z, relative intensity) 167 (M^+, 16), 149 (16), 142 (64), 113 (40), 95 (40), 69 (100), 55 (48).

(2*S*,4*Z*)-2-acetoxy-4-heptenenitrile (4d): The title compound was prepared from **3d** (75 mg, 0.75 mmol) according to general procedure B, using pH 5.0. **(*S*)-4d** was obtained as a clear oil (8 mg, 7 % yield, 91 % ee); R_f (PE/EA, 95 : 5) = 0.35; $[\alpha]^{22}_D$ = - 46 (*c*=1, $CHCl_3$); 1 H-NMR (250.13 MHz): δ = 1.02 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$), 2.05-2.11 (m, 2H, CH$_3$C*H*$_2$), 2.15 (s, 3H, C*H*$_3$C=O), 2.64-2.69 (m, 2H, C*H*$_2$CHCN), 5.29-5.41 (m, 2H, C*H*=C*H*), 5.66-5.76 (m, 1H, C*H*CN); ^{13}C-NMR (62.90 MHz): δ=13.3 (*C*H$_3$-CH$_2$), 20.3 (*C*H$_3$C=O), 20.3 (CH$_3$-*C*H$_2$), 30.1 (CH_2), 59.8 (*C*H-O), 116.6 (CN), 118.8 (*C*H-CH$_2$-CH), 137.8 (*C*H-CH$_2$-CH$_3$), 169.0 (C=O); MS ($C_9H_{13}O_2N$, m/z, relative intensity) 168 (M^++1, 2), 142 (16), 106 (84), 80 (64), 69 (100).

(2*S*,4*E*)-2-acetoxy-4-heptenenitrile (4e): The title compound was prepared from **3e** (75 mg, 0.75 mmol) according to general procedure B, using pH 4.0. **(*S*)-4e** was obtained as a clear oil (32.6 mg, 26 % yield, 87 % ee); R_f (PE/EA, 95 : 5) = 0.35; $[\alpha]^{22}_D$ = -32 (*c*=1, $CHCl_3$); ^{1}H-NMR (250.13 MHz): δ = 1.02 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$), 2.03-2.12 (m, 2H, CH$_3$C*H*$_2$), 2.15 (s, 3H, C*H*$_3$C=O), 2.56-2.62 (m, 2H, C*H*$_2$CHCN), 5.30-5.45 (m, 2H, C*H*=C*H*), 5.71-5.82 (m, 1H, C*H*CN); ^{13}C-NMR (100.61 MHz): δ = 13.2 (*C*H$_3$-CH$_2$), 20.1 (*C*H$_3$C=O), 25.4 (CH$_3$-*C*H$_2$), 35.4 (*C*H$_2$), 60.9 (*C*H-O), 116.4 (CN), 119.5 (*C*H-CH$_2$-CH), 139.0 (*C*H-CH$_2$-CH$_3$), 168.9 (C=O); MS ($C_9H_{13}O_2N$, m/z, relative intensity) 167 (M^+, 16), 149 (52), 142 (24), 106 (48), 83 (52), 69 (100).

(2*S*)-2-(*t*-butyldimethylsilanyloxy)-4-pentenenitrile (5a): A solution of recrystallised imidazole (0.87 g, 12.8 mmol) and *t*-butyldimethylsilyl chloride (2.11 g, 14 mmol) in 70 ml DMF was stirred at 0 °C for 20 min. The crude solution of **1a**, prepared from **3a** (844 mg, 11.7 mmol) according to general procedure B using pH 4 was added, the mixture was allowed to warm to RT and stirred overnight. The reaction mixture was diluted with 70 ml of water and extracted with diethyl ether (3 × 110 ml). The combined organic layers were washed with water (2 × 100 ml) and then with brine (1 × 100 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (PE/EA, 98 : 2) yielding **5a** as a clear oil (659 mg, 37 % yield, 95 % ee); R_f (PE/EA, 95 : 5) = 0.74; $[\alpha]^{22}_D$ = - 60 (*c* = 1, $CHCl_3$); ^{1}H-NMR (250 MHz): δ= 0.17 (s, 3H, C*H*$_3$Si), 0.22 (s, 3H, C*H*$_3$Si), 0.94 (s, 9H, *t*-but), 2.53-2.59 (m, 2H, C*H*$_2$CHCN), 4.47 (t, *J* = 6.5 Hz, 1H, C*H*CN), 5.23-5.30 (m, 2H, CH=C*H*$_2$), 5.84 (ddt, *J* = 17.4, 9.8 and 7.0 Hz, 1H, C*H*=CH$_2$); ^{13}C-NMR (62.90 MHz): δ = -4.93 (*C*H$_3$-Si), -4.73 (*C*H$_3$-Si), 18.49 (Si-*C*(CH$_3$)), 25.90 (Si-C(*C*H$_3$)), 41.11 (*C*H$_2$-CH-O), 62.31 (*C*H-O), 120.00 (CN), 120.51 (*C*H$_2$=CH), 131.40 (CH$_2$=*C*H); HRMS (EI) calculated for $C_{11}H_{21}NOSi$ (M^+) 211.1392 found 211.1409; MS ($C_{11}H_{21}NOSi$, m/z, relative intensity) 210 (M^+, 15), 156 (66), 126 (100), 73 (74).

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Chapter 3

The first encapsulation of hydroxynitrile lyase from *Hevea brasiliensis* in a sol-gel matrix

Summary: A straightforward process for the encapsulation of *Hb*HNL under low methanol conditions has been developed. By adding a sol, prepared by hydrolysis of TMOS/MTMS at pH 2.8 with continuous removal of methanol, to a stirred solution of the enzyme in a buffer at pH 6.5, at least 65 % of the activity of the free enzyme could be recovered after the encapsulation. The aquagels were successfully used in the synthesis of (*S*)-cyanohydrins.

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Introduction

Hydroxynitrile lyases (HNL's) are a class of enzymes that can be found in a wide range of plants, such as millet and the apple, almond, rubber and plum trees.[1] When the plant material is damaged, for instance by a herbivore, HNL catalyses the breakdown of a cyanohydrin into an aldehyde/ketone and toxic HCN. More interestingly, the enzyme may also catalyse the reverse reaction, enabling the synthesis of enantiopure cyanohydrins from aldehydes/ketones and HCN with excellent yields and enantioselectivities.[2,3,4,5,6,7,8] These enantiopure cyanohydrins can in turn readily be converted into a wide range of compounds that are versatile building blocks for the synthesis of fine chemicals, pharmaceuticals and agrochemicals.[2,9,10]

Recently, the immobilisation of enzymes has attracted much attention, increased stability when used in organic solvents and recyclability being the main research objectives. Cross-linked crystals and cross-linked enzyme aggregates of HNL's together with HNL's adsorbed on solid supports or encapsulated in sol-gels and polyvinyl alcohol (PVA)-gels have been used as catalysts in the synthesis of optically active cyanohydrins.[11,12,13,14,15,16] However, only the PVA-entrapped HNL from *Prunus amygdalus*, the cross-linked enzyme crystals from *Manihot Esculenta* and the cross-linked enzyme aggregates from *Prunus amygdalus* proved to be stable upon recycling. In this context it is important to notice that HNL's only have in common that they all catalyse the cyanogenesis. Structurally they can belong to different classes of enzymes and are therefore not always comparable. For example, HNL from *Hevea brasiliensis* is closely related to the α/β hydrolases, which also include lipases, while *Prunus amygdalus* is closely related to FAD dependent oxidoreductases.[17,18]

The (*S*)-Hydroxynitrile lyase from *Hevea brasiliensis* (*Hb*HNL) has been used for the addition of HCN to a wide range of aldehydes and ketones.[19,20,21,22,23,24] In spite of its versatility, the only immobilisation reported is the adsorption of *Hb*HNL onto celite.[12] When the immobilised enzyme was used in an organic solvent it was found that the maximum activity of the enzyme was only obtained when there was a discrete water layer surrounding the enzyme. This indicates that the enzyme is only active in a micro-aqueous environment inside the organic solvent. To assure this whilst avoiding a separate aqueous phase in the reaction, the *Hb*HNL can be encapsulated in a sol-gel matrix. The pores of such a matrix can

be filled with the aqueous buffer of choice and then used in an orgaic solvent. In this manner, the versatile *Hb*HNL will become available for an even wider range of reaction conditions.

The sol-gel technique allows the synthesis of chemically inert glasses, which in theory can be formed into any desired shape. They have high porosity (up to 98 % pore volume) and a relatively high mechanical and thermal resistance. In addition they can be produced under conditions that are relatively benign to enzymes. This technique has successfully been applied for the encapsulation of lipases into various sol-gel materials,[25,26,27,28,29] and as the *Hb*HNL is structurally related to lipases, we assumed that the encapsulation of *Hb*HNL should proceed in a similar manner.

As sol-gel encapsulated CAL-B (33 kDa) which belongs to the α/β hydrolase family just like the *Hb*HNL, has been recycled up to eight times without any loss of activity,[25] it is reasonable to assume that the dimer of *Hb*HNL (58.4 kDa) is equally well encapsulated in a sol-gel matrix (*Hb*HNL is a dimer in aqueous solutions[30]). In spite of this structural similarity, the *Hb*HNL is much more susceptible towards deviations from its optimum conditions.[31,32,33,34,35] Due to this, newly developed methodology rather than the standard immobilisation procedures, is applied.

Here we present the first successful encapsulation of the (*S*)-selective hydroxynitrile lyase from *Hevea brasiliensis* in a sol-gel matrix.

Results and discussion

In preliminary experiments, we encapsulated the *Hb*HNL into a sol-gel matrix following standard procedures.[25,27] In these procedures, the methanol released during the formation of the sol was not eliminated, which caused a complete deactivation of the *Hb*HNL during the gelation process. This is in line with the previously described methanol sensitivity of *Hb*HNL.[36] In another procedure, the alkoxy silanes are partially hydrolysed and then transesterified with glycerol. The methanol is removed from the sol, and then the sol is mixed with water containing the bio-molecules.[15] This method is so far the best method reported for the encapsulation of methanol-sensitive enzymes. Nevertheless, applied to *Hb*HNL it gave unsatisfactory results.

Therefore a new procedure was developed, in which the alkoxysilanes were almost 100 % hydrolysed by acid mediated hydrolysis, and the released methanol was removed by evaporation. The enzyme, dissolved in a buffer with pH 6.5, was then added to this precursor sol. At this pH, the gelation was catalysed and at the same time the enzyme was stabilized.[35,37] As soon as the gel was formed, it was submerged in the same buffer, pH 6.5, to remove any remaining methanol, possibly formed from hydrolysis of residual methoxy groups, by dialysis.

With this procedure, the aquagels[38] showed an activity of at least 65 % relative to the free enzyme in the standard aqueous activity test. The apparent decrease of activity is probably due to deactivation by residual methanol and diffusion limitations. Initial rate studies showed that the system is indeed limited by diffusion,[39] which indicates that the actual loss of activity during the encapsulation procedure is lower than 35 %. Due to changes of the specific particle size of the ground aquagels under the reaction conditions, quantification of the diffusion limitations was not pursued.

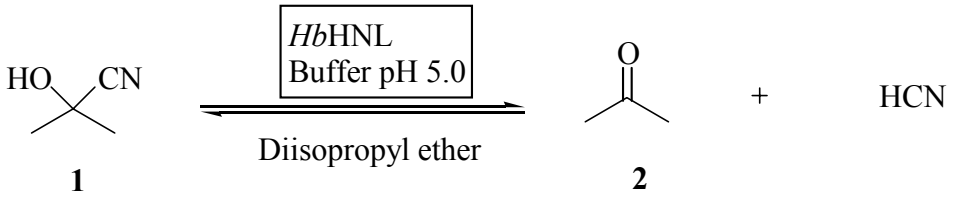
The use of poly vinyl alcohol (PVA) as an additive in the sol-gels is known to increase the activity of lipases in hydrophobic sol-gel materials.[26] Since the structure of *Hb*HNL is comparable with that of lipases, this possibility was also investigated, but, no effect of the PVA on the enzyme activity could be observed.[40] This indicates that the presence of PVA might only have an effect on the lipase activity after or during the drying of the gel.

Varying the MTMS (methyltrimethoxysilane)/TMOS (tetramethoxysilane) ratio is also known to change the activity of the sol-gel entrapped lipases.[41] Aquagels with a concentration of MTMS in TMOS varying from 0 to 50 volume % were prepared[42] and used in the addition of HCN to benzaldehyde, but, no difference in activity could be detected. No higher MTMS concentrations were examined since the gelation process then took several hours and the gels had a paste-like aspect. A mixture of 20 volume % MTMS in TMOS was chosen for further studies since this mixture gave the most convenient gelation time.

Attempts to dry the aquagels as aerogels and xerogels resulted in a total loss of activity. In the case of the xerogel there are two possible reasons for deactivation. When the gel is dried by evaporation of the water phase in open air (xerogels), capillary stress will cause partial collapse of the gel structure as the liquid-gas interface moves in through the gel. As the gel

shrinks, some of the enzymes will also be crushed. Secondly, it has been suggested that the exposure of *Hb*HNL to a gas-liquid interface drastically reduces the half-life of the enzyme.[35] In the preparation of the aerogels, the water in the aquagel is replaced with acetone, which again is replaced with CO_2 in an autoclave. When the supercritical conditions for CO_2 are reached, by increasing the temperature of the autoclave, the autoclave is slowly evacuated. The acetone is most likely causing the deactivation in this drying procedure. To verify whether this was the case, the buffer filling the pores of the aquagel was exchanged with acetone and then back to the buffer by dialysis. The resulting gel showed no activity, indicating that indeed acetone or the acetone water mixture did deactivate the *Hb*HNL. It has been shown that stirring *Hb*HNL in acetone containing 0.25 % water over 15 h at room temperature gives only 15 % loss of activity.[36] From this it can be concluded that it is the acetone-water mixture, rather than the acetone itself, that deactivates the enzyme. However, as it is known that *Hb*HNL is inactive at low water concentrations, it is not desirable to dry the gels, but rather to use them directly as aquagels. In this manner the enzyme will be completely hydrated with the buffer of choice (50 mM phosphate/citrate buffer pH 5.0) when suspended in the organic solvent. The buffer remains inside the pores of the aquagel and no separate macroscopic water phase is formed in the reaction mixture.

The encapsulated *Hb*HNL was applied in the enantioselective addition of HCN to benzaldehyde **3a**, furaldehyde **3b**, hexanal **3c**, *m*-phenoxybenzaldehyde **3d** and methyl isopropyl ketone **3e** to give their (*S*)-cyanohydrins **4a-e**. For safety reasons acetone cyanohydrin was used as the cyanide source, even though the liberated acetone has a negative effect on the enzyme (Scheme 1).



	R^1	R^2
a	Phenyl	H
b	2-Furyl	H
c	Hexyl	H
d	*m*-Phenoxyphenyl	H
e	Isopropyl	Me

Scheme 1. The synthesis of optically active cyanohydrins in diisopropyl ether using *Hb*HNL encapsulated in a sol gel matrix where the pores of the matrix is filled with a 50 mM phosphate/citrate buffer pH 5.0.

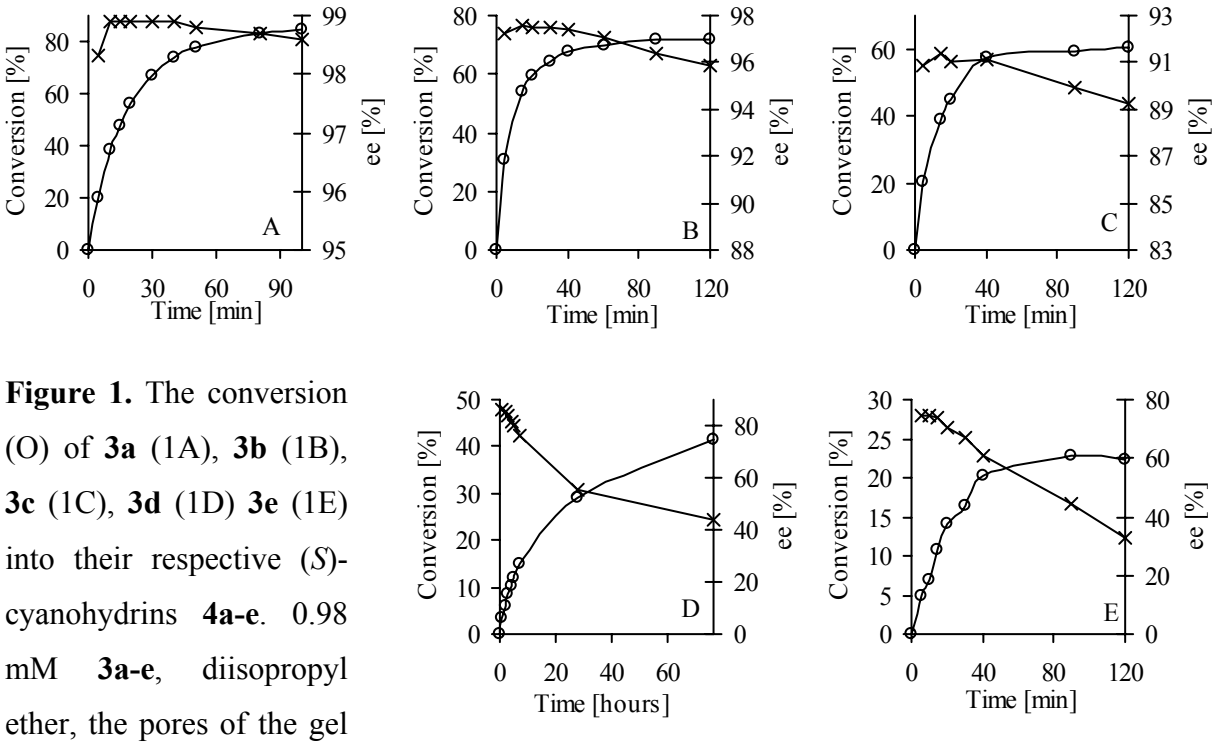


Figure 1. The conversion (O) of **3a** (1A), **3b** (1B), **3c** (1C), **3d** (1D) **3e** (1E) into their respective (*S*)-cyanohydrins **4a-e**. 0.98 mM **3a-e**, diisopropyl ether, the pores of the gel filled with a 50 mM phosphate/citrate buffer pH 5.0, 3 eq acetone cyanohydrin and 1.8 KU *Hb*HNL. The enantiomeric excess (×) was determined by chiral GC (see materials and methods).

As both, the generation of HCN and the addition of the HCN to the carbonyl group, are reversible reactions, the maximum conversion will be determined by the equilibrium constants of the two reactions and can, therefore, not be compared to the isolated yields (typically 95 - 99 %) that are obtained in the standard method where a five fold excess of free HCN is used.[22] When the amount of enzyme used in the literature procedure (2.5 times more) is taken into account, the reaction time that was observed, approximately 40-50 min (Figure 1), is comparable with that found in the literature (15 min).[22] The overall equilibrium in Scheme 1 was established rapidly in all cases except for **3d,** a substrate known to be "difficult" for HNL's.[3] A satisfactory result for this substrate has only been described in a highly enzyme loaded emulsion, using free HCN.[22]

The enantiomeric excess of the products is in line with those reported in the literature (>98 % for **4a-d**, and 75 % for **4e**)[22] for the same reactions. Small differences are due to the chemical background reactions (which form the racemic cyanohydrin) under the specific reaction conditions. To establish the stability of the sol-gel encapsulated *Hb*HNL, it was recycled 3 times in the reaction of HCN with benzaldehyde, using acetone cyanohydrin as the cyanide source. When the gels were washed with diisopropyl ether between each cycle, there was a rapid loss of activity and the biocatalysts were completely deactivated after the second recycle (Figure 2A). The deactivation was partially prevented when the gels were washed with a 50 mM phosphate buffer of pH 5.0 between each cycle. Then, the initial rate only dropped by around 50 % for each cycle. In all cases the enantiomeric excess of the product was higher than 98 % (Figure 2B).

A gel identical to the one used in the experiment described in Figure 2B was stirred with diisopropyl ether for 4 h (which is the same time as it took to perform the first three cycles of the experiment described in Figure 2B) before benzaldehyde and acetone cyanohydrin were added. The conversion observed for this gel was comparable with the conversion in cycle one of Fig 2B, indicating that there is no deactivation due to the buffer inside the gel or the solvent. Instead, the loss of activity must be caused by HCN, acetone, acetone cyanohydrin, the substrate, the product or leakage of the enzyme.

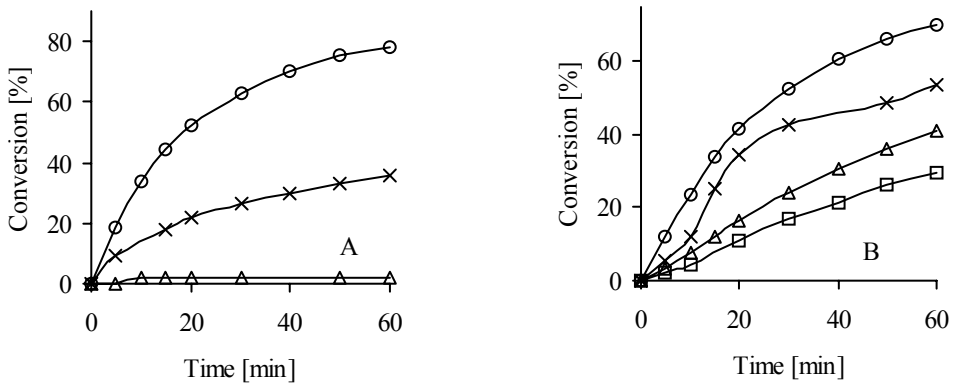


Figure 2. Recycling of the sol-gel encapsulated *Hb*HNL using benzaldehyde as the substrate in the standard reaction. In Figure A the gel was washed with diisopropyl ether between each cycle and in Figure B the gel was washed with a 50 mM phosphate buffer pH 5.0. Cycle 1 (O), cycle 2 (×), cycle 3 (Δ) and cycle 4 (□).

In the case of washing with diisopropyl ether, the deactivation is probably due to the acetone, which is formed during the reaction. Firstly, acetone is harmful for the enzyme, secondly, it changes the solubility of the solvent in the aqueous phase and vice versa. When the solvent is used for washing some of the water will be washed away from the gel, lowering the enzyme activity.

When the gels are washed with the buffer, the initial conditions for the enzyme are re-established and the activity is relatively higher than when washing with a solvent. The loss of activity will in this case, too, be due to the acetone formed, but also due to leakage of enzyme during the washing procedure.

In order to rule out any possible leakage of activity into the organic phase, the suspension of one reaction was filtered (taking care that no HCN could escape during the filtration) and the filtrate was monitored for activity. As expected the filtrate showed no activity (Figure 3). However, when the reaction was performed in water, it was found that up to 17 % of the observed activity derives from *Hb*HNL that had leached into the aqueous phase.[43]

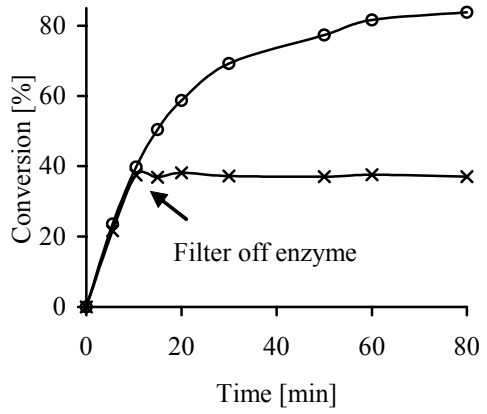


Figure 3: Test for leakage of activity from the gel into the organic phase. Unfiltered reaction (O) and filtered reaction (×). (See experimental part)

Encapsulated enzymes are not covalently bound to the sol gel surface. This means that if the gel breaks and the "capsule" around an enzyme opens, the enzyme will be washed away. Indeed, when the activity of an aquagel, which had been crushed into a fine powder was compared to an aquagel that had been crushed into a fine powder and then had been washed, a significant difference was found (Figure 4). The washed powder showed a decrease of the initial reaction rate by 48 % compared to the unwashed sol-gel powder. Even though the gels were shaken and not magnetically stirred in the recycling experiment, it is probable that some of the gels got further crushed leading to loss of activity in the subsequent washing.

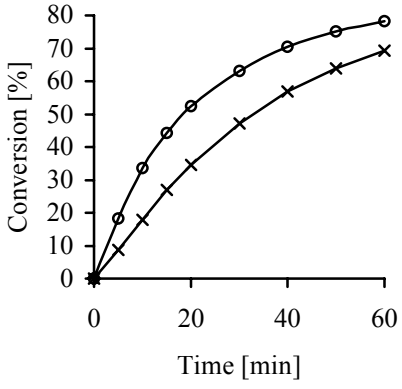


Figure 4: Test for leakage of activity during the washing procedure. Ground gel (O) and ground and washed gel (×). (See experimental part)

In order to characterise the gels, they were dried under supercritical conditions with CO_2 to give aerogels. Compared to open air drying which gives major structural changes during the drying process this technique will give virtually no or only minor structural changes. Nevertheless, the results from the characterisation should as a result only be considered as a strong indication. BET-analysis gave a surface area of 1000 m^2/g and a pore volume of 1.74 cm^3/g. The pore size distribution (Figure 5) is relatively wide, where the maximum pore radius is 5 nm. In comparison the dimension of the *Hb*HNL monomer is approximately 3.0×3.8×4.8 nm.[44] After the formation of the gel, the aquagel is usually aged for 12-72 hours in order to complete the hydrolysis and condensation.

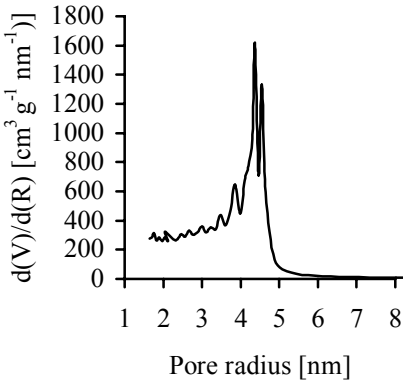


Figure 5: Pore size distribution of the sol-gel; the vertical axis is the differential dV/dR, where V is the adsorbed nitrogen gas volume, at standard conditions, per gram of gel [cm^3 /g] and R is the pore radius [nm].

To investigate if there is an optimal ageing time for the *Hb*HNL, we tested the activity of gels aged up to 16 days. The result (Figure 6) shows that there is a significant drop in activity during the first four to five days; then the activity stabilizes. The initial drop of activity is probably due to the formation of methanol during the beginning of the aging process and structural changes within the gel. The structural changes during the first days then level off.

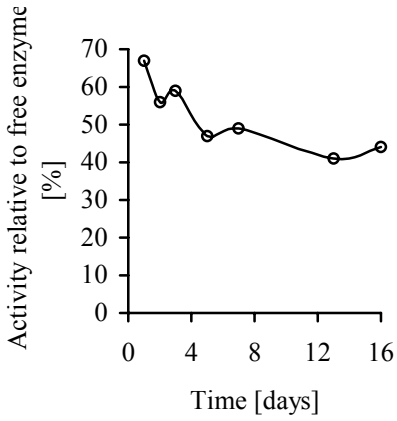


Figure 6: Activity of the encapsulated enyme as a function of the time of aging

Conclusion

*Hb*HNL, an enzyme very sensitive to organic solvents and requiring a near neutral pH, was successfully immobilized in a sol-gel. This low methanol immobilisation and first application of an aquagel holds great potential for the sol-gel encapsulation of many sensitive enzymes.

Experimental Section

General Remarks: *Hb*HNL was made available in a 25 mM potassium phosphate buffer pH 6.5 with 0.09 % sodium azide (3600 IU/ml) by Roche Diagnostics (Penzberg, Germany). The activity of the homogeneous enzyme was determined as described in the literature.[30] The reactants used in this study were poly vinyl alcohol with an average molar mass of M = 15 000 (Fluka), methyltrimethoxysilane (MTMS, 98 %, Aldrich) and tetramethoxysilane (TMOS, 98 %, Aldrich). Benzaldehyde, *m*-phenoxybenzaldehyde, furaldehyde, hexanal and methyl isopropyl ketone were all of analytical grade and distilled under a nitrogen atmosphere less than two hours before use. Mandelonitrile was purified by column chromatography at most 24 hours prior to use and stored under nitrogen at –4 °C. Acetone cyanohydrin was distilled and stored under nitrogen at 4 °C. Diisopropyl ether was of analytical grade and used without further purification. The derivatised samples were analysed on a β-cyclodextrin column (CP-Chirasil-Dex CB 25m × 0.25 mm) using a Shimadzu Gas Chromatograph GC-14B equipped with a FID detector and a Shimadzu Auto-injector AOC-20i, using N2 as the carrier gas. The conversion and the enantiomeric excess were calculated from the peak areas. The temperature programs and retention times are given in Table 1. UV measurements were performed on a UNICAM UV/Vis spectrometer. A Brunauer, Emmett

and Teller (BET) analysis of a gel dried as an aerogel, desorbed at 200 ºC, gave the specific surface area and the pore size distribution. Buffer A is a 25 mM potassium phosphate buffer pH 6.5 with 0.09 % sodium azide, buffer B is a 50 mM citrate/potassium phosphate buffer pH 5.0. Racemic reference compounds were prepared according to standard procedures[45] and their NMR-spectra were in accordance with literature.45,[46,47,48]

Table 1. Temperature programs and retention times for **3 a-e**, **(*R*)-4 a-e** and **(*S*)-4 a-e**

Substrate	Temperature program[a]	R_t[b] **3**	R_t[b] **(R)-4**	R_t[b] **(S)-4**
a	125 ºC (3 min) – 20 ºC/min – 200 ºC (0 min)	3.11	6.35	6.64
b	100 ºC (3 min) – 20 ºC/min – 200 ºC (0 min)	3.17	6.50	6.80
c	75 ºC (5 min) – 30 ºC/min – 200 ºC (1 min)	5.49	9.03	9.27
d	125 ºC (3 min) – 20 ºC/min – 200 ºC (13 min)	10.16	19.19	19.58
e	60 ºC (5 min) – 2 ºC/min – 98 ºC (0 min)	2.80	22.85	23.07

[a] Initial temperature (holding time) – Temperature gradient – Final temperature (holding time),

[b] Retention time (min).

General procedure A. Preparation of the sol-gel precursor: Acidic water (1.38 ml, pH adjusted to 2.85 by addition of HCl) was added to a mixture of MTMS (2.10 g, 15.4 mmol), TMOS (9.08 g, 58.5 mmol) and distilled water (10.4 ml) and stirred in a 100 ml round bottom flask until a homogenous mixture was obtained. The formed methanol was continuously removed on a rotary evaporator until the characteristic odours of MTMS, TMOS and MeOH were not detectable anymore. The mixture was then cooled to 0 °C and water was added until the total volume corresponded to the initial MTMS/TMOS - volume. The precursor (sol) was used immediately for the encapsulation of *Hb*HNLGeneral procedure B. Encapsulation of *Hb*HNL in a sol-gel matrix for standard activity test.

General procedure B. Encapsulation of *Hb*HNL in a sol-gel matrix for standard activity test: The stock solution of *Hb*HNL (100 mg, 3.6 KU/ml) was diluted to 6.0 g with buffer A. This solution (40 µl) was added to a mixture of the precursor (500 µl; prepared as described in general procedure A), and buffer A (460 µl) and stirred magnetically for 20 s. The stirring bar was removed and when the mixture gelled (4-5 min), the gel was submerged in buffer A and aged at 4 °C for 24 h. Buffer A was then replaced with distilled water and the gel was

aged further for 20 h at 4 °C. The aquagel tablet was ground in a morter into a fine powder and tested for catalytic activity.

General procedure C. Encapsulation of *Hb*HNL in a sol-gel matrix for synthetic reaction: A mixture of the stock solution of *Hb*HNL (0.5 ml, 3.6 KU/ml) and the precursor mixture (500 µl; prepared according to standard procedure A) was stirred magnetically for 20 sec. The stirring bar was removed and when the mixture gelled (4-5 min), the gel was submerged in buffer A and aged at 4 °C for 24 h. Then, the resulting gel-tablet was submerged in buffer B at 0 °C, and fixed above the magnet that stirred the buffer. After 1 h the surface of the aquagel was dried with a paper and the gel was rapidly ground into a fine powder which was used immediately for the synthesis of enantiopure cyanohydrins.

General procedure D. Standard aqueous activity test for the encapsulated enzyme in aqueous media: Mandelonitrile (80 µl) was dissolved in 10 ml of a citric acid/potassium phosphate buffer (3 mM, pH 3.5). This solution (1.4 ml), and all of the sol-gel encapsulated *Hb*HNL, prepared as described in general procedure B, were added to buffer B (4.9 ml) at 25 °C. The mixture was stirred magnetically for another 6 min, before the reaction was stopped by addition of concentrated HCl (2 drops). A sample was filtered through cotton and the UV-absorption of the supernatant was measured at 280 nm against a blank reaction. The activity was calculated according to the equation below.

$$Activity = \frac{V}{\varepsilon_{280} \times l \times S \times T} \Delta Abs / \min$$

Activity: The activity of the sample [U/ml]; *V*: Total reaction volume [ml]; *ε280*: 1.376 [l × $mmol^{-1}$ × cm^{-1}]; *l*: Path length in the UV-cell [cm]; *S*: Volume of enzyme solution added in the preparation of the gel [ml]; *T*: Reaction time [min].

General procedure E. Synthesis of optically active cyanohydrins: At 25 ˚C, all of the sol-gel encapsulated *Hb*HNL, prepared according to general procedure C (1.8 kU *Hb*HNL), was added to a magnetically stirred solution of the freshly distilled aldehyde/ketone (4.92 mmol) in diisopropyl ether (5 ml) which was saturated with buffer B. The reaction was started by the addition of acetone cyanohydrin (1.35 ml, 14.76 mmol). Samples of 10 µl were taken through the septum at different stages of conversion. The samples were added to a mixture of dichloromethane (0.5 ml), acetic anhydride (40 µl) and pyridine (40 µl). After at least 12 h at

room temperature the samples were analysed by chiral GC. The conversion and the enantiomeric excess were calculated from the relative peak areas of the aldehyde and the cyanohydrin derivative. The results are given in Figure 1.

Recycling, washing with diisopropyl ether saturated with a 50 mM citrate/phosphate buffer pH 5.0: The gels were prepared according to general procedure C and used in the addition of HCN to benzaldehyde according to general procedure E. The synthetic reaction was stopped after 1h and the gel was washed with diisopropyl ether saturated with buffer B before it was reused in a new cycle. The results of three cycles are given in Figure 2A.

Recycling, washing with a 50 mM citrate/phosphate buffer pH 5.0: The gels were prepared according to general procedure C and used in the addition of HCN to benzaldehyde according to general procedure E, with the following exception: The synthetic reaction was shaken orbitally and after one hour the gel was washed on a filter with a 50 mM phosphate buffer pH 5.0 (40 ml) and then reused in a new cycle. The results of four cycles are given in Figure 2B.

Test for leakage of activity to the organic solvent: Two gels were prepared according to general procedure C and used in the addition of HCN to benzaldehyde according to general procedure E, in two separate reactions A and B. After 10 min reaction B was filtered in a closed system to avoid any leakage of HCN. No more conversion could be detected in the supernatant. The conversion curves of the two reactions are given in Figure 3.

Test for leakage of activity by washing: Two gels were prepared according to general procedure C and used in the addition of HCN to benzaldehyde according to general procedure E, in two separate reactions A and B. The crushed gel used in reaction B was washed with buffer B prior to its use in the synthetic reaction. The conversion curves for the two reactions are given in Figure 4.

The effect of ageing on the gels: Seven gels were prepared according to general procedure B and stored for 1, 2, 3, 5, 7, 13 and 16 days respectively. The activity relative to the free enzyme was then measured according to general procedure D. The results are given in Figure 6.

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38) In an aquagel, the pores of the gel are filled with water.

39) Gels prepared according to standard procedure C were cut or ground into a particle size of 3-5 mm, 1-2 mm and < 0.1 and used in the addition of HCN to benzaldehyde according to standard procedure E. The gels showed relative initial rates of 0.2, 0.3 and 1, respectively.

40) Three gels were prepared according to procedure B where the 25 mM phosphate buffer pH 6.5 contained 0.0, 1.0 and 2.0 % PVA respectively. The gels were tested for activity according to procedure D.

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42) The gels were prepared according to standard procedure C with different MTMS/TMOS ratios and used in the addition of HCN to benzaldehyde according to standard procedure E.

43) Gels prepared according to standard procedure B and tested for activity according to standard procedure D. After 10 min the reaction mixtures were filtered and the activity of the filtrates were monitored.
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Chapter 4

Enantioselective synthesis of protected cyanohydrins via a kinetic resolution of cyanohydrin esters

Summary: A straightforward process for the preparation of optically active protected cyanohydrins, important building blocks for the synthesis of drugs and agrochemicals, has been established. Lipase B from *Candida Antarctica* (CAL-B) catalyses the kinetic resolution of racemic cyanohydrin acetates under mild conditions. The resulting labile cyanohydrins were re-protected either via an enzyme catalysed route, involving the addition of vinyl butyrate, or chemically after removal of the enzyme from the reaction mixture. This process gives access to both enantiomers in their pure form and in good yields, while reducing the risks due to HCN considerably. Moreover, a variety of different protection groups were introduced.

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Introduction

Cyanohydrins are versatile building blocks in organic synthesis.[1,2,3,4,5,6] They can readily be converted into a wide range of compounds such as α-hydroxy-acids[7], -esters[8], -ketones[9]-aldehydes[10] and β-hydroxy-amines.[11] Indeed, they have been utilised for the synthesis of, *e.g.* (*R*)-salbutamol,[12] (*R*)-terbutaline,[12] (*S*)-amphetamines (a family of drugs commonly referred to as Ecstasy),[13] as well as the Williamson glycine template.[14] In addition they are crucial components in pyrethroid insecticides synthesis.

Despite the fact that the first enantioselective hydroxynitrile-lyase-catalysed synthesis of cyanohydrins was already described in 1908[15] and despite their major role in the "synthon approach",[16] they are still not utilised to the extent that one might expect. Therefore, we aimed to develop a straightforward, enantioselective process for the synthesis of protected and, hence, stable cyanohydrins.

The kinetic resolution of cyanohydrin acetates has been investigated with a range of lipases.[17,18,19,20] Recently, the readily available *Candida antarctica* lipase B (CAL-B, "Novozyme 435" also known as chirazyme L-2, c.-f., C2, Lyo, which are both immobilised on a macroporous acrylic resin) has been used in the enantioselective de-acylation of mandelonitrile acetate **(*R*,*S*)-1a** to give (*R*)-mandelonitrile acetate **(*R*)-1a** and (*S*)-mandelonitrile cyanohydrin **(*S*)-2a**.[21] This procedure has been optimised for the kinetic resolution of a wide range of aromatic cyanohydrin acetates with excellent results.[22] However, the resulting (*S*)-cyanohydrins are relatively unstable. To avoid this problem, the kinetic resolution should ideally be coupled to a chemical or enzymatic protection reaction. This would yield both enantiomers of the cyanohydrin protected as two different and, therefore, readily separable derivatives.

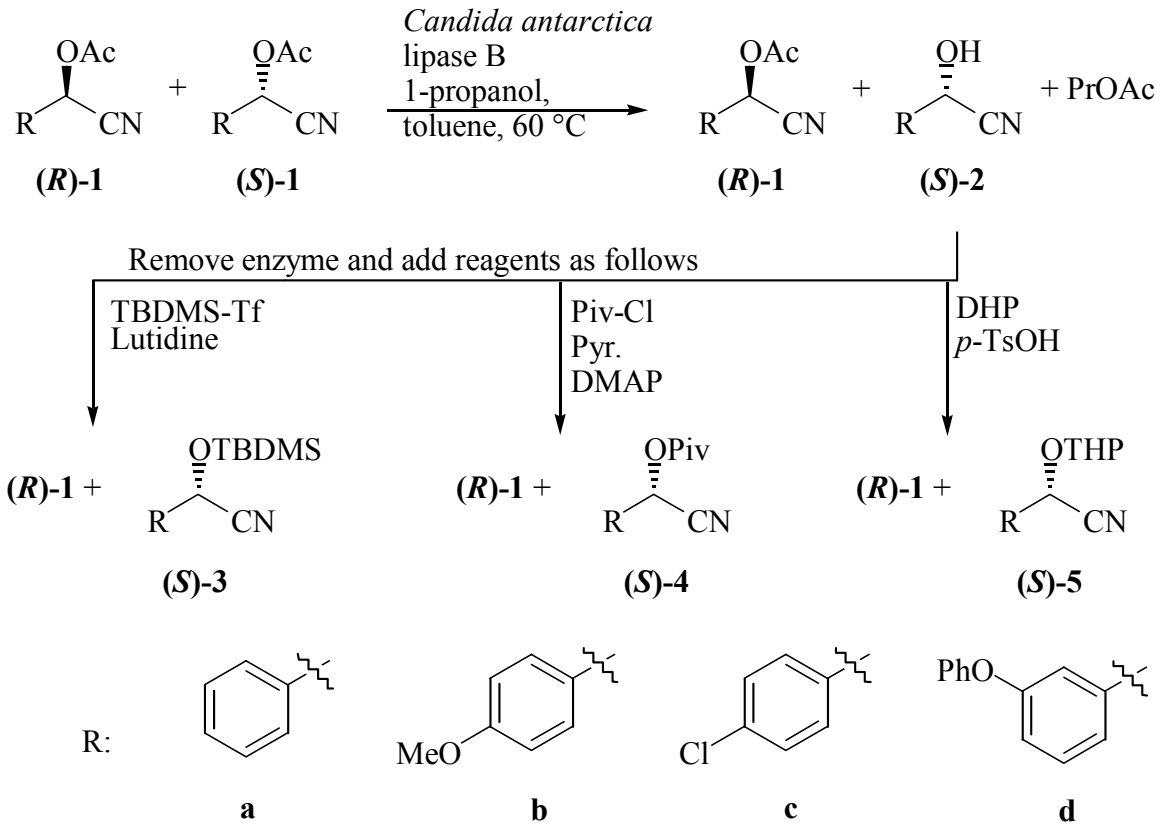
The *tert*-butyldimethylsilyl-group (TBDMS-group) and the pivaloyl group were chosen as protection groups that can be introduced under basic conditions. Both are known as versatile protecting groups in cyanohydrin chemistry and the TBDMS-group can be introduced without significant racemisation.[23] The tetrahydropyranyl-ethers (THP-ethers) were the obvious choice for a protection group that can be introduced under acidic conditions.[14]

By using the same enzyme (CAL-B) to protect the (*S*)-cyanohydrins directly after the kinetic resolution with a suitable acylating agent in a true one-pot reaction, esters can be formed under neutral conditions. This procedure should give access to a wide range of potentially interesting esters.

With the approaches described above, stable, protected and enantiopure cyanohydrin acetates may be obtained. Since the racemic cyanohydrin acetates can be prepared in a straightforward reaction of the appropriate aldehyde with sodium cyanide and acetic anhydride,[24] any risk of releasing HCN is minimised and the remaining cyanide ions can be destroyed by standard procedures.[25]

Results and Discussion

For the kinetic resolution of the racemic cyanohydrin acetates, the conditions described earlier were applied.[22] However, since the protection reaction has to be performed with the mixture obtained from the kinetic resolution, these conditions had to be newly investigated. For the protection of **(*S*) 2a-d** as TBDMS-ethers, several conditions were explored. TBDMS-triflate (TBDMS-Tf) in the presence of lutidine[26] gave higher yields than when Hünig's base[27] was employed. TBDMS-Cl in combination with imidazole[23] gave less favourable results, possibly due to solubility problems of imidazole in toluene. Pivalate was readily introduced by reaction with pivaloyl chloride in the presence of pyridine and dimethyl amino pyridine (DMAP),[28] and the THP-ethers were also formed with great ease under standard conditions (scheme 1). The introduction of the methoxyisopropyl and the benzyl ether groups (under acidic[29] and basic[30] conditions), however, failed.



Scheme 1: Kinetic resolution of cyanohydrin acetates and protection of the formed (*S*)-cyanohydrins

Four cyanohydrin acetates **(*R,S*) 1a-d** were subjected to a kinetic resolution followed by the chemical protection of the newly formed (*S*)-cyanohydrins **(*S*) 2a-d**. All the desired products **(*S*) 3a-d, (*S*) 4a-d** and **(*S*) 5a-d** were obtained in good yield and enantiomeric purity (Table 1, Scheme 1). Previous work in this group has shown a low selectivity for the kinetic resolution of aliphatic cyanohydrin acetates. This class of compounds is therefore not included in this work. However, if the kinetic resolution of aliphatic cyanohydrins should be desired other lipases have been used with excellent results.

In order to prevent any enzyme degradation, the protection reactions were performed after removing the enzyme from the reaction mixture. In the case of the synthesis of the TBDMS- and pivaloyl-derivatives, a straightforward column filtration gave the pure products. This was not the case for the THP-ethers since two diastereoisomers were formed and the THP-ethers had polarities comparable to the acetates. Conversions were, therefore, determined by ^{1}H-NMR. However, these conversions correspond to the isolated yield, within the margins of experimental error.

Table 1: Results of the kinetic resolution of cyanohydrin acetates and protection of the formed (*S*) cyanohydrins

R	Yield[a] (*ee*)[b] **(*S*)-3**	Yield[a] (*ee*)[b] **(*R*)-1**[d]	Yield[a] (*ee*)[b] **(*S*)-4**	Yield[a] (*ee*)[b] **(*R*)-1**[e]	Ratio[c] (*ee*)[b] **(*S*)-5**	Ratio[c] (*ee*)[b] **(*R*)-1**[f]	Ratio[c] Aldehyde[f,g]
A	88 (89)	93 (96)	99 (83)	98 (98)	96 (93)	100 (93)	4
B	79 (89)	90 (97)	81 (88)	90 (97)	84 (93)	100 (92)	16
C	78 (82)	96 (>99)	76 (78)	85 (>99)	86 (90)	99 (>99)	16
D	89 (-)	94 (87)	71 (81)	89 (90)	86 (84)	100 (91)	14

[a] Yields are isolated yields [%].

[b] Enantiomeric excess [%] was determined by chiral HPLC.

[c] Ratios of **5**, **1** and the aldehyde in the reaction mixture were determined by NMR.

[d]From protection as silyl-ether.

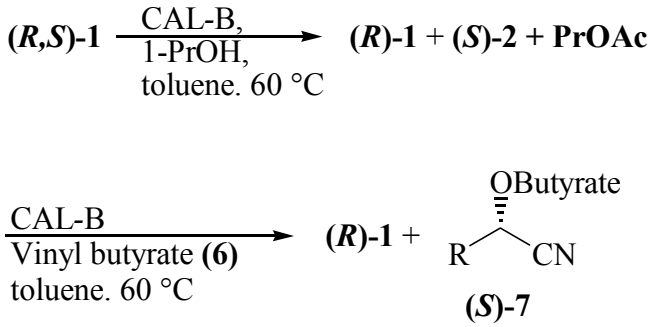
[e]From protection as pivaloyl-ester.

[f]From protection as THP-ether.

[g]Degradation product of cyanohydrin.

The results show that the *ee*'s of **(*S*) 3a-d** and **(*S*) 4a-d** are lower than for **(*S*) 5a-d**. This might be due to the instability of cyanohydrins under basic conditions where they slowly racemise. Indeed, for the silylation and the esterification a base is used. In contrast to **(*S*) 3a-d** and **(*S*) 4a-d** the formation of **(*S*) 5a-d** takes place under acidic reaction conditions. Then the racemisation is surpressed and the *ee*'s are, therefore, higher. However, when the kinetic resolution was performed without the coupling to a protection reaction[22] the *ee*'s of **(*S*) 2a-d** were even higher than those of **(*S*) 3a-d**, **(*S*) 4a-d** or **(*S*) 5a-d**.

In order to increase the optical purity of the protected **(*S*) 2a-d** to the level of the unprotected compounds, a different approach was explored. Instead of removing the enzyme and performing the protection chemically, the acylating agent vinyl butyrate (**6**) was added to the reaction mixture (Scheme 2).



Scheme 2: One pot synthesis of **(*S*)-7 a-d**

Under the same conditions as used for the kinetic resolution, **(*S*) 2a-d** was now acylated in the same pot by the same enzyme to give the (*S*)-cyanohydrin butyrates **(*S*) 7a-d** (table 2). (*S*)-Cyanohydrin decanoates were prepared in the same way with similar results, but with reaction times of several days.

Table 2: Results of the kinetic resolution of cyanohydrin acetates and the enzymatic protection of the formed (*S*)-cyanohydrins in a one-pot reaction.

R	Yield[a] (*ee*) [b] **(*S*)-7**	Yield[a] (*ee*) [b] **(*R*)-1**
a	85 (98)	93 (95)
b	80 (97)	81 (91)
c	73 (97)	93 (98)
d	67 (96)	92 (75)

[a]Yields are isolated yields (%).

[b]Enantiomeric excess (%) was determined by chiral HPLC.

With this truly one-pot procedure even better *ee*'s could be obtained for the re-protected cyanohydrins **(*S*) 7a-d**. The separation was, again, a straightforward column filtration, giving access to the desired products in good yields and with great ease. However, the *ee*'s of **(*R*) 1a-d** obtained from the enzymatic protection were lower than those observed after the chemical protection. This can be explained by the presence of propyl acetate formed during the kinetic resolution, which can also act as an acylating agent, (albeit slower than **6**), to give **(*S*) 1a-d** instead of **(*S*) 7a-d**. As a consequence the yields of the **(*S*) 7a-d** and the *ee*'s of **(*R*) 1a-d** decrease. In addition, any racemisation of **(*S*) 2a-d**, which would give lower *ee*'s in the chemical protection reactions, gives lower yields for the enzyme protections since **(*R*) 2a-d** is

not a substrate for the enzyme. Since the compounds **(*R*) 1a-d** and **(*S*) 7a-d** can both easily be racemised[31] and then again be submitted to the kinetic resolution, it is possible to obtain either enantiomer in close to quantitative yields with excellent selectivities.

With this CAL-B based kinetic resolution and protection sequence, protected aromatic cyanohydrins become readily available in high optical purity. In the case of aliphatic cyanohydrins CAL-B is significantly less enantioselective.[32] However, it is well known that Porcine pancreatic lipase and *Candida rugosa* lipase show excellent enantioselectivity for these substrates.[33,34]

To examine the recyclability of CAL-B, the kinetic resolution of racemic **(*R,S*)-1c** followed by the protection as THP-ether was repeated four times (Table 3). Even in the fifth run, no loss of activity or selectivity of the enzyme was observed. This proves the great versatility of CAL-B for the enantioselective synthesis of protected cyanohydrins.

Table 3: Recycling experiment

Cycle	0	1	2	3	4
Ratio[a] (% *ee*)[b] **(*R*)-1c**	98 (99)	96 (99)	98 (97)	98 (98)	98 (98)
Ratio (% *ee*) **(*S*)-5c**	86 (90)	86 (90)	88 (93)	90 (93)	91 (93)
Ratio 4-chlorobenzaldehyde **8**	16	18	14	12	10

[a]The ratios between **1c**, **5c** and **8** were determined in the reaction mixture by ^{1}H NMR (No other products were observed).

[b]Enantiomeric excess (%) was determined by chiral HPLC.

Conclusion

In summary we have developed a straightforward enzyme-based method to synthesise either enantiomer of differently protected cyanohydrins in excellent yield and optical purities (*R*: >91 % *ee*, *S*: >97 % *ee*.). This should help to further establish the cyanohydrins as versatile building blocks in organic synthesis.

Experimental Section

General Remarks: ^{1}H and ^{13}C-NMR spectra were recorded on a Varian VXR-400S (400 and 100, MHz, respectively) or a Varian Unity Inova 300 (300 MHz and 75, MHz, respectively),

instrument. Chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were determined on a VG 70 SE spectrometer working at 70 eV. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. Melting points are uncorrected. Column chromatography was carried out with silicagel 0.060-0.200 mm, pore diameter ca. 6 nm and with mixtures of petroleum ether (PE) and ethyl acetate (EtOAc) as solvent. TLC was performed on 0.20 mm silica gel and developed in a vanillin bath [vanillin (15 g) in ethanol (250 ml) + conc. H_2SO_4 (2.5 ml)] where all products containing a CN group gave orange spots except the THP-ethers which gave blue spots. Dry toluene and dry 1-propanol was purchased from Aldrich. Racemic cyanohydrin acetates[24] and cyanohydrins[35] were synthesised according to literature procedures. Immobilised Lipase B from *Candida antarctica* (CAL-B, Chirazyme L-2, c.-f., C2, Lyo) was a generous gift from Roche Diagnostics Penzberg (W. Tischer). HPLC analysis: The optical purity was determined by HPLC using a 4.6 × 250 mm 10 μ Chiracel OD column with a Waters 510 pump, and a Waters 486 UV detector. The eluent and the retention times for the different products are given in table 4. The flow was 0.8 ml min^{-1}.

Table 4: Eluents and retention times for HPLC analysis

Compound	Eluent Hexane : *i*-PrOH	R_t (*R*) [min.]	R_t (*S*) [min.]
(*R*,*S*)-1a	98 : 2	13.87	15.49
(*R*,*S*)-1b	90 : 10	10.55	11.94
(*R*,*S*)-1c	90 : 10	10.22	12.14
(*R*,*S*)-1d	90 : 10	19.40	15.28
(*R*,*S*)-3a	99.5 : 0.5	8.38	6.62
(*R*,*S*)-3b	99.5 : 0.5	9.36	8.34
(*R*,*S*)-3c	99.75 : 0.25	10.78	10.19
(*R*,*S*)-4a	99.5 : 0.5	9.36	10.34
(*R*,*S*)-4b	99.5 : 0.5	14.97	16.11
(*R*,*S*)-4c	99.5 : 0.5	12.43	14.51
(*R*,*S*)-4d	90 : 10	11.59	8.11
(*R*,*S*)-5a	98 : 2	10.12	10.65
(*R*,*S*)-5b	90 : 10	8.16	8.73
(*R*,*S*)-5c	90 : 10	7.28	8.35
(*R*,*S*)-5d	90 : 10	8.74	12.73
(*R*,*S*)-7a	95 : 5	7.90	8.45
(*R*,*S*)-7b	95 : 5	10.04	11.11
(*R*,*S*)-7c	95 : 5	9.11	10.47
(*R*,*S*)-7d	95 : 5	18.21	15.96

Enzyme activity test : Tributyrin (1.47 ml, 5.02 mmol) was added to 48.5 ml of a 10 mM Potassium phosphate buffer, pH 7.0 [10 mM potassium dihydrogen phosphate (100 ml) adjusted to pH 7.0 with 10 mM dipotassium hydrogen phosphate (~ 100 ml)] in a thermostated vessel at 25 °C and the mixture was stirred mechanically. The pH was maintained at 7.0 with an automatic burette, and when the pH stabilised, immobilised Lipase B from *Candida antarctica* CAL-B (9 mg) was added. The consumption of 100 mM sodium hydroxide was monitored over 40 min and plotted against time. The specific activity was calculated from the base consumption at the linear part of the graph. 1 μmol of NaOH consumed per min corresponds to 1 unit (1 U) of activity. The activity was found to be 3.8 KU/g dry carrier.

General procedure A: Kinetic resolution: Immobilised Lipase B from *Candida antarctica* CAL-B (368 mg) was dried overnight in a desiccator over SiO_2 and added to a mechanically stirred solution of **1** (3.68 mmol) in dry toluene (36 ml) under N_2 atm, at 25 °C. The reaction mixture was heated to 60 °C and then 1-propanol (0.54 ml, 7.36 mmol) was added. After stirring for 3h the reaction mixture was cooled to 0 °C and transferred through a thin canula into a new reaction vessel. The residual immobilised enzyme was washed with dry toluene (7 ml) to ensure complete transfer. The liquid phase was then treated according to the following general procedures.

General procedure B: Protection as TBDMS-ether: Lutidine (1.46 ml, 12.5 mmol) and TBDMS-Tf (2.11 ml, 9.2 mmol) were added to the liquid phase from the general procedure A and the mixture was stirred at ambient temperature under N_2 atm. for 3 h. The reaction was quenched with water (10 ml). The organic phase was washed with 1M HCl (25 ml), sat. $NaHCO_3$ (25 ml) and dried over $MgSO_4$. The solvent was removed under vacuum and the products were purified by column chromatography on silica gel, **(*S*) 3a-d** was eluted with PE/EtOAc (95:5) and **(*R*) 1a-d** with PE/EtOAc (90:10).

Racemic cyanohydrins protected as TBDMS-ethers: Racemic **(*R,S*) 3a-d** was prepared from racemic **(*R,S*) 2a-d** according to general procedure B with the following deviation. A solution of the cyanohydrin (1.84 mmol) in dry toluene (36 ml) and 1-propanol (0.54ml) was used instead of the filtrate from the kinetic resolution. The yields were **(*R,S*)-3a**: 71 %, **(*R,S*)-3b**: 78 %, **(*R,S*)-3c**: 92 % and **(*R,S*)-3d**: 98 %. NMR as below.

(*S*)-(−)-α-[(tert-butyldimethylsilyl)oxy]-benzeneacetonitrile (*S*)-3a and (*R*)-(+)-1-Cyano-1-phenylmethyl acetate (*R*)-1a: The title compounds were prepared from racemic **(*R,S*)-1a** according to general procedure B.

(*S*)-3a was obtained as a pale yellow oil (399 mg, 88 %, 89 % *ee*): $[\alpha]^{25}_D$ = –17.5 (c 1.0 $CHCl_3$) [lit.[23] $[\alpha]^{25}_D$ = +17.0 (c 1.0 $CHCl_3$) for the (*R*) enantiomer with *ee* >99 %]; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.15 (s, 3H, CH_3-Si), 0.23 (s, 3H, CH_3-Si), 0.94 (s, 9H, $(CH_3)_3C$), 5.52 (s, 1H, CH-O), 7.36-7.50 (m, 5H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = –5.2 (CH_3-Si), –5.1 (CH_3-Si), 18.2 (Si*C*$(CH_3)_3$), 25.5 (*C*$H_3)_3$C), 64.0 (CH-O), 119.3 (CN), 126.1 (C-2,6), 128.9 (C-3,5), 129.2 (C-4), 136.5 (C-1).

(*R*)-1a was obtained as a pale yellow oil (300 mg, 93 %, 96 % *ee*): $[\alpha]^{25}_D$ = +6.3 (c 1.0 $CHCl_3$) [lit.[36] $[\alpha]^{25}_D$ = –7.2 (c 2.3 $CHCl_3$) for the (*S*) enantiomer with >99 % *ee*]; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.14 (s, 3H, CH_3CO), 6.40 (s, 1H, CH-O), 7.20-7.58 (m, 5H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.5 (*C*H_3CO), 62.9 (CH-O), 116.2 (CN), 127.9 (C-2,6), 129.3 (C-3,5), 130.4 (C-4), 131.8 (C-1), 169.94 (C=O).

(*S*)-(–)-α-[(tert-butyldimethylsilyl)oxy]-(4-methoxy-phenyl)-acetonitrile (*S*)-3b and (*R*)-(–)-1-Cyano-1-(4-methoxyphenyl)methyl acetate (*R*)-1b: The title compounds were prepared from racemic **(*R,S*)-1b** according to general procedure B.

(*S*)-3b was obtained as a pale yellow oil (404 mg, 79 %, 89 % *ee*): $[\alpha]^{25}_D$ = –11.6 (c 1.0 $CHCl_3$) [lit.[23] $[\alpha]^{20}_D$ = + 16 (c 1.0 $CHCl_3$) for the (*R*) enantiomer with >99 % *ee*]; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.12 (s, 3H, CH_3-Si), 0.20 (s, 3H, CH_3-Si), 0.92 (s, 9H, $(CH_3)_3C$), 3.82 (s, 3H, OCH_3), 5.45 (s, 1H, CH-O), 6.92 (m, 2H, H-3,5), 7.38 (m, 2H, H-2,6); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = –5.1 ($(CH_3)_2Si$), 18.2 (Si*C*$(CH_3)_3$), 25.6 (*C*$H_3)_3C$), 55.4 (OCH_3), 63.7 (CH-O), 114.3 (C-3,5), 119.5 (CN), 127.7 (C-2,6), 128.7 (C-1), 160.3 (C-4).

(*R*)-1b was obtained as a pale yellow solid (343 mg, 90 %, 97 % *ee*): mp. 58.2-58.4 °C; $[\alpha]^{25}_D$ = – 20.0 (c 1.0 $CHCl_3$) [lit.[36] $[\alpha]^{20}_D$ = +19.0 (c 1.55 $CHCl_3$) for the (*S*) enantiomer with 95 % *ee*]; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.13 (s, 3H, CH_3CO), 3.82 (s, 3H, OCH_3), 6.35 (s, 1H, CH-O), 6.94 (m, 2H, aromatic), 7.44 (m, 2H, aromatic); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 20.5 (*C*H_3CO), 55.4 (OCH_3), 62.6 (CH), 114.6 (C-3,5), 116.4 (CN), 123.9 (C-1), 129.7 (C-2,6), 161.2 (C-4), 169.0 (C=O).

(*S*)-(–)-α-[(tert-butyldimethylsilyl)oxy]-(4-chloro-phenyl)-acetonitrile (*S*)-3c and (*R*)-(–)-1-Cyano-1-(4-chlorophenyl)methyl acetate (*R*)-1c: The title compounds were prepared from racemic **(*R,S*)-1c** according to general procedure B.

(*S*)-3c was obtained as a pale yellow oil (405 mg, 78 %, 82 % *ee*): $[\alpha]^{25}_D$ = –11.6 (c 1.0 $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.15 (s, 3H, CH_3-Si), 0.23 (s, 3H, CH_3-Si), 0.94 (s, 9H, $(CH_3)_3C$), 5.48 (s, 1H, CH-O), 7.4 (m, 4H, aromatic); ^{13}C-NMR (100 MHz, $CDCl_3$): δ =

–5.2 (CH_3-Si), –5.1 (CH_3-Si), 18.2 (Si*C*(CH_3)$_3$), 25.5 (*C*H_3)$_3$C), 63.4 (CH-O), 118.9 (CN), 127.4 (C-2,5), 129.2 (C-3,6), 135.1, 135.3 (C-1,4).

(*R*)-1c was obtained as a pale yellow oil (370 mg, 96 %, > 99 % *ee*): $[\alpha]^{25}_D$ = –10.5 (c 1.0 $CHCl_3$) [lit.[37] $[\alpha]^{25}_D$ = + 31.5 (c 1.17 benzene) for the (*S*) enantiomer with 84 % *ee*]; ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 2.17 (s, 3H, CH_3CO), 6.39 (s, 1H, CH-O), 7.4-7.5 (m, 4H, aromatic); ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 20.4 (*C*H_3CO), 62.2 (CH-O), 115.8 (CN), 129.3, 129.5 (C-2,3,5,6), 130.3 (C-1), 136.6 (C-4), 168.8 (C=O).

(*S*)-(–)-α-[(tert-butyldimethylsilyl)oxy]-(3-phenoxyphenyl)-acetonitrile (*S*)-3d and (*R*)-(–)-1-Cyano-1-(3-phenoxyhenyl)methyl acetate (*R*)-1d: The title compounds were prepared from racemic **(*R,S*)-1d** according to general procedure B.

(*S*)-3d was obtained as a pale yellow oil (555 mg, 89 %): $[\alpha]^{25}_D$ = –18.0 (c 1.0 $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 0.12 (s, 3H, CH_3-Si), 0.21 (s, 3H, CH_3-Si), 0.90 (s, 9H, (CH_3)$_3$C), 5.47 (s, 1H, CH-O), 6.98-7.40 (m, 9H, aromatic) ^{13}C-NMR (75 MHz, $CDCl_3$) δ = –5.3 (CH_3-Si), –5.1 (CH_3-Si), 18.1 (Si*C*(CH_3)$_3$), 25.5 (*C*H_3)$_3$C), 63.5 (CH-O), 115.8 (C-2'), 119.0 (CN), 119.1 (C-4'), 119.4 (C-2", 6"), 120.3 (C-6'), 123.9 (C-4"), 129.9 (C-3", 5"), 130.3 (C-5'), 138.4 (C-1'), 156.4 (C-3'), 158.1 (C-1").

(*R*)-1d was obtained as a pale yellow oil (461 mg, 94 %, 87 % *ee*): $[\alpha]^{25}_D$ = –7.1 (c 1.0 $CHCl_3$) [lit.[36] $[\alpha]^{25}_D$ = + 7.44 (c 0.75 $CHCl_3$) for the (*S*) enantiomer with 99 % *ee*]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 2.16 (s, 3H, CH_3CO), 6.36 (s, 1H, CH-O), 7.0-7.42 (m, 9H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 20.4 (*C*H_3CO), 62.4 (CH-O), 115.9 (CN), 117.7 (C-2'), 119.4 (C-2", 6"), 120.1 (C-4'), 122.1 (C-6'), 124.1 (C-4"), 130.0 (C-3", 5"), 130.6 (C-5'), 133.5 (C-1'), 156.2 (C-3'), 158.2 (C-1"), 168.8 (C=O).

General procedure C: Protection as pivaloyl-ester: DMAP (5 mg, 0.04 mmol), pivaloylchloride (1.36 ml, 11.04 mmol) and pyridine (0.71 ml, 8.83 mmol) were added to the fitrate from the general procedure A and the mixture was stirred at ambient temperature under N_2 atm for 14 h. 1M HCl (25 ml) was added and the organic phase was washed with sat. $NaHCO_3$ (25 ml) and dried over $MgSO_4$. Solvents were removed under vacuum and the crude products were purified by column chromatography on silica gel PE/EtOAc (90:10).

Racemic cyanohydrins protected as pivaloyl-esters: Racemic **(*R*,*S*) 4a-d** were prepared from racemic **(*R*,*S*) 2a-d** according to general procedure C with the following deviation. A solution of the cyanohydrin (1.84 mmol) in dry toluene (36 ml) and 1-propanol (0.54 ml) was used instead of the filtrate from the kinetic resolution. The yields were **(*R*,*S*)-4a**: 76 %, **(*R*,*S*)-4b**: 47 %, **(*R*,*S*)-4c**: 84 % and **(*R*,*S*)-4d**: 78 %.

(*S*)-(–)-1-Cyano-1-phenylmethyl pivaloate (*S*)-4a and (*R*)-(+)-1-Cyano-1-phenylmethyl acetate (*R*)-1a: The title compounds were prepared from racemic **(*R*,*S*)-1a** according to general procedure C.

(*S*)-4a was obtained as a pale yellow oil (395 mg, 99 %, 83 % *ee*): $[\alpha]^{25}_{D}$ = –5.9 (c 1.0 $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.24 (, s, 9H, $(CH_3)_3C$), 6.41 (s, 1H, CH-O), 7.41-7.51 (m, 5H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 26.9 ($CH_3)_3C$), 38.9 (*C*-CO), 62.8 (CH-O), 116.3 (CN), 127.5 (C-2,6), 129.2 (C-3,5), 130.2 (C-4), 132.1 (C-1), 176.4 (C=O).

(*R*)-1a was obtained as a pale yellow oil (313 mg, 97 %, 98 % *ee*): $[\alpha]^{25}_{D}$ = +5.6 (c 1.0 $CHCl_3$). NMR data as above.

(*S*)-(+)-1-Cyano-1-(4-methoxyphenyl)methyl pivaloate (*S*)-4b and (*R*)-(+)-1-Cyano-1-(4-methoxyphenyl)methyl acetate (*R*)-1b: The title compounds were prepared from racemic **(*R*,*S*)-1b** according to general procedure C.

(*S*)-4b was obtained as a pale yellow solid (371 mg, 81 %, 88 % *ee*): mp. 40.2-41.4 °C; $[\alpha]^{25}_{D}$ = +0.4 (c 1.0 $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.22 (s, 9H, $(CH_3)_3C$), 3,81 (s, 3H, CH_3O), 6.35 (s, 1H, CH-O), 6.94 (m, 2H, aromatic), 7.43 (m, 2H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 27.0 ($CH_3)_3C$), 39.0 (*C*-CO), 55.5 (OCH_3), 62.8 (CH-O), 114,7 (C-3,5), 116.7 (CN), 124.3 (C-1), 129.5 (C-2,5), 161.2 (C-4), 176.7 (C=O); MS: m/z 247(43), 163(14), 146(100), 135(33), 57(64); $C_{14}H_{17}NO_3$ (247.12): calcd. C 68.00, H 6.93, N 5.66; found C 67.76, H 6.94, N 5.61; HRMS: calc 247.1208; found 247.1209.

(*R*)-1b was obtained as a pale yellow solid (340 mg, 90 %, 88 % *ee*.): $[\alpha]^{25}_{D}$ = +13.6 (c 1.0 $CHCl_3$). NMR data as above.

(*S*)-(+)-1-Cyano-1-(4-chlorophenyl)methyl pivaloate (*S*)-4c and (*R*)-(–)-1-Cyano-1-(4-chlorophenyl)methyl acetate (*R*)-1c: The title compounds were prepared from racemic **(*R,S*)-1c** according to general procedure C.

(*S*)-4c was obtained as a pale yellow oil (352 mg, 76 %, 78 % *ee*): $[\alpha]^{25}_D$ = +2.8 (c 1.0 $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.23 (s, 9H, $(CH_3)_3C$), 6.38 (s, 1H, CH-O), 7.44 (m, 4H, aromatic); ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 26.6 ($(CH_3)_3C$), 38.6 (*C*-CO), 62.0 (CH-O), 115.8 (CN), 128.8, 129.3 (C-2,3,5,6), 130.5 (C-1), 136.1 (C-4), 176.0 (C=O); MS : m/z 251(28), 167(13), 150(50), 139(90), 111(60), 85(25), 57(100); $C_{13}H_{14}ClNO_2$ (251.07): calcd. C 62.03, H 5.61, N 5.56; found C 62.44. H 5.76, N 5.41; HRMS: calcd. 251.0713; found 251.0713.

(*R*)-1c was obtained as a pale yellow oil (328 mg, 85 %, >99 % *ee*): $[\alpha]^{25}_D$ = –13.9 (c 1.0 $CHCl_3$). NMR data as above.

(*S*)-(+)-1-Cyano-1-(3-phenoxyhenyl)methyl pivaloate (*S*)-4d and (*R*)-(–)-1-Cyano-1-(3-phenoxyphenyl)methyl acetate (*R*)-1d: The title compounds were prepared from racemic **(*R,S*)-1d** according to general procedure C.

(*S*)-4d was obtained as a pale yellow oil (419 mg, 73 %, 81 % *ee*): $[\alpha]^{25}_D$ = +0.2 (c 1.0 $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.23 (s, 9H, $(CH_3)_3C$), 6.35 (s, 1H, CH-O), 7.0-7.2 (m, 9H, aromatic); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 26.8 ($(CH_3)_3C$), 38.9 (*C*-CO), 62.3 (CH-O), 116.0 (CN), 117.0 (C-2'), 119.5 (C-2", 6"), 119.9 (C-4'), 121.6 (C-6'), 124.1 (C-4"), 130.0 (C-3", 5"), 130.6 (C-5'), 133.8 (C-1), 156.2 (C-3'), 158.3 (C-1"), 176.3 (C=O).

(*R*)-1d was obtained as a pale yellow oil (416 mg, 84 %, 90 % *ee*): $[\alpha]^{25}_D$ = –7.2 (c 1.0 $CHCl_3$). NMR data as above.

General procedure D: Protection as THP-ether: *p*-TsOH (5 mg, 0.028 mmol) was added to the filtrate from general procedure A at 0 °C. Then DHP (0.75 ml, 8.24 ml) was added dropwise over a period of 10 min. The reaction was then stirred under N_2 for 2 h at ambient temperature and quenched with sat $NaHCO_3$ (25 ml). The organic phase was dried over

$MgSO_4$. Solvents were removed under vacuum and the crude products were analysed by ^{1}H-NMR.

Racemic cyanohydrins protected as THP-ethers: Racemic **(*R,S*) 5a-d** were prepared from racemic **(*R,S*) 2a-d** according to general procedure D with the following deviation. A solution of the cyanohydrin (1.84 mmol) in dry toluene (36 ml) and 1-propanol (0.54 ml) was used instead of the filtrate from the kinetic resolution. The products were purified by column chromatography on silica gel PE/EtOAc (90:10). The yields were **(*R,S*)-5a**: 92 %, **(*R,S*)-5b**: 96 %, **(*R,S*)-5c**: 94 % and **(*R,S*)-5d**: 92 %.

(*2S*)-phenyl-[(tetrahydro-pyran-2-yloxy)]-acetonitrile (*S*)-5a and (*R*)-(–)-1-Cyano-1-phenylmethyl acetate (*R*)-1a: The title compounds were prepared from racemic **(*R,S*) 1a** according to general procedure D. The ratio of aromatic components in the reaction mixture was determined by ^{1}H-NMR (300 MHz, $CDCl_3$); **(*S*)-5a** (47 %, δ = 5.42 and 5.59, CH-O), **(*R*)-1a** (51 %, δ = 6.40, CH-O) and benzaldehyde (2 %, δ = 10.03, HC=O); **(*S*)-5a**: 93 % *ee*; **(*R*)-1a**: 93 % *ee*.

Characterisation of racemic **(*R,S*)-5a** : ^{1}H-NMR (300 MHz, $CDCl_3$): *Diastereoisomer A*: δ = 1.44-1.95 (m, 6H, THP), 3.63 (m, 1H, OCH_2), 4.01 (m, 1H, OCH_2), 4.74 (m, 1H, OCHO), 5.42 (s, 1H, CH-O), 7.38-7.56 (m, 5H, aromatic), *Diastereoisomer B*: δ = 1.44-1.95 (m, 6H, THP), 3.63 (m, 1H, OCH_2), 3.79 (m, 1H, OCH_2), 5.11 (m, 1H, OCHO), 5.59 (s, 1H, CH-O), 7.38-7.56 (m, 5H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$) *Diastereoisomer A*: δ = 18.7, 25.1, 29.8 (THP), 62.4 (OCH_2), 65.8 (OCHO), 96.8 (CH-O), 117.6 (CN), 127.4 (C-2, 6), 129.1 (C-3, 5), 129.8 (C-4), 133.9 (C-1), *Diastereoisomer B*: δ = 18.2, 25.1, 29.8 (THP), 62.0 (OCH_2), 66.5 (OCHO), 97.5 (CH-O), 118.3 (CN), 127.4 (C-2, 6), 129.0 (C-3, 5), 129.6 (C-4), 133.7 (C-1).

(*2S*)-(4-methoxy-phenyl)-[(tetrahydro-pyran-2-yloxy)]-acetonitrile (*S*)-5b and (*R*)-(–)-1-Cyano-1-(4-methoxyphenyl)methyl acetate (*R*)-1b: The title compounds were prepared from racemic **(*R,S*)-1b** according to general procedure D. The ratio of aromatic components in the reaction mixture was determined by ^{1}H-NMR (300 MHz, $CDCl_3$); **(*S*)-5b** (42 %, δ = 5.36 and 5.53, CH-O), **(*R*)-1b** (50 %, δ = 6.35, CH-O) and anisaldehyde (8 %, δ = 9.89, HC=O); **(*S*)-5b**: 93 % *ee*; **(*R*)-1b**: 92 % *ee*.

The reaction mixture was purified by column chromatography PE/EtOAc (90:10) to give *diastereoisomer A* and a mixture of *diastereoisomer B* together with **(*R*)-1b**. The two last compounds were separated by an additional purification by column chromatography (toluene 100 %)

Diastereoisomer A: A pale yellow oil (218 mg, 48 %) : ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.50-1.85 (m, 6H, THP), 3.62 (m, 1H, OCH_2), 3.78 (m, 1H, OCH_2), 3.83 (s, 3H, MeO), 5.08 (m, 1H, OCHO), 5.53 (s, 1H, CH-O), 6.94 (m, 2H, aromatic), 7.45 (m, 2H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.7, 25.1, 29.8 (THP), 55.4 (MeO), 62.4 (OCH_2), 65.5 (OCHO), 96.8 (CH-O), 114.4 (C-3, 5), 117.8 (CN), 125.8 (C-1), 129.0 (C-2, 6), 160.6 (C-4).

Diastereoisomer B: A pale yellow solid (164 g, 36 %): mp 56.2-56.8 °C ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.50-1.90 (m, 6H, THP), 3.62 (m, 1H, OCH_2), 4.00 (m, 1H, OCH_2), 5.08 (m, 1H, OCHO), 5.36 (s, 1H, CH-O), 6.94 (m, 2H, aromatic), 7.40 (m, 2H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.3, 25.2, 29.8 (THP), 55.4 (MeO), 62.0 (OCH_2), 66.0 (OCHO), 96.9 (CH-O), 114.4 (C-3, 5), 118.5 (CN), 125.9 (C-1), 129.1 (C-2, 6), 160.7 (C-4); MS: m/z 247(9), 205(8), 163(29), 146(100), 107(12), 85(86), 55(27); HRMS calcd. for $C_{14}H_{17}NO_3$: 247.1208; found 247.1216.

(*R*)-1b: A pale yellow solid (375 mg, 99 %): $[\alpha]^{25}_{D}$ = +17.0 (c 1.0 $CHCl_3$). NMR data as above.

(*2S*)-(4-chloro-phenyl)-[(tetrahydro-pyran-2-yloxy)]-acetonitrile (*S*)-5c and (*R*)-(–)-1-Cyano-1-(4-chlorophenyl)methyl acetate (*R*)-1c: The title compounds were prepared from racemic **(*R,S*) 1c** according to general procedures D. The ratio of aromatic components in the reaction mixture was determined by ^{1}H-NMR (300 MHz, $CDCl_3$); **(*S*)-5c** (43 %, δ = 5.39 and 5.57, CH-O), **(*R*)-1c** (49 %, δ = 6.38, CH-O) and 4-chlorobenzaldehyde (8 %, δ = 9.98, HC=O); **(*S*)-5c**: 90 % *ee*; **(*R*)-1c**: >99 % *ee*.

Characterisation of racemic **(*R,S*)-5c** : ^{1}H-NMR (400 MHz, $CDCl_3$): *Diastereoisomer A*: δ = 1.50-1.90 (m, 6H, THP), 3.63 (m, 1H, OCH_2), 4.00 (m, 1H, OCH_2), 4.73 (m, 1H, OCHO), 5.39 (s, 1H, CH-O), 7.39-7.49 (m, 4H, aromatic), *Diastereoisomer B*: δ = 1.50-1.90 (m, 6H, THP), 3.63 (m, 1H, OCH_2), 3.75 (m, 1H, OCH_2), 5.09 (m, 1H, OCHO), 5.57 (s, 1H, CH-O),

7.35-7.49 (m, 4H, aromatic); ^{13}C-NMR (100 MHz, $CDCl_3$) *Diastereoisomer A and B*: δ = 18.2, 18.7, 25.0, 25.1, 29.7(2C) (THP), 62.1, 62.5 (OCH_2), 65.1, 65.8 (OCHO), 96.9, 97.6 (CH-O), 117.2, 118.0 (CN), 128.8(2C), 129.2, 129.3 (C-2,6 or 3,5), 132.2, 132.4 (C-1), 135.7, 135.8 (C-4).

(*2S*)-(3-phenoxy-phenyl)-[(tetrahydro-pyran-2-yloxy)]-acetonitrile (*S*)-5d and (*R*)-(–)-1-Cyano-1-(3-phenoxyhenyl)methyl acetate (*R*)-1d: The title compounds were prepared from racemic **(*R,S*)-1d** according to general procedure D. The ratio of aromatic components in the reaction mixture was determined by ^{1}H-NMR (300 MHz, $CDCl_3$); **(*S*)-5d** (43 %, δ = 5.36 and 5.54, CH-O), **(*R*)-1d** (50 %, δ = 6.36, CH-O) and 3-phenoxybenzaldehyde (7 %, δ = 9.95, HC=O); **(*S*)-5d**: 84 % *ee*; **(*R*)-1d**: >99 % *ee*.

Characterisation of racemic **(*R,S*)-5d**: ^{1}H-NMR (300 MHz, $CDCl_3$): *Diastereoisomer A*: δ = 1.40-1.90 (m, 6H, THP), 3.60 (m, 1H, OCH_2), 3.70 (m, 1H, OCH_2), 5.08 (m, 1H, OCHO), 5.54 (s, 1H, CH-O), 6.95-7.36 (m, 9H, aromatic), *Diastereoisomer B*: δ = 1.40-1.90 (m, 6H, THP), 3.60 (m, 1H, OCH_2), 3.95 (m, 1H, OCH_2), 4.72 (m, 1H, OCHO), 5.36 (s, 1H, CH-O), 6.95-7.36 (m, 9H, aromatic); *Diastereoisomer A*: ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.5, 25.0, 29.7 (THP), 62.2 (OCH_2), 65.3 (OCHO), 96.7 (CH-O), 118.1 (CN), 119.2 (C-2",6"), 119.3 (C-2'), 121.7 (C-4'), 123.8 (C-4"), 129.9 (C-3",5"), 130.3 (C-5'), 135.6 (C-1'), 156.4 (C-3'), 157.9 (C-1") *Diastereoisomer B*: δ = 18.2, 25.1, 29.7 (THP), 62.0 (OCH_2), 66.1 (OCHO), 97.7 (CH-O), 117.3 (CN), 119.2 (C-2",6"), 119.4 (C-2'), 121.7 (C-4'), 123.8 (C-4"), 129.9 (C-3",5"), 130.4 (C-5'), 135.9 (C-1'), 156.4 (C-3'), 158.0 (C-1"). MS *Diastereoisomer A*: m/z 309(7), 225(14), 198(100), 169(50), 141(40), 115(17), 85(54); *Diastereoisomer A* $C_{19}H_{19}NO_3$ (309.14): calcd. C 73.77, H 6.19, N 4.53; found C 73.54, H 6.26, N 4.32; HRMS: calcd. 309.1365; found 309.1374.

General procedure E: Enzyme catalysed protection as butyryl-ester: The kinetic resolution was performed as in general procedure A, but instead of cooling the reaction and separating the enzyme from the reaction mixture, vinyl butyrate **6** (2.8 ml, 18.4 mmol) was added. After stirring at 60 °C overnight the reaction mixture was filtered. 1M HCl (25 ml) was added to the filtrate and the organic phase was washed with sat. $NaHCO_3$ (25 ml) and dried over $MgSO_4$. Solvents were removed under vacuum and the crude products were purified by column chromatography on silica gel PE/EtOAc (95:5).

Racemic cyanohydrins protected as butyryl-esters: Racemic **(*R*,*S*) 7a-d** were prepared from racemic **(*R*,*S*) 2a-d** according to general procedure C with the following deviation. A solution of the cyanohydrin (1.84 mmol) in dry toluene (36 ml) and 1-propanol (0.54 ml) was used instead of the filtrate from the kinetic resolution, and butyryl chloride (11.4 mmol) was used instead of pivaloyl chloride. The yields were **(*R*,*S*)-7a**: 62 %, **(*R*,*S*)-7b**: 84 %, **(*R*,*S*)-7c**: 74 % and **(*R*,*S*)-7d**: 84 %.

(*S*)-(−)-1-Cyano-1-phenylmethyl butyrate (*S*)-7a and (*R*)-(+)-1-Cyano-1-phenylmethyl acetate (*R*)-1a: The title compounds were prepared from racemic **(*R*,*S*)-1a** according to general procedure E.

(*S*)-7a was obtained as a pale yellow oil (316 mg, 85 %, 98 % *ee*): $[\alpha]^{25}_{D}$ = –7.0 (c 1.0 $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 0.94 (t, 3H, *J* = 7.5 Hz, CH_3), 1.70 (tq, 2H, *J* = 7.4 Hz, C*H*$_2$-CH_3), 2.38 (dt, 2H, *J* = 2.8, 7.5, CH_2CO), 6.44 (s, 1H, CH-O), 7.41-7.58 (m, 5H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.5 (CH_3), 18.2 (*C*H_2-CH_3), 35.6 (*C*H_2CO), 62.6 (CH-O), 116.2 (CN), 127.8 (C-2,6), 129.2 (C-3,5), 130.3 (C-4), 131,9 (C-1), 171,6 (C=O).

(*R*)-1a was obtained as a pale yellow oil (302 mg, 93 %, 95 % *ee*): $[\alpha]^{25}_{D}$ = +5.9 (c 1.0 $CHCl_3$). NMR data as above.

(*S*)-(+)-1-Cyano-1-(4-methoxyphenyl)methyl butyrate (*S*)-7b and (*R*)-(+)-1-Cyano-1-(4-methoxyphenyl)methyl acetate (*R*)-1b: The title compounds were prepared from racemic **(*R*,*S*)-1b** according to general procedure E.

(*S*)-7b was obtained as a pale yellow oil (364 mg, 80 %, 97 % *ee*): $[\alpha]^{25}_{D}$ = +12.0 (c 1.0 $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.94 (t, 3H, *J* = 7.5, CH_3), 1.68 (tq, 2H, *J* = 7.4, C*H*$_2$-CH_3), 2.37 (dt, 2H, *J* = 2.9, 7.5, CH_2CO), 3.83 (s, 3H, CH_3O), 6.38 (s, 1H, CH-O), 6.94 (m, 2H, aromatic), 7.45 (m, 2H, aromatic); ^{13}C-NMR (100MHz, $CDCl_3$): δ = 13.5 (CH_3), 18.2 (*C*H_2-CH_3), 35.6 (*C*H_2CO), 55.4 (OCH_3), 62.3 (CH-O), 114.5 (C-3,5), 116.4 (CN), 124.03 (C-1), 129.6 (C-2,6), 161.1 (C-4), 171,7 (C=O); MS: m/z 233(26), 163(32), 146(100), 135(77), 77(37); $C_{13}H_{15}NO_3$ (233.11): calcd. C 66.94, H6.48, N 6.00; found C 67.22, H 6.45, N 5.64; HRMS: calcd. 233.1052; found 233.1049;

(*R*)-1b was obtained as a pale yellow solid (306 mg, 81 %, 91 % *ee*): $[\alpha]^{25}_D$ = –17.5 (c 1.0 $CHCl_3$). NMR data as above.

(*S*)-(+)-1-Cyano-1-(4-chlorophenyl)methyl butyrate (*S*)-7c and (*R*)-(–)-1-Cyano-1-(4-chlorophenyl)methyl acetate (*R*)-1c: The title compounds were prepared from racemic **(*R,S*)-1c** according to general procedure E.

(*S*)-7c was obtained as a pale yellow oil (338 mg, 73 %, 97 % *ee*): $[\alpha]^{25}_D$ = +6.7 (c 1.0 $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.95 (t, 3H, *J* = 7.4, CH_3), 1.68 (tq, 2H, *J* = 7.4, C*H*$_2$-CH_3), 2.39 (dt, 2H, *J* = 3.0, 7.4, CH_2CO), 6.41 (s, 1H, CH-O), 7.40-7.49 (m, 4H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.5 (CH_3), 18.2 (*C*H_2-CH_3), 35.5 (*C*H_2CO), 61.9 (CH-O), 115.8 (CN), 129.2, 129.5 (C-2,6,3,5), 130.5 (C-4), 136.6 (C-1), 171,5 (C=O); MS : m/z 237(16), 167(45), 150(76), 139(26), 71 (100); $C_{12}H_{12}ClNO_2$ (237.05): calcd. C 60.64, H 5.09, N 5.89; found C 60.89, H 5.16, N 5.80; HRMS: calcd. 237.0557; found 237.0558.

(*R*)-1c was obtained as a pale yellow oil (357 mg, 93 %, 98 % *ee*): $[\alpha]^{25}_D$ = –9.9 (c 1.0 $CHCl_3$). NMR data as above.

(*S*)-(+)-1-Cyano-1-(3-phenoxyphenyl)methyl butyrate (*S*)-7d and (*R*)-(–)-1-Cyano-1-(3-phenoxyhenyl)methyl acetate (*R*)-1d: The title compounds were prepared from racemic **(*R,S*)-1d** according to general procedure E.

(*S*)-7d was obtained as a pale yellow oil (388 mg, 67 %, 96 % *ee*): $[\alpha]^{25}_D$ = +4.2 (c 1.0 $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.95 (t, 3H, *J* = 7.4, CH_3), 1.68 (tq, 2H, *J* = 7.4, C*H*$_2$-CH_3), 2.39 (dt, 2H, ^2J = 11.7, ^3J = 7.4, CH_2CO), 6.38 (s, 1H, CH-CN), 7.00-7.42 (m, 9H, aromatic); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 13.5 (CH_3), 18.2 (*C*H_2-CH_3), 35.5 (*C*H_2CO), 62.2 (CH-O), 115.9 (CN), 117,5 (C-2'), 119,4 (C-2",6"), 120.1 (C-4'), 122.0 (C-6'), 124.1 (C-4"), 130.0 (C-3",5"), 130.6 (C-5'), 133.6 (C-1'), 156.2 (C-3'), 158.2 (C-1"), 171.5 (C=O).

(*R*)-1d was obtained as a pale yellow oil (464 mg, 92 %, 75 % *ee*): $[\alpha]^{25}_D$ = –5.1 (c 1.0 $CHCl_3$). NMR data as above.

Recycling experiment: The experiment yielding **(*S*)-5c** and **(*R*)-1c** was repeated four times using the same enzyme. The reactions were performed as described in the general procedure D with the following exceptions: In the four repetitions the enzyme was not dried but directly used in the next cycle after it had been washed with dry toluene (7 ml). Toluene, 1-propanol and racemic **(*R,S*)-1c** were then added to the enzyme. The ratio of aromatic components in the reaction mixture was determined by integration of the following ^{1}H-NMR-signals (300 MHz, $CDCl_3$): **(*S*)-5c** (δ 5.39 and 5.57, CH-O), **(*R*)-1c** (δ 6.38, CH-O) and **8** (δ 9.98, HC=O). Cycle zero: **(*S*)-5c** (49 %, *ee* 99 %), **(*R*)-1c** (43 %, *ee* 90 %) and **8** (8 %); cycle one: **(*S*)-5c** (48 %, *ee* 99 %), **(*R*)-1c** (43 %, *ee* 90 %) and **8** (9 %); cycle two: **(*S*)-5c** (49 %, *ee* 97 %), **(*R*)-1c** (44 %, *ee* 93 %) and **8** (7 %); cycle three: **(*S*)-5c** (49 %, *ee* 98 %), **(*R*)-1c** (45 %, *ee* 93 %) and **8** (6 %); cycle four: **(*S*)-5c** (49 %, *ee* 98 %), **(*R*)-1c** (45 %, *ee* 93 %) and **8** (5 %).

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Chapter 5

Enantioselective synthesis of aromatic cyanohydrin esters via a dynamic kinetic resolution

Summary: The base and lipase catalysed enantioselective synthesis of cyanohydrin esters was investigated, and the problem of previously reported low yields due to residual water in the reaction mixture was addressed. When the lipase was immobilised on celite R-633 as a carrier, both the enantioselectivity and the reaction times for this dynamic kinetic resolution were improved, thus enabling a highly enantioselective synthesis of aromatic and heteroaromatic cyanohydrin acetates.

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Introduction

Chiral cyanohydrins can be converted into a wide range of compounds, which are versatile building blocks for the synthesis of fine chemicals, pharmaceuticals and agrochemicals, and they are therefore an important subject of research.[1,2,3] There are four main approaches for the synthesis of enantiopure cyanohydrins: either chemically, using cyclic dipeptides or transition metal complexes, or enzymatically, using hydroxynitrile lyases (HNL's) or lipases.[1]

The enzyme-catalysed methods are of great interest due to their low costs and environmentally friendly aspects. In the HNL catalysed additions of HCN to aldehydes and ketones, both excellent yields and enantiomeric purities were achieved within short reaction times (< 1h).[4,5,6,7,8,9] However, the reaction is mostly performed in a two-phase system, so water sensitive and water insoluble substrates can only be used with difficulties. Since the reaction is an equilibrium reaction, a large excess of HCN or of the less toxic acetone cyanohydrin is needed to obtain high conversions. Furthermore, not all substrates are accepted by the HNL's and some of the resulting cyanohydrins racemise very easily. As an alternative to the HNL's, one can use lipases. They have been applied both in the kinetic and in the dynamic kinetic resolution (DKR) of cyanohydrins (Scheme 1).[1,10] While the kinetic resolution has a maximum yield of 50 % only, and involves at least two separate steps to reach higher yields, the DKR starting from a prochiral aldehyde yields theoretically 100 % in just one step.[11]

The DKR combines a base catalysed equilibrium between an aldehyde, acetone cyanohydrin and the resulting cyanohydrin of the aldehyde, and a lipase-catalysed acylation of one enantiomer of this cyanohydrin. As the remaining enantiomer of the cyanohydrin is racemised by the base, one can theoretically obtain a 100 % yield of the acylated cyanohydrin. This reaction was already published in 1991,[12,13,14] but, in spite of its elegance, it has only been used successfully in a limited number of cases[15,16,17,18,19] This might be due to the fact that hydroxynitrile lyase-catalysed cyanohydrin synthesis tends to be faster and to give higher yields and enantiomeric purities for most of the substrates that have been studied.[4] But this could also be caused by the unsuccessful examples of the DKR that have been reported.[20,21,22]

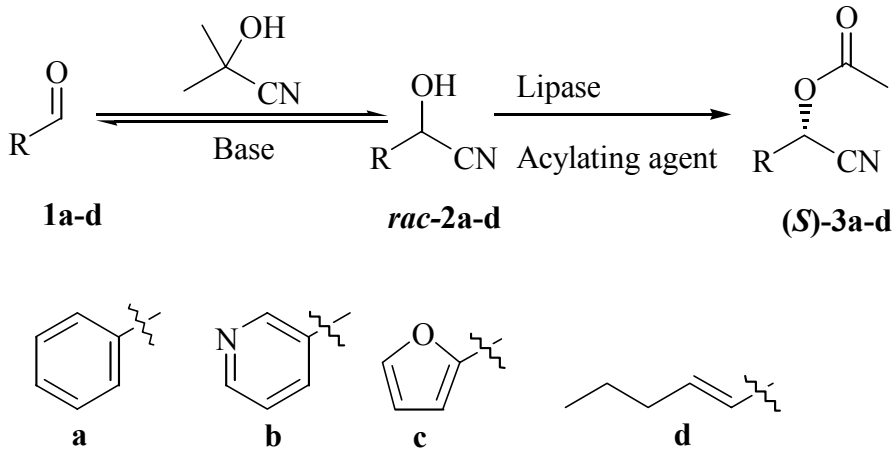
It has previously been shown that one of the most widely applied lipases in synthesis, *Candida antarctica* lipase B immobilised on a methacrylate polymer (Novozyme 435, or Chirazyme L-2, c.-f., C2, Lyo; both abbreviations stand for the same enzyme preparation), is particularly enantioselective in the kinetic resolution of cyanohydrins.[23,24] In order to increase the utility of the DKR, we recently investigated the reaction. Starting from **1a,** using Novozyme 435, basic Amberlite was used as the racemisation catalyst.[22] The racemisation of mandelonitrile (**2a**), the trans-cyanation between the acetone cyanohydrin and the aldehyde, as well as the kinetic resolution of mandelonitrile via acylation, all worked well separately. However, when the three reactions were combined, the reaction hardly even went to 16 % conversion in the DKR. It was concluded that the water present in the system hydrolysed the acylating agent, yielding an acid, which in turn neutralised the base that catalyses the dynamic equilibrium. Eventually, the acid might even render the enzyme inactive. HCN formed during the reaction could have an equivalent effect. If simply more base was added, another problem arose, namely the base-catalysed polymerisation of HCN. These polymers inhibited the enzyme and led to a full stop in the reaction.

Results and Discussion

In this work we now look at five different possibilities to solve this problem. Solid buffers have previously proven to be useful in establishing and maintaining optimum acid-base conditions for enzymes in organic media.[25] We use the shorthand "pH" to refer to this, although in the absence of a liquid water phase, simple pH is not the appropriate parameter. If solid buffers are used in the DKR, they should help in ensuring a favourable "pH", buffering any acetic acid formed. Another option is to use cyanide salts as bases. They should also neutralise any acetic acid formed in the reaction, yielding HCN that in turn should add to the aldehyde. In the first description of the DKR, molecular sieves were used to dry the reaction.[12,13,14] We look at this possibility, too, even though molecular sieves also work as ion exchangers, and therefore may alter the "pH" of the reaction in an unfavourable direction.[26] The carrier of the enzyme may also be of importance. As Celite has been used as the carrier in all the successful applications of the DKR, it is also of interest to compare Novozyme 435 with CAL-B immobilised on Celite. In addition it is known that celite R-640 can be used to control water activities in organic media.[27] It can therefore be expected that the Celite used for the immobilisation has this effect on the reaction, too. We chose celite R-633 in this study since we have applied it successfully in the immobilisation of *Hb*HNL before.[28] The acylating

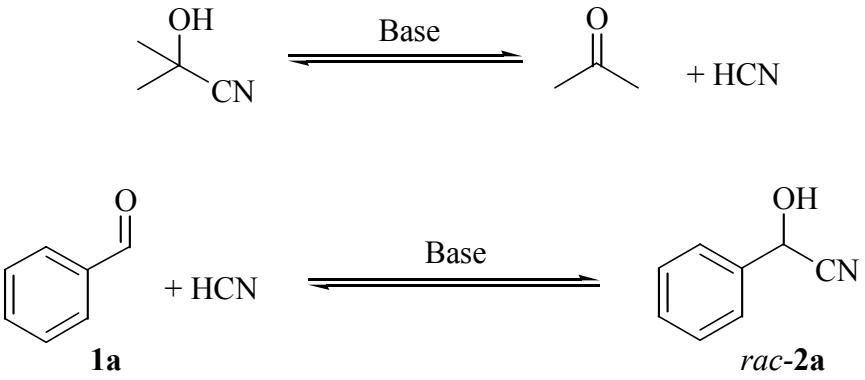
agent is known to have an effect on both the enantioselectivity and the activity of the enzyme and the possibility of using other acylating agents was explored.[10]

Based on the first DKR reported,[12,13,14] 1 mmol of aldehyde, 2 mmol of acetone cyanohydrin, and 3 mmol of acetylating agent were used in combination with various amounts of base and Novozyme 435 in 4 ml of toluene at 40 ºC. In our previous work we could show that 380 U of Novozyme 435 were sufficient for the kinetic resolution of **2a** to proceed to 50 % conversion within 4 h and with an excellent E (> 100). Therefore 380 U/mmol substrate were employed for the reaction.[22] (Scheme 1)



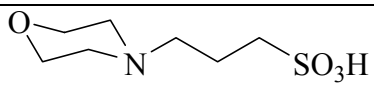
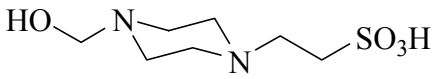
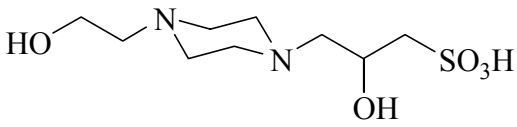
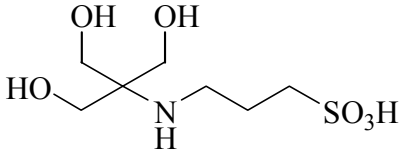
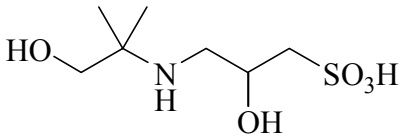
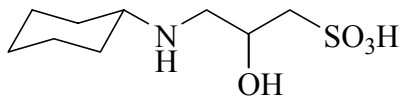
Scheme 1: Enantioselective synthesis of cyanohydrin esters via a dynamic kinetic resolution.

It was investigated whether solid buffers could replace the traditionally used Amberlite IRA-904 in OH^--form (Amberlite)[14,18] Since fast racemisation of the non-acylated enantiomer of **2** is essential for the DKR (to reach 100 % conversion with a high enantioselectivity), we tested various solid buffers (MOPS, HEPES, HEPPSO, AMPSO and CAPS) as catalysts for the dynamic equilibrium. (Scheme 2, Table 1). Only CAPS gave results that were similar to Amberlite, but CAPSO too catalysed this reaction within a reasonable time frame. Based on these results, CAPS, CAPSO and amberlite were used for our further studies. The active catalyst in the buffer pair is probably the solid Na salt, which has a free basic amino group.



Scheme 2: Base-catalysed equilibrium between acetone cyanohydrin, HCN and acetone and between **1a**, HCN and **2a**.

Table 1: pK_a of the buffers and time to reach the base-catalysed equilibrium between **1a**, acetone cyanohydrin, **2a** and acetone.

Solid buffer		pK_a	T_{eq} (min)[a]
MOPS		7.2	no reaction
HEPES		7.5	> 30 h
HEPPSO		7.8	no reaction
TAPS		8.4	24 h
AMPSO		9.0	30 h
CAPSO		9.6	5h
CAPS		10.4	10 min
Amberlite	IRA-904		20 min

[a] Time to reach equilibrium with 100 mg of buffer.

In order to find the optimum quantity of base to use in the DKR starting from **1a** and **1b**, various amounts of CAPSO, CAPS (40, 60, 80, 100, 120 mg of each salt of the buffer pair), amberlite (20, 40, 100, 200, 300 mg, 1 mmol OH^-/g Amberlite) and 380 U of Novozyme 435 per mmol substrate were used. The reactions were analysed after 7 days.

In the case of CAPSO, an optimum conversion is reached for both **1a** (conv. 100 %, ee 91 %) and **1b** (conv. 66 %, ee 74 %) when 100 mg of each salt of the pair was used. With CAPS the maximum conversions were already achieved when only 60 mg of each salt of the pair were used (**1a:** conv. 98 %, ee 91 % and **1b:** conv. 57 %, ee 26 %). However, for **1b** the conversions and ee's were slightly lower than with CAPSO. Amberlite is a strong base and in the case of **1a** and **1b**, the conversion drops slightly when more base is added. This is probably due to polymerisation of HCN and a consequential deactivation of the enzyme.

For **1a**, the ee is generally only slightly reduced if more base is used in the reactions. But in the case where the amount of amberlite OH^- utilised in combination with **1b** increases from 40 mg to 200 mg, the ee for **3b** decreases from 70 % to 20 %. This might be due to the fact that **1b** is also a base in itself, aiding the chemical background reaction or the base catalysed racemisation of the product.

All the experiments with the solid buffers were repeated using molecular sieves 4A to dry the reaction. However, independent of how much buffer or amberlite was used, the molecular sieves did not influence the reaction significantly: neither the conversion nor the ee obtained showed much deviation from the results obtained without molecular sieves.

In the original paper describing the DKR, both amberlite conditioned with NaOH and NaCN were used. Even though the two procedures gave comparable results, the OH^- form was chosen because it gave a slightly higher ee. Instead of using CN^- conditioned amberlite, we explored the possibility of employing different cyanide salts. The idea is that any acetic acid formed as a side product in the reaction will be neutralised by the cyanide salt. The HCN formed will then add to the aldehyde while the metal salt of the acetate precipitates. In order to pair the soft cyanide anion with a hard, intermediate and soft acid, we chose for the salts NaCN, $Zn(CN)_2$ and CuCN respectively, and used these as bases/salts in parallel with Amberlite OH^- and NaOAc in the DKR starting from **1a** and **1b** (Table 2). The composition of reagents remained unchanged; *i.e.* 2 equivalents acetone cyanohydrin were added.

Table 2: The use of salts as bases in the DKR starting from 0.98 mmol **1a** and **1b**, using 380 U/mmol substrate of Novozyme 435, after 6 days.

Compound	NaCN[a,b]	$Zn(CN)_2$[a,b]	CuCN[a,b]	NaOAc[a.b]	Amberlite[a,c]
1a	90 (36)	24 (94)	0 (-)	41 (89)	55 (90)
1b	96 (0)	25 (85)	3 (91)	32 (35)	63 (57)

[a] Conversion (ee) [%], [b] 1 eq., [c] 0.3 eq.

As expected there is a correlation between the hardness of the acid and the enantioselectivity. According to the hard soft acid base principle, the bond in CuCN has a covalent character, NaCN is completely ionic while $Zn(CN)_2$ is an intermediate between the two. Consequently, the CN^- is more "available" as a base in NaCN than in CuCN. As predicted this trend is evident in the results. NaCN catalyses the reaction, but in the case of the more reactive substrates the ee is low due to the base catalysed acylation of the cyanohydrin or the base catalysed racemisation of the product. The CuCN is a poor catalyst, and, it only works in the case of the **1b**. However, as **1b** is a base in itself, the observed conversion might also be auto-catalysed. $Zn(CN)_2$ gives by far the best ee's but only a moderate conversion. Both NaOAc and Amberlite gave higher conversions than $Zn(CN)_2$, but, the ee's were lower.

These experiments were repeated in the presence of molecular sieves (4 Å) as drying agents. As expected there was no significant difference between the two sets of experiments, whether molecular sieves are used or not. Any acid is neutralised by the cyanide salt to form HCN and the corresponding salt of the acid. As long as there is cyanide salt present, the "pH" of the reaction should be constant and the molecular sieves should not act as ion exchangers.

In all the successful, albeit sometimes slow, DKR of cyanohydrins, the lipases were immobilised on Celite. Celites are natural silicates, and the porous ones can adsorb large amounts of water. They bind this water tightly *via* hydrogen bridges, so that the water inside the pores has a reduced mobility and is released with difficulty even by drying at high temperatures. Indeed Celite R-640 can be used to efficiently control low water activities in organic solvents, since it can adsorb more than 90 % of the Celite weight.[27] In comparison, Novozyme 435 is adsorbed on a divinylbenzene-crosslinked, hydrophobic macroporous polymer based on methyl and butyl methacrylic esters.[29] This lipophilic material will readily release any water that is attached to it into the dry reaction mixture, thereby enabling the

hydrolysis of both the product and the acyl donor. Not all types of Celites are suitable for enzyme immobilisations and care has been taken to choose a suitable one. We immobilised CAL-B on Celite Bio-catalyst carrier R-633 according to standard procedures[30] and tested it with substrates **1a**, **1c**, **1d** and the bases NaCN, $Zn(CN)_2$, NaOAc and Amberlite. The results obtained after 6 days are shown in table 3.

Table 3: The use of salts as bases in the DKR starting from **1a**, **1c** and **1d** using CAL-B on Celite R-633 (380 U), after 6 days.

Compound	NaCN [a,b]	$Zn(CN)_2$ [a,b]	Amberlite[c]	NaOAc[a,b]
1a	-	18(92)	87(95)	56(91)
1c	69(4)	23(75)	97(86)	71(84)
1d	76(11)	10(81)	71(79)	28(74)

[a] Conversion (ee) [%], [b] 1 eq., [c] 0.3 eq.

The observed trend is the same as when Novozyme 435 was used. However, if one considers both conversion and enantiomeric excess, Amberlite is clearly the best base to use. The result we obtained for **1c** is a significant improvement over what has earlier been reported. In the original work on the DKR, a conversion of 73 % of **1c** to its corresponding cyanohydrin acetate **3c** was achieved with an ee of 47 %, respectively, in 6 days using Amberlite as the base. The results that are reported here for **1a** are also an improvement compared to those obtained earlier with Novozyme 435, both in terms of conversion and enantiopurity.

In order to see how CAL-B on Celite R-633 (910 KU/mmol substrate) performed in combination with the solid buffers, it was used for the synthesis of **3a**, together with various amounts of base (CAPSO, CAPS) (Table 4).

Table 4: The use of various amounts of CAPSO and CAPS as bases in the DKR starting from **1a** (0.98 mmol) using CAL-B on Celite R-633 (910 U/mmol substrate) with a reaction time of 48 h.

Mass acid/base [mg]	CAPSO[a]	CAPS[a]	Amberlite[a,b]
-/30	-	-	83 (97)
40/40	48 (95)	87 (91)	-
60/60	52 (95)	85 (82)	-
80/80	78 (91)	85 (79)	-
100/100	86 (92)	86 (77)	-

[a]Conversion (ee) [%], [b] 0.3 eq.

Although CAPSO gave fairly high ee's, a substantial amount of buffer is necessary to obtain the same conversion as with CAPS. The conversion that is obtained with CAPS is not influenced by how much base is used, however, the ee drops as a function of the amount of base added. These differences can be rationalised by the base strength of the solid buffers. More CAPSO is needed for the racemisation since it is the weaker base, while larger amounts of the stronger base CAPS lead to product racemisation. When performing the same reaction using 30 mg of Amberlite, 83 % conversion and an ee of 97 % were obtained. This is better than what was obtained with both CAPSO and CAPS. These results cannot be directly compared with those obtained with Novozyme 435 since less units of the enzyme were employed in those experiments.

However, it is clear that Amberlite in combination with CAL-B on Celite R-633 not only gives good yields but also an excellent enantioselectivity. In order to directly compare the two different lipase immobilisations, we tested various amounts of both the CAL-B on Celite R-633 and Novozyme 435 in the synthesis of **3a** (Figure 1).

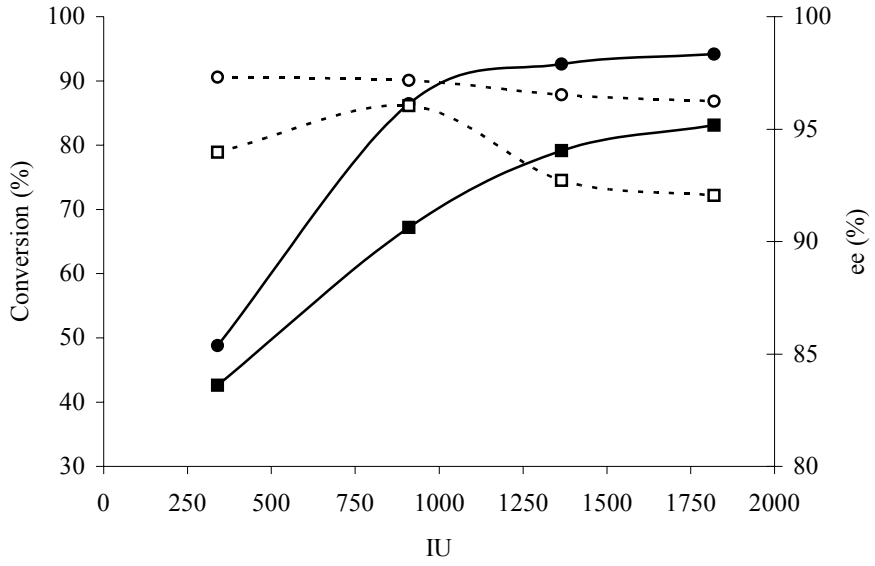


Figure 1: The yield of **3a** after 3 days using Amberlite OH^- as the base, isopropenyl acetate as the acylating agent, and various amounts of Novozyme 435 (conversion ■, ee □) were tested vs. CAL-B on Celite R-633 (conversion ●, ee ○).

The results clearly demonstrate that even if the same amount of activity is used, the CAL-B adsorbed on Celite R-633 performs significantly better, both in respect to yield and enantioselectivity. In the case of CAL-B on Celite R-633 the conversion increases with an increasing amount of enzyme, until a plateau is reached, indicating that the enzymatic reaction is no longer the rate-limiting step. The slight drop in ee for **3a** is consistent with this, indicating that the dynamic equilibrium now is the rate-limiting step.[31] It also shows that the 380 U, which were enough for the kinetic resolution of **2a** to proceed rapidly, are not enough for the DKR to proceed smoothly: at least 1.3 KU/mmol substrate have to be used. To ensure that it is really the effect of the carrier that causes the difference described in Figure 1, and not the immobilisation procedure, we stirred the Novozyme 435 in the same buffer-sucrose solution that was used for the immobilisation of CAL-B on Celite R-633 and dried it in the same way. The treated Novozyme 435 showed no difference compared to the untreated Novozyme 435 preparation.

Even though vinyl acetate and vinyl butanoate give acetaldehyde as a side product, which can in general be harmful for enzymes,[10] and can act as a substrate for the DKR, they have still been the acylating agents of choice in several of the reported successful applications of the DKR. Their success can probably be ascribed to the celite carrier which is reported to reduce

the negative effect of acetaldehyde on the enzyme.[32] We tested isopropenyl acetate, vinyl acetate and vinyl butanoate with CAL-B adsorbed on Celite R-633, and, after 16 h the conversions and ee's were 35 (98), 13 (83) and 14 (92) respectively. Since CAL-B in combination with isopropenyl acetate gives both the best conversion and enantiomeric excess, all further studies were performed with this acylating agent.

The reaction was performed with 1350 U/mmol CAL-B on Celite R-633 on a preparative scale, starting from **1a** and **1c**; using Amberlite as the base and isopropenyl acetate as the acylating agent (Table 5).

Table 5: Results of the DKR starting from **1a** and **1c** using optimised reaction conditions

Substrate	Time [days]	Yield [%]	ee [%]
1a	4.0	97	98
1c	4.5	92	89

For both **1a** and **1c** the preparative reaction proceeded within four days, which is faster than described earlier (4 and 4.5 days rather than 6 days for both **3a** and **3c** respectively). More importantly, the enantioselectivity for both compounds was significantly improved. For **1c** the yield has increased from 57 % to 92 %, and the enantiomeric excess has increased from 47 % to 89 %. For **1a**, it is now an almost enantiospecific reaction (ee = 98 %), instead of an enantioselective reaction (ee = 84 %).[13] When comparing it to the vanadium-salen catalysed formation of **3a** from **1a**, KCN and acetic anhydride it also is a significant step forward. Although the vanadium-catalysed reaction proceeds faster (10 h), the yield (88 %) and the enantioselectivity (ee = 90 %) are lower.[33]

As the optimum conditions described in this chapter did not work satisfactory for aliphatic substrates, they are treated in chapter 6.

Conclusion

This work describes the synthesis of cyanohydrins via a DKR using the readily available lipase B from *Candida antarctica*. The problems experienced earlier, of low conversions and long reaction times due to hydrolysis of the acylating agents, have been addressed and solved.

Varying the nature and the amount of base used in the DKR of cyanohydrins already gave improvements compared to previous work using Novozyme 435, although a straightforward change in the carrier of the enzyme had the largest effect on the reaction. Whereas Novozyme 435 probably releases water from the carrier into the reaction media, causing hydrolysis which subsequently stopped the reaction, Celite R-633 suppressed the negative side effects of water most likely by binding it. With lipase B from *Candida antarctica* adsorbed on Celite R-633, both the conversion and the ee was improved significantly when compared to earlier results.

Experimental

General Remarks: ^{1}H and ^{13}C-NMR spectra were recorded on a Varian Unity Inova 300 (300 MHz and 75, MHz, respectively), instrument. Chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. Column chromatography was carried out using silica gel with a particle size of 0.060-0.200 mm, pore diameter ca. 6 nm. TLC was performed on 0.20 mm silica gel and developed in a vanillin bath [vanillin (15 g) in ethanol (250 ml) + conc. H_2SO_4 (2.5 ml)] where all products containing a CN group gave orange spots. Dry toluene was purchased from Aldrich. Lyophilised lipase B from *Candida antarctica* was purchased from Roche. Immobilised lipase B from *Candida antarctica* (CAL-B, Novozyme) was a generous gift from Novo Nordisk (Dr. Deussen). Lyophilised CAL-B was adsorbed on Celite Bio-Catalyst Carrier R-633 from World Minerals.[30] In the original procedure Celite® (Filter agent) from Aldrich was used. The activity of the enzymes used was determined as described earlier.[24] Amberlite IRA-904 was conditioned with NaOH[13], and the capacity of the conditioned ion exchanger was found to be 1 mmol OH^-/g. All aldehydes, acetone cyanohydrin and isopropenyl acetate were distilled prior to use and stored under nitrogen. The racemic cyanohydrin acetates **3a-f** were prepared according to a standard procedure[34] and their spectroscopic data correspond to those in the literature.[14,24,35,36,37] All reactions were performed in a 10 ml glass vial equipped with a silicone supported teflon septum and a screw cap. 1,3,5-Triisopropylbenzene was used as an internal standard in the reactions. HPLC analysis: The HPLC analysis were performed using a 4.6 × 250 mm Chiracel OB-H column with a Waters 510 pump, and a Waters 486 UV detector. The eluent was a 90:10 mixture of hexane and 2-propanol containing 0.1 % acetic acid. The flow was 0.8 ml min^{-1}. GC analysis:

The conversion and enantiomeric purity was determined by chiral GC using a β-cyclodextrin column (CP-Chirasil-Dex CB 25m × 0.25 mm) using a Shimadzu Gas Chromatograph GC-17A equipped with a FID detector and a Shimadzu Auto-injector AOC-20i, using He with a linear gas velocity of 75 cm/s as the carrier gas. The temperature programs and retention times are given in Table 6.

Table 6: Temperature programs and retention times for **1 a-d**, **(*R*)-3 a-d** and **(*S*)-3 a-d**.

Substrate	Temp. [ºC][a]	R_t [min] **1**	R_t [min] **(*R*)-3**	R_t [min] **(*S*)-3**
a	145	1.00	2.62	3.12
b	145	0.99	3.89	5.05
c	120	1.00	3.04	3.83
d	120	0.99	2.96	3.91

[a] All analyses were performed isothermally.

Base catalysed trans-cyanation from acetone cyanohydrin to benzaldehyde: Acetone cyanohydrin (0.17 ml, 1.84 mmol) was added to a stirred mixture of benzaldehyde (98 mg, 0.92 mmol) and either the solid buffer (50 mg of both the acid and its corresponding sodium salt) or Amberlite (30 mg), in toluene (4 ml) at 40 ºC. Samples (10 µl) were diluted in hexane and analysed by HPLC.

General procedure A: The DKR of cyanohydrins, catalytic scale: Acetone cyanohydrin (0.17 ml, 1.84 mmol) was added to a stirred mixture of the aldehyde (0.98 mmol), the acylating agent (2.76 mmol) the immobilised enzyme (as stated in the text) and the base (as stated in the text) in dry toluene (4 ml). The reaction was stirred magnetically at 40 ºC. Samples (10 µl) were diluted in acetone and centrifuged. The supernatant was analysed by chiral GC.

General procedure B: The DKR of cyanohydrins, preparative scale: Acetone cyanohydrin (0.86 ml, 9.4 mmol) was added to a stirred mixture of the aldehyde (4.7 mmol), isopropenyl acetate (1.62 ml, 14.9 mmol), CAL-B on Celite R-633 (6.83 KU) and Amberlite IRA-904 in OH^- form (150 mg) in dry toluene (20 ml). When the reaction was completed, the enzyme and the resin was filtered off and washed with toluene (2 x 10 ml). The solvents were

removed under vacuum and the residue was purified by column chromatography on silica gel (PE/EtOAc, 90:10).

(*S*)-(-)-2-Acetoxy-2-phenylethanenitrile [(*S*)-3a]: The title compound was prepared from benzaldehyde (499 mg, 4.7 mmol), following general procedure B. **(*S*)-3a** was isolated as a clear oil (798 mg, 97 % yield, 98 % ee): $[\alpha]^{D}_{25}$ = -6.2 (c 1, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.14 (s, 3H, CH_3CO), 6.40 (s, 1H, CH-O), 7.20-7.58 (m, 5H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.5 (*C*H_3CO), 62.9 (CH-O), 116.2 (CN), 127.9 (C-2,6), 129.3 (C-3,5), 130.4 (C-4), 131.8 (C-1), 169.9 (C=O).

(*S*)-(-)-2-Acetoxy-2-(2-furyl)ethanenitrile [(S)-3c]: The title compound was prepared from furaldehyde (452 mg, 4.7 mmol), following general procedure B. **(*S*)-3d** was isolated as a clear oil (718 mg, 92 % yield, 89 % ee): $[\alpha]^{D}_{25}$ = +22.4 (c 1.0 $CHCl_3$); ^{1}H-NMR (300 mHz, $CDCl_3$): δ = 2.14 (s, 3H, CH_3C=O), 6.47 (m, 2H, CH-CN and C(3)-H), 6.69 (m, 1H, C(2)-H), 7.51 (m, C(4)-H); ^{13}C-NMR (75 mHz, $CDCl_3$): δ = 20.2 (CH_3), 55.8 (CH-O), 111.2 and 112.7 (C-2 or C-3), 114.3 (CN), 144.2 (C-1), 145.1 (C-4), 168.8 (C=O).

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Chapter 6

Enantioselective synthesis of aliphatic cyanohydrin esters via a dynamic kinetic resolution

Summary: When the standard conditions for the enantioselective synthesis of cyanohydrin acetates via a DKR are applied to aliphatic substrates, only a kinetic resolution is observed. However, by exchanging the base (Amberlite IRA-904 in OH^- form) against NaCN, quantitative conversions and good enantioselectivities could be obtained.

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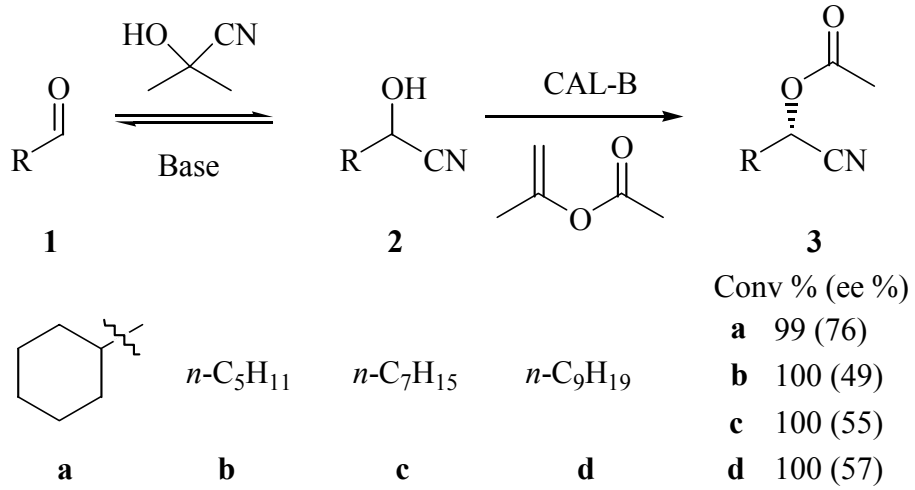
Introduction

Enantiopure cyanohydrins are highly versatile building blocks in organic synthesis. [1,2,3] A variety of chemical and enzymatic approaches to prepare them have been described, the dynamic kinetic resolution (DKR) being particularly attractive.[4] It combines a base-catalysed equilibrium between an aldehyde, acetone cyanohydrin and the resulting racemic cyanohydrin of the aldehyde, with a lipase-catalysed acylation of one enantiomer of this cyanohydrin. As the remaining enantiomer of the cyanohydrin is racemised by the base, yields of up to 100 % can be obtained (Scheme 1).



Scheme 1 The dynamic kinetic resolution of aliphatic cyanohydrins.

In spite of its elegance and successful use,[5,6,7,8] this method has gained rather moderate attention compared with the Hydroxynitrile lyase (HNL) catalysed addition of HCN to aldehydes and ketones.[9,10,11] In the case of aliphatic substrates, this could be due to the poor results which were communicated in the first description of the reaction.[12,13,14] There, **1b** and isobutyraldehyde gave 63 and 85 % conversion to the corresponding cyanohydrin acetates with ee's of 51 and 15 %, respectively. This is comparable to what can be obtained from a poor kinetic resolution (KR), indicating that the base (Amberlite IRA-904 in OH^--form) fails to racemise the cyanohydrin.

Previously we succeeded in significantly improving the DKR of aromatic cyanohydrins,[15,16] we therefore set out to apply our findings to aliphatic aldehydes. They have not only proven to be troublesome substrates for the DKR, among the chemical routes towards acylated aliphatic cyanohydrins, there are only a few successful examples with high enantioselectivities.[17,18,19]

Results and discussion

In our previous work on the optimisation of the enantioselective synthesis of aromatic cyanohydrin esters, we found that cyanide salts were capable of catalyzing the DKR starting from aromatic aldehydes, however with unsatisfactory results. Some salts *eg*. NaCN were too basic and catalyzed mainly the chemical acylation, resulting in a racemic product, while CuCN gave nearly no conversion at all.[16] In contrast to the aromatic cyanohydrins, stronger bases are needed to racemise the aliphatic cyanohydrins, making the solid cyanide salts potential bases for this reaction. To confirm this, we used NaCN, KCN and $Zn(CN)_2$ as bases/salts and compared them with Amberlite OH^- and NaOAc in the synthesis of **3a** and **3b**.[20] Since it has earlier been demonstrated that the carrier of the enzyme can have a significant influence on the reaction, two different immobilisates of *Candida antarctica* lipase B (CAL-B), an enzyme that has been shown to be particularly selective both in the KR and the DKR of cyanohydrins,[21,22,23] were employed (Table 1).

Table 1: The use of cyanide salts as bases in the DKR using 380 U/mmol of *Candida antarctica* lipase B, reaction time 6 days.

Substrate	Enzyme	NaCN[a,b]	KCN[a,b]	$Zn(CN)_2$[a,b]	NaOAc[a,b]	Amberlite[a,c]
1a	Novozyme 435	99 (76)	78 (58)	47 (82)	54 (74)	70 (74)
1a	CAL-B on Celite	100 (42)	86 (29)	32 (92)	31 (89)	46 (90)
1b	Novozyme 435	100 (49)[d]	n.d	79 (62)	68 (27)	73 (28)
1b	CAL-B on Celite	97 (46)[d]	93 (29)	60 (68)	51 (56)	68 (52)

[a] Conversion into **3a** and **3b**, % (ee, %). [b] 1 eq. [c] 0.3 eq. [d] Reaction time 2 days.

For the reactions reported in entry 1 of table 1, we also monitored the ee of **2a** during the reaction,[24] and after 6 days we found ee's of 71, 81 and 80 % for the reactions using $Zn(CN)_2$, NaOAc and Amberlite respectively. This is good evidence that the racemisation of **2** is too slow for a successful DKR. The results in entry 2, 3 and 4 also indicate that these three bases are too weak and only give a KR without any racemisation of the intermediate cyanohydrin **2**.

In the case of NaCN and KCN, no ee for **2a** could be observed, demonstrating that these bases are indeed strong enough to efficiently racemise the cyanohydrin. However, the ee's in the reactions using these two salts are lower than those that can be observed when using

$Zn(CN)_2$, NaOAc and Amberlite. One reason for this could be the base-catalyzed racemisation of **3b**, but, this is unlikely since no decrease in ee could be observed for a reaction that was stirred for another four days after its completion. As was the case for the aromatic aldehydes, some degree of base-catalysed acylation of **2b** might cause this lower ee of **3b**.[16]

$Zn(CN)_2$, NaOAc and Amberlite gave the highest ee's for **3** when they were used in combination with the Celite R-633 immobilized enzyme (Table 1). NaCN and KCN gave the best results in combination with Novozyme 435. Probably Celite R-633 has a higher affinity to water and binds it, while the methacrylate polymer carrier of CAL-B in Novozyme 435 more readily releases the water bound to the carrier into the reaction media.[16] Since NaCN and KCN will neutralise any acetic acid formed by hydrolysis of the acylating agent, it is less critical for the reaction to use Celite R-633 as the carrier for CAL-B. The formed HCN will add to the aldehyde to form **2** and the metal acetate, which acts as a mild base.

On the other hand when NaOAc and Amberlite are used, it is more important to use a carrier that does not release any water into the reaction mixture. Amberlite will neutralise the acid but at the same time forms a new molecule of water. NaOAc will form a buffer with the acetic acid but the capacity of this buffer might be exceeded.

As expected $Zn(CN)_2$ seems to follow the trend of NaOAc and Amberlite, rather than that of the two other cyanide salts. This shows that the $Zn(CN)_2$ is a weaker base than KCN or NaCN and therefore less efficient in the neutralisation of the acid.

In order to probe whether a higher ee could be obtained with an aldehyde containing a longer chain than **1b**, we also tested **1c** and **1d** as substrates for the reaction but neither the reaction rate nor the enantioselectivity of the reaction changed significantly.[25]

When the reaction was scaled up[26] using Novozyme 435 in combination with NaCN, **3a** and **3b** were isolated with a yield of 92 and 74 %, and an ee of 78[27] and 50 %[28] respectively. This is a significant improvement of the first DKR of aliphatic cyanohydrins.

Conclusion

In conclusion, by exchanging the Amberlite OH^-, which is commonly used as a base in the DKR of cyanohydrins against NaCN, a true DKR of the aliphatic cyanohydrins could be developed. In addition, by using NaCN, the reaction also became less sensitive towards water that is present in the reaction mixture. The ee's of the products were; however, lower than what could be expected, most likely due to a small degree of base-catalysed chemical acylation.

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24) Conversion and enantiomeric purity were determined by chiral GC using a β-cyclodextrin column (CP-Chirasil-Dex CB 25m × 0.25 mm) using He with a linear gas velocity of 75 cm/s.

Temperature programs and retention times for **1 a-d**, **(*R*)-3 a-d** and **(*S*)-3 a-d**.

Substrate	Temp. [ºC][a]	R_t [min] **1**	R_t [min] **(*R*)-3**	R_t [min] **(*S*)-3**
A	145	0.97	2.60	2.95
B	154	0.75	1.41	1.57
C	145	0.87	2.60	3.03
D	165	0.99	2.79	3.05

[a] All analyses were performed isothermally.

25) Racemic **3c**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.89 (t, J = 7.0 Hz, 3H, CH_3-CH_2), 1.25-1.40 (m, 8H, CH_3-CH_2-CH_2-CH_2-CH_2), 1.50 (m, 2H, CH_2-CH_2-CH), 1.9 (m, 2H, CH_2-CH), 2.13 (s, 3H, CH_3-C=O), 5.31 (t, J = 6.8 Hz, 1H, C*H*-CN); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.0 (C8), 20.4 (*C*H3-C=O), 22.6 (C7), 24.6 (C6), 28.8 (C5), 29.0 (C4), 31.6 (C3), 32.3 (C2), 61.2 (C-O), 117.0 (CN), 169.2 (C=O). Racemic **3d**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, J = 6.8 Hz, 3H, CH_3-CH_2), 1.20-1.40 (m, 10H, CH_3-CH_2-CH_2-CH_2-CH_2-CH_2), 1.50 (m, 2H, CH_2-CH_2-CH), 1.9 (m, 2H, CH_2-CH), 2.12 (s, 3H, CH_3-CO), 5.30 (t, J = 6.8 Hz, 1H, C*H*-CN); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.1 (C10), 20.4 (*C*H_3-C=O), 22.7 (C9), 24.6 (C8), 28.9 (C7), 29.3 (C6), 29.3 (C5), 29.4 (C4), 31.9 (C3), 32.3 (C2), 61.2 (C-O), 117.0 (CN), 169.2 (C=O).

26) Procedure as in the preparative scale experiment in chapter 5.

27) **(*S*)-3a**: $[\alpha]_D^{25}$ = - 47,7 (*c* 1, MeOH); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.10-1.40 (m, 5H, ring CH and CH_2), 1.70-1.90 (m, 6H, ring CH_2), 2.14 (s, 3H, CH_3), 5.17 (d, J = 6.0 Hz, 1H, C*H*-CN); ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.3 (CH_3), 25.3, 25.4, 25.8, 28.0, 28.1 and 40.5 (ring C), 65.6 (CH-O), 116.2 (CN), 169.3 (C=O).

28) **(*S*)-3b**: $[\alpha]_D^{25}$ = - 36.9 (*c* 1, MeOH); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.90 (m, 3H, CH_3-CH_2), 1.33 (m, 4H, CH_3-CH_2-CH_2), 1.50 (m, 2H, CH_2-CH_2-CH), 1.9 (m, 2H, CH_2-CH), 2.14 (s, 3H, CH_3-CO), 5.31 (t, J = 6.8 Hz, 1H, C*H*-CN); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 13.9 (C6), 20.4 (*C*H_3-C=O), 22.3 (C5), 24.2 (C4). 30.9 (C3), 32.3 (C2), 61.2 (CH), 117.0 (CN), 169.2 (C=O).

Chapter 7

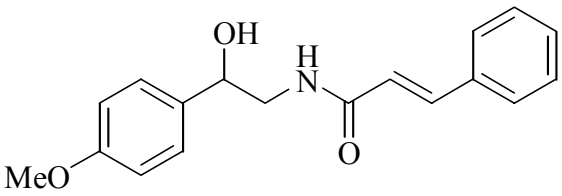
Catalytic hydrogenation of cyanohydrin esters

Summary: The catalytic hydrogenation of acylated cyanohydrins followed by an intra-molecular migration of the acyl group to yield pharmaceutically interesting *N*-acyl *β*-amino alcohols, is shown to be a successful one-pot preparation method. The combination of a multistep Design of Experiment (DoE) approach and high-throughput methodology proved to be an effective strategy for the optimization of the reaction. With the favoured catalyst/solvent combination nickel-on-alumina in dioxane, both hydrogenation and acyl-group migration proceeded smoothly, giving the *N*-acyl *β*-amino alcohols in yields of up to 90 % for aliphatic, and up to 50 % for benzylic substrates the latter beeing more prone to side reactions. When enantiopure cyanohydrin esters were used, no racemisation was found to occur at the chiral centre of an aliphatic molecule, though a minor decrease in ee was observed for a benzylic substrate.

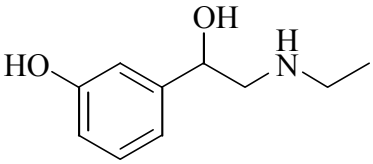
This work has been performed in collaboration with Silvia R. M. Pereira and the chapter is in press: L. Veum, S. R. M. Pereira, J. C. van der Waal, U. Hanefeld, *Eur. J. Org. Chem.* **2006**, *Accepted.*

Introduction

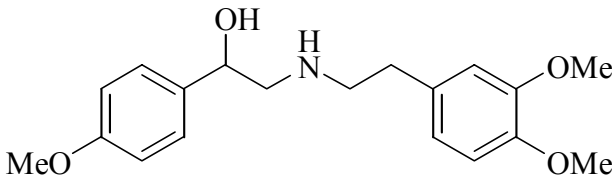
N-acylated *β*-aminoalcohols such as aegeline (see Figure 1) occur in nature and can readily be converted into *β-sec*-aminoalcohols, an important class of compounds in the pharmaceutical and agrochemical industries. Some representative examples of the numerous biologically active *β*-amino alcohols are Aegeline, Etilefrine, Bamethane, and Denopamine (Figure 1). An established route to the *N*-acyl-*β*-aminoalcohols is the reduction of the free cyanohydrin, followed by acylation of the amino group.[1,2,3] The reduction is usually performed using stoichiometric amounts of either $LiAlH_4$ or BH_3, but it can also be achieved by catalytic hydrogenation under strongly acidic conditions.[4,5,6,7,8] If enantiopure substrates are used, the stereocentre remains intact during all these reactions. Given the low atom efficiency of aluminium and boron hydride reductions, and the strongly acidic conditions required for the catalytic hydrogenations, a different approach has been investigated, with the overall aim of integrating the reduction and acylation steps in a one-pot procedure under mild conditions.



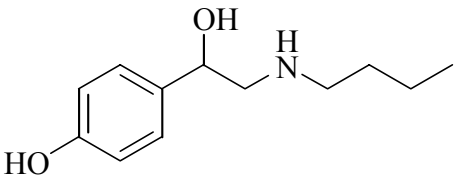
Aegeline



Etilefrine



Denopamine

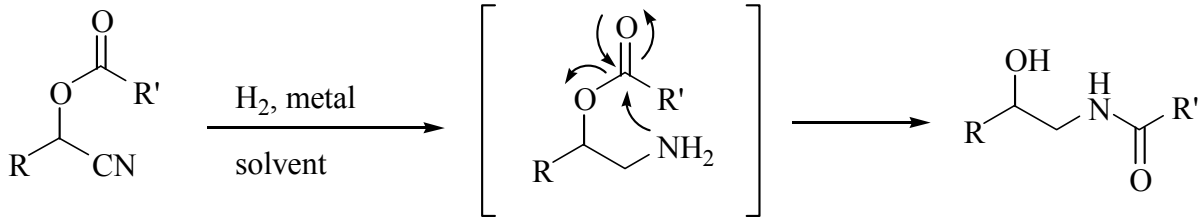


Bamethane

Figure 1: *N*-acyl-*β*-aminoalcohols and *β-sec*-aminoalcohols showing high biological activity.

The unprotected cyanohydrins that are commonly used as starting materials are relatively unstable and racemise easily. In contrast to this, cyanohydrin esters are stable and do not racemise. Moreover, they are readily prepared, both in their racemic[9] and enantiopure

forms.[10,11,12] In addition, the acyl group of the protected cyanohydrins is a potential intra-molecular acyl-donor (see Scheme 1). Following the catalytic hydrogenation of the nitrile group, the newly formed amine, as a strong nucleophile, can immediately react with the neighbouring acyl group via a five-membered transition state to yield the *N*-acyl *β*-amino alcohol. This type of intramolecular acyl migration has previously been described in the $NaBH_3(OCOCF_3)$ reduction of an acylated cyanohydrin to yield Denopamine,[13] suggesting that it should proceed equally well following catalytic hydrogenation of the nitrile.



1 **2**

Scheme 1: The hydrogenation of acylated cyanohydrins with subsequent acyl-migration.

Earlier reports of the catalytic hydrogenation of acylated cyanohydrins, and in particular of mandelonitrile esters, describe the application of Pd/C or PtO_2 under strongly acidic conditions.[14,15] The primary product obtained was not the *N*-acyl-*β*-amino-alcohol but *β*–phenyl-ethyl-amine, owing to the facile hydrogenation of the benzylic C-O bond over platinum or palladium catalysts. In this case the amine was the desired product.[14] However, in the present work the objective is to maximize the yield of the *N*-acylated *β*–aminoalcohol and the reductive cleavage of the benzylic C-O bond needs to be avoided. This investigation of a selective, catalytic route for the direct conversion of acylated cyanohydrins (**1**) into *N*-acylated *β*–aminoalcohols (**2**) employed high-throughput methods for the screening of catalysts, solvents and reaction conditions.

The large number of parameters to be investigated suggested a Design-of-Experiment (DoE) approach. DoE methodologies[16,17,18] are superior to traditional methods where one factor at a time is varied while the remaining factors are held constant. Such a traditional approach do not take intrerations of the various parameters into account and the path towards the real optimum might be troublesome or only found by pure luck. The DoE methodology involves the consecutive and simultaneous optimization of the various parameters; they make it possible to maximize the amount of information that can be obtained from the results, while

minimizing the number of experiments, and increase the possibility of establishing the true optimum within the search space. The experiments to be performed are chosen statistically in order to cover the whole search space as efficiently as possible. The size of the design (selected number of reactions) depends on the kind of information that is desired. In the present case a strategy of three sequential small designs was adopted. This enables the information obtained from the first to be used to improve the subsequent designs.[19] Preliminary small designs (typically less than 25 % of the possible number of reactions) are sufficient to distinguish between significant and insignificant parameters and are therefore well suited in the early stages of the research effort to reduce the search space. By continuous refinement of the conditions in subsequent optimisation designs, the most influential parameters can then be studied and further optimization achieved. Since the number of parameters to investigate has been reduced, the number of experiments per parameter can be increased. In this way, more information on the main effects and especially the interactions of the parameters can be obtained. In such designs more than 50 % of the possible number of reactions is typically performed. Such a sequence of DoE's is supposed to be a better strategy than one large one, because the information obtained from one design is used to improve the following.

Results and discussion

As shown by Hartung,[14] the hydrogenation of benzylic cyanohydrin acetates easily yields products such as *β*-phenylethylamines by reductive cleavage of the benzylic C-O bond. In aliphatic substrates, on the other hand, the C-O bond is more stable and resistant to cleavage even under drastic conditions. Different conditions are likely to be required for the selective hydrogenation of acylated aliphatic and benzylic cyanohydrins and, therefore, it was chosen to optimise the reactions for mandelonitrile acetate (**1a**), representative for the benzylic substrates, and 2-cyanohexyl acetate (**1c)** (see Scheme 2), representative for the aliphatic substrates, separately.

First design

The use of a DoE strategy requires as the first step the compilation of all potentially important parameters, based on previous experience, the literature or chemical intuition. The initial search space should be broad enough to assure that the real optimum of the reaction is included. In the hydrogenation of nitriles the main factor influencing the reaction rate and the

product distribution is the metal of the hydrogenation catalyst. Most commonly, Raney nickel, Raney cobalt, Pd/C, Pt/C, Ru/C, and Rh/C are used.[20] The same metals but supported on SiO_2 and Al_2O_3 are often employed as well.[20] Normally Rh, Pd and Pt tend to give more secondary and tertiary amines than Co, Ni and Ru. As the migration of the acyl group might suppress the formation of secondary and tertiary amines, these metals were also included in the investigation. For the initial screening, Ni, Pd, Rh, Pt and Ru, on carbon and Al_2O_3 as carriers, were selected as representative catalysts.

The solvent forms a second important parameter. The most commonly used solvents for the hydrogenation of nitriles are protic solvents such as methanol and ethanol. However, since the envisaged reaction involves a migration of the acyl-group, solvents with a broader range of properties were selected: 2-propanol, a protic but less polar solvent than methanol; dioxane, an aprotic, polar ether; and toluene, a relatively apolar solvent.

It is known that the addition of ammonia and of water can change the distribution ratio of the products of nitrile hydrogenation.[20] Ammonia is a commonly used additive, favouring the formation of primary amines, though in the present case reaction with the ester group is a possible side reaction. Reports on the effect of water are conflicting; several cases have been reported where water is added to promote the formation of both primary and secondary amines[21,22] but it was also claimed that water does not change the product distribution but instead increases the reaction rate.[23] The effects of both these additives were studied in the initial screening.

The levels of each parameter, which determine the parameter space for the initial screen is summarised in Table 1. The reaction temperature was varied over two levels. The small reactors of the high-throughput unit did not permit independent variation in pressure, which was kept constant at 20 bar H_2. Using a d-Optimal algorithm a selection of 24 reactions out of the total of 320 possible combinations was made for each of the two substrates.[24] This design is sufficient to study the main parameters which affect the process, while a more detailed subsequent study would then allow for further optimisation. Acidic conditions were not included since any formation of the amino salts would prevent the intramolecular migration of the acyl group. Furthermore, in contrast to the free cyanohydrins, the cyanohydrin esters (**1**) are more stable towards possible basic side products in the reaction.

Table 1: The parameter space to be investigated for substrates **1a** and **1c**.

Parameter	Level 1	Level 2	Level 3	Level 4	Level 5
Temperature (ºC)	90	120	-	-	-
Reaction time (h)	3	24	-	-	-
Support	Alumina	Carbon	-	-	-
Solvent	2-propanol	Toluene	Dioxane	Methanol	-
Additive	No additive	H_2O	NH_3	$H_2O + NH_3$	-
Metal	Ni	Pd	Pt	Rh	Ru

After executing the 2 × 24 reactions, *N*-acyl-*β*-aminoalcohols **2a** and **2c** were identified among the products in two of the experiments for each substrate, showing the hydrogenation indeed to have been followed by intra-molecular acyl-migration in a one-pot procedure. The conditions for the four successful reactions are given in Table 2. This result already shows the advantage of using DoE with successive small designs as an approach towards the optimisation of a new reaction. This made it possible to investigate a large parameter space and identify a narrow region of interest for further exploration, even though only 8 % of the possible number of reactions was executed. If a single large DoE design had been chosen, a large number of unnecessary reactions would have been performed.

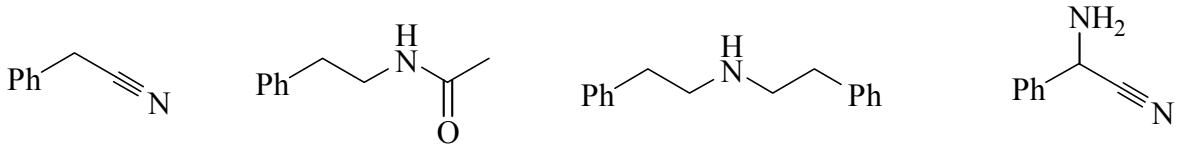
When using such a small design it is important to realise that each result is extremely influential in the calculation of the main effects of the parameters. These calculations will become increasingly inaccurate with a growing number of “zero-yield” reactions or failed experiments. In this case the number of reactions leading to the desired product is so low, i.e. 2 per design, that a statistical evaluation of the effect of the parameters on the yield would not be meaningful. The results doe however enable the identification of unfavourable factors and their exclusion from the next screening phase.

Table 2: Conditions for the reactions yielding the right product in the initial screening.

Substrate	Metal	Support	Temp [°C]	Solvent	Additive	Conversion of **1** [%]	Yield[a] **2** [%]
1a	Ni	C	120	Dioxane	NH_3	100	33
1a	Rh	Al_2O_3	120	Dioxane	NH_3+H_2O	100	24
1c	Ni	Al_2O_3	120	Dioxane	H_2O	100	65
1c	Rh	C	120	2-propanol	H_2O	100	48

[a] According to GC

The reactions in Table 2 were all run for 24 h at 120 ºC, with either dioxane or 2-propanol as the solvent, and ammonia or water as the additive. The successful metals were Ni and Rh, supported on either carbon or alumina. From the reactions which did not yield the desired product, the following trends could be observed: the reactions using Ru or toluene gave low conversions, while the reactions performed in MeOH in all cases gave complete conversions, but, with a wide range of side-products. Since the intention of the first screening was to reduce the parameter space, none of the side-products of the reactions were isolated. However, GC-MS enabled the identification of several side-products (**3** to **6**).



3 **4** **5** **6**

Figure 2: Identified side-products in the hydrogenation of **1a**.

The presence of **5** shows that the secondary amine is formed in some cases. Products **3** to **5**, in which the benzylic alcohol group has been removed, were particularly dominant when platinum was used as the catalyst, which is to be expected from the application of platinum catalysts for the cleavage of this type of bond. Equivalent by-products could also be identified in the case of the aliphatic substrate, though in much smaller amounts, which is in accordance with the greater stability of the C-O bond. The fact that even the aliphatic C-O bond can be cleaved, can be attributed to stabilizing effect of the nitrile group on the intermediate radical formed during the cleavage.

In the successful reactions of **1a** (see Table 2) ammonia was present as additive, but analysis of the results did not show unambiguously that the presence of ammonia was essential. Since the formation of **6** indicates that ammonia also reacts with the substrate it was chosen, in order to avoid this side reaction, to further optimise the conditions in the absence of ammonia.

Samples taken after 3 hours showed only low conversions and there was no formation of **2a** or **2b** in any other reaction than under the conditions reported in Table 2. The long reaction time could be due to an initial activation period for the catalyst but the study of this was deferred to a later stage and a reaction time of 24 h was maintained for the second design.

Based on the results of the first design, the second one was conducted with the parameters indicated in Table 3. In order to further study the effect of the carrier silica was included in this design. Since the parameter space was now considerably reduced, a full factorial design, i.e. 36 combinations, became feasible for each substrate. All the reactions were performed at 120 °C and 20 bar H_2, with a reaction time of 24 h.

Table 3: Conditions and parameters in the second screening round

Parameter	Level 1	Level 2	Level 3
Additive	No additive	H_2O	-
Solvent	2-propanol	Dioxane	-
Metal	Ni	Rh	Ru
Support	Alumina	Carbon[a]	Silica

[a]In the case of nickel, Raney-nickel was used instead of nickel on carbon.

The results of this screening are presented in Figure 3 and Figure 4. The conversions of **1a** and **1c** were in all cases 100 %, except for the reactions where Ru-alumina and Ru-silica was used. For those two catalysts no conversion was observed. In sharp contrast to the first screening, where only a few reactions yielded the *N*-acyl-*β*-aminoalcohols, all active catalysts now yielded the desired products. A statistical evaluation of the results from Figure 3 and Figure 4, with respect to the main effects of the parameters and their interactions, is presented in Figure 5 and Figure 6.

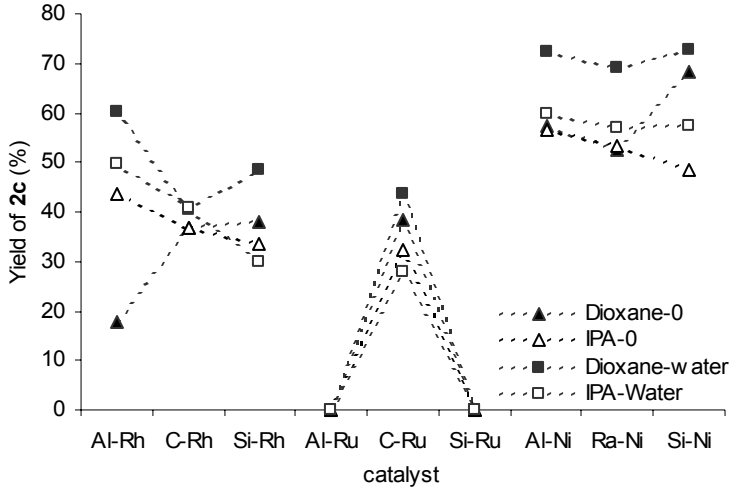


Figure 3: Graphical representation of the results of the second screening with substrate **1c**

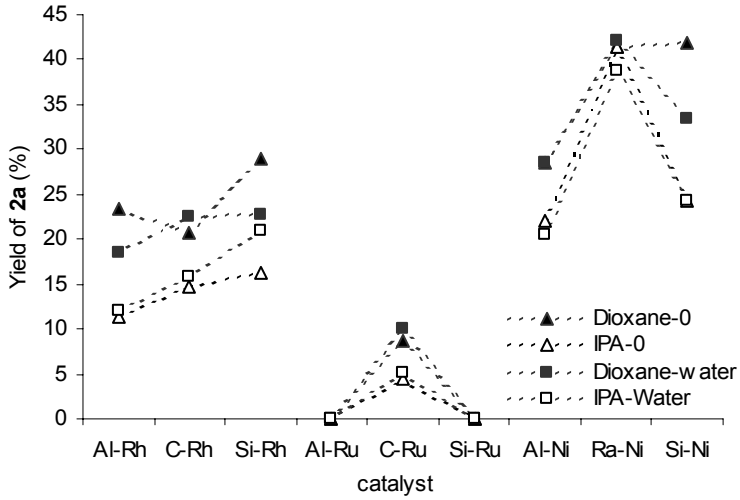


Figure 4: Graphical representation of the results of the second screening with substrate **1a**.

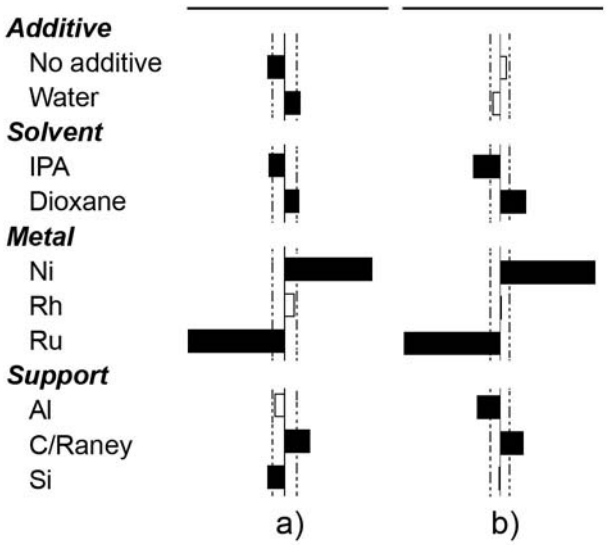


Figure 5: Effect of the main parameters in the second screening: a) aliphatic substrate **1c**; b) benzylic substrate **1a**. The lengths of the bars show the relative influence of the main parameters on the yield of **2a** and **2c**. The bars directed to the right have a positive relative effect and those to the left a negative. The dotted lines represent the 95 % confidence interval calculated from the estimated experimental variance. Effects higher than this confidence interval are considered significant and are represented in black ('Al' = alumina, 'Si' = silica).

When evaluating the main parameters in Figure 5, many similarities for the two different substrates are noted. The most important parameter in both cases is the type of metal, with nickel being the best followed by rhodium. For ruthenium, the poor results from the initial screening are confirmed. In the initial screening, the successful reactions included those in which water was used as an additive. A small but statistically significant positive effect of water is indeed observed in the case of the aliphatic, though not with the benzylic, substrate. Although the difference between the two solvents is small, dioxane is statistically significantly better than 2-propanol for both substrates. With respect to the effects of the catalyst carriers, the apparent superiority of carbon is based solely on the fact that ruthenium gives the product only in combination with carbon while, in addition, nickel on carbon was not available and Raney-Ni was used instead, so that no conclusion on carrier effects can be drawn from this second design.

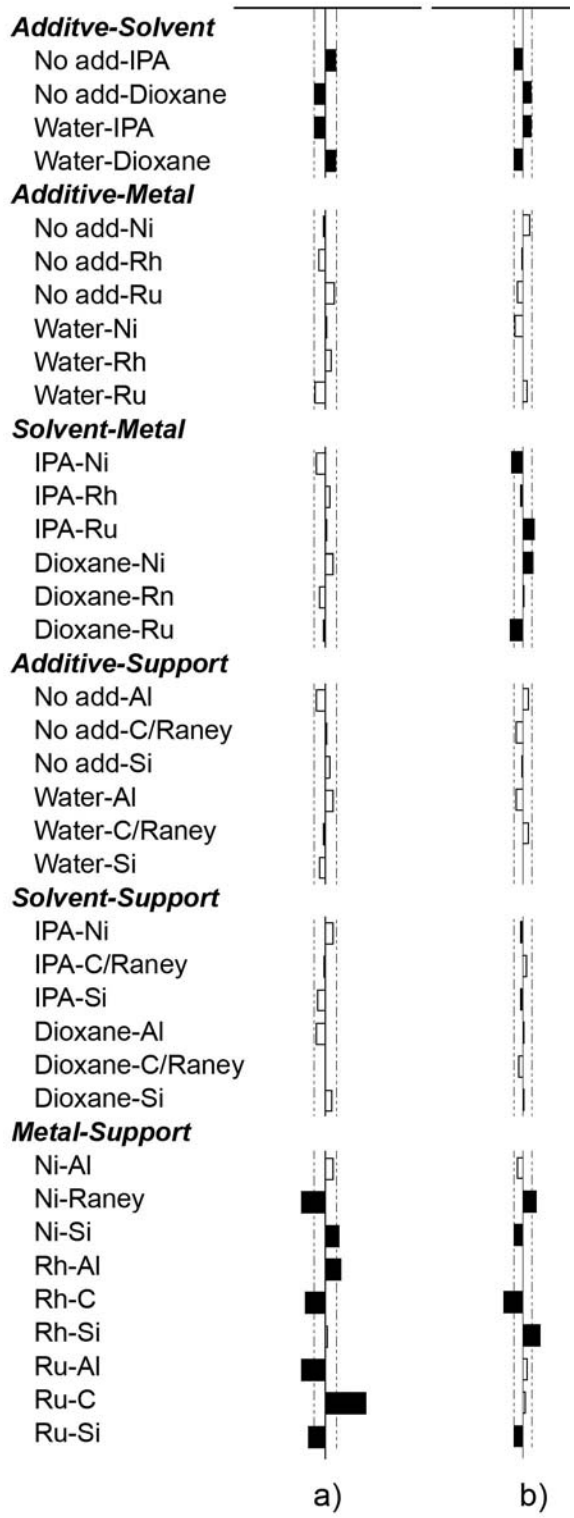


Figure 6: Interaction effects between the parameters of the second screening: a) aliphatic substrate **1c**; b) benzylic substrate **1a**. The lengths of the bars show the relative influence of the interaction effects, between the different parameters, on the yield of **2a** and **2c**. The bars directed to the right have a positive relative effect and to those the left a negative one. The dotted lines represent the 95 % confidence interval calculated from the estimated experimental variance. Effects higher than this confidence interval are considered significant and are represented in dark. ('Al' = alumina, 'Si' = silica.)

With respect to the interactions between the parameters presented in figure 6 it was noticed that, for both substrates, there are significant additive/solvent and metal/support interactions, while in the case of the benzylic substrate a solvent/metal interaction also exists. However, the effects of these interactions are relatively small in comparison with the main effect of the metal itself. For the aliphatic substrates, nickel on silica, using dioxane as the solvent and water as additive, is the combination of choice, while for the benzylic substrate Raney-nickel is the indicated catalyst, in dioxane without addition of water.

In preparation for this, a test was conducted of whether the results with the nickel catalysts could be improved by activation with H_2 prior to the catalytic test. By activating the catalysts at 140 ˚C for 12 h at 40 bar H_2, nickel on alumina gave similar yields to those of Raney-nickel and it was found convenient to use this catalyst for further optimisation. In the case of the aliphatic substrate the reaction time was reduced to two hours and, for the benzylic substrate, to three hours. Once again, dioxane proved to be slightly superior to 2-propanol As a result of this, it was chosen to perform the third round of screening, for the optimisation of temperature and pressure, using activated nickel on alumina in dioxane. In the case of the aliphatic substrate **1c**, water was used as an additive.

All the reactions from these two screenings were performed in the "Quick Catalyst Screening 96" platform. This equipment has a maximum pressure limit of 20 bar and no individual temperature control for the reactors. Further optimisation regarding pressure and temperature was for that reason performed in a conventional autoclave.

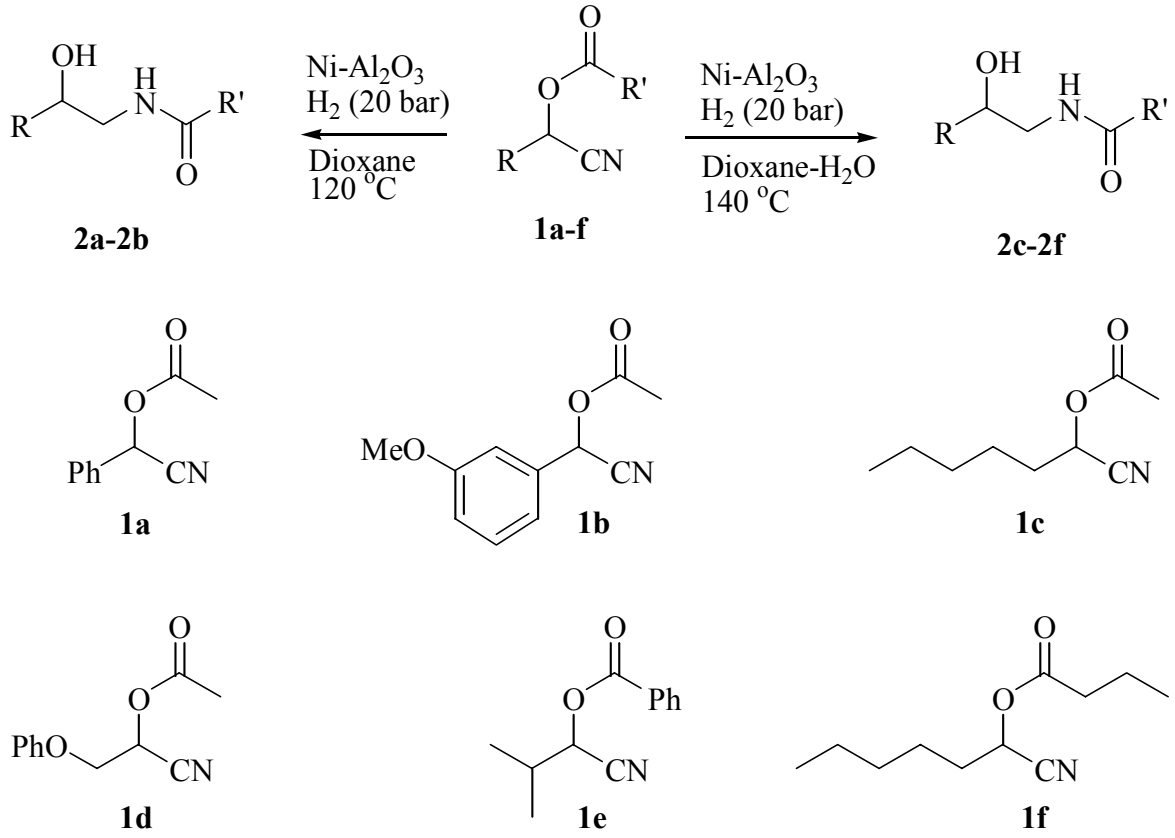
Third design

A temperature range of 80 to 160 ˚C and a pressure range from 5 to 40 bar was tested. Only minor differences in the yield (± 7 % for the aliphatic, ± 5 % for the aromatic) were observed, except for reaction temperatures below 90 ˚C, where hardly any reaction occurred. Despite the small differences in the observed yields an optimum of 10 bar H_2 at 140 ˚C is found for the aliphatic substrate **1c** and 20 bar H_2 at 120 ˚C is found for the benzylic substrate **1a**.

Other substrates

In order to establish the versatility of the reaction, these optimised conditions were applied to a number of other acylated cyanohydrins: a substituted benzylic ester (**1b**), an aliphatic substrate with an aromatic side chain (**1d**), and aliphatic substrates with a variety of acyl

groups (**1e**, **1f**) (Scheme 2). All these substrates were successfully hydrogenated to yield the desired *N*-acyl-*β*-aminoalcohols **2a-f**. The conversion of the substrates was in all cases 100 %. The benzylic substrates **1a** and **1b** gave more side products than the aliphatic compounds **1c-f**. This difference between the substrates is in accordance with the unstable benzylic C-O bond. In the case of **1c** and **1f**, the products were isolated by crystallisation from the reaction mixtures.



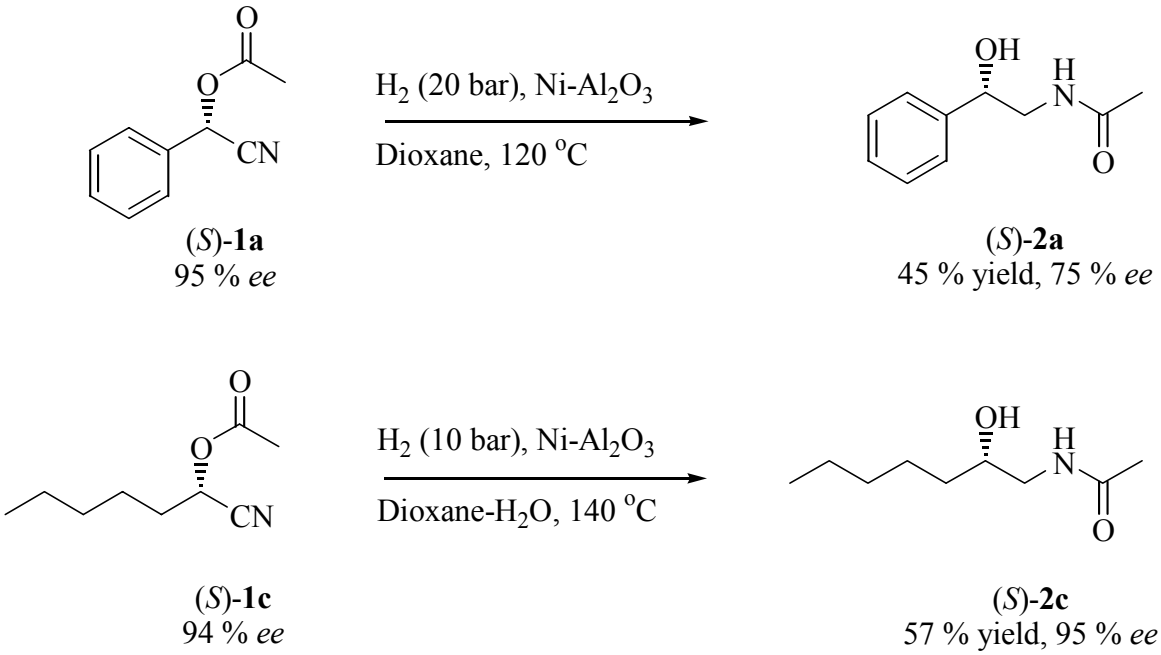
Scheme 2: The catalytic hydrogenation of cyanohydrin esters.

Table 4: Conversion and yield from the hydrogenation of acylated cyanohydrins **1a-f**.

	Conversion of **1** [%]	NMR-Yield of **2** [%]	Isolated yields of **2** [%]
1a	100	n.d	49[a]
1b	100	n.d	50[a]
1c	100	74	57[b]
1d	100	91	72[a]
1e	100	~75	58[a]
1f	100	83	30[b]

[a] Isolated by column chromatography. [b] Isolated by recrystallisation from ethyl acetate, not optimised.

The hydrogenation was also performed on the optically active substrates (*S*)-**1a** (95 % ee) and (*S*)-**1c** (94 % ee). As expected the chiral centre of (*S*)-**1c** was found to remain unchanged during both the hydrogenation and the intramolecular migration. This was not the case with (*S*)-**1a,** however, and the isolated (*S*)-**2a** had an ee of only 75 % (see Scheme 3). This decrease in ee might be explained by a base-catalysed racemisation of the substrate; the base being either ammonia released in the formation of the secondary amine side-product, or the secondary amine itself.



Scheme 3: Catalytic hydrogenation of enantiopure acylated cyanohydrins

Conclusion

The catalytic hydrogenation of acylated cyanohydrins (**1**) with subsequent intra-molecular migration of the acyl group constitutes a valuable one-pot route to the pharmaceutically important *N*-acyl*β*-aminoalcohols (**2**). Both the hydrogenation and the migration proceeded smoothly and the desired product could be obtained in yields of up to 90 % for the aliphatic substrates and up to 50 % for the more sensitive benzylic substrates. The application to a range of aliphatic and aromatic substrates with different acyl groups was demonstrated. When enantiopure substrates are employed the stereocentre remains unaltered for aliphatic substrates and only a small amount of racemisation is observed for benzylic substrates. Given the straightforward access to the (chiral) starting materials and the mild, catalytic reaction conditions this one-pot sequence represents a significant step forward.

A multistep DoE approach proved an efficient method for the optimization of the reaction. From more than 2000 possible combinations of the parameters requiring to be studied, it proved possible to effect the optimization using only 70 experiments for each substrate. This shows the great advantage of the DoE approach towards the optimisation of a new reaction, enabling a large parameter space to be investigated and the most interesting range within the parameter space to be identified.

Experimental section

General: ^{1}H and ^{13}C-NMR spectra were recorded on a Varian VXR-400S (400 and 100 MHz, respectively) or a Varian Unity Inova 300 (300 and 75 MHz, respectively), instrument. Chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quadriplet) and m (multiplet). Mass spectra were determined on a VG 70 SE spectrometer operating at 70 eV. GC-MS was measured by means of a VG 250 SE instrument equipped with a CP Sil 8 CB column of 25 m × 0.25 mm and 0.4 μm DF. A Varian Star 3600 – GC equipped with a CP Sil 5CB column with 50 m × 0.55 mm and 1 μm DF, was used to determine the conversions in the crude reaction mixtures. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. Melting points are uncorrected. Column chromatography was carried out with silica gel packing of 0.060-0.200 mm, pore diameter ca. 6 nm and with mixtures of petroleum ether (PE), methanol (MeOH) and ethyl acetate (EtOAc) as solvent. TLC was performed on 0.20 mm silica gel. The nickel catalysts were all purchased from commercial sources and activated at 140 ˚C for 12 h at 40 bar H_2 before use in the general procedures B, C and D described below. All other catalysts, and the solvents employed, were used as received from commercial sources. For all the supported catalysts the metal loading was 5 %, except for Rh-Silica (1 %), Ni-Alumina (50 %) and Ni-Silica (66 %). Racemic[9] and enantiopure cyanohydrin acetates[11,25] were synthesised according to literature procedures. The optical purity of **2a** was determined by HPLC employing a Waters 510 pump, a 4.6 × 250 mm 10 μ Chiracel OJ column and a Waters 486 UV detector. The eluant was a mixture of hexane and 2-propanol (90:10) with a flow of 0.8 ml min^{-1}. The optical purities of **1a**, **1c** and **2c** were determined by chiral GC using a Shimadzu Gas Chromatograph GC-17A equipped with a *β*-cyclodextrin column (CP-Chirasil-Dex CB 25m × 0.25mm). A Shimadzu Auto-injector AOC-20i and FID detector were employed, and He with a linear gas velocity of 75 cm/s formed the carrier gas. The Avantium "Quick Catalyst Screening 96" platform was used to

perform the reactions of the first and second experimental designs. This equipment has a maximum pressure limit of 20 bar and the temperature is controlled for all reactors simultaneously. Otherwise, a 100 ml Parr autoclave was used. The elemental analysis was performed on a Elementar Vario EL III analyser.

General procedure A: Screening in the Avantium "Quick Catalyst Screening 96" platform: The various supported metal catalysts (5 mg) were weighed into the autoclaves and added to a 1.7 M solution of the substrate in the desired solvent (1.5 ml). When water was used as an additive, 10 μl was added. In the case ammonia was used as additive, the concentration of ammonia in the reaction mixture was 0.5 M. After stirring the reaction at 90/120 ºC and 20 bar H_2, for 3 or 24 h, the reaction mixture was centrifuged and the supernatant liquid analysed by GC and GC-MS.

General procedure B: Screening for temperature and pressure in the Parr autoclave: The pre-activated 50 % Ni on alumina (100 mg) was added to a solution of **1a** or **1c** (5.7 mmol) in dioxane (30 ml). In the case of **1c**, water (0.2 ml) was also added. After stirring the reaction at 80, 100, 120, 140, or 160 ºC and 5, 10, 20, 30 or 40 bar H_2, for 2 hours the reaction mixture was filtered. The filtrate was analysed by GC.

General procedure C. Reductions in the Parr autoclave with optimized conditions for substrates prepared from aromatic aldehydes: Activated 50 % Ni on alumina (100 mg) was added to a solution of the substrate (5.7 mmol) in dioxane (30 ml). After stirring the reaction at 120 ºC and 20 bar H_2, the reaction mixture was filtered. A sample of 2 ml was taken from the filtrate and the solvents from this sample were removed under vacuum. The sample was then analysed by ^{1}H-NMR. The combined filtrate and NMR-sample was then evaporated to dryness to yield the oil or solid products.

***N*-(2-hydroxy-2-phenylethyl) acetamide 2a**: The solid prepared from **1a** according to general procedure C was purified by column chromatography (silica, EtOAc/MeOH, 95:5, R_f = 0.27). Yield of **(*S*)-2a**: 503 mg (49 %) as a white solid; m.p. 125-126 ºC; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 ºC, TMS): δ = 2.01 (s, 3H, C*H*$_3$-C=O), 3.32 (ddd, *J* = 5.0, 7.9, 14.1 Hz, 1H, C*H*$_2$-N), 3.70 (ddd, *J* = 3.3, 7.0, 14.1, 1H, C*H*$_2$-N), 4.85 (dd, *J* = 3.3, 7.9, 14.1 Hz, 1H, C*H*-O), 5.92 (s, 1H, N*H*), 7.28-7.38 (m, 5H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 ºC, TMS):

δ = 23.1 (*C*H_3), 47.6 (*C*H_2-N), 73.6 (*C*H-O), 125.8, 128.8, 128.5 and 141.8 (aromatic), 171.6 (*C*=O); IR (KBr): ν = 3300, 3080, 1648, 1547, 1295 cm^{-1}; MS (70 eV, EI): *m/z* (%): 179 (1) [M^+], 161 (3) [M^+-H_2O], 120 (14), 107 (21), 79 (31), 77 (31), 73 (100); elemental analysis calculated (%) for $C_{10}H_{13}NO_2$ (179.22): C 67.02, H 7.31, N 7.82; found: C 67.00, H 7.49, N 7.81.

(*S*)-*N*-(2-hydroxy-2-phenylethyl) acetamide (*S*)-2a: The solid prepared from **(*S*)-1a** (95 % ee) according to general procedure C was purified by column chromatography (silica, EtOAc/MeOH, 95:5, R_f = 0.27). Yield of **(*S*)-2a**: 0.454 mg (45.4 %) as a white solid; *ee* = 75 %, $[\alpha]_D^{20}$ = + 8.1 (*c* = 1.0 in MeOH); other spectroscopic data as for **2a**.

***N*-[2-hydroxy-2-(3-methoxyphenyl)ethyl] acetamide 2b**: The solid prepared from *rac*-**1b** according to general procedure C was purified by column chromatography (silica, EtOAc/MeOH, 95:5, R_f = 0.25). Yield of **2b**: 0.570 mg (57 %) as a white solid; m.p. 123-124 ºC; ^{1}H-NMR (300 MHz, CD_3OD, 25 ºC, TMS): δ = 1.93 (s, 3H, C*H*$_3$-C=O), 3.28 (dd, *J* = 7.9, 13.7 Hz, 1H, C*H*$_2$-N), 3.45 (dd, *J* = 4.6, 13.5 Hz, 1H, C*H*$_2$-N), 3.78 (s, 3H, OC*H*$_3$), 4.71 (dd, *J* = 4.6, 7.9 Hz, 1H, C*H*-O), 6.81 (ddd, J = 0.9, 2.6, 8.2 Hz, 1H, C4-*H*), 6.95 (m, 2H, C2-H, C6-*H*),), 7.24 (apparent t, J = 7.9 Hz, 1H, C5-*H*); ^{13}C-NMR (75 MHz, CD_3OD, 25 ºC, TMS): δ = 22.5 (*C*H_3-CO), 48.3 (*C*H_2-N), 55.6 (O*C*H_3), 73.5 (*C*H-O), 112.6 (*C*2), 114.1 (*C*4), 119.4 (*C*6), 130.3 (*C*5), 145.5 (*C*1), 161.2 (*C*3), 173.6 (*C*=O); IR (KBr): ν = 3290, 1634, 1596, 1552, 1259, 1066 cm^{-1}; MS (70 eV, EI): *m/z* (%): 209 (7) [M^+], 191 (3) [M^+-H_2O], 150 (31), 109 (25), 73 (87), 62 (46), 45 (100); elemental analysis calcd (%) for $C_{11}H_{15}NO_3$ (209.24): C 63.14, H 7.23, N 6.69; found: C 61.41, H 7.57, N 6.50.

General procedure D. Reductions in the Parr autoclave with optimised conditions for substrates prepared from aliphatic aldehydes: Activated 50 % Ni on alumina (100 mg) was added to a solution of the substrate (5.7 mmol) in dioxane (30 ml) and water (0.2 ml). After stirring the reaction at 140 ºC and 10 bar H_2, the reaction mixture was filtered. A sample of 2 ml was taken from the filtrate and the solvents from this sample were removed under vacuum and the sample was then analysed by ^{1}H-NMR. The combined filtrate and NMR-sample was then evaporated to dryness to yield the oil or solid products.

***N*-(2-hydroxyheptyl) acetamide 2c**: The oil prepared from **1c** according to general procedure D was purified by recrystallisation from EtOAc. Yield of **2c**: 454 mg (56 %) as a white solid; m.p. 75-76 ºC; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 ºC, TMS): δ = 0.89 (m, 3H, C*H*$_3$-CH$_2$), 1.29-1.47 (m, 8H, CH$_3$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$), 2.00 (s, 3H, C*H*$_3$-C=O), 3.08 (ddd, *J* = 5.0, 7.9, 13.7 Hz, 1H, C*H*$_2$-N), 3.45 (ddd, *J* = 2.9, 6.6, 13.9 Hz, 1H, C*H*$_2$-N), 3.45 (s, 1H, O*H*), 3.69 (m, 1H, C*H*-O), 6.49 (s, 1H, N*H*); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 ºC, TMS): δ = 14.0 (*C*H$_3$-CH$_2$), 22.6 (CH$_3$-*C*H$_2$), 23.2 (*C*H$_3$-CO), 25.2 (CH$_3$-CH$_2$-*C*H$_2$), 31.8 (*C*H$_2$-CH$_2$-CH), 35.0 (*C*H$_2$-CH), 45.9 (*C*H$_2$-N), 71.2 (*C*H-O), 171.4 (*C*=O); IR (KBr): ν = 3425, 3279, 1661, 1627, 1586, 1569, 1136 cm^{-1}; MS (70 eV, EI): *m/z* (%): 174 (3) [M$^+$+1], 102 (10), 73 (100); elemental analysis calcd (%) for $C_9H_{19}NO_2$ (173.25): C 62.39, H 11.05, N 8.08; found: C 62.01, H 11.67, N 8.04.

(*S*)-*N*-(2-hydroxyheptyl) acetamide (*S*)-2c: The oil prepared from **(*S*)-1c** (5.4 mmol, 94 % *ee*) according to general procedure D was purified by recrystallisation from EtOAc. Yield of **(*S*)-2c**: 533 mg (57 %) as a white solid; *ee* = 95 %; m.p. 75-76 ºC; $[\alpha]_D^{20}$ = + 14.1 (*c* = 1.0 in MeOH); other spectroscopic data as for **2a**.

***N*-(2-hydroxy-3-phenoxypropyl) acetamide 2d**: The oil prepared from *rac*-**1d** according to general procedure D was purified by column chromatography (silica, EtOAc/MeOH, 95:5, R_f = 0.29). Yield of **2d**: 967 mg (72 %) as a white solid; m.p. 49-50 ºC; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 ºC, TMS): δ = 1.99 (s, 3H, C*H*$_3$-C=O), 3.36 (ddd, *J* = 5.5, 6.8, 6.8 Hz, 1H, C*H*$_2$-N), 3.59 (ddd, *J* = 3.3, 6.1, 14.0 Hz, 1H, C*H*$_2$-N), 3.92 (d, *J* = 5.5 Hz, 2H, C*H*$_2$-O), 4.09 (m, 1H, C*H*-O), 4.18 (s, 1H, O*H*), 6.58 (s, 1H, N*H*), 6.87 (m, 2H, aromatic), 6.95 (m, 1H, aromatic), 7.26 (m, 2H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 ºC, TMS): δ = 23.0 (*C*H$_3$-C=O), 43.0 (*C*H$_2$-N), 69.5 (*C*H-OH and *C*H$_2$-O), 114.5, 121.2, 129.6, and 158.4 (aromatic), 171.9 (*C*=O); IR (KBr): ν = 3384, 3299, 1630, 1601, 1571, 1284, 1118, 751 cm^{-1}; MS (70 eV, EI): *m/z* (%): 209 (3) [M$^+$], 191 (32) [M$^+$-H$_2$O], 148 (7), 116 (100); elemental analysis calcd (%) for $C_{11}H_{15}NO_3$ (209.24): C 63.14, H 7.23, N 6.69; found: C 61.99, H 7.22, N 6.46.

***N*-(2-hydroxy-3-methylbutyl) benzamide 2e**: The solid prepared from *rac*-**1e** according to general procedure D was purified by column chromatography (silica, EtOAc/PE, 45:55, R_f = 0.30). Yield of **2e**: 681mg (58 %) as a white solid; m.p. 116-117 ºC; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 ºC, TMS): δ = 0.97 (dd, *J* = 6.8, 9.0 Hz, 6H, 2 C*H*$_3$), 1.73 (m, 1H, C*H*-(CH$_3$)$_2$),

3.06 (s, 1H, O*H*), 3.30 (ddd, *J* = 4.6, 8.61, 13.7 Hz, 1H, C*H*$_2$-N), 3.50 (m, 1H, C*H*-O), 3.72 (ddd, *J* = 2.8, 6.8, 13.7 Hz, 1H, C*H*$_2$-N), 6.86 (s, 1H, N*H*), 7.38 (m, 2H, aromatic), 7.46 (m, 1H, aromatic), 7.77 (m, 2H, aromatic); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 ºC, TMS): δ = 17.9 (*C*H$_3$), 18.6 (*C*H$_3$), 32.3 (*C*H-(CH$_3$)$_2$), 44.1 (CH$_2$-N), 76.3 (*C*H-O), 127.0, 128.5, 131.5, and 134.3 (aromatic), 168.5 (*C*=O); IR (KBr): ν = 3398, 3319, 1633, 1578, 1541, 1057, 697 cm^{-1}; MS (70 eV, EI): *m/z* (%): 207 (1) [M$^+$], 189 (3) [M$^+$-H$_2$O], 164 (16), 134 (89), 122 (29), 105 (100); elemental analysis calcd (%) for $C_{12}H_{17}NO_2$ (207.27): C 69.54, H 8.27, N 6.76; found: C 68.75, H 8.61, N 6.68.

***N*-(2-hydroxyhepyl) butanamide 2f**: The oil prepared from *rac*-**1f** according to general procedure D was purified by recrystallisation from EtOAc. Yield of **2f**: 345 mg (30 %) as a white solid; m.p. 62-63 ºC; ^{1}H-NMR (300 MHz, $CDCl_3$, 25 ºC, TMS): δ = 0.89 (m, 3H, pentyl-C*H*$_3$), 0.95 (t, *J* = 7.5 Hz, 3H, propyl-C*H*$_3$), 1.29-1.44 (m, 8H, CH$_3$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$), 1.67 (sextet, *J* = 7.4 Hz, 2H, C*H*$_2$-CH$_2$-C=O), 2.18 (t, *J* = 7.4 Hz CH$_2$-C*H*$_2$-C=O), 2.94 (s, 1H, O*H*), 3.11 (ddd, *J* = 4.9, 7.7, 13.0 Hz, 1H, C*H*$_2$-N), 3.47 (ddd, *J* = 2.7, 6.2, 13.7 Hz, 1H, C*H*$_2$-N), 3.70 (m, 1H, C*H*-O), 6.15 (s, 1H, N*H*); ^{13}C-NMR (75 MHz, $CDCl_3$, 25 ºC, TMS): δ = 13.8 (propyl-*C*H$_3$), 14.0 (pentyl-*C*H$_3$), 19.2 (*C*H$_2$-CH$_2$-C=O), 22.6 (CH$_3$-*C*H$_2$-CH$_2$-CH$_2$), 25.2 (CH$_3$-CH$_2$-*C*H$_2$-CH$_2$), 31.8 (*C*H$_2$-CH$_2$-CH), 35.0 (*C*H$_2$-CH), 38.6 (*C*H$_2$-C=O), 45.7 (*C*H$_2$-N), 71.5 (*C*H-O), 174.2 (*C*=O) ; IR (KBr): ν = 3418, 3283, 2964, 2919, 1657 ,1624, 1566 cm^{-1}; MS (70 eV, EI): *m/z* (%): [M$^+$] could not be identified, 130 (17), 101 (100); elemental analysis calcd (%) for $C_{11}H_{23}NO_2$ (173.25): C 65.63, H 11.52, N 6.96; found: C 64.78, H 12.03, N 6.83.

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Summary

This thesis deals with various enzyme-catalysed approaches towards enantiopure cyanohydrins, which are important building blocks in both the pharmaceutical and agricultural industry. Common for the methods that have been investigated is that enzymes were used in combination with chemical catalysts in cascade reactions, either in one pot or in consecutive reactions without isolation of the intermediates. Chapter 1 contains an overview of this work, emphasizing the importance of immobilising, either of the enzyme or of the chemical catalyst, for these cascades to be successful.

The preparation of (*S*)-γ,δ-unsaturated cyanohydrins in a two-step cascade reaction from γ,δ-unsaturated alcohols is described in chapter 2. First the alcohol is oxidised to the corresponding aldehyde utilizing catalytic amounts of immobilised TEMPO, and $PhI(OAc)_2$ as the stoichiometric oxidant. Due to the instability of the resulting aldehyde, the reaction mixture is used directly for the next step after filtering off the TEMPO and removing the acetic acid formed during the oxidation. The (*S*)-γ,δ-unsaturated cyanohydrins were obtained in a *Hb*HNL-catalysed hydrocyanation of the aldehyde. Only by removing the TEMPO prior to the enzyme reaction, the products could be obtained in excellent optical purity.

In chapter 3 a straightforward process for the encapsulation of *Hb*HNL in sol-gels under low methanol conditions is developed. By adding a sol that was prepared by hydrolysis of TMOS/MTMS at pH 2.8 with continuous removal of methanol to a stirred solution of the enzyme in a buffer at pH 6.5, at least 65 % of the activity of the free enzyme could be recovered after the encapsulation. These aquagels were successfully used in the synthesis of (*S*)-cyanohydrins. Although the recycling of the gels failed, the method holds great potential for the encapsulation of other methanol sensitive enzymes.

Chapter 4 describes a straightforward process for the preparation of optically active protected cyanohydrins. Lipase B from *Candida antarctica* (CAL-B) catalyses the kinetic resolution of racemic cyanohydrin acetates under mild conditions. The resulting labile cyanohydrins were re-protected either via an enzyme-catalysed route, involving the addition of vinyl butyrate, or chemically after removal of the enzyme from the reaction mixture. This process gives access to both enantiomers in their pure form and in good yields, while reducing the risks due to HCN considerably. Moreover, a variety of different protection groups were introduced.

The base- and lipase-catalysed enantioselective synthesis of cyanohydrin esters via a DKR was investigated (chapter 5) and the problem of previously reported low yields due to residual water in the reaction mixture was addressed. When the lipase was immobilised on Celite R-633 as a carrier, both the enantioselectivity and the reaction times for this dynamic kinetic resolution improved, thus enabling a highly enantioselective synthesis of aromatic and heteroaromatic cyanohydrin acetates.

When the standard conditions for the DKR described in chapter 5 were applied to aliphatic substrates, only a kinetic resolution was observed. This problem was solved (chapter 6) by exchanging the base (Amberlite IRA-904 OH^- form) against NaCN. Then, both quantitative conversions and good enantioselectivities could be obtained.

This far, most of the work in the thesis has been focused on the synthesis of enantiopure cyanohydrins and mainly protected as their acetates. In chapter 7 a novel application of these compounds is investigated. The catalytic hydrogenation of acylated cyanohydrins, with subsequent intra-molecular migration of the acyl group to yield pharmaceutically interesting β-hydroxyamides, is shown to be a successful one-pot preparation method. The combination of a multistep DoE approach and high-throughput methodology proved to be an effective strategy for the optimization of the reaction. With the favoured catalyst/solvent combination nickel on alumina in dioxane, both hydrogenation and acyl-group migration proceeded smoothly, giving the β-hydroxyamides in yields of up to 90 % for aliphatic, and 50 % for benzylic substrates, which are more prone to side reactions. When enantiopure cyanohydrin esters were used, no racemization was found to occur at the chiral centre of an aliphatic molecule, though a minor decrease in ee was observed for a benzylic substrate.

Samenvatting

In dit proefschrift komen diverse enzym-gekatalyseerde benaderingen van enantiozuivere cyaanhydrinen aan de orde. Dit zijn belangrijke bouwstenen in de farmaceutische industrie en voor landbouwchemicaliën. Gemeenschappelijk voor de methodes die onderzocht zijn is dat de enzymen werden gebruikt in combinatie met chemische katalysatoren in cascadereacties, òf in één pot òf in opeenvolgende reacties zonder isolatie van de tussenproducten. Hoofdstuk 1 bevat een overzicht van het beschreven werk, met nadruk op het belang van de immobilisatie van het enzym of van de chemische katalysator, voor een succesvolle cascadereactie.

In hoofdstuk 2 wordt de bereiding van (*S*)-γ,δ-onverzadigde cyaanohydrinen met behulp van een cascadereactie in twee stappen uitgaande van γ,δ-onverzadigde alcoholen beschreven. Eerst wordt deze alcohol geoxideerd tot het overeenkomstige aldehyde met gebruik van katalytische hoeveelheden geïmmobiliseerd TEMPO, en $PhI(OAc)_2$ als stoichiometrisch oxidatiemiddel. Wegens de instabiliteit van de resulterende aldehydes, werd het reactiemengsel, na het filtreren van de TEMPO katalysator en het verwijderen van het tijdens de oxidatie gevormde azijnzuur, direct ingezet in de volgende stap. (*S*)-γ,δ-onverzadigde cyaanhydrinen werden verkregen in een *Hb*HNL-gekatalyseerde hydrocyanering van de aldehydes. Slechts door het voorafgaande verwijderen van TEMPO werden na de enzymreactie producten met uitstekende optische zuiverheid verkregen.

In hoofdstuk 3 wordt een eenvoudig proces voor de inkapseling van *Hb*HNL in sol-gels bij lage methanolconcentraties beschreven. Door een sol, dat verkregen werd door hydrolyse van TMOS/MTMS bij pH 2,8 onder continue verwijdering van methanol, toe te voegen aan een geroerde oplossing van het enzym in een buffer bij pH 6.5, behoudt het ingekapselde enzym 65 % van de activiteit van het vrije enzym. Deze aqua-gels werden met succes gebruikt in de synthese van (*S*)-cyaanhydrinen. Hoewel de recycling van de gels te wensen overlaat, is de methode veelbelovend voor de inkapseling van andere methanolgevoelige enzymen.

Hoofdstuk 4 beschrijft een eenvoudig proces voor de bereiding van optisch actieve beschermde cyaanhydrinen. Lipase B van *Candida antarctica* (CALB) katalyseert de kinetische resolutie van racemische cyaanhydrin acetaten onder milde condities. De resulterende labiele cyaanhydrinen werden her-beschermd via een enzym-gekatalyseerde route (met vinylbutyraat als acyl-donor) of chemisch na verwijdering van het enzym uit het

reactiemengsel. Op deze manier kon er een reeks van verschillende beschermingsgroepen geïntroduceerd worden. Met deze methodes konden de twee enantiomeren met hoge zuiverheid en in goede opbrengsten worden verkregen. Tegelijkertijd zijn de risico's op vorming van HCN aanzienlijk verminderd.

De base- en lipase-gekatalyseerde enantioselective synthese van cyaanhydrine esters via een dynamische kinetische resolutie (DKR) werden onderzocht (hoofdstuk 5) en het probleem van de eerder gerapporteerde lage opbrengsten, toe te schrijven aan overblijvend/ten gevolge van restanten van water in het reactiemengsel, werd opgehelderd. Door immobilisatie van het lipase op Celite R-633 als drager werden zowel de enantioselectiviteit als ook de reactietijden van de DKR worden verbeterd. Hierdoor wordt een bijna geheel enantiospecifieke synthese van aromatische en heteroaromatische cyaanhydrine acetaten mogelijk.

Toen de standaardcondities voor de DKR beschreven in hoofdstuk 5 werden toegepast op alifatische substraten, werd slechts een kinetische resolutie geobserveerd. Dit probleem werd opgelost (hoofdstuk 6) door de base Amberlite door basisch NaCN te vervangen. Op deze manier kunnen zowel kwantitatieve omzettingen als ook goede enantioselectiviteiten worden verkregen.

Tot zo ver is het grootste deel van het werk in het proefschrift geconcentreerd op de synthese van enantiozuivere cyaanhydrinen, hoofdzakelijk beschermd als acetaten. In hoofdstuk 7 wordt een nieuwe toepassing van deze verbindingen beschreven. De katalytische hydrogenering van geacyleerde cyaanhydrinen, gevolgd door een intramoleculaire migratie van de acylgroep kon succesvol uitgevoerd worden in een één-pot. Zo kunnen farmaceutisch interessante β-hydroxyamiden eenvoudig gemaakt worden. De combinatie van een multistep DoE benadering en een "high throughput methodiek" bleek een efficiënte strategie voor de optimalisering van de reactie te zijn. Met de optimale combinatie van katalysator en oplosmiddel, nikkel op alumina in dioxaan, verliep zowel de hydrogenering als de acyl-groep migratie gemakkelijk, en werden de β-hydroxyamiden werd verkregen in opbrengsten van 90 % voor alifatische, en 50 % voor benzylische substraten. De laatste zijn gevoelig voor nevenreacties. Bij gebruik van de esters van enantiomeer zuivere cyaanhydrinen, werd bij benzylische substraaten een geringe daling van de ee waargenomen. Alifatische substraten gaven helemaal geen racemisatie.

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So, as the Dutch say,
Hartelijk bedankt hoor!

Curriculum Vitae

Lars Veum was born in Voss, Norway, on December 15, 1975. Following the graduation from "Voss Gymnas" in 1994, he started his chemistry studies at the "Norges Teknisk-Naturvitenskaplige Universitet" in Trondheim. From 1997 to 1998 his studies had a one year interruption by the service the Royal Norwegian Navy. He graduated in 2000, and his final research project, "New Chiral Ferrocenyl Ligands for Homogeneous Catalysis" was carried out under the supervision of Dr Marc Larchevêque at the "Ecole Nationale Supérieure de Chimie de Paris". In September 2000 he started his PhD research at the "Technische Universiteit Delft" under the supervision of Dr. Ulf Hanefeld, in the group of Prof. dr. Thomas Maschmeyer, later Prof. dr. Roger Sheldon. From 2003 to 2004 an additional year to his PhD studies was spent working on the encapsulation of *Hb*HNL in Sol-Gels at the "Institut de Recherches sur la Catalyse", (Lyon) under the supervision of Prof. dr. Alain Pierre. The results of his PhD research are presented in this thesis.