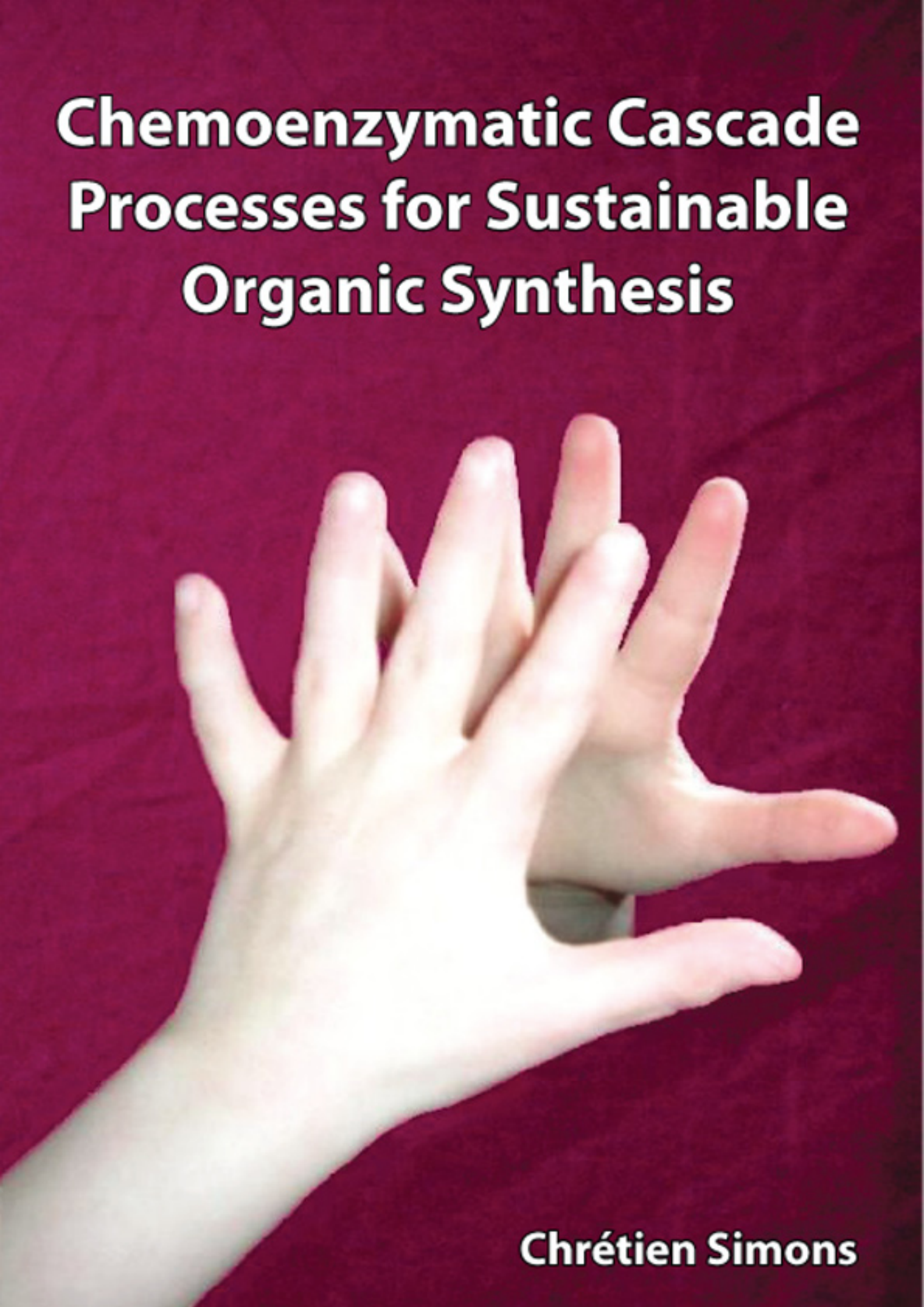
Chemoenzymatic Cascade Processes for Sustainable Organic Synthesis



Chrétien Simons

Stellingen behorende bij het proefschrift

Chemoenzymatic cascade processes for sustainable organic synthesis

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1. Als belangenorganisaties blijven volharden in hun standpunten tegen kernenergie, dan valt zonne-energie strikt genomen af als geschikt alternatief voor de fossiele brandstoffen.

2. Waar toxicologen vooral problemen zien, zien chemici juist kansen.

3. Bij de betiteling van nanotechnologie als innovatie wordt er voorbijgegaan aan de moleculaire dimensies en de rijke historie van de chemie.

   Kabinetsvisie Nanotechnologie, Tweede Kamer, vergaderjaar **2006–2007**, *29 338*, nr. 54

4. De teruglopende kwaliteit van stellingen hangt samen met het feit dat de tijd die vroeger besteed werd aan het bedenken van goede stellingen, nu gebruikt wordt om een wetenschappelijk verantwoord proefschrift af te leveren.

   Het Promotiereglement van de TU Delft: Opponeerbaarheid van stellingen.

5. De oplossingen voor de milieuproblematiek druisen in tegen de natuurlijke aard van de mens.

6. De mono- en tricyclogepalladeerde dendritische systemen ontwikkeld voor de aldol condensatie zijn praktisch zeer beperkt bruikbaar.

   G. Rodriguez, M. Lutz, A.L. Spek, G. van Koten, *Chemistry*, **2002**; *8*, 45-57

7. Vanuit een katalytisch perspectief is er geen voordeel als de hydrogeneringsactiviteit, verkregen door de verankering van een rhodium complex in de actieve site van papaïne, volledig ten koste gaat van de enzymatische activiteit.

   L. Panella, J. Broos, J. Jin, M. W. Fraaije, D. B. Janssen, M. Jeronimus-Stratingh, B. L. Feringa, A. J. Minnaard, J. G. De Vries, *Chem. Commun*, **2005**, 5656-5658

8. Alleen aanhangers van “intelligent design” zullen verbaasd zijn over “catalytic promiscuity” bij biokatalysatoren, immers voor aanhangers van de evolutietheorie behoort dit evident te zijn.

   U. T. Bornscheuer, R. J. Kazlauskas, *Angew. Chem. Int. Ed.*, **2004**, *43*, 6032-6040

9. Door vast te houden aan de validatievoorwaarde, dat het eindresultaat van een alternatief en de oorspronkelijke dierproef volledig overeenkomen, wordt er een kans gemist om de toxicologische beoordeling van stoffen te verbeteren.

10. De ontdekking van de computer betekende voor de mensheid een grote sprong vooruit, maar daar merkt men in het dagelijks leven weinig van.

Deze stellingen worden opponeerbaar en verdedigbaar geacht en zijn als zodanig goedgekeurd door de promotor Prof. dr. R. A. Sheldon

Propositions belonging to the thesis

Chemoenzymatic cascade processes for sustainable organic synthesis

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1. If non-governmental organizations persist in their position against nuclear energy, solar energy will be lost as a suitable alternative for fossil fuels.

2. Where toxicologists see mainly difficulties, chemists see opportunities.

3. Labeling nanotechnology as an innovation fails to take into account the molecular dimensions and the rich history of chemistry.

   Kabinetsvisie Nanotechnologie, Tweede Kamer, vergaderjaar **2006–2007**, *29 338*, nr. 54

4. The diminishing quality of propositions can be explained by the fact that the time that was formerly utilized to devise high-quality propositions is now used to create a scientifically sound thesis.

   Het Promotiereglement van de TU Delft: Opponeerbaarheid van stellingen.

5. The solutions for the various environmental issues are in conflict with human nature.

6. The mono- and tricyclopalladated dendritic systems developed for the aldol condensation have a very limited practical value.

   G. Rodriguez, M. Lutz, A.L. Spek, G. van Koten, *Chemistry*, **2002**; *8*, 45-57

7. From a catalytic point of view no benefits are gained when the hydrogenation activity obtained by anchoring a rhodium complex in the active site of papain is at the expense of the entire enzymatic activity.

   L. Panella, J. Broos, J. Jin, M. W. Fraaije, D. B. Janssen, M. Jeronimus-Stratingh, B. L. Feringa, A. J. Minnaard, J. G. De Vries, *Chem. Commun*, **2005**, 5656-5658

8. Only supporters of “intelligent design” will be surprised by “catalytic promiscuity” in biocatalysts, since it should be evident for supporters of the evolution theory

   U. T. Bornscheuer, R. J. Kazlauskas, *Angew. Chem. Int. Ed.*, **2004**, *43*, 6032-6040

9. Maintaining the validation requirement, that the results of an alternative procedure correspond completely with the original animal test, results in a lost opportunity to improve the toxicological assessment of substances.

10. The discovery of the computer meant a great leap forward for mankind, however in daily live this is hardly noticeable.

These propositions are considered opposable and defendable and as such have been approved by the supervisor Prof. dr. R. A. Sheldon

Chemoenzymatic cascade processes for sustainable organic synthesis

Chrétien Simons

Front: Sarolta's representation of chirality
Back: Jarco's representation of chirality
The combination of front and back represents an asymmetric catalytic cascade

Cover design by Pieke Simons

Chemoenzymatic cascade processes for sustainable organic synthesis

Proefschrift

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus prof. dr. ir. J. T. Fokkema,
voorzitter van het College voor Promoties,
in het openbaar te verdedigen op 12 juni 2007 om 12:30

door

Chrétien SIMONS

doctorandus in de scheikunde
geboren te Zoetermeer

Dit proefschrift is goedgekeurd door de promotoren:

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Prof. dr. I. W. C. E. Arends

Samenstelling promotiecomissie:

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Dr. P. Gamez	Universiteit Leiden

Reservelid:
Prof. dr. W. R. Hagen

Dr. U. Hanefeld heeft als directe begeleider in belangrijke mate bijgedragen aan de tot standkoming van dit proefschrift

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Aan mijn vrouw
Pieke
mijn inspiratie

Aan mijn kinderen
Sarolta en Jarco
mijn motivatie

Contents

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Chemoenzymatic Cascade Processes for Sustainable Organic Synthesis

Introduction

Catalysis has been an essential component in the production of bulk chemicals for almost a century, but its application in the synthesis of fine chemicals/pharmaceuticals has been far less pronounced. This difference originates from the more complex nature of fine chemical synthesis and the need for a fast and flexible synthesis that is competitive in terms of 'time-to-market' pressures. The complexity results either in an absence of effective catalytic systems for the desired transformation or in highly specialised catalysts, which require a significant amount of time and experience to achieve the desired result. However, during the last decades catalysis has increasingly matured into a valued element in the syntheses of fine chemicals and is finding wider recognition by the synthetic organic chemist.

In addition to the ubiquitous pursuit of profit, the main driving forces for this recent upsurge are the growing environmental awareness and the rapid discovery of new catalysts and catalytic transformations. For that latter point, the developments in homogeneous transition metal catalysis and biocatalysis are of great significance and

logically these two specialities have greatly contributed to the possibilities for catalysis in the production of fine chemicals.

In this introduction a personal view of highlights in both worlds of transition metal catalysis and biocatalysis will be presented. The aim is to demonstrate the various advantages and disadvantages of their application in organic synthesis. Based on these separate discussions, the possibilities inherent in a merger between these two fields, leading to chemo-enzymatic cascades for the production of fine chemicals, will be examined. Finally the current state-of-the-art in chemo-enzymatic cascades will be presented, followed by the justification of this thesis.

Transition Metals in Organic Synthesis

The success of transition metals in organic synthesis can to a high degree be attributed to the great versatility of the complexes these metals tend to form. Virtually every organic functional group will interact with a transition metal. Generally these interactions dramatically alter the stability/reactivity of this coordinated functional group, opening up numerous novel reactions, which are impossible to achieve by conventional synthetic methods. This alteration of reactivity is dependent on the type of metal as well as the oxidation state, geometry and coordination number of the metal complex, resulting in a seemingly infinite number of possible reactions. More importantly, the reactivity can be fine-tuned by the ligands surrounding the metal, giving the chemist control over the course of the reaction. By changing the electronic and steric properties of these ligands, catalysts can in principle be tailor-made to perform just about any desired transformation.

An excellent demonstration of this ability is the pioneering work of Knowles on asymmetric hydrogenation (Figure 1).[1] Initial experiments on the Rh-catalysed hydrogenation of 2-acetamidocinnamic acid using methylisopropylphenylphospine as ligand led to an enantiomeric excess of 28%. Upon replacing the propyl group with *o*-anisyl (PAMP) the ee values dramatically improved to 58%. Further modification of the substituents gave the first industrially applied ligand, *o*-anisylcyclohexylmethylphosphane (CAMP) inducing an ee of 88%. Thus by relatively small modifications of the ligand, the reaction could be fine-tuned to give the desired enantioselectivity. The continuation of Knowles' search for better catalysts, perfectly demonstrates the difficulty of predicting the influence of ligand alterations. Dimerisation of PAMP, led to the second commercially applied ligand, DiPAMP. With this molecule 2-acetamidocinnamic acid could be reduced with an ee of 95%, an increase of 27

percentage points. Simple intuition would dictate that a dimerisation of CAMP would result in even higher values, but unfortunately this is not the case. The dimer of CAMP, DiCAMP, produces *N*-acylphenylalanine with a disappointing selectivity of 60-65%, far inferior to the 88% of the monomer. In spite of the rapid increase in knowledge of transition metal catalysis, catalyst design remains predominantly a matter of trial and error.

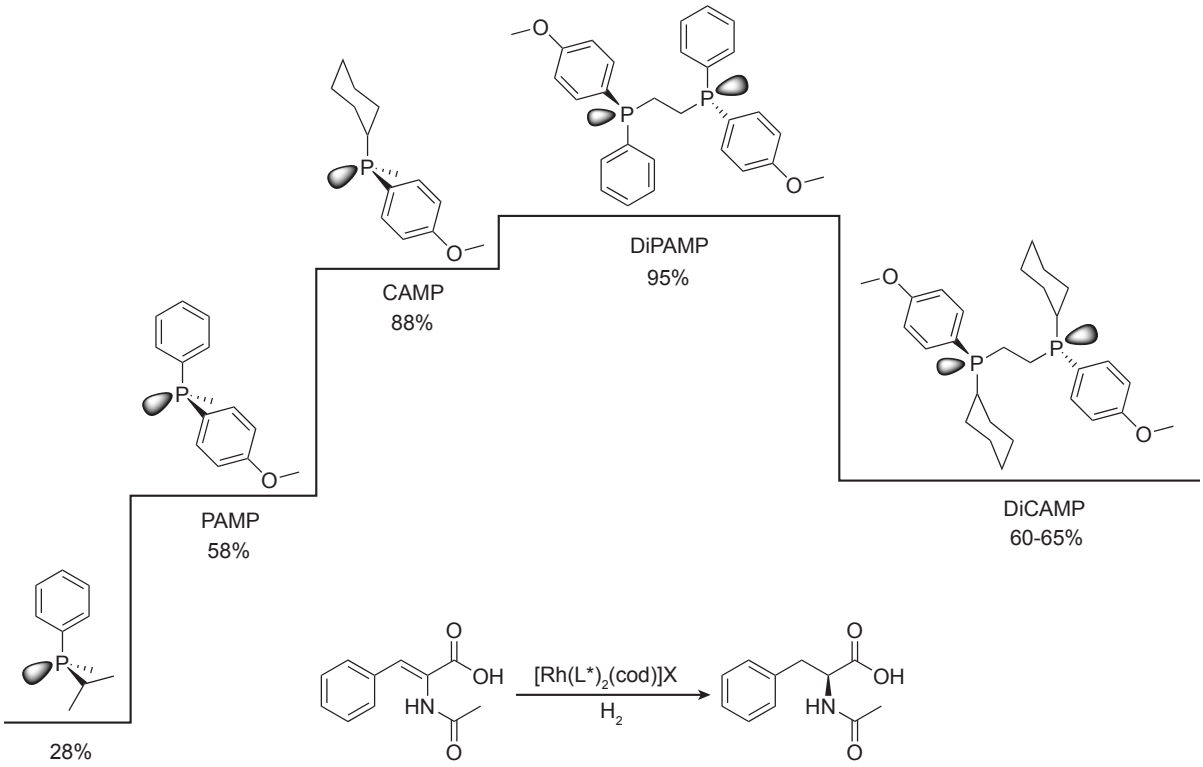


Figure 1. Influence of the ligand on the enantioselectivity of the Rh-catalysed hydrogenation of 2-acetamidocinnamic acid.

The great versatility of the transition metal complexes is reflected by the diversity of their application in organic synthesis. In Figure 2 the main types of transition metal-catalysed reactions used to produce fine chemicals/specialty chemicals are listed.

This list contains many reactions without which the modern chemist would be rendered powerless: Oxidation, reduction and the most important reaction in organic synthesis C-C bond formation. To clarify the importance and potential of this type of catalysis in modern synthetic chemistry, some illustrative examples from the current literature are given. A more comprehensive overview of this topic is provided in the excellent work of M. Beller and C. Bolm.[2]. For asymmetric syntheses the book of Ojima is an outstanding reference.[3]

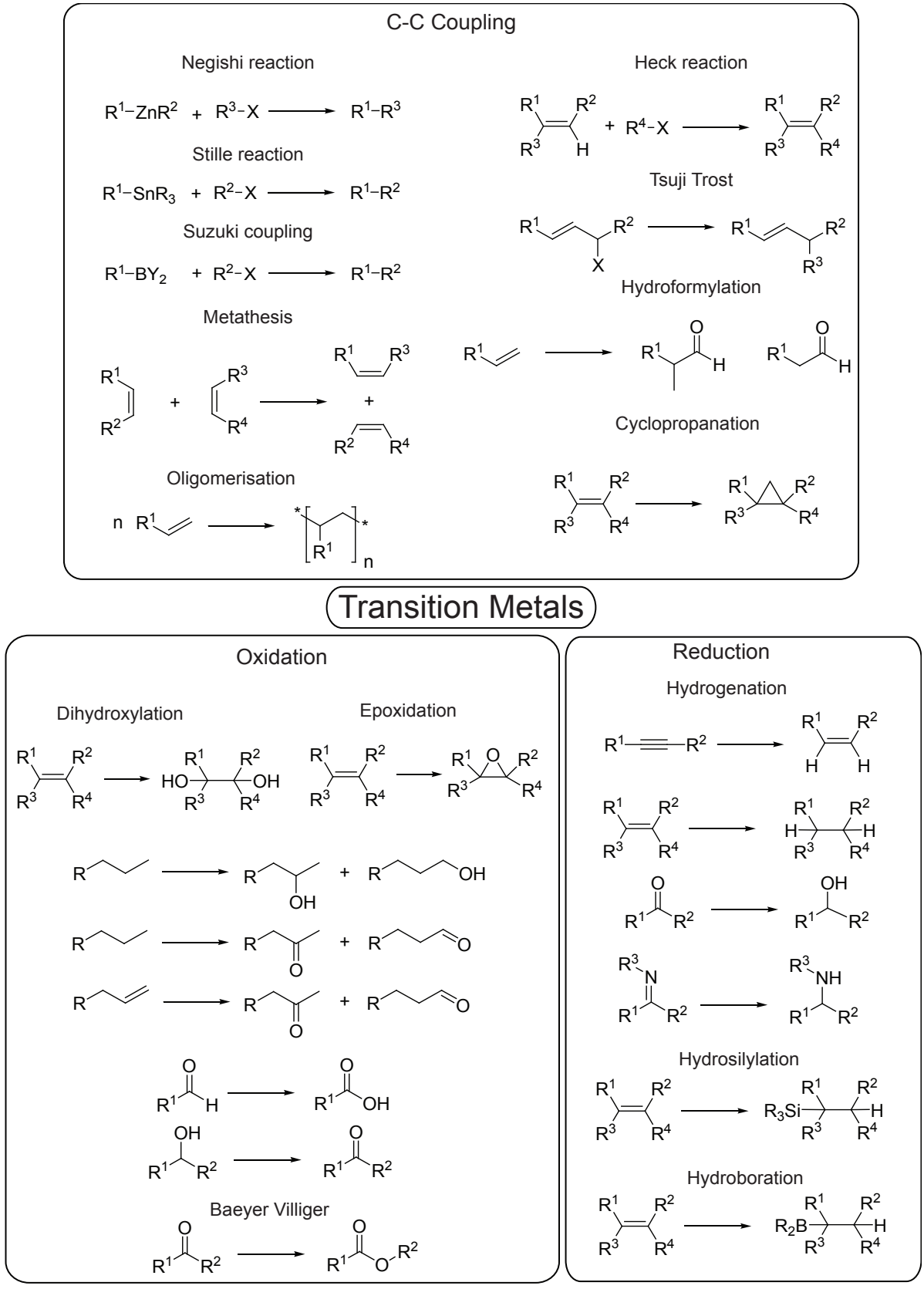


Figure 2. A selection of transition metal catalysed reactions in organic synthesis.

Oxidation

Oxidation is the foremost technology to introduce functionalities into organic molecules and thus of great importance for the synthesis of chemicals. It comprises a wide range of reactions, e.g. epoxidation, dihydroxylation, Wacker oxidation, Baeyer-Villiger oxidation, oxidative cleavage of olefins, oxidative coupling and alcohol oxidation, a majority of which can be catalysed or initiated by transition metals. Despite the wide range of possibilities of catalytic oxidation, synthetic organic chemists still persist in the use of stoichiometric oxidants, like $KMnO_4$ and $K_2Cr_2O_7$. This is a difference like night and day, as compared to the manufacture of bulk chemicals, where catalytic oxidation with dioxygen has been routinely applied for decades. This hesitation to use catalytic oxidations originates from the lack of selectivity with dioxygen, due to the presence of autoxidation and overoxidation. Fortunately, these problems can, to a large extent, be circumvented by employing other oxidants, like H_2O_2, RO_2H, R_3NO and NaClO, which are acceptable for fine chemicals on account of the high-value products. With the continuous improvement of selectivity and the growing possibilities of catalytic oxidation, synthetic chemists will hopefully abandon the wasteful stoichiometric oxidation reagents.

One of the most potent transition metal-catalysed oxidations for complex organic synthesis is the Sharpless asymmetric epoxidation (SAE),[4] the importance of which was recognised by the Nobel committee in 2001.[5] This potential is nicely demonstrated by the total syntheses of laulimalide (**1**),[6-10] a powerful microtubule stabilizing antitumor agent belonging to the same family as the frontline anticancer drug Taxol.[11] In most of the total syntheses of this unique and complex synthetic target, the final step consists of the selective introduction of the sensitive epoxide at C16-C17 employing SAE, $Ti(O^iPr)_4$-(+)-tartrate and *t*-Butylhydroperoxide (TBHP) (Figure 3).[6] The precursor, deoxylaumalide (**2**), possesses two allylic alcohols at C15 and C20, which are *pseudo*-enantiomeric and the (+)-tartrate-Ti-catalyst is extremely efficient in discriminating between them.[7-9] The C20 alcohol forms a mismatch with the (+)-tartrate catalyst,[4, 12] resulting in slow epoxidation whereas the C15 alcohol matches, yielding fast and selective formation of the 16,17-epoxide. Employing (-)-tartate as ligand generates selectively the 21,22-epoxide. Furthermore, the use of the Ti-catalyst eliminates the necessity to protect the C20 hydroxyl and avoids the easy isomerization of **1** to isolaulimalide (**3**), which can occur during deprotection. Thus, SAE is a highly regio and stereoselective oxidation, which does not effect numerous sensitive functionailities, even the very delicate 2,3-*cis*-enoate moiety is unscathed by this transformation.

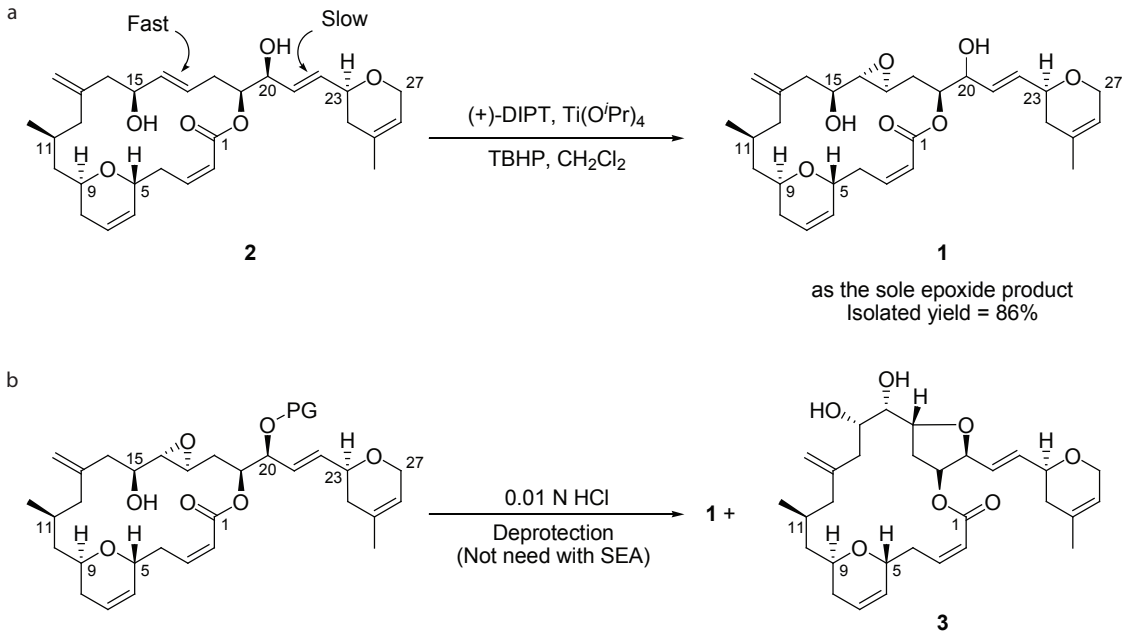


Figure 3. a) The final step in the total synthesis of laulimalide (**1**), demonstrating the potential of the Ti-catalysed Sharpless asymmetric epoxidation ((+)-DITP= (+)-(*R*,*R*)-diisopropyl tartrate). b) Isomerization of **1** to isolaulimalide (**3**), which can occur during deprotection. This competing side reaction is avoided, since the total synthesis using SEA eliminates the deprotection step.

Sharpless received the Nobel prize not only for his development of the asymmetric epoxidation, but also for his discovery of the asymmetric dihydroxylation (AD).[13] The conversion of olefins to vicinal diols via this methodology has become a valuable tool for the synthesis of polyoxygenated natural products. This is nicely illustrated by the total synthesis of Uvaricin (**4**)[14], a member of the Annonaceous acetogenins family that are known not only for their antitumor activity but also for being potent antimalarial and pesticidal agents (Figure 4).[15] All six asymmetric centers of the C15-C24 fragment of **4** were introduced by three distinct AD steps. The synthesis directly begins with the creation of 2 of those chiral centers. **5** is converted by osmium-catalysed AD to **6** using the commercially available AD-mixture-β,[16] consisting of $(DHQD)_2$-PHAL (= 1,4-bis(9-*O*-dihydroquinidine)-phthalazine), $K_3Fe(CN)_6$, K_2CO_3 and $K_2OsO_2(OH)_4$, with excellent yield and selectivity. An inversion of one of the chiral alcohols, followed by a sequence of steps yields diene **7**, which plays a key role in this synthetic approach toward **4**. This diene undergoes two consecutive AD reactions to incorporate the complementary stereocenters. Firstly the more electron rich double-bond is regioselectively dihydroxylated using AD-mixture-α,[16] which contains the other diastereomer of the cinchona alkaloid units in the PHAL-ligand, $(DHQ)_2$-PHAL (=

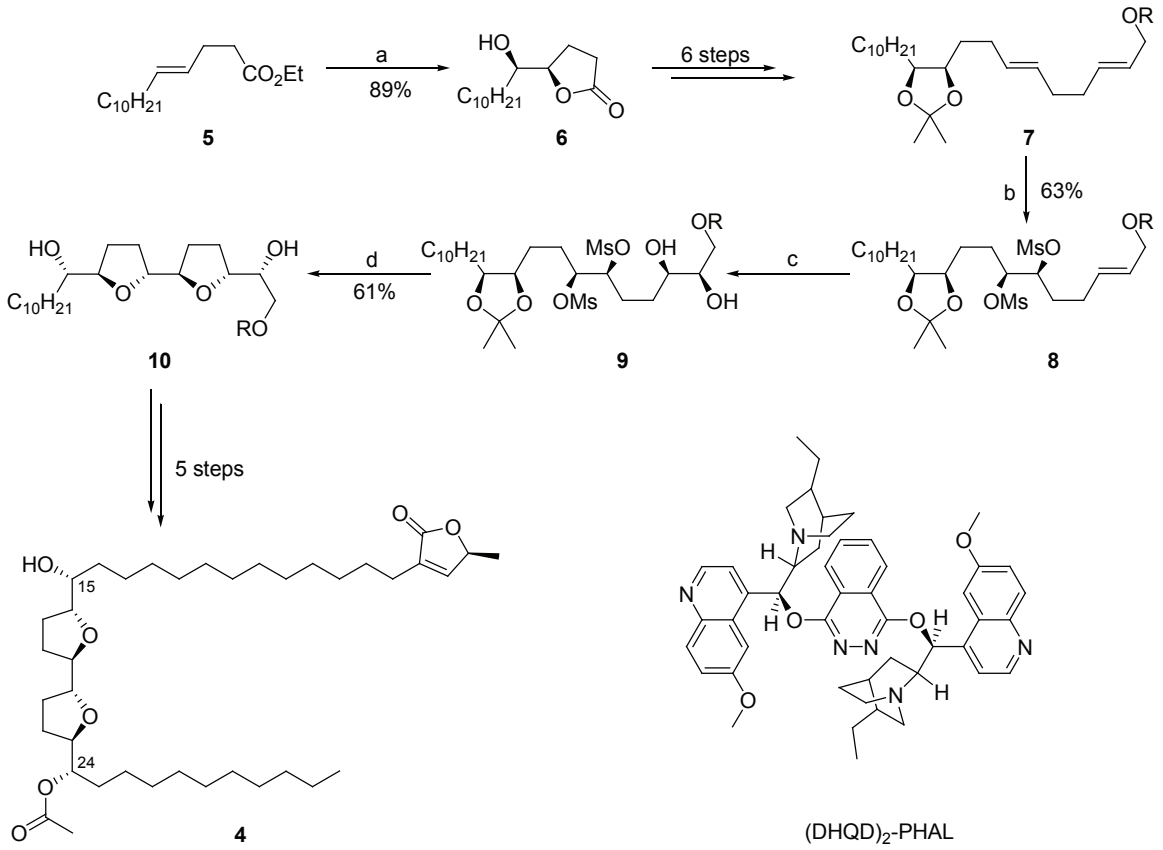


Figure 4. The total synthesis of Uvaricin (**4**) by Keinan et al,[14] utilizing three AD steps to introduce the six asymmetric centers of the C15-C24 fragment. a) i. AD-mix-β, $MeSO_2NH_2$, ii. 3N KOH then 3N HCl, iii. TsOH (5%). b) i. AD-mix-α, $MeSO_2NH_2$, ii. MsCl, Et_3N. c) AD-mix-α, $MeSO_2NH_2$, 3,4 g crude product starting from 3.07 g **9** d) i. MeOH, TsOH, ii. pyridine. AD-mix-β is a commercially available AD mixture consisting of $(DHQD)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3 and $K_2OsO_2(OH)_4$. AD-mix-α contains the other diastereomer of the cinchona alkaloid units in the PHAL-ligand, $(DHQ)_2$-PHAL.

1,4-bis(9-*O*-dihydroquinine)-phthalazine). The regioselectivity of this transformation is complete, since the only observed byproduct is the tetrol, obtained from overoxidation of **7**. After protection of the two hydroxyls, **8** undergoes a second AD, using the AD-mixture, of the slightly less reactive double bond and yields **9** with all chiral centres of the C15-C24 fragment properly assembled. Finally a straightforward multiple ring closure produces the Bis-THF fragment of **4**. This example clearly demonstrates the ease with which some of the transition metal catalysts can be used. This complicated catalytic system is commercially available as a ready-made stable solution, eliminating the need to weigh out each component for small scale reactions and making the asymmetric dihydroxylation a very straightforward procedure, which actually requires water and is

insensitive to oxygen. Another benefit, which this example reveals, is the accessibility of both enantiomers of a product by simple replacement of the chiral ligand.

The last example of transition-metal-catalysed oxidation, the asymmetric oxidation of sulfides in the production of esomeprazole (**11**), demonstrates its industrial relevance. Esomeprazole, an antiulcer agent, is the active pharmaceutical ingredient of Nexium with annual sales of $ 1.2 billion (5.5% of the global market 2004). It is produced by AstraZeneca as depicted in Figure 5. The crucial step in the manufacture of this enantiopure drug is the enantioselective introduction of the sulfoxide group. The Ti-diethyl tartrate (DET) complex oxidizes the sulfide with an enantiomeric excess of 93% in excellent yield (>90%). This is a dramatic improvement to the initially investigated resolution of the racemate, consisting of 5 additional steps, including a chromatographic separation, with an overall yield of 21%. Key factors for the success of this asymmetric sufide oxidation are the addition of Hünig's base and cumene hydroperoxide as oxidant, as well as the ease with which it could be fitted into the existing racemic production process. The use of a transition metal catalyst led to a full scale catalytic process operating on the multi-ton volume per annum, which is far superior in terms of yield and E-factor to the diastereomeric resolution. This is a key example of commercially applied catalysis in the production of fine chemicals, even though there is still room for improvement, especially with respect to the high catalyst loading (~4-16mol%).

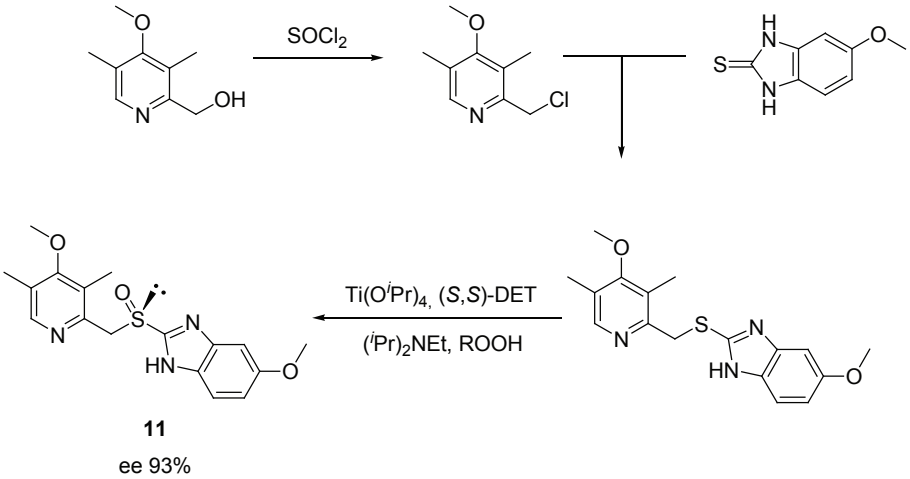


Figure 5. Production process of esomeprazole (**11**) as manufactured by AstraZeneca (DET = diethyl tartrate).

Hydrogenation

Hydrogenations of unsaturated organic compounds are, in contrast to most of the aforementioned oxidations, clean and selective reactions. This difference results from the fact that hydrogen is a rather unreactive molecule under ambient conditions. Nevertheless, it is easily activated by transition metals, creating numerous possibilities for reductions utilizing H_2, e.g. of C=O, C=C, and C=N bonds. Frequently they proceed quantitatively without the formation of side products and waste, making them ideal reactions in terms of ecology and (atom) economy. Consequently hydrogenation is a popular reaction for the chemical industry. For industrial applications heterogeneous catalysts are preferably applied due to the ease of separation and the possibility to recycle the often expensive catalysts. Homogeneously catalysed hydrogenations are only worth the effort if there are other advantages, e.g. with respect to selectivity. Encouragingly the selectivity alone is reason enough for the existence and development of homogeneous catalysts. For example, whereas heterogeneous catalysts will frequently also reduce accompanying functional groups, like ketones, arenes, and aromatic nitro groups, in addition to the desired group, the homogeneous variant will generally leave other functional groups untouched. In addition, homogeneous catalysis also offers the possibility of employing other hydrogen donors (formic acid, 2-propanol, benzyl alcohol, etc.) in the so-called transfer hydrogenations, which complement the hydrogenation with respect to selectivity. One type of hydrogenation in which the metal complexes truly dominate is the asymmetric hydrogenation, the reduction of a prochiral double bond, yielding enantiopure product. It was in this field that for the first time a man-made catalyst could match the selectivity of enzymes. Now, asymmetric hydrogenation has developed into a mature and powerful technology, which is by far the most dominant technique in industrial enantioselective catalysis.[17, 18] Its discovery and development was regarded equally important as the asymmetric oxidations; this is reflected by honouring the inventors, Knowles and Noyori, with half of the Nobel prize of 2001.[1, 19]

It only seems fitting that the first example demonstrating the usefulness of hydrogenation is the Monsanto L-DOPA process, the first industrial catalytic asymmetric synthesis (Figure 6). This success story started in mid-1960's with the discovery that the L-enantiomer of (3,4-dihydroxyphenyl)alanine (L-DOPA) was effective in the treatment of Parkinson's disease, which led to the demand for an efficient manufacturing process of this enantiopure amino acid. The development of methods for chiral phosphane preparation by Mislow[20, 21] and Horner[22, 23] in the same period, combined with the Wilkinson catalyst, $Rh(PPh_3)_3Cl$,[24], gave Knowles the idea to prepare L-DOPA by

means of asymmetric hydrogenation, starting from the Erlenmeyer azlactone (**12**).[25] This azlactone, which is readily prepared from vanillin and acetylglycine, gives after a straightforward hydrolysis the prochiral precursor (**13**) for L-DOPA. After a careful screening of various ligands, which is described on page 2, CAMP was initially selected as the ligand of choice in combination with rhodium. By employing [Rh((*R*)-CAMP)$_2$(cod)]BF$_4$, **13** could be hydrogenated with an enantioselectivity of 88%, which was unprecedented at that time and a major breakthrough for enantioselective catalysis. Shortly thereafter CAMP was replaced by DiPAMP and even higher ee's were obtained (95%). This step is highly sustainable, since no-side products are formed and the reaction is 100% atom efficient, an achievement that even nowadays most reactions cannot match. Unfortunately the elegance of the entire process is partially offset, due to the last step in the synthesis, a harsh and wasteful chemical hydrolysis, necessary to cleave off the protecting groups. Currently, the Monsanto L-DOPA process is no longer operational and L-DOPA is produced via various biocatalytic methodologies.

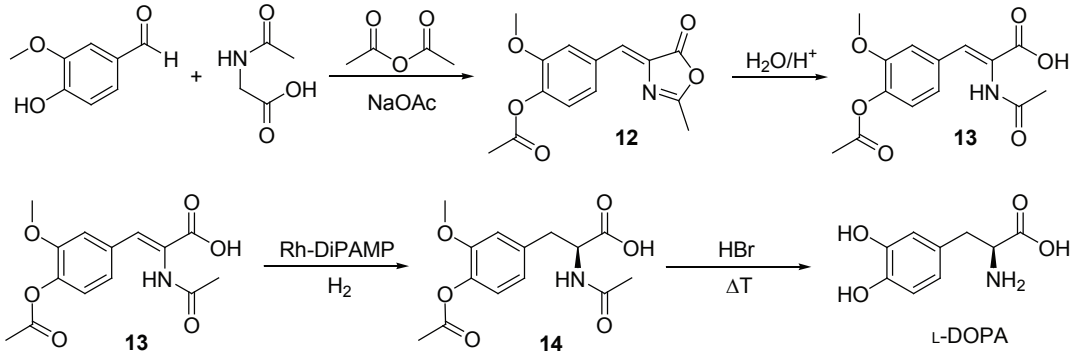


Figure 6. Monsanto L-DOPA process.

In addition to its great historical value, this process has been crucial for our fundamental understanding of (enantioselective) catalysis and as asymmetric hydrogenation makes up a great part of this thesis, a short discussion of the mechanism of asymmetric hydrogenation will be given.

The reaction involving DiPAMP complexes has extensively been studied by Halpern et al. and this has led to the generally accepted mechanism, which is depicted in Figure 7.[26] The most interesting mechanistic discovery of this study is the fact that the most stable alkene adduct (major manifold) is not the one responsible for the major product. The origin of the enantioselectivity lies in the markedly higher reactivity of the minor intermediate towards H_2. The initial step in the mechanism is the coordination of the substrate, the enamide, to the Rh, which gives predominately the major substrate adduct,

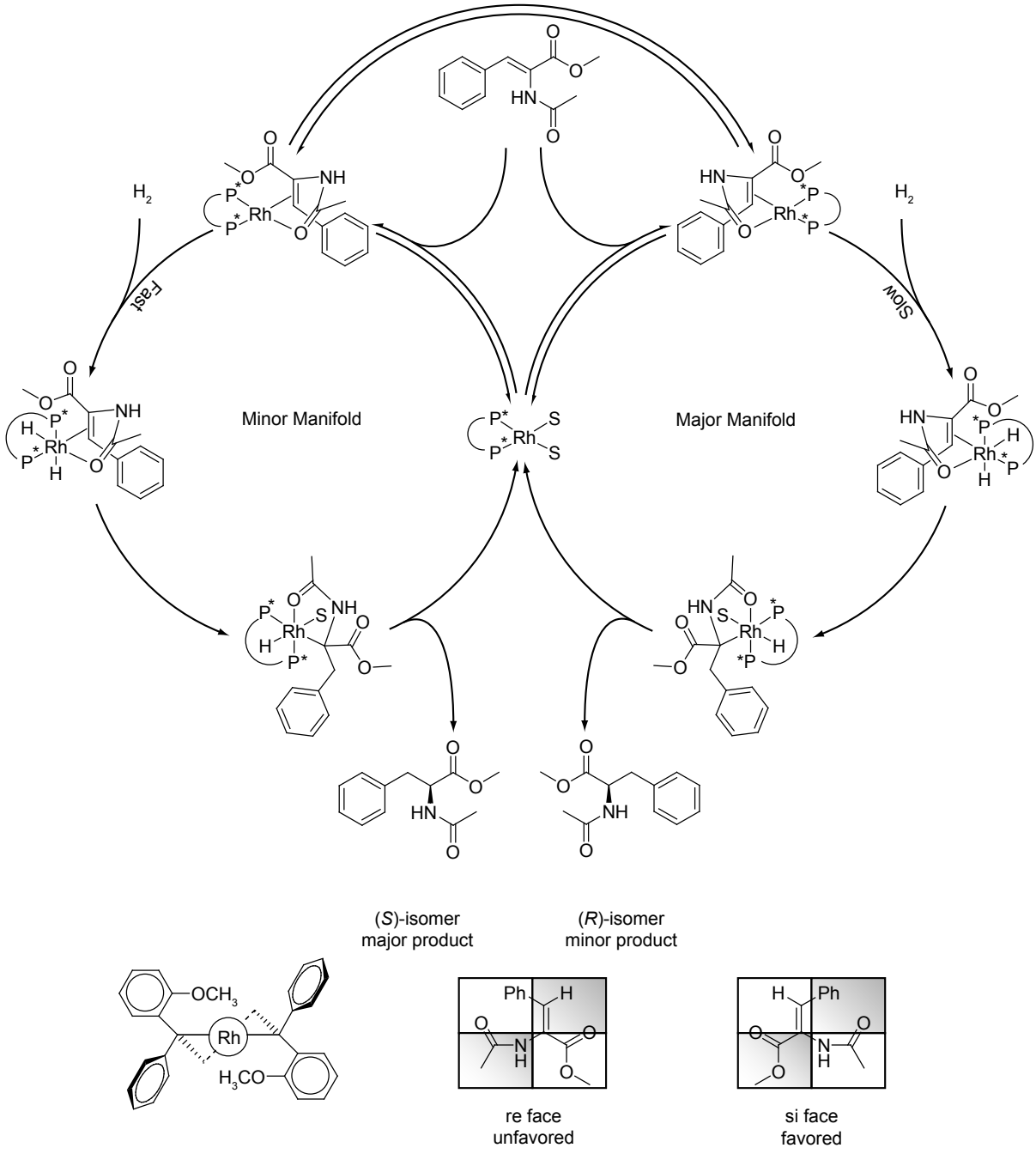


Figure 7. Halpern mechanism of asymmetric hydrogenation.

but also a small amount of the faster reacting minor adduct. The difference between the stability of these intermediates results from a dissimilar spatial arrangement of the substrate in relation to the chiral ligand (Figure 7 bottom). These two equilibrating intermediates undergo a rate-determining irreversible oxidative addition of H_2 with different reactivities. The difference in reactivity in this step, which signifies a $\Delta\Delta G^{\ddagger}$ of 3.7 kcal/mol, accounts for the enantioselectivity of 96% of the Rh-DiPAMP

hydrogenation. The remaining steps, migration/insertion and reductive elimination, proceed smoothly and produce the product.

Recently Imamato and Gridnev revealed another mechanism for electron-rich phosphine Rh complexes.[27, 28] With these complexes oxidative addition of H_2 can occur before the substrate coordination. The enantio-determining step in this mechanism is the migratory insertion and the intermediates that provide different stereoselection in all the previous stages of the catalytic cycle are in equilibrium with one another. This shows that even 10 years after the generally accepted elucidation of the mechanism, new discoveries regarding this intriguing reaction are still being reported and many questions remain to be answered.

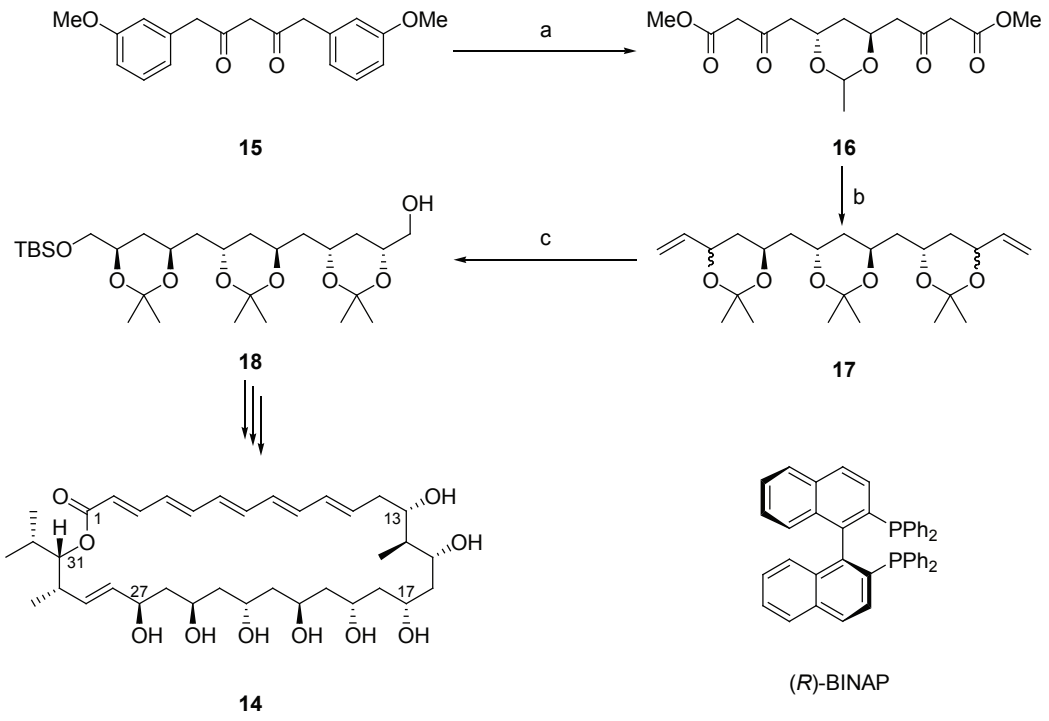


Figure 8. Total synthesis of the macrolide antibiotic (+)-Mycoticin A (14) by Schreiber et al.[33] a) i. $Ru_2Cl_4[(R)\text{-BINAP}]_2(Et_3N)$ (10 mol%), H_2 (100atm), 59%, ii. $(EtO)_2CHCH_3$, H^+, 90%, iii. Li/NH_3, iv. O3, 60%. b) i. $Ru_2Cl_4[(R)\text{-BINAP}]_2(Et_3N)$ (10 mol%), H_2 (100atm), ii. $(CH_3O)_2C(CH_3)_2$, H^+, iii. DIBALH, iv. Vinyl Grignard, v. $(CH_3O)_2C(CH_3)_2$, H^+, 30% (in 4 steps).

The second example of asymmetric hydrogenation demonstrates the impact of the work of Noyori, the third Nobel Laureate of 2001. With his discoveries of the atropisomeric chiral diphosphine, BINAP (Figure 8) and the corresponding Ru complexes,[29-32] he greatly extended the scope of asymmetric hydrogenations.

Enantioselectivities were further improved to values exceeding 99% and substrates like α,β-unsaturated ketones (carbonyl selectivity), functionalised ketones, α,β- and β,γ-unsaturated carboxylic acids could now also be reduced enantioselectively. An interesting application of these discoveries is the total synthesis of the macrolide antibiotic (+)-Mycoticin A (**14**) by Schreiber et al., in which 4 of the 6 chiral hydroxyl groups of the C17-C27 fragment are introduced by means of the Noyori-Akutagawa catalyst, $Ru_2Cl_4[(R)\text{-BINAP}]_2(Et_3N)$ (Figure 8).[33] Two-directional catalytic asymmetric reduction of the diketon, **15**, produced the desired diol in a good 60% isolated yield. The creation of two chiral hydroxyl groups is followed by protection, dissolved metal reduction and ozonolysis to afford the bis-β-keto ester **16**. The conversion of **16** to the tris-acetonide **17**, was achieved in four steps (30% overall yield), including a second double catalytic asymmetric reduction. No information is given about the exact value of the enantioselectivity, but with an isolated yield of 30% over 4 steps including a difficult two-directional Grignard addition, it should be regarded as good. The final two chiral centres of the C17-C27 fragment are introduced by a double ozonolysis followed by a base-catalysed epimerisation, to provide **18** a key intermediate in this synthetic approach towards **14**. This total synthesis clearly shows that Noyori's catalyst is a valuable tool in the synthesis of polyols, an important component in many biologically active compounds. In this example the catalyst loadings are quite high, 10%, but this is not a general requirement for these catalytic systems. Industrial reductions with these catalytic systems frequently reach TON's of >20000.[18]

C-C coupling

As already mentioned, carbon-carbon bond formations are among the most important reactions in organic synthesis. Transition metal catalysis, which facilitates a very wide spectrum of this type of reaction, is arguably the most powerful methodology for this objective. It comprises reactions such as: hydroformylation, cross-coupling reactions (e.g. Heck, Suzuki, Tsuji-Trost and Sonogashira reactions), metathesis, cyclomerisation, oligomerisation and hydrocyanation, which form the backbone of innumerable syntheses of fine chemicals and natural products. The importance of these transformations becomes immediately evident upon examining natural product syntheses of the last 15 years, a large majority of which contain one or more steps employing a metal catalysed C-C coupling.[34, 35] This new ability to forge C-C bonds between or within functionalized and sensitive substrates has dramatically enhanced the prowess of the synthetic chemist and permanently changed the retrosynthetic analysis of complex molecular frameworks. Their impact on organic chemistry is to a

great extent a result of their versatility, robustness and efficiency. Also this application of transition metals in organic chemistry is considered to be of such importance as to award 3 scientists the 2005 Nobel prize for their work on one of these modern transition metal mediated cross coupling reactions, viz. metathesis. Yves Chauvin was awarded the prize for unravelling the mechanism, in doing so opening the possibility to rationally design better catalysts. Richard R. Schrock and Robert H. Grubbs received their parts of the prize for the creation of practical catalysts.

The remarkable level of both chemoselective and stereoselective control, that is possible in these C-C couplings, is clearly illustrated by the total synthesis of sanglifehrin A (**19**). **19** is a promising immuno-suppressant, i.e. a compound able to suppress host rejection of transplants, with a spirolactam and a 22-membered unsaturated, sensitive macrocycle as key features. A synthesis was devised with the central role for

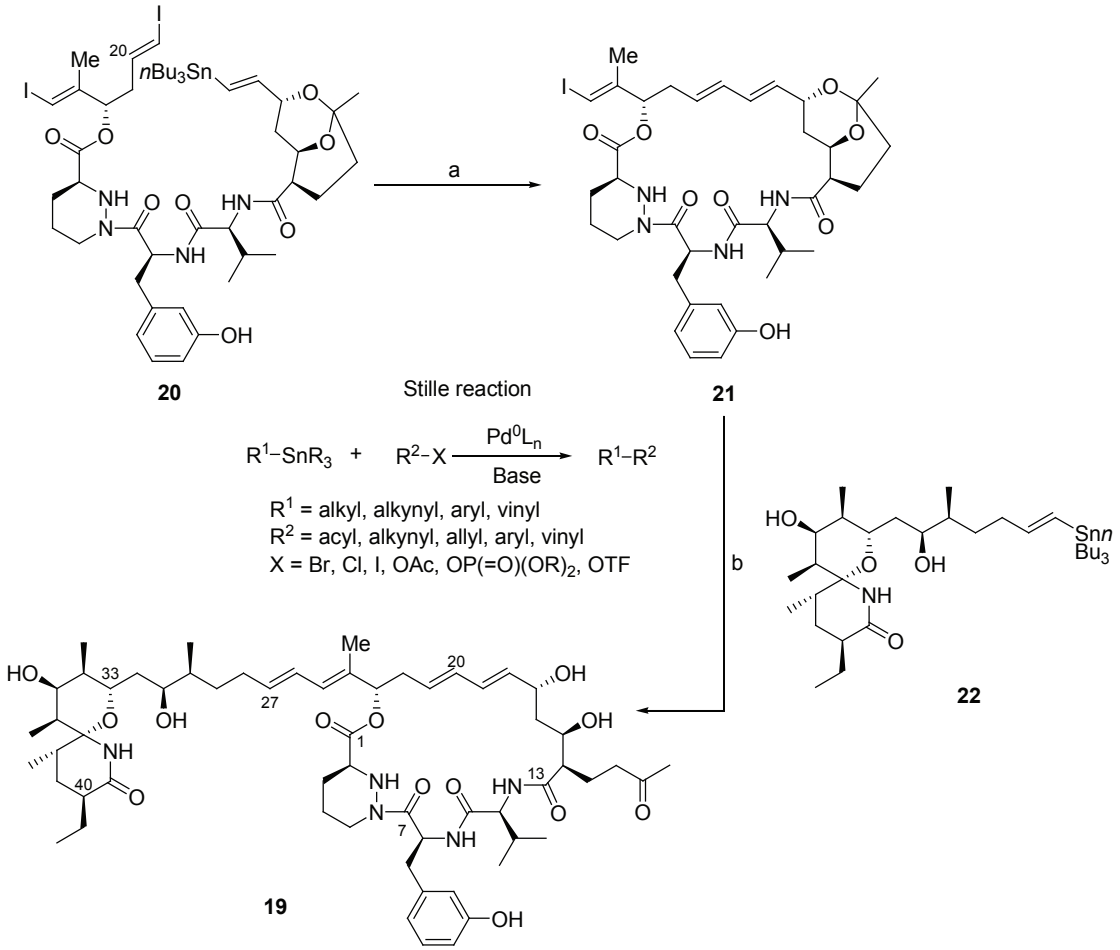


Figure 9. Chemoselective intra- and intermolecular Stille coupling in the total synthesis of sanglifehrin A (**19**) by Nicolaou et al. a) $[Pd_2(dba)_3]CHCl_3$ (0.15 eq.), $AsPh_3$ (0.6 eq), iPr$_2$NEt, DMF, 25 °C (62%). b) i. $[Pd_2(dba)_3]CHCl_3$ (0.1 eq.), $AsPh_3$ (0.2 eq), iPr$_2$NEt, DMF, 40 °C (45%), ii. 2 N H_2SO_4, THF/H_2O (33%).

a chemoselective intramolecular Stille macrocyclization,[36] followed by a second Stille coupling[37] between the macrocycle and spirolactam moiety (Figure 9). It was anticipated that the reactivity of two vinyl iodides within structure **20,** differ to such a degree, due to the dissimilar steric hindrance, such as to allow for this proposed sequential construction. This rather daring manoeuver paid dividends, as it was found that treatment of a dilute solution of **20** in DMF with $[Pd_2(dba)_3]CHCl_3$, $AsPh_3$ and iPr_2NEt at 25 °C led to the exclusive formation of the desired sanglifehrin cycle intermediate, **21,** in an isolated yield of 62%. The second Stille coupling, treatment of a mixture of the vinylstannane spirolactam **22** and **21** with a catalytic amount of in-situ generated Pd(0) tetrakistriphenylarsine in DMF at a slightly elevated temperature, followed by cleavage of the acetal protection group, completes the synthesis of sanglifehrin A.

The syntheses by Baldwin et al.[38] of two other immunosuppresants, SNF4435 C (**23**) and SNF4435 D (**24**), which were isolated from *Streptomyces spectabilis*, verify the claim of the phenomenal synthetic potential of modern transition-metal-mediated cross-coupling reactions (Figure 10). Baldwin et al. recognised that another metabolite from this organism, spectinabilin (**25**), was a constitutional isomer for **23** and **24**. The proof for this biogenetic hypothesis was provided by the synthesis of **25,** followed by a biomimetic conversion to **23** and **24** through a cascade of *E*/*Z*-isomerisations and electrocyclizations. This elegantly devised route towards **25** consists entirely of metal catalysed C-C bond forming steps, namely a metathesis,[39] a Suzuki coupling and finally a Negishi coupling.[38] The synthesis starts with a Ru-catalysed cross metathesis between **26** and **27**, using the 2nd generation Grubbs catalyst to introduce the functionality necessary for the Suzuki coupling. **28** is prepared in excellent yield (98%) but with moderate stereoselectivity. This low selectivity is acceptable, since the preparation of synthetically useful vinyl boronate species, like **28**, is inaccessible by more conventional means. The subsequent palladium-catalysed Suzuki coupling with the dibromide, **29**, yields **30** with complete selectivity with respect to both coupling partners. Finally, the Negishi-type coupling of (*Z*)-**30** with Me_2Zn catalysed by $[Pd(PtBu_3)_2]$ proceeded with full retention of stereochemistry and efficiently afforded pure **25**. By facilitating the requisite *E* to *Z* isomerisation with a Pd catalyst $[PdCl_2(MeCN)_2]$, spontaneous electrocyclizations occurred to produce **23** and **24**, consequently proving the biogenetic hypothesis.

Apparently the possibilities of transition metal catalysts are only limited by the imagination and dedication of the chemist, but of course there are also drawbacks. A disadvantage which becomes quite apparent from the aforementioned examples is the amount of catalyst that is used in most syntheses (10 mol% of complex) can hardly be

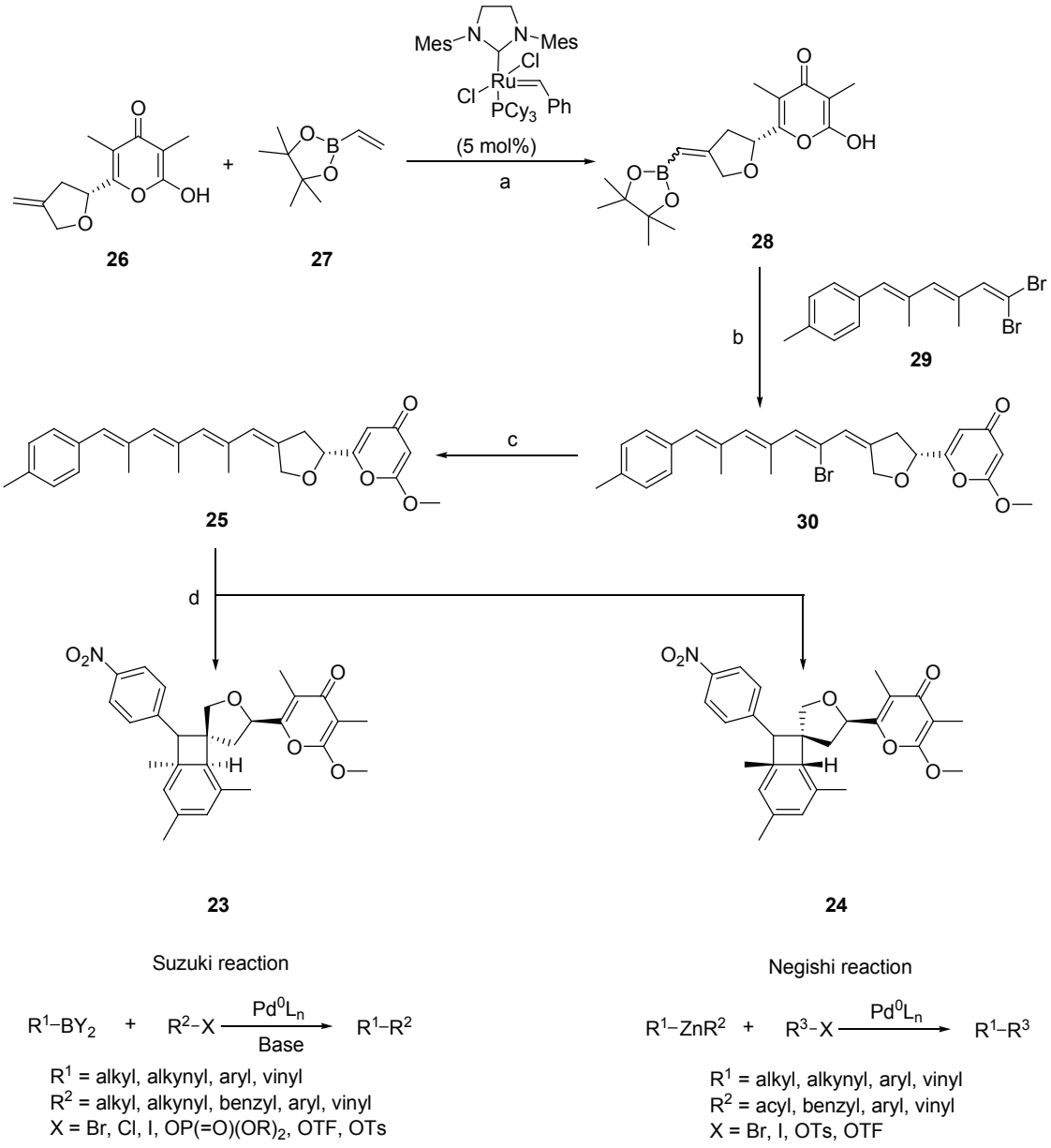


Figure 10. Total synthesis of spectinabilin (**25**) and its biomimetic conversion to SNF4435 C (**23**) and SNF4435 D (**24**) by Baldwin et al. a) CH_2Cl_2, reflux (98%, *E*/*Z* 1:1.2). b) $Pd(PPh_3)_4$ (10 mol%), TlOEt, aq. THF (64%) c). Me_2Zn $Pd(tBu_3P)_2$, THF (89%). d) $PdCl_2(MeCN)_2$, 70 °C(22%, 23/24 2.5:1).[38]

called catalytic. Although this is not a major concern for small scale synthesis, it becomes a big issue when developing an industrial process, especially since these complexes are expensive. A remark has to be added regarding the high catalyst loadings in total synthesis. The catalysts are normally employed under non-optimised conditions. So in most cases there is still ample room for improvement.

This directly brings up another concern with transition metal catalysts. Most

catalytic systems are normally only tested on “easy” model compounds, consequently these systems may not be suitable for real substrates. The development of new efficient systems for real substrates is a time-consuming process, whereas the time-to-market for most fine chemicals and pharmaceuticals is short. This often results in the decision to pursue a non-catalytic synthetic route, since the development of a profitable catalytic route requires too much time.

In addition to the above mentioned drawbacks, the sensitivity of most complexes, e.g. towards oxygen or water, makes transition metal catalysts difficult to handle. These and other impurities can severely reduce the activity and selectivity of the catalyst. Finally, most of the advanced catalytic transformations employ homogeneous catalysts, which have the intrinsic disadvantage of difficult separation from the desired product. Since most transition metals are toxic and the product is often destined for in-human application, this can be a significant impediment for application. A potential solution for this issue is presented in this thesis.

Biocatalysts in Organic Synthesis

In comparison to transition-metal catalysis, biocatalysis is a relatively unexplored field in organic synthesis. The difference in the level of application is, to a large extent, a result of their origin in different fields of science. Whereas organic chemistry and transition metal catalysis are both chemical disciplines of science, biocatalysis had originally more affinity with biology. Consequently, chemists were traditionally untrained in biocatalysis. In addition, neither the different terminology, nor the perception to have to work with living organisms helped to encourage chemists to enter this new field.

These perceptions, however, are largely based on misconceptions, since isolated enzymes can be handled like any other chemical catalyst. It is anyhow a misapprehension to think that chemical catalysis and biocatalysis are two fundamentally different sciences. Biocatalysis is actually a specialisation of (chemical) catalysis and logically the same principles apply. This is sometimes not immediately apparent since these principles are often labelled differently. Metalloenzymes are, in principle, transition metal catalysts with polypeptide ligands. Of course, enzymes require special conditions, but so do most of the aforementioned chemical catalysts. Like enzymes, chemical catalysts become pH dependent when employed in water. So biocatalysts can in principle be just as valuable as transition metals for the synthesis of chemicals.

Organic chemists started to recognize the potential of biocatalysis in the 1980s and

enzymes in organic chemistry are now a flourishing research area. Apart from the ever-growing knowledge of biocatalytic systems, several forces stimulated the application of enzymes in organic synthesis. In the first place the new classes of compounds, e.g. carbohydrates and nucleic acids, that are becoming key targets of molecular research have induced many chemists to turn to enzymes. These new compounds are natural targets for biocatalysts and, consequently, they can be efficiently synthesised and manipulated by enzymes, whereas chemical alternatives towards this end do not exist. The increasing demands for ecologically acceptable processes also motivates the use of Nature's catalysts, which operate under mild conditions and with environmentally benign reagents, to save energy and avoid hazardous waste. The motivation to apply biocatalysts is fuelled by the rapid developments in genetic engineering, which finally give scientists the possibility to modify enzymes for the desired transformation. Rational design[40] and especially directed evolution[41, 42] have become powerful tools to design new catalysts and to improve their activity, selectivity and/or stability. A good example of the potential of genetic engineering is the induction of enantioselectivity in a lipase by Reetz et al.[43] The wild-type lipase, PAO1 from bacterium *Pseudomonas aeruginosa*, exhibited an enantioselectivity of 2% in the hydrolysis of racemic *p*-nitrophenyl 2-methyldecanoate. By using the error-prone polymerase chain reaction (epPCR) the lipase gene was subjected to random mutagenesis with a low mutation frequency resulting in a substitution of 1-2 amino acids of the original 285. The best mutant of the first generation already showed an enantioselectivity of 31%, a 15 fold increase. In the following generations the clone with the highest enantioselectivity was chosen for subsequent mutagenesis, which ultimately led to a fourth generation mutant with an enantioselectivity of 81% (conversion range 20-30%). This result becomes even more impressive, when realizing that the applied techniques are very convenient. To quote Reetz during the congress New frontiers in biocatalysis (Noordwijkerhout, 2005): "epPCR is as easy as column chromatography", provided of course that one has the right equipment at one's disposal and a suitable and rapid screening method is available.

The most important applications of enzymes in organic transformations are depicted in Figure 11. Just as for transition metal catalysis, the usefulness of enzymes will be demonstrated by illustrative examples. These examples will be grouped according to type of enzyme rather than reaction. For a more comprehensive overview of this topic the reader is redirected to the outstanding work of K. Drauz and H. Waldmann.[44] A good introduction for organic chemists to this topic is provided by the excellent textbook of K. Faber.[45]

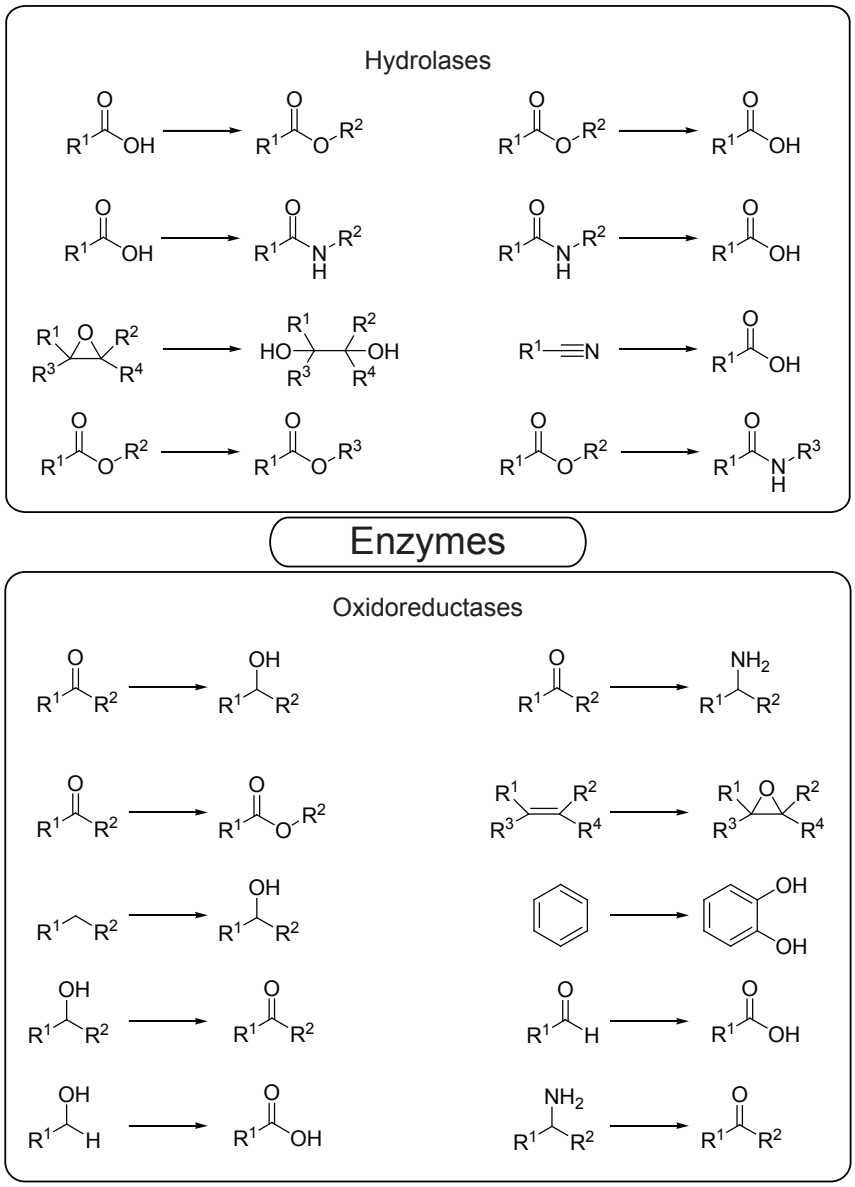


Figure 11. A selection of enzyme-catalysed reactions in organic synthesis.

Hydrolases

Among the biocatalysts in organic synthesis, hydrolases (enzymes that catalyse hydrolysis reactions) are the most commonly applied. This class of enzymes consists primarily of lipases, esterases, proteases and amidases, of which many are readily accessible, also for synthetic chemists. Their popularity stems from their ability to catalyse a wide range of reactions[46] and their tolerance of organic solvents. This immediately refutes one of the arguments not to use biocatalysts in organic chemistry, namely the misconception that enzymes only operate in water. Most lipases, which catalyse the hydrolysis of lipids to glycerol and fatty acids, actually require a hydrophobic

interface for productive binding of the substrate. A detailed discussion of the cause will be given below. Biocatalysts thus can be used in organic solvents with the accompanying advantages, like reactants not having to be water-soluble and suppression of unwanted side reactions. Nevertheless, all enzymes require a minimal amount of water to be active, the so-called structural water, but the exact quantity of water required varies significantly between species. Most lipases, esterases and some proteases are stable and active in neat organic solvents, whereas other enzymes deactivate dramatically in the presence of small amounts of organic solvents.

The fact that most hydrolytic enzymes function so well in organic solvents makes these biocatalysts very versatile in organic transformations, since it allows them to be used for synthetic reactions. In the absence of bulk water hydrolases also catalyse the reverse reaction, e.g. esterification, amide formation, and transesterification (i.e. cleavage using an alcohol instead of water). Furthermore, unlike other types of enzymes, all these reactions are catalysed without the need of expensive co-factors. In addition to this extensive reaction scope, hydrolases also possess a broad substrate specificity. They often accept various synthetic intermediates as substrates while they maintain their high stereoselectivity, in some cases even if its structure is far removed from the natural substrate.

The main application of hydrolases lies in the field of asymmetric synthesis, predominantly (dynamic) kinetic resolutions and desymmetrization, followed by their application in chemo and regioselective condensations/hydrolysis, notably the selective introduction and removal of protective groups. A good example for hydrolases applied in the field of asymmetric synthesis is the asymmetric total synthesis of Fredericamycin A (**31**) by Kita et al. (Figure 12).[47] **31** is a potent and unique quinone antitumor antibiotic, isolated from *Streptomyces griseus*. The pivotal intermediate for its synthesis was the elusive chiral dione (**32**). This compound presented significant problems with its difficult construction of the quaternary carbon centre and facile racemization. The solution to these problems lay in the lipase catalysed desymmetrization of a prochiral diol (**33**). The initially investigated desymmetrization of the corresponding diester, also catalysed by a lipase, failed due to spontaneous decarboxylation of the monoester. The enantiotopic selective acylation of **33** using *Candida rugosa* lipase, however, yielded the product (**34**) with good enantiomeric excess (83% ee, 57% yield). Crucial to this success was the acyl donor 1-ethoxyvinyl 2-furoate (**35**), since the more common vinyl acetate gave an adduct much more susceptible to racemization, and the solvent. The best results were obtained with a 10:1 mixture of *i*Pr_2O and MeCN with a fraction of water. The optical purity of **34** was further improved by a sequential enzymatic step,

i.e. a kinetic resolution with ethoxyvinyl butylate and *Pseudomonas aeruginosa* lipase. This lipase selectively acylated the undesired *S* enantiomer resulting in an enantiopurity of 97% ee for the *R* enantiomer. After protection of the remaining hydroxyl group with TBSOTf and alcoholysis of the ester group, the elusive chiral dione (**32**) was prepared by a series of reaction steps comprising an oxidation and, a methylation followed by another oxidation. With this pivotal intermediate finally in hand, the asymmetric synthesis of Fredericamycin A could be completed via intramolecular cycloaddition, followed by the aromatic Pummerer-type reaction.[48, 49] In conclusion, by combining two enzymatic transformations, a difficult key-intermediate, for which there were no other suitable synthetic pathways, could be synthesised in optically pure form.

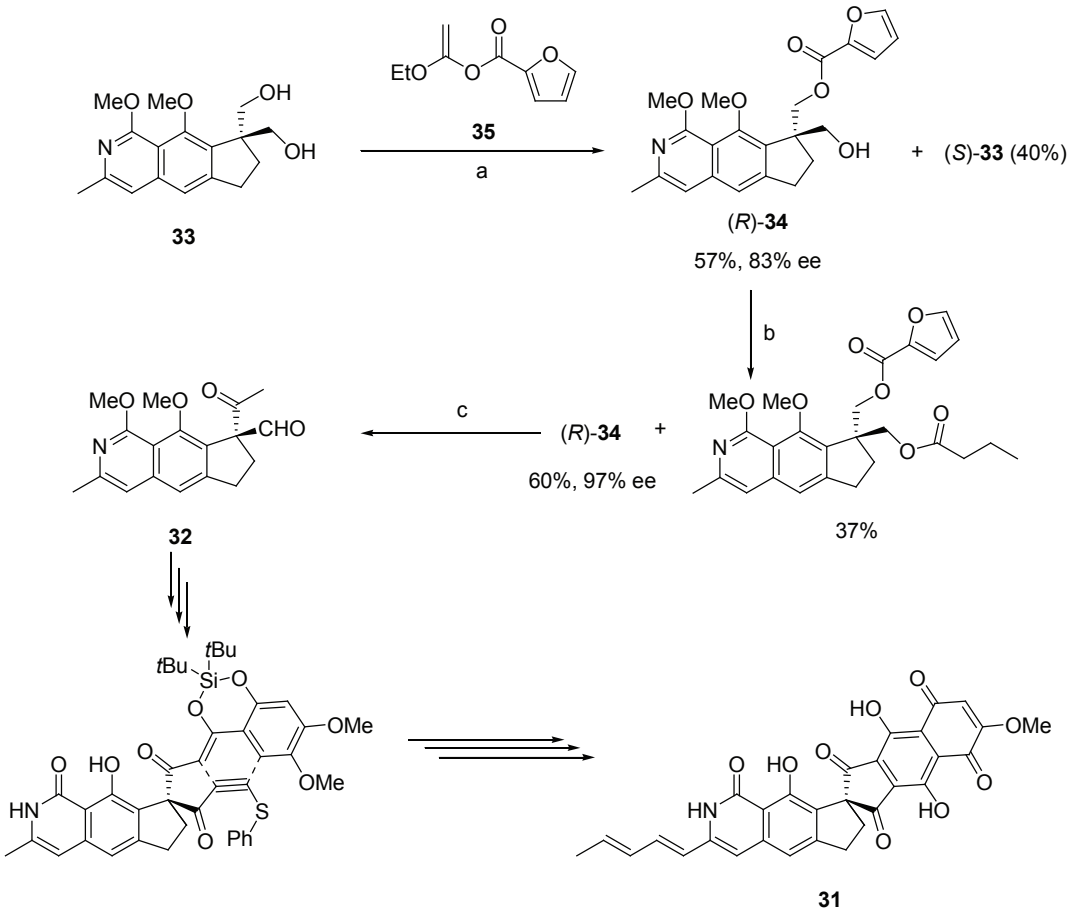


Figure 12. Asymmetric total synthesis of Fredericamycin A (**31**) by Kita et al. (Figure 12).[47] via the optically pure dione intermediate, **32.** a) *Candida rugosa* lipase (Meito MY), *i*PrO$_2$/MeCN/H$_2$O (1000:100:1), 40 °C. b) *Pseudomonas aeruginosa* lipase (Toyobo-LIP), ethoxyvinyl butylate, *i*PrO$_2$, 40 °C. c) i. TBSOTf, Na$_2$CO$_3$, THF, ii. K$_2$CO$_3$, MeOH, iii. Dess-Martin periodinane, MeCN, iv. MeLi, THF, v. Bu$_4$NF, THF, vi. Swern oxidation.

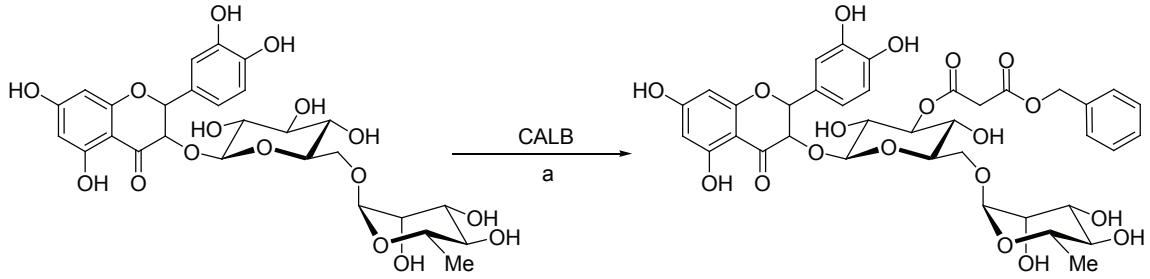


Figure 13. Selective malonylation of rutin (**36**) at 3"-position of the glycoside. a) *Candida antarctica* lipase (Novozym 453), acetone/pyridine (9:1), dibenzyl malonate, 45 °C.[50]

An impressive example of a selective enzymatic condensation/protection is the acylation of rutin (**36**) by *Candida antarctica* lipase (Figure 13).[50] Rutin, which is found in many plants, is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose. Consequently, it possesses 10 free hydroxyl groups (six secondary and four phenolic). Treatment of **36** with dibenzylmalonate in the presence of *Candida antarctica* lipase yielded only the monoester at the 3" of the glucose moiety. This degree of selectivity is difficult if not impossible to achieve by any other means.

Due to their advantageous properties, hydrolases have logically also found their way to industry. The artificial non-carbohydrate sweetener, aspartame (**37**) for instance is produced by the thermolysin catalysed coupling of protected L-aspartic acid with L-phenylalanine methyl ester (Figure 14).[44, 51] The use of an enzyme in this process has

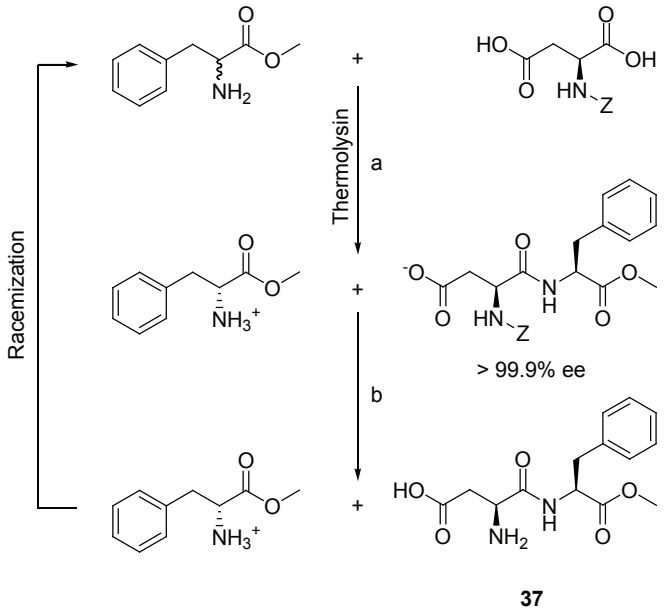


Figure 14. Biocatalytic synthesis of aspartame (**37**). a) thermolysin from *Bacillus proteolicus*, pH 7. b) i. HCl, ii. H_2, Pd/C, MeOH.

several distinct advantages. First of all the high regioselectivity of thermolysin eliminates the need to protect the β-carboxyl group of the aspartic acid. In the chemical synthesis this protection is required, because otherwise the bitter tasting β-aspartame would be formed. Secondly, the enzyme-catalysed reaction takes place in aqueous media under mild conditions. Normally, a condensation reaction in water would be severely limited by its equilibrium, but since the process employs an excess of racemic phenylalanine methyl ester, which causes the formation and precipitation of the insoluble salt between the aspartame precursor and D-phenylalanine methyl ester, the equilibrium is shifted completely towards the product. The use of racemic phenylalanine methyl ester is possible due to the enantiospecificity of thermolysin.

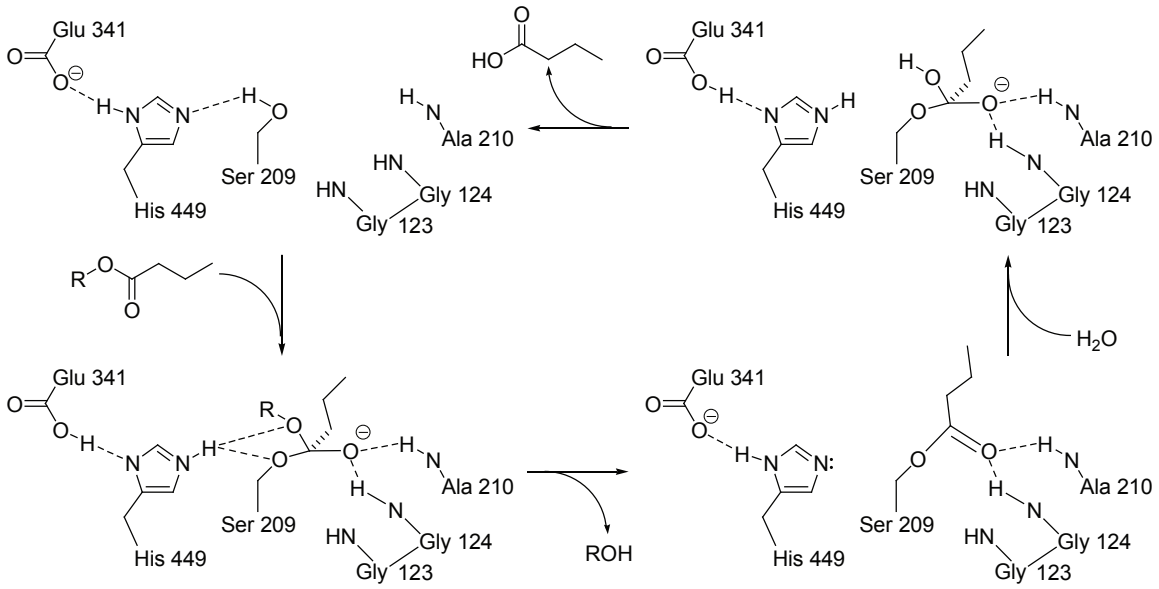


Figure 15. The catalytic triad of lipases and its operation in the hydrolysis of a butyric acid ester. The amino acid numbering corresponds to the active site of *Candida rugosa lipase* (CRL).[46]

Since lipases and acylases play an important role in chapter 5 and 6 their mechanisms will be briefly discussed here. The active site of most lipases consists of a triad of Ser, His and Asp(or Glu) together with several oxyanion stabilising residues (Figure 15). Consequently, lipases are called serine esterases. These residues of the active site occur in the same order in all lipase amino acid sequences and are oriented in the same 3D fashion in all structures known to date. The 3D orientation of this catalytic machinery is approximately the mirror image of that in serine proteases, e.g. subtilisin and chymotrypsin. With most lipases this catalytic triad is blocked by a helical segment,

usually referred to as the lid, when they are dissolved in aqueous solutions. Upon binding to a hydrophobic interface, such as a lipid droplet, the lid opens, exposing the active site to the substrate. Additionally, the opening of the lid positions one of the oxyanion stabilising residues in its proper catalytic orientation. This entire process is called interfacial activation.

After exposing the catalytic triad, the ester binds to the lipase as the serine residue attacks the carbonyl group. The tetrahedral intermediate, which is formed, is stabilised by hydrogen bonding with the residues in the oxyanion cavity and with the His-residue which delocalises its positive charge on the third residue of the triad (which in this case, Figure 15, is a Glu residue). Release of the alcohol results in an acyl enzyme intermediate which is subsequently attacked by water or another nucleophile. Via a similar tetrahedral intermediate the acid is finally released from the active site and the enzyme is restored to its original state.

Aminoacylases are part of the metallo-protease family, which utilize a Zn^{2+} ion to act as Lewis acid for the activation of the nucleophilic water and the carbonyl group of the scissile bond. Although the exact mechanism is as yet unknown, the general assumption is that the reaction proceeds as depicted in Figure 16.[53, 54] The carboxylate group of a Glu residue in the active site facilitates the nucleophilic attack of water. For the hydrolysis of *N*-acyl amino acids it was reported that its terminal carboxylate group is essential for productive binding.[55]

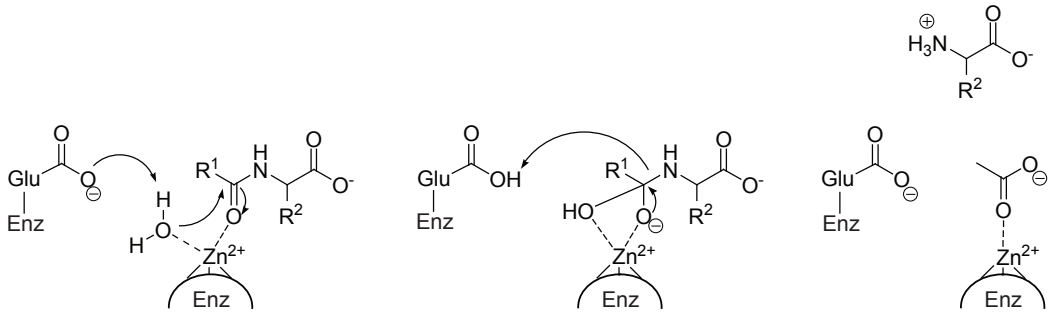


Figure 16. Proposed mechanism for Aminoacylase.[53, 54]

Oxidoreductases

Oxidoreductase is the generic term for all enzymes that catalyse redox reactions. This group consists of dehydrogenases, oxidases, monooxygenases, dioxygenases and peroxidases. In contrast to hydrolases these enzymes are rarely applied in organic synthesis. In the exceptional case that oxidoreductases are employed, it involves generally whole cell systems. The application of whole cells for organic transformations

is an interesting subject, but is outside the scope of this thesis. The big contrast in applicability between hydrolases and oxidoreductases has mainly two underlying causes. Most oxidoreductases (dehydrogenases and monooxygenases) require stoichiometric amounts of expensive and relatively unstable co-factors, i.e. NAD(H), NADP(H), FAD or PQQ. Replacement of these reductants/oxidants with more economical man-made alternatives is often not viable, thus these processes usually require complicated co-factor recycling to become feasible. This naturally also explains why whole cells are more commonly applied in these transformations, since living organisms come with their own built-in regeneration systems and can grow on cheap media. The best alternatives for whole cells are the coupled substrate process and the coupled enzyme process (Figure 17). In the coupled substrate process a second auxiliary substrate regenerates the co-factor by action of the enzyme that also converts the main substrate, e.g. alcohol dehydrogenase from *Thermoanaerobium brockii* in combination with isopropanol.[56, 57] The coupled enzyme process utilizes different enzymes for the reduction of the substrate and the co-factor, e.g. formate/formate dehydrogenase for the recycling of $NAD(P)^+$.[58, 59] Secondly the application of oxidoreductases lags behind other catalytic systems due to their instability. The isolated enzymes are often inactivated by the reactive radical intermediates generated in the reaction process. Due to their high costs it is difficult to develop economically feasible processes.

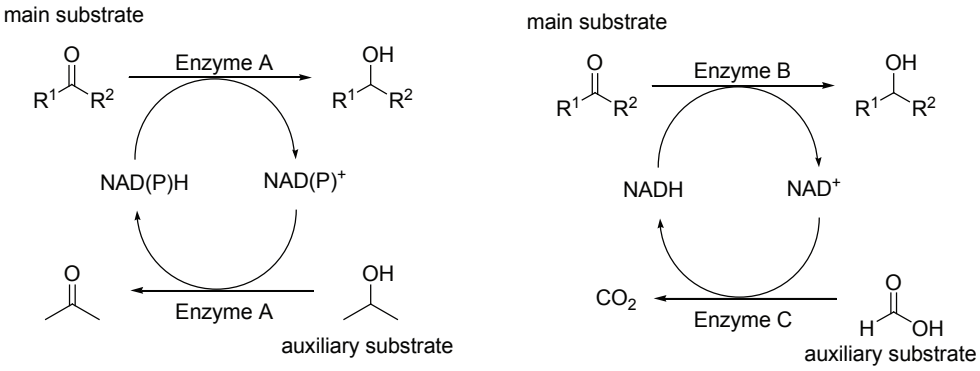


Figure 17. Regeneration of NAD(P)H: a) coupled substrate process (enzyme A = e.g. alcohol dehydrogenase from *Thermoanaerobium brockii*). b) coupled enzyme process (enzyme C = formate dehydrogenase)

Despite these drawbacks oxidoreductases are an interesting class of enzymes with a noteworthy potential. As already mentioned, selective oxidations in general are difficult reactions, especially when employing green and cheap oxidants, like O_2 and H_2O_2. Any tool that can perform these reactions with high selectivity is a valuable addition to

the arsenal of the chemist and the oxidoreductases fulfil this criterion perfectly. In addition enzymatic oxidation allows the direct oxyfunctionalization with O_2 or H_2O_2 of unreactive organic substrates such as alkenes, which remains a largely unresolved challenge to synthetic chemistry.

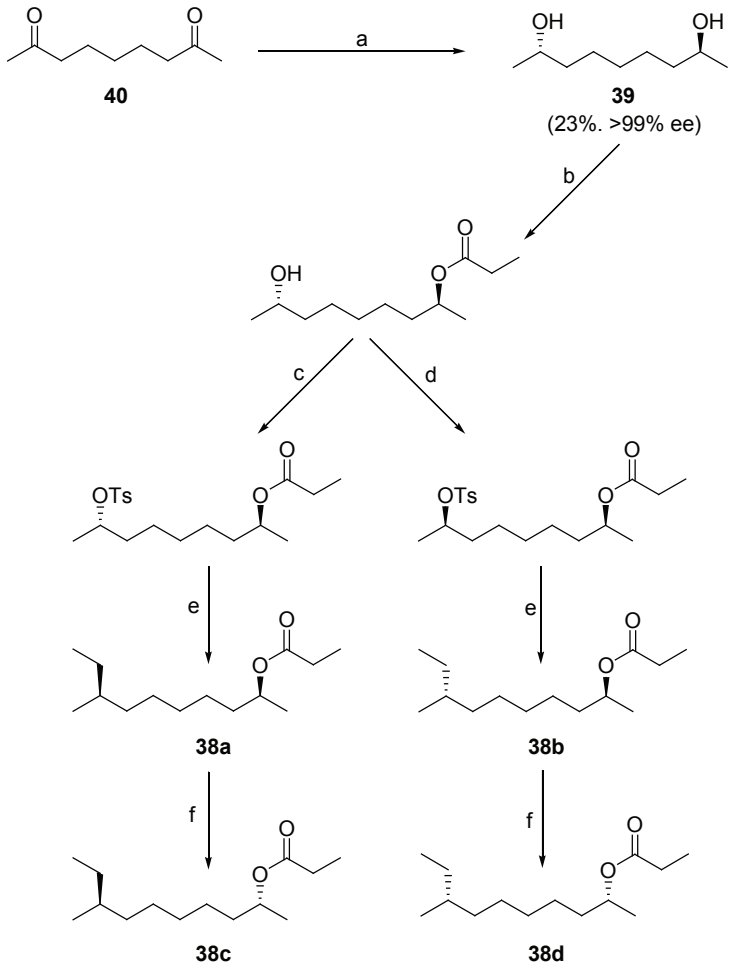


Figure 18. Synthesis of all four stereoisomers of the four isomers of the western corn rootworm sex pheromone (**38**): a) TBADH, $NADP^+$, 2-propanol. b) BuLi, $(EtCO)_2O$. c) TsCl, pyridine. d) PPh_3, DEAD, $Zn(OTs)_2$. e) $Et_2CuMgBr$. f) i. KOH, H_2O/MeOH, ii. PPh_3, DEAD, $EtCO_2H$.

In the rare occasions that isolated oxidoreductases have been utilised for synthetic application, they have given a good impression of their value. For instance, in enantioselective reductions of ketones with alcohol dehydrogenases, the enantiomeric excess frequently exceeds 97%. Furthermore, oxidative coupling, catalysed by peroxidases has found increasing interest as a potential substitute for using toxic formaldehyde in resin manufacturing.[44] The first type of utilization is nicely illustrated

by the synthesis of all four isomers of the western corn rootworm sex pheromone, **38** (Figure 18).[60] The (*S*,*S*)-diol (**39**), from which all isomers were prepared, was obtained by the enantioselective reduction of 2,8-nonandione (**40**) catalysed by alcohol dehydrogenase from *Thermoanaerobium brockii* (TBADH). The dehydrogenase reduces both carbonyls of this substrate with enantioselectivities >99%. As simple as this substrate may seem, no readily available alternative exists for its chiral preparation as is often the case with chiral molecules composed of essentially non-interacting carbon skeletons. The disappointing yield is due to the lower reaction rate of the intermediary hydroxyketone, which was isolated with a 50% yield and >99% ee. Interestingly $NADP^+$ was added in a catalytic amount and regenerated by the coupled substrate process using 2-propanol. Partial esterification, followed by a tosylation either with retention or inversion of the configuration at the carbinol centre, and finally an alkylation, provided two of the sex pheromone isomers. These two isomers could be epimerised to the two remaining stereomers using the Mitsunobu procedure.

Even though enzymes are increasingly being used in synthesis, there is still a long way to go before their application for the preparation of organic molecules is common practice. Next to the aforementioned co-factor-dependence and stability problems, which certainly do not only apply to oxidoreductases, there are several other issues that need to be addressed. First of all a significant part of the enzymes are limited by a narrow substrate specificity. Furthermore, most enzymes or even the organisms that produce the desired biocatalyst are unavailable for chemists to investigate their synthetic usefulness, either due to patents, lack of commercial sources or simple because nobody grows them anymore. Unlike chemical catalysts, biocatalysts can simply not be synthesised, making the inaccessibility of the organism a major obstacle. Related to this is that nature only produces one enantiomer of the enzyme and due to the lack of artificial syntheses there is no general way of creating mirror-image enzymes. Consequently, it is impossible to invert the chiral induction of a given enzymatic reaction by choosing the other enantiomer of the biocatalyst, a strategy which is possible with chemical catalysts as already demonstrated. Thus, to gain access to the opposite enantiomeric product of a biocatalytic process, one has to follow once more an uncertain path in search of a new and suitable enzyme and just like with transition metal catalysts the development of a new system is a time-consuming process.

In addition to these issues, the purity of enzyme preparations can also be a cause for concern. Biocatalysts are almost always supplied as crude mixtures, which contain a lot of residues of the organism, including carbohydrates, peptides and other enzymes. These impurities can complicate the production process and/or workup of the product

and even destroy the selectivity of the wanted enzyme.

To overcome all these obstacles intense collaboration between the various disciplines of science is required, since an expert in organic chemistry normally is not an expert in enzymology. If this collaboration is accompanied by a continuous effort with the current dedication, enzymes will surely have a bright future in chemistry.

Chemo-Enzymatic cascades

After having analysed all the aspects -advantages and disadvantages- of transition metal catalysis as well as biocatalysis, one might be tempted to single out one of the two as being the most promising catalytic methodology. However this would be a big mistake as no method is superior in a general sense. For each transformation it has to be separately evaluated to decide what is the best methodology. This evaluation should also include all other forms of catalysis and even classical chemistry and take into account all aspects of the process, economical as well as environmental. When studying the various examples given in this introduction it will already become quite apparent that biocatalysis and transition metal catalysis perfectly complement each other. For example: transition metals are very versatile for oxidations and reductions (tasks often difficult to perform with enzymes, due to problems regarding co-factor regeneration), whereas enzymes readily perform hydrolytic reactions and their reverse (here, a chemo-catalytic approach often requires drastic conditions and generates large amounts of salts as waste). Furthermore, enzymes are generally far superior in the synthesis of natural products, whereas chemical catalysts are excellently suited to introduce non-natural functional groups.

Most syntheses, especially those of fine chemicals, do not consist of only one transformation, but require multiple steps to obtain the desired product. In many of those cases both enzymes and transition metals have to be utilised to obtain the most efficient route/process. Frequently, only by combining different catalytic systems, be they enzymes, solid metal oxides or complexes, will it be possible to design economic fully catalytic syntheses. If chemists succeed in the popularisation of this type of appraoch, chemistry will have made a giant leap towards sustainability.

But even when syntheses are fully catalytic, there may still be room for improvement, since this will not solve the other major issue of multistep syntheses, namely the elaborate and wasteful work-up of each individual reaction step. At present most complex molecules are still prepared in a step-by-step approach, which means that in order to convert a starting material A into the desired product D, the intermediates B and C also

need to be isolated and purified before continuing with the next reaction step (Figure 19a). This in combination with the remaining stoichiometric conversion steps can result in a waste generation of 100kg/kg product or more.[61] Other disadvantages of the step-by-step approach are low space-time yields (kg/L/h), high energy consumption and high costs, e.g. due to the expensive installations needed for purification. Obviously, the elimination of these intermediate purification steps, giving a so-called multistep-cascade as depicted schematically in Figure 19b, would have very significant vast benefits. This type of approach is by no means new. In living organisms, where countless chemical reactions take place, not a single intermediate is purified. In a cell the starting material A is converted into the final product D, without separation of the intermediates B and C. An additional advantage of nature's approach of coupling the individual steps is that equilibria of reaction pathways, involving high-energy intermediates, can be driven towards the desired product. Furthermore, these high energy intermediates are very reactive and tend to give side reactions. By directly converting them into the desired product, the formation of byproducts is greatly suppressed.

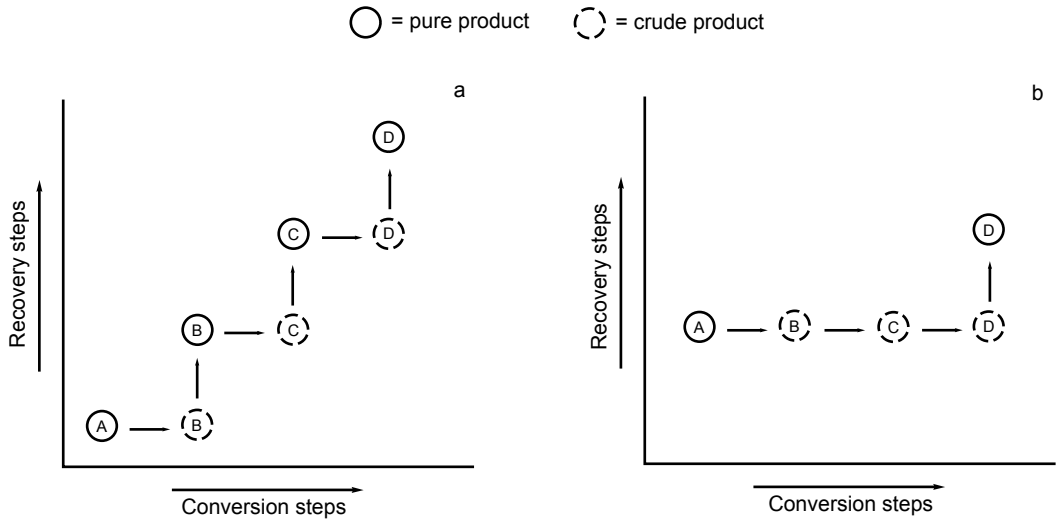


Figure 19. Schematic representation of a) the step-by-step and b) multistep-cascade approach as used in the synthesis of substances.[62]

So why do chemists utilize the step-by-step approach, when the advantages of the multistep-cascade approach are considerable? The popularity of the step-by-step approach among chemists lies in the incompatibility of the individual reaction steps with each other, making a multistep-cascade approach impossible. Unlike a living organism, which performs every reaction at similar conditions (37 °C, pH 7 and in water), chemists utilize a broad range of conditions, which are custom made for each

type of transformation. These conditions often do not combine well with each other. For example, whereas the first reaction step in the synthesis of compound D requires acidic conditions to proceed, the reagent used in the second step is unstable at a pH below 7. There are numerous other reasons, like incompatible solvents, temperature and reagents, which make it generally impossible to straightforwardly apply the multistep-cascade approach in the synthesis of fine chemicals. In addition, the optimisation necessary to obtain the desired results of a single chemical transformation is already a challenging process without having to take into account the requirements of the next reaction step. In short, the conditions of a reaction cannot easily be adjusted for the sake of another transformation.

Consequently, the multistep cascade approach is in practice a very difficult concept to realise. Either one is fortunate that all the reaction steps in the desired synthesis sequence are compatible or one has to devise smart solutions to circumvent any incompatibilities. Since practitioners of chemistry cannot count on Fortuna, as many chemistry students have quickly discovered during their practical courses, they have to depend on their ingenuity. Luckily Nature gives the chemist some clues as to what these smart solutions might be. Although organisms are fortunate that their numerous reactions proceed under similar conditions, Nature has also found ingenious ways to deal with the existing incompatibilities. Besides the highly selective natural catalysts—the aforementioned enzymes— Nature make use of coupled syntheses, protein-regulated transport and compartmentalisation utilizing membranes to achieve its successful multistep-cascades. Consequently, some of the multistep cascades, which have been developed are inspired by systems present in living organisms.

Terminology

Before giving a short overview of the published efforts to integrate chemical and enzymatic synthetic steps in cascade procedures, the terminology used to classify this type of reactions needs to be clarified. In surveying the literature on this topic the terms cascade, tandem, domino and one-pot are frequently encountered. Whilst these near-synonymous terms are used interchangeably, they represent different classes of multistep cascades. In the review by D. E. Fogg et al.[63] a comprehensive 'taxonomy' is given and the definitions therein are used throughout this thesis with some minor additions. In Figure 20 a flowchart is provided to aid the classification of multistep cascades.

All combined reactions without intermediate isolation/purification can be classified as (multistep-) cascade conversions.[62] If the cascade involves multiple catalytic steps,

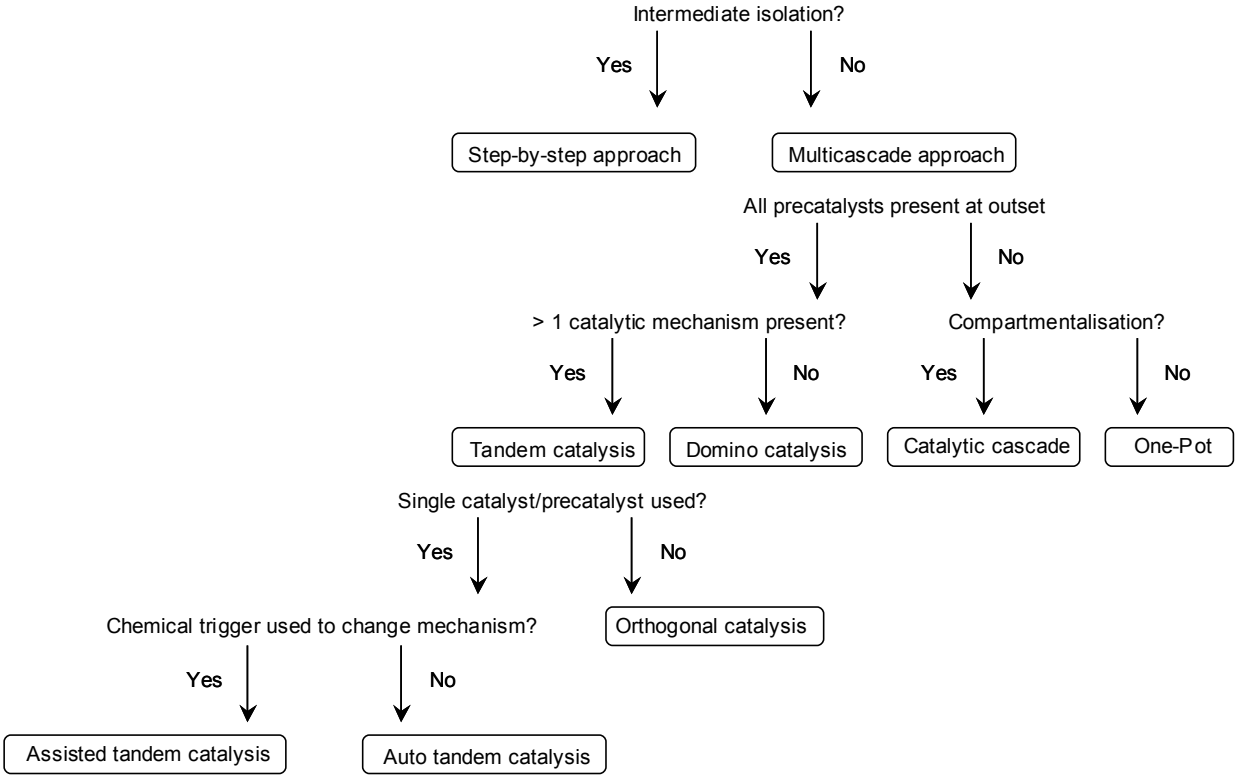


Figure 20. Flowchart for the classification of multistep cascade processes.

it is often referred to as a catalytic cascade. This is a rather general term, which can be subdivided into several more specific classes. First of all, there is the subclass of one-pot reactions. The only requirement for this type is that all the various reaction steps of the cascade take place in the same reaction vessel. In the one-pot as well as the cascade classification, the various steps in the multistep synthesis may be carried out as one transformation at a time, i.e. catalysts and/or reagents may be added after a chemical transformations is completed. A one-pot reaction, which has at least all the catalytic species—whether masked or apparent—present from the outset, is defined as either a tandem or domino reaction. To distinguish between these two remaining classes of multistep-cascades, the number of catalytic mechanisms has to be taken into account. If the previous requirements are met and the sequential transformation exploits two or more mechanistically distinct processes, the catalytic reaction is classified as tandem catalysis. Three categories of tandem catalysis may be further distinguished: orthogonal, assisted and auto, as indicated in Figure 20, which differ in the way they switch between the mechanisms. However, if the multiple transformations are effected via a single catalytic mechanism, the reaction is classified as domino reaction.[‡] Tietze defines a domino reaction as a process involving two or more bond-forming transformations, which take place under the same conditions without adding additional

‡ Confusingly the term cascade is sometimes used to exclusively refer to domino reactions.

reagents and catalysts and in which the subsequent reactions result as a consequence of the functionality formed in the previous step.[64] To illustrate this definition, one can imagine the domino reaction as falling domino pieces. The first reaction, which is initiated by a catalyst, sets in motion a series of bond-forming reactions. In contrast, the tandem reaction needs multiple catalytic interventions, pushes so to speak, to keep the "dominoes" falling.

Overview

The integration of multiple chemical synthetic steps in cascade procedures is an emerging field in Chemistry. Its potential is increasingly being recognized, which is reflected by the growing number of publications on this topic each year. By now numerous papers have been published on cascades. An excellent, but very concise review is given by Bruggink et al.[62] This overview will only focus on transition-metal and enzyme combinations, since these are the most relevant for the research presented in this thesis. In addition, these combinations have in theory a very broad scope, since the diversity of these individual classes of catalysts is already enormous, let alone if they are combined.

Upon examining numerous publications, dealing with this topic, it becomes quite apparent that the development of transition metal-enzyme cascades is still in a very early stage. Because, whilst the quantity of successful cascades is already impressive, the diversity in these cascades is very limited. The majority of publications concern dynamic kinetic resolutions, e.g. the chemocatalysed racemization combined with enzymatic resolution. Co-factor regeneration is also a discipline in which enzyme and transiton metal cascades are quite frequently reported, but other applications of transition metal-enzyme cascades are rare and often limited to a single publication.

The success of dynamic kinetic resolutions (DKR), is undoubtedly related to its significance in the synthesis of enantiopure compounds for which there is an increasing interest in the pharmaceutical and agricultural industries. With DKR a wide variety of optically pure compounds can be produced. The principle behind DKR is the selective conversion of one of the two enantiomers (i.e. resolution), whilst at the same time the opposite enantiomer is continuously racemized Figure 21. This has the major advantage over a kinetic resolution with a maximum yield of only 50%, that all substrate can converted into the desired enantiomer.

The very first example of a one-pot reaction, involving the action of an enzyme and a metal catalyst, was in fact a dynamic kinetic resolution. In 1980 Van Bekkum pioneered this field with the one-pot conversion of glucose into mannitol using a

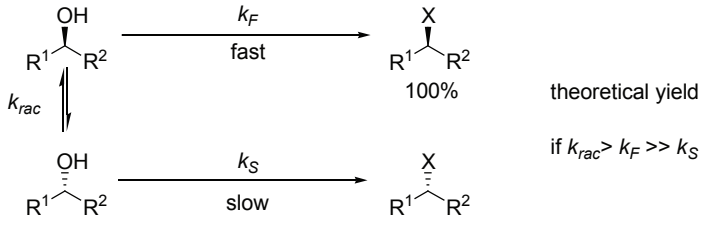


Figure 21. The principle of dynamic kinetic resolution using alcohols as an example.

glucose-isomerase and copper-on-silica catalyst.[65, 66] The isomerase enzyme converts glucose into a 1:1 glucose-fructose mixture and maintains this equilibrium, while the copper catalyst preferentially hydrogenates fructose into mannitol, which is 3-fold more expensive than glucitol, the sole product from common glucose hydrogenation. Yields of 62-66% were obtained, which is a significant improvement compared to the yields of the commercial catalytic hydrogenation of glucose/fructose mixtures. This example already demonstrates the benefits of the one-pot approach. However, it also demonstrated that there are obstacles. The greatest obstacle that had to be overcome was the inhibition of both catalysts by each other. Poisoning of the hydrogenation catalyst by the enzyme was prevented by immobilisation of the enzyme on silica and the enzyme was protected from the copper by addition of EDTA.

After this first milestone, it took until the mid-90s before other successful DKRs utilizing an enzyme and a metal catalyst were reported. Eventually, Williams et al., and shortly thereafter Bäckvall et al., demonstrated that the concept of chemo-enzymatic DKR was not restricted to a single example.

Williams *et al.* successfully combined a palladium-catalysed racemisation of an allylic acetate and the enantioselective hydrolysis of this acetate by *Pseudomonas fluorescens* lipase to produce the corresponding allylic alcohol in good yields (81%) and excellent ee (96%).[67] The same group also reported the DKR of (±)-1-phenylethanol utilizing the transferhydrogenation catalysts, Rh/phenanthroline (**41**) or Ir/phenanthroline, for the racemisation and *Pseudomonas fluorescens* lipase (PFL) for the enantioselective acylation (76% conversion with 80% ee).[68]

At the same time Bäckvall et al. developed a more efficient system for the DKR of (±)-1-phenylethanol and its derivates.[69,70] The key factors in this system were the robust, non-interfering binuclear Ru complex **42** (depicted in Figure 22) and the compatible acyldonor *p*-chlorophenyl acetate. Utilizing **42** and the acyldonor in combination with the immobilised *Candida antarctica* lipase B (Novozym 435), a variety of racemic secondary alcohols were transformed to the corresponding enantiomerically pure acetates with, in most cases, enantiomeric excesses of >99% and yields of 78-92%.

These successes significantly stimulated the development of other DKR systems.

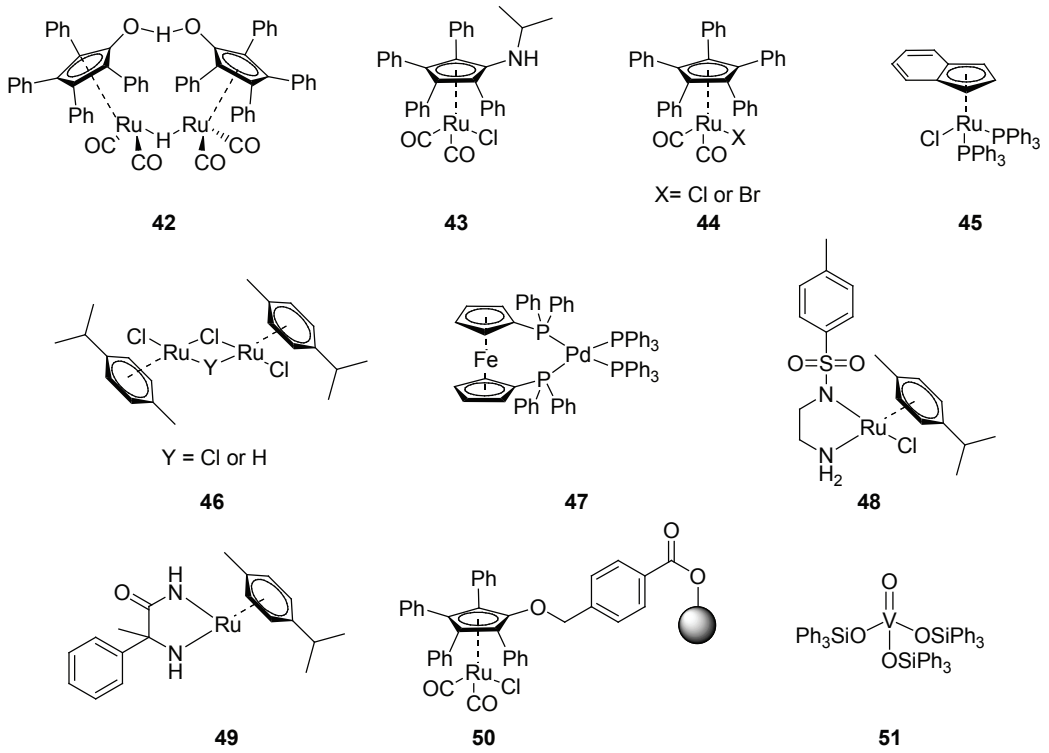


Figure 22. Transition metal complexes, which have been successfully applied for in situ racimization in a chemo-enzymatic DKR.

For example Kim et al. combined the *Pseudomonas cepacia* lipase-catalysed transesterification with a racemization catalysed by **46** for the deracemization of allylic alcohols.[71] This system generally produces the acetylated allylic alcohol with an enantiomeric excess >99% in isolated yields of 85%.

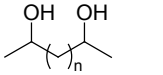
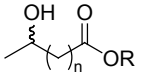
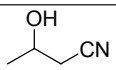
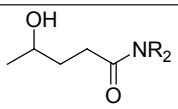
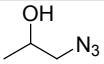
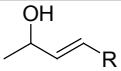
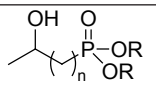
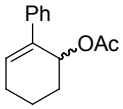
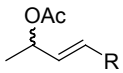
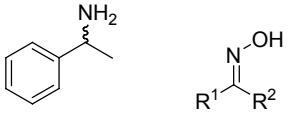
Sheldon et al. reported another successful combination of transition metals and enzymes for the DKR of (±)-1-phenylethanol.[72] Their novel ruthenium based catalytic system, **48** , in combination with TEMPO as oxidant was capable of catalysing the in situ racemization during enzymatic resolution to produce the acetate in good conversions and enantioselectivity.

Meanwhile, the chemo-enzymatic DKR has also found its way to industrial application. In 2002 a large scale industrial process for DKR of secondary alcohols was developed at DSM by Verzijl et al. in which they used a modified Noyori type Ru-catalyst (**49**) together with immobilized CALB.[73]

Besides these examples, numerous other transition metal and enzyme combinations have been developed to produce a wide range of enantiopure compounds as can been seen in Table 1 and the excellent review of Bäckvall et al.[74]

The DKR employing catalyst **50** is a noteworthy entry in Table 1, since it involves an immobilised ruthenium complex. This has the potential benefit of easy separation

Table 1. Efficient Chemo-enzymatic DKR's for various substrates.

Substrate	Catalyst	Enzyme[a]	Yield (%)	Ee (%)	Ref.
	**41**	PFL	60; 76	98; 80	[68]
	42	CALB	60-88	91->99	[69, 70]
	45	PCL	60-98	82-99	[76]
	48	CALB	76	>99	[72]
	43	CALB	95	>99%	[77, 78]
	43	Subtilisin	82-98	52-92	[79]
	44	CALB	83-99	91->99	[80, 81]
	44	Subtilisin	70-97	87-99	[82]
	50	CALB	85-99	79->99	[75]
	**42**	CALB	47-90	96->99	[83, 84]
	44	CALB	26-95	81->99	[85]
	42	PCL	91-97	95->99	[86]
	**42**	PCL	60-80	30-98	[86-88]
	**42**	PCL	48-79	95-99	[89]
	**42**	CALB	72-98	44-99	[90, 91]
	**42**	CALB	62-94	92->99	[92]
	**42**	PCL	63-93	85-97	[93]
	**46**	PCL	84-92	95->99	[71]
	51	CALB	81-96	91-99	[94]
	**42**	CALB	84->99	>99	[95]
	$PdCl_2(MeCN)_2$	PFL			[67]
	**47**	CALB	67-78	97->99	[96]
	Pd/C	CALB	70-89	94-99	[97, 98]
	**42**	CALB			[99]

[a] PFL = *Pseudomonas fluorescens* lipase; CALB = *Candida antarctica* lipase B; PCL = *Pseudomonas cepacia* lipase.

and recyclability. This polymer-bound catalyst was successfully applied in the DKR of various secondary alcohols, displaying the same catalytic activity as its homogeneous counterpart.[75] Unfortunately the goal of recyclability was not entirely met.

In conclusion by combining transition metals and enzymes in a cascade the DKR has become a powerful tool to produce enantiopure compounds, which will find an even more universal application when several remaining issues, e.g. high catalyst loading and the large amount of additives, have been resolved.

Following the success in DKR, the combination of enzymes and metals has very recently found its application in the synthesis of polymers. By using a chemo-enzymatic cascade Meijer et al. devised a new methodology, iterative tandem catalysis, to prepare enantiopure polymers.[100] Utilizing one of the reported systems for the DKR of alcohols, namely **49** and CALB, they synthesised an enantiopure oligoester from a racemic monomer, 6-methyl-ε-carpolactone (**52**) as depited in Figure 23. The principle behind this new methodology is exactly the same as for the DKR. After ring-opening of the lactone the enzyme selectively esterifies the OH-group of the *R*-enantiomer with an other lactone (*S* o*r R*). If the attached lactone is *R* the polymerisation continues, however, the enzyme does not propagate the polymerisation on the *S* alcohol. For this reason the racemisation catalyst **49** is added to the reaction mixture, which makes it possible to polymerise all the available starting material by converting the *S* into the *R* enantiomer. Following this first paper, Heise et al. applied this methodology to actually build up polymers of significant molecular weight and high optical purity.[101]

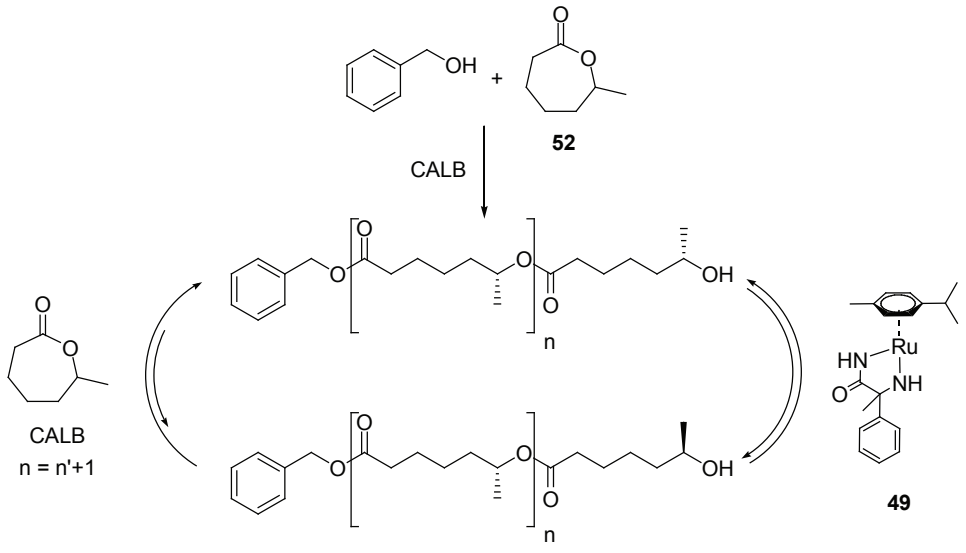


Figure 23. Synthesis of enantiopure oligoester from a racemic monomer, 6-methyl-ε-carpolactone (**52**) by iterative tandem catalysis.[100]

Another application of chemoenzymatic cascades in polymer chemistry is the synthesis of chiral block copolymers also by Meijer et al as depicted in Figure 24.[102] To synthesise these copolymers without intermediate workup, Meijer et al. combined two different, consecutively proceeding polymerization reactions, namely the CALB-catalysed Ring Opening polymerisation (ROP) and Ni-catalysed atom transfer controlled radical polymerisation (ATRP). The key to the successful combination of these two types of polymerisations was a novel initiator (**53**) possessing both a ROP and ATRP moiety. This initiator is first used to initiate the ROP of 4-methyl-ε-carpolactone (**54**) to generate the polyester part of the block copolymer. With a demonstrated initiator efficiency of >95%, **53** ensures that almost all polyester chains contain the initiator molecule, which is important to realize a high block copolymer yield. Subsequently, the polymerised initiator is used to initiate the $Ni(PPh_3)_2Br_2$ catalysed ATPR of methyl methacrylate, resulting in the formation of the desired block copolymer (**55**). Besides catalysing the ATPR, the nickel also inhibits the still present CALB. This inhibition was exploited to stop the ROP at the desired lactone conversion so as to ensure a high ee of the polymer.

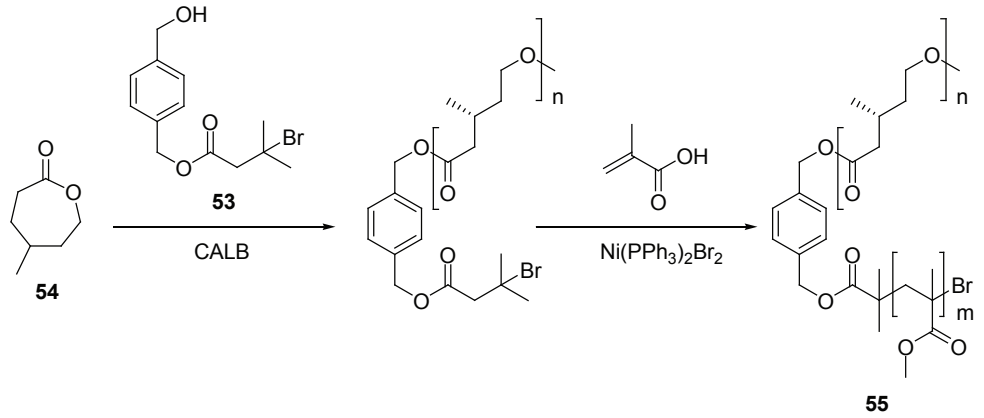


Figure 24. Cascade approach to a chiral block copolymer, combining enantioselective ROP of **54** and ATRP of methyl methacrylate.

As mentioned earlier, co-factor regeneration is another part of chemistry in which chemoenzymic cascades are relatively common. The origin of this lies in the need for practical methods for the regeneration of the expensive co-factors and the excellent redox properties of transition metals. Co-factors are usually either oxidised or reduced, when used by an enzyme, and consequently transition metals are excellently suited to convert these co-factors back to their original state. Whilst in theory these cascades sound perfect, they are quite difficult to realise, since they involve many different

components that should all be compatible to make the system exploitable. Nevertheless, various research groups have developed in situ co-factor regeneration systems that employ a transition metal.[103-110] In general the metal acts as a mediator for the electron transfer between the consumed co-factor and a low cost redox equivalent. In Figure 25 an example is given to demonstrate the mechanism of these co-factor regeneration cascades. The actual redox equivalent can be chemical, photochemical as well as electrochemical.

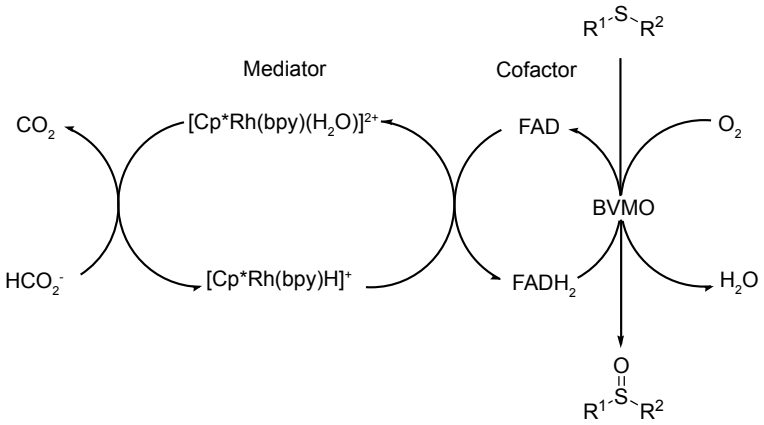


Figure 25. Enzymatic oxidation of organic sulfides catalysed by Baeyer-Villiger monooxygenase (BVMO) as an example of transition-metal-mediated co-factor regeneration.[104]

Various Rh-complexes have been described as specific and efficient catalysts for the in situ regeneration of NAD(P)H. The most noteworthy of these complexes is $[Cp^*Rh(bpy)(H_2O)]^{2+}$, which was introduced by Steckhan et al.[111-113] This hydride transfer catalyst has, in contrast to most other hydride transfer donors, an exceptionally high regiospecificity for the pyridinium ring of NAD^+ and $NADP^+$. For example, this complex was used for the in situ NAD^+ regeneration in the enzymatic chiral reduction of 4-phenyl-2-butanone[113] and pyruvate[112] with horse liver alcohol dehydrogenase, employing formate as reductant. Recently, the preparative value of this regeneration system was demonstrated by Hollmann et al. in the production of chiral alcohols.[105] Using *Thermus sp*. alcohol dehydrogenase, 1.3 g of enantiopure (1*S*, 3*S*)-3-methylcyclohexanol (ee>97%) could be produced in a 750 ml-scale emulsion process. The catalytic performance of $[Cp^*Rh(bpy)(H_2O)]^{2+}$ in this study is the highest reported for a non-enzymatic nicotamide regeration system so far, in terms of TON (up to 1500) and TOF (>400h^{-1}). Finally $[Cp^*Rh(bpy)(H_2O)]^{2+}$/formate has also been applied as NAD^+ and flavine regenerator in the enzymatic oxidation of organic sulfides

catalysed by flavin-monooxygenases (Figure 25).[104]

Another system to catalyse the reduction of NAD^+, developed by Whitesides et al.[103], involves the combination of a water-soluble hydrogenation catalyst with two enzymes. Instead of using formate as reductant this system employs H_2, which has the advantages of being the lowest-cost reductant and yielding no requisite by-products. The H_2 however is not used to directly reduce the NAD^+. A bis(phosphine) rhodium complex utilises the H_2 to first reduce the intermediate hydride carrier, pyruvate. The produced lactate is the actual reductant for the regeneration of NAD^+. This regeneration is facilitated by the enzyme, lactate dehydrogenase (LDH). Finally the generated NADH is used by horse liver alcohol dehydrogenase (HLAD) to reduce the substrate, (±)-2-norbornanone. With this complicated system 72% *endo*-norbornanol and 28% *exo*-norbonanol could be produced with very high TON's for the enzymes (2.5×10^6 for LDH and 7.5×10^4 for HLAD) and a good TON for the Rh complex (1470). However this system has several practical disadvantages due to the rhodium catalyst: limited lifetime and low activity towards the pyruvate. Just recently, a direct reaction of H_2 and $NADP^+$ catalysed by $[RuCl_2(TPPTS)_2]_2$ (TPPTS = tris(*m*-sulfonatophenyl)phosphine) for the in situ regeneration of co-factors was reported, but again this system is inferior to the existing enzyme/enzyme regeneration systems.[110]

In most of the previous examples the authors selected their reagents and conditions to be compatible for the development of their chemo-enzymatic cascade. However, this is not always possible for instance when the multistep synthesis requires both a Brønsted acid and a Brønsted base. Gelman et al nicely demonstrated that this type of incompatibility could be overcome by entrapping both the acid and base in a sol-gel matrix.[114] In a following publication they extended their sol-gel methodology of one-pot sequences with opposing reagents to an enzyme/metal-complex pair.[115] $RhCl[P(C_6H_5)_3]_3$ was immobilised in a sol-matrix, the porosity of which allows the substrate to reach the catalyst but prevent the catalyst from reaching the enzyme. This function of the sol gel matrix is quite similar to that of a membrane in a living cell. To prove the feasibility of this concept the immobilized catalyst was combined with an immobilised lipase (*Mucor miehei)* for one-pot esterification and C-C double bond hydrogenation reactions. The one-pot procedure with the two immobilised catalyst lead to saturated esters in good yields. In contrast, when only the enzyme was immobilized yields decreased almost 7-fold due to inhibition of the enzyme. This concept should be applicable generally to other enzyme/ transition metal combination, since the sol-gel entrapment does not require specific functionalities of the catalysts.

The last example of a successful chemo-enzymatic cascade in this introduction is

the one-pot four step catalytic cascade involving an enzyme, a homogeneous and a heterogeneous catalyst by Schoevaart et al.[116] This highly innovative catalytic cascade reaction converts galactose (**55**) to the 4-deoxy sugar (**59**) without the need for any protection groups (Figure 26). In the first step galactose oxidase together with catalase and O_2 catalyses the selective oxidation of the primary alcohol group to the corresponding hydrated aldehyde (**56**) at the 6-position. Subsequent treatment of **56** with L-proline results in the elimination of H_2O, yielding the unsaturated **57**. Catalytic hydrogenation with Pd/C, followed by a $NaBH_4$ reduction of the carbonyl, gave the desired product **59**. The overall yield of this cascade was >90% with a product-to-waste ratio of 10:1, whereas the traditional step-by-step synthesis using protective groups gave a yield of <30% with a 1:10 product-to-waste ratio. In addition the time required to perform the sequence was reduced to 24 h as compared to the many days commonly required by avoiding the time consuming protection/deprotection and purification steps.

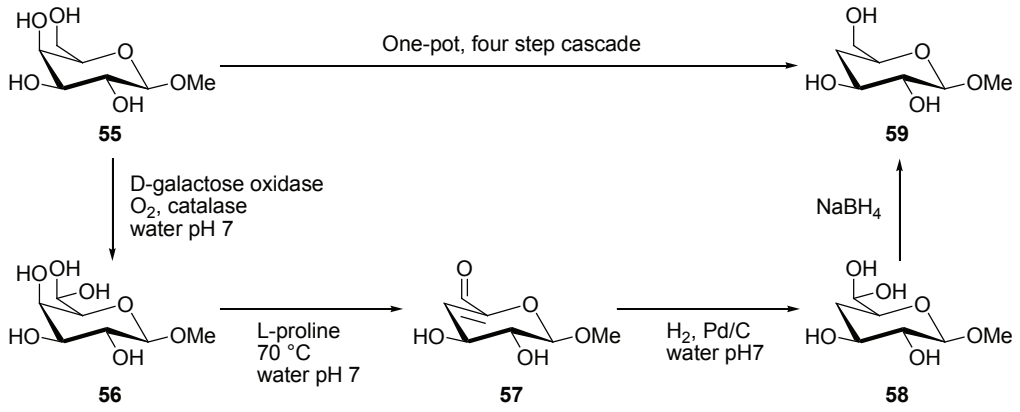


Figure 26. The one-pot four step catalytic cascade for the synthesis of **59** by Schoevaart *et al.*[116]

Hence, this final example clearly proves that the claims made in the beginning of this introduction about cascade syntheses are feasible and that investments made to develop these systems can be very beneficial. Also the other cascades have proven that the cascade approach improves the quality of the product and lowers the total amount of waste per kg of product. The chemo-enzymatic DKR gives twice the yield of the classic kinetic resolution ,while at the same time eliminating the waste represented by the "wrong" enantiomer. In the co-factor regeneration cascades, the process requires only a fraction of the expensive co-factor needed for the enzyme-only transformation. The use of both enzymes and transition metals in polymer chemistry has opened up ways to novel materials.

Despite these successes, chemo-enzymatic cascades have a long way to go before they are commonly applicable in synthesis. At the moment the systems that are successful in combining transition metals and enzymes with similar or superior results to the step-by-step approach are an exception. That cascades in general are still in the early phase of their development, is also reflected by the number of transformation steps that are performed in most of the cascades. In most cases this does not exceed two different transformations, which is only a small improvement when the synthesis of a fine chemical consists of, say, seven steps. In addition, comparison of cascades with the transformations performed by transition metals and biocatalyst individually reveals that the products synthesised by cascades are relatively simple. However, this is not surprising since the step-by-step approach is over one-hundred years old and cascades have only found more widespread recognition since the 1990's. The advancement made in this short time and the benefits gained should motivate scientists to intensify their efforts towards catalytic cascades.

Aim of the thesis

The research described in this thesis deals with the development of a new catalytic cascade for the synthesis of enantiopure amino acids. The starting point of the research is the benchmark Monsanto L-DOPA process: an elegant enantioselective chemical reduction, followed by a wasteful chemical hydrolysis. A chemo-enzymatic cascade should greatly improve the sustainability of this type of amino acid synthesis. Before starting the work on the chemo-enzymatic cascade, the individual transformations of the L-DOPA process, an enantioselective hydrogenation and an amide hydrolysis, are optimized to minimize waste and improve the quality of the product.

In Chapters 2-4 the enantioselective hydrogenation step is improved by immobilizing the catalyst on a solid support, which results in a recyclable catalyst that is readily separated from the product. More specifically, Chapter 2 describes the development of a new anionic support material for the non-covalent anchoring of two well-established asymmetric hydrogenation catalysts and the catalytic performance of these new heterogeneous catalysts. In Chapter 3 the scope of the anionic support material is extended to Rh complexes with monodentate ligands and in Chapter 4 the immobilisation properties of the novel support are compared to those of three other types of anionic supports.

The optimisation of the amide hydrolysis is addressed in Chapter 5. This chapter describes the screening of various enzymes for the hydrolysis of the *N*-acyl group,

resulting in multiple promising hits. Finally the best results of the previous chapters are combined to generate a successful chemo-enzymatic cascade procedure for the synthesis of enantiopure amino acids in chapter 6.

References

[1] W. S. Knowles, *Angew. Chem. Int. Ed.*, **2002**, *41*, 1998.

[2] M. Beller, C. Bolm, *Transition Metals for Organic Synthesis: Building Blocks and Fine Chemicals Second Edition*, **2004**.

[3] I. Ojima, *Catalytic Asymmetric Synthesis, Second Edition*, Wiley-VCH, New York, **2000**.

[4] R. A. Johnson, K. B. Sharpless, *Catalytic Asymmetric Synthesis (2nd Edition)*, **2000**, 231.

[5] K. B. Sharpless, *Angew. Chem. Int. Ed.*, **2002**, *41*, 2024.

[6] J. Mulzer, E. Oehler, *Chem. Rev.*, **2003**, *103*, 3753.

[7] A. Ahmed, E. K. Hoegenauer, V. S. Enev, M. Hanbauer, H. Kaehlig, E. Oehler, J. Mulzer, *J. Org. Chem.*, **2003**, *68*, 3026.

[8] J. Mulzer, E. Ohler, *Angew. Chem. Int. Ed.*, **2001**, *40*, 3842.

[9] I. Paterson, C. De Savi, M. Tudge, *Org. Lett.*, **2001**, *3*, 3149.

[10] A. K. Ghosh, Y. Wang, *J. Am. Chem. Soc.*, **2000**, *122*, 11027.

[11] S. L. Mooberry, G. Tien, A. H. Hernandez, A. Plubrukarn, B. S. Davidson, *Cancer research*, **1999**, *59*, 653.

[12] Y. Gao, J. M. Klunder, R. M. Hanson, H. Masamune, S. Y. Ko, K. B. Sharpless, *J. Am. Chem. Soc.*, **1987**, *109*, 5765.

[13] R. A. Johnson, K. B. Sharpless, *Catalytic Asymmetric Synthesis (2nd Edition)*, **2000**, 357.

[14] A. Yazbak, S. C. Sinha, E. Keinan, *J. Org. Chem.*, **1998**, *63*, 5863.

[15] L. Zeng, Q. Ye, N. H. Oberlies, G. Shi, Z. M. Gu, K. He, J. L. McLaughlin, *Natural product reports*, **1996**, *13*, 275.

[16] K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K. S. Jeong, H. L. Kwong, K. Morikawa, Z. M. Wang, et al., *J. Org. Chem.*, **1992**, *57*, 2768.

[17] H.-U. Blaser, *Chem. Commun.*, **2003**, 293.

[18] H. U. Blaser, F. Spindler, A. Studer, *Appl. Catal. A*, **2001**, *221*, 119.

[19] R. Noyori, *Angew. Chem. Int. Ed.*, **2002**, *41*, 2008.

[20] O. Korpiun, R. A. Lewis, J. Chickos, K. Mislow, *J. Am. Chem. Soc.*, **1968**, *90*, 4842.

[21] O. Korpiun, K. Mislow, *J. Am. Chem. Soc.*, **1967**, *89*, 4784.
[22] L. Horner, *Pure Appl. Chem.*, **1964**, *9*, 225.
[23] L. Horner, H. Winkler, A. Rapp, A. Mentrup, H. Hoffmann, P. Beck, *Tetrahedron Lett.*, **1961**, 161.
[24] J. A. Osborn, F. H. Jardine, J. F. Young, G. Wilkinson, *J. Chem. Soc. (A) Inorg. Phys. Theor.*, **1966**, 1711.
[25] J. S. Buck, W. S. Ide, *Org. Synth.*, **1933**, *13*, 8.
[26] C. R. Landis, J. Halpern, *J. Am. Chem. Soc.*, **1987**, *109*, 1746.
[27] I. D. Gridnev, Y. Yamanoi, N. Higashi, H. Tsuruta, M. Yasutake, T. Imamoto, *Adv. Synth. Catal.*, **2001**, *343*, 118.
[28] I. D. Gridnev, N. Higashi, K. Asakura, T. Imamoto, *J. Am. Chem. Soc.*, **2000**, *122*, 7183.
[29] M. Kitamura, T. Ohkuma, S. Inoue, N. Sayo, H. Kumobayashi, S. Akutagawa, T. Ohta, H. Takaya, R. Noyori, *J. Am. Chem. Soc.*, **1988**, *110*, 629.
[30] A. Miyashita, A. Yasuda, H. Takaya, K. Toriumi, T. Ito, T. Souchi, R. Noyori, *J. Am. Chem. Soc.*, **1980**, *102*, 7932.
[31] T. Ohta, H. Takaya, R. Noyori, *Inorg. Chem.*, **1988**, *27*, 566.
[32] T. Ohkuma, H. Ooka, S. Hashiguchi, T. Ikariya, R. Noyori, *J. Am. Chem. Soc.*, **1995**, *117*, 2675.
[33] C. S. Poss, S. D. Rychnovsky, S. L. Schreiber, *J. Am. Chem. Soc.*, **1993**, *115*, 3360.
[34] K. C. Nicolaou, P. G. Bulger, D. Sarlah, *Angew. Chem. Int. Ed.*, **2005**, *44*, 4490.
[35] K. C. Nicolaou, P. G. Bulger, D. Sarlah, *Angew. Chem. Int. Ed.*, **2005**, *44*, 4442.
[36] M. A. J. Duncton, G. Pattenden, *Perkin Trans. 1*, **1999**, 1235.
[37] J. K. Stille, *Angew. Chem.*, **1986**, *98*, 504.
[38] F. Jacobsen Mikkel, E. Moses John, M. Adlington Robert, E. Baldwin Jack, *Org. Lett.*, **2005**, *7*, 2473.
[39] M. F. Jacobsen, J. E. Moses, R. M. Adlington, J. E. Baldwin, *Org. Lett.*, **2005**, *7*, 641.
[40] F. Cedrone, A. Menez, E. Quemeneur, *Current Opinion in Structural Biology*, **2000**, *10*, 405.
[41] K. A. Powell, S. W. Ramer, S. B. Del Cardayre, W. P. C. Stemmer, M. B. Tobin, P. F. Longchamp, G. W. Huisman, *Angew. Chem. Int. Ed.*, **2001**, *40*, 3948.
[42] F. Valetti, G. Gilardi, *Natural Product Reports*, **2004**, *21*, 490.
[43] M. T. Reetz, A. Zonta, K. Schimossek, K. Liebeton, K.-E. Jaeger, *Angew. Chem. Int. Ed.*, **1997**, *36*, 2830.

[44] K. Drauz, H. Waldmann, Editors, *Enzyme catalysis in organic synthesis: A comprehensive handbook, Volume I-III*, **2002**.
[45] K. Faber, Editor, *Biotransformation in Organic Chemistry, 3rd Completely Revised Edition*, **1997**.
[46] U. Bornscheuer, R. Kazlauskas, *Hydrolases in Organic Synthesis: Regio- or Stereoselective Biotransformations*, **1999**.
[47] S. Akai, T. Tsujino, N. Fukuda, K. Iio, Y. Takeda, K.-i. Kawaguchi, T. Naka, K. Higuchi, E. Akiyama, H. Fujioka, Y. Kita, *Chem. Eur. J.*, **2005**, *11*, 6286.
[48] S. Akai, Y. Takeda, K. Iio, K. Takahashi, N. Fukuda, Y. Kita, *J. Org. Chem.*, **1997**, *62*, 5526.
[49] Y. Kita, Y. Takeda, K. Iio, K. Yokogawa, K. Takahashi, S. Akai, *Tetrahedron Lett.*, **1996**, *37*, 7545.
[50] S. Riva, B. Danieli, M. Luisetti, *J. Nat. Prod.*, **1996**, *59*, 618.
[51] R. A. Sheldon, *Chirotechnology: Industrial Synthesis of Optically Active Compounds*, **1993**.
[52] I. Alfonso, V. Gotor, *Chem. Soc. Rev.*, **2004**, *33*, 201.
[53] D. W. Christianson, W. N. Lipscomb, *Accounts of Chemical Research*, **1989**, *22*, 62.
[54] C. H. Wong, G. M. Whitesides, *Enzymes in synthetic organic chemistry, Vol. 12*, Pergamon, **1994**.
[55] H. K. Chenault, J. Dahmer, G. M. Whitesides, *J. Am. Chem. Soc.*, **1989**, *111*, 6354.
[56] E. Keinan, K. K. Seth, R. Lamed, *J. Am. Chem. Soc.*, **1986**, *108*, 3474.
[57] E. Keinan, E. K. Hafeli, K. K. Seth, R. Lamed, *J. Am. Chem. Soc.*, **1986**, *108*, 162.
[58] A. F. Bückmann, M. R. Kula, R. Wichmann, C. Wandrey, *J. Appl. Biochem.*, **1981**, *3*, 301.
[59] R. Wichmann, C. Wandrey, A. F. Bückmann, M. R. Kula, *Biotechnol. Bioeng.*, **1981**, *23*, 2789.
[60] E. Keinan, S. C. Sinha, A. Sinha-Bagchi, *J. Org. Chem.*, **1992**, *57*, 3631.
[61] R. A. Sheldon, *Chemtech*, **1994**, *24*, 38.
[62] A. Bruggink, R. Schoevaart, T. Kieboom, *Org. Process Res. Dev.*, **2003**, *7*, 622.
[63] D. E. Fogg, E. N. dos Santos, *Coord. Chem. Rev.*, **2004**, *248*, 2365.
[64] L. F. Tietze, *Chem. Rev.*, **1996**, *96*, 115.
[65] M. Makkee, A. P. G. Kieboom, H. Van Bekkum, *Carbohydr. Res.*, **1985**, *138*, 237.

[66] M. Makkee, A. P. G. Kieboom, H. Van Bekkum, J. A. Roels, *Chem. Commun.*, **1980**, 930.
[67] J. V. Allen, J. M. J. Williams, *Tetrahedron Lett.*, **1996**, *37*, 1859.
[68] P. M. Dinh, J. A. Howarth, A. R. Hudnott, J. M. J. Williams, W. Harris, *Tetrahedron Lett.*, **1996**, *37*, 7623.
[69] B. A. Persson, A. L. E. Larsson, M. Le Ray, J.-E. Bäckvall, *J. Am. Chem. Soc.*, **1999**, *121*, 1645.
[70] A. L. E. Larsson, B. A. Persson, J.-E. Bäckvall, *Angew. Chem. Int. Ed.*, **1997**, *36*, 1211.
[71] D. Lee, E. A. Huh, M.-J. Kim, H. M. Jung, J. H. Koh, J. Park, *Org. Lett.*, **2000**, *2*, 2377.
[72] A. Dijksman, J. M. Elzinga, Y.-X. Li, I. W. C. E. Arends, R. A. Sheldon, *Tetrahedron: Asymmetry*, **2002**, *13*, 879.
[73] G. K. M. Verzijl, J. G. De Vries, Q. B. Broxterman, *WO 01090396*, **2001**.
[74] O. Pamies, J.-E. Bäckvall, *Chem. Rev.*, **2003**, *103*, 3247.
[75] N. Kim, S.-B. Ko, M. S. Kwon, M.-J. Kim, J. Park, *Org. Lett.*, **2005**, *7*, 4523.
[76] J. H. Koh, H. M. Jung, M.-J. Kim, J. Park, *Tetrahedron Lett.*, **1999**, *40*, 6281.
[77] J. H. Choi, Y. H. Kim, S. H. Nam, S. T. Shin, M.-J. Kim, J. Park, *Angew. Chem. Int. Ed.*, **2002**, *41*, 2373.
[78] J. H. Choi, Y. K. Choi, Y. H. Kim, E. S. Park, E. J. Kim, M.-J. Kim, J. Park, *J. Org. Chem.*, **2004**, *69*, 1972.
[79] M.-J. Kim, Y. I. Chung, Y. K. Choi, H. K. Lee, D. Kim, J. Park, *J. Am. Chem. Soc.*, **2003**, *125*, 11494.
[80] B. Martín-Matute, M. Edin, K. Bogar, J.-E. Bäckvall, *Angew. Chem. Int. Ed.*, **2004**, *43*, 6535.
[81] B. Martín-Matute, M. Edin, K. Bogar, F. B. Kaynak, J.-E. Bäckvall, *J. Am. Chem. Soc.*, **2005**, *127*, 8817.
[82] L. Borén, B. Martin-Matute, Y. Xu, A. Cordova, J.-E. Bäckvall, *Chem. Eur. J.*, **2005**, *12*, 225.
[83] B. A. Persson, F. F. Huerta, J.-E. Bäckvall, *J. Org. Chem.*, **1999**, *64*, 5237.
[84] B. Martín-Matute, J.-E. Bäckvall, *J. Org. Chem.*, **2004**, *69*, 9191.
[85] M. Edin, B. Martín-Matute, J.-E. Bäckvall, *Tetrahedron: Asymmetry*, **2006**, *17*, 708.
[86] M.-J. Kim, Y. K. Choi, M. Y. Choi, M. J. Kim, J. Park, *J. Org. Chem.*, **2001**, *66*, 4736.
[87] O. Pamies, J.-E. Bäckvall, *J. Org. Chem.*, **2002**, *67*, 1261.

[88] A.-B. L. Runmo, O. Pamies, K. Faber, J.-E. Backvall, *Tetrahedron Lett.*, **2002**, *43*, 2983.
[89] A.-B. L. Fransson, L. Boren, O. Pamies, J.-E. Bäckvall, *J. Org. Chem.*, **2005**, *70*, 2582.
[90] O. Pamies, J.-E. Bäckvall, *Adv. Synth. Catal.*, **2001**, *343*, 726.
[91] O. Pamies, J.-E. Bäckvall, *Adv. Synth. Catal.*, **2002**, *344*, 947.
[92] O. Pamies, J.-E. Bäckvall, *J. Org. Chem.*, **2001**, *66*, 4022.
[93] O. Pamies, J.-E. Bäckvall, *J. Org. Chem.*, **2002**, *67*, 9006.
[94] S. Akai, K. Tanimoto, Y. Kanao, M. Egi, T. Yamamoto, Y. Kita, *Angew. Chem. Int. Ed.*, **2006**, *45*, 2592.
[95] O. Pamies, J. E. Baeckvall, *J. Org. Chem.*, **2003**, *68*, 4815.
[96] Y. K. Choi, J. H. Suh, D. Lee, I. T. Lim, J. Y. Jung, M.-J. Kim, *J. Org. Chem.*, **1999**, *64*, 8423.
[97] M. T. Reetz, K. Schimossek, *Chimia*, **1996**, *50*, 668.
[98] Y. K. Choi, M. J. Kim, Y. Ahn, M.-J. Kim, *Org. Lett.*, **2001**, *3*, 4099.
[99] J. Paetzold, J. E. Baeckvall, *J. Am. Chem. Soc.*, **2005**, *127*, 17620.
[100] B. A. C. van As, J. van Buijtenen, A. Heise, Q. B. Broxterman, G. K. M. Verzijl, A. R. A. Palmans, E. W. Meijer, *J. Am. Chem. Soc.*, **2005**, *127*, 9964.
[101] I. Hilker, G. Rabani, G. K. M. Verzijl, A. R. A. Palmans, A. Heise, *Angew. Chem. Int. Ed.*, **2006**, *45*, 2130.
[102] J. Peeters, A. R. A. Palmans, M. Veld, F. Scheijen, A. Heise, E. W. Meijer, *Biomacromolecules*, **2004**, *5*, 1862.
[103] O. Abril, G. M. Whitesides, *J. Am. Chem. Soc.*, **1982**, *104*, 1552.
[104] G. de Gonzalo, G. Ottolina, G. Carrea, M. W. Fraaije, *Chem. Commun.*, **2005**, 3724.
[105] F. Hollmann, A. Kleeb, K. Otto, A. Schmid, *Tetrahedron: Asymmetry*, **2005**, *16*, 3512.
[106] F. Hollmann, A. Schmid, *Biocatal. Biotransform.*, **2004**, *22*, 63.
[107] H. C. Lo, R. H. Fish, *Angew. Chem. Int. Ed.*, **2002**, *41*, 478.
[108] R. Miyatani, Y. Amao, *Biotechnol. Lett.*, **2002**, *24*, 1931.
[109] R. Miyatani, Y. Amao, *J. Mol. Catal. B: Enzym.*, **2004**, *27*, 121.
[110] P. S. Wagenknecht, J. M. Penney, R. T. Hembre, *Organometallics*, **2003**, *22*, 1180.
[111] R. Ruppert, S. Herrmann, E. Steckhan, *Chem. Commun.*, **1988**, 1150.
[112] R. Ruppert, S. Herrmann, E. Steckhan, *Tetrahedron Lett.*, **1987**, *28*, 6583.
[113] D. Westerhausen, S. Herrmann, W. Hummel, E. Steckhan, *Angew. Chem.*, **1992**,

104, 1496.
[114] F. Gelman, J. Blum, D. Avnir, *Angew. Chem. Int. Ed.*, **2001**, *40*, 3647.
[115] F. Gelman, J. Blum, D. Avnir, *J. Am. Chem. Soc.*, **2002**, *124*, 14460.
[116] R. Schoevaart, T. Kieboom, *Top. Catal.*, **2004**, *27*, 3.

2

Non-Covalent Anchoring of Asymmetric Hydrogenation Catalysts on a New Mesoporous Aluminosilicate: Application and Solvent Effects

Introduction

Transition metal catalysed asymmetric hydrogenation is becoming increasingly important for the production of enantiopure pharmaceuticals and agrochemicals. The Monsanto L-DOPA process[1] represents one of the most prominent examples of the successful implementation of this technology. In recent years highly enantioselective catalysts for a broad range of substrates (olefins, ketones, imines, etc.)[2] have been developed. However, large-scale applications of this mature methodology are often hampered by the difficult removal of the homogeneous catalysts. Heterogenisation of the metal complexes provides a way to greatly ease this separation and to improve the recycling of the expensive catalyst. A commonly applied technique is the covalent binding of the complex to a solid support.[3] Serious drawbacks of this approach are the time-consuming and difficult ligand modification as well as not always predictable effects on activity and selectivity. Augustine et al. reported a very elegant method for the heterogenisation of ionic transition metal complexes, which did not require any modification of the complexes and additionally, in some cases, improved their catalytic

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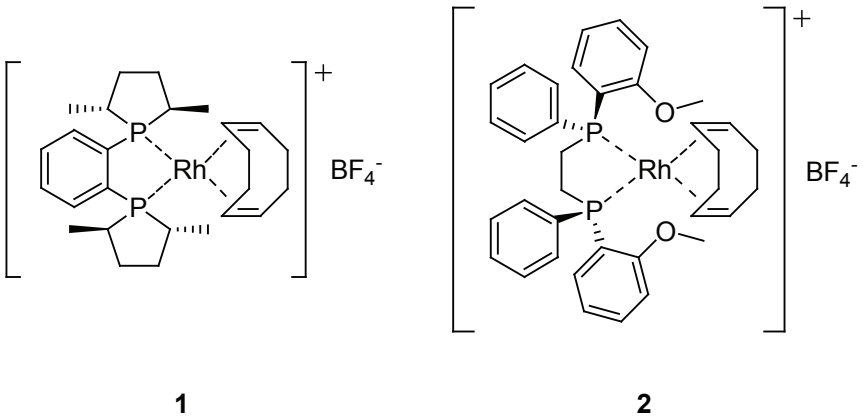
activity and selectivity.[4] This method utilised the cationic character of the complex to bind it non-covalently to an inorganic support, employing heteropoly acids as the anchors. Variations of this approach using different surface modification strategies to anchor via electrostatic binding have been reported by Hölderich et al.,[5] Hems et al.[6] and Broene et al.[7]

Inspired by these new anchoring techniques, we set out to utilise the new mesoporous aluminosilicate, AlTUD-1, as a support for chiral rhodium complexes. Here, we describe the preparation of this new aluminosilicate and its use in the immobilisation of two established asymmetric hydrogenation catalysts, [Rh^{I}(cod)((*R,R*)-MeDuPHOS)]BF$_4$ (**1**)[8] and [Rh^{I}(cod)((*S,S*)-DiPAMP)]BF$_4$ (**2**),[9] wherein cod is 1,5-cyclooctadiene (Figure 1). The application of these new heterogeneous catalysts in asymmetric hydrogenation and the striking influence of the solvent, a factor often ignored, were investigated.



1 **2**

Figure 1. The asymmetric hydrogenation catalysts: [Rh^{I}(cod)((*R,R*)-MeDuPHOS)]BF$_4$ (**1**) and [Rh^{I}(cod)((*S,S*)-DiPAMP)]BF$_4$ (**2**).

Results and Discussion

The starting point for the development of the mesoporous aluminosilicate (AlTUD-1, pore diameter 20-500 Å) was the recent discovery of a new templating method for mesoporous networks.[10] This novel approach uses inexpensive, non-surfactant chemicals to produce mesoporous materials with high surface areas (up to ca. 1000 m^2/g) and three-dimensional connectivities. The 3D pores should allow for a better accessibility of the catalyst compared to one-dimensional pore systems as found in materials such as MCM-41.[11]

For the purpose of immobilizing cationic complexes on the material an unusually

low Si/Al ratio of *ca.* 4 is desirable. Preferably, to ensure a high Brønsted acidity, the aluminium should be coordinated tetrahedrally. The templates described have the ability to stabilise metal alkoxides by complexation,[12] and thus seemed ideally suited to produce the desired aluminosilicate.

Initial experiments with the most frequently reported template triethanolamine, did not give satisfactory results. However, with tetraethyleneglycol (TEG)[13] as a template a white solid, denoted as AlTUD-1, was obtained. The complete removal of the template was confirmed by IR and the structural properties of AlTUD-1 were investigated with X-ray powder diffraction and N_2 physisorption.

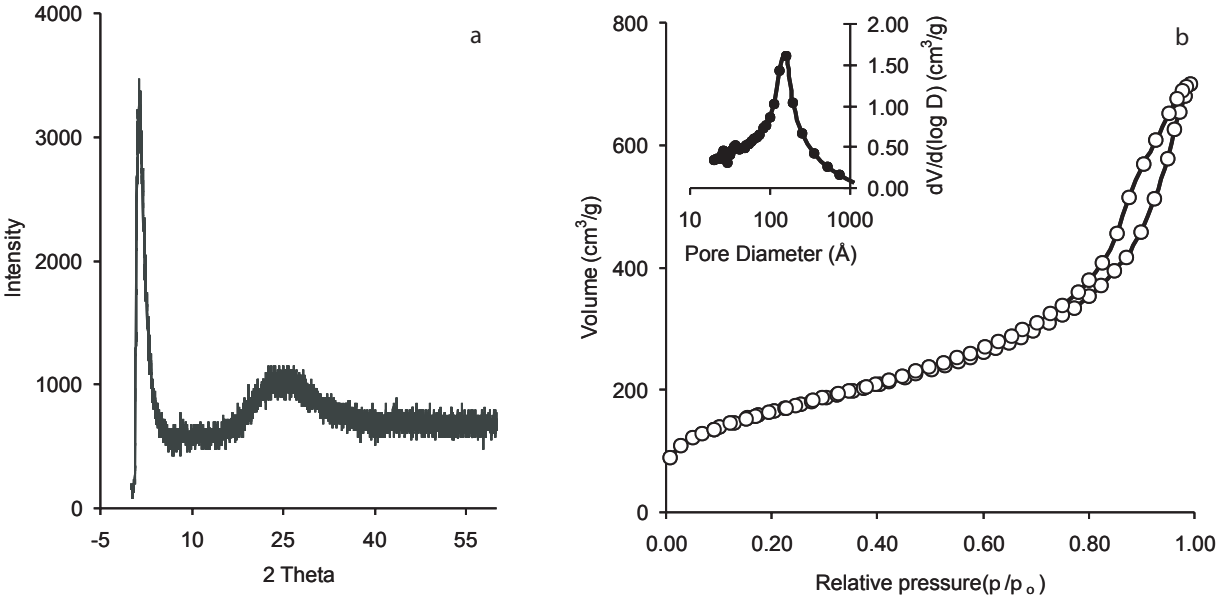


Figure 2. a) Powder XRD ($Cu_{K\alpha}$) pattern of AlTUD-1; b) Nitrogen sorption isotherms of AlTUD-1; Inset: corresponding pore size distribution.

The XRD pattern in Figure 2a shows one dominant signal, an intense peak around 0.65° θ, indicating that AlTUD-1 is a mesostructured material. The N_2 sorption isotherms, in Figure 2b, also show the mesoporous texture in what is a typical Type IV isotherm with a type H1 hysteresis loop, characteristic for mesoporous materials. Additional data, derived from the isotherm, illustrate that AlTUD-1 has a large surface area of ca. 600 m^2/g and a total pore volume of 1.1 cm^3/g. The pore size distribution is fairly broad and shows a maximum at 150 Å (inset). In the synthesis of the purely siliceous mesoporous silica (TUD-1) by this templating method, the pore size distribution could be tuned by variation of the hydrothermal treatment time: a longer duration increased the pore diameter. For AlTUD-1, variation in the hydrothermal treatment time had

little to no effect on the pore size distribution. This is, to a large degree, due to the faster formation of Al-O-Si bonds compared to Si-O-Si bonds, rendering the overall system less dynamic and, therefore, less sensitive towards changes in pore size with temperature. Increasing the time over a range of 3 h to 24 h gave a pore diameter of 150 Å to 250 Å, all with the same broad distribution, as shown in Figure 2b inset. Similarly, the surface area increased only marginally when prolonging the hydrothermal treatment from 3h to 24h (~500 to 625 m^2/g). It can be assumed reasonably that AlTUD-1 exhibits a 3-dimensional structure due to the synthesis method used. Furthermore, all analyses of AlTUD-1 indicate that it is consistent with a TUD-1 like structure.

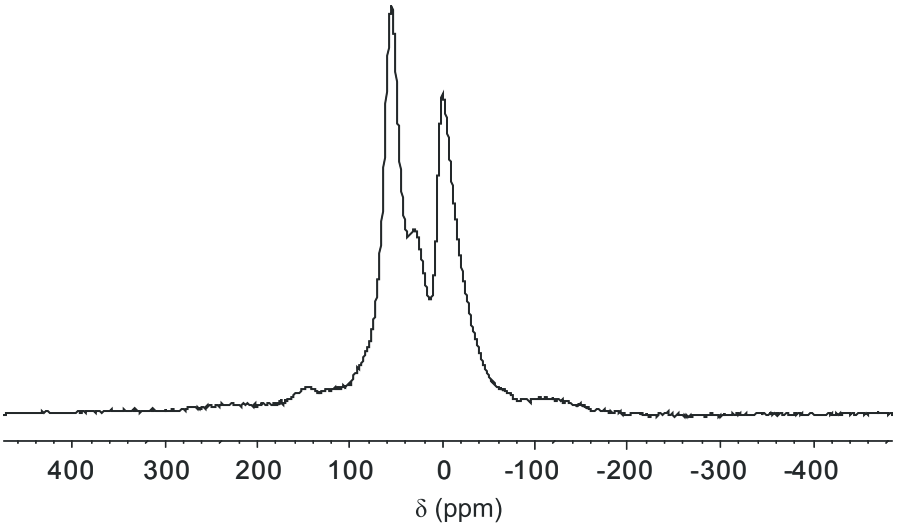


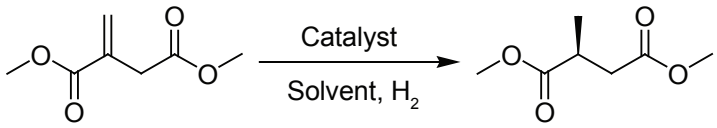
Figure 3. ^{27}Al-NMR spectrum of AlTUD-1.

The nature of Al in AlTUD-1 was investigated using ^{27}Al-NMR (Figure 3). The spectrum exhibits a strong resonance at 55 ppm, which can be assigned to the desired Brønsted acidic, tetrahedrally coordinated Al ($Al_{tetrahedral}$). The signals at 31 ppm and 0 ppm can be ascribed to pentacoordinated Al and hexacoordinated Al, respectively. It follows from the integration that approximately 43% of the Al is coordinated tetrahedrally. Although the addition of TEG was not able to completely suppress the formation of hexacoordinated Al, it did allow for the formation of a mesoporous aluminosilicate with a high surface area and a $Si/Al_{tetrahedral}$ ratio of 9 (overall Si/Al = 4). This new material AlTUD-1 with its large surface area, mesoporous structure and high proportion of Brønsted acidic Al combines all the desired properties for an anionic carrier.

Complexes **1** and **2** were immobilised by straightforward ion exchange, using the three-dimensional mesoporous aluminosilicate (AlTUD-1). A high $Al_{tetrahedral}$/Rh ratio

of approximately 10 was chosen, so that any cationic complex that is inadvertently mobilised during the hydrogenation reaction is surrounded by many vacant acidic sites, thereby increasing the chances to be immobilised again. Both pre-formed complexes and those prepared in situ can be immobilised. The resulting immobilised catalysts are expected to have a virtually unmodified, and possibly even improved, catalytic behaviour. The immobilised catalysts, denoted as **1**-AlTUD-1 and **2**-AlTUD-1 respectively, were washed with ethanol or 2-propanol to remove any non-anchored catalyst. Typically, a loading of 1 wt% Rh was obtained.

Table 1. Asymmetric hydrogenation of **3** in various solvents.[a]



3

Entry	Catalyst	Solvent	Conv. (%)	**3**/Rh ratio	TOF ($molmol^{-1}h^{-1}$)	Ee (%)	Rh loss (mg/L) [%][c]
1[b]	**1**	MeOH	100	1250	>1000	96	-
2[b]	**1**-AlTUD-1	MeOH	100	1250	>1000	98	2.00 [23]
3[b]	**1**-AlTUD-1	2-PrOH	100	200	>1000	96	0.35 [1.4]
4	**1**-AlTUD-1	MeOH	22	250	51	97	4.5 [15]
5	**1**-AlTUD-1	2-PrOH	32	325	105	96	0.78 [2.5]
6	**1**-AlTUD-1	CH_2Cl_2	26	250	62	98	0.29 [0.7]
7	**1**-AlTUD-1	EtOAc	11	175	19	98	0.29 [0.5]
8	**1**-AlTUD-1	MTBE	10	250	25	96	0.04 [0.1]
9	**1**-AlTUD-1	Toluene	0	250	0	n.d.	n.d.

[a] Reaction was performed using the Avantium Quick Catalyst Screen platform; conditions: ~6 mg supported catalyst, $p_{initial}(H_2)$= 5 bar, volume 1.5 ml, [substrate]= 0.1 M, time = 60 min, *S* major enantiomer with (*R,R*)-MeDuPHOS) as ligand.

[b] Reaction was performed in a Parr hastelloy C autoclave; conditions: 100 mg supported catalyst, 50 ml solvent, $p(H_2)$= 5 bar, [substrate]= 0.1 M, time = 30 min, *S* major enantiomer with (*R,R*)-MeDuPHOS) as ligand.

[c] Percentage of total amount Rh.

For a direct comparison of the immobilised and homogeneous catalysts, the catalytic behaviour of **1**-AlTUD-1 was studied in the asymmetric hydrogenation of dimethyl itaconate (**3**) (Table 1). No difference was found between chiral catalysts that were immobilised as synthesised and those that were prepared by addition of a solution

of bis(1,5-cyclooctadiene)rhodium tetrafluoroborate and the chiral ligand to AlTUD-1. The comparison between the homogeneous catalyst (entry 1, Table 1) and **1**-AlTUD-1 (entry 2, Table 1) under identical conditions reveals that there is no decrease in either selectivity or activity upon anchoring **1** on AlTUD-1. However, significant leaching of Rh was observed, casting doubt on the heterogeneity of the reaction. Therefore, other solvents were screened using the *Avantium Quick Catalyst Screen platform* (entry 4-9, Table 1) to reduce this problem. When switching from the mechanically stirred autoclave to the magnetically stirred Quick Catalyst Screen platform, a significant drop in activity was observed (entry 3 vs entry 5, Table 1), while the enantioselectivity was hardly affected by the change of reaction vessel and stirring mode. The reduction in activity is principally due to a reduced mass-transfer of hydrogen from the gas to the liquid phase, caused by the different reaction vessel design. Reduction in the hydrogen uptake slows down the reaction, since hydrogen is involved in the rate-determining step.[14a] Nevertheless, this system is suited to find trends in the leaching of Rh. The lack of activity in toluene is not surprising, since aromatic compounds tend to form stable η^6 arene complexes with Rh^I.[15]

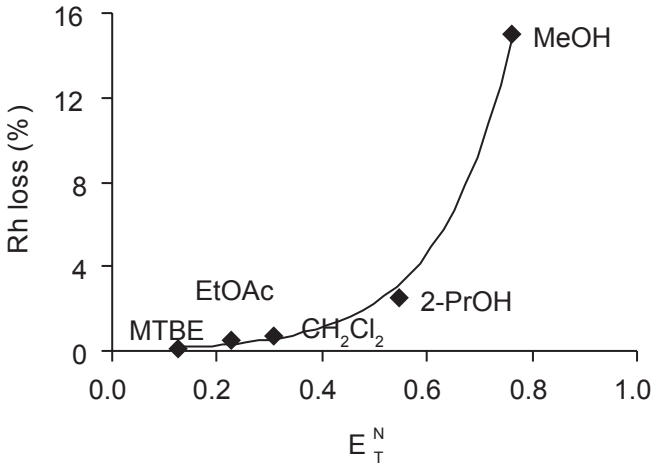


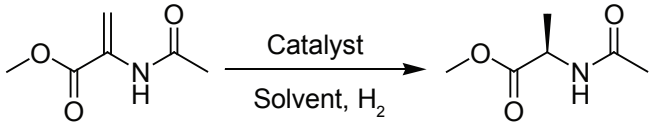
Figure 4. The correlation between the polarity of the solvent (E_T^N) and the loss of Rh (in percentage of total amount of Rh).

The screening revealed a similar loss of Rh with methanol as solvent, when compared to the original experiment. As expected, the Rh loss could largely be overcome by switching to less polar solvents. With the less polar, but still protic, 2-propanol as solvent, leaching of Rh could already be reduced by a factor 6. Using dichloromethane or ethyl acetate, both regarded as polar aprotic solvents, the Rh in solution could be reduced to 0.29 mg/L, corresponding to 0.5-0.7% of the total amount of Rh. Minimal

leaching (0.04 mg/L, 0.1% of the original Rh) was obtained with the much less polar and aprotic MTBE (*t*-butylmethyl ether). Simply by switching the polarity of the solvent, the leaching of the catalyst could be reduced by a factor 150.

A good measure for solvent polarity is the normalised empirical parameter E_T^N,[16] that is based on the transition energy for the longest-wavelength solvatochromic absorption band of a pyridinium *N*-phenolate betaine dye. This parameter also takes into account specific solute/solvent interactions, like hydrogen bonding and electron pair donating/electron pair accepting interactions. The correlation between this parameter and loss of Rh is given in Figure 4. This exponential correlation can be rationalised by the increasing ability to stabilise charged species with increasing polarity. While MTBE has almost no possibility to stabilise charges, ethyl acetate has the ability to stabilise positive charges by lone pair donation and its dipolar moment. However, ethyl acetate is far less efficient in the stabilisation of negative charges. Methanol on the other hand has the capability to stabilise both, explaining the large amounts of Rh in solution.

Table 2. Asymmetric hydrogenation of **4** in various solvents.[a]



4

Entry	Catalyst	p(H_2)[b] (bar)	Solvent	Conv. (%)	TOF ($molmol^{-1}h^{-1}$)	Ee (%)	Rh loss (mg/L) [%][c]
1	**1**	1	MeOH	100	>350	>98	-
2	**1**-AlTUD-1	1	MeOH	100	>350	>98	4.9 [17]
3	**1**-AlTUD-1	1	MTBE	100	>350	90	0.01 [0.05]
4	**1**-AlTUD-1	1	EtOAc	100	>350	84	0.01 [0.05]
5	**1**-AlTUD-1	1	2-PrOH	100	>350	75	0.4 [1.6]
6	**2**	3	MeOH	100	>200		-
7	**2**-AlTUD-1	3	MeOH	100	>200	92	4.6 [20]
8	**2**-AlTUD-1	3	EtOH	100	>200	79	0.9 [4]
9	**2**-AlTUD-1	3	Water	81	159	64	1.2 [5.6]
10	**2**-AlTUD-1	3	2-PrOH	26	69	44	1.3 [7.5]
11	**2**-AlTUD-1	3	MTBE	54	103	26	0.02 [0.09]
12	**2**-AlTUD-1	3	EtOAc	35	75	30	0.09 [0.45]

[a] Reaction was performed in a Parr hastelloy C autoclave, conditions: 50 ml solvent, [**4**] = 0.025 M, 0.1 g catalyst, **4**/Rh ratio = 100, *R* major enantiomer with (*R,R*)-MeDuPHOS) or (*S,S*)-DiPAMP) as ligand, reaction time: 20 min for **1** and 30 min for **2**.

[b] Initial pressure.

[c] Percentage of total amount Rh.

The screening of various solvents also shows that the enantioselectivity in the hydrogenation of **3** with **1**-AlTUD-1 is independent of the solvent. **1**-AlTUD-1 exhibits excellent enantioselectivities of up to 98% in all solvents. The enantioselectivity fluctuated only within 1 to 2% between different solvents.

The encouraging results with **3** as substrate motivated us to investigate the asymmetric hydrogenation of the higher functionalised substrate methyl 2-acetamidoacrylate (**4**) (Table 2, the reaction times are close to the shortest reaction times in which 100% conversion could be obtained for the best solvent/catalyst combination – all reactions gave quantitative yields when the reaction time was extended).

Again, **1**-AlTUD-1 in methanol gave results similar to the homogeneous catalyst, as was the case for **2**-AlTUD-1. Interestingly, the asymmetric hydrogenation of **4** with **2**-AlTUD-1 proceeded even in water. The homogeneous catalyst is poorly soluble in this solvent, but when using the immobilised catalyst, reasonable TOF's with moderate ee were obtained (entry 9, Table 2). Thus, immobilisation on AlTUD-1 also broadens the range of solvents for asymmetric hydrogenation.

Once more, significant leaching was observed with MeOH as the solvent. Analogous to the experiments with **3**, this leaching could be reduced by a change of solvent. The leaching could even be suppressed to <0.1%. Loss of Rh is slightly higher for **2**-AlTUD-1, especially when 2-propanol is used as solvent. Surprisingly, the amount of Rh leached in water is considerably lower when compared to methanol (entry 7 and 9, Table 2), although its E_T^N value is higher (1.00). This is due to the hydrophobic character of the cation.

The results with **2**-AlTUD-1 clearly show that the solvent also has an influence on the activity of the catalyst, where the TOF drops from >200 for methanol and ethanol (entries 7 and 8, Table 2) to 69 for 2-propanol (entry 10, Table 2). An obvious explanation could be the different solubility of H_2 in the various solvents. However, there is no correlation between the hydrogen solubility and the TOF (Table 3).

Table 3. The mole fraction solubilities χ_{H_2} of hydrogen and the TOF in the asymmetric hydrogenation of **4** using **2**-AlTUD-1 as catalyst in various solvents.

Solvent	$\chi_{H_2}(10^{-4})$[a]	TOF (mol mol^{-1}h^{-1})
Methanol	15	>200
Ethyl acetate	3.5	75
2-Propanol	2.7	69
Ethanol	2.1	>200
Water	0.14[b]	159

[a] χ_{H_2} at 10 bar H_2 and 25 °C.[17] [b] χ_{H_2} at 1 bar H_2 and 25 °C.[17b]

Unexpectedly, the solvent also had a significant influence on the enantioselectivity of **1**-AlTUD-1 and **2**-AlTUD-1 in the hydrogenation of **4**. While **3** was hydrogenated with excellent enantioselectivity using **1**-AlTUD-1 in all solvents screened, the ee in the reduction of **4** varied between >98% for methanol and 75% for 2-propanol. In contrast, Burk et al. reported essentially identical enantiomeric excesses for the homogeneous hydrogenation of **4** in the solvents methanol, THF, dichloromethane, ethanol, 2-propanol and ethyl acetate.[8] The interaction between the support and the catalyst, which varies for different solvents, seems to influence the enantioselectivity in the hydrogenation of **4**. From these results, MTBE appears to be the ideal solvent when using **1**-AlTUD-1, since it combines high ee with virtually no loss of Rh for either substrate.

With regard to leaching, MTBE is also the solvent of choice for **2**-AlTUD-1, but the enantioselectivity of **2-**AlTUD-1 drops dramatically (entry 11, Table 2). It appears that for this catalyst the solvent dependence of the enantioselectivity is even greater, ranging from 92 to 26 ee%. Once again the homogeneous complex shows a different behavior. Whereas Knowles reports a marginally better efficiency in higher alcohols[1], here the enantiomeric excess decreases with higher alcohols. As for **1**-AlTUD-1, the interaction between support and catalyst seemingly plays a significant role. However, the relation between solvent and enantiomeric excess is not identical for both catalysts, which becomes particularly apparent for MTBE, ethyl acetate and 2-propanol. For **1**-AlTUD-1 2-propanol is the least suitable solvent, while MTBE is the second best. For **2-**AlTUD-1 MTBE is by far the poorest solvent, while it performs reasonably well in

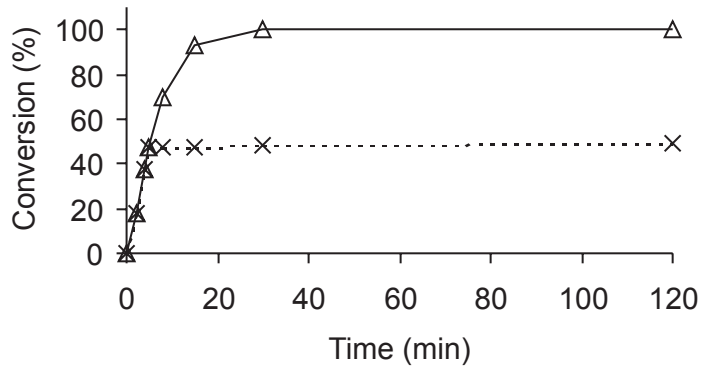


Figure 5. Determination of the heterogeneity of the AlTUD-1 supported catalysts by a filtration test. Lines: (Δ) conversion of **4** in MeOH, with **2**-AlTUD-1 as catalyst (entry 7, Table 2); (X) conversion of **4** in methanol, where the catalyst, **2**-AlTUD-1, was removed after 5 min.

2-propanol.

This dependence of the enantioselectivity on the solvent is unexpected. It is, however, not entirely surprising, since the energy difference responsible for an enantiomeric excess of 99.9% is only ca. 4 kcal/mol.[14] This energy difference is similar to that existing between solvated species, making the ee quite dependent on the solvent.

To confirm that the catalytic hydrogenation is indeed heterogeneous, the residual activity of the filtrate was measured in a filtration test.[18] A few minutes after the start of a normal hydrogenation procedure, **2**-AlTUD-1 was removed and the reaction was continued with the filtrate only. There is no additional conversion after the removal of the catalyst, (Figure 5), which clearly demonstrates that it is the heterogeneous catalyst that catalyses the reaction and that any Rh leached is inactive. This was also confirmed

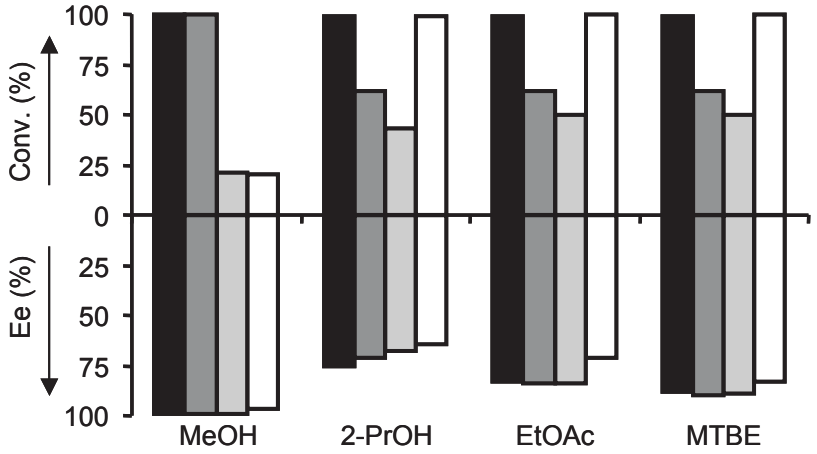


Figure 6. Recycling of **1**-AlTUD-1 in the asymmetric hydrogenation of **4** using conditions described in Table 2. Different bars represent consecutive runs. For run 4 modified conditions were used: $p_{initial}(H_2)$= 5 bar, time 120 min.

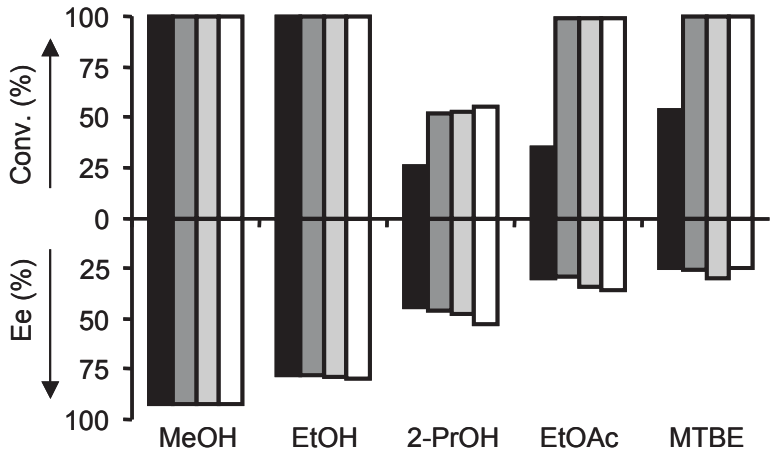


Figure 7. Recycling of **2**-AlTUD-1 in the asymmetric hydrogenation of **4** using conditions described in Table 2. Different bars represent consecutive runs.

for **1**-AlTUD-1.

The recyclability of **1**-AlTUD-1 and **2**-AlTUD-1 has been studied for all experiments described; results are depicted in Figure 6 and Figure 7. In all solvents, with the exception of methanol, the activity of **1**-AlTUD-1 drops in the second run and in the third run. But with prolongation of the reaction time, 100% conversion could again be achieved in the 4th run, stressing the importance of short reaction times when comparing activities in consecutive runs. The enantioselectivity remains almost constant upon re-use and decreases only slightly in run 4, which can partially be explained by the altered reaction conditions. In MeOH the catalyst retains its activity in run 2, but is almost inactive in runs 3 and 4. The different behaviour in MeOH can be ascribed to the considerable leaching in this solvent. However, **2**-AlTUD-1 could be recycled without loss of activity or selectivity, even in MeOH.[‡] In some cases the activity increased after run 1, which could be rationalised by the induction period, needed to form the active species.[19]

The deactivation of **1**-AlTUD-1 cannot only be ascribed to the decreasing amount of Rh in successive runs, since this effect should be equal for **1**-AlTUD-1 and **2**-AlTUD-1. Another reason why this cannot be the only explanation is that the magnitude of deactivation is almost independent of the solvent. Even in MTBE, in which leaching is <0.1%, the same decrease of activity is observed. The dissimilarity in recyclability between the two catalysts should most likely be attributed to their different stabilities. The instability of Rh-DuPHOS complexes has been described earlier.[20] Probably the catalyst decomposes at the end of the reaction or during the recycling procedure.

Conclusions

The ability to readily separate and recycle homogeneous catalysts was achieved by non-covalent anchoring of these types of catalysts on a new aluminosilicate. These new catalysts showed a virtually identical behaviour to their homogeneous counterparts. Upon recycling, the immobilised catalyst **2**-AlTUD-1 displayed neither loss of activity nor of selectivity. **1**-AlTUD-1 was not fully recyclable, which is in line with the known instability of the homogeneous catalyst. The advantage of not having to modify the complex for the immobilisation and the absence of a negative influence of the immobilisation make this methodology fast and reliable for positively charged, proven homogeneous systems.

The choice of solvent is extremely important when applying this methodology. This factor not only influences the activity, but also the enantioselectivity of the catalyst as well as the leaching of Rh. The re-mobilisation of the Rh complex from the support shows an

‡ Mass Transfer limitations were investigated, but did not seem to occur.

exponential increase with increasing polarity of solvent. To minimise leaching, apolar solvents are recommended, but solvents like ethyl acetate and dichloromethane already give satisfactory results. Furthermore, this new immobilisation of the catalysts gives the possibility to combine catalysts and substrates, which are normally incompatible due to different solubilities. Thus, a new carrier material allows the straightforward immobilisation of transition metal catalysts, whilst simultaneously broadening their applicability.

Experimental section

General

Reactions and manipulations involving air-sensitive compounds were performed under an atmosphere of dry nitrogen using standard Schlenk-type techniques. Dry solvents were purchased from Aldrich and flushed with nitrogen for an hour before use. Dimethyl itaconate (DMI) from Acros was purified by crystallisation from methanol by cooling to −78 °C. Bis(1,5-cyclooctadiene)rhodium tetrafluoroborate was prepared according to a literature procedure.[21] Chloro(1,5-cyclooctadiene)rhodium dimer was purchased from Strem. All other reagents were purchased from Aldrich, Acros or Fluka and used without further purification. Hydrogenations were performed in a 100 ml Parr hastelloy C autoclave (A1128HC) or using the Avantium Quick Catalyst Screen platform: 96 small scale pressure reactors with a volume of 8 ml in parallel. These reactors are equipped with a Teflon insert and utilise magnetic stirring. IR spectra were recorded on a Perkin Elmer "Spectrum One" FT-IR spectrometer in KBr from 4000-450 cm^{-1}. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Inova 300 MHz or a Varian VXR-400S spectrometer, relative to TMS. ^{31}P NMR spectra were recorded on a Varian Inova 300 MHz relative to 1% H_3PO_4 and were ^{1}H decoupled. ^{27}Al MAS experiments were performed at 9.4 T on a Varian VXR-400 S spectrometer operating at 104.2 MHz with pulse width of 1ms. 4 mm zirconia rotors were used with a spinning speed set to 6 kHz. The chemical shifts are reported with respect to $Al(NO_3)_3$ as external standard at 0 ppm. The rhodium content of the immobilised catalysts were measured using instrumental neutron activation analysis (INAA), which was performed at the Interfaculty Reactor Institute (IRI), Delft. The "Hoger Onderwijs Reactor" nuclear reactor, with a neutronflux of 10^{17} neutrons s^{-1} cm^{-2}, was used as a source of neutrons and the gammaspectrometer was equipped with a germanium semiconductor as detector. Rhodium leaching was determined by analysing the reaction filtrates with graphite AAS on a Perkin Elmer 4100ZL. N_2 desorption isotherms were measured on a Quantachrome Autosorb-6B at

77 K and X-ray powder diffraction patterns were recorded by using $Cu_{K\alpha}$ radiation on a Philips PW 1840 diffractometer equipped with a graphite monochromator. Conversions of the hydrogenation reactions were determined by ^{1}H-NMR and GC analysis, using a Varian Star 3400 CX GC with a CP wax 52 CB column (50 m x 0.70 mm, df = 2.0 μm), on column injection, FID at 250 °C and Nitrogen as carrier gas (10 psi). Oven program for **3** and its products: 60 °C (2 min), 5 °C/min to 185 °C (3 min). Oven program for **4** and its products: 60 °C (2 min), 10 °C/min to 200 °C (6 min). Enantiomeric excesses in the hydrogenation of **3** were determined by chiral HPLC using a Chiralcel OD column (250 x 4.6 mm) with 2-propanol/ hexane (2:98) as eluens, a flow of 0.8 ml/min and UV detection at 215 nm. Retention times (min): (*R*)-dimethyl 2-methylsuccinate (10), dimethyl itaconate (15) and (*S*)-dimethyl 2-methylsuccinate (19). Enantiomeric excesses in the hydrogenation of **4** were determined by chiral GC using a Shimadzu GC-17A, equipped with a Chiralsil DEX CB column (25 m x 0.32 mm, df = 0.25 μm), He as carrier gas, split injector (36/100) at 220 °C and FID at 220 °C. Retention times (min) at 95 °C isotherm: 2-acetamidoacrylate (5.4), (*S*)-methyl 2-acetamidopropanoate (7.5) and (*R*)-methyl 2-acetamidopropanoate.

Synthesis of [Rh^1(cod)((*R*,*R*)-MeDuPHOS)]BF_4 (1)

[Rh((*R,R*)-MeDuPHOS)(COD)]BF_4 was prepared by a slightly modified literature procedure.[8] [Rh(cod)$_2$]BF_4 (0.12 g, 0.29 mmol) and (*R,R*)-MeDuPHOS (0.09 g, 0.29 mmol) were dissolved in CH_2Cl_2 (8 ml) and stirred for 30 min. Slowly diethyl ether (28 ml) was added, yielding an orange precipitate, which was collected by filtration. Yield 0.10 g (59%)

^{1}H NMR ($CDCl_3$): δ 1.03 (dd, ^3J(H,H) = 6.6, ^3J(P,H) = 15.0, 6H, CH_3), δ 1.46 (dd, ^3J(H,H) = 7.2 Hz, ^3J(P,H) = 18.3, 6H, CH_3), δ 1.63 (m, 4H, CH_2), δ 1.93 (m, 2H, CH, CH_2), δ 2.30-2.80 (m, 12 H, CH_2, CH), δ 2.61 (m, 2H, CH, CH_2), δ 2.71 (m, CH, CH_2), δ 5.07 (br, 2H, CH=C), δ 5.63 (br, 2H, CH=C), δ 7.70 (m, 4H, Ph); ^{31}P NMR ($CDCl_3$): δ 77.15 (d, ^1J(Rh,P) = 148.1).

Synthesis of [Rh^1(cod)((*S*,*S*)-DiPAMP)]BF_4 (2)

[Rh(cod)((*S,S)*-DiPAMP)]BF_4 was synthesised according to the procedure of Knowles et al.[9] To (*S,S)*-DiPAMP (0.50 g, 1.1 mmol) in 90% MeOH was added [Rh(cod)Cl]$_2$ (0.27 g, 0.55 mmol). The slurry became orange and, after 1h stirring, gave a red-orange solution. The complex was precipitated by the slow addition of $NaBF_4$ (0.18 g) in water (1.37 ml). After 1 h of additional stirring a red-orange solid was obtained by filtration. The solid was washed with water and recrystallised from ethanol. Yield 0.69 g (84%)

^{1}H NMR ($CDCl_3$): δ 2.32-2.39 (m, CH_2, 12H), δ 3.62 (s, 6H, OCH_3), δ 4.64 (br, 2H, CH=C), δ 5.30 (br, 2H, CH=C), δ 6.93-7.04 (m, 6H, Ar), δ 7.50 (m, 2H, Ar), δ 7.66 (m, 6H, Ar), δ 7,97 (m, 4H, Ar); ^{31}P NMR ($CDCl_3$): δ 48.5 (dd, J(Rh,P) = 151 Hz, ^{2}J(P,P) = 38 Hz)

Synthesis of methyl 2-acetamidoacrylate (4)

2-Acetamidoacrylic acid was methylated by the procedure of Gladiali et al.[22] 2-Acetamidoacrylic acid (6.45 g, 50 mmol) was added to acetone (300 ml), followed by K_2CO_3 (13.82 g, 100 mmol). The mixture was stirred mechanically and heated to 60-65°C. Iodomethane (10.64 g, 75 mmol) was added slowly and the suspension was stirred overnight at the same temperature. The precipitate was removed by filtration and the acetone was removed by evaporation. The residue was dissolved in a small amount of ethyl acetate/petroleum ether (7:3) and filtered over silica. The volatiles were removed by evaporation and the residue was crystallised from *n*-hexane. Yield 5.86 g (82%) of a colourless solid.

m.p: 50-51 °C; ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3H, CH_3CO), δ 3.85 (s, 3H, OCH_3), δ 5.88 (d, ^{2}J(H,H) = 1.2, 1H, *H*CH), δ 6.60 (d, ^{2}J(H,H) = 1.2, 1H, HC*H*); ^{13}C NMR ($CDCl_3$): δ 24.7 (CH_3(CO)), δ 53.0 (CH_3O), δ 108.7 (CH_2), δ 130.9 (CCH_2), δ 164.6 ($COOCH_3$), δ 168.9 ((CO)N)

Preparation of AlTUD-1

At 45 °C aluminium isopropoxide (6.12 g, 0.03 mol) was added into a mixture of absolute ethanol (27.6 g, 0.60 mol), anhydrous 2-propanol (27.05 g, 0.45 mol) and tetraethyl orthosilicate (25.0 g, 0.12 mol) under stirring. This was followed by the addition of tetraethylene glycol (29.1 g, 0.15 mol). Finally a solution of absolute ethanol (27.6 g, 0.60 mol), anhydrous 2-propanol (27.05 g, 0.45 mol) and H_2O (5.41 g, 0.30 mol) was added dropwise to this mixture. The resulting mixture was stirred for ½ h at RT, followed by aging without stirring for 6h, also at RT. The obtained wet gel was dried at 70 °C for 21 h, at 98 °C for 2 h and hydrothermally treated at 160 °C for 3-21 h in an autoclave with Teflon insert. Finally the solids were calcined (with 1 °C/min to 550 °C, 4 h at 550 °C, with 1 °C/min to 600, 10 h at 600 °C). Elemental analysis gave a Si/Al ratio of 3.8-4. $Al_{tetrahedral}$/Si ratio = 0.11 determined with ^{27}Al MAS (see Figure 3). For other analyses see Figure 2 in results and discussion.

Immobilisation procedure for [Rh^{I}(cod)((*R*,*R*)-MeDuPHOS)]BF$_4$ (1)

AlTUD-1 (1.1 g) was dried at 200 °C under vacuum for 2h. To the dried support was added 2-propanol (45 ml). After 30 min stirring, **1** (88.4 mg, 0.146 mmol) in 2-propanol (20 ml) was added and the resulting suspension was stirred for 3h. The solid was collected by filtration and washed thoroughly with portions of 2-propanol (30 ml) until the washings were colourless (approx. 5 times). Finally the catalyst was dried at 55 °C under vacuum for 2h. Rh loading was determined by INAA: 11.5 mg Rh/g support, which corresponds to an $Al_{tetrahedral}$/Rh ratio of approximately 10.

Immobilisation procedure for [Rh^{I}(cod)((*S*,*S*)-DiPAMP)]BF$_4$ (2)

AlTUD-1 (1.1 g) was dried at 200 °C under vacuum for 2h. To the dried support was added ethanol absolute (45 ml). After 30 min stirring, **2** (166.0 mg, 0.219 mmol) in absolute ethanol (20 ml) was added and the resulting suspension was stirred for 3h. The solid was collected by filtration and soxhlet extracted with absolute ethanol overnight. Finally the catalyst was dried at 55 °C under vacuum for 2h. Rh loading was determined by INAA: 12.2 mg Rh/g support, which corresponds to an $Al_{tetrahedral}$/Rh ratio of approximately 10.

Typical Hydrogenation reaction

All hydrogenation experiments were performed with 0.1 g of immobilised catalyst (~1 w% Rh). The catalyst was transferred to the autoclave under a nitrogen atmosphere, followed by 50 ml of substrate solution (concentrations and solvents given in Table 1 and Table 2). The sealed autoclave was purged with hydrogen by pressurizing to 7 bars while stirring at 300 rpm, followed by release of pressure. This cycle was repeated 5 times and finally the desired pressure was applied and the stirring speed was increased to 1000 rpm. At the end of the reaction the remaining hydrogen pressure was released and the autoclave was purged three times with nitrogen, pressurizing to 5 bars while stirring at 300 rpm, followed by release. Under a nitrogen atmosphere the solution was separated from the catalyst by a syringe equipped with an acrodisc GF syringe filter (1.0 μm pore size). After removal of the solution, fresh substrate solution was added to the used catalyst and the hydrogenation procedure was repeated. All catalysts were reused in this way several times.

Hydrogenation using the Avantium Quick Catalyst Screen

The small scale pressure reactors were charged with **1**-AlTUD-1 (6 mg), followed

by 1.5 ml of a 0.1 M solution of **3**. The following solvents were screened in parallel: methanol, ethyl acetate, dichloromethane, 2-propanol, MTBE and toluene. The reactors were simultaneously pressurized to 5 bars, followed by release of pressure to purge the system with hydrogen. This cycle was repeated 5 times, after which the reactors were again pressurized to 5 bars and stirred at 1500 rpm for 1 hour.

Filtration test

To determine the heterogeneity of the reaction the activity of the filtrate was measured using a filtration test. A hydrogenation reaction was carried out according to the typical hydrogenation procedure as described above. After 5 min (17-25% of normal reaction time) the hydrogenation reaction was stopped by releasing the hydrogen pressure and purging with nitrogen. The solution was withdrawn from the autoclave with a syringe equipped with an acrodisc GF syringe filter (1.0 μm pore size) and the solution was stored under nitrogen. The catalyst was removed from the autoclave and the stored solution was transferred back into the autoclave under a nitrogen atmosphere. Then the hydrogenation reaction was continued using the typical hydrogenation procedure. After filtration no additional conversion was observed.

References

[1] W. S. Knowles, *Acc. Chem. Res.*, **1983**, *16*, 106-112.

[2] a) I. Ojima, *Catalytic Asymmetric Synthesis, Second Edition*, Wiley-VCH, New York, **2000**; b) H.U. Blaser, E. Schmidt, Asymmetric Catalysis on Industrial Scale, Wiley-VCH, Weinheim, 2004.

[3] a) Q. H. Fan, Y. M. Li, A. S. C. Chan, *Chem. Rev.*, **2002**, *102*, 3385-3466; b) N. E. Leadbeater, M. Marco, *Chem. Rev.*, **2002**, *102*, 3217-3273; c) W. Dumont, J. C. Poulin, T.-P. Dang, H. B. Kagan, *J. Am. Chem. Soc.*, **1973**, *95*, 8295-8299; d) T. Ohkuma, H. Takeno, Y. Honda, R. Noyori, *Adv. Synth. Catal.*, **2001**, *343*, 369-375.

[4] R. Augustine, S. Tanielyan, S. Anderson, H. Yang, *Chem. Commun.*, **1999**, 1257-1258.

[5] H. H. Wagner, H. Hausmann, W. F. Hölderich, *J. Catal.*, **2001**, *203*, 150-156.

[6] W. P. Hems, G. J. Hutchings, WO 2002036261, **2002**.

[7] F. M. de Rege, D. K. Morita, K. C. Ott, W. Tumas, R. D. Broene, *Chem. Commun.*, **2000**, 1797-1798.

[8] M. J. Burk, J. E. Feaster, W. A. Nugent, R. L. Harlow, *J. Am. Chem. Soc.*, **1993**,

115, 10125-10138.

[9] B. D. Vineyard, W. S. Knowles, M. J. Sabacky, G. L. Bachman, D. J. Weinkauff, *J. Am. Chem. Soc.*, **1977**, *99*, 5946-5952.

[10] a) J. C. Jansen, Z. Shan, L. Marchese, W. Zhou, N. van der Puil, T. Maschmeyer, *Chem. Commun.*, **2001**, 713-714; b) Z. Shan, T. Maschmeyer, J. C. Jansen, WO 2001017901, **2001**.

[11] J. S. Beck, J. C. Vartuli, W. J. Roth, M. E. Leonowicz, C. T. Kresge, K. D. Schmitt, C. T. W. Chu, D. H. Olson, E. W. Sheppard, S. B. McCullen, J. B. Higgins, J. L. Schlenker, *J. Am. Chem. Soc.*, **1992**, *114*, 10834-10843.

[12] a) Z. Shan, E. Gianotti, J. C. Jansen, J. A. Peters, L. Marchese, T. Maschmeyer, *Chem. Eur. J.*, **2001**, **7**, 1437-1443; b) Z. Shan, J. C. Jansen, L. Marchese, T. Maschmeyer, *Microporous Mesoporous Mater.*, **2001**, *48*, 181-187.

[13] Z. Shan, J. C. Jansen, W. Zhou, T. Maschmeyer, *Applied Catalysis, A: General*, **2003**, *254*, 339-343.

[14] a) C. R. Landis, J. Halpern, *J. Am. Chem. Soc.*, **1987**, *109*, 1746-1754; b) S. Feldgus, C. R. Landis, *J. Am. Chem. Soc.*, **2000**, *122*, 12714-12727.

[15] D. Heller, H.-J. Drexler, A. Spannenberg, B. Heller, J. You, W. Baumann, *Angew. Chem.*, **2002**, *114*, 814-817; *Angew. Chem. Int. Ed.*, **2002**, *41*, 777-780.

[16] a) C. Reichardt, *Chem. Rev.*, **1994**, *94*, 2319-2358; b) C. Reichardt, E. Harbusch-Goernert, *Liebigs Ann. Chem.*, **1983**, 721-743.

[17] a) E. Toukoniitty, P. Mäki-Arvela, J. Kuusisto, V. Nieminen, J. Päivärinta, M. Hotokka, T. Salmi, D. Y. Murzin, *J. Mol. Catal. A: Chem*, **2003**, *192*, 135-151; b) P. Purwanto, R. M. Deshpande, R. V. Chaudhari, H. Delmas, *J. Chem. Eng. Data*, **1996**, *41*, 1414-1417; c) Q. Liu, F. Takemura, A. Yabe, *J. Chem. Eng. Data*, **1996**, *41*, 1141-1143.

[18] H. E. B. Lempers, R. A. Sheldon, *J. Catal.*, **1998**, *175*, 62-69.

[19] D. Heller, H.-J. Drexler, J. You, W. Baumann, K. Drauz, H.-P. Krimmer, A. Börner, *Chem. Eur. J.*, **2002**, *8*, 5196-5203.

[20] a) S.-G. Lee, Y. J. Zhang, J. Y. Piao, H. Yoon, C. E. Song, J. H. Choi, J. Hong, *Chem. Commun.*, **2003**, 2624-2625; b) S. Guernik, A. Wolfson, M. Herskowitz, N. Greenspoon, S. Geresh, *Chem. Commun.*, **2001**, 2314-2315.

[21] T. G. Schenck, J. M. Downes, C. R. C. Milne, P. B. Mackenzie, T. G. Boucher, J. Whelan, B. Bosnich, *Inorg. Chem.*, **1985**, *24*, 2334-2337.

[22] S. Gladiali, L. Pinna, *Tetrahedron Asymm.*, **1991**, *2*, 623-632.

3

Efficient Immobilisation of Rh-MonoPhos on the Aluminosilicate AlTUD-1

Introduction

Recently it was shown that rhodium complexes with chiral monodentate ligands such as phosphoramidites (MonoPhos)[1], phosphites[2] or phosphonites[3] are very powerful tools for reductions[4] and conjugate additions.[5] Contrary to common expectations, these catalysts are highly enantioselective. Since the development of (*R,R*)-DIOP in 1971,[6] it was assumed that a conformationally rigid symmetric bidentate diphosphine ligand is required for effective asymmetric induction.[7] The great advantage of these monodentate ligands compared to bidentate ones is their greater ease of synthesis. However, although more accessible, there are intrinsic difficulties with regard to recycling as the system is homogeneous in nature.

The immobilisation of transition metal catalysts is a well-established approach to improve their recyclability.[8] Although the Rh-MonoPhos catalyst was only reported late in 2000,[1] its first immobilisation was already described by 2003: complexes prepared from p-vinylaniline and 3-vinyl-8-quinoline containing ligands (homogeneous and polystyrene incorporated) were investigated.[9] The homogeneous

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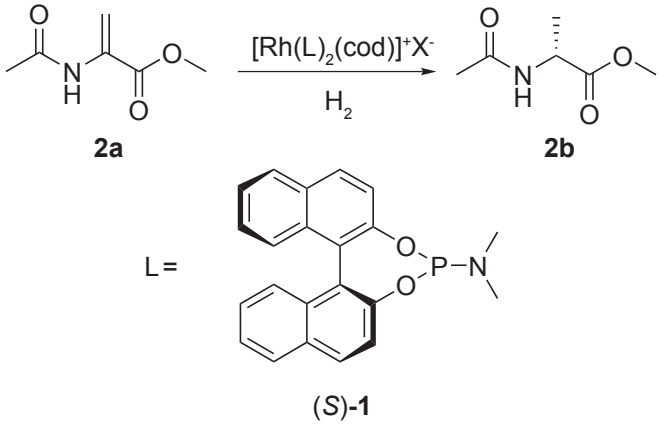
and the heterogeneous pair of Rh-catalysts, performed equally well in the reduction of itaconic acid and **2a** (Scheme 1). Although the target of recyclability was met, the ligand modification had a negative influence on the catalyst. Both pairs of Rh-catalysts, gave enantioselectivities of only approx. 70%. This is well below the ee's of > 95% that are normally achieved with the archetypal MonoPhos **1** as ligand.[1]



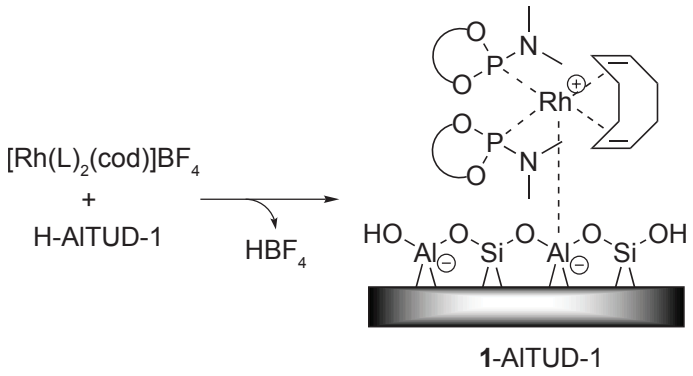
Scheme 1. Model reaction for the Rh-phosphoramidite catalysed asymmetric hydrogenation.

It has been demonstrated that ionic complexes can successfully be immobilised on ionic carriers. An important advantage of this methodology is that no ligand modifications are necessary. Moreover, the heterogenised catalysts tend to retain their full activity and selectivity.[8,10] In this way, the homogenous catalyst can be immobilised without the need of a difficult and time-consuming modification, making this approach very versatile. We recently developed as an ionic carrier a Brønsted acidic aluminosilicate, AlTUD-1, with the ideal characteristics for catalyst immobilisation: a three-dimensional mesoporous structure and a high surface area. It was applied successfully in the ionic immobilisation of two well-established Rh hydrogenation catalysts, Rh-DuPHOS and Rh-DIPAMP.[11] Both could readily be re-used and only very little leaching was observed, proving the potential of AlTUD-1.

Based on these results we reasoned that it should be possible to immobilise the ionic Rh-MonoPhos catalyst without modifications of the ligand, while at the same time ensuring recyclability and possibly expanding the range of solvents in which the catalyst can be used.

Results and Discussion

The enantiopure Rh precursor complex, [Rh(**1**)$_2$(cod)]BF$_4$, was immobilised on AlTUD-1 by a straightforward ion-exchange procedure yielding the supported catalyst (**1**-AlTUD-1, 1wt% Rh; Scheme 2).[11]



Scheme 2. Immobilisation of [Rh(L)$_2$(cod)]BF$_4$, wherein L is (*S*)-**1**, on AlTUD-1 according to ref 11.

This heterogeneous catalyst was tested in the asymmetric hydrogenation of methyl-2-acetamidoacrylate (**2a**). The comparison between the homogeneous (X^- = BF_4^-) catalyst and **1**-AlTUD-1 in the same solvent CH_2Cl_2 (Table 1, entries homogeneous and 1) reveals that the high activity of the catalyst is retained upon immobilisation (TOF of ~2100 h^{-1}). The immobilised catalyst also shows good enantioselectivity, although somewhat lower than the homogeneous catalyst. By changing to other solvents, such as 2-propanol (entries 5 and 6) and MTBE (entries 7 and 8), excellent enantioselectivities (ee up to 97%) are regained. Entry 4 shows that high TON (up to 7100) can be achieved with **1**-AlTUD-1 without compromising the enantiomeric excess. Surprisingly the hydrogenation even proceeds in water, a solvent not commonly used for hydrogenations, with excellent enantioselectivity (95%) and good activity (entries 9 and 10). Normally, asymmetric hydrogenations in water require specially designed water-soluble ligands to proceed and there are only few examples were the obtained enantioselectivities can match those of the corresponding transformation in organic solvents.[12] By immobilising [Rh(**1**)$_2$(cod)]$^+$ on AlTUD-1 the problem of solubility of the Rh complex in water is circumvented, while maintaining the catalyst's activity and outstanding selectivity.

Table 1. Asymmetric hydrogenation of **2a** using 1-AlTUD-1 as catalyst.[a]

Entry	Solvent	Time (min)	Conversion (%)	Ee[c] (%)	Rh loss (mg/L) [%][d]
Homogeneous	CH_2Cl_2	7	100	97	-
1	CH_2Cl_2	7	96	83	0.76 [2.9]
2	EtOAc	7	70	92	1.27 [5.5]
3	EtOAc	11	100	92	1.04 [4.5]
4[b]	EtOAc	1200	71	94	1.08 [35]
5	2-PrOH	7	39	91	2.30 [9.4]
6	2-PrOH	25	100	97	2.06 [8.4]
7	MTBE	7	11	94	0.32 [1.3]
8	MTBE	30	91	94	0.45 [1.8]
9	Water	35	75	95	0.21 [0.6]
10	Water	60	100	95	0.11 [0.3]

[a] 5 bar H_2, 50 ml solvent, [**2a**] = 0.05 M, 0.1g catalyst with 1wt% Rh.

[b] 10 bar H_2, 50 ml solvent, [**2a**] = 0.2 M, 0.01g catalyst with 1wt% Rh.

[c] ee's were determined as described in ref 11.

[d] Percentage of total amount of Rh determined by AAS of the filtrate.

With regard to leaching of the catalyst from the support, water again is an excellent solvent (entries 9 and 10). In water the loss of Rh is less than 1%. MTBE, too, is very suitable in this respect, with a loss of Rh of less than 2%. In 2-propanol, however, leaching is considerable, i.e. almost 10%. The heterogeneity of the system has been probed by a filtration test.[13] Using the conditions of entry 3; the catalyst was removed under inert atmosphere by filtration after 4 min, and the reaction was continued using the remaining filtrate. This test revealed that the system is indeed heterogeneous: no activity was found in the filtrate.

$[Rh(\mathbf{1})_2(cod)]^+$ was immobilised on AlTUD-1 in order to obtain a recyclable Rh-MonoPhos catalyst. The results of the recycling experiments are given in Figure 1. The reaction times chosen are close to the minimum time needed to obtain 100% conversion (see Table 1). From this data, it becomes apparent that the catalyst is recyclable without any appreciable loss of activity and enantioselectivity in almost all solvents. Even in water, the catalyst can be re-used without significant deterioration, proving that the phosphoramidite **1** (MonoPhos) is very stable under aqueous reaction conditions. The increase of activity after run 1, which is observed for CH_2Cl_2 and water, can be explained by the slow reduction of the cod ligand; only after its complete removal the catalyst displays its full activity. In several cases the enantioselectivity also increased upon recycling. For CH_2Cl_2, MTBE and EtOAc the ee improved from 83 to 88%, 91 to 94% and 92 to 94% respectively. This might also be due to changes of the catalytic

species concurrent with the reduction of cod. In 2-propanol, however, the activity decreases significantly upon reuse. The enantioselectivity also decreases, although less dramatically than the activity (from 97% to 92%). The reduced activity can be rationalised by the considerable leaching in this solvent.

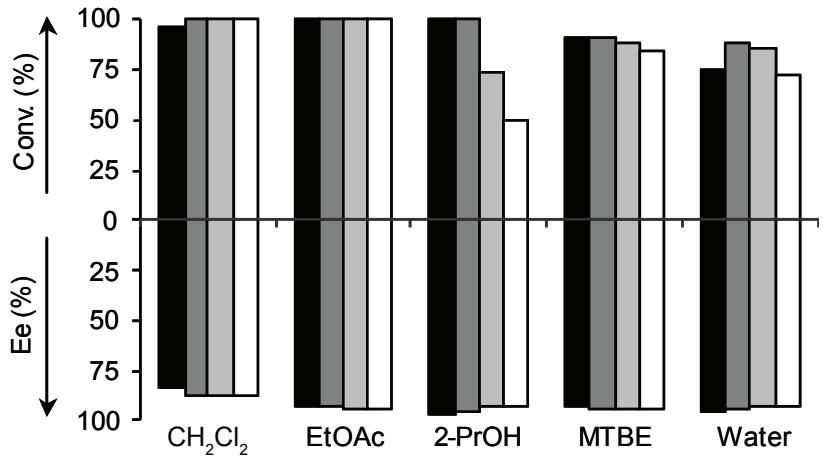


Figure 1. Recycling of 1-AlTUD-1 in the asymmetric hydrogenation of **2a** using conditions a and the reaction times in line with those for 100 % conversion in Table 1. Different bars represent consecutive runs.

Conclusion

We have demonstrated that the Rh complexes with monodentate ligands can be immobilised via ionic interaction with the same success as complexes based on bidentate ligands. The activity is hardly affected by the support and the excellent enantioselectivity of these catalysts is maintained, even upon re-use. This, once more, demonstrates the broad scope of immobilisation via ionic interactions and of AlTUD-1 as carrier material. The ability to use the heterogeneous catalyst in water, a solvent normally considered as difficult for asymmetric hydrogenations, significantly broadens the scope of the reduction, enabling its application under ecologically sound conditions.

References

[1] M. van den Berg, A. J. Minnaard, E. P. Schudde, J. van Esch, A. H. M. de Vries, J. G. de Vries and B. L. Feringa, *J. Am. Chem. Soc.*, **2000**, *122*, 11539-11540.

[2] M. T. Reetz and G. Mehler, *Angew. Chem. Int. Ed.*, **2000**, *39*, 3889-3890.

[3] C. Claver, E. Fernandez, A. Gillon, K. Heslop, D. J. Hyett, A. Martorell, A. G.

Orpen and P. G. Pringle, *Chem. Commun.*, **2000**, 961-962.

[4] M. van den Berg, A. J. Minnaard, R. M. Haak, M. Leeman, E. P. Schudde, A. Meetsma, B. L. Feringa, A. H. M. de Vries, C. E. P. Maljaars, C. E. Willans, D. Hyett, J. A. F. Boogers, H. J. W. Henderickx and J. G. de Vries, *Adv. Synth. Catal.*, **2003**, *345*, 308-323.

[5] A. Duursma, L. Lefort, J. A. F. Boogers, A. H. M. de Vries, J. G. de Vries, A. J. Minnaard and B. L. Feringa, *Org. Biomol. Chem.*, **2004**, *2*, 1682-1684.

[6] (a) T.-P. Dang and H. B. Kagan, *Chem. Commun.*, **1971**, 481; (b) H. B. Kagan and T.-P. Dang, *J. Am. Chem. Soc.,* **1972**, *94*, 6429.

[7] T. Ohkuma, M. Kitamura and R. Noyori, in Catalytic Asymmetric Synthesis, 2nd ed.; I. Ojima Ed., Wiley, New York, **2000**; (b) M. J. Burk and F. Bienewald in Transition Metals for Organic Synthesis, M. Beller and C. Bolm, Eds., Wiley-VCH, Weinheim **1998**, Vol. 2.

[8] P. McMorn and G. J. Hutchings, *Chem. Soc. Rev.*, **2004**, *33*, 108-122.

[9] S. Doherty, E. G. Robins, I. Pal, C. R. Newman, C. Hardacre, D. Rooney and D. A. Mooney, *Tetrahedron Asymm.*, **2003**, *14*, 1517-1527.

[10] (a) R. Augustine, S. Tanielyan, S. Anderson and H. Yang, *Chem. Commun.*, **1999**, 1257-1258; (b) H. H. Wagner, H. Hausmann and W. F. Hölderich, *J. Catal.*, **2001**, *203*, 150-156; (c) F. M. de Rege, D. K. Morita, K. C. Ott, W. Tumas and R. D. Broene, *Chem. Commun.*, **2000**, 1797-1798.

[11] C. Simons, U. Hanefeld, I. W. C. E. Arends, R. A. Sheldon and T. Maschmeyer, *Chem. Eur. J.*, **2004**, *10*, 5829.

[12] (a) U. M. Lindstrom, *Chem. Rev.*, **2002**, *102*, 2751-2772; (b) R. Selke in Asymmetric Catalysis on Industrial Scale: Challenges, Approaches and Solutions, H. U. Blaser and E. Schmidt, Eds., Wiley-VCH, **2004**, Chapter 2.

[13] H. E. B. Lempers and R. A. Sheldon, *J. Catal.*, **1998**, *175*, 62-69.

4

Comparison of Supports for the Electrostatic Immobilisation of Asymmetric Homogeneous Catalysts

Introduction

The immobilisation of homogeneous catalysts on insoluble supports is a well-established methodology which enables the combination of the advantages of a homogeneous catalyst with those of a heterogeneous catalyst.[1, 2] Heterogeneous catalysts have the benefit of easy separation and recyclability, while homogeneous catalysts display, in general, higher activities and superior selectivities. The merger of these two worlds is especially important in enantioselective catalysis, since this field is dominated by homogeneous catalysts. These catalysts normally consist of noble, albeit toxic, metals and expensive chiral ligands. Therefore, catalyst recyclability is a major concern. One of the most attractive strategies for immobilisation relies on electrostatic interactions between the catalyst and the support rather than on covalent tethering, since it circumvents the need for time-consuming and often difficult ligand modification.[3, 4]

The important influence of these electrostatic interactions on activity and selectivity has long been recognized in homogeneous catalysis. One of the most striking examples

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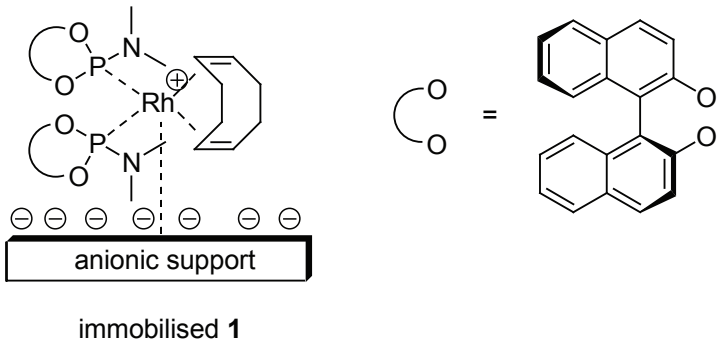
is the effect of different halide ligands on the rhodium-catalysed asymmetric ring opening of oxabenzonorbornadiene,[5] where the enantiomeric excess increased from 45% to 98% simply by replacing Cl^- with I^-. Very recently Pfaltz *et al.* also demonstrated the importance of the anion for enantioselective hydrogenation.[6] His study revealed that in the asymmetric hydrogenation of (*E*)-1,2-diphenyl-1-propene with Ir-PHOX the reaction rate strongly decreases across the series $[Al\{OC(CF_3)_3\}_4]^- > [B(C_6F_5)_4]^- > PF_6^- >> BF_4^- > CFSO_3^-$. Surprisingly, in the electrostatic anchoring of asymmetric homogeneous catalysts the study of the anion/anionic support has been neglected. While most authors compare their immobilised catalyst with its homogeneous counterpart, the comparison with other anionic support materials is lacking. The absence of a systematic study on the influence of the anionic support becomes even more surprising, when recognizing that the anion also affects characteristics like leaching, diffusion and the embedding of the catalyst. This absence of data leaves an important question, what is the best support for this application, unanswered.



immobilised **1**

Figure 1. Schematic representation of Rh-MonoPhos immobilised on an anionic support.

To fill this void for the heterogenisation of asymmetric hydrogenation catalysts, Rh-MonoPhos (**1**) (depicted in Figure 1), was immobilised on four different anionic carrier materials and the behaviour of the four resulting heterogeneous catalysts was systematically investigated for the hydrogenation of methyl 2-acetamidoacrylate (MAA). We selected the three-dimensional mesoporous aluminosilicate, AlTUD-1[4, 7] to represent the silicate-based materials used for immobilisation purposes. As second support material we selected the Augustine system, phosphotungstic acid (PW) on alumina, since it set the standard for ionic anchoring. Nafion was selected to represent the ionic exchange resins, as it showed promising results in the field of asymmetric hydrogenation.[8] A drawback of this resin is its low surface area and for this reason

SAC-13 was selected as an additional support. SAC-13 is a commercially available Nafion silica composite,[9] which largely overcomes the above mentioned drawback of Nafion.

Results and Discussion

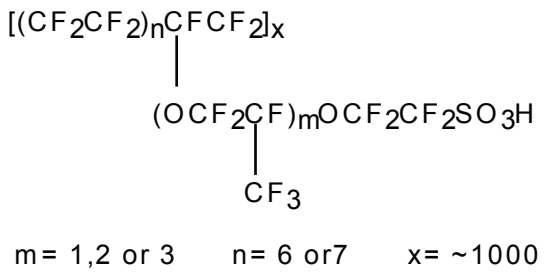
Support structures

The four carriers selected differ significantly in their structure. The first carrier, AlTUD-1 is a purely inorganic material. This aluminosilicate, with a Si/Al ratio of 4, has a three-dimensional mesoporous structure and shows a maximum in the pore size distribution at 150 Å. The effective $Si/Al_{tetrahedral}$ ratio for the immobilisation of cations is 9. In addition, AlTUD-1 has a large surface area of ca. 600 m^2/g and a total pore volume of 1.1 cm^3/g. Details of the analysis are given elsewhere.[4]

The second system consists of a heteropoly acid, in this case phosphotungstic acid, $H_3PO_{40}W_{12}$ (PW), supported on alumina.[3] Alumina was preferred over silica, since prior unpublished investigations showed that the interaction between the heteropoly acid and silica is inadequate, resulting in considerable leaching of PW from the surface. In contrast the alumina-PW interaction was much better and no leaching did occur for alumina. Instead of the commercial alumina, a mesoporous alumina, which structurally closely resembles AlTUD-1 in structure, was chosen to enable a more accurate comparison between these two inorganic supports. This material, denoted as TUD-Al_2O_3, was prepared by a similar procedure to that used for AlTUD-1,[10] which resulted in a surface area of 313 m^2/g and a maximum in the pore size distribution at 60 Å. Since it is described in the literature, that the presence of PW was crucial for the immobilisation of Rh complexes on alumina, TUD-Al_2O_3 was not selected as a separate support.[11, 12] The ratio of PW to TUD-Al_2O_3 was fixed at 19 w% PW. This system will be referred to as PWTUD.

Nafion as the solid equivalent of triflic acid is the only fully organic support of the four. The triflate group ($CF_3SO_3^-$) is attached to the Teflon-backbone (Scheme 1) of Nafion. The resin has a surface area of typically 0.02 m^2/g[9] and an equivalent weight of 1100 (molar mass divided by its valence). It consists of clusters (diameter 40-50 Å) of the sulphonate-terminated groups separated by channels (diameter 10 Å) within the hydrophobic matrix.[13] Although the surface area of Nafion is low the triflate anion, a very popular and versatile anion in homogeneous catalysis, makes it a promising carrier.

SAC-13 overcomes the drawback of low surface area of Nafion by dispersing



Scheme 1. Chemical structure of Nafion.

nanosized particles (200-600 Å) of Nafion in a porous silica matrix. The surface area of the composite used was 102 m^2/g with a very broad pore size distribution, ranging from 60 Å to 2000 Å, as determined by N_2 physisorption. The Nafion content of the material, which was analysed by thermogravimetric analysis (TGA), was 16w% which results in an equivalent weight of ~6900.

Initial experiments with Nafion demonstrated that the strongly acidic sites had a negative influence on the catalyst's performance and consequentially only the sodium forms of Nafion and SAC-13 were used.

Immobilisation of Rh-MonoPhos

Rh-MonoPhos was immobilised by straightforward ion exchange. In this procedure an anionic site/Rh ratio in the range of 7-10 was selected so as to ensure the re-immobilisation of any Rh species mobilised during catalysis and also to prevent catalyst clustering. The only exception to this is PWTUD: for this support the procedure of Augustine et al. was adopted,[14] which utilizes a PW/Rh ratio of 1, corresponding to 3 negative charges per Rh.

Table 1. The results of the immobilisation of Rh-MonoPhos on various supports.[a]

Support	Rh loading (mg/g)	Rh Uptake[b] (%)	Anionic site/Rh
AlTUD-1	12	66	15
PWTUD[c]	1.4	29	9
SAC-13	1.4	66	11
Nafion	0.5	4	175
Nafion[d]	7.9	52	15

[a] Immobilisations were performed in 2-propanol, see experimental section for details.

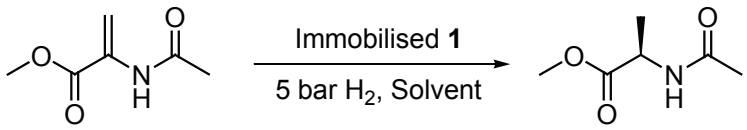
[b] The percentage of Rh immobilised relative to the Rh amount during the ion exchange.

[c] W loading = 90 mg/g.

[d] Immobilisation was performed in methanol.

The results of the immobilisations are given in Table 1. The immobilisations were performed in accordance with procedures from the literature, i.e. our previously published procedure[4] for AlTUD-1, SAC-13 and Nafion, and the procedure for Augustine *et al.*[14] for PWTUD. This resulted in Rh loadings varying from 1 w% for AlTUD-1 to 0.05 w% for Nafion. AlTUD-1 is the best support with respect to Rh loading as well as Rh uptake. These high values are consistent with a material that was developed for the purpose of catalyst immobilisation. The extremely low loading of Nafion however was surprising. This is emphasised by the anion to Rh ratio. While all other supports achieved comparable anion to Rh ratios, ranging from 9 to 15, Nafion displayed a ratio at least 10 times higher. Especially the large difference with SAC-13 is noteworthy, since it contains the same type of anionic sites. This strongly indicates that the low loading of Nafion is due to the poor accessibility of the triflate groups. The dependence of the morphology of Nafion on the solvent is well-known.[13] In an effort to improve the accessibility of the negative charges and increase the Rh loading, the immobilisation on Nafion was repeated in methanol, a solvent which causes more swelling of the resin.[15] Using this solvent, the Rh content could be increased 16-fold and an anion to Rh ratio equal to the other supports was obtained. As mentioned in the experimental section, the catalysts resulting from immobilisation in methanol and 2-propanol will be referred to as NafionC1 and NafionC3 respectively.

PWTUD is a three-component system and, therefore, not only the Rh-loading but also the loading of PW is important. During ion exchange 75 % of the PW present in the mixture settles on the alumina, giving a PW loading of 117 mg/g. Although the Rh uptake is quite low (29 %), it has the lowest anion to Rh ratio, i.e. 3. This is in line with the results described by Augustine.[14]



Scheme 2. Test reaction for asymmetric hydrogenation catalysed by immobilised **1.**

Hydrogenation with supported Rh-MonoPhos

The different catalysts were tested in the hydrogenation of methyl 2-acetamidoacrylate (MAA, Scheme 2) and the results are given in Tables 2 to 5. The reported turnover frequencies (TOF) are all lower estimates as they are derived from the conversion at the corresponding reaction time. Not all catalysts were tested in CH_2Cl_2 , since it is an

Table 2. Results of the asymmetric hydrogenation of MAA catalysed by **1** immobilised on AlTUD-1.[a]

Entry	Solvent	Time (min)	Conv. (%)	TOF[b] ($molmol^{-1}h^{-1}$)	ee (%)	Rh loss (mg/L) [%][c]
Homogeneous	CH_2Cl_2	7	100	>1700	97	-
1	MTBE	7	20	350	94	0.32 [1.3]
2	MTBE	30	91	450	94	0.45 [1.8]
3	EtOAc	7	70	1300	92	1.27 [5.5]
4	EtOAc	10	99	1300	93	1.04 [4.5]
5	CH_2Cl_2	7	96	1600	83	0.76 [2.9]
6	2-PrOH	7	39	800	91	2.30 [9.4]
7	2-PrOH	25	100	>500	97	2.06 [8.4]
8	Water	35	75	290	95	0.21 [0.6]
9	Water	60	100	>220	95	0.11 [0.3]

[a] Initial pressure 5 bar H_2, 20 °C, 50 ml solvent, [MAA] = 0.05 M, 0.1g catalyst with 1wt% Rh.

[b] TOF = mol substrate converted/mol catalyst*h, calculated at the indicated time.

[c] Percentage of total amount of Rh determined by AAS of the filtrate.

Table 3. Results of the asymmetric hydrogenation of MAA catalysed by **1** immobilised on PWTUD.[a]

Entry	Solvent	Time (min)	Conv. (%)	TOF[b] ($molmol^{-1}h^{-1}$)	ee (%)	Rh loss (mg/L) [%][c]	W loss (mg/L) [%][d]
1	MTBE	30	97	750	96	<0.05 [0.4]	<0.1 [<0.01]
2	EtOAc	7	83	2300	97	0.10 [0.7]	<0.1 [<0.01]
3	2-PrOH	25	96	800	93	0.18 [1.2]	2.6 [0.2]
4	Water	30	98	750	96	0.29 [2.0]	3.3 [0.26]

[a] Initial pressure 5 bar H_2, 20 °C, 50 ml solvent, [MAA] = 0.05 M, 0.7 g catalyst with 0.14 wt% Rh.

[b] TOF = mol substrate converted/mol catalyst*h, calculated at the indicated time.

[c] Percentage of total amount of Rh determined by AAS of the filtrate.

[d] Percentage of total amount of W determined by ICP of the filtrate.

undesirable solvent for industrial application.

The first parameter of interest is the activity of the catalyst. For comparison of the carrier-dependent activities of MonoPhos, the TOFs were plotted versus carrier and solvent (Figure 2). This reveals that in all tested solvents PWTUD (Table 3) gives the most active catalyst, corresponding to the activation of the catalysts by the PW (as reported by Augustine *et al.*[3]). Also consistent with Augustine, PWTUD gave a discoloration after hydrogenation of MAA, which none of the other supports showed. Augustine explained this discoloration by partial reduction of the tungsten in PW, which according to him may be the cause of the activation.[16] These results support this hypothesis, since structurally AlTUD-1 and PWTUD are quite similar, both possess

Table 4. Results of the asymmetric hydrogenation of MAA catalysed by **1** immobilised on SAC-13(Na^+).[a]

Entry	Solvent	Time (min)	Conv. (%)	TOF[b] ($molmol^{-1}h^{-1}$)	ee (%)	Rh loss (mg/L) [%][c]
1	MTBE	30	55	275	92	0.21 [0.9]
2	EtOAc	30	97	460	98	2.5 [12]
3	CH_2Cl_2	7	55	1180	86	1.8 [8.8]
4	CH_2Cl_2	20	93	700	87	2.2 [11]
5	2-PrOH	30	70	332	96	3.5 [17]
6	Water	30	100	508	97	0.04 [0.12]

a) Initial pressure 5 bar H_2, 20 °C, 50 ml solvent, [MAA] = 0.05 M, 0.7 g catalyst with 0.14 wt% Rh.

b) TOF = mol substrate converted/mol catalyst*h, calculated at the indicated time.

c) Percentage of total amount of Rh determined by AAS of the filtrate.

Table 5. Results of the asymmetric hydrogenation of MAA catalysed by **1** immobilised on Nafion.[a]

Entry	Catalyst	Time (min)	Solvent	Conv. (%)	TOF[b] ($molmol^{-1}h^{-1}$)	ee (%)	Rh loss (mg/L) [%][c]
1	NafionC3	30	EtOAc	12	970	98	0.04 [3.0]
2	NafionC3	900	EtOAc	42	110	97	0.06
3	NafionC3	900	MeOH	2	5.9	-	0.9 [78]
4	NafionC3	30	Water	23	1850	96	0.01 [0.7]
5	NafionC3	900	Water	100	>270	92	0.01
6	NafionC1	900	MTBE	6	1.4	-	<0.005 [0.03]
7	NafionC1	30	EtOAc	16	80	98	0.02 [0.09]
8	NafionC1	1200	EtOAc	95	11	97	0.03
9	NafionC1	30	2-PrOH	5	27	-	0.15 [0.80]
10	NafionC1	1200	2-PrOH	30	4	91	0.17
11	NafionC1	30	MeOH	32	215	79	6.0 [40]
12	NafionC1	900	MeOH	100	>22	75	5.7
13	NafionC1	900	Water	100	>17	90	0.01 [0.05]

a) Initial pressure 5 bar H_2, 20 °C, 50 ml solvent, [MAA] = 0.05 M, 0.1 g catalyst.

b) TOF = mol substrate converted/mol catalyst*h, calculated at the indicated time.

c) Percentage of total amount of Rh determined by AAS of the filtrate.

three-dimensional connectivity, but AlTUD-1 exhibits a lower activity although it should have a better accessibility (higher S_{BET}, total pore volume and pore diameter).

The Nafion supports (Tables 4 and 5) also lack this additional interaction, since the triflate moiety is a textbook example of a non-coordinating ligand. Their activities do not even remotely match those obtained with PWTUD. In fact, both types of Nafion supports also perform worse than AlTUD-1, especially the pure Nafion resin.

The TOFs obtained with NafionC1 are only a fraction of those obtained with other

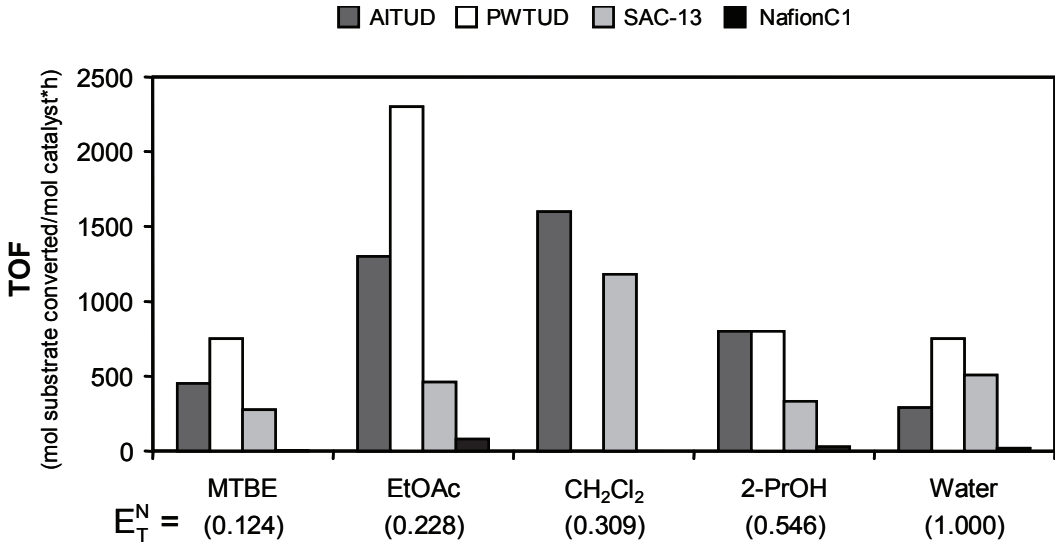


Figure 2. TOFs of the various supported catalysts in several solvents. The normalised empirical parameter, E_T^N[23]‡ is good measure for solvent polarity.

supports. The low values of Nafion resin are most likely caused by poor swelling of the Nafion, which greatly hampers the accessibility of the catalyst inside the particles. To test this hypothesis, the hydrogenation with NafionC1 was also performed in MeOH (Table 5, entries 11 and 12), a solvent omitted for the other carriers due to the low selectivity of the Rh-MonoPHOS catalyst in this solvent. Indeed, in MeOH, TOFs approaching those of the other carriers are reached. When the TOFs of the various solvents are weighed against the corresponding solvent uptake of Nafion (Na^+-form), as determined by Yeo et al.,[15] a dependence is observed: Nafion takes up 4 times as much methanol than water or 2-propanol, which corresponds fairly well to the difference in TOF.

The relation between poor activity and accessibility of Nafion is further stressed by the results obtained with NafionC3, the catalyst with low loading due to poor accessibility. Although the conversions with this catalyst are very low, the initial TOFs are very high. In water (Table 5, entry 4) it is even the most active catalyst per Rh. These results can be rationalised by the fact that on this support all the Rh complexes are immobilised on the outer anionic groups, due to the immobilisation for NafionC3 being performed in 2-PrOH, hence the Rh complex could not enter the particles, resulting in the predominant loading at the external surface. Thus, for NafionC3 all active particles are highly accessible to the substrate, which leads to the initial high activity.

By incorporating Nafion into a silica matrix, as in the case of SAC-13, these accessibility problems are greatly reduced as can be derived from the difference in TOFs. But SAC-13 is still outperformed by the PWTUD supported catalyst by more than a factor 2 in most solvents.

‡ E_T^N is based on the transition energy for the longest-wavelength solvatochromic absorption band of a pyridinium *N*-phenolate betaine dye.

Figure 2 also clearly shows that the activity is strongly dependent on the solvent. This dependence appears to be the same for all supports with the exception of Nafion, due to the reasons mentioned above. In CH_2Cl_2 the highest activity is observed. When changing to a more polar solvent or less polar solvent (a higher or lower E_T^N-value[‡]) the TOF decreases. Thus, both MTBE and water give lower activities.

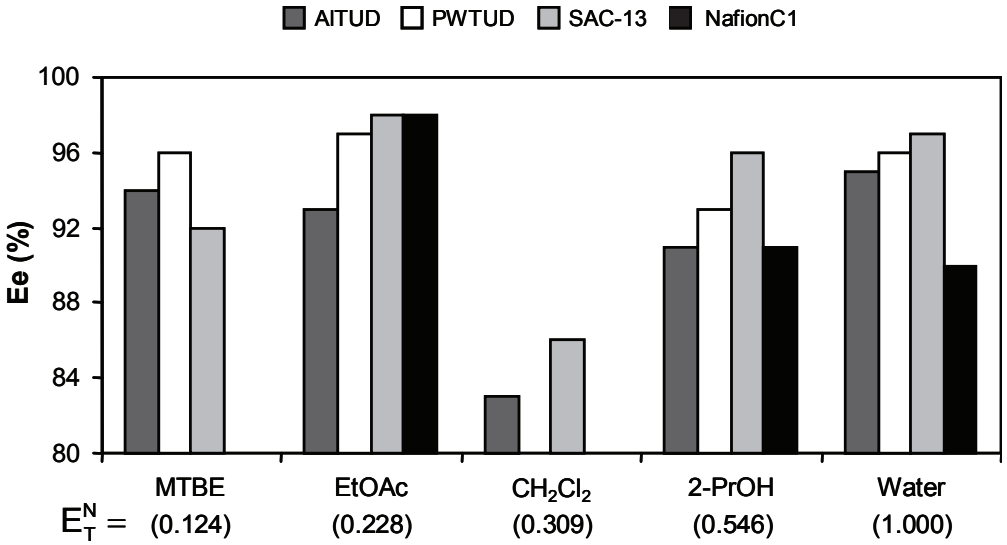


Figure 3. ee of the various supported catalysts in several solvents. The normalised empirical parameter, E_T^N[23] is a good measure for solvent polarity.

The influence of the anionic supports on the selectivity (Figure 3) of the catalyst is less pronounced than their influence on the TOF (Figure 2). Again PWTUD (Table 3) appears to be an excellent choice, although SAC-13 (Table 4) is marginally better in most solvents. AlTUD-1 (Table 2) gives somewhat lower, but still very good enantioselectivities. With CH_2Cl_2 as solvent all catalysts display relatively low selectivities that do not surpass 87%. This minimum is unexpected, since CH_2Cl_2 together with EtOAc was the best solvent for hydrogenation with the homogeneous catalyst **1**.[17] EtOAc, thus, remains the solvent of choice for the hydrogenation with the supported catalysts.

In order to investigate how well the catalyst is immobilised, leaching studies were performed. Previously we have shown that this is greatly dependent on the reaction solvent.[4] Here again, a distinct correlation between leaching and solvent is observed. (Figure 4). In MTBE and water all materials are equally suitable as support, since virtually no leaching is found in these solvents. In other solvents large differences are observed between the materials. As for the other parameters discussed previously, PWTUD (Table 2) exhibits the best properties. Virtually no loss of Rh in a wide range

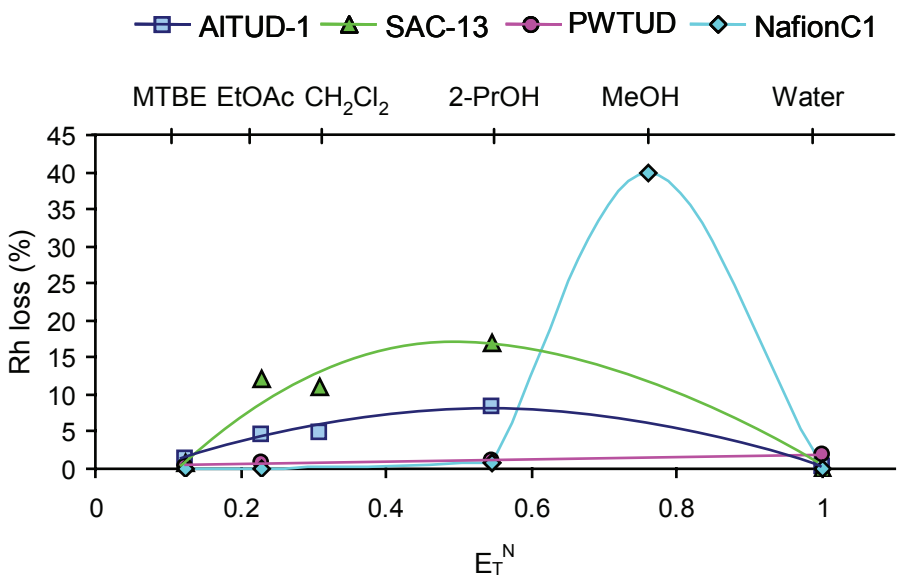


Figure 4. The amount of Rh found in solution after hydrogenation and removal of the supported catalyst.

of solvents is found, making it the support of choice. Surprisingly NafionC1 (Table 5) performs almost as well. In methanol (Table 5, entry 11), however, Nafion lacks the ability to retain the Rh-complex. This sharp contrast between methanol and the other solvents is rooted in the swelling properties of this support. As discussed above, Nafion has a more open structure in methanol compared to that observed in the other solvents and this results in a high level of leaching. Thus, it can be concluded that the immobilisation of Nafion is only partially based on ionic interaction and for a significant part on encapsulation.

This conclusion is supported by the leaching characteristics of SAC-13 (Table 4). Whilst Nafion and SAC-13 have the same anionic sites, the loss of rhodium shows a different dependence on polarity, especially for the range of MTBE to 2-PrOH. Whereas Nafion exhibits an almost flat solvent/leaching dependence over this range, SAC-13 exhibits a logarithmic dependence, similar to AlTUD-1. The open structure of SAC-13 decreases the possibility for encapsulation significantly. Consequently the influence of the solvent on the support structure is negligible which accounts for the observed differences in leaching behaviour between the two nafion-based supports.

As mentioned above, the curves in Figure 4 for SAC-13 and AlTUD-1 are very similar, consistent with a similar type of immobilisation between support and catalyst, although there is a significant difference in the strength of this interaction. AlTUD-1 is approximately twice as effective in retaining the catalyst complex. In these materials the Rh loss initially increases as the solvent stabilizes charges more readily. However,

when the solvent is too polar it cannot solubilise the catalyst anymore, due to the hydrophobic ligands, consequently leaching in water is very low.

As stated earlier, PWTUD demonstrates a high stability against leaching in all solvents, presumably also in MeOH as can be derived from the work of Brandts *et al.*[18] In their investigation on the immobilisation of Rh-MeDuPHOS on Al_2O_3/PW, the immobilised catalyst exhibited a Rh loss of 2.3% in MeOH in the hydrogenation of dimethyl itaconate as opposed to ca. 1% in 2-PrOH. PWTUD thus shows a distinctly different behaviour to the other supports, i.e. an almost linear dependence of the loss of rhodium on the solvent. This difference may well be caused by an additional interaction between PW and Rh, namely a Rh-O bond. Evidence for this additional interaction can be found in the UV data of $Rh(DiPAMP)^+$ with various counterions, as reported by Augustine et al.[16] The complexes with non-coordinating counterions, such as BF_4^-, SbF_6^- and $CF_3SO_3^-$, had spectra distinguished by absorption doublets at 340 and 460 nm. The complexes with Cl^- and AcO^- have these counterions bound directly to the Rh and showed only a single adsorption bond around 280 nm or 285, respectively. When these complexes were treated with PW and washed thoroughly, they all showed only a single absorption at 285 nm. This similarity with the AcO^- complex suggests the presence of a bond between Rh and the oxygen of PW. A bonding interaction of this type has also been observed directly in solution between heteropoly acids and $[Rh(cod)(CH_3CN)_2]BF_4$.[19] The multiply charged anion PW, thus, has distinctly different properties to the singly charged sulfonate groups of Nafion and SAC-13 or the evenly dispersed charges of AlTUD-1. Tungsten leaching in 2-PrOH is slightly higher compared to values reported by Brandts,[18] which may be caused by differences between the surface of TUD-Al_2O_3 and γ-Al_2O_3.

The final factor, which is of interest for immobilised catalysts, is their recyclability. Representative results of the recycling experiments are given in Figure 5. Every catalyst was recycled four times in all solvents reported. The catalysts exhibited almost complete retention of selectivity and activity upon reuse in almost all solvents, bar those which caused substantial leaching. Furthermore, the heterogeneity of the various catalyst systems was confirmed by a so-called filtration test in which the solid catalyst is removed under an inert atmosphere at the reaction temperature (20 °C) before completion of the reaction, followed by continuation of the hydrogenation to screen for any remaining activity in solution.[20] While the nature of the anionic support had a significant effect on the activity and selectivity of the catalyst, as well as on its leaching characteristics, no influence on its recyclability was detected.

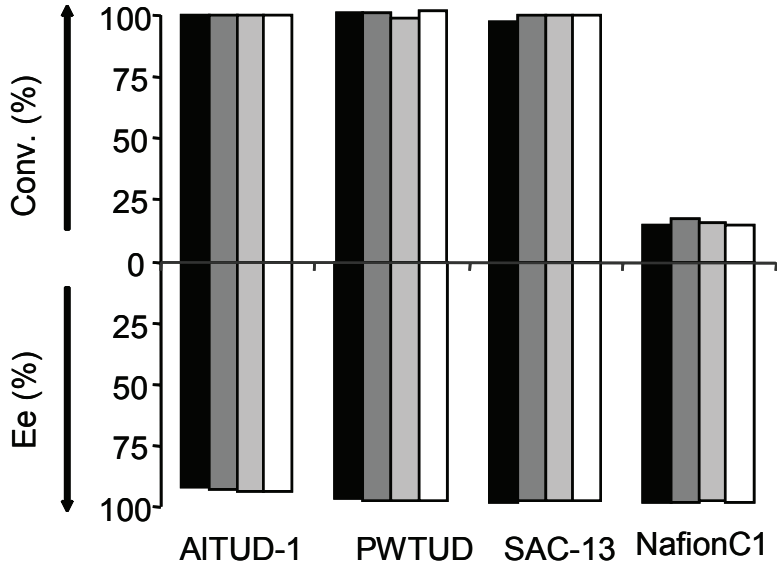


Figure 5. Recycling of **1** immobilised on the various supports in the asymmetric hydrogenation of MAA in EtOAc. Reaction times are in line with those in the corresponding table. Different bars represent consecutive runs.

Conclusions

The objective of this study was to select the best support for the non-covalent anchoring of asymmetric hydrogenation catalysts. We can safely conclude that PWTUD outperforms the other supports in almost every aspect. Immobilisation of Rh-MonoPhos on this support resulted in the catalyst with the highest activity. But especially with respect to leaching this is by far the best anionic carrier. Virtually no leaching in any solvent was observed, while the enantioselectivity remained excellent. The cause of its superior anchoring abilities lies in the type of bonding between Rh and the phosphotungstic acid, which is thought to be partially covalent. AlTUD-1, a good second choice as support material, lacks this additional binding interaction and is thus much more sensitive to the polarity of the solvent with respect to leaching. SAC-13 behaves similarly with respect to leaching, but is far inferior in terms of activity compared to AlTUD-1. The immobilisation of complexes on the Nafion resin, in contrast, relies on encapsulation rather than ionic interactions. Its encapsulating properties not only prevent the complex from going into solution in all solvents except methanol, but also prevent the substrate from reaching the catalytic site.

This systematic investigation towards support effects clearly demonstrates the need to study all aspects of catalysis with immobilised catalysts. Research should not only be focused on the catalytic complex, when searching for the best catalyst. The type of support greatly influences all the relevant parameters associated with asymmetric

hydrogenation. The solvent of the reaction can make the difference between a moderate catalyst and an exceptional one. A better understanding of these and other interactions involved in catalysis will greatly facilitate the ongoing search for better catalysts.

Experimentals and methods

General

Reactions and manipulations involving air-sensitive compounds were performed under an atmosphere of dry nitrogen using standard Schlenk-type techniques. Dry solvents were purchased from Aldrich and deoxygenated by flushing with nitrogen for an hour before use. Methyl 2-acetamidoacrylate,[4] Bis(1,5-cyclooctadiene)rhodium tetrafluoroborate,[21] (*R*)-MonoPhos,[22] [Rh^{I}(cod)((*R*)-MonoPhos)$_2$]BF_4,[17]§ and AlTUD-1 (S_{BET} 600 m^2/g, total pore volume 1.1 cm^3/g and pore diameter 15nm)[4] were prepared according to literature procedures. Chloro(1,5-cyclooctadiene)rhodium dimer was purchased from Strem. All other reagents were purchased from Aldrich, Acros or Fluka and used without further purification.

Hydrogenations were performed in a 100 ml Parr hastelloy C autoclave (A1128HC). The rhodium and tungsten content of the immobilised catalysts were measured using instrumental neutron activation analysis (INAA), which was performed at the Interfaculty Reactor Institute (IRI), Delft. The "Hoger Onderwijs Reactor" nuclear reactor, with a neutronflux of 10^{17} neutrons s^{-1} cm^{-2}, was used as a source of neutrons and the gammaspectrometer was equipped with a germanium semiconductor as detector. Rhodium leaching was determined by analysing the reaction filtrates with graphite AAS on a Perkin Elmer 4100ZL. Tungsten leaching was determined by analysing the reaction filtrates with ICP on a PerkinElmer Optima 5300DV. The Nafion content of SAC-13 was determined by thermogravimetric analysis on a Cahn TG-131 (5 °C/min to 800 °C). N_2 desorption isotherms were measured on a Quantachrome Autosorb-6B at 77 K.

Conversions of the hydrogenation reactions were determined by ^{1}H-NMR and GC analysis, using a Varian Star 3400 CX GC with a CP wax 52 CB column (50 m x 0.70 mm, df = 2.0 μm), on column injection, FID at 250 °C and nitrogen as carrier gas (10 psi). Oven program for MAA and its products: 60 °C (2 min), 10 °C/min to 200 °C (6 min). Enantiomeric excesses in the hydrogenation of MAA were determined by chiral GC using a Shimadzu GC-17A, equipped with a Chiralsil DEX CB column (25 m x 0.32 mm, df = 0.25 μm), He as carrier gas, split injector (36/100) at 220 °C and FID at 220 °C. Retention times (min) at 95 °C isotherm: 2-acetamidoacrylate (5.4), (*s*)-methyl 2-

§ Uncharacterised complex and it is probably a mixture of various species.

acetamidopropanoate (7.5) and (*R*)-methyl 2-acetamidopropanoate.

Preparation TUD-Al_2O_3

TUD-Al_2O_3 was prepared according to the procedure of Shan *et al.*[10] At 45 °C 15.32 g aluminium isopropoxide (75 mmol) was added into a mixture of 13.8 g absolute ethanol (300 mmol), 13.5 g anhydrous 2-propanol (225 mmol) under stirring. This was followed by the addition of 14.6 g tetraethylene glycol (75 mmol). Finally a solution of 13.8 g absolute ethanol (300 mmol), 13.5 g anhydrous 2-propanol (225 mmol) and 2.7 g H_2O (150 mmol) was added dropwise to this mixture. The resulting mixture was stirred for ½ h at RT, followed by aging without stirring for 6 h, also at RT. The obtained wet gel was dried at 70 °C for 21 h, at 98 °C for 2 h and it was hydrothermally treated at 160 °C for 3-21 h in an autoclave with Teflon insert. Finally the solids were calcined (with 1 °C/min to 550 °C, 4 h at 550 °C, with 1 °C/min to 600 °C, 10 h at 600 °C). TUD-Al_2O_3 was analysed by N_2 physisorption (Figure 6) from which the following characteristics could be derived: S_{BET} 313 m^2/g, total pore volume 0.61 cm^3/g and pore diameter 6nm. PWTUD is made *in situ* from TUD-Al_2O_3 during the immobilisation so no explicit structural data is available for this material. However exploratory research showed that the presence of PW on the support only slightly reduced the surface area and pore volume in accordance with the increase of the sample weight.

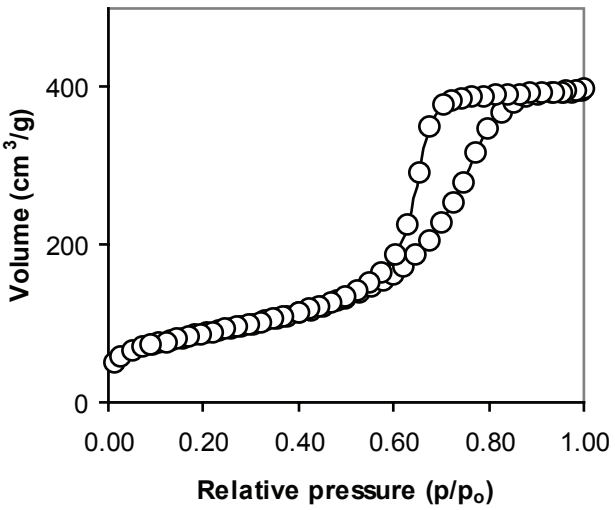


Figure 6. Nitrogen sorption isotherms of TUD-Al_2O_3.

Immobilisation procedures

[Rh^{I}(cod)((R)-MonoPhos)$_2$]BF$_4$ on AlTUD-1

1.0 g AlTUD-1 was dried at 200 °C under vacuum for 2 h. To the dried support was added 45 ml 2-propanol. After 30 min stirring, 0.15 g [Rh^{I}(cod)((*R*)-MonoPhos)$_2$]BF$_4$ (0.15 mmol) in 40 ml 2-propanol was added and the resulting suspension was stirred for 3 h. The solid was collected by filtration and washed thoroughly with portions of 30 ml 2-propanol until the washings were colourless (approx. 5 times). Finally the catalyst was dried at 55 °C under vacuum for 2 h. The resulting catalyst loading was 11 mg Rh/g support, which corresponds to an $Al_{tetrahedral}$/Rh ratio of approximately 10.

[Rh^{I}(cod)((R)-MonoPhos)$_2$]BF$_4$ on PWTUD

TUD-Al_2O_3 was dried for 2 h under vacuum at 200 °C and phosphotungstic acid (PW) was dried for 12 h under vacuum at 100 °C. To 1.9 g TUD-Al_2O_3 was added 40 ml 2-propanol, followed after 5 min stirring by 0.358 g PW in 20 ml 2-propanol. The mixture was stirred for 30 min after which 0.106 g [Rh^{I}(cod)((*R*)-MonoPhos)$_2$]BF$_4$ was added. After 3 h stirring, the solid was collected by filtration and washed thoroughly with portions of 30 ml 2-propanol until the washings were colourless (approx. 5 times). Finally the catalyst was dried at 55 °C under vacuum for 2 h. The resulting catalyst loading was 1.4 mg Rh/g and 93 mg_W/g.

[Rh^{I}(cod)((R)-MonoPhos)$_2$]BF$_4$ on Nafion

The sodium form of Nafion was prepared by washing the resin with 2 M NaCl until neutral, followed by thorough washing with water. Finally the solid was dried at 150 °C under vacuum for 4 h. To 1.0 g Nafion (Na^+) was added 35 ml methanol, followed after 10 min stirring by 0.15 g [Rh^{I}(cod)((*R*)-MonoPhos)$_2$]BF$_4$ dissolved in 35 ml methanol. The resulting suspension was stirred for 3 h. The solid was collected by filtration and washed thoroughly with portions of 30 ml methanol until the washings were colourless (approx. 5 times). Finally the catalyst was dried at 55 °C under vacuum for 2 h. The resulting catalyst loading was 7.9 mg Rh/g. Initially immobilisation of [Rh^{I}(cod)((*R*)-MonoPhos)$_2$]BF$_4$ on Nafion was performed in 2-propanol, which resulted in low loading as explained in results and discussion. The catalysts resulting from immobilisation in methanol and 2-propanol will be referred to as NafionC1 and NafionC3 respectively.

[Rh^{I}(cod)((R)-MonoPhos)$_2$]BF$_4$ on SAC-13

The sodium form of SAC-13 was prepared by washing the composite with 2 M NaCl

until neutral, followed by thorough washing with water. Finally the solid was dried at 150 °C under vacuum for 4 h. To 6.34 g SAC-13 (Na^+) was added 30 ml 2-propanol, followed after 10 min stirring by 0.13 g $[Rh^I(cod)((R)\text{-MonoPhos})_2]BF_4$ in 30 ml 2-propanol. The resulting suspension was stirred for 3h. The solid was collected by filtration and washed thoroughly with portions of 30 ml methanol until the washings were colourless (approx. 5 times). Finally the catalyst was dried at 55 °C under vacuum for 2 h. The resulting catalyst loading was 1.4 mg Rh/g.

Typical Hydrogenation reaction

All hydrogenation experiments were performed at 20 °C and using the amount of a supported catalyst corresponding with approximately 1 mg rhodium, with the exception of NafionC3. Due to the low loading with this catalyst only 0.06 mg Rh was used. The catalyst was transferred to the autoclave under a nitrogen atmosphere, followed by 50 ml of substrate solution (0.05 M). The sealed autoclave was purged with hydrogen by pressurizing to 7 bars while stirring at 300 rpm, followed by release of pressure. This cycle was repeated 5 times and finally the desired pressure, 5 bars, was applied and the stirring speed was increased to 1000 rpm. At the end of the reaction the remaining hydrogen pressure was released and the autoclave was purged three times with nitrogen, pressurizing to 5 bars while stirring at 300 rpm, followed by release. Under a nitrogen atmosphere the solution was separated from the catalyst by a syringe equipped with an acrodisc GF syringe filter (1.0 μm pore size). After removal of the solution, fresh substrate solution was added to the used catalyst and the hydrogenation procedure was repeated.

References

[1] J. A. Gladysz, *Chem. Rev.*, **2002**, *102*, special issue.

[2] P. McMorn, G. J. Hutchings, *Chem. Soc. Rev.*, **2004**, *33*, 108.

[3] R. Augustine, S. Tanielyan, S. Anderson, H. Yang, *Chem. Commun.*, **1999**, 1257.

[4] C. Simons, U. Hanefeld, I. W. C. E. Arends, R. A. Sheldon, T. Maschmeyer, *Chem. Eur. J.*, **2004**, *10*, 5829.

[5] M. Lautens, K. Fagnou, *J. Am. Chem. Soc.*, **2001**, *123*, 7170.

[6] S. P. Smidt, N. Zimmermann, M. Studer, A. Pfaltz, *Chem. Eur. J.*, **2004**, *10*, 4685.

[7] C. Simons, U. Hanefeld, I. W. C. E. Arends, A. J. Minnaard, T. Maschmeyer, R. A. Sheldon, *Chem. Commun.*, **2004**, 2830.

[8] I. Toth, B. E. Hanson, M. E. Davis, *J. Organomet. Chem.*, **1990**, *397*, 109.
[9] M. A. Harmer, W. E. Farneth, Q. Sun, *J. Am. Chem. Soc.*, **1996**, *118*, 7708.
[10] Z. Shan, J. C. Jansen, W. Zhou, T. Maschmeyer, *Appl. Catal. A*, **2003**, *254*, 339.
[11] M. J. Burk, A. Gerlach, D. Semmeril, *J. Org. Chem.*, **2000**, *65*, 8933.
[12] P. Goel, S. Anderson, J. J. Nair, C. Reyes, Y. Gao, S. K. Tanielyan, R. L. Augustine, R. Appell, C. Goralski, D. Hawn, *Chem. Ind.*, **2003**, *89*, 523.
[13] T. D. Gierke, G. E. Munn, F. C. Wilson, *J. Polym. Sci., Part B: Polym. Phys.*, **1981**, *19*, 1687.
[14] S. K. Tanielyan, R. L. Augustine, (Seton Hall University,USA). WO, **1998**, p. 66.
[15] R. S. Yeo, C. H. Cheng, *J. Appl. Polym. Sci.*, **1986**, *32*, 5733.
[16] R. L. Augustine, S. K. Tanielyan, S. Anderson, H. Yang, Y. Gao, *Chem. Ind.*, **2001**, *82*, 497.
[17] M. van den Berg, A. J. Minnaard, R. M. Haak, M. Leeman, E. P. Schudde, A. Meetsma, B. L. Feringa, A. H. M. de Vries, C. E. P. Maljaars, C. E. Willans, D. Hyett, J. A. F. Boogers, H. J. W. Henderickx, J. G. de Vries, *Adv. Synth. Catal.*, **2003**, *345*, 308.
[18] J. A. M. Brandts, P. H. Berben, *Org. Process Res. Dev.*, **2003**, *7*, 393.
[19] M. Pohl, D. K. Lyon, N. Mizuno, K. Nomiya, R. G. Finke, *Inorg. Chem.*, **1995**, *34*, 1413.
[20] H. E. B. Lempers, R. A. Sheldon, *J. Catal.*, **1998**, *175*, 62.
[21] T. G. Schenck, J. M. Downes, C. R. C. Milne, P. B. Mackenzie, T. G. Boucher, J. Whelan, B. Bosnich, *Inorg. Chem.*, **1985**, *24*, 2334.
[22] R. Hulst, N. Koen de Vries, B. L. Feringa, *Tetrahedron: Asymmetry*, **1994**, *5*, 699.
[23] C. Reichardt, *Chem. Rev.*, **1994**, *94*, 2319.

5

Unexpected Lipase-Catalysed Deprotection of *N*-Acyl Amino Acids

Introduction

The enantioselective synthesis of amino acids has long been a topic that has attracted the attention of many chemists. Countless methods have been developed ranging from transition metal catalysed reactions to the *bis* lactim ether approach.[1-5] Most of these, very different methods, have one thing in common: The enantiopure amino acid is not synthesised as the free compound but it is masked by an attached chiral auxiliary or protecting group. Consequently the very elegant enantioselective syntheses are often followed by rather harsh deprotection reactions.

This disadvantage of most amino acid syntheses can, however, also be taken advantage of: When the protection group is removed with a chiral reagent, it is possible to further amplify the enantiopurity of the target molecule. Lipases and acylases fulfil all the criteria that these reagents have to conform to: high enantioselectivity, low substrate specificity and high activity under very mild reaction conditions. So far only the acylases, particularly from *Aspergillus oryzae* and pig kidneys were shown to be able to readily remove *N*-acyl protections groups.[6-8] The very selective hydrolysis by these

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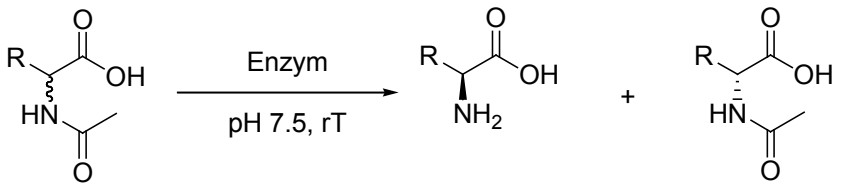
enzymes is also applied by Taneba[9, 10] and Degussa for the multi ton scale resolution of several amino acids.[9, 11, 12] For *N*-formyl protecting groups the available enzymes, for deprotection are even fewer. To the best of our knowledge the only example in organic synthesis of a enzymatic removal of a *N*-formyl group is by Sonke *et al* utilizing a peptide deformylase.[13]

Commonly it is assumed that lipases cannot cleave amides.[14] As they were optimised by nature for the cleavage of esters they supposedly were not able to attack the thermodynamically more stable amide bond. Recently, the very elegant *p*-acetoxybenzyloxycarbonyl (AcOZ) group was introduced, which can be removed by lipases. [15] However, here the lipase does not directly hydrolyse the amide bond, but the amine group is liberated via a relay reaction.

In order to ensure that the full potential of the hydrolases is utilised for the gentle removal of the two above-mentioned amine-protecting groups a wider screen of hydrolases was deemed necessary. We therefore studied 15 different, commercially available lipases, acylases, proteases and esterases for hydrolyses of *N*-acyl and *N*-formyl protecting groups.

Results and discussion

N-acyl amino acids were chosen as model substrates to screen these 15 enzymes (Scheme 1), since the *N*-acyl group is a common functionality/protection moiety in the synthesis of amino acids which is commonly removed under harsh deprotection conditions. Refluxing in concentrated acid is not uncommon. It was already mentioned that aminoacylases are ideally suited for the cleavage of the *N*-acyl group, but they have also drawbacks. As with most enzymes, aminoacylases have limited substrate tolerance and only two of them are commercially available. Furthermore these commercially available acylases are both L-specific, ruling out their application for the deprotection of D-amino acid.



1 (L)-**2** (D)-**1**

1-2a R = CH_3 **1-2b** R = CH_2Ph

Scheme 1

This justifies the screening of a broad range of enzymes for their hydrolysis capabilities of the amide bond in *N*-acyl amino acids. An accurate comparison between different families of enzymes, e.g. lipases and acylases, however is complicated, since the activity tests for those families are unrelated. To obtain the best possible assessment, the activities of the lipases and esterase were determined with the hydrolysis of tributyrin[16] and those of the acylases with the hydrolysis of *N*-acyl-L-methionine.[17] The amidases and proteases gave no practical activity values when the *N*-acyl-L-methionine test was used, thus the activity tests recommended by their suppliers were used.

The 15 commercially available enzymes were first screened for their hydrolysis activity of the amide bond in *N*-acyl-D,L-alanine (**1a**) (Table 1). The acylases from porcine kidney and *Aspergillus melleus,* are clearly the best enzymes, with respect to activity and selectivity. This is not surprising, since nature created these enzymes for this purpose. The activity of some of the lipases and PLE however is astonishing, since it is commonly thought that lipases are not able to catalyse the hydrolysis of an amide bond.[14] One exception to this is the CAL-B catalysed hydrolysis of *N*-acyl 1-

Table 1. Enzyme catalysed hydrolysis of N-acyl-D,L-alanine (**1a**)[a]

Enzyme[b, c]	Reaction time 3h			Reaction time 24h		
	Conv. (%)	Ee_p (%)	*E*	Conv. (%)	Ee_p (%)	*E*
RML	traces	-	-	traces	-	-
RML[d]	3	n.d.	-	11	65 (L)	5.1
PLE	25	77 (L)	9.9	50	79 (L)	20
HLL	3	n.d	-	3	n.d	-
TL	10	67 (L)	5.4	13	65 (L)	5.2
TL[d]	15	70 (L)	6.4	25	68 (L)	6.5
Trypsin IX	2	n.d.	-	4	n.d.	-
PenG amidase	12	80 (L)	10	50	88 (L)	45
Acylase 1 AM	48	99	>600	58	72 (L)	62
Acylase 1 PK	53	90 (L)	>110	53	90 (L)	>110

a) 333 units, 1.5 mmol substrate, 30 mL potassium phosphate buffer (0.1 M, pH 7.5), rT.

b) RML = *Rhizomucor miehei* lipase; PLE = porcine liver esterase; HLL = *Humicola Lanuginose* lipase; TL = *Pseudomonas stutzeri* lipase; Trypsine IX = Trypsine type IX from porcine pancreas; PenG amidase = penicillin G amidase; Acylase 1 AM = *Aspergillus melleus* acylase 1; Acylase 1 PK = porcine kidney acylase 1.

c) *Achrobacter* sp. lipase, *Candida antartica* lipase B, *Candida rugosa* Lipase, *Pseudomonas fluorescences* lipase, *Alcaligenes* sp. lipase, porcine pancreas Trypsin II, bovine pancreas α-Chymotrypsin, *Bacillus licheniformis* Subtilisin were also screened but no activity was observed.

d) 10-fold amount of enzymes added; 3333 units, 1.5 mmol substrate, 30 mL potassium phosphate buffer (0.1 M, pH 7.5), rT.

arylethylamines.[18] However CAL-B is not among the active lipases for the hydrolysis of *N*-acylalanine. RML and TL are the ones that demonstrate a modest activity for the amide hydrolysis on the condition that significant amount of enzyme (3333 units) is added. Still these two lipases and especially PLE outperform most of the tested proteases, which nature engineered to hydrolyse amides. Trypsin XI gives very poor conversions, while the other trypsin II, chymotrypsin and subtilisin show no activity at all. The only serine protease, which was able to catalyse the hydrolysis sufficiently, was Pen G amidase.

All active enzymes show selectivity for the L-substrate. The acylases give, as expected, very high *E*-values (>110), the amidase is second best with an *E*-value of 45. PLE also displays a reasonably high preference for the L-substrate (*E*-value = 20), especially when taking into account that its activity was unexpected. The lipases demonstrate only modest selectivity for the L-amino acid. In the acylation of primary amines, lipases generally follow Kazlauskas' rule,[19, 20] where the enantiopreference is determined by the steric bulk of the substituents (Fig 1). In the hydrolysis of **1a** the selectivity, opposite to the one predicted by Kazlaukas' rule, is observed, suggesting that electronic factors dominate the enantiodiscrimination of alanine.

L-Alanine Kazlauskas' rule

Figure 1. Graphical presentation of the Kazlauskas' rule to determine the enantioselectivity of lipases in the hydrolysis of amides

The enzymes were also tested with *N*-acyl-D,L-phenylalanine (**1b**) as substrate (Table 2). Most of the enzymes, which showed activity for **1a** e.g. PLE and TL, are not able to hydrolyse **1b**. Both the acylases show a significant hydrolysis rate for **1b**, but acylase I PK is considerably slower for this substrate then for **1a**. This drop in activity for acylase I PK when changing from alanine to phenylalanine was also observed by others.[6, 21, 22] Both acylases show an excellent selectivity for the L-enantiomer of **1b**. Besides the acylases, RML is the only enzyme tested that is able to hydrolyse the amide bond in **1b**. Once more this lipase demonstrates a superior activity in the hydrolysis of amides as compared to the proteases. The *N*-acyl group is removed by RML with excellent enantioselectivity (*E* = 61).

Table 2. Enzyme catalysed hydrolysis of N-acyl-D,L-phenylalanine (2a)[a]

Enzyme[b, c]	Reaction time 3h			Reaction time 24h		
	Conv. (%)	Ee (%)	*E*	Conv. (%)	Ee (%)	*E*
RML[d]	3	n.d.	-	19	96	61
PLE	0	-	-	0	-	-
HLL[d]	0	-	-	0	-	-
TL[d]	0	-	-	0	-	-
Acylase 1 AM	49	99	>600	54	83	46
Acylase 1 PK	5	96	51	37	99	>300

a) 333 units, 1.5 mmol substrate, 30 mL potassium phosphate buffer (0.1 M, pH 7.5), rT.

b) RML = *Rhizomucor miehei* lipase; PLE = porcine liver esterase; HLL = *Humicola Lanuginose* lipase; TL = *Pseudomonas stutzeri* lipase; Acylase 1 AM = *Aspergillus melleus* acylase 1; Acylase 1 PK = porcine kidney acylase 1.

c) *Achrobacter* sp. lipase, *Candida antartica* lipase B, *Candida rugosa* Lipase, *Pseudomonas fluorescences* lipase, *Alcaligenes* sp. lipase, porcine pancreas Trypsin II, Trypsine type IX from porcine pancreas, bovine pancreas α-Chymotrypsin, *Bacillus licheniformis* Subtilisin were also screened but no activity was observed.

d) 10-fold amount of enzymes added; 3333 units, 1.5 mmol substrate, 30 mL potassium phosphate buffer (0.1 M, pH 7.5), rT.

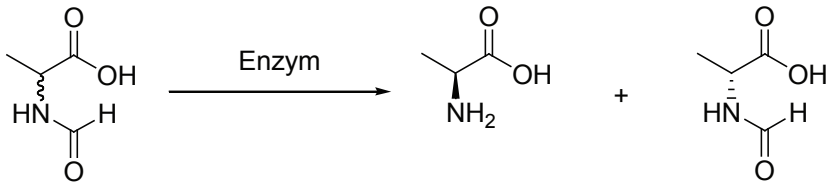
To investigate the potential of these 15 commercially available enzymes in the removal of the *N*-formyl protection groups, the *N*-formyl derivative of D,L-alanine (**3a**) was selected as model substrate (Table 3). From these 15 enzymes, three were found to be active in the hydrolysis of **3a**. This is the first example where enzymes, other than deformylases, are employed to deprotect *N*-formyl amides.

The *N*-formyl is evidently more challenging to remove for most of the tested enzymes. The two lipases, which demonstrated reasonable activity in the *N*-acyl deprotection, do not hydrolyse the *N*-formylamide of **3a.** This is unexpected since *N*-formyl is more readily hydrolysed with H^+ and thus less stable. [23] Two of the active enzymes, i.e. PLE and Acylase 1 PK, also hydrolyse the *N*-formyl amide with a significant lower rate as compared to the *N*-acyl group. Only acylase 1 AM demonstrates no noticeable difference in activity between these substrates. The enantioselectivity of acylase 1 AM however is slightly diminished. Be that as it may, acylase 1 AM is an excellent new catalyst for the hydrolysis of *N*-formylalanine.

Conclusions

In conclusion, we have demonstrated for the first time that PLE and the lipases RML

Table 3. Enzyme catalysed hydrolysis of N-formyl-D,L-alanine (3a)[a]



3a (D)-**3a**

Enzyme[b, c]	Reaction time 3h			Reaction time 24h		
	Conv. (%)	Ee (%)	*E*	Conv. (%)	Ee (%)	*E*
RML[d]	0	-	-	0	-	-
PLE	3	n.d.	-	10	n.d.	-
HLL[d]	0	-	-	0	-	-
TL[d]	0	-	-	0	-	-
Acylase 1 AM	50	91	67	47	90	58
Acylase 1 PK	21	n.d.	-	50	95	145

a) 333 units, 1.5 mmol substrate, 30 mL potassium phosphate buffer (0.1 M, pH 7.5), rT.

b) RML = *Rhizomucor miehei* lipase; PLE = porcine liver esterase; HLL = *Humicola Lanuginose* lipase; TL = *Pseudomonas stutzeri* lipase; Acylase 1 AM = *Aspergillus melleus* acylase 1; Acylase 1 PK = porcine kidney acylase 1.

c) *Achrobacter* sp. lipase, *Candida antartica* lipase B, *Candida rugosa* Lipase, *Pseudomonas fluorescences* lipase, *Alcaligenes* sp. lipase, porcine pancreas Trypsin II, Trypsine type IX from porcine pancreas, bovine pancreas α-Chymotrypsin, *Bacillus licheniformis* Subtilisin were also screened but no activity was observed.

d) 10-fold amount of enzymes added; 3333 units, 1.5 mmol substrate, 30 mL potassium phosphate buffer (0.1 M, pH 7.5), rT.

and TL are able to hydrolyse *N*-acyl amides. The ability of these lipases to hydrolyse this very stable amide proves that the dogma "lipases cannot hydrolyse amides" is incorrect. The scope of enzymes is not limited to their natural role. Their catalytic promiscuity should be investigated in more detail. This could extend their usefulness in organic synthesis significantly.

We have also demonstrated for the first time that several enzymes other than deformylases are capable of deprotecting *N*-formylalanine. PLE, acylase 1 PK and especially acylase 1 AM are quite efficient in the hydrolysis of the *N*-formyl bond. The fact that these new active enzymes are commercially available opens up new possibilities for the use of enzymes in protection/deprotection chemistry. Hopefully this will further promote the use of enzymes in organic synthesis, since replacing chemical deprotection with biocatalytic deprotection is a major progress towards sustainability.

Experimental section

General

Achrobacter sp. Lipase (lipase AL, Meito Sangyo), *Alcaligenes* sp. lipase (lipase AL, Meito Sangyo), *Aspergillus melleus* acylase 1 (Fluka), *Candida antartica* lipase B (Novozymes 435, Novozymes), *Candida rugosa* lipase (type VII, Sigma), α-Chymotrypsin (from bovine pancreas, Sigma), *Humicola Lanuginose* lipase (SP523, Novo Nordisk), penicillin G amidase (PGA-450, an immobilised *E* coli penicillin G acylase on a specially developed organic polymer containing 59% water, Roche Diagnostics), porcine kidney acylase 1 (Sigma), *Pseudomonas fluorescences* lipase (Fluka), *Pseudomonas stutzeri* lipase (lipase TL, Meito Sangyo), *Rhizomucor miehei* lipase (SP524, Novo Nordisk), Subtilisin (protease (subtilisin Carlsberg) from *Bacillus licheniformis*, Sigma), Trypsin II-S (Porcine Pancreas, Sigma), Trypsin IX-S (Porcine Pancreas, Sigma) were obtained from their respective suppliers. NMR spectra were recorded on a Varian Inova 300 MHz or a Varian VXR-400S spectrometer relative to *t*-BuOH.

N-acyl-D,L-alanine

D,L-Alanine (10 g , 76.3 mmol) and acetic anhydride (28 mL, 0.3 mol) were stirred in methanol (50 mL) for 6h under reflux, after which all volatiles were removed by evaporation. The crude product was triturated with ethyl acetate. The white solid was collect by filtration and dried under vacuum. Yield 98% (14 g, 74.8 mmol)

1H NMR (300MHz, D_2O): δ 4.29 (q, J = 7.2 Hz, 1H, C*H*CH_3), δ 1.98 (s, 3H, $COCH_3$), δ 1.38 (d, J = 7.2 Hz, 3H, C*H*$_3$CH).

N-formyl- D,L-alanine

N-formylalanine was prepared as described by Kolb et al.[24] D,L-Alanine (3.8g , 42.7 mmol), formic acid (8.6 g, 0.19 mol), and acetic anhydride (17 g, 0.17 mol) were stirred in acetic acid (100 mL) for 4h at rT, after which all volatiles were removed by evaporation. The crude product was recrystallised from ethyl acetate. The resulting solid was further purified by ion-exchange. The DOWEX-50 (H^+) column was rinsed with water until neutral, followed by ammonia (1M). The ammonia layer was lyophilised. Yield 40% (2g, 17 mmol)

1H NMR (D_2O): δ 8,04 (s, 0.9H, HCO, rotamer 1), δ 8.00 (s, 0.1H, HCO rotamer 2), δ 4.42 (q, J = 7.2 Hz, 0.9H, C*H*CH_3, rotamer 1), δ 4.38 (q, J = 7.2 Hz, 0.1H, C*H*CH_3, rotamer 2), δ 1.40 (d, J = 7.2 Hz, 2.7H, C*H*$_3$CH, rotamer 1), δ 1.43 (d, J = 7.2 Hz, 0.3H,

CH_3CH, rotamer 2)

N-acyl-D,L-phenylalanine

α-Acetamidocinnamic acid (5,0 g, 24,4 mmol) was hydrogenated with 10% Pd/C under 1 bar H_2 in ethanol (300 mL). After the reaction was finished (ca 5 h) and filtered, the volatiles were removed by evaporation. The crystals were obtained by filtration and dried under vacuum.

Yield 95% (4.8 g, 23.2 mmol)

1H NMR (D_2O): δ 7.33-7.20 (m, 5H, C_6H_5), δ 4.63 (dd, J = 5.0 Hz, J = 9.1, 1H, C*H*CH_2), δ 3.18 (dd, J = 5.1 Hz, J = 14.0, 1H, CHC*H*$_2$), δ 2.92 (dd, J = 9.1 Hz, J = 14.0 1H, CHC*H*$_2$), δ 1.88 (s, 3H, $COCH_3$).

Activity assessments

Lipases and esterases

The activities of the lipases and esterases were determined as described by Veum et al.[16] 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 of potassium dihydrogen phosphate (100 mL) adjusted to pH 7.0 with 10 mM of dipotassium hydrogen phosphate (ca.100 mL)] in a thermostatted 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 had stabilised, the enzyme was added (for example, 9 mg of CAL B). The consumption of 100 mM sodium hydroxide was monitored over 40 min and plotted against time. 1 μmol of NaOH consumed per min corresponds to 1 unit (1 U) of activity.

Acylases, amidases and proteases

The activities of the acylases were determined as described by Bakker et al.[17]

N-Acetyl-L-methionine (15.7 mM) was dissolved in a Tris buffer (5 ml, 50 mM, pH 7.5), the pH was adjusted with NaOH (1 M) and enzyme was added. The reaction was quenched after 1 h by adding HCl (1 M, 5 ml) and the conversion was measured by reversed-phase HPLC using a custom-packed Symmetry C18 cartridge (Waters Radial-Pak, 8 × 100 mm, 7 μm) acetonitrile/phosphate buffer (50 mM, pH 2.2) of 7.5:92.5 (v: v) as eluent (flow 1.5 mL/min for the first 4 min followed by 3 mL/min), with detection at 210 nm. 1 unit (U) is the amount of enzyme hydrolyzing 1 μmol of N-acetyl-L-methionine per minute.

The activity of pen G acylase was determined as described by Van Langen et al.[25] To a 2% solution of penicillin-G potassium salt in 0.1 M phosphate buffer at pH 8.0

and 34°C enzyme was added. During the hydrolysis the pH was maintained at 8.0 with automated NaOH titration. 1 unit (U) of penicillin-G acylase liberates 1 μmol of phenylacetic acid per min.

The activities of subtilisin and the various trypsins were determined as described by their supplier.

Hydrolysis of N-Acyl-d,l-amino acids and N-formyl-d,l-alanine

To 1.5 mmol subtrate in 30 mL potassium phosphate buffer (0.1 M, pH 7.5) was added 333 units of enzyme. The mixture was shaken for the desired duration, after which samples were taken. The samples were adjusted to pH 5 with 1M HCl, heated to 60 °C with Norit, filtered over Celite and lyophilized. Conversions were determined by ^{1}H-NMR. Before determining the enantioselectivity, the samples were passed over a DOWEX-50 (H^+) column, which was rinsed with water until neutral, followed by 1M ammonia. The ammonia layer was again lyophilised.

Enantiomeric excesses of alanine were determined by chiral HPLC using a Crownpak CR (+) column (150 x 4 mm) with $HClO_4$ (pH = 1) as eluens, a flow of 0.5 mL/min at 0 °C and UV detection at 215 nm. Retention times (min): D-alanine (4.6) and L-alanine (10.8). For phenylalanine the enantiomeric excesses were also determined by chiral HPLC using a Crownpak CR (+) column (150 x 4 mm) using a different eluens. $HClO_4$ (pH = 2), a flow of 0,8 mL/min at 25 °C and UV detection at 215 nm. Retention times (min): D-phenylalanine (9.2) and L-phenylalanine (11.7).

References

[1] J.-A. Ma, *Angew. Chem. Int. Ed.*, **2003**, *42*, 4290.
[2] U. Schöllkopf, U. Groth, K. O. Westphalen, C. Deng, *Synthesis*, **1981**, 969.
[3] K. Maruoka, T. Ooi, *Chem. Rev.*, **2003**, *103*, 3013.
[4] R. M. Williams, J. A. Hendrix, *Chem. Rev.*, **1992**, *92*, 889.
[5] R. M. Williams, *Synthesis of Optically Active a-Amino Acids*, Pergamon Press, Oxford, **1989**.
[6] H. K. Chenault, J. Dahmer, G. M. Whitesides, *J. Am. Chem. Soc.*, **1989**, *111*, 6354.
[7] K. Faber, Editor, *Biotransformation in Organic Chemistry, 3rd Completely Revised Edition*, **1997**.
[8] A. S. Bommarius, K. Drauz, K. Gunther, G. Knaup, M. Schwarm, *Tetrahedron:*

Asymmetry, **1997**, *8*, 3197.
[9] K. Drauz, H. Waldmann, Editors, *Enzyme catalysis in organic synthesis: A comprehensive handbook, Volume I-III*, Wiley-VCH, Weinheim, **2002**.
[10] T. Takahashi, K. Hatano, *EP 304021 A2*, **1989**.
[11] W. Leuchtenberger, M. Karrenbauer, U. Ploecker, *Ann. N. Y. Acad. Sci.*, **1984**, *434*, 78.
[12] C. Wandrey, R. Wichmann, W. Leuchtenberger, M. R. Kula, A. Bueckmann, *EP 23346*, **1981**.
[13] T. Sonke, B. Kaptein, A. F. V. Wagner, P. J. L. M. Quaedflieg, S. Schultz, S. Ernste, A. Schepers, J. H. M. Mommers, Q. B. Broxterman, *J. Mol. Catal. B: Enzym.*, **2004**, *29*, 265.
[14] I. Alfonso, V. Gotor, *Chem. Soc. Rev.*, **2004**, *33*, 201.
[15] D. Kadereit, H. Waldmann, *Chem. Rev.*, **2001**, *101*, 3367.
[16] L. Veum, M. Kuster, S. Telalovic, U. Hanefeld, T. Maschmeyer, *Eur. J. Org. Chem.*, **2002**, 1516.
[17] M. Bakker, F. van De Velde, F. van Rantwijk, R. A. Sheldon, *Biotechnol. Bioeng.*, **2000**, *70*, 342.
[18] H. Smidt, A. Fischer, P. Fischer, R. D. Schmid, *Biotechnol. Tech.*, **1996**, *10*, 335.
[19] U. Bornscheurer, R. Kazlauskas, *Hydrolases in Organic Synthesis: Regio- or Stereoselective Biotransformations*, Wiley-VCH, Weinheim, **1999**.
[20] F. van Rantwijk, R. A. Sheldon, *Tetrahedron*, **2004**, *60*, 501.
[21] S. M. Birnbaum, L. Levintow, R. B. Kingsley, J. P. Greenstein, *J. Biol. Chem.*, **1952**, *194*, 455.
[22] I. Gentzen, H. G. Loffler, F. Schneider, *Z. Naturforsch.*, **1980**, *35*, 544.
[23] L. Panella, A. M. Aleixandre, G. J. Kruidhof, J. Robertus, B. L. Feringa, J. G. De Vries, A. J. Minnaard, *J. Org. Chem.*, **2006**, *71*, 2026.
[24] J. J. Kolb, G. Toennies, *J. Biol. Chem.*, **1942**, *144*, 193.
[25] L. M. van Langen, E. de Vroom, F. van Rantwijk, R. Sheldon, *FEBS Lett.*, **1999**, *456*, 89.

6

A Successful Enantioselective Chemoenzymatic Cascade in Water

Introduction

During the last decades an increasing urgency has arisen to develop greener and economically competitive processes for the industrial synthesis of chemicals.[1] Especially in the production of pharmaceuticals or agrochemicals, where the waste generation can surpass 100 kg/kg product, this is a pressing necessity.[2] A very important tool in the chemist's arsenal to achieve more environmentally benign processes is catalysis. Not only does the remarkable progress in (enantioselective) chemocatalysis continue, but the potential of biocatalysts is also increasingly being recognized by the fine chemical industry.[3] However, either of these catalysts only solve part of the problem, since they are usually part of a complex multistep synthesis where the majority of reaction steps still consist of classic stoichiometric chemistry. Additionally, these processes usually require wasteful and expensive isolation as well as purification of intermediates. For genuinely sustainable processes, the majority of steps should be catalytic and, ideally, intermediate purification and isolation steps should be circumvented. Cascade reactions offer a unique opportunity to address these issues, in particular when

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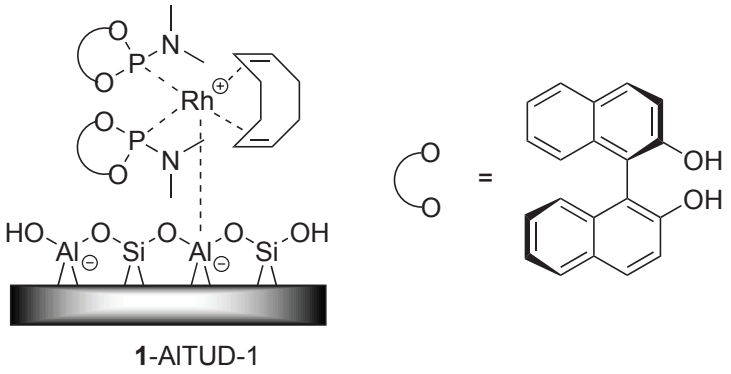
carefully orchestrated, involving enzymes and/or chemocatalysts.[4-6] These two types of catalysts complement each other: transition metals are very versatile for oxidations and reductions (tasks often difficult to perform with enzymes, due to problems with cofactor regeneration) and enzymes readily perform hydrolytic reactions and their reverse (whereas a chemo-catalytic approach often requires drastic conditions and generates large amounts of salts as waste).

To overcome the common incompatibility of reagents and conditions, smart solutions need to be found: immobilization of the catalyst, as a form of compartmentalization or in combination with other compartmentalization approaches, is often an efficient strategy. A noteworthy example was recently published by Gelman *et al.*[7] By immobilizing a lipase and a rhodium complex in two separate sol-gel matrices they were able to perform a one-pot esterification and hydrogenation reaction leading to saturated esters in good yields. In contrast, when only the enzyme was immobilized yields decreased almost 7-fold. Thus, the support of the catalyst in this example has a similar function to a membrane in a cell – it inhibits the interaction of the incompatible reagents.



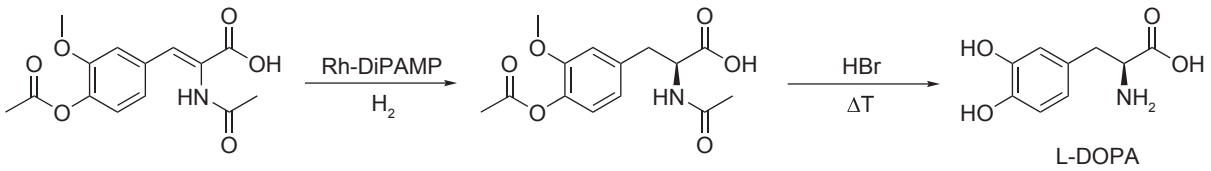
1-AlTUD-1

Figure 1. Rh-MonoPhos immobilised on AlTUD-1.

Recently, we reported in a communication the successful immobilization of the asymmetric hydrogenation catalyst, Rh-MonoPhos, on AlTUD-1 ([**1**-AlTUD-1] Figure 1).[8] This catalyst is based on the synthetically readily accessible MonoPhos ligand,[9] which was immobilized *via* straightforward ionic interactions with the surface of the three-dimensional mesoporous material, AlTUD-1.[10] In this manner, the need for modification of the ligand prior to immobilization is circumvented. In addition to the obvious advantages of easy separation and improved recyclability of the catalyst, this methodology also opened up the possibility to use water as a reaction medium, thereby creating the unique opportunity for a chemo-enzymatic cascade, since water is the ideal

solvent for enzyme-catalyzed hydrolyses. We now report on the successful combination of the chemocatalytic asymmetric hydrogenation with enzymatic hydrolysis of the product to afford an one-pot green synthesis of enantiopure amino acids in water.

The benchmark Monsanto *L*-DOPA process (Scheme 1) perfectly illustrates the problem: an elegant enantioselective chemical reduction is followed by a waste generating chemical hydrolysis.[11] A chemo-enzymatic cascade would greatly improve the sustainability of this type of amino acid synthesis: the ideal process would be entirely catalytic, require only one work-up and eliminate the use of organic solvents and the stoichiometric formation of salts.



Scheme 1. The Monsanto *L*-DOPA process.

Results and Discussion

To test our concept we chose the synthesis of *L*-alanine, starting from methyl 2-acetamidoacrylate (see Figure 2). The ideal candidates for the enzymatic part of the cascade are the well-known aminoacylases,[12] since they were evolved to catalyze this type of hydrolysis. We selected both *Aspergillus melleus* (AM) and porcine kidney (PK) as sources for this type of enzyme, since these acylases are highly active,[13] commercially available and, in the case of *Aspergillus melleus*, inexpensive.

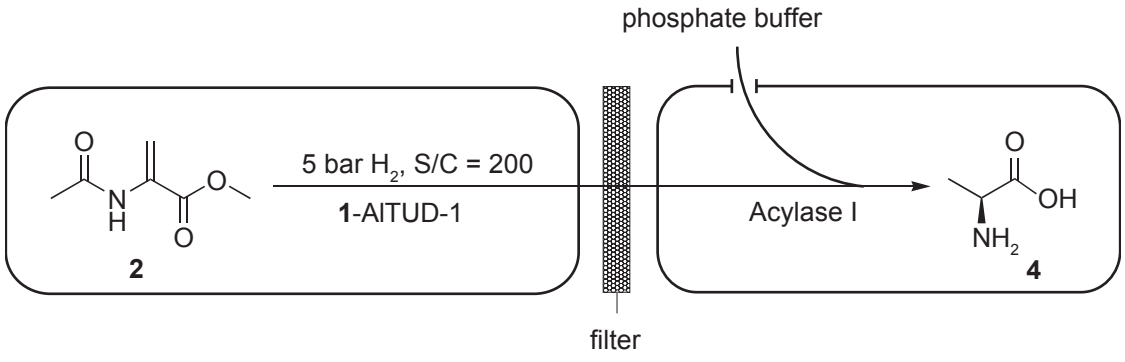


Figure 2. Schematic representation of the filtered cascade.

In the first form of cascade, which we investigated, the hydrogenation and hydrolysis reactions were separated by a straightforward filtration. A schematic representation of this setup is given in Figure 2. The results of the hydrogenations as well as the hydrolyses can be found in Table 1.

Table 1. Results of the various chemo-enzymatic cascades in the asymmetric synthesis of **4**.

Enzyme[a]	Conversion of **2** [%]	Ee of **3a** [%]	Conversion[b] of **3a** [%]	Ratio **4**/**3b**[b]	Ee of **4**[b] [%]
AM filtered	100	95	98 (98)	100:0 (89:11)	>98 (>98)
PK filtered	100	95	82 (16)	87:13 (63:37)	>98
AM unfiltered	100	95	98 (98)	100:0 (85:15)	>98 (>98)
PK unfilterd	100	95	40 (14)	98:2 (85:15)	>98

[a] AM = Acylase I from *Aspergillus melleus* (175 U); PK = Acylase I from porcine kidney (700 U); filtered and unfiltered refer to the respective protocol.‡

[b] After 24h of hydrolysis; results in brackets are after 4h of hydrolysis.

In the first step, i.e. the asymmetric hydrogenation, the intermediate **3a** is produced with an enantiomeric excess (ee) of 95% as reported earlier.[8] The second step is initiated by simply passing the reaction mixture through the filter and adding the enzyme with a concentrated phosphate buffer (10v%, pH = 7.5, 1.1 M). Given that the substrate of the hydrolysis reaction (**3a**) has 2 functional groups that can be hydrolyzed, namely the amide group and the ester group, several products (Figure 3) can be formed. From these possible products, **3c** was never detected, corresponding to the earlier findings that acylase I requires a terminal carboxylate group for its hydrolytic action on the *N*-acyl group.[13] Advantageously, both acylases demonstrated ester hydrolysis activity for the *N*-acyl amino acid esters, giving rise to **3b**. This monohydrolyzed product is sequentially and efficiently converted by both enzymes into the desired free amino acid, **4**. A similar sequence was observed by Liljeblad *et al.* in the hydrolyses of the methyl esters of racemic *N*-acylvaline and *N*-acylmethionine.[14] AM is clearly more active in

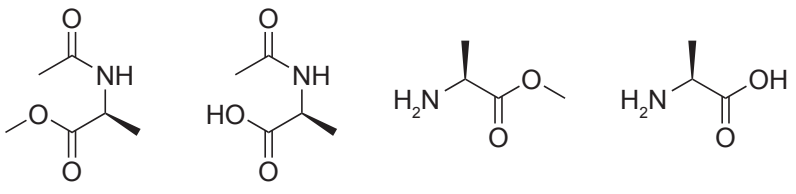


Figure 3. Substrate and possible products of the hydrolysis step.

‡ 1 U corresponds to the amount of enzyme which hydrolyses 1µmol N-acetyl-*L*-methionine per minute at pH 7.5 and 22 °C.

this hydrolysis sequence than PK, even though more units for PK were used. AM reaches a conversion of **3a** of 98% within 4h, whereas PK only achieves 14% in the same time-frame. With both enzymes there is still a significant quantity of **3b** present after 4 h. The conversion of **3a** with AM remains at 98% after 24 h, but now **4** is the only product. As is described in literature acylase I demonstrates high enantioselectivity for the ester as well as the amide hydrolysis,[14, 15] resulting in a maximum conversion of 98%, since the remaining 2% of the substrate is the wrong enantiomer. The enantioselectivity of the hydrolysis is confirmed by the enantiopurity of **4** (ee of >98%, as determined by chiral HPLC). The same analysis for PK showed that this enzyme also exhibits a very high selectivity (>98% ee). Thus, by using this chemo-enzymatic cascade, we not only reduced the total number of steps and made the synthesis entirely catalytic, but we also enhanced the enantiopurity of the product.

In our systematic exploration towards a genuine cascade process for the asymmetric synthesis of the amino acid, we also investigated the possibility to perform the reaction without intermediate removal of the rhodium catalyst. The hydrolysis is simply initiated by addition of enzyme and buffer. Consequently, the hydrogenation catalyst is still present in the hydrolytic phase. The activity and selectivity of AM was unaffected by **1**-AlTUD-1 (Table 1). In contrast, PK exhibited significantly lower conversions, especially after 24 h. The selectivity of both enzymes on the other hand remained excellent. Thus, the unfiltered system provides the same advantages as the filtered protocol, e.g. enhanced enantiopurity, compared to the stepwise synthesis. Additionally, the process is further simplified by eliminating the filtration step.

We also investigated if it would be possible to perform a genuine cascade process with all the ingredients, rhodium-catalyst as well as buffer and enzyme, already present from the beginning. Under these conditions **2** was subjected to hydrogenation at 5 bar H_2 and 22 °C. However, after 24 h only a negligible amount of hydrogen was consumed. Visual inspection of the one-pot reaction revealed that the enzyme had been adsorbed onto the support of the hydrogenation catalyst, thereby greatly diminishing accessibility of the catalyst. To determine whether the buffer also influenced the hydrogenation activity, the hydrogenation of **2** utilizing **1**-AlTUD-1 was conducted separately in the phosphate buffer. This revealed that the phosphate buffer is indeed part of the problem. In this medium 24% conversion with an ee of 74% was reached, compared to a conversion of 100% with an enantioselectivity of 95% in water.

In order to circumvent this problem, we modified the substrate. By switching from the ester to the sodium salt of **3a**, the hydrolysis reaction becomes pH neutral, thus eliminating the need for a buffer.§ Furthermore, immobilization of the enzyme will

§ AM requires the presence of buffer for the hydrolysis of **3a**. The drop in pH caused by the ester hydrolysis completely deactivates AM in non-buffered media.

overcome its adsorption on the TUD-1 surface. After screening several immobilized acylases, the cross-linked enzyme aggregate (CLEA) of AM was selected[16] due to its superior activity. This modified one-pot reaction is depicted schematically in Figure 4.

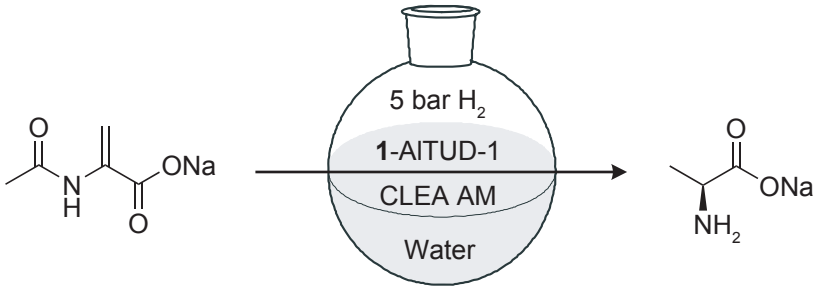


Figure 4. Schematic representation of the modified one-pot procedure.

Unfortunately, the hydrogen consumption was very slow under these conditions. After 24 h merely 7% conversion of the substrate was reached. However, the product consisted entirely of **4**, demonstrating that the enzyme does not lack activity in this system, even though accurate data on the enzyme activity cannot be derived from these results. Surprisingly, the enzyme did not hydrolyse the remainder of the substrate, the unhydrogenated **2**, as no side products were detected in the reaction mixture. The spatial requirements of the unsaturated amino acid most likely hinder the productive docking of the substrate into the active site. Apparently the activity of **1**-AlTUD-1 in this system is still inhibited, despite the absence of phosphate buffer or free enzyme. An explanation for these results is suggested by the report of Malmström et al.,[17] who demonstrated that the rates of rhodium catalyzed hydrogenation of 2-acetamidoacrylic acid are strongly dependent on the pH of the mixture. They showed that the steepest change occurred between pH of 4.5 and 3.2, which coincides with the pK_a-value of 2-acetamidoacrylic acid. From their NMR data it could be deduced that by going from protonated to completely unprotonated substrate, the coordination mode of the olefin changes. At low pH the chelate complex involves the double bond and the amide carbonyl, while at higher pH it consists of the double bond and carboxylate anion. This carboxylate complex undergoes oxidative addition of hydrogen much slower, which explains the poor hydrogenation activity in our modified one-pot setup.

Conclusion

We have successfully combined immobilized Rh-MonoPhos (**1**-AlTUD-1) and Acylase I, leading to a chemo-enzymatic cascade for the enantioselective synthesis of amino acids. This entirely catalytic sequence offers several major advantages compared to the classic amino acid synthesis via asymmetric hydrogenation followed by chemical hydrolysis. By employing this cascade, the formation of stoichiometric amounts of salts and the use of organic solvents are eliminated and the number of reaction steps is reduced to one. In addition to creating a genuinely sustainable process, this methodology also produces a superior product, since the enantiopurity is greatly enhanced. This truly demonstrates the power of smart reaction design. The key to the successful combination is the compartmentalization of both catalysts. We have demonstrated the feasibility of a one-pot procedure, superior results being obtained in a sequential protocol. Even when comparing the two investigated sequential protocols, with and without filtration, the one with the higher degree of compartmentalization (filtered) is preferred, since it offers the possibility to recycle the hydrogenation catalyst. Having demonstrated the effectiveness of compartmentalization by catalyst immobilization in the amino acid synthesis to achieve sustainable processes, we believe it to be widely applicable in chemical synthesis. We also note that the process is amenable to operation in a membrane reactor in which the organometallic catalyst and the enzyme are compartmentalized on different sides of an ultrafiltration membrane.

Experimental section

General

Reactions and manipulations involving air-sensitive compounds were performed under an atmosphere of dry nitrogen using standard Schlenk-type techniques. Dry solvents for the synthesis of the catalysts were purchased from Aldrich and deoxygenated before use. Solvents used in de hydrogenation were also de-oxygenated before use. Methyl 2-acetamidoacrylate,[10] Bis(1,5-cyclooctadiene)rhodium tetrafluoroborate,[18] (*R*)-MonoPhos[19], $[Rh^{I}(cod)((R)\text{-MonoPhos})_2]BF_4$ [9] and acylase CLEA[16] were prepared according to literature procedures. Chloro(1,5-cyclooctadiene)rhodium dimer was purchased from Strem. Acylase I from *Aspergillus melleus* was obtained from Fluka and Acylase I from porcine kidney grade II from Sigma. All other reagents were purchased from Aldrich, Acros or Fluka and used without further purification. Hydrogenations were performed in a 100 ml Parr hastelloy C autoclave (A1128HC). NMR spectra were

recorded on a Varian Inova 300 MHz or a Varian VXR 400s spectrometer, relative to TMS. Enantiomeric excesses of **3a** were determined by chiral GC using a Shimadzu GC-17A, equipped with a Chiralsil DEX CB column (25 m x 0.32 mm, df = 0.25 μm), He as carrier gas, split injector (36/100) at 220 °C and FID at 220 °C. Retention times (min) at 95 °C isotherm: 2-acetamidoacrylate (**2**) (5.4), (*S*)-methyl 2-acetamidopropanoate (*S*-**3a**) (7.5) and (*R*)-methyl 2-acetamidopropanoate (*R*-**3a**) (8.1). Enantiomeric excesses of **4** were determined by chiral HPLC using a Crownpak CR (+) column (150 x 4 mm) with $HClO_4$ (pH = 1) as eluens, a flow of 0.5 ml/min at 0 °C and UV detection at 215 nm. Retention times (min): D-alanine (4.6) and L-alanine (10.8). The rhodium content of the immobilized catalysts were measured using instrumental neutron activation analysis (INAA), which was performed at the Interfaculty Reactor Institute (IRI), Delft. The "Hoger Onderwijs Reactor", with a neutronflux of 10^{17} neutrons s^{-1} cm^{-2}, was used as a source of neutrons and the gammaspectrometer was equipped with a germanium semiconductor as detector.

Immobilization procedure for $[Rh^{I}(cod)((R)\text{-MonoPhos})_2]BF_4$

AlTUD-1 (1.0 g) was dried at 200 °C under vacuum for 2 h. To the dried support 2-propanol (45 ml) was added. After 30 min stirring, Rh-MonoPhos (0.15 g, 0.15 mmol) in 2-propanol (40 ml) was added and the resulting suspension stirred for 3 h. The solid was collected by filtration and washed thoroughly with portions of 30 ml 2-propanol until the washings were colorless (approx. 5 times). Finally, the catalyst was dried at 55 °C under vacuum for 2 h. Rh loading was determined by INAA: 11 mg Rh/g support, which corresponds to an $Al_{tetrahedral}$/Rh ratio of approximately 10.

Hydrogenation reaction of cascade.

All hydrogenation experiments were performed with 0.1 g of immobilized catalyst (~1 w% Rh). **1**-AlTUD-1 (0.1 g) was transferred to the autoclave under a nitrogen atmosphere, followed by substrate solution (50 ml water, 0.05 M **2**). The sealed autoclave was purged with hydrogen by pressurizing to 7 bars while stirring at 300 rpm, followed by release of pressure. This cycle was repeated 5 times and finally the desired pressure was applied and the stirring speed was increased to 1000 rpm. After 1 h the remaining hydrogen pressure was released and the autoclave was purged three times with nitrogen.

Enzymatic hydrolysis reaction of cascade

After an optional filtration of the hydrogenation mixture (50 ml), phosphate buffer (5 ml, pH 7.5, 1.1 M) was added followed by AM (205 mg, 175 U) or PK (90 mg, 700 U). This mixture was shaken for 24 h during which samples were obtained. The samples were adjusted to pH 5 with 1 M HCl, heated to 60 °C with Norit, filtered over Celite and lyophilized. Conversions were determined by ^{1}H-NMR. Before determining the enantioselectivity, the samples were passed over a DOWEX-50 (H^{+}) column, which was rinsed with water until neutral, followed by ammonia (1 M). The ammonia layer was again lyophilized.

One-Pot procedure

1-AlTUD-1 (0.1 g) and Acylase I from *Aspergillus melleus* (82 mg) were transferred to the autoclave under a nitrogen atmosphere, followed by substrate solution [50 ml, 0.05 M in phosphate buffer (pH 7.5, 0.1 M) for the one-pot or in water for the modified one-pot protocol respectively]. The sealed autoclave was purged with hydrogen by pressurizing to 7 bars while stirring at 300 rpm, followed by release of pressure. This cycle was repeated 5 times and finally the desired pressure was applied and the stirring speed was increased to 1000 rpm. After 24 h the remaining hydrogen pressure was released and the autoclave was purged three times with nitrogen.

References

[1] M. Poliakoff, J. M Fitzpatrick, T. R. Farren, P. T. Anastas, *Science*, **2002**, *297*, 807.

[2] R. A. Sheldon, *Chemtech*, **1994**, *24*, 38.

[3] M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer, T. Zelinski, *Angew. Chem. Int. Ed.*, **2004**, *43*, 788.

[4] A. Bruggink, R. Schoevaart, T. Kieboom, *Org. Process Res. Dev.*, **2003**, *7*, 622.

[5] O. Pamies, J.-E. Bäckvall, *Chem. Rev.*, **2003**, *103*, 3247.

[6] R. Schoevaart, T. Kieboom, *Top. Catal.*, **2004**, *27*, 3.

[7] F. Gelman, J. Blum, D. Avnir, *J. Am. Chem. Soc.*, **2002**, *124*, 14460.

[8] C. Simons, U. Hanefeld, I. W. C. E. Arends, A. J. Minnaard, T. Maschmeyer, R. A. Sheldon, *Chem. Commun.*, **2004**, 2830.

[9] M. van den Berg, A. J. Minnaard, R. M. Haak, M. Leeman, E. P. Schudde, A. Meetsma, B. L. Feringa, A. H. M. de Vries, C. E. P. Maljaars, C. E. Willans, D. Hyett, J. A. F. Boogers, H. J. W. Henderickx, J. G. de Vries, *Adv. Synth. Catal.*,

2003, *345*, 308.
[10] C. Simons, U. Hanefeld, I. W. C. E. Arends, R. A. Sheldon, T. Maschmeyer, *Chem. Eur. J.*, **2004**, *10*, 5829.
[11] W. S. Knowles, *Angew. Chem. Int. Ed.*, **2002**, *41*, 1998.
[12] K. Faber, Editor, *Biotransformation in Organic Chemistry, 3rd Completely Revised Edition*, Springer, Berlin, **1997**.
[13] H. K. Chenault, J. Dahmer, G. M. Whitesides, *J. Am. Chem. Soc.*, **1989**, *111*, 6354.
[14] A. Liljeblad, R. Aksela, L. T. Kanerva, *Tetrahedron: Asymmetry*, **2001**, *12*, 2059.
[15] M. I. Youshko, L. M. van Langen, R. A. Sheldon, V. K. Svedas, *Tetrahedron: Asymmetry*, **2004**, *15*, 1933.
[16] M. L. Bode, F. van Rantwijk, R. A. Sheldon, *Biotechnol. Bioeng.*, **2003**, *84*, 710.
[17] T. Malmström, O. F. Wendt, C. Andersson, *Dalton Trans.*, **1999**, 2871.
[18] T. G. Schenck, J. M. Downes, C. R. C. Milne, P. B. Mackenzie, T. G. Boucher, J. Whelan, B. Bosnich, *Inorg. Chem.*, **1985**, *24*, 2334.
[19] R. Hulst, N. Koen de Vries, B. L. Feringa, *Tetrahedron: Asymmetry*, **1994**, *5*, 699.

Summary

Chemical production processes often require wasteful and expensive isolation as well as purification of intermediates. Catalytic cascades offer a unique opportunity to eliminate these inefficient and polluting steps, in particular when carefully orchestrated, involving enzymes and chemocatalysts.

This thesis describes our efforts towards a genuinely sustainable chemo-enzymatic cascade for the synthesis of enantiopure amino acids, based on the benchmark Monsanto L-DOPA process. By replacing the wasteful chemical hydrolysis step of the L-DOPA process with an enzymatic reaction and by combining this with the sequential enantioselective catalytic reduction in one step a sustainable cascade process should be plausible.

Chapter 1 begins with a short overview of the possibilities as well as limitations of transition metal catalysis and biocatalysis. The various aspects of these types of catalysis are clarified with some illustrative examples. Based on these separate discussions, the opportunities in a merger between these two fields, leading to chemo-enzymatic cascades for the production of fine chemicals, is examined. Finally the current state-of-the-art in chemo-enzymatic cascades is reviewed.

Chemo-enzymatic cascades are often hampered by the incompatibility of the various catalysts, reagents and conditions. In Chapter 2 the incompatibility issues for the asymmetric hydrogenation step are addressed by the non-covalent anchoring of the cation catalytic complexes on a solid support. To this end a new Brønsted acidic aluminosilicate, AlTUD-1, with ideal characteristics for catalyst immobilisation (mesoporous structure, high surface area and high $Al_{tetrahedral}$/Si ratio), was developed. The two well-established asymmetric hydrogenation catalysts: [Rh^{I}(cod){(*R*,*R*)-MeDuPHOS}]BF_4 and [Rh^{I}(cod){(*S*,*S*)-DiPAMP}]BF_4 were successfully immobilised on this new support, resulting in highly active and selective catalysts for the asymmetric reduction of dimethyl itaconate and methyl 2-acetamidoacrylate, giving enantiomeric excesses of up to >98%. Leaching of Rh showed a significant dependence on the polarity of the solvent in which the catalysis was performed.

In Chapter 3 the catalytic performance of the immobilised hydrogenation catalyst is further improved by substituting the Rh-complexes with [Rh((*S*)-MonoPhos)$_2$

(cod)]BF_4. Like the previously immobilised catalysts, the new catalyst was highly active and selective with the additional advantage of improved stability and a less solvent dependent enantioselectivity. The immobilised MonoPhos catalyst could even be used in water. In addition this is the first time that a monodentate phosphoreamidite Rh complex was anchored by a non-covalent approach.

In Chapter 4 the influence of the support on the asymmetric hydrogenation of dehydroamino acids using non-covalently immobilised catalysts was investigated. Rh-MonoPhos was successfully immobilised on four different anionic carrier materials: a mesoporous aluminosilicate (AlTUD-1), phosphotungstic acid on alumina (PWTUD), Nafion, and a Nafion silica composite (SAC-13). These heterogeneous catalysts were evaluated in the asymmetric reduction of methyl-2-acetamidoacrylate. Although most of the catalysts were highly selective, the activity and the loss of rhodium were strongly dependent on the type of support. PWTUD appeared to be the best support for this application, because it gave the catalyst with the highest activity and virtually no leaching in any solvent. Its superior anchoring ability derives from the additional bonding between the Rh and the phosphotungstic acid. Nafion, on the other hand, was by far the poorest support, giving very low activity. Immobilisation with this support relies on encapsulation rather than on ionic interactions. Its encapsulating properties not only prevent the complex from going into solution, but also prevent the substrate from reaching the catalytic site.

After having optimised the catalyst for the hydrogenation step, Chapter 5 describes the work done to find the ideal candidate for the enzymatic hydrolysis in the cascade procedure. To ensure that the full potential of hydrolases was utilized 15 different commercially available lipases, acylases, proteases and an esterase were studied for the removal of *N*-acyl and *N*-formyl protecting groups in amino acid derivatives. In addition to the well-known acylases from porcine kidney and *Aspergillus melleus*, this screening revealed that porcine liver esterase (PLE) and the lipases from *Rhizomucor miehei* and *Pseudomonas stutzeri* are also efficient catalysts for the hydrolysis of *N*-acylalanine. Furthermore, three enzymes were found to be active in the hydrolysis of *N*-formylalanine, i.e. PLE and the two acylases. This is the first example where enzymes, other than deformylases, are employed to deprotect *N*-formyl amides.

Finally in Chapter 6, the two optimised catalytic transformations are combined to afford a successful chemoenzymatic cascade process. The combination of immobilised

Rh-MonoPhos and acylase I produced amino acids enantioselectively in water, without the need for isolation of intermediates. In addition, the enzymatic hydrolysis increases the enantiopurity of the product from 95% ee to >98% ee. Compatibility studies revealed that for optimum results compartmentalisation of the catalysts is required.

Samenvatting

Doorgaans vereisen chemische productieprocessen verspillende en kostbare isolatie en zuivering van intermediairen. Katalytische cascades zijn bij uitstek geschikt om deze inefficiënte en vervuilende stappen te elimineren. In het bijzonder als ze zorgvuldig worden ontworpen door gebruik te maken van zowel enzymen als chemokatalysatoren.

Dit proefschrift beschrijft onze pogingen om een werkelijk duurzame chemo-enzymatische cascade, die gebaseerd is op het Monsanto L-DOPA proces, te ontwikkelen voor de synthese van enantiozuivere aminozuren. Door de verspillende chemische hydrolyse van het L-DOPA proces te vervangen door een enzymatische reactie en deze in een stap te combineren met de daaropvolgende enantioselectieve katalytische hydrogenering, ligt een duurzame chemo-enzymatische cascade binnen handbereik.

Hoofdstuk 1 begint met een kort overzicht van zowel de mogelijkheden als de beperkingen van overgangsmetaalkatalyse en biokatalyse. De verschillende aspecten van elk van deze typen katalyse worden verduidelijkt met enkele voorbeelden. Na de afzonderlijke besprekingen worden de mogelijkheden van een fusie tussen deze twee gebieden om te komen tot chemo-enzymatische cascades behandeld. Tot slot wordt de huidige stand van zaken met betrekking tot chemo-enzymatische cascades besproken.

Chemo-enzymatische cascades worden vaak belemmerd door incompatibiliteit van de diverse katalysatoren, reagentia en reactiecondities. In Hoofdstuk 2 worden de incompatibiliteitsproblemen voor de asymmetrische hydrogeneringsstap aangepakt door het kationisch katalysatorcomplex niet-covalent te verankeren op een vast dragermateriaal. Hiertoe werd een nieuw anionisch aluminosilicaat, AlTUD-1, ontwikkeld met ideale karakteristieken voor katalysator immobilisatie (mesoporeuze structuur, groot oppervlak en relatief hoge $Al_{tetrahedral}$/Si verhouding). De twee gevestigde asymmetrische hydrogeneringskatalysatoren, $[Rh^{I}(cod)\{(R,R)\text{-MeDuPHOS}\}]BF_4$ en $[Rh^{I}(cod)\{(S,S)\text{-DiPAMP}\}]BF_4$, zijn met succes op het nieuwe dragermateriaal geïmmobiliseerd en dit resulteerde in zeer actieve en selectieve katalysatoren voor de enantioselectieve reductie van dimethylitaconaat en methyl 2-acetamidoacrylaat met een enantiomere overmaat tot >98%. Het verlies van Rh was sterk afhankelijk van de polariteit van het gebruikte oplosmiddel.

In hoofdstuk 3 wordt de prestatie van de geïmmobiliseerde hydrogeneringskatalysator verder verbeterd door het Rh-complex te vervangen door [Rh^{I}((*S*)-MonoPhos)$_2$(cod)] BF_4. Even zoals de eerder geïmmobiliseerde katalysatoren, was de nieuwe katalysator zeer actief en selectief met als bijkomstig voordeel een verbeterde stabiliteit en een minder oplosmiddel-afhankelijke enantioselectiviteit. De geïmmobiliseerde Rh-MonoPhos katalysator was zelfs toepasbaar in water en het is daarnaast ook de eerste keer dat een monodentate fosforamidiet Rh complex niet-covalent is verankerd.

In hoofdstuk 4 wordt de invloed van de drager bestudeerd op de asymmetrische hydrogenering van dehydroaminozuren door niet-covalent gebonden katalysatoren. Rh-MonoPhos werd met success geïmmobiliseerd op vier verschillende anionische dragermaterialen: mesoporeuze aluminosilicaat (AlTUD-1), fosforwolfraamzuur op alumina (PWTUD), Nafion, en een Nafion silica composiet (SAC-13). Deze heterogene katalysatoren werden getest in de asymmetrische hydrogenering van methyl-2-acetamidoacrylaat. Ook al waren de meeste katalysatoren zeer selectief, de activiteit en het verlies van rhodium waren sterk afhankelijk van het type drager. PWTUD bleek de meest geschikte drager te zijn voor deze toepassing, aangezien het de hoogste activiteit opleverde en nagenoeg geen verlies van rhodium ten gevolge had in de diverse oplosmiddelen. Zijn superieur bindend vermogen wordt ontleend aan de extra binding tussen Rh en fosforwolfraamzuur. Nafion daarentegen is met grote afstand de slechtst presterende drager, mede door de extreem lage activiteit. Bij deze drager berust de immobilisatie op insluiting in plaats van ionische interactie. De insluiting verhindert niet alleen dat het complex in oplossing gaat, maar ook dat het substraat het actieve centrum bereikt.

In navolging van de optimalisatie van de katalysator voor de hydrogeneringsstap, beschrijft hoofdstuk 5 de zoektocht naar de ideale kandidaat voor de enzymatische hydrolyse. Om te garanderen dat het volledige arsenaal van de hydrolases wordt benut, zijn er 15 verschillende, commercieel verkrijgbare lipases, acylases, proteases en een esterase bestudeerd voor de ontscherming van de *N*-acyl en *N*-formyl groep bij aminozuurderivaten. Naast de bekende acylases van *Aspergillus melleus* en de varkensnier, heeft deze screening aangetoond dat ook varkenslever esterase (PLE) en de lipases van *Rhizomucor miehei* en *Pseudomonas stutzerii* effectieve katalysatoren zijn voor de hydrolyse van *N*-acylalanine. Tevens bleken drie enzymen actief te zijn in de hydrolyse van *N*-formylalanine, namelijk PLE en de twee acylases. Dit is de eerste melding van andere enzymen dan de deformylases, die actief zijn in de ontscherming

van *N*-formylamides.

Tot slot worden in hoofdstuk 6 de twee geoptimaliseerde katalytische transformaties met succes gecombineerd tot een chemo-enzymatisch cascade proces. De combinatie van de geïmmobiliseerde Rh-MonoPhos en acylase I leidde tot een enantioselectieve productie van aminozuren in water, zonder dat de intermediairen behoeven te worden geïsoleerd. Daarnaast verhoogde de enzymatische hydrolyse de enantiozuiverheid van 95% ee naar >98% ee. Compatibiliteitstudies toonden aan dat voor de beste resultaten fysieke scheiding van de katalysatoren is vereist.

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Chrétien
(Eindelijk klaar)

Curriculum Vitae

Chrétien Simons werd op 6 juni 1977 in Zoetermeer geboren. In 1995 werd het VWO diploma behaald aan het Pallas College te Zoetermeer, waarna werd begonnen aan de studie Scheikundige technologie aan de Technische Universiteit Delft. De studie werd in september 1996 voort gezet aan de Rijksuniversiteit Leiden in de richting Scheikunde. In de doctoraal fase van deze studie werd als specialisatie coordinatie chemie en homogene katalyse gekozen met als afronding een afstudeerproject in de vakgroep Coordinatie en Bioanorganische Chemie van Prof. dr. J. Reedijk. Het onderzoek betrof de optimalisatie van de Cu-gekatalyseerde oxidatieve polymerisatie van 2,6-dimethylphenol voor de productie van de thermoplast PPE. In januari 2001 werd het doctoraal examen met succes afgelegd en in datzelfde jaar werd onder begeleiding van prof. dr. Thomas Maschmeyer en prof. dr. Roger A. Sheldon begonnen aan het promotieonderzoek, dat in dit proefschrift staat beschreven. Per januari 2006 is hij in dienst van de Nederlandse Cosmetica Vereniging als technisch medewerker.