DISSERTATION

Evaluation of an enzyme library of reductases for application in bioactive compound synthesis

ausgeführt zum Zwecke der Erlangung des akademischen Grades eines Doktors der technischen Wissenschaften unter der Leitung von

Ao. Univ. Prof. Dipl.-Ing. Dr.
Marko D. Mihovilovic

E 163

Institut für Angewandte Synthesechemie

Eingereicht an der Technischen Universität Wien
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Für meiner Mutter

Ich möchte mich bei meinen Eltern für die fortwährende finanzielle und moralische Unterstützung während der Zeit meines Studiums bedanken und dafür, dass sie mir eine Universitätsausbildung so selbstverständlich erscheinen ließen.
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Ein weiterer Dank gilt meiner Tante für die jahrelange Untersützung ebenso wie meiner Schwester.

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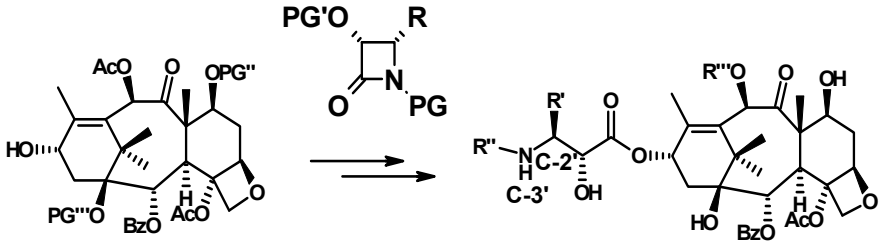
Legend:

All compounds prepared or used as starting material are numbered in bold Arabic numbers. Compounds known in the literature are additionally underlined.

Literature citations are indicated as subscript Arabic letters.

Deutsche Kurzfassung

Taxol ist eines der vielversprechendsten Krebstherapeutika, speziell die C-13 Seitenkette lässt eine hohe Flexibilität für chemische Veränderungen zu, jedoch limitieren Nebeneffekte die Anwendung.
Das Ziel der vorliegenden Arbeit war die Untersuchung der Bioreduktion von verschiedenen β-Lactamen mittels Hefereduktasen, die in *E.coli* kloniert wurden. Durch Einsatz dieser Reduktasen als chirale Katalysatoren können nicht racemische chirale Alkohole erzeugt werden. Mit diesen Intermediaten sollte die enantioselektive Synthese von modifizierten C-13 Seitenketten verwirklicht werden.

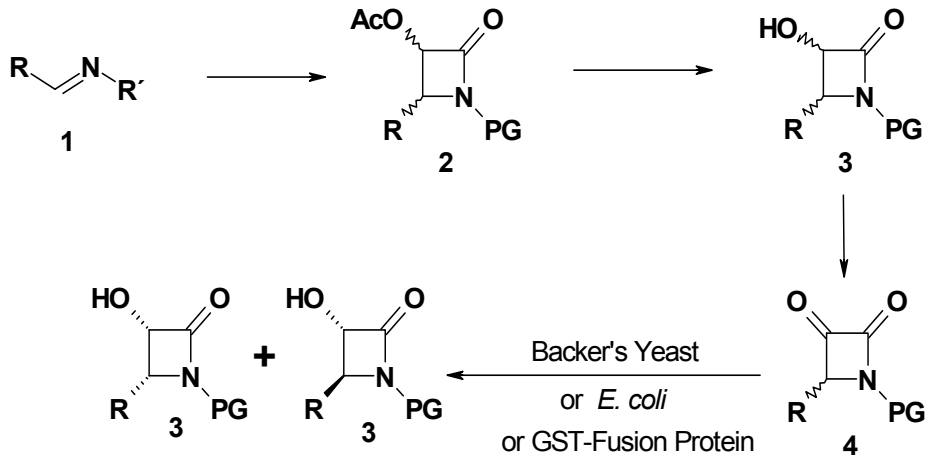


Baccatin III

Taxol derivative

In dieser Arbeit wird versucht eine schon bekannte und schnelle Methode weiter zu optimieren um α-Keto-β-lactame mit unterschiedlichen Resten (R) und verschiedenen Schutzgruppen (PG) herzustellen.

Von Imin (**1**) ausgehend wurden über eine [2+2] Staudinger-cyclisierung mittels Acetoxyessigsäurechlorid die unterschiedlichen Azetidine (**2**) synthetisiert. Mittels Hydrolyse wurden die verschiedenen Alkohole (**3**) hergestellt und durch anschließende Oxidation die Vorstufen (**4**) für die Bioreduktionsversuche erzeugt.



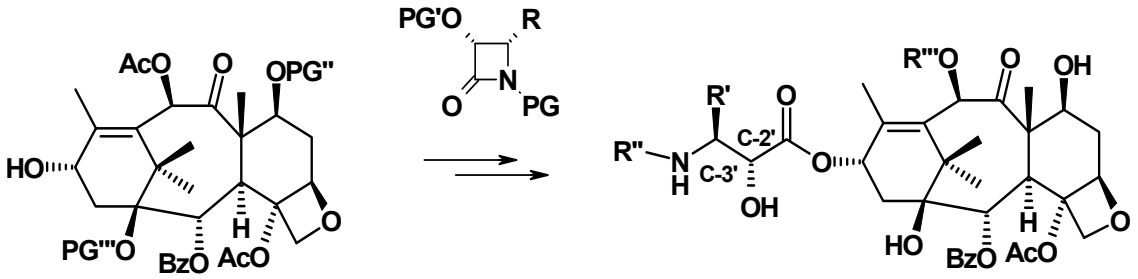
R= Ph, $CH_2C(CH_3)CHC_6H_5$, t-butyl, thiophen, furan
PG= -PMP, $-CH_2-C_6H_5$, $-CH_2-CH=CH_2$, $-C-(CH_3)_3$, $-CH_3$

Diese Vorstufen wurden auf biokatalytische Akzeptanz mit Hilfe eines Hefe-screenings getestet. Dabei konnte erfreulicherweise festgestellt werden, dass sich alle entwickelten Edukte für eine Biotransformation eignen, da sie von Hefe transformiert wurden. Jedoch konnte mit der Hefereduktion keine Enantiomerenreinheit, sondern nur Racemate oder Produkte mit begrenzter optischer Reinheit erhalten werden.
Da aber für die weitere Verwendung der synthetisierten Produkte Enantiomerenreinheit nötig ist, wurden weitere Versuche mit einzelnen in *E.coli* klonierten Hefereduktasen durchzuführen um optisch reine Produkte zu erhalten.
Ziel dieser Dissertation war die Untersuchung von 23, im Rahmen der Arbeit dargestellten Edukte, auf Substratakzeptanz durch handelsübliche Hefe, sowie von 19 Ganz-Zell-Systemen, als auch 15 in *E.coli* klonierte, zur Homogenität aufgereinigte, Hefereduktasen (GST-Fusionsproteine) zu überprüfen.

Abstract

Taxol is one of the most important anticancer drugs, especially the C-13 side chain tolerates a variety of modifications, but unwanted side effects limit the application.

Our aim is to modify this side chain to suppress the high hydrophobicity and the development of resistance. Within an ongoing research program we are searching for more suitable analoges of taxol by developing bioreductive approaches to optically pure C-13 side chain precursors. One appealing strategy in this context is the utilization of α-keto-β-lactams, which can be coupled with Baccatin III.

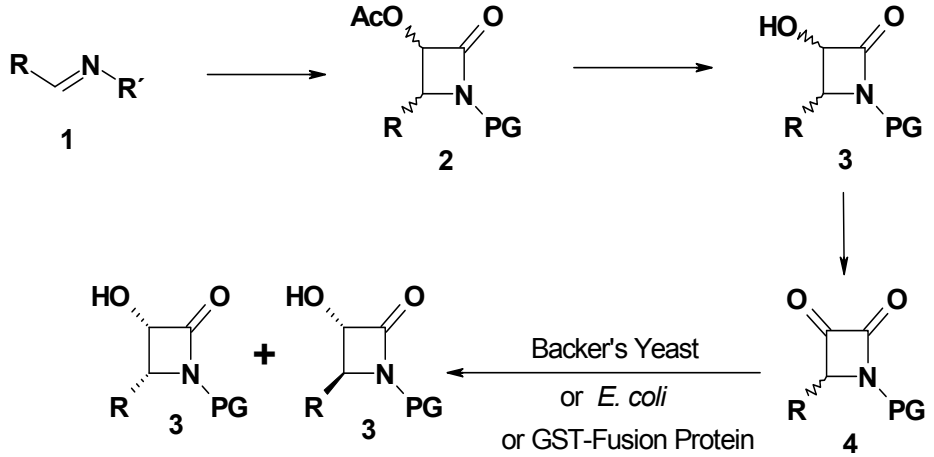


Baccatin III

Taxol derivative

In this PhD thesis we present the elaboration of a recently outlined facile and efficient method for the synthesis of α-keto-β-lactams by extension to diverse substituents R and various protecting groups (PG) at the nitrogen atom.

Starting from different imines **1** a [2+2] Staudinger type cycloaddition with activated carboxylic acid chloride was performed which leads to azetidines **2**. Straight forward functional group transformation enables access to ketolactams **3** as pivotal precursors **4** for bioreduction studies.



R= Ph, $CH_2C(CH_3)CHC_6H_5$, t-butyl, thiophen, furan
PG= -PMP, $-CH_2-C_6H_5$, $-CH_2-CH=CH_2$, $-C-(CH_3)_3$, $-CH_3$

These precursors were tested of their suitability as substrates for yeast reductases. The results were promising, because every substrate could by transform in the biotransformation. However, no enantiopure compound could be obtained; only racemic products or products with limited optically purity could be prepared.
Due to the fact, that only optically pure products are interesting for the C-13 side chain, some other experiments were realized. A collection of 15 reductases originating from yeast have been overexpressed in *Escherichia coli (E. coli)* as gluthatione (S)-transferase fusion proteins (GST-Fusion Protein). With these reductases we checked their ability as enantioselective reducing agents.

The aim of this PhD-Thesis was the detailed study of the 23 synthesized educts for substrate acceptance by the 15 yeast reductases by means of whole cell systems as well as with purified GST-Fusion-Proteins.

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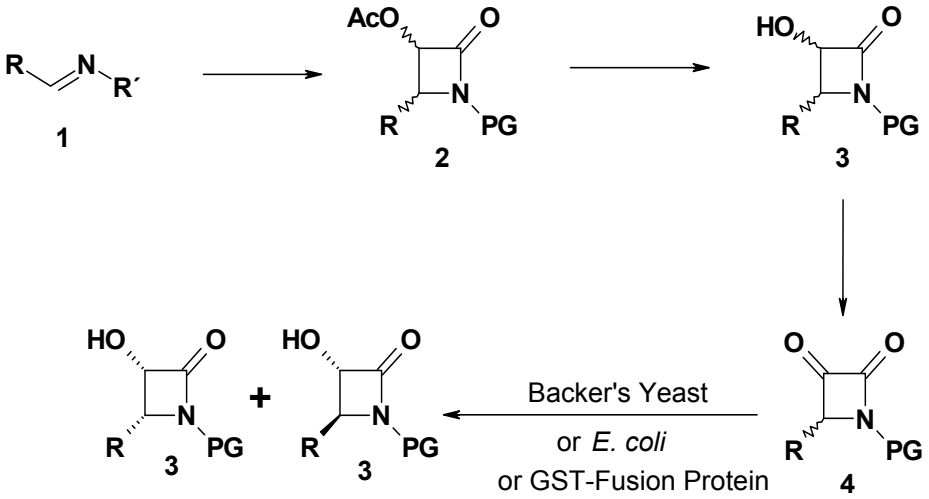
GENERAL SCHEMES

β-Lactam Method



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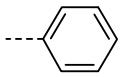
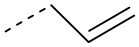
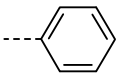
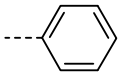
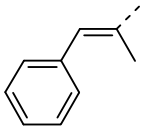
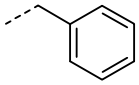
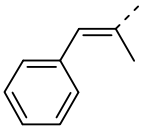
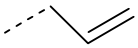
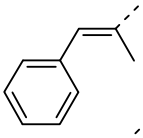
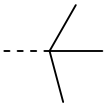
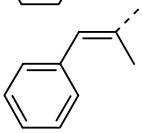
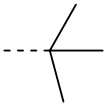
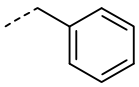
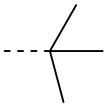
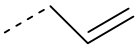
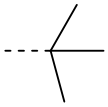
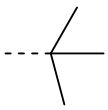
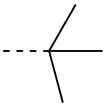
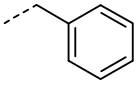
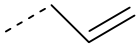
a		
b		
c		
d		
e		
f		
g		
h		
i		
j		
k		
l		
m		

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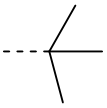
n		
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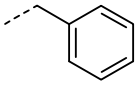
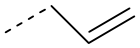
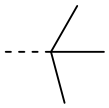
p		
q		
r		
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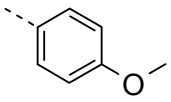
t		
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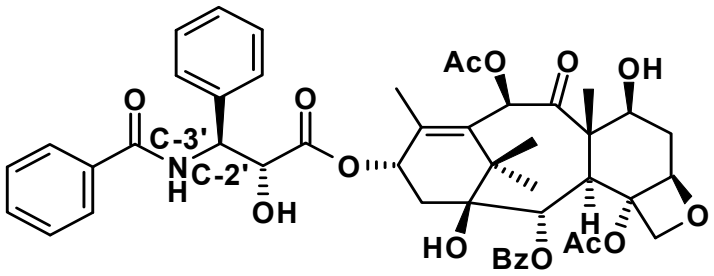
1. INTRODUCTION

1.1 Taxol and Taxol Derivatives

Medicinal plants are most promising sources for novel lead structures in modern drug synthesis. Many activity studies were made from the different active ingredients from plant sources, but the stock of useful compounds can have some few problems. For example the growth of the plant is too low; the quantity of the required chemical substance is limited; the active compound is only localized in specific organs and harvesting leads to the destruction of the natural sources[1]. Therefore it is very difficult to have a stable commercial supply of the active compound.

Paclitaxel, one of the most important anticancer drugs, which is commonly called Taxol, is a good example for a drug with such supply problems which is clearly reflected in the historic development of the drug. A closely reputed anticancer drug is docetaxel, in generally called Taxotere. They are effective agents for the treatment of ovarian and metastatic breast cancer, AIDS-related Kaposi's sarcoma[2] and non small cell lung cancer[3]. And it is being tested for the treatment of numerous cancer types in combination therapies with other antineoplastic agents[4].



Taxol

Taxol was collected from the bark of the Pacific yew tree (*Taxus brevifolia*). Unfortunately the concentration of Taxol in the bark is quite low, the product is contaminated, so that a purification step is necessary to obtain Taxol in pure form. Between 2000-4000 trees are needed to produce 1kg of pure Taxol. A vast spectrum of synthetic strategies have been developed for the synthesis of the taxane skeleton

[1] Frense, D. *Appl. Microbiol. Biotechnol*. **2007**, *73*, 1233-1240
[2] Rowinsky, E.K.; Cazenave, L.A.; Donehower, R.C. *J. Natl. Cancer Inst*. **1990**, *82*, 1247
[3] Spencer, C.M.; Faulds, D. *Drugs* **1994**, *48*,794-847
[4] Goldspiel, B.R. *Pharmacotherapy* **1997**, *17*, 110

but only moderate success has been reached in the total synthesis[5] of the naturally source taxanes with respect to possible commercialization.
The Holton Taxol total synthesis in 1994 was the first total synthesis of Taxol (generic name: paclitaxel).[6]

The Holton Taxol total synthesis is a good example of a linear synthesis starting from commercially available natural compound patchoulene oxide. This epoxide can be obtained in two steps from the terpene patchoulol and also from borneol. The reaction sequence is also enantioselective, synthesizing (+)-Taxol from (-)-patchoulene oxide or (-)-Taxol from (-)-borneol with a reported specific rotation of + and -47 (c=0.19 / MeOH). The Holton sequence to Taxol is relatively short compared to that of the other groups with an estimated 37 step not counting the addition of the amide tail. One of the reasons is that the patchoulol starting compound already contains 15 of the 20 carbon atoms required for the Taxol ABCD ring framework.
Other raw materials besides the already mentioned patchoulene oxide required for this synthesis are 4-pentenal, m-chloroperoxybenzoic acid, methyl magnesium bromide and phosgene. Two key chemical transformations in this sequence are a Chain rearrangement and a sulfonyloxaziridine enolate oxidation.

The β-lactam approach pioneered by Holton, Ojima and Georg remains the most widely used method, and this method is currently used by Bristol-Myers Squibb for the commercial synthesis of Taxol. As one example, Holton used the β-lactam methodology to prepare a number of analoges such as the furan and the thiophen derivatives.[7]
Another β-lactam approach has been developed by Holton that uses coupling of a suitably derivatized β-lactam with 7-(triethylsilyl)-baccatin III, followed by acid hydrolysis, to give Taxol in excellent yield.[8]

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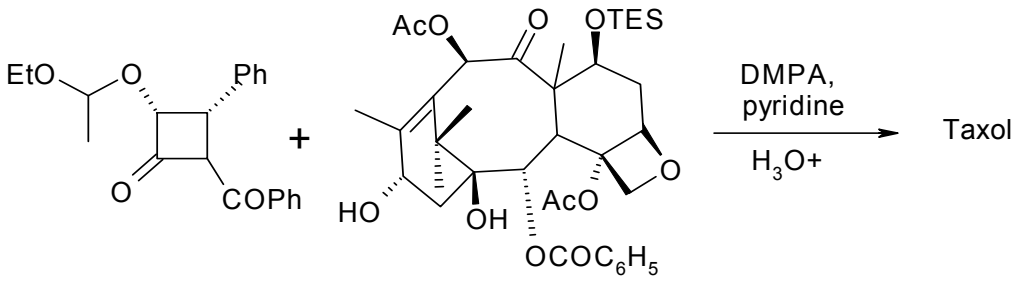
[5] a) Holton, R.A.; Somoza C.; Kim, H.B.; Liang, F.; Biediger, R.J; Boatman, P.D.; Shindo, M.; Smith, C.C.; Kim, S.J. *Am. Chem. Soc.* **1994**, *116(4)*, 1597-1598 b) Nicolaou, K.C ;Yang, Z.; Liu, J.J.; Ueno, H.; Nantermet, P.G.; Guy, R.; Claiborne, C.F.; Renaud, J.; Couladouros, E.A.; Paulvannan, K.; Sorensen, E.J. *Nature* **1994**, *367*, 630 - 634 c) Kingston, D.G.I. *J. Nat. Prod* Review, **2000**, *63*, 726-734 d) Fritzpatrick F.A., Wheeler, R. *Intern. Immunopharma*. **2003**, *3*, 1699-1714

[6] a) Robert A. Holton, R.A.; Kim, H.B.; Somoza, C.; Liang, F.; Biediger, R.J.; Boatman, P.D.; Shindo, M.; Smith, C.C.; Kim, S. *J. Am. Chem. Soc.*, 1994, 116(4), 1599 - 1600 b) Holton,R.A.; Juo, R.R.; Kim, H.B.; Williams, A.D.; Harusawa, S.; Lowenthal, R.E.; Yogai S. *J. Am. Chem. Soc.* 1988, 110(19), 6558-6560

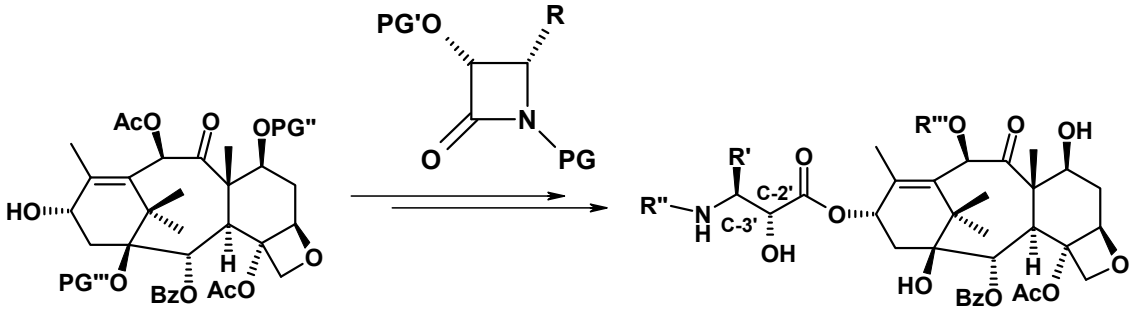
[7] Zechmeister, L.; Glasenapp-Breiling, M.; Herz W. *Progress in the Chemistry of Organic Natural Products*, Springer-Verlag, **2002**, *1-253*

[8] Holton, R.A. Eur. Pat. Appl.EP 400,971, **1990**: Chem. Abst. **1990**, 114, 164568q



Holton's approach requires 5 equivalents of β-lactam. Furthermore, this reaction is very slow and is performed under almost neat conditions. Ojima and collaborators have developed optimal conditions for this method.[9]
The β-lactam synthon method was also used by several other groups.[10] They all tried different syntheses of different β-lactam derivatives and their coupling with suitable protected baccation III derivatives in order to synthesize Taxol or Taxotere.

But this process has recently received competition by biological methods of production, in fact direct extraction from the Chinese yew tree (*Taxus chinensis or Taxus yunnanensis*) and plant cell culture fermentation processes without the need of synthetic intervention, furnish industrial quantities of Taxol.



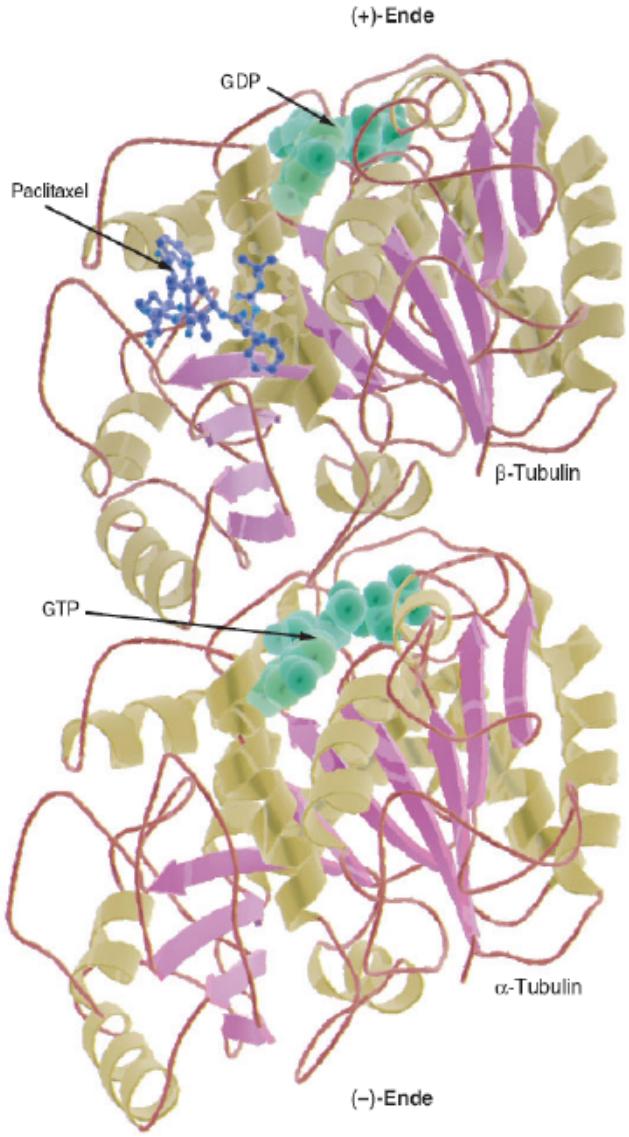
Baccatin III

Taxol derivative

[9] Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y.H.; Sun, C.M.; Brigaud, T. *Tetrahedron*, **1992**, *48*, 6985-7012

[10] a) Brieva, R.; Crich, J.Z.; Sih, C.J. *J. Org.Chem.* **1993**, *58*, 1068-1075 b) Georg, G.I.; Cheruvallath, Z.S.; Harriman, G.C.B.; Hepperle, M.; Park, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2467-2470 c) Farina, V.; Hauck, S.I.; Walker, D.G. *Synlett* **1992**, *1*, 761-763 d) Ojima, I.; Pack, Y.M.; Sun, C.M.; Brigaud, T.; Zhao, M. *Tetrahedron Lett.* **1992**, *33*, 5737-5740 e) Annunziata, R.; Benaglia, M.; Cinquini, M.; Cozzi, F.; Ponzini, F. *J. Org. Chem.* **1993** *58*, 4746-4748 f) Palomo, C.; Aizpurua, J.M.; Miranda, J.I.; Mielgo, A.; Odriozola, J.M. *Tetrahedron Lett.* **1993** *34*, 6325-6328 g) Brown, S.; Jordan, A.M.; Lawrence, N.J.; Pritchard, R.G.; McGown, A.T. *Tetrahedron Lett.* **1998** *39*, 3559-3562 h) Ojima, I.; Wang, T.; Delaloge, F. *Tetrahedron Lett.* **1998**, *39*, 3663-3666 i) Ojima, I.; Delaloge F. *Chem. Soc. Rev.* **1997** *26*, 377-386 j) Ojima, I.; Pack, Y.M.; Zucco, M.; Duclos, O.; Kuduk, S.A. *Tetrahedron Lett.* **1993**, *34*, 4149-4152

Taxol acts primarily through an interaction with tubulin. Taxol has been widely used as anti-microtubule agents.



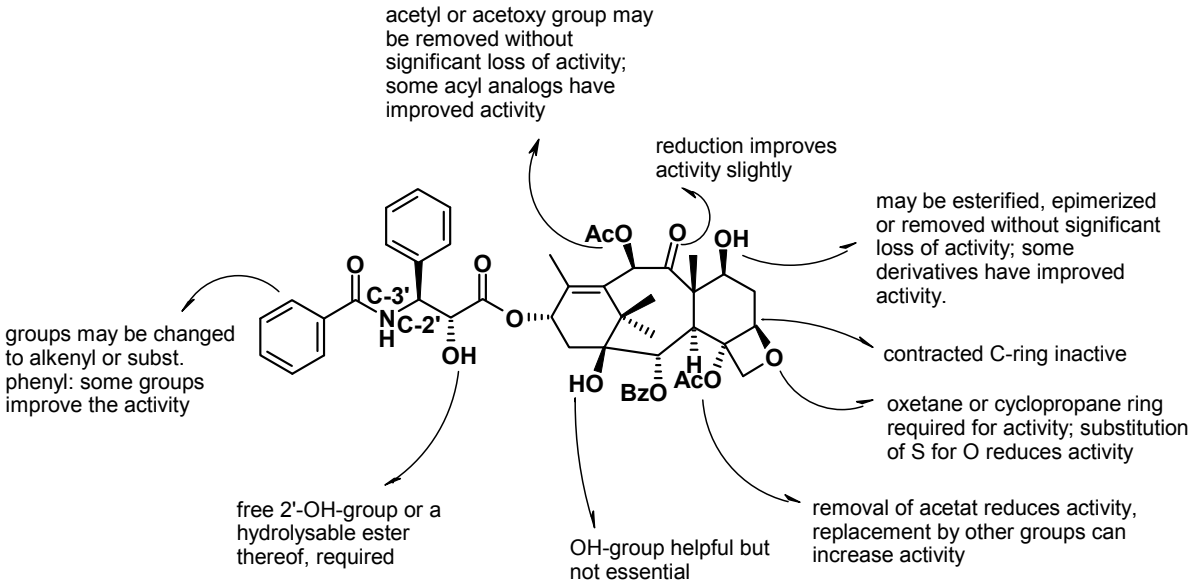
Tubulin is the “building block” of microtubules is a critical target molecule of anti-microtubule agents. During the cell cycle, the tubulin function depends on tubulin polymerization or depolymerization. When microtubule dynamics are disrupted with anti-microtubule agents in dividing cells, metaphase arrest or/and apoptosis occur[11]. Anti-microtubule agents can be classified into two types according to their action mechanism: microtubule-stabilizing agents and microtubule-destabilizing agents.

[11] Shin, D.-Y., Choi, t.-S. *J. Reprod. Develop.* **2004**, *50*, 647-652

Taxanes belong to the stabilizing group[12]. Paclitaxel interferes with the normal function of microtubule growth: the microtubule is influenced in their function by hyper-stabilizing their structure. This destroys the cell's ability to use its cytoskeleton in a more flexible manner. Specifically, paclitaxel binds to the tubulin protein of microtubules and locks them in place. This resulting paclitaxel/microtubule complex does not have the ability to disassemble. This adversely affects cell function because the shortening and lengthening of microtubules is necessary for their function as a transportation highway for the cells. This indicates that, due to this mechanism, the drug is not free of side effects, as all dividing cells may be influenced. Cancer cells are affected in a much higher range than other cells since cancer cells divide much faster than non-cancerous cells, they are far more susceptible to paclitaxel treatment. Research has also indicated that paclitaxel induces programmed cell death (apoptosis) in cancer cells by binding to an apoptosis stopping protein called Bcl-2 (B-cell leukemia 2) and thus arresting its function.
But how the interaction of the drug and the receptor works exactly is still matter of scientific debate. It would provide an insight into the pharmacophore of Taxol (the conformation of taxol in the binding pocket on tubulin) and why some structural changes are beneficial and why others are not for the taxols activity. In addition, perhaps in part the presence of a large number of non-productive conformers is due to that Taxol has a relatively weak association with tubulin and these binding conformations could lead to the design of improved analoges[13].

Due to paclitaxel drug-resistance it is important to evaluate new analoges to afford the inability of paclitaxel to cross the blood brain barrier and to enable the oral bioavailability[14].
Taxol possesses 8 oxygenated positions around the ring system, at position 1, 2, 4, 5, 7, 9, 10 and 13. When the C-7 hydroxyl group is removed a 7-deoxytaxol is created, but this compound shows cytotoxicity comparable to that of Taxol. The same work result by changes at the 10 position (10-deacetoxytaxol). Trials on the "southern" part of Taxol leads to 4-deacetyltaxol and 4-deacetoxytaxol but both were less active than Taxol. Further vast numbers of structure-activity studies led to the conclusion that changes in the polycyclic taxane part of taxol decrease the biological activity as cytotoxin in the majority of cases[13].

[12] Mooberry, S.L., Tien, G., Hernandez, A.H., Plubrukarn, A., Davidson, B.S. *Cancer Research*, **1999**, *59*, 653-660
[13] Kingston, D.G.I. *Photochemistry* **2007**, *68*, 1844-1854
[14] Ge, H.; Spletstoser, J.T.; Yang, Y.; Kayser. M.; Georg, G.I. *J. Org, Chem*. **2007**, *72*, 756-759



While the side chain is also essential for the biological activity, there exists some flexibility for modifications in this part of the molecule[15]. For example, by coupling Baccatin III with aliphatic groups, such as activated β-lactam derivatives or functionalized acetic acid derivatives, Taxoid analogues were obtained.

This methodology allowed the preparation of precursors for 2nd generation taxoids. Common side effects include nausea, vomiting, loss of appetite, change in taste, thinned or brittle hair, pain in the joints of the arms or legs lasting 2-3 days, changes in color of the nails, tingling in the hands or toes. But more serious side effects such as unusual bruising or bleeding, pain/redness/swelling at the injection side, change in normal bowel habits for more than 2 days, fever, chills, cough, difficulty swallowing, dizziness, shortness of breath, severe exhaustions, skin rash, facile flushing and chest pain can also occur. A number of these side effects are associated with the recipient used, Cremophor EL, a polyoxyethylated castor oil. Allergies to drugs such as cyclosporine, teniposide and drugs containing polyoxyethylated castor oil may indicate increased risk of adverse reactions to paclitaxel. Dexamethasone is given prior to beginning paclitaxel treatment to mitigate some of these side effects.

Several investigators have studied the NMR spectra of taxol in solution to determine the solution conformation. In non-polar solvents, taxol exists primarily in a “non-polar” conformation in which the N-benzoyl and 2-benzoylgroup are associated. In polar aqueous solvents, it adopts a set of “polar” or “hydrophobic collapsed” conformation in which the 3’-phenyl group is oriented towards the 2-benzoyl group.[16] To overcome these and some of the major disadvantages of Taxol in order to increase the biological activity especially against resistant cancer cell lines a series of novel “second generation” taxoids were developed. Taxoid derivatives with incorporation of

[15] Wani, M.C.; Taylor H.L.; Wall, M.E.; Coggon P.; McPhail A.T. *J. Am. Chem. Soc.***1971**, *93*, 2325
[16] Kingston, D.G.I. *Photochemistry* **2007**, *68*, 1844-1854

bulky groups such as *tert*-butyl, branched butyl and butenyl, cyclohexyl gave good results with respect to the antitumor activity and a better solubility in aqueous solvents[17].

In this thesis we tried in part to modify the C-13 side chain to repress the hydrophobic collapse in polar solvents, especially in water, by big bulky groups, as well as to push back the hydrophilic by introducing polar groups.
At the hydrophobic collapse the C-13 side chain flaps under the polycyclic core structure, with some side valences between the phenylrest from Taxol and the lipophile groups from the polycyclic core structure occur.

1.1.1 β-Lactam

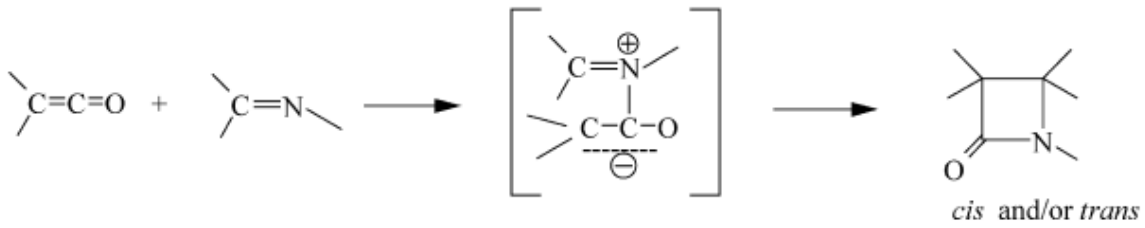
Besides their biological relevance, the importance of β-lactams as synthetic intermediates has been recognized in organic synthesis. The β-lactam (azetidin-2-one) is an interesting ring system found in penicillin, one of the most employed antibacterial in clinical medicine. A particularly interesting example has been outlined for the assembly of the C-13 side chain in the anti-tumor drug paclitaxel[18], in the asymmetric synthesis of human leukocyte elastase inhibitors[19] and as key intermediates for penems and carbapenems[20]. Within a long-term project we are interested in utilizing these building blocks for accessing novel side chain precursors for modified taxoids as potential cytostatics via a bioreductive process[21].
Besides, 2-azetidinones can be also used as starting material to prepare bis-γ-lactams, pyrrolizidines, pyrrolidines, piperidines, indolizidines, cyclic enaminones, pyridones, oxazinones, and other complex natural products. Also other form of compounds of biological and medicinal interest has been accomplished through N1-

[17] a) Ojima, I.; Wang, T.; Miller, M. L.; Lin, S.; Borella, C. P.; Geng, X.; Pera, P.; Bernacki, R. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3423-3428 b) Ojima, I.; Lin, S. *J. Org. Chem.* **1998**, *63*, 224-225 c) Yamaguchi, T.; Harada, N.; Ozaki, K.; Arakawa, H.; Oda, K.; Nakanishi, N.; Tsujihara, K.; Hashiyama, T. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1639-1644.
[18] a) Kayser, M.M.; Yang, Y.; Mihovilovic, M.D.; Feicht, A.; Rochon, F.D. *Can. J. Chem*. **2002**, *80*, 796-800 b) Kayser, M.M.; Mihovilovic, M.D.; Kearns, J.; Feicht, A.; Stewart, J.D. *J. Org. Chem*. **1999,** *64*, 6603 c) Kingston, D.G.I. *Phytochemistry* 2007, *68*, 1844 d) Frense, D. *Appl. Microbiol. Biotechnol*. **2007**, *73,* 1233 e) Kayser, M.M.; Drolet, M.; Stewart, J.D. *Tetrahedron Asym.* **2005**, 16, 4004
[19] Cvetovich, R.J.; Chartrain, M.; Hartner, Jr. F.W.H.; Roberge, C.; Amato, J.S.;Grabowski, E.J.J. *J. Org. Chem.* **1996**, *61*, 6575
[20] Seki, M.; Fututani, T.;Miyake, T.; Yamanaka, T.; Ohmizu, H. *Tetrahedron: Asym.* **1996**, *7*, 1241
[21] a) Yang, Y.; Kayser, M.M.; Rochon, F.D.; Rodriguez, S.; Steward, J.D. *J. Mol. Catal*. **2005**, *32*, 167 b) Kayser, M.M.; Drolet, M.; Steward, J.D. *Tetrahedron. Assym.* **2005**, *16*, 4004 c) Kayser, M.M.; Mihovilovic, M.D.; Kearns, J.; Feicht, A.; Steward, J.D. *J. Org. Chem*. **1999**, *64*, 6603 d) Mihovilovic, M.D.; Kayser, M.M.; Rodriguez, S.; Steward, J.D. *215th ACS National Meeting Dallas* **1998** March 29-April 2.

C2 bond breakage coupled to rearrangement reactions[22]. The rigidity of this four-membered ring makes this methodology often highly selective.

1.1.2 Cyclization

Owing the biological importance and the synthetic application of hydroxyl-β-lactams several different synthestic strategies were developed, and a large range of functionalities and substitution patterns at these compounds was proposed[23].
There are numerous methods available for the construction of the β-lactam ring[24].
The classical routes to 2-azetidinones can be generally classified as [2+2] cycloaddition, cyclization reactions of β-aminoacids and esters and carbene insertion reactions[25]. A widely used method is via the [2+2] cyclocondensation of ketenes and imines, the Staudinger reaction[26].
The Staudinger type cyclization ([2+2] ketene-imine cycloaddition reaction) has been largely used for the synthesis of β-lactam derivatives, which are important intermediates in pharmaceutical and synthetic chemistry array.[27] The reaction of an imine and a ketene can produce cis- or trans-isomers as well as mixtures of both.
The Staudinger reaction[28] is a transformation involving nucleophilic attack of an imine to a ketene which leads to a zwitterionic intermediate, followed by direct ring closure to produce a *cis* β-lactam product.



Alternatively, it undergoes an isomerization of the imine moiety in the zwitterionic intermediate to form a sterically more favorable intermediate, which forms the *trans*

[22] Alcaide, B.; Almendros, P.; Aragoncillo, C. *Chem. Rev.* **2007**, *107*, 4437-4492
[23] For a comprehensive compilation of relevant literature see: Yang, Y., Wang, J; Kayser, M. *Tetrahedron: Asym.* **2007**, *18,* 2021-2129
[24] a)Singh, G.S. *tetrahedron* **2003**, *59*, 7631-7649 b) France, S.; Shah, M.H.; Weatherwax, A.; Wack, H.; Roth, J.P.; Lectka, T.*J. Am. Chem. Soc*. **2005**, *127,*1206-1215 c) Ardura, D.; Lopez, R. *J. Org. Chem.* **2007**, *72*, 3259-3267 d) Alcaide, B., Almendros, P.; Martinez del Campo, T.; Rodriguez-Acebes, R. *Adv. Synth. Catal.* **2007**, *349*, 749-758 e) Palomo, C.; Aizpurua, J.M., Balentova, E., Jiminez, A.; Oyarbide, J.; Fratila, R.M.; Miranda, J.I. *Org. Letters* **2007**, *9*, 101-104 f) Coyne, A.G.; Müller-Brunz, H.; Guiry, P.J. *Tetrahedron.Asymmetry* **2007**, *18*, 199-207
[25] Alcaide, B.; Almendros, P. *Chem. Soc. Rev.* **2001**, *30*, 226-240
[26] Ojima, I.; Delaloge, F. *Chem. Soc. Reviews* **1997** *26*, 377-386
[27] Hu, L.; Wang, Y.; Bonan, L.; Da-Ming, D.; Xu, J. *Tetrahedron* **2007**, *63*, 9387-9392
[28] Singh, G.S. *Tetrahedron* **2003**, *59*, 7631-7649

β-lactam product after ring closing. This competition between direct ring closing and isomerization of the intermediate affects the diastereoselectivity[29]. Stereochemistry is predominately controlled by the electronic effects of the imine and the ketene substituents, the steric hindrance of the imine N-substituent and the reaction temperature. Minor effects on the diastereoselectivity are imposed by solvents, additives, reaction rates, ketene generations and order of the addition of the reagents[30].

Different methods to generate ketenes from activating agents such as 1,1-carbonyldi-imidazol[31], triphosgene[32], ethyl chloroformate[33], trifluoracedic anhydride[34], *p*-toluene-sulfonyl chloride[35], phosphorus derived reagents[36], cyanuric chloride[37] and the Mukaiyama reagent[38] has been developed. But some of these reagents are toxic, or to expensive or the yield of the product is low.

The Vilsmeier reagent (chloromethylenedimethylammonium chloride) serves also as an activating reagent for carboxylic acids to give esters, amides and acid chlorides and for alcohols to give alkyl chlorides, esters, alkyl arylsulfides and imides[39]. Sharma and coworkers have used this reagent for the synthesis of β-lactams from β-amino acids[40]. It can be prepared from N,N-dimethylformamide and a chlorinating reagent.[41] The Vilsmeier reagent was prepared from DMF and oxalyl chloride or thionyl chloride in dry CH_2Cl_2. The imines were treated with different substituted acetic acids and the reagent in dry CH_2Cl_2 at 0°C in the presence of Et_3N to give β-lactams.

De Risi *et al.*[42] have described a new synthetic approach to 2-azetidinones starting from β-enamino ketoesters with key intermediate β-aminoester.

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[29] Jiao, L.; Liang, Y.;Xu, J.X. *J. Am. Chem. Soc.* **2006**, *128*, 6060-6069

[30] Georg, G.I.; Ravikumar, V.T. In The Organic Chemostry of β-Lactams; George, G.I., Ed.; Verlag Chemie: New York, 1993; 295-381

[31] Nahmany, M.; Melman, A. *J. Org. Chem.* **2006**, *71*, 5804-5806

[32] a)Deshmukh, A.R.A.S.; Krishnaswamy, D.;Govande, V.V.; Bhawal, B.M.; Gumaste, V.K. *Tetrahedron* **2002**, *58*, 2215-2225 b) Deshmukh, A.R.A.S.; Krishnaswamy, D.; Bhawal, B.M. *Tetrahedron Lett*. **2000**, *41*, 417-419

[33] Bose, A.K.; Manhas, M.S.; Amin, S.G.; Kapur, J.C.; Kreder, J.; Mukkavilli, L.; Ram, B.; Vincent, J.E. *Tetrahedron Lett*.**1979**, 2771-2774

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[36] a)Bhalla, A.; Venugopalany, N.; Bari, S.S. *Tetrahedron* **2006**, *62*, 8291-8302 b) Farouz-Grant, F.; Miller, M.J. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 2423-2428

[37] Manhas, M.S.; Bose, A.K.; Khajavi, M.S. *Synthesis* **1981**, 209-211

[38] Matsui, S.; Hashimoto, Y.; Saigo, K. *Synthesis* **1998**, 1161-1166 b) George, G.I.; Mashava, P.M.; Guan, X. *Tetrahedron Lett*. **1991**, *32*, 581-584

[39] Barrett, A.G.M.; Braddock, D.C.; James, R.A.; Procopiou, A. *J. Chem. Soc., Chem. Commun*. **1997**, 433-434

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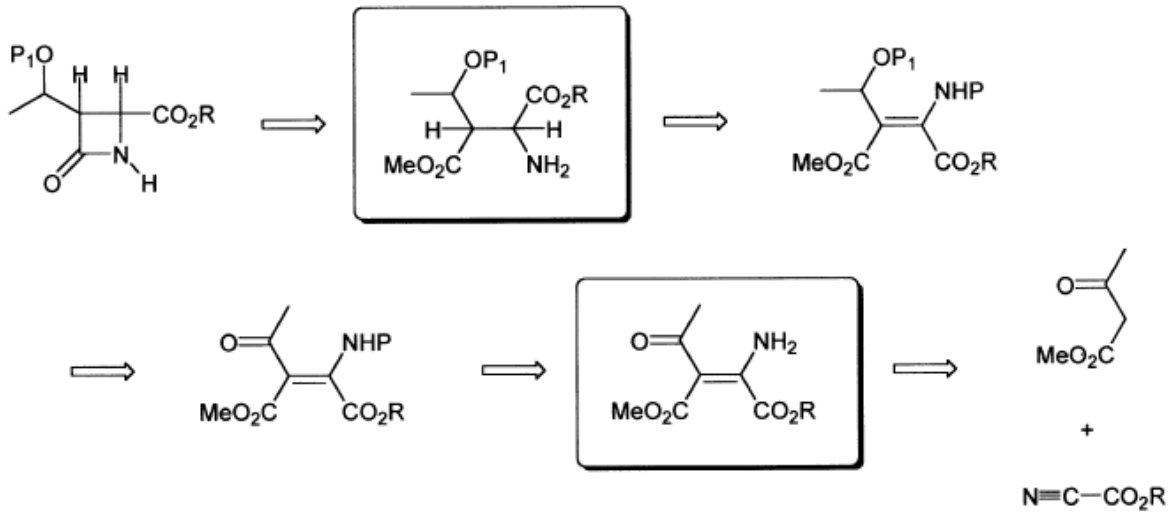
[41] Eilingsfeld, H.; Seefelder, M.; Weidinger, H. *Angew. Chem*. **1960**, *72*, 836-845

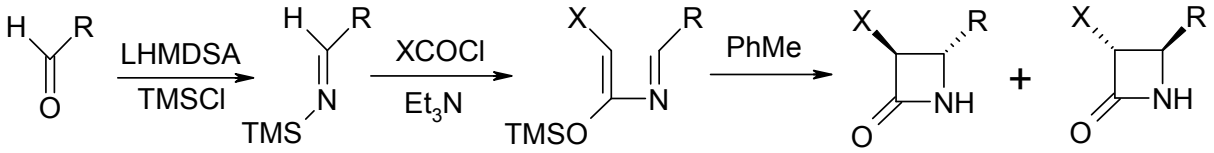
[42] De Risi, C.; Pollini, G.P.; Veronese, A.C.; Bertolasi, V. *Tetrahedron*, **2001**, *57*, 10155-10161



β-Enamino ketoesters reacted with benzyl chloroformate in the presence of sodium hydride to give N-protected enamino ketoesters. The next step was the reduction of the carbonyl group with $NaBH_4$, followed by the transformation of the secondary hydroxyl group with TBDMSCl into the corresponding *tert*.-butyldimethylsilyl ether. Reduction of the carbon-carbon double bound was achieved by catalytic hydrogenation with Raney-Nickel. Now, the stage for the cyclization step for the construction of the 2-azetidinone ring was set and performed by trimethylsilyl chloride with Et_3N followed by addition of *tert*.-butyl magnesium chloride with 75% yield[43].

In the "acid chloride-imine" reaction[44] acid chloride was annelated with dithiocarbonimidates in the presence of Et_3N to form 4,4-bis(alkylthio)azetidin-2-ones. It may be noted that this product can also been synthesized by treating the Schiff's' bases with diphenylacetyl chloride and Et_3N. The oxidative hydrolysis with *N*-bromosuccinimide (NBS) in aqueous MeCN furnished nearly about 90% yield.
Other methods for the preparation of β-lactams have been developed over the years, for example the ketene-imine cycloaddition[45], metalloester enolate-imine condensation[46], isocyanate-alkene cycloaddition[47] and alkyne-nitrone cycloaddition[48]. Panunzio and co-workers have found the utilization of 2-aza-1,3-dienes.

[43] Bari, S.S.; Trehan, I.R.; Sharma, A.K.; Manhas, M.S. *Synthesis* **1992**, 439-442
[44] Bari, S.S.; Trehan, I.R.; Sharma, A.K.; Manhas, M.S. *Synthesis* **1992** 439-442
[45] Palomo, J.M.; Aizpurua, I.G.; Oiarbide, M. *Eur. J. Org. Chem*. **1999**, 3223
[46] Benaglia, M.; Cinquini, M.; Cozzi, F. *Eur. J. Org. Chem*. **2000**, 563
[47] Chmielewski, M.; Kaluza, Z.;Furman, B. *Chem. Commun*. **1996**
[48] a)Basak, A.; Ghosh, S.C.; Bhowmick, T.; Das, A.K.; Bertolasi, V. *Tetrahedron. Lett*. **2002**, *43*, 5499
b) Lo, M.M.-C.; Fu, G.C. *J. Am. Chem. Soc.* **2002**, *124*, 4572



The reaction starts from a ketone with LHMDSA; TMSCl and ClCOCl or BrCOCl in the presence of Et_3N over 1-halo-3-aza-4-alkyl-1,3-dienes followed by conrotatory ring closure to the 3-halo-4-arylazetidin-2-ones. By dehalogenation using tris(trimethylsilyl)-silane a useful modification of the azetidinones is obtained[49].
Another method was the treatment of a ketal or thioketal protected glyoxylic acid esters with a strong base such as *tert*-BuOK or BuLi providing anions that were condensed with imines to ketal or thioketal protected β-lactam[50].
Using the Staudinger reaction Panunzio and co-workers[51] isolated O-silylated intermediates from a ketene (R-CH=C=O) with *N*-silylimines as *trans*-product. Arrieta and co-workers found that the energy of activation of polymerization was lower than that for the formation of the C-N bound[52].
By addition via a [2+2] cycloaddition the adduct of the Lawesson's Reagent (LR) with 2,2'-dithiobisbenzothiazol (MBTS) gives the MBTS-TPP coupling reaction. This addition adducts reacts with carboxylic acid functional group of the amino acid to obtain the activated ester. Attack of the benzothiazole sulfide anion on the carbonyl carbon affords the formation of β-amino thioester. Finally, intramolecular attack on the carbonyl carbon of the thioester by the amino nitrogen with tertiary base triethylamine (TEA) leads to the desired the β-lactam.

Mihovilovic et al.[53] tried an alternative approach to treat a ketal or thioketal protected glyoxylic acid esters with strong base such as *tert*-BuOK or BuLi providing anions that were condensed with imines to ketal or thioketal protected β-lactams.

Sterically demanding imines are inert under this reaction conditions due to sterical hindrance in the initiating addition across the C=N bond. Perhaps this is due to the reaction mechanism. After treatment with the base LDA the formed anion attacks the imine carbon. The incurred intermediate cyclizes with concomitant elimination of ethanol. In the case of the sterically very demanding imines with a *tert*.-butyl group next to the reaction centre the initial attack is hindered.

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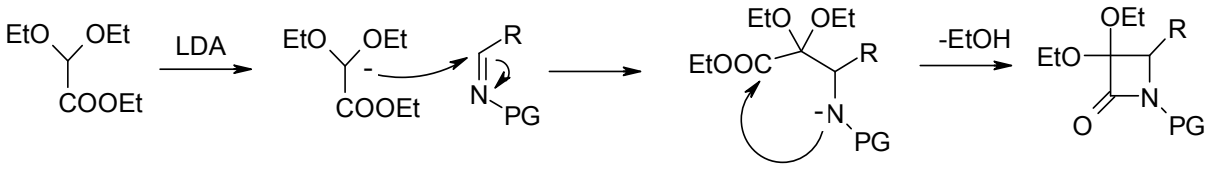
[49] a)Bacchi, S.; Bongini, A.; Panunzio, M.; Villa, M. *Synlett* **1998**, 843-849 b) Martelli, G.; Spunta, G.; Panuntio, M. *Tetrahedron Lett.* **1998**, *39*, 6257-6262
[50] Cainelli, G.; Panunzio, M.; Giacomini, D.; DiSimone, B.; Camarini, R. *Synthesis*, **1994**, 805
[51] Panunzio, M.; Bachi, S.; Campana, E.; Fiume, L.; Vicennati, P. *Tetrahedron Lett.* **1999**, *40*, 8495-8499
[52] Arrieta, A.; Cossio, F.P.; Lecea, B. *J. Org. Chem.* **2000**, *65*, 8458-8463



However, the main problem of this synthetic strategy was the cleavage of O,O- and S,S- ketals when heterocycles such as thiophen or pyridine were present, which are sensitive to the required strong acidic or oxidative reaction conditions. The necessary low pH conditions led to polymerization or sulfonation of the material. For example in the case of a naphthyl side chain substantial decomposition was observed and no traces of ketone could be observed. The removal of the PMP-protective group seems to deactivate the ketal since the product where PG is a phenyl could not be converted to the carbonyl compound under similar conditions.

Generally, thioketals are much more resistant to hydrolysis than the corresponding *O,O*-ketals. The major system to recover the carbonyl functionality is an oxidative cleavage. In this process one or both sulfur atoms are oxidized to sulfoxides or sulfones increasing their ability to act as leaving groups.
In the literature there are a few indications that ketals adjacent to a carbonyl or carboxyl group tend to be difficult to deprotect[54]. In previous studies conducted in this research group it was found that this seemed to be even more so the case for spiro compounds. Several methods either were too mild - resulting in no conversion – or too strong – leading to polymerization of the starting material. No applicative reaction conditions could be found to recover the carbonyl functionality for diverse substitution patterns.

Due to the preliminary successful work on the Staudinger type reaction in this working group, we decided to follow this straight forward strategy. This concept was previously used for the preparation of similar compounds. This method is a milder procedure and was adapted for the preparation of α-keto-β-lactams[55] and it is the most widely used reaction for the preparation of β-lactam ring systems.

[53] a)Mihovilovic, M.D. *Post Doc-Report* **1997** Univ. New Brunswick-Saint John Campus; b) Ojima, I.; Delaloge, F. *Chem. Soc. Reviews* **1997** *26*, 377-386 c) Alcaide, B.; Almendros, P. *Chem. Soc. Rev.* **2001**, *30*, 226-240
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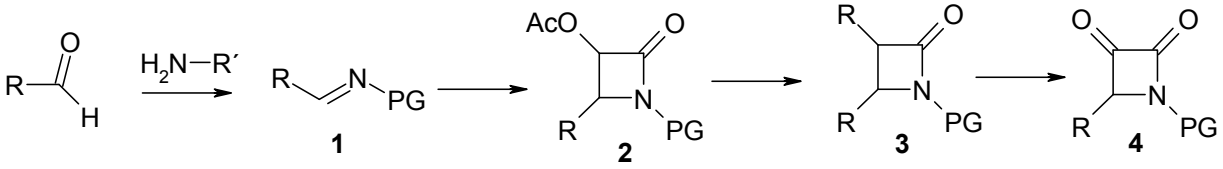
[55] Mihovilovic, M.D.; Feicht, A.; Kayser, M.M. *J. Prakt. Chem*. **2000**, *342*, 585-590



R= Ph, $CH_2C(CH_3)CHC_6H_5$, t-butyl, thiophen, furan
R'= -PMP, $-CH_2-C_6H_5$, $-CH_2-CH=CH_2$, $-C-(CH_3)_3$, $-CH_3$

The starting material for the synthesis of 3-hydroxy-ß-lactams was different aldehyds. A Staudinger type cycloaddition by condensation with activated carboxylic acids (with protected imines) leads to product **2**. This α-acetoxy-ß-lactams were hydrolyzed by KOH under mild conditions **3**. Subsequent oxidations were performed by the treatment of DMSO in the presence of P_2O_5 to give the α-Keto-ß-lactams **4**.

The goal for this PhD thesis was to develop an easy and efficient synthesis for enantiopure 3-hydroxy-β-lactams.

1.2 Biocatalysis

Biocatalysis represents a very environmentally friendly and cost efficient method for the synthesis of chiral compounds for the pharmaceutical industry.
Especially since the Thalidomide incident, it is absolutely essential to create pharmaceutical substances with chiral centers in optically pure form.
To complement the arsenal of catalytic methods, enzymes were used in organic chemistry to enable a variety of new and enantioselective transformations. The asymmetric reduction of ketones is one of the most relevant, basic and applied reactions for the synthesis of non-racemic chiral alcohols, which can be converted into different functionalities without racemization. This is important for the formation of industrially important chemicals such as pharmaceuticals, agrochemicals and natural products. The catalysts for the asymmetric reduction of ketons can be divided into two categories: chemical and biological methodologies.

Biocatalysts have typical characteristics when they were compared with homogeneous or heterogeneous chemical catalysis. Some features that differentiate biocatalysts from chemical catalysts:

The selectivity: Very high enantio-, regio- and chemoselectivities can be obtained due to the strict recognition of the substrate by the enzyme. For example chemical catalysts can perform highly enantioselective reductions only if the two substituents at the carbonyl carbon of the ketones are significantly different, whereas for biocatalysis the two groups can be quite similar to still give high enantioselectivities.

The safety of the reaction: Biocatalytic reductions are generally safe. The solvent is usually water, reaction conditions are mild, and dangerous reagents are not necessary. For example ethanol and glucose is used as hydrogen source instead of hydrides or explosive hydrogen gas.

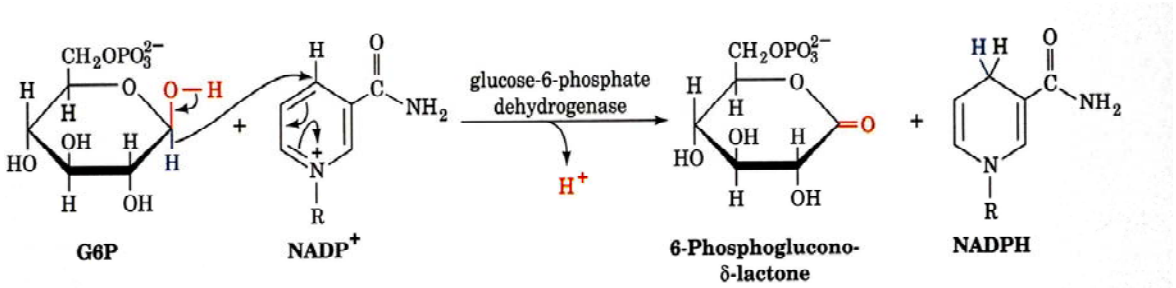
The catalyst preparation: Some of the biocatalysts for reductions, whole cells and isolated enzymes, are commercially available and ready to employ like chemical catalysts. Commercially available biocatalysts are baker's yeast, and its alcohol dehydrogenases, horse liver and hydroxysteroid dehydrogenase from *Pseudomonas testosterone* and *Bacillus spherisus*. To obtain other variants, it may be necessary to cultivate cells from seed cultures that perhaps are commercially purchasable.

Easily disposable: Biocatalysts are microorganisms, plants, animals or their isolated enzymes. They can be decomposed in the environment after use without major problems.

Large scale synthesis: This is one of the disadvantages of using biocatalysts. For the scale up synthesis the workup procedures might be complicated, a large operational volume for the cultivation may be necessary and the space-time yields

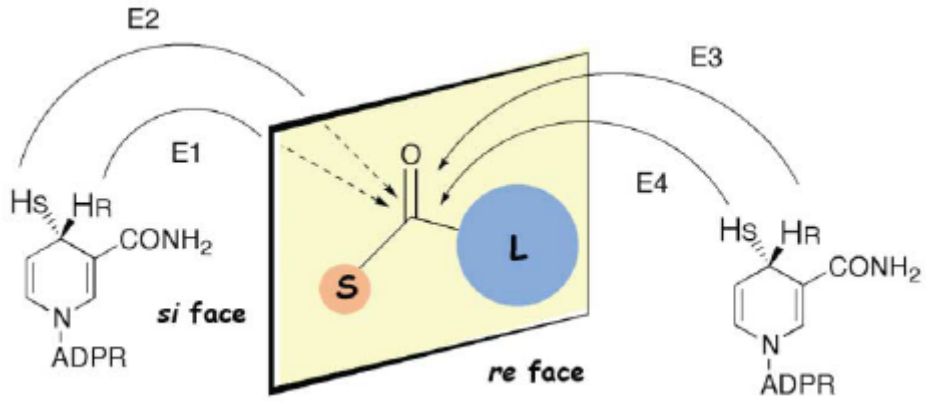
are not as high due to the long reaction times and the low substrate concentration. These disadvantages have been overcome by using genetic methods and by exploring the reaction conditions.

The reductions of carbonyl groups[56] are catalyzed by dehydrogenases and reductases. They were classified as E.C.1.1.1.x enzymes. The natural substrates of these biocatalysts are alcohols, such as ethanol, glycerol lactate and the corresponding carbonyl compounds, where the redox reaction can proceed in both directions. Also unnatural substrates can be reduced enantioselectively. To display catalytic activities, the enzymes need a coenzyme such as NADH or NADPH from which a hydride is conveyed to the substrate carbonyl carbon. There are four stereochemical patterns that allow the transfer of the hydride from the coenzyme, NAD(P)H, to the substrate[57].

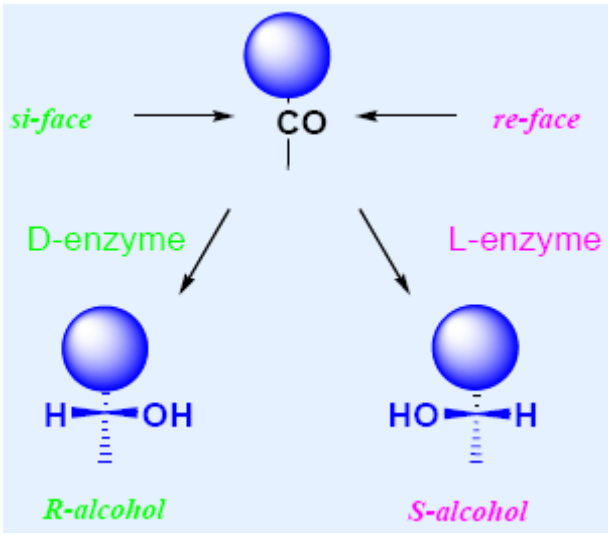


Effective application of whole yeast cells or isolated enzymes in reductive biotransformation requires a method for regeneration of the cofactor. In whole yeast cells the regeneration takes place directly in the cell as long as the organism is metabolically active, for isolated enzymes the mostly used method for NADPH regeneration utilize glucose-6-phosphate (G-6-P) by glucose-6-phosphate dehydrogenase (G-6-PDH) as regeneration system[58]. With the enzymes *Pseudomonas* sp. alcohol dehydrogenase, *Lactobacillus kefir* alcohol dehydrogenase (E1), *Geotrichum candidum* glycerol dehydrogenase, and *Mucor javanicus* dihydroxyaceton reductase (E2) the hydride attacks the si-face of the carbonyl group (resulting in formation of R-alcohols), whereas with yeast- and horse liver alcohol dehydrogenase and *Moraxella* sp. alcohol dehydrogenase (E3) the hydride attacks the re-face giving S-products. On the other side, E1 and E3 enzymes transfer the pro-(R)-hydride of the coenzyme, and (E2) enzymes use the pro-(S)-hydride.

[56] Schomburg, D.; Salzmann, M. *Enzyme Handbook*, springer verlag, Berlin, D, **1990**
[57] Bradshaw, C.W.; Fu, H.; Shen, G.-J.; Wong C.-H. *Org. Chem.* **1992**, *57*, 1526-1532
[58] Ward, O.P.; Young, C.S. *Enzyme Microb. Technol.* **1990**, *12,* 482-493



Generally, when the attack comes from the si-face of the molecule, the R-alcohol is formed. The other way round, when the attack takes place from the re-face, the S-alcohol is build.



Advantages of using enzymes in biotransformations include their capability to carry out a wide range of organic reactions, often with much higher reaction rates than those observed in classical organic chemistry and their selectivity with respect to reactions and substrate type and their general regiospecific and stereospecific nature. The products of biotransformations may be present in a highly pure form.

During this PhD-Thesis the utilization of reductases originating from yeast was investigated using whole cells as well as isolated enzymes.

1.2.1 Advantage or Disadvantage of Whole-cell and Isolated Enzymes:

The use of biocatalysis in the preparation of enantiopure compounds is a facile and attractive alternative to other chemical methods[59]. Baker's yeast, the most popular whole-cell biocatalyst, has been used in various transformations as an environmentally benign reagent in organic chemistry. Using whole cells for the chiral reduction is economically attractive, due to availability, low costs, ease to handle, and simple disposal. In most cases an aqueous solvent has been employed as reaction media[60]. Certain disadvantages are associated to employing such solvents: the solubility of the organic substrate, the possibility of undesired side reactions such as hydrolysis (caused by excess water) and possible separation problems of the product.

Baker's yeast is a rich source of reducing enzymes and was specifically used for asymmetric reduction of carbonyl compounds. It tolerates a large variety of carbonyl substrates. This large substance acceptance is due to the presence of a broad number of reductase enzymes. Several of them have been isolated and characterized.[61] Some of these reductases possess overlapping substrate specificities, but with inverse enantioselectivities. Unfortunately, yeast catalyzed biotransformations sometimes result in low optical purities as a result of the presence of enzymes with overlapping substrates specifities but different stereoselectivities[62]. This reduces the optical purities of the alcohol product. To overcome these problems, some methodologies such as modification of substrate structure, concentration, use of organic solvents, heat treatment and addition of exogenous modifiers can be attempted[63].

Enzyme catalysts have enormous potential for the development of stereo selective reagents for chiral synthesis. Baker's yeast has been the most widely used whole cell biocatalyst, primarily for the asymmetric reduction for carbonyl compounds such as β-keto-esters[64]. Eight reductases have been identified as major β-keto ester reductases in baker's yeast: fatty acid synthetase, aldo-keto reductase, α-acetoxy keto reductase, aldose reductase, *D*-hydroxy acid Dehydrogenase, medium-chain

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dehydrogenase, short-chain dehydrogenase superfamilies and so on[65]. Fatty acid synthetase accepts only α-unsubstituted β-keto esters and gives the corresponding (R)-alcohols. Aldo-keto reductase and α-acetoxy keto reductase accept α-substituted β-keto esters and yields the corresponding (S) alcohols. From the aldose reductase superfamily, four reductases are high selective. Two catalyze the si-face and two the re-face hydride transfer. Ybr149w and Ycr107w proteins gave primarily *cis*-alcohol whereas Yjr096w and Ydl124w gave the *trans*-alkohol.[66] Some problems can appear by the separation of the product from the biomass, but their advantage is that they utilize simple carbohydrates for cofactor regeneration, so that their economic value is still given.

The key step of the synthetic elaboration of β-lactams in context of this project is the stereoselective enzymatic reduction. When yeast is used a whole-cell biocatalyst a mixture of products may be the result due of the large numbers of genes (≥ 49), each of them operate with different stereoselectivities and substrate specificities. Consequently, in modern biocatalysis it is more promising to examine each enzyme separately. In this context it is most convenient, that the yeast genome[67] is fully characterized and that a large number of reductases with proven or tentative biocatalytic activity (based on sequence homology) have become available by genetic engineering[68]. In context of this project, a first try to investigate the prospect of yeast based reductases for the production of Taxol side chain derivatives was to utilize *Escherichia coli* based overexpression of yeast reductases as whole cell biocatalysts for the reduction of azetidin-2-one. Kayser *et al.*[69] found that enzyme Ara1p which was diastereoselective towards aryl substituted substrates and allowed a dynamic kinetic resolution that leads to a highly enantioselective alcohol[70].
Whole cell based biotransformations can be limited in their efficiency by membrane permeability and toxicity, isolated enzyme assays provide a more reliable assessment of the potential of a particular enzyme for application in synthetic chemistry. Consequently, an expression library for 19 yeast reductases in *E. coli* was created and utilized for facile enzyme purification by creating glutathione (S)-transferase (GST) fusions proteins the gene for the reductase. This method allows

[65] Nakamura, K.; Kawai, Y.; Miyai, T.; Honda, S.; Nakajima, N.; Ohno, A. *Bull. Chem. Soc. Jpn*. **1991,** *64,* 1467-1470
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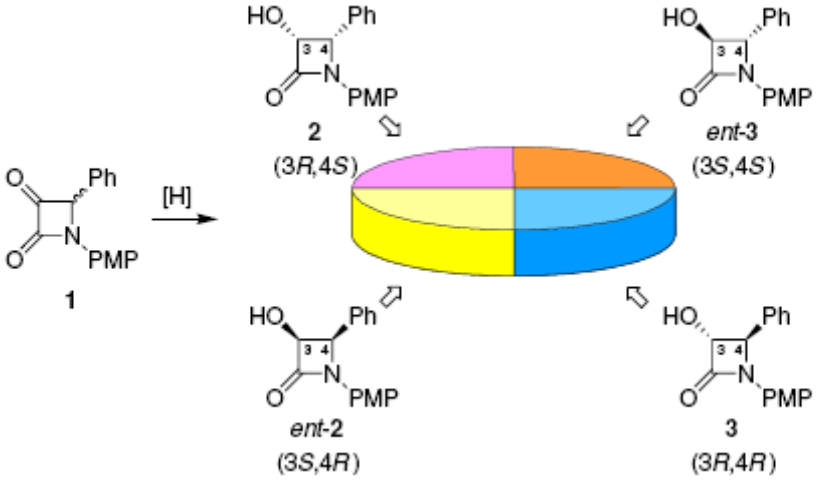
for easy purification by a common affinity chromatography[71]. For each of these 19 GST-fusion proteins NADPH is needed as cofactor. The cofactor itself was regenerated by glucose-6-phosphat coupled with glucose-6-phosphat dehydrogenase within biotransformations at pH 7.
This approach has been utilized to identify suitable enzymes for the production of the natural side chain of Taxol in both antipodal forms[72].



Another way to synthesize stereochemically pure β-lactams is to convert suitable substrates by lipases[73] of different origin (PS-30, Novozym-435, AK, PPL) in a deprotection step.
The lipase catalyzed the reaction of racemic acetates to enantioselective alcohol via hydrolysis in an aqueous puffer within a kinetic resolution prcess. It was found, that lipase PS-30 is a suitable calatyst for enantioselective hydrolysis of racemic β-lactams. The rate of acetate hydrolysis was higher using more lipase in the reaction. For all tested substrates the lipase-catalyzed hydrolysis provides access to the 3S enantiomer with high enantioselectivity.

[71] a) Kaluzna, I.; Andrew, A. A.; Bonilla, M.; Martzen, M. R.; Stewart, J. D. *J. Mol. Cat. B* **2002**, *17*, 101-105 b) Kaluzna, I. A.;Matsuda, T.; Sewell, A.K.; Stewart, J.D. *J. Am. Chem. Soc.* **2004**, *126,* 12827-12832 c) Wolberg, M.; Kaluzna, I. A.; Müller, M.; Stewart, J. D. *Tetrahedron: Asymmetry* **2004**, *15*, 2825-2828 d) Kaluzna, I. A.; Feske, B. D.; Wittayanan, W.; Ghiviriga, I.; Stewart, J. D. *J. Org. Chem.* **2005**, *70*, 342-345
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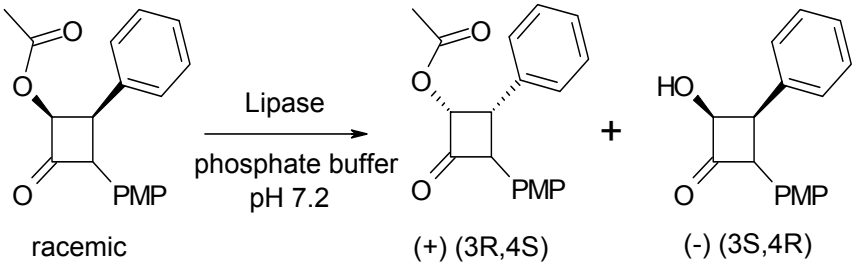


Different methods are recently developed to find the most suitable reaction and to control enantioselectivities of those reaction. They can be classified in three approaches:

a) Search and creation of the biocatalyst

Screening: Screening for a novel enzyme is still one of the most powerful tools to find a biocatalytic reduction system[74]. It is possible to find a suitable biocatalyst by applying the latest screening and selection technologies, enable rapid identification of enzyme activities from diverse sources. These sources used for screening of reduction can be soil samples, commercial enzymes, culture sources, a clone bank, etc. Biocatalyst candidates can origin from animals, microorganism or plants as long as suitable sequence data is available and the aimed for catalysts can be expressed in easy to culture host organisms. In particular the approach based on the exploitation of metagenome libraries represents the substantial advantage, that it is not necessary to cultivate the original enzyme producer. It should be noted that less than 5% of all microorganisms can currently be cultivated and proliferated in an artificial environment. Consequently, direct access to biocatalyst candidates via DNA libraries (as it is the case in the metagenome approach) offers access to a much larger diversity of possible catalysts.

Overexpression: Microorganisms can be transformed and modified for different purposes,[75] for example to improve enzyme production in a cell, to provide coenzyme regenerating enzymes in the same cell, to improve low enantioselectivities due to the presence of many enzymes in a cell with overlapping substrate specifities but different enantioselectivities or the problem of overmetabolism.

Directed evolution: the directed evolution of enzymes has been used to enhance the reducing function of the enzyme. For example, this method was used to eliminate the co-factor requirement of *Bacillus stearothermophillus* lactate dehydrogenase, which is activated in the presence of fructose-1,6-biphosphate.[76] But, the activator is

[74] a) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Takahashi, S.; Wada, M.; Kataoka, M.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 847-851 b) Wada, M.; Kataoka, M.; Kawabata, H.; Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Shimizu, S. *Biosci. Biotech. Biochem.* **1998**, *62*, 280-285

[75] a) Kizaki, N.; Yasohara, Y.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **2001**, *55*, 590-595 b) Itoh, N.; Yoshida, K.; Okada, K. *Biosci. Biotech. Biochem.* **1996**, *60*, 1826-1830

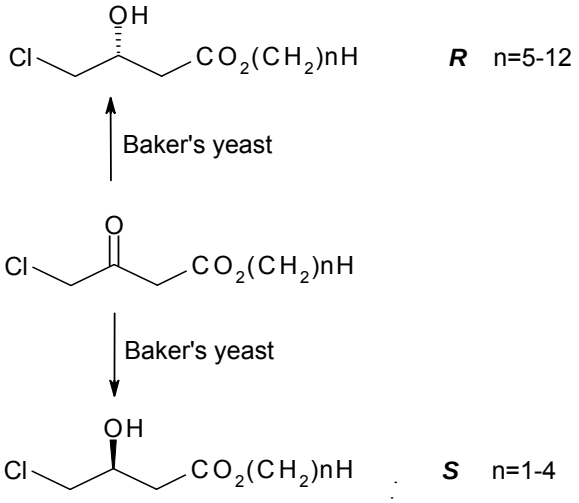
[76] Allen, S.J.; Holbrook, J. *J. Protein Eng.* **2000**, *13*, 5-7

expensive and representative of a sort of co-factor complications that are undesirable in industrial process.[77]

b) Modification of the substrate

The enantioselectivity of a biocatalytic reduction can be affected by substrat modification because the enantioselectivity of the reduction reaction is profoundly defined by the substrate's structure.[78]

For example, in the reduction of 4-chloro-3-oxobutanoate by baker's yeast, the length of the ester moiety controlled the stereochemical course of the reduction.[79] When the ester moiety was smaller than a butyl group, (S)-alcohols were obtained, and when it was larger than a pentyl group, (R)-alcohols were obtained.



The enantioselectivity of aromatic ketones can also be controlled by the modification of substrates. Baker's yeast reduction of hydroxyl ketone afforded the (R)-alcohol, whereas acetoxy ketone afforded the (S)-alcohol.[80]

[77] Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron:*Asymmetry, **2003**, *14*, 1659-2681

[78] a) Fujisawa, T.; Itoh, T.; Sato, T. *Tetrahedron Lett.* **1984**, *25*, 5083-5086 b) Nakamura, K.; Ushio, K.; Oka, S.; Ohno, A.; Yasui, S. *Tetrahedron Lett.* **1984**, *25*, 3979-3982

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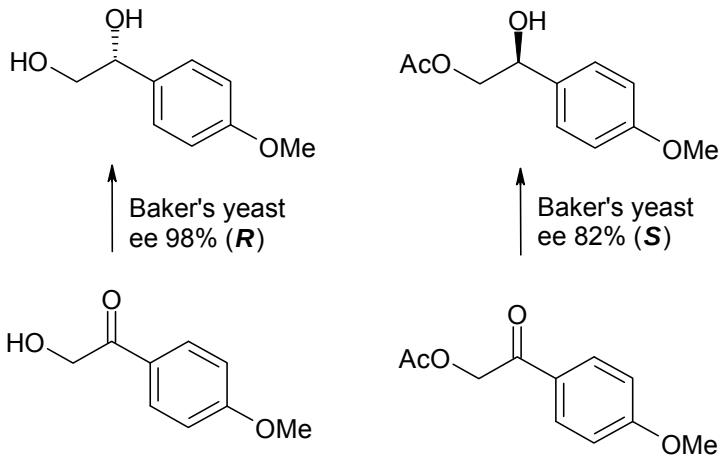

c) Optimization of the reaction conditions

Since dried cell mass can be stored for a longer time and can be used when ever needed, this simple to access biocatalyst variant is often used for a reduction.

Solvent engineering: Biocatalysis can be performed in various solvents[81]. Non-aqueous solvents can be used to enhance the solubility of substrates as well as to control enantioselectivities. Organic solvents, for example benzene, have been used to control enantioselectivities in some case. Ionic liquids have also received substantial attention in the last years as environmentally friendly solvents for bioreductions[82].

Additives such as cyclodextrin and hydrophobic polymers have also been used to control the concentration of the substrate or product[83]. Addition of β-cyclodextrin decreases the substrate concentration in order to control the enantioselectivities. This strategy is particularly useful in whole-cell mediated reactions.

Reaction temperature: The temperature is one of the parameters affected the enantioselectivity of a reaction.

Inhibitors: When poor overall enantioselectivities were observed due to the presence of multiple competing enzymes with diverse enantioselectivities, one of the most useful methods is to use an inhibitor for the unwanted enzymes. Inhibitors of enzymes in baker's yeast were ethyl chloroacetate, methyl vinyl ketones, sulphur compounds, Mg^{2+}, Ca^{2+}, allyl bromide, etc[84].

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[81] a)Panza, J.L.; Russel A.J.; Beckmann, E.J. *Tetrahedron* **2002**, *59,* 4091-4104 b) Howarth, J.; James, P.; Dai, J. *Tetrahedron. Lett.* **2001**, *42*, 7517-7519 c) Conceicao, G.J.A.; Moran, P.J.S.; Rodriguez, J.A.R. *Tetrahedron: Asymmetry* **2003**, *14*, 43-45 d) Zelinski, T.; Kula, M.-R. *Biocatal. Biotransformation* **1997,** *15,* 57-74

[82] Howarth, J.; James, P.; Dai, J. *Tetrahedron Lett.* **2001**, *42,* 7517-7519

[83] a) Nakamura, K.; Takenaka, K.; Fujii, M.; Ida, Y. *Tetrahedron Lett.* **2002**, *43*, 3629-3631 b) D'Arrigo, P.; Fuganti, C.; Fantoni, G.P.; Servi, S. *Tetrahedron* **1998**, *54*, 15017-15026

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However, the most important approach to affect stereoselectivity in modern biocatalysis is represented by genetic engineering. In particular in the case of overlapping and divergent enantioselectivities of competing enzymes, careful selection of the host organism is mandatory. In addition, routine molecular biology techniques are available nowadays to both overexpress a required biocatalyst (even in the presence of competing enzymes) in order to increase its concentration by several orders of magnitude as well as to de-active competitors by gene knock-out which shuts off efficiently the production of catalytically active proteins. These strategies can be applied individually or in combination within whole-cell biocatalysis depending on the particular situation.

Using the β-lactam approach to obtain Paclitaxel derivatives from Baccatin III needs an efficient synthetic process for the preparation of the *cis*-precursors.
The biocatalytic part of this PhD-Thesis involved the screening of wild-type and recombinant whole-cells as well as isolated enzymes[85] from overexpression systems. Those screenings should allow finding either the best strains or the best enzymes able to reduce enantioselectively the target compounds into the required enantiomers, introducing thus the correct configuration in the side chain.
An enzymatic screening was considered because of some previous results observed on some other β-lactams. Indeed, this previous study pointed out that the recombinant wholes-cells could lead to the formation of by-products. While the enzymatic screening should allow an accurate substrate acceptance profiling, the microbial biotransformation is most suitable for up-scaling of fermentations involving enzymatic redox systems.

[85] Kaluzna, I.; Andrew, A. A.; Bonilla, M.; Martzen, M. R.; Stewart, J. D. *J. Mol. Cat. B* **2002**, *17*, 101-105

2. RESULTS AND DISCUSSION

2.1 Synthesis of β-lactams

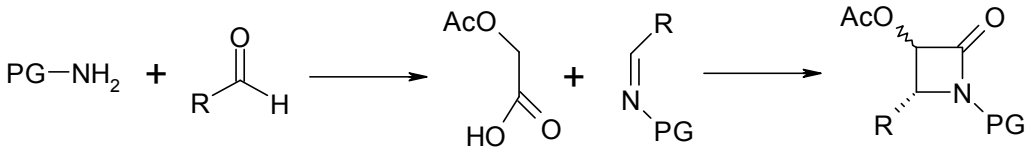
The synthesis of racemic β-lactams was performed via a three-step reaction sequence.

2.1.1 Cyclization

The classical route to 2-azetidinones can be generally classified as [2+2] cycloaddition, cyclization reaction of β-aminoacis and esters, and carbene insertion reactions.
The Staudinger reaction is a stepwise reaction including nucleophilic attack of an imine to a ketene, leading to a zwitterionic intermediate, which undergoes directly a ring closure to produce a *cis*-lactam product, or undergoes an isomerization of the imine moiety in the zwitterionic intermediate to form a sterically more favorable *trans*-lactam product. The competition between direct ring closure and isomerization of the zwitterionic intermediate controls the diastereoselectivity.
The different *cis/trans* ratio of β-lactam products produced from different ketenes may vary with temperature[27].

The selected Staudinger reactions were conducted in different solvent at reflux, 0°-5°C and at -78°C. The ratio of *cis*- and *trans*-β-lactam products was detected via ^{1}H NMR spectra of the crude reaction mixture.

The required starting materials acetoxyacetic acid chloride[86] and imines **1a-1s**[87] were synthesized according to the literature.



R= Ph, $CH_2C(CH_3)CHC_6H_5$, t-butyl, thiophen, furan
R'= -PMP, $-CH_2-C_6H_5$, $-CH_2-CH=CH_2$, $-C-(CH_3)_3$, $-CH_3$

To optimize the effectiveness of the cyclization protocol, various reaction conditions were applied for this step:

[86] Mihovilovic, M.D.; Spina, M.; Stanetty, P. *Arkovic* **2005**, *33*
[87] Mihovilovic, M.D.; Feicht, A.; Kayser, M.M. *J. Prakt. Chem.* **2000**, *6,* 585-590

Method A:

Precursors **1a, 1b, 1d, 1e, 1h, 1i, 1l, 1m, 1o, 1p, 1q or 1s** were dissolved in toluene under Ar, then 3 equiv. Et_3N were added. The reaction mixture was heated to reflux and carefully 1.5 equiv. acetoxyacetic acid chloride were added. The mixture was allowed to stir overnight at reflux and was monitored by TLC until full consumption of starting material was observed. The reaction was cooled to rt and extracted three times with 2N hydrochloric acid and three times with satd. sodium bicarbonate solution. Purification of the products was carried out by flash chromatography.

Method B:

Starting materials **1b, 1c, 1f, 1g, 1j, 1k, 1n or 1r** were dissolved in methylene chloride under Ar, then 3 equiv. Et_3N was added. The reaction mixture was cooled to 0-5°C and slowly 1.3 equiv. acetoxyacetic acid chloride were added. The mixure was stirred till the monitoring by TLC indicated full consumption of starting material. The reaction was allowed to warm to room temperature. The workup procedure included extraction with 2N hydrochloric acid (3x) and once with satd. sodium bicarbonate solution. Purification of the products was conducted by flash chromatography[88].

Method C:

Compounds **1b, 1n, 1o or 1r** were dissolved in methylene chloride under Ar, then 3 equiv. Et_3N was added. The reaction mixture was cooled to 0-5°C and slowly 1.15 equiv. acetoxyacetic acid chloride was added. The mixure was stirred till the monitoring by TLC showed full consumption of starting material. The reaction was allowed to warm to room temperature. The workup procedure was done by extraction three times with 2N hydrochloric acid, once with satd. sodium bicarbonate solution and once with brine. Purification of the products was done by flash chromatography.[89]

[88] Del Buttero, P.;Baldoli, C.; Molteni, G.,;Pilati; T. *Tetrahedron:Asymmetry* **2000**, *11,* 1927-1941

[89] Mastalerz, H.; Cook, D.; Fairchild, C.R.; Hansel, S.; Johnson, W.; Kadow, J.F.; Long, B.H.; Rose, W.C.; Tarrant, J.; Wu, M.-J.;Xue, M.Q.; Zhang, G.; Zoeckler, M.; Vyas, D.M. *Bioorg. Med. Chem.* **2003**, *11*, 4315-4323

[82] This product is literature known, but was synthetized by a different method than previously reported

Product yields are shown in table 1 (only successful experiments are included):

Imine	R	PG	Method[a]	Product	Yield [%]	*cis/trans* ratio
1a	phenyl	-allyl	A	**2a**[82]	73%	50:50
1b	phenyl	t.-butyl.	B	**2b**	90%	100:0
			C		89%	100:0
			A		83%	100:0
1c	phenyl	$-CH_3$	B	**2c**	96%	100:0
1d	C_6H_5-CH=C(CH_3)	-bn	A	**2d**	79%	100:0
1e	C_6H_5-CH-C(CH_3)	-allyl	A	**2e**	68%	83:17
1f	C_6H_5-CH-C(CH_3)	t.-butyl	B	**2f**	96%	100:0
1g	C_6H_5CH-C(CH_3)	$-CH_3$	B	**2g**	99%	100:0
1h	t.-butyl	-bn	A	**2h**	81%	72:28
1i	t.-butyl	-allyl	A	**2i**	82%	70:30
1j	t.-butyl	t.-butyl	B	**2j**	62%	100:0
1k	t.-butyl	$-CH_3$	B	**2k**	81%	100:0
1l	thiophene	-bn	A	**2l**	87%	56:44
1m	thiophene	-allyl	A	**2m**	84%	65:35
1n	thiophene	t.-butyl	B	**2n**	82%	100:0
			C		67%	100:0
1o	thiophene	$-CH_3$	A	**2o**	73%	42:58
			C		- [b]	
1p	furan	-bn	A	**2p**	92%	50:50
1q	furan	-allyl	A	**2q**	74%	53:47
1r	furan	t.-butyl	B	**2r**	68%	100:0
			C		69%	100:0
1s	furan	$-CH_3$	A	**2s**	95%	12:88

[a] Method A: cyclized in toluene under reflux, Method B: cyclized in CH_2Cl_2 at 0-5°C, Method C: cyclized in CH_2Cl_2 at –78°C
[b] Decomposition

table 1

The results indicate that there are some effects caused by the temperature differences. By method B, where the reaction temperature is 0-5°C, only the *cis*-products were formed.

The cyclization of starting material **1b** was investigated under different conditions. The best yield was obtained with method B (90%), nearly the same yield for the method C (89%) and a somewhat lower result was obtained with method A (83%). All three cases were selective for favoring the formation of the *cis*-product. For imine **1n** method B gave 93%, whereas method C yielded only 67% of cyclized material. In both cases the *cis*-β-lactam product was favored. The yield for the lactam **2r** was independent from the method, if cyclizations were performed according to protocol C (69%) or method B (68%). In both cases only the *cis* product was formed. Due to the

easier handling of the method B, the larger scale conversion was performed according to this procedure.

All other cyclizations were done by two different methods:
So the imines **1a, 1d, 1e, 1h, 1i, 1l, 1m, 1o, 1p, 1q** and **1s** were cyclized in a Staudinger type [2+2] cycloaddition following the reaction conditions of method A and for the imines **1b, 1c, 1f, 1g, 1j, 1k, 1n** and **1r** method B were chosen. The selected method was depending from the different substituent R and the nitrogen protecting group PG, but also the stability was a limiting factor for the method A.
All products were obtained in good yields, **2a, 2d, 2e, 2i, 2l, 2m, 2p** and **2q** were purified by column chromatography, **2b, 2c, 2f, 2g, 2h, 2j, 2k, 2n, 2o, 2r** and **2s** were used without further purification for the subsequent transformation as the crude material was sufficiently pure. Method C was never used for the big scale, because of the decomposition of some products or because nearly the same yield was reached compared to methods A or B (and these protocols are less complex).
Within the Staudinger cyclization reaction usually *cis* and *trans* ß-lactams were formed. The ratio between *cis* and *trans* products was determined by ^{1}H-NMR spectroscopy. According to the literature[90], β-lactams prepared by method B afford *cis* ß-lactams. Precursors **1b, 1c, 1f, 1g, 1j, 1k, 1n** and **1r** gave the corresponding *cis* ß-lactams **2b, 2c, 2f, 2g, 2j, 2k, 2n** and **2r** via this methodology and the stereochemistry was confirmed by analysis of the coupling constants in the NMR spectra of the obtained products.
The *cis* and *trans* isomers can be distinguished by the coupling constants as well as by chemical shift.
The coupling constants values (*J*) for the *cis*-compounds are generally larger, than for the *trans* products. In the literature the coupling constant for the *cis*-products are reported around 5Hz and for the *trans* product around 2Hz.[55]
^{1}H NMR spectra of the products revealed the larger coupling constants for *cis* (4.2Hz-5.7Hz) than for *trans* (1.2-1.9Hz).

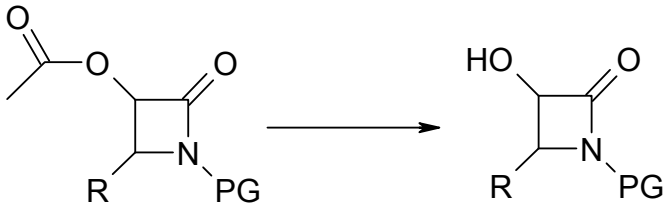
For example, the signals of the *cis* H-3/H-4 are shifted slightly upfield (by about 0.3 ppm) compared to *trans* H-3/H-4. The shift difference between the peaks of the *cis* 3- or 4-protons is suitable for distinguishing *cis*/*trans* isomers, with the difference being greater for *trans*.

Product **2d** gave also only the *cis*-product *via* method A. In all other cases diastereomeric mixtures were obtained. Products **2e, 2h, 2i** and **2m** were obtained predominantly as *cis*-isomers accompanied by 20-30% of the *trans* product.

[90] Del Buttero, P.; Baldoli, C.; Molteni, G.; Pilati T. *Tetrahedron Asymm*. **2000**, *11*,1927

Compounds **2a**, **2l, 2o, 2p** and **2q** were produced in a 50:50 ratio. In contrast, product **2s** was formed in higher *trans* ratio with only 10% of the *cis*-compound. For the next step, the hydrolysis of the ester protective group, no significant change in isomeric ratio was observed. For the correct correlation of *cis* and *trans* isomers the coupling constants in the ^{1}H-NMR were calculated.
A series of additional products **2t-2y** were already reported in the literature and were synthesized according to this procedure; experimental details are not included in this thesis.[55]

2.1.2 Hydrolysis



2a-s **3a-s**

The next step was a mild hydrolysis avoiding eventual ring opening of the heterocyclic core. The acetyl group was removed with KOH in THF to give the desired 3-hydroxy-ß-lactams (**3a-3s**).

A 5% solution of the compounds **2a-s** were dissolved in THF, cooled to 0°C and slowly treated with half of the volume 2N KOH, so that the temperature did not increase. This mixture was stirred at 0°C till TLC indicated complete conversion (2-3h). The reaction was quenched at 0°C by addition of water and extracted with EtOAc washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness. Pure products were obtained, so no further purification was necessary.

Product yields are shown in table 2:

precursor	R	PG	product	yield	*cis/trans* ratio
2a	phenyl	-allyl	**3a**	97%	53:47
2b	phenyl	-t. butyl	**3b**	88%	100:0
2c	phenyl	$-CH_3$	**3c**	76%	100:0
2d	C_6H_5-CH-C$(CH_3)_3$	-bn	**3d**	99%	100:0
2e	C_5H_6-CH-C$(CH_3)_3$	-allyl	**3e**	99%	91:9
2f	C_6H_5-CH-C$(CH_3)_3$	-t. butyl	**3f**	91%	94:6
2g	C_6H_5-CH-C$(CH_3)_3$	$-CH_3$	**3g**	85%	100:0
2h	t.-butyl	-bn	**3h**	99%	69:31
2i	t.-butyl	-allyl	**3i**	83%	79:21
2j	t.-butyl	-t. butyl	**3j**	82%	63:38
2k	t.-butyl	$-CH_3$	**3k**	67%	100:0
2l	thiophene	-bn	**3l**[91]	99%	66:33
2m	thiophene	-allyl	**3m**	98%	59:41
2n	thiophene	-t. butyl	**3n**	85%	100:0
2o	thiophene	$-CH_3$	**3o**	71%	33:66
2p	furan	-bn	**3p**[91]	97%	50:50
2q	furan	-allyl	**3q**	83%	50:50
2r	furan	-t. butyl	**3r**	90%	100:0
2s	furan	$-CH_3$	**3s**	74%	20:80

table 2

The stereochemistry of the resulting alcohols was predominantly in favor of the *cis*-compounds. Only exception was educt **2s** which leads to a 1:4 mixture and educt **2o** with leads to a 1:2 mixture with *trans*-alcohol as predominant isomer; however, this reflects essentially the isomeric distribution after the cyclization reaction. Hence, the hydrolysis does not affect the isomeric distribution as obtained in the previous step to a significant extent.

The hydrolysis of products **3b, 3c, 3d, 3g, 3k, 3n** and **3r** leads only to a *cis*-alcohol conformation.

The stereochemistry of the alcohol **3a, 3p and 3q was** formed in a 1:1 mixture of both isomers.

The products **2e** and **2f** were obtained in a ratio of 9:1 with higher proportion of the *cis*-alcohol. Product **3i** was obtained in a *cis-trans*-mixture 8:1. Educt **2h** was transformed in a *cis-trans*-mixture of 7:1. Products **2j, 2l** and **2m** were converted with the ration *cis:trans* = 3:1.

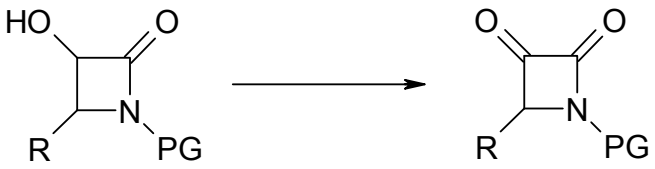
The alcohols were formed in excellent yields and no further purification was required.

[91] Alicade, B.; Biurrun, C.; Martinez, A.; Plumet, J. *Tetrahsdron Lett.* **1995**, *36*, 5417-5420,

Only with the products **3c**, **3k**, **3o** and **3s** the yield was relatively lower compared to the majority of transformations, however, still in a quite good range. This may be associated to the presence of an N-Me group, and all compounds of this series displayed significantly higher water solubility; consequently, the slightly lower yield could be an effect of the work-up protocol.

The products **3t-3y** were literature known and were synthesized according to this procedure.[55]

2.1.3 Oxidation Step



3a-r **4a-r**

Phosphorus pentoxide (1.5 equiv) was added carefully to dry dimethylsulfoxide at 0-5°C under Ar and stirred at this temperature for 10-15 minutes. The mixture was then allowed to warm to room temperature. The alcohols **3a-3r** diluted in little dry dimethylsulfoxide were added and stirred at room temperature until TLC indicated complete conversion.
In a first series of experiments the reaction time was 12-24 hours as the reaction showed complete consumption of starting material according to TLC. Prolonged reaction times up to 72 hours were required to achieve full conversion *and* good yields. This indicates the formation of an intermediate difficult to detect by TLC. After hydrolysis with cooled satd. sodium bicarbonate solution and extraction with EtOAc, the solution was washed three times with brine, dried over Na_2SO_4, filtered and evaporated to dryness.
Purification of the products was carrid out by flash chromatography.

Product yields are shown in table 3:

precursor	R	PG	product	yield	reaction time
3a	phenyl	-allyl	**4a**	52%	68h
3b	phenyl	t. butyl	**4b**	84%	68h
3c	phenyl	$-CH_3$	**4c**	74%	68h
3d	C_6H_5-CH-C(CH_3)	-bn	**4d**	81%	68h
3e	C_5H_6-CH-C(CH_3)	-allyl	**4e**	41%	68h
3f	C_6H_5-CH-C(CH_3)	t. butyl	**4f**	96%	68h
3g	C_6H_5-CH-C(CH_3)	$-CH_3$	**4g**	62%	68h
3h	t.-butyl	-bn	**4h**	87%	68h
3i	t.-butyl	-allyl	**4i**	93%	68h
3j	t.-butyl	t. butyl	**4j**	90%	68h
3k	t.-butyl	$-CH_3$	**4k**	92%	68h
3l	thiophene	-bn	**4l**	83%	68h
3m	thiophene	-allyl	**4m**	70%	68h
3n	thiophene	t. butyl	**4n**	89%	68h
3o	thiophene	$-CH_3$	**4o**	52%	68h
3p	furan	-bn	**4p**	65%	68h
3q	furan	-allyl	**4q**	45%	68h
3r	furan	t. butyl	**4r**	86%	68h

table 3

The fact that, after 72h stirring at rt the solution was dark brown to black, the mixture was dissolved in EtOAc and activated carbon was added. This solution was stirred for 5min at rt, filtered over two paper filters and worked up as usual.

The yield of the oxidation step is over all good. Again some variations in isolated yields were observed, which may be attributed to product solubility. In particular lipophilic compounds (R = t.-butyl and/or PG = t.-butyl) were usually obtained in high yields, while more polar products (esp. all N-Me ketones) often gave less satisfying results

Another problem was allyl-protected systems (products **4a**, **4e** and **4q**), where generally also low yields were observed. The difficulties in making these products available is most likely associated to limited stability of the compounds due to the unsaturation in the protecting group. Very limited storage stability was observed for these compounds even at reduced temperatures (-20°C in a freezer) and this may be a result of partial polymerization.

Interestingly, product **4m** showed good yield (70%) however the product **4q** gave only a yield about 45%. Both products have the same protecting group (PG = allyl) and an aromatic system as substituent. The only difference is the heteroatom. In the case of thiophen the yields are generally a little bit higher compared to the furan ring

system. Hence, it can be concluded that the thiophen system is better compatible with the reaction conditions of the oxidation step than the furan ring system.
The oxidation of product **3s** did not work. The educt was destroyed during the reactionprocess.

The ultimate oxidation step after alcohol deprotection is usually performed by treatment with P_2O_5/DMSO to give the α-keto-β-lactam. In this context, it was also the aim of the synthetic part of this thesis to investigate alternate oxidation reactions for the synthesis of some ketones, as the P_2O_5/DMSO methodology sometimes displayed only moderate reproducibility and gave somewhat lower yields.

The oxidation step with educt **3o** did not work with high yield. One problem was, that this product is highly water soluble, so the work up has to be changed. The product loss was very high in the step of hydrolysis. The solution was not washed with brine, thereby many impurities could not be extracted.
To avoid the problem of removing dimethylsulfoxide from the mixture, the solvent was changed. Dimethylsulfoxide was only added in needed quantity. The solvents tried were acetone with 1equiv. and 5equiv. dimethylsulfoxide and dioxane with 2equiv. and 5equiv. dimethylsulfoxide. In both cases no product was formed, only starting material was recovered.

Due to these problems, another oxidation procedure was tried.

The typical oxidation reaction is the Swern-oxidation:[92]

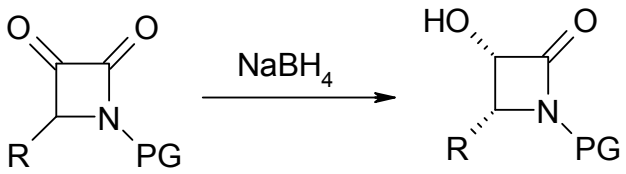
Under Ar a solution of oxalyl chloride (0.06mL, 3.7equiv.) in 1.4mL anhydrous methylenchloride was cooled to -70°C. Dimethylsulfoxide (0.06mL, 4equiv.) in 0.3mL anhydrous methylenchloride was added drop wise. After 30min a solution of alcohol **3o** (0.1g, 0.55mmol) in 0.5mL anhydrous methylenchloride was added. The mixture was stirred at low temperature for 15min and then triethylamine (0.39mL, 6equiv.) was added. The solution was kept at -40°C for 1h and then allowed to warm to room temperature.
After flash chromatography (PE:EtOAc 7:1) 43% of product **4o** were obtained.
The educt **3o** was also not really accessible for the Swern-oxidation, normally yields up to 80% yield are achieved with this reaction method.
This oxidation was also tried for the educt **4v**, but here no conversion could be achieved.

[92] a) Marx, M.; Tidwell, T.T. *J. Org. Chem.* **1984**, *49*, 788-793 b) Ireland R.E.; Norbeck, D.W. *J. Org. Chem.* **1985**, *50*, 2198-2200

Another method is the oxidation with $Na_2Cr_2O_7.2H_2O$:[93]
Alcohol **3o** (0.1g, 0.55mmol) was diluted in 0.27mL ether. The solution was warmed up to 25°C. To this mixture $Na_2Cr_2O_7.2H_2O$ (54.5mg, 67mmol) was added. The solution was stirred for 15min, and then 0.04mL conc. H_2SO_2 in 0.27mL H_2O was added.
The monitoring by TLC showed only starting material. Also with this method no conversion could be achieved for the educt **3o**.

The products **4t-4y** were literature know and were synthesized according to this procedure.[55]

2.1.4 Reduction with $NaBH_4$



4a-s **3a-s**

We know from previous studies, that the reduction of α-keto-β-lactams with $NaBH_4$ is highly diastereoselective and gives only the *cis*-3-hydroxy-ß-lactams (however, in racemic form)[94].

The products **5a-s** were prepared according to a literature protocol[95] by chemical reduction in order to provide reference material for reverse and straight phase chromatographic analysis of the subsequent bioreduction. These products were analyzed by HPLC to optimize the separation of the racemic *cis*-products on a chiral column.

The ketones were dissolved in ethanol, the mixture was cooled to 5°C and 1 equiv. $NaBH_4$ was added. This mixture was stirred for one hour, hydrolyzed with 2N HCl and stirred for additional 3 hours. After TLC indicated complete conversion, the mixture was extracted with EtOAc, dried over Na_2SO_4, filtered and evaporated to dryness.

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[93] a)Schwetlick, K. et al *Organicum* 21.Auflage, 430-436 b) Garg, C.P. *J. Org. Chem. Soc.* **1961**, *83*, 2952-2953
[94] Kayser, M.M.; Yang, Y.; Mihovilovic, M.D.; Feicht, A.; Rochon, F.D. *Can. J. Chem*. **2002**, *80*, 796-800
[95] Mihovilovic, M.D. *Post Doc-Report* **1997** Univ. New Brunswick-Saint John Campus

Product yields are shown in table 4:

precursor	R	PG	product	yield	*cis:trans ratio*
4a	phenyl	-allyl	**3a**	94%	100:0
4b	phenyl	-t. butyl	**3b**	97%	100:0
4c	phenyl	$-CH_3$	**3c**	98%	100:0
4d	C_6H_5-CH-$C(CH_3)_3$	-bn	**3d**	97%	100:0
4e	C_5H_6-CH-$C(CH_3)_3$	-allyl	**3e**	98%	100:0
4f	C_6H_5-CH-$C(CH_3)_3$	-t. butyl	**3f**	99%	100:0
4g	C_6H_5-CH-$C(CH_3)_3$	$-CH_3$	**3g**	98%	100:0
4h	t.-butyl	-bn	**3h**	99%	100:0
4i	t.-butyl	-allyl	**3i**	96%	100:0
4j	t.-butyl	-t. butyl	**3j**	99%	100:0
4k	t.-butyl	$-CH_3$	**3k**	99%	100:0
4l	thiophene	-bn	**3l**	98%	100:0
4m	thiophene	-allyl	**3m**	98%	100:0
4n	thiophene	-t. butyl	**3n**	99%	100:0
4o	thiophene	$-CH_3$	**3o**	99%	100:0
4p	furan	-bn	**3p**	95%	100:0
4q	furan	-allyl	**3q**	96%	100:0
4r	furan	-t. butyl	**3r**	99%	100:0
4t	Phenyl	-PMP	**3t**	99%	100:0
4u	Phenyl	Bn	**3u**	98%	100:0
4v	C_6H_5-CH-$C(CH_3)_3$	Bn	**3v**	100%	100:0
4w	t.-butyl	PMP	**3w**	100%	100:0
4x	thiophen	PMP	**3x**	99%	100:0
4y	furan	PMP	**3y**	99%	100:0

table 4

This reaction worked without any problems in very high yields. Every starting material was transformed to the *cis*-alcohol in high selectivity. No further purification was necessary, because of the very high product quality after work up, which was even sufficient for subsequent chiral HPLC analysis. All samples were analyzed by reverse phase and straight phase HPLC for reference purposes.

2.1.5 Conclusion

The Staudinger reaction still plays a central role among synthetic methods for azetidinone synthesis, and the only disadvantage is the difficult to control diastereoselectivity of the transformation. Employing different conditions and reagents we are able to enable stereoselective reactions. Every product cyclized with method B yielded only the *cis*-products. When applying method A the stereoselectivity was in a range of 1:1 up to 100:0 in favor of the *cis*-product. With this method the formation of the *cis*-product was enhanced. Method C gave also only the *cis*-product. Summarizing, method B and C can be carried out under mild conditions and preferably provide gave *cis*-products. In contrast, educts cyclized by method A under hasher conditions gave mixtures of *cis* and *trans* isomers with the only exeption of two products (in the case of **2b** and **2d** only *cis*-products were obtained). The yields for this step were all over good to excellent (only yields for products **2e**, **2j** and **2r** were moderate).

The hydrolysis of the alcohols was performed under very mild conditions with excellent yields and without any complications.

Subsequent oxidation to ketones was the most problematic step. With some products very good yields could be achieved, but with some other products, only moderate yields were obtained. For example for products **4a**, **4e**, **4g**, **4o**, **4p** and **4q** only very moderate yields around 40-60% were attained. Our attempts to increase the yields by applying differnt oxidation reactions proved to be difficult. Changing the solvent and varying the equivalents of DMSO and P_2O_5 showed no success, only starting material could be detected. Among the “typical” oxidation reactions, the Swern-oxidation did not improve isolated yields, at all; only low conversions were obtained. Also, oxidation with $Na_2Cr_2O_7.2H_2O$ did not give efficient conversion and only starting material was recovered.

The subsequent reduction from the ketones to alcohols was conducted chemically to obtain reference material for the biotransformation.
In conclusion, new β-lactams were obtained in over all good yields. The easily available starting materials and the simplicity of all the synthetic steps make this approach very practical for the synthesis of 2-azetidiones. New building blocks for the synthesis of Paclitaxel analogues and other pharmaceutically important compounds were prepared.

2.2 Enzymatic Reduction to the Alcohol

Biotransformations require relatively mild physical conditions of pH and temperature, which preserve the functional integrity of the catalysts and are advantageous when labile substrates or products are involved[96]. The possible sensitivity of enzymes to denaturation by reactant and/or product demanded that biotransformation processes be designed to prevent inactivation of the biocatalysts employed. Where cells are used as biocatalytic reagents, the system must allow adequate rates of penetration and diffusion of substrate into, and product from, the cells. And enzyme reactions involving formation of undesirable by-products or degeneration of the desired product have to be inhibited or minimized. Two-phase aqueous-organic solvents systems can facilitate the performance of enzyme-catalysed reactions including substrates that are relatively insoluble in water[97]. The enzyme is in the water phase and the substrate is in the organic phase. The enzyme reaction takes places at the boundary between the phases. The enzymes can also act in the organic solvents provided the enzyme remains surrounded by a monolayer of water[98].

2.2.1 Analytics

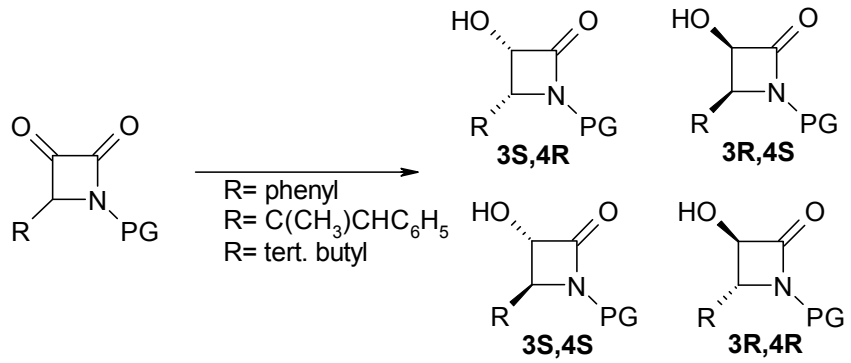
None of the β-lactams investigated within this thesis could be measured on the GC-MS. The spectra shows only fragments, which leads to the conclusion, that the four ring system was cleaved during the recording.
The best method to monitore the conversion was by reverse phase HPLC. For all components which contain aromatic systems, spectra were measured at 254nm.
For all other compounds, the max-plot spectra were consulted for the interpretation.
For the effective separation on the straight phase HPLC it was necessary to equip the chiral column with a non-chiral pre-column in order to enable efficient separation. Spectra were recorded at the same wavelength as for the reverse phase.
The detection of the ketons was general very difficult. On the reverse phase peak width was sometimes problematic (over 2min), so that signal overlapping with contaminants made interpretation of the data more difficult.
Another aspect, that complicated analyses, was the high sensitivity of the available columns and instrumentation to minor changes in concentration and analytical matrix. We observed significant differences in the elution behavior of reaction partners for the same transformation, depending on the type of biocatalyst (whole cell, isolated

[96] Crueger, W. and Crueger, A. *Biotechnology:A Textbook of Industrial Microbiology, science Tech. Madison,* **1984**
[97] Lilly, M.D. *J. Chem. Tech. Biotechnol.* **1982**, *32*, 162-169
[98] Klibanov, A.M.*Chemtech.* **1986**, *16*, 354-359

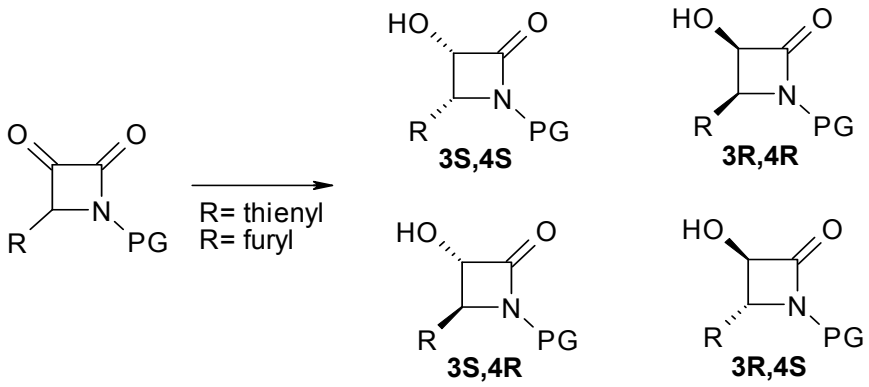
enzyme); obviously, matrix contaminants led to shifts in retention times. Consequently, excessive use of reference HPLC-runs was required, since otherwise peak assignment would not have been possible. Still, due to significant shifts in retention behavior even within 2-3 samples, interpretation of the analytical data was tedious and highly time consuming.
Some particular technical problems were encountered:
Ketone precursors usually gave very broad signals in chiral-phase HPLC; only RP-HPLC gave reasonable signals and allowed analysis of reaction progress.
Sometimes, *trans*-products co-eluted with the EtOAc solvent peak. Changing the extraction solvent to methylene chloride alleviated the signal overlap, as it is not UV-active; a novel disadvantage was that some products could not be extracted from the supernatant efficiently with this solvent. Most of the experiments were done in screening scale, so that a loss of product in part may compromise the exact values in conversions as well as the *cis/trans* ratio (although the extraction behavior of isomers was usually highly similar).
For the conversion only the reverse phase HPLC gave highly reproducible and reliable data. This was the only opportunity were both, the ketons and the alcohols, could be detected.
With the sodium borohydrate reduction, pure standards for all *cis*-products were achieved. After the column purification from the up-scaling products of the baker's yeast reduction, some product could be separated in *cis* and *trans*. So, for some products also a *trans*-reference material was available. However, not for all products a separation of *cis/trans* could be obtained on preparative scale. For all *cis*-products and in every case, were the *trans*-product could be isolated, a ^{1}H NMR spectra was recorded to characterize and conform the isomers.

2.2.2 Stereochemistry

Depending on the substituent R four diastereoisomers can be produced in the bioreduction step with the configurations outlined in the following schemes; the configuration is independent from all PG used.
When the R is a phenyl or alkyl substituent, the configuration is shown in scheme



If the rest R is 2-thienyl or 2-furyl the conformation is shown in scheme.



In the discussion of these products, for easier handling, the numbers were omitted, but the sequence of stereocenters is always position 3 prior to position 4.

2.2.3 Baker's Yeast Reduction

In an initial attempt to obtain optically pure 3-hydroxy-β-lactams, we performed baker's yeast reduction of the racemic 3-oxo-β-lactams.

2.2.4 Screening Reactions with Yeast

Commercial yeast (1g) was added to 25mL of a 20% glucose solution in a 50mL baffled Erlenmeyer flask and incubated at 30°C in an orbital shaker. After 15min 10mg of substrate (**4a-4y**) diluted in 100µL dioxane and 1 equiv. of β-cyclodextrin (to increase the solubility) were added. After completion of fermentation (48h) the biomass was separated by centrifugation. The supernatant was saturated with sodium chloride, to avoid emulsion formation and extracted with EtOAc and brine.

All newly synthesized and literature known substrates **4a-4y** were accepted as substrates in a first screening reaction using yeast.

With every product full conversion was achieved after 48h. The only exception was product **3w** where the reverse phase HPLC (RP HPLC) showed only a conversion of 58%.
Some previous studies in this field were conducted by Kayser et al.[21] and these results were highlighted in blue color in table 5; this data also served for reference purposes in the assignment of configuration.

The entries are arranged according to substituent R starting with phenyl, C_6H_5-CH=C(CH_3), tert. butyl, thiophen and furan.

Representative experiments are summarized in table 5:

Pro-duct	Chiral HPLC								RP HPLC	
	trans SS	*trans* RR	*ee*	*Σ trans*	*cis* SR	*cis* RS	*ee*	*Σ cis*	*trans*	*cis*
3t	1.2	23.5	*90*	24.7	24.4	50.9	*35*	75.3	31	69
Lit.3t	<1	38	-	<39	14	48	*55*	59	-	-
3u	18.6	19.8	*3*	38.4	36.8	24.8	*19*	61.6	39.5	60.5
3a	5.9	-	*100*	5.9	49.8	44.3	*6*	94.1	4.3	95.7
3b	12.7	15.5	*10*	28.2	45.8	26	*28*	71.8	13.1	86.9
3c	-	-	-	-	-	100	*100*	100	-	100
3v	-	-	-	-	28.4	71.6	*43*	100	-	100
3d	0.8	6.2	*79*	7	72.9	20.1	*57*	93	8.8	91.2
3e	1.7	46.1	*93*	47.8	30.5	21.7	*17*	52.2	49.5	50.5
3f	3.9	-	*100*	3.9	79.6	16.5	*66*	96.1	-	100
3g	-	35.8	*100*	35.8	64.2	-	*100*	64.2	44.8	55.2
3w	2.5	36.8	*77*	39.3	28.8	31.2	*5*	60.7	45.6	54.4
Lit.3w	<1	43	-	<44	2	55	*93*	57	-	
3h	-	-	-	-	55.8	44.2	*12*	100	-	100
3i	-	-	-	-	75.7	24.3	*51*	100	-	100
3j	-	-	-	-	66.4	33.6	*33*	100	-	100
3k	-	-	-	-	94.4	5.6	*89*	100	-	100
	trans SR	*trans* RS	*ee*	*Σ trans*	*cis* SS	*cis* RR	*ee*	*Σ cis*	*trans*	*cis*
3x	9.5	19.4	*34*	28.9	23.3	47.8	*34*	71.1	37.1	62.9
Lit.3x	<1	37	-	<38	12	51	-	63	-	-
3l	11.1	26.9	*41*	38	50.2	11.8	*62*	62	45.8	54.2
3m	26.9	12.5	*36*	39.4	48.1	12.5	*59*	60.6	40.9	59.1
3n	-	45.5	*100*	45.5	49.3	5.2	*81*	54.5	47.4	52.6
3y	3.1	39.5	*85*	42.6	12	45.4	*58*	57.4	46.8	53.2
Lit. 3y	<1	40	-	<41	18	41	-	59	-	-
3p	2.9	35.2	*85*	38.1	46.4	15.5	*50*	61.9	46.5	53.5
3r	43.6	15.8	*47*	59.5	33.6	7	*66*	40.5	54.8	45.2

table 5

Kayser et al.[21] conducted also experiments in this field using some substituted lactams usually with a PMP nitrogen protecting group. Their results are also shown in table 5. Comparative experiments were conducted with ketones **4t**, **4w**, **4x** and **4y** for reference purposes and also to investigate possible differences in commercial Baker's yeast strains in North America and Central Europe. With all substrates approximately the same results were obtained as in the literature published, the only exception was product **3w**. The results from the reverse phase match together with the literature, but the measurement of the straight phase are different in the specification of the four possible alcohol stereoisomers.

As can be seen in table 5 only six products could be transformed enantioselectively. For the products **3c, 3v, 3h, 3i, 3j** and **3k** baker's yeast gave high selectivity for the *cis*-alcohol. Only **4c** was transformed to enantiopure *cis*-(3R,4S)-alcohol. Nearly the same results were observed for product **3k**. Here the *cis*-(3S,4R)-alcohol was formed with more than 94%. Compound **4h** was nearly transformed in a 50:50 of the *cis* isomers (SR:RS). Product **3v** was obtained as *cis* (SR:RS) in a 1:3 ratio and product **3i** was produced in a 3:1 (SR:RS). The last product which was only transformed as *cis*-product was **3j** where a ratio of 2:1 was obtained.
Products **3a**, **3d** and **3f** gave primarily the *cis*-alcohol with 93-96%, were only **3f** gave 66% ee. With product **3b** a 7:3 ratio between *cis* and *trans* alcohols was obtained. The ee of the *cis*-alcohol was very low with 28%.

For all other products the stereoselectivity was generally modest. Product **3u** gave a 3:2 ratio between *cis* and *trans.*
Products **3g**, **3w, 3l, 3m**, **3n**, **3p** and **3r** were achieved in around 50:50 ratio of *cis* and *trans.* For compound **3n** only one *trans* alcohol (RS) was received and the ee for the *cis*-alcohol was about 81%. Compound **3g** was the only product where only a single enantiomer *cis* and *trans* alcohol was obtained. As shown in the table 36% of the RR-alcohol and 65% of the (SR)-alcohol were received. For product **3q** it was not possible to get any results, because of the fast polymerization of the ally-group. The experiment was carried out twice, but in both case an interpretation was not possible.

2.2.5 Scale-up (50mg) Reaction with Yeast:

In order to obtain also synthetic quantities of reference material and for subsequent comparison to the isolated fusion-enzyme biotransformations, several reactions were repeated in a 50mg scale with work-up and subsequent column chromatography. Consequently, almost a complete set of *trans*-alcohols became available as reference for the HPLC-measurements.

Commercial yeast (2g) was added to 50mL of a 20% glucose solution in a 250mL baffled Erlenmeyer flask and incubated at 30°C in an orbital shaker. After 15min 50mg of substrate (**4a-4y**) diluted in 300µL dioxane and 1 equiv. of β-cyclodextrin (to increase the solubility) were added. After completion of fermentation (48h) the biomass was separated by centrifugation. The supernatant was saturated with sodium chloride to avoid emulsion formation and extracted with EtOAc. Purification of the products was conducted by flash chromatography.

Table 6 shows the results for the scale-up yeast reduction with work up:

Product	Chiral HPLC								RP HPLC	
	trans SS	*trans* RR	*ee*	*Σ trans*	*cis* SR	*cis* RS	*ee*	*Σ cis*	*trans*	*cis*
3t	7.1	21.6	52	28.7	23.4	47.9	68	71.3	24	76
Lit.3t	<1	38	-	<39	14	48	55	69	-	-
3u	17.2	22.3	6	39.5	32.6	27.9	16	60.5	49.5	50.5
3a	8.9	9.5	14	18.4	39.3	42.3	4	81.6	14.8	85.2
3b	3.7	23.4	85	27.1	43.8	29.1	19	72.9	27.1	72.9
3c	-	-	-	-	-	100	100	100	-	100
3v	-	-	-	-	30	70	40	100	-	100
3d	0.2	0.8	68	1	70.4	28.6	17	99	0.5	99.5
3e	1.7	50.5	95	52.2	25.3	22.5	6	47.8	46.6	53.4
3f	2.8	-	100	2.8	58.5	38.7	25	97.1	1.6	98.4
3g	-	28.1	100	28.1	71.9	-	100	71.9	41.5	58.5
3w	-	45.2	100	45.2	19.9	34.9	27	54.8	37.6	62.4
Lit.3w	<1 SS	43	-	<44	2	55	93	57	-	
3h	-	-	-	-	56	44	12	100	-	100
3i	-	-	-	-	61	39	21	100	-	100
3j	-	-	-	-	65.0	35.5	30	100	-	100
3k	-	-	-	-	83.6	16.4	64	100	-	100
	trans SR	*trans* RS	*ee*	*Σ trans*	*cis* SS	*cis* RR	*ee*	*Σ cis*	trans	*cis*
3x	10.5	36.7	55	47.3	13.7	39.0	48	52.7	39.1	60.9
Lit.3x	<1	37	-	<38	12	51	-	63	-	-
3l	3.8	37.2	82	41	39.9	19.1	35	59	43.3	56.7
3n	0.5	25.5	96	25.9	60.9	13.1	64	74.06	25.9	74.06
3y	14.7	34.4	37	49.1	15.6	35.3	38	50.9	31	69
Lit. 3y	<1	40	-	<41	18	41	-	59	-	-
3p	7.6	15.6	35	23.2	56.1	20.7	52	76.8	31.7	68.3
3q	22.1	31.2	34	53.27	31.4	15.3	35	46.7	47.6	52.4
3r	33.1	20.5	24	53.6	38.4	8	66	46.4	54.8	45.2

table 6

Confirming the validity of screening results and scale-up transformations, in both settings (10mg and 50mg), the majority of results only deviated within usual experimental error limits and shows no significant differences. Changes were observed for the following products: With compound **3a** only the SS *trans* product was observed in the screening reaction, while in the reaction set-up including work up nearly a 1:1 ratio of both *trans* alcohols was found. For compound **3d** a shift favouring the *cis*-alcohol was observed in the bigger scale experiments with 99% diastereoselectivity; the *cis* product (RS) was formed in higher quantities than in the screening.

The biggest deviation was found for product **3n**: In the screening reaction nearly a 1:1 of the *cis* (RS) and the *trans* (SS) alcohols was obtained. However, in the larger scale experiment the equilibrium shifted to the *cis*-alcohol. A 1:3 ratio was observed and all four possible alcohol stereoisomers were obtained. For product **3m** no straight phase results could be obtained, due to the polymerisation of the allyl-rest.

In table 7 the work up yields were summarized:

product	YIELD		Yield *Trans*		Yield *cis*		Mixture *trans/cis*	
	Yield [mg]	Yield [%]	Yield *trans* [mg]	Yield *trans* [%]	Yield *cis* [mg]	Yield *cis* [%]	Yield mixture [mg]	Yield mixture [%]
3t	24.7	49	5.6	22.6	16.2	65.6	2.9	11.7
3u	21.1	42	10.4	49.3	10.6	50.2	1.0	0.5
3a	32.1	64	4.7	14.6	27.3	85.0	1.0	3.1
3b	16	32	5.7	35.6	10.3	64.4	-	-
3c	35.5	70	-	-	35.3	100	-	-
3v	25.2	50	-	-	25.2	100	-	-
3d	41.3	80	0.7	1.3	40.6	98.7	-	-
3e	20.2	40	9.2	45.5	10.6	52.5	0.4	2.0
3f	15.5	31	0.3	2.1	15.2	97.9	-	-
3g	29	57	0.7	24.1	22.0	75.9	-	-
3w	26.6	73	10	37.6	16.6	62.4	-	-
3h	16.4	33	-	-	16.4	100	-	-
3i	15.2	30	-	-	15.2	100	-	-
3j	34.4	61	-	-	34.4	100	-	-
3k	13.3	27	-	-	13.3	100	-	-
3x	35.5	70	9.6	27.2	10.9	30.9	14.8	41.9
3l	29.8	59	8.4	28.2	11.2	37.6	10.2	34.2
3n	47.8	98	12.4	25.9	35.4	74.1	-	-
3y	33.5	66	5.7	17.0	19.7	58.8	8.1	24.2
3p	32.6	65	7.2	21.8	21.2	65.0	-	-
3q[99]	34.8	69	-	-	-	-	-	-
3r	49.2	97	13.1	26.6	29.6	60.2	6.5	13.2

table 7

For products **3t, 3u, 3a, 3e, 3x, 3l, 3y** and **3r** mixture fraction of *cis* and *trans* were obtained.

The highest yield, up to 80%, was achieved with products **3d**, **3n** and **3r**. Good yields after work-up were obtained for product **3a**, **3c, 3g, 3w, 3j, 3x, 3y, 3p** and **3q**. With all other products yields were moderate. It is interesting to note that all products with R =

[99] No separation of *cis* and *trans* on preparative scale

tert. butyl showed very low yields (**3h, 3i, 3k**). Similarily, products (**3b** and **3f**) with tert. butyl as protecting group also gave yields of only around 30%.

2.2.6 Screening Overexpressed Yeast Reductases in *E. coli* (whole cell)

Due to these favorable results obtained with commercial yeast, the next step was the study of whole cell biotransformation of recombinant overexpression systems of particular reductases under growing conditions.
The whole cell screening may again be complicated because of toxicity and limited membrane permeability. After the first two screening series with substrates **4t** and **4v** the idea was rejected to test all precursors with overexpressed yeast reductases used as whole cells.

2.2.6.1 *cis*-3-Hydroxy-1-(4-methoxyphenyl)-4-phenyl-azetidin-2-one (3t, $C_{16}H_{15}NO_3$)

Expression strains harboring pIK 2-32 were grown on LB_{kan} agar plates for 15-20 hours at 37°C, then LB_{kan}-media (10mL) was inoculated with a single colony and incubated in a baffled Erlenmeyer flask at 37°C for 1 day in an orbital shaker. These experiments were done in 24 well plates: therefore, 20mg substrate (**4t** or **4v**) dissolved in 150µL dioxane and 4 µL IPTG-stock was added to 1mL of the preculture. Reaction control was performed by reverse phase HPLC analysis. After 72h the conversion was usually low.

The conversion for substrate **4t** is shown in table 8:

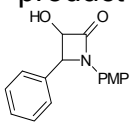
product 	pIK i	conv. HPLC	rev. phase HPLC	
			trans	cis
3t	pIK 3	25.4	56.6	43.4
3t	pIK 4	4.0	32.9	67.1
3t	pIK 5	16.3	46.7	53.3
3t	pIK 6	20.0	73.6	26.4
3t	pIK 7	24.4	38.7	61.3
3t	pIK 8	20.0	42.2	57.8
3t	pIK 9	6.7	-	100
3t	pIK 10	19.7	37.3	62.7
3t	pIK 11	20.9	38.2	61.8
3t	pIK 12	21.1	40.0	60.0
3t	pIK 13	42.8[100]	54.4	45.6
3t	pIK 15	24.1	41.8	58.2
3t	pIK 18	24.5	44.9	55.1
3t	pIK 23	22.3	46	54
3t	pIK 25	27.1	46.5	53.5
3t	pIK 29	30.7	48.2	51.8
3t	pIK 30	22.3	48.5	51.5
3t	pIK 31	18.5	50.1	49.9
3t	pIK 32	22.4	49.3	50.7
3t	pAKS1	21.9	49.8	50.2
3t	pTM3	17.3	33.3	66.7

table 8

With the enzyme pIK 29 the highest conversion (30%) was achieved, except the enzyme pIK 13, where the spectrum was not baseline separated.
Only with one enzyme (pIK 9) the *cis*-product could be generated exclusively. A *trans/cis*-ration of 3:7 was obtained with pIK 4, whereas with pIK 6 the ratio was also 7:3 but more *trans*-product was obtained. For nearly all other products the ratio between *trans* and *cis* was in the range of 2:3 up to 1:1.
But it is difficult to draw detailed conclusions as the results are based on low to moderate (at best) conversions.

[100] The chromatogramms of pIK13 could not be exactly integrated, because the peaks were not baseline separated.

2.2.6.2 *cis*-3-Hydroxy-1-(4-methoxyphenyl)-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3v, $C_{19}H_{19}NO_3$)

The screening was realized with educt **4v** too. This product gives approximately equivalent result. The conversion is as low as with substrate **4t**.

The conversion was shown in table 9:

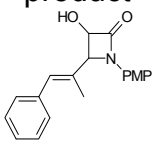
product 	pIK i	conv. HPLC	rev. phase HPLC	
			trans	cis
3v	pIK 3	31.9	38.4	61.6
3v	pIK 4	21.6	44.2	55.8
3v	pIK 5	20.6	44.1	55.9
3v	pIK 6	11.8	62.7	37.3
3v	pIK 7	21.8	49.8	50.2
3v	pIK 8	19.8	46.5	53.5
3v	pIK 9	18.5	48.5	51.5
3v	pIK 10	12.9	44.9	55.1
3v	pIK 11	20.6	48.9	51.1
3v	pIK 12	19.9	48.9	51.1
3v	pIK 13	24.3	45.6	54.4

table 9

Having the same low conversion, this screening was stopped by enzyme pIK 13.
All enzymes converted the educts to a *trans:cis* ratio between 2:3 up to 1:1. Only exception was the enzyme pIK 6 where the *trans*-product was favored in a ratio 3:2.

The fact that these results were not really satisfying, the main view was focused on the GST fusion proteins in isolated form; this approach offered the prospect of direct analysis of the particular biocatalysts without eventual side-reactions by the host organism and was expected to give a better evaluation of suitable catalysts for the preparation of optically enriched lactams for novel taxoids.

2.2.7 GST-Fusion Proteins:

A library of 15 yeast reductases was expressed in *Escherichia coli* as glutathione (S)-transferase (GST) fusion protein[69], which can be purified by a common affinity matrix. This allows the properties of each catalyst to be defined in the absence of competitors.

Kayser et al.[101] also conducted some experiments related to this thesis while these studies were in progress.
Consequently, a collection of 15 yeast reductases, expressed in *E. coli* as glutathione (S)-transferase (GST) fusion protein, was isolated and purified.

Reductases available within the research group:

Yeast gene	Plasmid	Kind of reduktase	purification	Size*
	pIK2	glutathion without yeast gene	y	
YOL151w	pIK3	methylglyoxal reductase	y	38.2kDa
YDR368w	pIK4	2-methylbutyraldehyde reductase	y	34.7kDa
YDR541c	pIK5	like dihydroflavonol-4-reductase	y	38.6kDa
YGL039w	pIK6	oxidoreductase	y	38.2kDa
YGL157w	pIK7	oxidoreductase	y	38.1kDa
YDL124w	pIK8	α-ketoamide reductase	y	35.5kDa
YJR096w	pIK9	xylose und arabinose reductase	y	32.3kDa
YCR107w	pIK10	arylalkohol dehydrogenase		40.9kDa
YNL331c	pIK11	like arylalkohol dehydrogenase		42.0kDa
YBR149w	pIK12	D-arabinose dehydrogenase	y	38.9kDa
YNL274c	pIK13	putative hydroxyacid dehydrogenase	y	38.8kDa
YPL113c	pIK15	putative hydroxyacid dehydrogenase	y	45.0kDa
YPL275w	pIK18	NAD^+-dependent formate dehydrogenase		26.5kDa
YLR070c	pIK23	xylitol dehydrogenase		38.6kDa
YMR318c	pIK25	like cinnamyl alcohol dehydrogenase		39.6kDa
YHR104w	pIK29	aldose reductase	y	37.1kDa
YOR120w	pIK30	galactose induced protein of aldo/keto-red.	y	35.1kDa
YJR159w	pIK31	sorbitol dehydrogenase	y	38.2kDa
YCR105w	pIK32	like cinnamyl alcohol dehydrogenase	y	39.3kDa
YGL185c	pAKS1	putative hydroxyacid dehydrogenase		43.0kDa
YAL060w	pTM3	sterosp. (2R, 3R)-2,3-butandiol dehydrogenase		41.5kDa

*Size without GST fusion

table 10

[101] Kayser, M.M.; Drolet, M.; Stewart, J.D. *Tetrahedron:Asymmetry* **2005**, *16*, 4004-4009

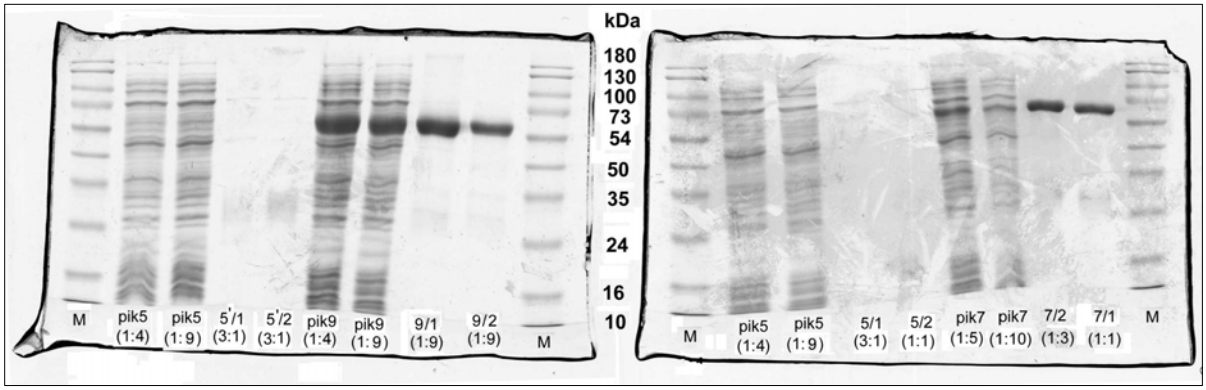
The focus was now on the purification of these 19 yeast reductases by a common affinity matrix. Only 15 enzymes could be purified in sufficient quality and quantities. The purified enzymes were indicated with a “y” in table 10.

2.2.7.1 Purification of Overexpressed Yeast Reductases in *E. coli*

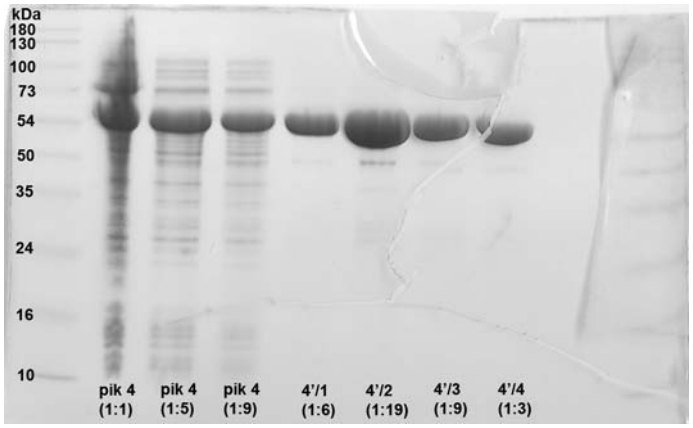
In order to avoid competing side reactions by other enzymes, purification of the GST-fusion biocatalysts was conducted; recombinant expression strains for yeast reductases in *E.coli* were cultivated and broken by ultrasound followed by affinity chromagrography to isolate the pure proteins. As can be seen in the following table, purification was successful only for some of the available expression systems. For all indicated biocatalysts the expression gave a protein of correct size which was also catalytically active.

Isolated GST-fusion proteins

Example of an SDS-Page (12% gel) of the reductases from the expressionsystem with pIK 4, 5, 7 and 9.



Purity of the collected fractions:
The prestrained marker was labeled with M. The enzymes pIK 5, 9 and 7 were shown as crude lysate after sonication in different dilutions as indicated. The samples of purified fusion proteins was indicated by an apostrophe (pIK 5’/1), where the slash indicates the collected fraction from the glutathione sepharose filled column.
The molecular weights of the yeast reductase are in the expected range of 54-73kDa. As can be seen from this scheme pIK 7 and pIK 9 could be purified with high concentration. For example the band at 60kDa displays the purified enzyme pIK 7 with the GST-fusion protein. Comparing this band to the band of pIK 5 the band is pale. The concentration of pIK 5 is very low.



Also pIK 4 could be purified with high concentration, the band at 60kda is very intensive.

In table 11 the concentration of the collected fractions of each fusion protein is disclosed:

Fraction	1	2	3	4	5	6	7
pIK							
2	8.89	26.97	18.79	8.07			
3		1.81	2.09	0.68			
4 (I)	14.77	37.75	19.71	8.64			
4 (II)	1.877	3.095	4.5914	2.5904	1.3202	1.268	
5	0.35	0.82					
6		0.9648	0.746	0.6938			
7	7.46	1.46	0.7				
8	7.149	7.532	2.59	1.35			
9	15.35	15.50	1.88	2.15			
12	2.3294	9.8114	11.7428	3.3560			
13	3.5996	9.9854	7.0622	4.9568	3.5996	2.7644	
15		0.329	0.262	0.156	0.196	0.162	
29	3.0602	3.4778	3.3734	2.7818	2.8166	2.9558	2.834
30	5.096	5.7398	5.0612	3.5648	2.7122	2.834	2.5034
31	3.0254	2.7818	1.9118	1.6508	1.355		
32	3.4778	5.096	4.8002	3.9128	2.9732	2.8166	

Protein concentrations [mg/2mL] for collected samples according Bradford protocol

table 11

All samples were stored at –20°C in 20% glycerol to avoid loss of activity during storage.

2.2.7.2 Screening Reaction with GST-Fusion Protein

The screening reactions were performed for the substrates **3t, 3e**, **3w**, **3h**, **3x**, **3l**, **3n** and **3p**:

The reaction mixture were implemented in 24 well plates containing β-lactam substrate (10mM), purified GST-fusion protein (20-200µL, including 10-100µg of protein), NADP (0.40µmoles, 0.30mg), glucose-6-phosphate (0.28µmoles, 8.6mg), glucose-6-phosphat dehydrogenase (10µg) in 2.0mL of 100mM KP_i, pH 7.0 (1M K_2HPO_4 + 1M KH_2PO_4). Reactions were incubated at 30°C for 48h.
A part of the reaction solution (700µL) was extracted with an equal volume of EtOAc, dried with Na_2SO_4 and sampled for HPLC.

2.2.7.2.1 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3t, $C_{19}H_{19}NO_3$)

Results were shown in table 12:

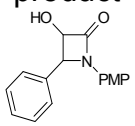
product 	pIK i	conv. HPLC	rev. phase HPLC		str. phase HPLC			
			trans	cis	trans RR	trans SS	cis SR	cis RS
3t	pIK 2	73	45	55	9.4	37.3	1.4	51.9
3t	pIK 3	36	90	10	47.5	40.4	5.8	6.3
3t	pIK 4	60	42	58	25.9	17.4	12.9	43.9
3t	pIK 4	49	60	40	41.3	25.6	4.9	28.2
3t	pIK 5	41	75	25	49.9	40.5	0.7	8.9
3t	pIK 7	37	90	10	52.3	39.4	4	4.3
3t	pIK 8	73	51	49	12.3	42.3	1.6	43.8
3t	pIK 9	29	91	9	48.6	40.4	6	5
3t	pIK 12	64	49	51	38.2	11.4	48.1	2.3
3t	pIK 13	64	46	54	39.5	7.1	50.9	2.5
3t	pIK 15	36	86	14	47.4	39.9	7.2	5.5
3t	pIK 29	32	91	9	49	40.2	5.9	4.9
3t	pIK 30	41	72	28	45.7	28.8	8.5	17
3t	pIK 31	32	90	10	40.4	49.3	5.6	4.7
3t	pIK 32	34	91	9	49.8	41.6	4.7	3.9

table 12

The conversion measured on the HPLC was quite comparable with exception of enzymes pIK 9 and 12 where significantly lower conversions were obtained relative to the literature[64].

Good conversions, between 60% and 73%, were obtained with the enzymes pIK 2, pIK 4, pIK6, pIK 8, pIK 12 and pIK 13. With all other enzymes the conversion was relatively low. With pIK 3 and pIK 8 the *cis/trans*-ratio was nearly 1:1. In both cases the SS *trans* and the RS *cis* was favored. A 1:1 ratio is also attained with the enzymes pIK12 and pIK13 but here the RR *trans* and the SR *cis* were preferred.. This reaction was synthesized twice and in both cases the spectra shows only background noise. Strain pIK 4 transformed educt **3t** with a 3:2 *trans/ cis*-ration were the selectivity was toward the RR *trans* and the RS *cis* product.

This experiment served as reference to previously published literature results[69] as compiled in table 13 using compound **4t** as precursor.

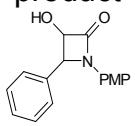
product 	pIK i	conv. GC	Chiral HPLC				
			conv. HPLC	trans RR	trans SS	cis SR	cis RS
3t	pIK 3	70	32	<1	32	55	13
3t	pIK 4	52	68	<1	9	43	47
3t	pIK 5	60	36	<1	24	47	28
3t	pIK 6	60	53	<1	14	42	44
3t	pIK 7	66	37	<1	25	43	32
3t	pIK 8	68	90	<1	10	7	83
3t	pIK 9	63	83	<1	8	15	77
3t	pIK 12	73	92	<1	4	93	3
3t	pIK 13	66	71	<1	8	25	66
3t	pIK 15	65	30	<1	24	49	27
3t	pIK 29	67	30	<1	24	50	26
3t	pIK 30	62	39	<1	17	53	30

table 13

As it can be seen by comparison of the two previous tables, the results do not really match well.

For the enzymes pIK 5, 6, 9, 15 and 30 the results of the biotransformations (GC literature and straight phase HPLC) were exactly inverted. For example with pIK 5 the relation between *trans*:*cis* was 3:1, but in the literature the relation is specified with 1:3.

All other results were not really compatible with the results from the literature.

For the straight phase as well as for the reverse phase HPLC reference material was injected, to confirm correct assignment of *cis* and *trans* products. The reference material was also verified by ^{1}H NMR. This may lead to the suggestion, that the data

published in the literature is based on incorrect assignment of the peaks; this is supported to a certain extent by the fact, that in literature reports in all cases (in particular in cases with almost complete conversion) a significant imbalance between *cis* and *trans* products is observed. In addition, the massive deviation of conversion determined by GC and HPLC as reported in the literature indicates some problems in the analytical investigations of this group.

The assignment of the four diastereomers was confirmed by some earlier studies on this compound and was verified with the data published in the literature[69] from biotransformation with yeast. Certainly, massive deviations between the findings within this thesis and the literature data can be a result of largely different conversions. The large difference between GC and HPLC analysis in the literature makes it impossible to draw any detailed conclusions from literature data to the results obtained within this thesis.

In any case, the data presented for substrate **4t** as given in this work was confirmed by independent assignment and analysis of both *cis* and *trans* products by NMR and HPLC as well as by independent bioreduction using yeast.

2.2.7.2.2 1-Allyl-3-hydroxy-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3e, $C_{15}H_{17}NO_2$)

In the following table 14 the results for the product **3e** is reported:

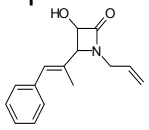
product 	pIK i	conv. HPLC	rev. phase HPLC	
			trans	cis
3e	pIK 2	5.9	11.5	88.5
3e	pIK 3	5	21	79
3e	pIK 4	11	53.6	46.4
3e	pIK 5	6.3	13.1	86.9
3e	pIK 6	6.3	16.1	83.9
3e	pIK 7	5.8	10.9	89.1
3e	pIK 8	6.5	10.1	89.9
3e	pIK 9	5.2	13	87
3e	pIK 12	5.7	9.9	90.1
3e	pIK 13	4.5	8.6	91.4
3e	pIK 15	4.8	10.1	89.9
3e	pIK 29	5	11.8	88.2
3e	pIK 30	17.4	79.4	20.6
3e	pIK 31	4.7	17.8	82.2
3e	pIK 32	4.8	11.3	88.7

table 14

It can be seen in the table the conversion for the substrate **4e** is very moderate and below 18%. Only biotransformations with enzymes derived from pIK 4 and pIK 30 exceeded the average conversion of approx. 6%. So nearly every enzyme gave primarily the *cis*-alcohol, except pIK 4 and pIK 30. With pIK 4 we got approximately a 1:1 conversion between *cis* and *trans* and with pIK 30 the selectivity was higher to the *trans* alcohol. The reason can be of the higher conversion with these enzymes.

The spectra for the straight phase HPLC could not be interpreted, because the EtOAc-peak was overlapping with the *trans*-product **3e**. Based on the very low conversions, this enzyme library did not offer a synthetically useful catalysts for this reduction; consequently, the enantioselectivity of the biocatalsts were not of sufficient interest to merit elaborate optimization of the analytical protocol.

2.2.7.2.3 3-Hydroxy-1-(4-methoxyphenyl)-4-tert-butyl-azetidin-2-one (3w, $C_{14}H_{19}NO_3$)

In the following table 15 the results for the product **3w** is reported:

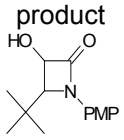
product 		rev. Phase HPLC		
	pIK i	conv. HPLC	trans	cis
3w	pIK 2	73.2	52.4	47.6
3w	pIK 3	9.4	28	72
3w	pIK 4	9.1	33.6	66.4
3w	pIK 5	8.1	31.5	68.5
3w	pIK 6	9.7	28.2	71.8
3w	pIK 7	10.1	30.5	69.5
3w	pIK 8	76.5	77.7	22.3
3w	pIK 9	9.5	31.8	68.2
3w	pIK 12	8.3	31.4	68.6
3w	pIK 13	10.3	26.3	73.7
3w	pIK 15	9.6	31	69
3w	pIK 29	9.6	30	70
3w	pIK 30	14.6	48.8	51.2
3w	pIK 31	11.9	30	70
3w	pIK 32	11.9	30.7	69.3

table 15

Also for substrate **4w** usually low conversions were observed for the majority of biocatalysts of the investigated collection. However, two candidates displayed good

substrate acceptance and these biotransformations were investigated in more detail. Two enzymes pIK 2 and pIK 8 are the exception with a high conversion around 75%. The major group of proteins displaying poor turnover gave alcohol **3w** in almost identical ratios of approx. 7:3 favoring formation of *cis* products. This ratio was inverted for biotransformations using the enzyme derived from pIK 8, now leading predominantly to formation of the *trans* alcohol. In the case of bioreductions with fusion proteins isolated from pIK 2 and 30 the ratio of diastereomeric alcohols was approx. 1:1.

Straight phase HPLC:

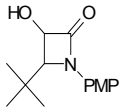
str. Phase HPLC									
Product 	pIK i	trans 3R,4R	trans 3S, 4S	ee	TRANS	cis 3S,4R	cis 3R,4S	ee	CIS
3w	pIK 2	56.4	-	100	56.4	24.7	18.9	13	43.6
3w	pIK 8	-	73.6	100	73.6	1.3	25.1	90	26.4

table 16

Due to the poor conversion and difficult separation of enantio- and regioisomers in straight phase HPLC only those experiments (pIK 2 and pIK 8) were analyzed which led to a reasonable conversion to alcohol products. With both enzymes formation of only one *trans* isomer was found. It is very interesting to note, that the enzyme originating from pIK 2 exclusively gives the 3R,4R configuration, while the pIK 8 derived biocatalyst provides access to the antipodal product (3S,4S). For the concomitantly produced *cis*-products in both case a mixture of the 3S,4R and the 3R,4S enantiomers was observed. With the pIK 2 gave a 2/1 ratio favoring the 3S,4R isomer, again the enzyme pIK 8 displayed a complementary behaviour with a ratio of nearly 1/20.

2.2.7.2.4 1-Benzyl-4-tert-butyl-3-hydroxy-azetidin-2-one (3h, $C_{14}H_{19}NO_2$)

In the following table 17 the results for the product **3h** were reported:

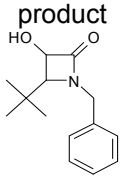
product 	pIK i	rev. Phase HPLC		
		conv. HPLC	TRANS	CIS
3h	pIK 2	94.7	51.8	48.2
3h	pIK 3	2.9	-	100
3h	pIK 4	1.2	-	100
3h	pIK 5	1.3	-	100
3h	pIK 6	1.5	-	100
3h	pIK 7	1.2	-	100
3h	pIK 8	95.3	51.7	48.3
3h	pIK 9	1.5	-	100
3h	pIK 12	4.61	-	100
3h	pIK 13	18.52	4.8	95.2
3h	pIK 15	1.7	-	100
3h	pIK 29	0.8	33.2	66.8
3h	pIK 30	5.5	46.4	53.6
3h	pIK 31	1.7	41	59
3h	pIK 32	1.9	37.1	62.9

table 17

Also in the case of substrate **4h** the majority of fusion biocatalysts displayed a very poor conversion with a few clear exceptions. Again, enzymes obtained from expression strains for pIK 2 and 8 led to high conversion (approx. 95%), followed by pIK 13 with a conversion around 18%.

This was the first biotransformation where diaselective formation of the *cis*-alcohol in high optical purities was observed with eight enzymes (pIK 3-7, 9, 12 and 15), however, only with biocatalysts that gave a very low conversion. But on the other hand 4 enzymes with also low conversion were not selective with a *cis:trans* ratio between 1:1 and 2:1.

With the enzyme 13/2 we reached high stereoselectivity toward the *cis*-alcohol.

Straight phase HPLC:

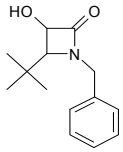
str. Phase HPLC									
Product 	pIK i	trans RR	trans SS	ee	TRANS	cis SR	cis RS	ee	CIS
3h	pIK 2	51.5	-	100	51.5	48.5	-	100	48.5
3h	pIK 8	52.9	-	100	52.9	47.1	-	100	47.1
3h	pIK13	6.3	-	100	6.3	93.7	-	100	93.7

Table 18

Again, only those biocatalysts were investigated in more detail by normal phase chiral HPLC displaying a conversion that offers the perspective for subsequent synthetic exploitation. The straight phase gave nearly the same ratio between *trans* and *cis* compared to the reverse phase, validating the analytical protocol.
With all three enzymes formation of single enantiomers for the *cis*- and the *trans*-product was observed. For the enzyme pIK 2 and 8 the ratio can be esteemed as 1:1 ratio between *cis* and *trans*. For the enzyme pIK 13 almost the cis product was obtained.

2.2.7.2.5 3-Hydroxy-1-(4-methoxyphenyl)-4-(2-thienyl)-azetidin-2-on (3x, $C_{14}H_{13}NO_3S$)

The results for the products **3x** are summarized in this table 19:

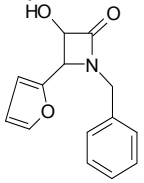
product 		rev. Phase HPLC			str. Phase HPLC							
	pIK i	conv. HPLC	trans	cis	trans SR	trans RS	ee	TRANS	cis SS	cis RR	ee	CIS
3x	pIK 2	100	37.2	62.8	36.8	8.0	64	44.8	4.6	50.6	83	55.2
3x	pIK 3	100	88.1	11.9	46.7	39.2	9	85.9	-	14.1	100	14.1
3x	pIK 4	100	22.1	77.9	3.2	30.1	81	33.3	4.3	62.4	9	66.7
3x	pIK 5	94.1	83.2	16.8	27.2	60.8	38	88	12	-	100	12
3x	pIK 6	94.5	80.4	19.6	54.5	28.6	31	83.1	16.9	-	100	16.9
3x	pIK 7	85.3	92.1	7.9	32.4	57.3	26	89.7	10.3	-	100	10.3
3x	pIK 8	99.8	50	50	56.9	-	100	56.9	-	43.1	100	43.1
3x	pIK 9	96.6	98.2	1.8	37.4	54.3	18	91.7	-	8.3	100	8.3
3x	pIK 12	99.7	46	54	2.5	44.2	89	46.7	49.3	4	85	53.3
3x	pIK 13	99.8	53.1	46.9	3.3	52.1	93	55.4	42.7	1.9	95	44.6
3x	pIK 15	94.6	90.6	9.4	21.4	65.5	51	86.9	-	13.1	100	13.1
3x	pIK 29	94.4	94.9	5.1	55.4	36.1	21	91.5	-	8.5	100	8.5
3x	pIK 30	96.7	65.3	34.7	14.3	54.1	58	68.4	16.7	14.9	5	31.6
3x	pIK 31	93.4	95.1	4.9	45.8	41.1	5	86.9	13.1	-	100	13.1
3x	pIK 32	92.1	92.7	7.3	61	35.6	26	96.6	-	3.4	100	3.4

Table 19

In contrast to the previous results almost complete conversion was observed for this product for almost every biocatalyst (minimum conversion 85%).

As can be seen from the data compiled in the table the majority of enzymes afforded the *trans*-product as a main bioreduction product. Two enzyme groups represented exceptions to this trend: While biocatalysts obtained from expression constructs pIK 2 and pIK 4 predominantly gave *cis* alcohols (in 4:1 and 3:2 ratios), purified enzyme pIK 8 and pIK 12 led to an alcohol formation with a balanced *cis/trans* ratio (approx. 50:50).
With three enzymes (pIK 9, pIK 29 and pIK 31) the *trans*-alcohol was obtained almost exclusively in 95-99% yield determined by HPLC.

By the straight phase HPLC the same ratio for the conversion was obtained as for the reverse phase.

With pIK12 and 13 the RS *trans*-product was obtained in a high optical purity, the SR product represents only 2-3%.

With enzyme pIK 8 only the SR *trans*-product was obtained, and for the *cis*-product also only the RR was produced in nearly 1:1 ratio.
By the *cis*-product it was interesting that for the enzymes pIK 3, 5, 6, 8, 9, 15, 29 and 31 only one *cis*-product was formed. A high selectivity for the SS *cis*-product was achieved with the enzymes pIK 12 and 13. With the enzymes pIK 2 and 4 the selectivity was rather to the RR *cis*-product.

2.2.7.2.6 1-Benzyl-3-hydroxy-4-thiophen-2-yl-azetidin-2-on (3l ,$C_{14}H_{13}NO_2S$)

Here the results for the product **3l**:

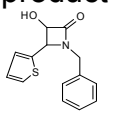
product 		rev. Phase HPLC			str. Phase HPLC							
	pIK i	conv. HPLC	trans	cis	trans SR	trans RS	ee	TRANS	cis SS	cis RR	ee	CIS
3l	pIK 2	94.4	32.1	67.9	8.4	31.1	58	39.5	42.6	17.9	41	60.5
3l	pIK 3	89.3	51.5	48.5	7.5	39	68	46.5	38.7	14.8	45	53.5
3l	pIK 4	100	28.3	71.7	7.9	30.5	60	38.4	40.6	21	31	61.6
3l	pIK 5	87.9	59.9	40.1	7.9	43.2	69	51.1	45.3	3.6	85	48.9
3l	pIK 6	89.6	51.5	48.5	29.8	19.3	22	49.1	45.6	4.4	81	50.9
3l	pIK 7	87.7	59	41	8.2	52.5	73	60.7	33.4	4.9	67	39.3
3l	pIK 8	98.9	33.7	66.3	4.7	35.9	68	40.6	35.5	23.9	17	59.4
3l	pIK 9	88.2	60.6	39.4	6.2	64	83	70.2	24.0	5.8	58	29.8
3l	pIK 12	99	57.3	42.7	7.7	54.1	75	61.8	37.8	0.4	98	38.2
3l	pIK 13	99.4	55.5	44.5	0.7	49.4	97	50.1	49.7	0.2	99	49.9
3l	pIK 15	88	61.3	38.7	8.3	57	75	65.3	34.1	0.6	96	34.7
3l	pIK 29	88.6	61.9	38.1	11	40.1	57	51.1	48.3	0.6	97	48.9
3l	pIK 30	95	70.7	29.3	7.8	53.3	74	61.1	38.2	0.7	93	38.9
3l	pIK 31	86.3	51.8	48.2	7.5	53.1	75	60.6	37.9	1.5	92	39.4
3l	pIK 32	85.4	58.9	41.1	8.2	52.6	73	60.8	38.9	0.3	98	39.2

table 20

Also with substrate **4l** good conversions were observed for the majority of enzymes. The *cis* products reached a high enantioselectivity with the enzymes pIK 5, 12-32. An ee between 85 and 99 was attained. For the *trans* product it was usually moderate, only exception was pIK 13 with an ee of 97.
In most of this case the *trans*-alcohol was formed preferrably, only exceptions are enzymes 2-4 and 8.
A ratio of 7:3 (*trans:cis*) was obtained with the pIK 30 and with pIK 5, pIK 7, pIK 9, pIK 12, pIK 15, pIK 29 and pIK 32 we obtained a 3:2 relation between *trans:cis*.
Four enzymes pIK 3, pIK 6, pIK 13 and pIK 31 transformed the educt **4l** in a 1:1 ratio.

Three enzymes were rather selective toward the *cis*-alcohol: pIK 8 reached a ratio of 1:3 as well as pIK 2 and pIK 4 a ratio 3:7 between *trans:cis*.

Straight phase HPLC indicated, that this product is preferrably converted to the RS *trans*-product and to the SS *cis*-product. The only exception is the enzyme pIK 6 were the SR *trans*-product was produced in higher yield.
For the enzymes pIK 5, 6, 7, 12, 13, 15, 29, 30, 31 and 32 the RR *cis*-product was formed below 5%. The SR *trans*-product was lower than 8.5% for nearly every enzyme.

2.2.7.2.7 1-tert-Butyl-3-hydroxy-4-thiophen-2-yl -azetidine-2-one (3n, $C_{11}H_{15}NO_2S$)

In this table the results for the product **3n** was shown:

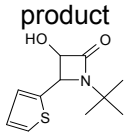
product 		rev. Phase HPLC			str. Phase HPLC							
		conv. HPLC	TRANS	CIS	trans SR	trans RS	ee	TRANS	cis SS	cis RR	ee	CIS
3n	pIK 2	100	55.1	44.9	50.9	0.4	98	51.3	46.8	1.9	92	48.7
3n	pIK 4	100	53.9	46.1	50.7	0.3	99	51.0	37.0	12.0	51	49.0
3n	pIK 8	100	55.6	44.4	51.4	-	100	51.4	45.6	3.0	87	48.6
3n	pIK 12	100	55.2	44.8	55.1	-	100	55.1	3.5	41.4	85	44.9
3n	pIK 13	100	55.2	44.8	56.5	-	100	56.5	2.1	41.4	90	43.5

table 21

Also for the product **3n** the conversion was very good, but substrate and product could not be baseline separated; hence, trance amounts of substrate could eventually compromise to a certain extent the exact data for the *trans* alcohol.

Only a selected group of reductases was investigated with this substrate. In any case, both diastereomers were usually produced from **3n** in almost equal amounts.
Based on the straight phase HPLC results, it can be deduced that with the enzymes pIK 8, 12 and 13 only the SR *trans*-product was formed. Nearly the same with the enzymes pIK 2 and 4 where only less that 0.4% of the other *trans*-product was formed.
The enzymes pIK 12 and 13 predominantly produced the RR *cis*-product whereas enzymes pIK 2 and 8 gave the SS *cis*-product.

2.2.7.2.8 1-Benzyl-4-furan-2-yl-3-hydroxy-azetidin-2-on (3p, $C_{14}H_{13}NO_3$)

The last substrate which was screened was **4p**. The results were presented in the table:

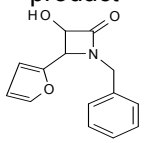
product 		rev. Phase HPLC			str. Phase HPLC							
	pIK i	conv. HPLC	TRANS	CIS	trans RS	trans SR	ee	TRANS	cis SS	cis RR	ee	CIS
3p	pIK 2	100	36.6	63.4	40.2	-	100	40.2	59.8	-	100	59.8
3p	pIK 3	97.1	22.8	77.2	22.9	-	100	22.9	76.4	0.7	98	77.1
3p	pIK 4	100	56	44	60.3	-	100	60.3	8.3	31.4	58	39.7
3p	pIK 5	97.6	23.8	76.2	24.3	-	100	24.3	-	75.7	100	75.7
3p	pIK 6	100	19.7	80.3	24.5	-	100	24.5	12.4	63.1	67	75.5
3p	pIK 7	100	22.5	77.5	30.4	-	100	30.4	12.3	57.3	65	69.6
3p	pIK 8	100	42.7	57.3	9.8	31.3	52	41.1		58.9	100	58.9
3p	pIK 9	97.3	25.2	74.8	-	25.3	100	25.3	44.4	30.3	19	74.7
3p	pIK 12	100	53.1	46.9	53.9	-	100	53.9	-	46.1	100	46.1
3p	pIK 13	100	51.3	48.7	55.2	-	100	55.2	44.8	-	100	44.8
3p	pIK 15	100	25	75	14	13.6	1	27.6		72.4	100	72.4
3p	pIK 29	100	27.4	72.6	20.9	-	100	20.9	4.7	74.4	63	79.1
3p	pIK 30	93.1	66.1	33.9	32.4	34.4	3	66.8	16.7	16.5	1	33.2
3p	pIK 31	80.2	15.2	84.8	18.4	-	100	18.4	2.2	79.4	95	81.6
3p	pIK 32	75.9	15.3	84.7	21.7	-	100	21.7	18.6	59.7	53	78.3

table 22

In a last case study, precursor **4p** was inverstigated and reverse phase HPLC showed generally a very high conversion with more than 93%. Only with pIK 31 and pIK 32 the conversion was somewhat lower with 75-80%.

Enzymes afforded in nearly all cases the *cis*-product as a major product, except for the enzyme pIK 4 and pIK 30 which gave a 3:2 and a 7:3 ratio of *trans:cis* and the *trans* isomers were dominant.

With 2 enzymes (pIK 12 and pIK 13) a 50:50 ratio between *trans* and *cis* alcohols was found.

With six enzymes (pIK 3, pIK 5, pIK 6, pIK 7, pIK 31 and pIK 32) we reached mainly the *cis*-alcohol. We reached with these enzymes nearly 80% of the *trans*-product. Also a high stereoselectivity toward the *cis* was also reached with the enzymes pIK 9, pIK 15 and pIK 29 with a ratio about 1:3.

The last two enzymes pIK 2 and pIK 8 transformed the educt **4p** in a relation 1:2 and 2:3 of *trans:cis*.

Straight phase HPLC:

For this product, the selectivity of this enzyme was very high for the RS *trans*-product. The enzymes pIK 2, 3, 4, 5, 6, 7, 12, 13, 29, 31 and 32 were only producing one *trans* enantiomer. The enzyme pIK 9 was selective for the SR product. A 1:1 ratio of the *trans*-products was obtained with the enzymes pIK 15 and 30.

The selectivity for the RR *cis*-product was only given by the enzymes pIK 5, 8, 12 and 15. A high selectivity toward this *cis* was obtained by the enzymes pIK 29 and 31. But the enzyme pIK 2 and 13 were selective for the SS *cis*-product. A high selectivity could also be reached with enzyme pIK 3. A 1:1 ratio was given with the enzymes pIK 30.

2.2.9 Conclusion

Biotransformation studies using native Baker's yeast indicated the principal suitability of reductases to reduce lactam precursors for modified taxoids. The selectivity of the biotransformations however was limited due to the presence of multiple enzymes with overlapping substrate specificities and stereoselectivities. Nevertheless, these biotransformations were successfully applied to prepare a large collection of optically enriched *cis*- and *trans*-alcohols from keto-lactam precursors. Together with the strictly *cis*-selective (but racemic) chemical reduction, a set of reference compounds could be prepared and analytical protocols for monitoring of biotransformations, determination of diastereomers (based on reverse phase HPLC) as well as enantioselectivity (normal/straight phase HPLC) were developed.

In order to obtain a better assessment of an expression library of various reductases originating from yeast for their suitability as biocatalysts for the transformation of interest, whole-cell bioreductions using *E.coli* expression strains were attempted. However, these biotransformations turned out to be quite inefficient.

In contrast, bioreductions using isolated GST-fused biocatalsts were significantly more successful. In this context, a sub-set of enzymes were isolated from the available library using affinity tag purification methods. Subsequent bioredutions on a selection of the available substrate library indicated suitable enzymes for particular compounds in almost all cases. Within the screening experiments conducted, certain enzymes displayed diastereoselective reductions in some cases, and in many cases enantioselective formation of *cis*- and/or *trans*-alcohols could be found. A most appealing finding was the fact, that in some cases, even enantiocomplementary products could be obtained by using two different candidates from the reductase library.

In light of the tedious analyses (combined reverse and straight phase HPLCs) and the large number of substrates and enzymes, only a selection of substrates were fully investigated on the complete set of available purified GST-fusion reductases due to time contraints in the implementation of the biocatalysis part of this thesis. However, interesting trends could be already identified for these systems, which open the door for future studies.

3. EXPERIMENTAL PART

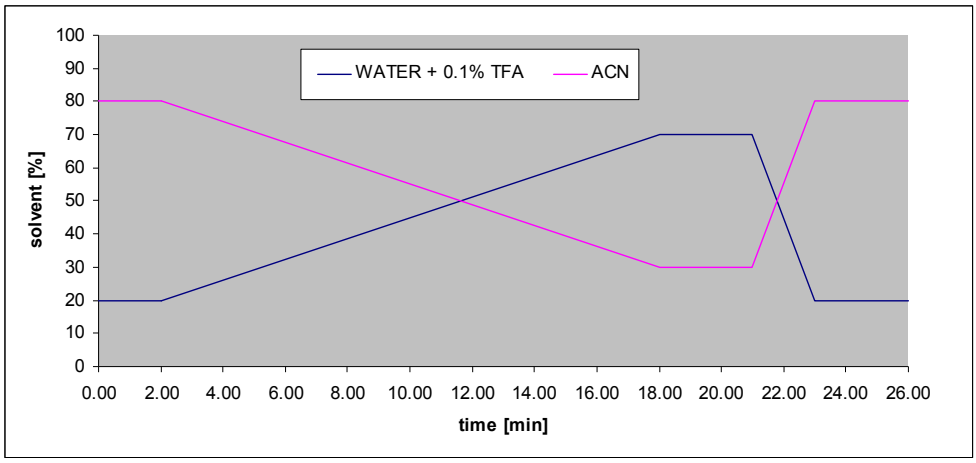
3.1 General

Unless otherwise noted, chemicals and microbial growth media were purchased from commercial suppliers and used without further purification. All solvents were distilled prior use; commercial Baker's yeast was purchased in a regular supermarket (Billa). All moisture-sensitive reactions were carried out under dry Ar.
Flash chromatography was carried out using Merck silica gel 60 from Merck (40-63µm). The ratio of crude material to silicagel was 1:30 in the described column chromatography purifications. Melting points were determined using a Kofler-type Leica Galen III micro hot stage microscope and are uncorrected.

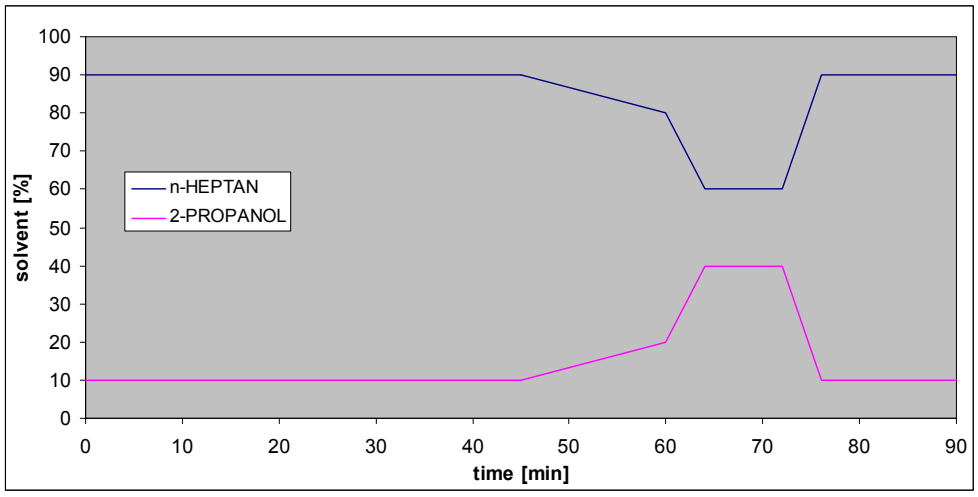
Proton and carbon NMR spectra were recorded on a BRUKER AC 200 FT-NMR spectrometer from $CDCl_3$ or d_6-DMSO solutions. Chemical shifts are reported in ppm using TMS as internal standard.
Combustion analysis was carried out in the Microanalytic Laboratory, University of Vienna.

Specific rotations $[\alpha]_D^{25}$ were measured at 25°C on a Perkin-Elmer 241 Polarimeter with a cont. Na-lamp (594nm) by following equation: $[\alpha]_D^{25} = 100^* \alpha/(c^*l)$; c[g/100mL], l[dm].

HPLC analysis's were recorded on a Thermo Finnigan Surveyor Plus using a Phenomenex Luna column C18(2) (10µ, 250x4.60mm). Detection was performed on a Thermo Finnigan PDA Plus Photodiode Array Detector. Following elouent was used: H_2O with 0.1% trifluoracedic acid and acetonitrile (ACN). Std-method: 1mL/min and the gradient indicated in the below scheme.



The chiral HPLC measurements were conducted of a Thermo Finnigan Surveyor Plus with a gradient starting at 90:10 n-heptan and 2-propanol. A LiChrosphor Si 60-5µ with the size 4.6x250mm was used prior to the separation column, for which a Daicel chemical industries LTD (DAIC 14325) Chiralcel OD-H column with the size 250x4.60mm filled with Cellulose tris (3,5-dimethylphenylcarbamate) coated on 5µm silica-gel was used. The flow was 0.5ml/min and the gradient can be seen out from the scheme.



Abbreviations:

DMSO	dimethylsulfoxide
Et_3N	triethylamine
THF	tetrahydrofuran
PE	petroleum ether
EtOAc	ethyl acetate
TLC	thin layer chromatography

Media for biotransformations:

LB_{kan}-Medium

5.0 g peptone
2.5 g yeast extract
5.0 g NaCl
500 mL deion. H_2O
autoklaved at 121°C for 20 minutes
1.5 mL kanamycine stock solution, added at about 40°C

for the plate culture media 15g/L agar were added.

Kanamycine – stock solution
50mg/ mL in deion. water, sterilized by filtration (0.2µm)

IPTG – Solution
200mg/ mL in deion. water, sterilized by filtration (0.2µm)

Glucose – Solution
4g/L glucose in deion. Water
autoclaved at 121°C for 20 minutes

pK_i - Buffer
5.356g of K_2HPO_4
2.619g of KH_2PO_4
500mL deion. H_2O
autoclaved at 121°C for 20min

Media for the protein purification:

10xPBS buffer

NaCl	1.4M	41g
KCl	27mM	1g
Na_2HPO_4	101mM	9g
KH_2PO_4	18mM	1.2g
		filled to 450mL H_2O

pH adjusted to 7.3 with conc. HCl, filled up to 500mL und autoclaved at 121°C for 20 min, stored in the fridge at 4°C

1xPBS/Triton buffer

10x PBS-Puffer diluted with dist. water and addition of 1% Triton-X-100, autoclaved at 121°C for 20 min, stored in the fridge at 4°C

Equilibration buffer

10mL 1xPBS/Triton buffer + 10µL Proteaseinhibitor

Protease inhibitor (storage –20°C):

1000×Pepstatin A / 1 mL

Pepstatin A	1 mM	
96 % EtOH		filled to 1 mL

1000×Leupeptin / 1 mL

Leupeptin	2 mM	
Sterilwater		filled to 1 mL

1000×PMSF / 1mL

PMSF	10 mM	
96 % EtOH		filled to 1 mL

50mM Tris·HCl/ 100mL

Tris base	50mM	0.608g
H_2O		filled to 80 mL

pH adjusted to 8, filling up to 100mL und autoclaved at 121°C for 20 min

Elution buffer

0.056g Glutathion (reduced),

+10mL 50mM Tris·HCl

+10µL Protease inhibitor

3.2 Cyclization Reactions

Method A:

GP I: A 10% solution of imines **1a**, **1d**, **1e**, **1h**, **1i**, **1l**, **1m**, **1o-1q** and **1s** in toluene was cyclized with acetoxyacedic acid chloride (1.5equiv.) and dry triethylamine (3equiv.) under reflux. After complete addition the reaction mixture was refluxed for 30min and slowly cooled to room temperature and stirred for 24h. The solution was hydrolyzed with 2N HCl and extracted with CH_2Cl_2; the combined organic layers were washed with saturated sodium bicarbonate solution, dried over sodium sulfate, filtered and concentrated.

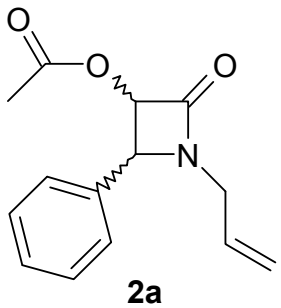
Method B:

GP II: A 10% solution of the corresponding imines **1b**, **1c**, **1f**, **1g**, **1j**, **1k**, **1n** and **1r** (1equiv.) in dry CH_2Cl_2 was cooled to approximately 0-5°C and treated with acetoxyacedic acid chloride (1.3equiv.) and dry triethylamine (3equiv.) under argon atmosphere. After complete addition the solution was stirred at room temperature for 24h. The reaction mixture was hydrolyzed with 2N HCl and extracted with CH_2Cl_2; the combined organic layers were washed with saturated sodium carbonate solution, dried over sodium sulfate, filtered and concentrated.

Method C:

GP III: A 5% solution of the corresponding imines **1b**, **1n**, **1o** and **1r** in dry CH_2Cl_2 was cooled to approximately -78°C and treated with acetoxyacedic acid chloride (1.15equiv.) and dry triethylamine (2.3equiv.). After complete addition the solution was slowly warmed to room temperature and then stirred over night. The reaction mixture was hydrolyzed with 2N HCl and extracted with CH_2Cl_2; the combined organic layers were washed with saturated sodium carbonate solution and with brine, dried over sodium sulfate, filtered and concentrated.

3.2.1 3-Acetoxy-1-allyl-4-phenyl-azetidin-2-one (2a, $C_{14}H_{15}NO_3$)



2a

Method A: via **GP II**

Imine **1a** (4g, 2.75mmol) was reacted with dry triethylamine (8.36g, 82.2mmol) and acetoxyacedic acid chloride (5.64g, 41.5mmol) to give 5.95g (73%) of **2a**[102].

Yield: 5.95g (73%), colorless oil

Cis/trans: 50.50

Cis:

^{1}H-NMR (400MHz, $CDCl_3$):

δ = 1.66 (s, 3H, CH_3COO), 2.16 (s, 2H, -CH_2-), 3.30-3.57 (m, 1H, -CH_2-b), 4.18 (m, 1H, -CH_2-a), 4.93 (d, *J*=4.7Hz, 1H, H-4), 5.10-5.23 (m, 2H, =CH_2), 5.62-5.79 (m, 1H, -CH=), 5.88 (d, *J*=4.5Hz, 1H, H-3), 7.13-7.46 (m, 5H, arom. H) ppm.

^{13}C-NMR ($CDCl_3$):

δ = 18.8 (q, CH_3COO), 42.1 (t, -CH_2-), 60.2 (d, C-4), 76.8 (d, C-3), 118.3 (t, =CH_2), 125.8 (d, C-4'), 127.7 and 129.5 (2d, C-3' and C-2'), 129.5 (d, -CH=), 131.7 (s, C-α), 134.1 (s, C-1'), 163.7 (s, COO), 168.1 (s, C-2) ppm

Trans:

^{1}H-NMR (400MHz, $CDCl_3$):

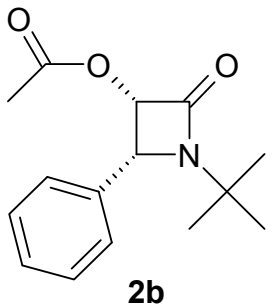
δ = 1.66 (s, 3H, CH_3COO), 2.16 (s, 2H, -CH_2-), 3.30-3.57 (m, 1H, -CH_2-b), 4.18 (m, 1H, -CH_2-a), 4.53 (d, *J*=1.6Hz, 1H, H-4), 5.10-5.23 (m, 2H, =CH_2), 5.62-5.79 (m, 1H, -CH=), 5.30 (d, 1H, H-3), 7.13-7.46 (m, 5H, arom. H) ppm

^{13}C-NMR ($CDCl_3$):

δ = 19.5 (q, CH_3COO), 42.2 (t, -CH_2-), 61.9 (d, C-4), 81.4 (d, C-3), 118.4 (t, =CH_2), 127.3 (d, C-4'), 127.7 and 128.0 (2d, C-3' and C-2'), 129.6 (d, -CH=), 131.7 (s, C-α), 134.1 (s, C-1'), 163.7 (s, COO), 168.6 (s, C-2) ppm.

[102] Banik, B.K.; Samajdar, S.; Banik, I.; NG, S.S.; Hann, J. *Heterocycles* **2003**, *61*. 97-100

3.2.2 *cis*-3-Acetoxy-1-tert.-butyl-4-phenyl-azetidin-2-one (2b, $C_{15}H_{19}NO_3$)



2b

Method A: via **GP I**

Imine **1b** (0.50g, 3.3mmol) was reacted with dry triethylamine (0.94g, 9.3mmol) and acetoxyacedic acid chloride (0.64g, 4.7mmol) to give 0.68g (83%) of **2b**.

Yield: 0.68g (83%), beige solid

*Cis/trans:*100:0

Method B: via **GP II**

Imin **1b** (0.50g, 3.3mmol) was reacted with dry triethylamine (0.94g, 9.3mmol) and acetoxyacedic acid chloride (0.55g, 4.0mmol) to give 0.72g (89%) of **2b**.

Yield: 0.73g (90%), brown solid (Purity 95% according to NMR)

Cis/trans: 100:0

Method C: via **GP III**

Imin **1b** (0.50g, 3.3mmol) was reacted with dry triethylamine (0.72g, 7.1mmol) and acetoxyacedic acid chloride (0.49g, 3.6mmol) to give 0.72g (89%) of **2b**.

Yield: 0.72g (89%), beige solid

Cis/trans: 100:0

M.p.: 143-145°C

Cis:

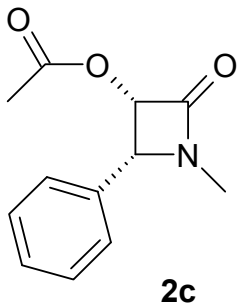
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.30 (s, 9H, $C(CH_3)_3$), 1.65 (s, 3H, CH_3), 4.94 (d, *J*=4.7Hz, 1H, H-4), 5.66 (d, *J*=4.9Hz, 1H, H-3), 7.28-7.39 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 18.9 (q, CH_3COO), 27.1 (q, $C(\underline{C}H_3)_3$), 53.8 (s, $\underline{C}(CH_3)_3$), 60.0 (d, C-4), 74.3 (d, C-3), 127.1 (d, C-4'), 127.4 and 127.5 (2d, C-3' and C-2'), 133.9 (d, C-1'), 163.5 (s, COO), 168.2 (s, C-2) ppm.

3.2.3 *cis*-3-Acetoxy-1-methyl-4-phenyl-azetidin-2-one (2c, $C_{12}H_{13}NO_3$)



2c

Method B: via **GP II**

Imin **1c** (0.50g, 4.2mmol) was reacted with dry triethylamine (1.27g, 12.6mmol) and acetoxyacedic acid chloride (0.75g, 5.5mmol) to give 0.88g (96%) of **2b**.

Yield: 0.88g (96%), brown oil (Purity 90% according to NMR)

Cis/trans: 100:0

Cis:

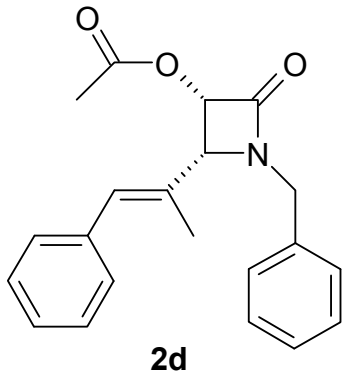
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.65 (s, 3H, CH_3COO), 2.87 (s, 3H, N-CH_3), 4.89 (d, *J*=4.5Hz, 1H, H-4), 5.82 (d, *J*=4.5Hz, 1H, H-3), 7.21-7.40 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.1 (q, CH_3), 27.5 (q, N-CH_3), 62.8 (s, C-4), 77.4 (s, C-3), 127.9 (d, C-4'), 128.2 and 128.5 (2d, C-3' and C-2'), 135.5 (s, C-1'), 165.1 (s, COO), 169.1 (s, C-2) ppm.

3.2.4 *cis*-3-Acetoxy-1-(4-benzyl)-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (2d, $C_{21}H_{21}NO_3$)



2d

Method A: via **GP I**

Imin **1d** (4.00g, 17.0mmol) was reacted with dry triethylamin (5.17g, 51.0mmol) and acetoxyacedic acid chloride (3.48g, 25.5mmol) to give 4.52g (79%) of **2d** as yellow oil after flash chromatography (PE:EtOAc 8:1).

Yield: 4.52g (79%), yellow oil

Cis/trans: 100:0

Cis:

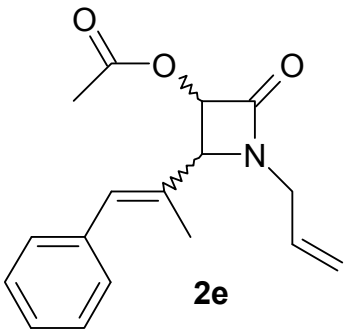
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.78 (s, 3H, -CH_3), 2.06 (s, 3H, CH_3COO), 4.03 (d, *J*=14.7Hz, 1H, CH_2-b), 4.21 (d, *J*=4.7Hz, 1H, H-4), 4.74 (d, *J*=14.7Hz, 1H, CH_2-a), 5.76 (d, *J*=4.7Hz, 1H, H-3), 6.37 (s, 1H, H-β), 7.12-7.30 (m, 10H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ= 15.4 (q, CH_3), 44.1 (t, N-CH_2), 65.6 (d, C-4), 77.7 (d, C-3), 126.5 (d, C-β), 127.3 and 127.9 (2d, C-4' and C-4''), 128.1, 128.2, 128.5, and 128.7 (4d, C-2', C-2'', C-3' and C-3''), 133.5 (s, C-α), 136.1 and 136.9 (2s, C-1' and C-1''), 169.0 (s, C-2) ppm.

3.2.5 3-Acetoxy-1-allyl-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (2e, $C_{17}H_{19}NO_3$)



2e

Method A: via **GP I**

Imine **1e** (1.00g, 5.4mmol) was reacted with dry triethylamine (1.64g, 16.2mmol) and acetoxyacedic acid chloride (1.11g, 8.1mmol) to give 1.04g (68%) of **2e** as colorless oil after flash chromatography (PE:EtOAc 6:1).

Yield: 1.04g (68%), colorless oil

Cis/trans: 83:17

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.86 (s, 3H, -CH_3), 2.05 (s, 3H, CH_3COO), 3.51-3.65 (m, 1H, -CH_2-b), 3.98-4.26 (m, 1H, -CH_2-a), 4.47 (d, J=4.9Hz, 1H, H-4), 5.04-5.33 (m, 2H, =CH_2), 5.65-5.86 (m, 1H, -CH=), 5.88 (d, J=4.7Hz, 1H, H-3), 6.50 (s, 1H, H-β), 7.17-7.41 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 15.6 (q, CH_3), 20.4 (q, CH_3COO), 43.6 (t, -CH_2-), 64.2 (d, C-4), 76.7 (d, C-3), 119.4 (t, =CH_2), 126.4 (d, C-4'), 128.3 and 128.9 (2d, C-3' and C-2'), 130.6 (s, C-1'), 130.7 (d, -CH=), 131.1 (d, C-β), 136.6 (s, C-α), 164.8 (s, COO), 169.4 (s, C-2) ppm.

Trans:

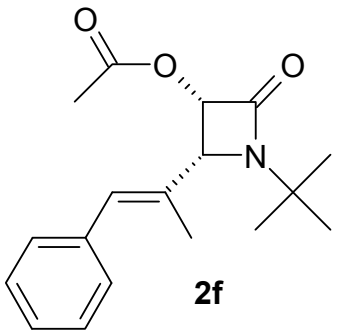
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.93 (s, 3H, CH_3), 2.16 (s, 3H, CH_3COO), 3.51-3.65 (m, 1H, -CH_2-b), 4.11 (d, *J*=1.9Hz, 1H, H-4), 3.98-4.26 (m, 1H, -CH_2-a), 5.04-5.33 (m, 2H, =CH_2), 5.46 (d, *J*=1.6Hz, 1H, H-3), 5.65-5-86 (m, 1H, -CH=), 6.74 (s, 1H, H-β), 7.17-7.41 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 15.6 (q, CH_3), 20.6 (q, CH_3COO), 43.6 (t, -CH_2-), 66.5 (d, C-4), 78.9 (d, C-3), 119.4 (t, =CH_2), 126.6 (d, C-4'), 128.3 and 128.9 (2d, C-3' and C-2'), 130.6 (s, C-1'), 130.7 (s, -CH=), 131.1 (s, C-β), 136.6 (s, C-α), 164.8 (s, COO), 169.4 (s, C-2) ppm.

3.2.6 *cis*-3-Acetoxy-1-*tert.*-butyl-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (2f, $C_{18}H_{23}NO_3$)



2f

Method B: via **GP II**

Imine **1f** (1.90g, 9.4mmol) was reacted with dry triethylamine (2.86g, 28.3mmol) and acetoxyacedic acid chloride (1.93g, 14.2mmol) to give 2.74g (96%) of **2f**.

Yield: 2.74g (96%), brown oil (Purity 92% according to NMR)

Cis/trans: 100:0

Cis:

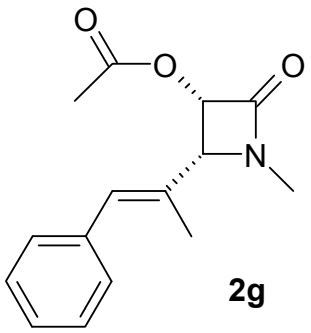
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.36 (s, 9H, $C(CH_3)_3$), 1.86 (s, $-CH_3$), 1.96 (s, 3H, CH_3COO), 4.46 (d, *J*=5.1Hz, 1H, H-4), 5.62 (d, *J*=5.1Hz, 1H, H-3), 6.50 (s, 1H, H-β) 7.15 -7.39 (m, 5H, arom. H) ppm

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 15.7 (q, $-CH_3$), 20.4 (q, CH_3COO), 27.8 (q, $C(\underline{C}H_3)_3$), 54.5 (s, $\underline{C}(CH_3)_3$), 64.1 (d, C-4), 74.9 (d, C-3), 127.0 (d, C-4'), 128.3 and 128.8 (2d, C-3'and C-2'), 130.0 (s, C-1'), 133.2 (d, C-β), 136.8 (s, C-α), 164.5 (s, COO), 169.4 (s, C-2) ppm.

3.2.7 *cis*-3-Acetoxy-1-methyl-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (2g, $C_{15}H_{17}NO_3$)



2g

Method B: via **GP II**

Imine **1g** (0.50g, 3.1mmol) was reacted with dry triethylamine (0.95g, 9.4mmol) and acetoxyacedic acid chloride (0.56g, 4.1mmol) to give 0.80g (99%) of **2g**.

Yield: 0.80g, (99%), brown oil (Purity 95% according to NMR)

Cis/trans: 100:0

Cis:

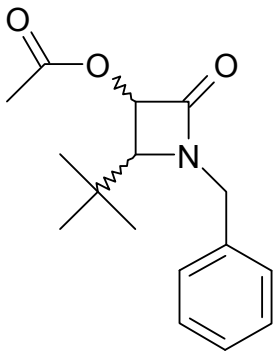
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.86 (s, 3H, -CH_3), 2.05 (s, 3H, CH_3COO), 2.90 (s, 3H, N-CH_3), 4.39 (d, *J*=4.7Hz, 1H, H-4), 5.88 (d, *J*=4.7Hz, 1H, H-3), 6.49 (s, 1H, H-β), 7.22-7.42 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 15.4 (q, CH_3), 20.3 (q, CH_3COO), 27.3 (q, N-CH_3), 65.8 (d, C-4), 77.0 (d, C-3), 127.1 (d, C-4'), 128.3 and 128.9 (2d, C-3' and C-2'), 130.5 (s, C-1'), 130.8 (d, C-β), 136.6 (s, C-α), 165.3 (s, COO), 169.4 (s, C-2) ppm.

3.2.8 3-Acetoxy-1-benzyl-4-tert-butyl-azetidin-2-one (2h, $C_{16}H_{21}NO_3$)



2h

Method A: via **GP I**

Imine **1h** (1.00g, 5.7mmol) was reacted with dry triethylamine (1.73g, 17.1mmol) and acetoxyacedic acid chloride (1.17g, 8.6mmol) to give 1.28g (81%) of **2h**.

Yield: 1.28g, (81%), colorless oil.

Cis/trans: 72:28

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 0.95 (s, 9H, $C(CH_3)_3$), 2.14 (s, 3H, CH_3), 3.45 (d, *J*=5.3, 1H, H-4), 4.00-4.16 (m, 1H, CH_2-b), 4.86-5.00 (m, 1H, CH_2-a), 6.00 (d, *J*=4.9Hz, 1H, H-3), 7.15-7.41 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.8 (q, CH_3COO), 26.7 (q, $C(\underline{C}H_3)_3$), 33.2 (s, $\underline{C}(CH_3)_3$), 46.1 (t, NCH_2), 66.1 (d, C-4), 74.2 (d, C-3), 127.9 (d, C-4'), 128.2 and 128.8 (2d, C-2' and C-3'), 134.9 (s, C1'), 166,8 (s, COO), 169.3 (s, C-2) ppm.

Trans:

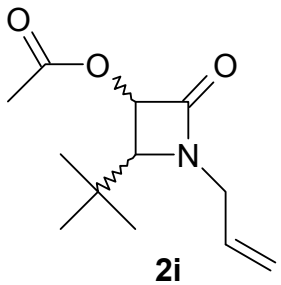
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 0.92 (s, 9H, $C(CH_3)_3$), 2.12 (s, 3H, CH_3), 3.32 (d, *J*=1.8Hz,1H, H-4), 4.00-4.16 (m, 1H, CH_2-b), 4.86-5.00 (m, 1H, CH_2-a), 5.60 (d, *J*=1.8Hz, 1H, H-3), 7.15-7.41 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz,$CDCl_3$):

δ = 20.7 (q, CH_3COO), 26.1 (q, $C(CH_3)_3$), 32.0 (s, $C(CH_3)_3$), 46.1 (t, NCH_2), 68.9 (d, C-4), 75.2 (d, C-3), 127.9 (d, C-4'), 128.2 and 128.8 (2d, C-2' and C-3'), 135.1 (s, C1'), 166,1 (s, COO), 169.3 (s, C-2) ppm.

3.2.9 3-Acetoxy-1-allyl-4-*tert.*-butyl-azetidin-2-one (2i, $C_{12}H_{19}NO_3$)



2i

Method A: via **GP I**

Imine **1i** (3.60g, 28.7mmol) was reacted with dry triethylamine (8.72g, 86.2mmol) and acetoxyacedic acid chloride (5.89g, 43.4mmol) to give 5.30g, (82%) of **2i** as yellow oil after flash chromatography (PE:EtOAc 8:1).

Yield: 5.30g, (82%), yellow oil

Cis/trans: 70:30

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 0.93 (s, 9H, $C(CH_3)_3$), 2.07 (s, 3H, CH_3COO), 3.48-3.65 (m, 2H, -CH_2-b and H-4), 4.16-4.28 (m, 1H, -CH_2-a), 5.09-5.24 (m, 2H, CH_2=), 5.65-5.80 (m, 1H, -CH=), 5.94 (d, *J*=5.3Hz, 1H, H-3) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.8 (q, CH_3COO), 26.7 (q, $C(\underline{C}H_3)_3$), 33.3 (q, $\underline{C}(CH_3)_3$), 44.7 (t, -CH_2-), 66.5 (d, C-4), 74.1 (d, C-3), 119.3 (t, =CH_2), 131.1 (d, CH=), 166.3 (s, COO), 169.3 (s, C=O) ppm.

Trans:

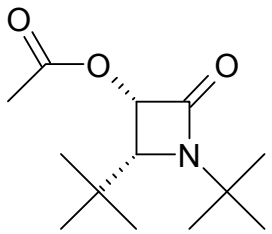
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 0.93 (s, 9H, $C(CH_3)_3$), 2.06 (s, 3H, CH_3COO), 3.38 (d, *J*=2.0Hz, 1H, H-4), 3.48-3.65 (m, 1H, -CH_2-b), 4.16-4.28 (m, 1H, -CH_2-a), 5.09-5.24 (m, 2H, CH_2=), 5.47 (d, *J*=1.4Hz, 1H, H-3), 5.65-5.80 (m, 1H, -CH=), 5.94 (d, *J*=5.3Hz, 1H, H-3) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.7 (q, CH_3COO), 26.1 (q, $C(CH_3)_3$), 32.1 (q, $C(CH_3)_3$), 44.8 (t, $-CH_2-$), 69.1 (d, C-4), 75.2 (d, C-3), 119.0 (t, $=CH_2$), 131.4 (d, CH=), 165.6 (s, COO), 169.3 (s, C=O) ppm

3.2.10 *cis*-3-Acetoxy-1,4-di-*tert.*-butyl-azetidin-2-one (2j, $C_{13}H_{23}NO_3$)



2j

Method B: via **GP II**

Imine **1j** (2.00g, 14.2mmol) was reacted with dry triethylamine (4.30g, 42.5mmol) and acetoxyacedic acid chloride (2.51g, 21.2mmol) to give 2.59g, (62%) of **2j**.

Yield: 2.59g, (62%), brown solid (Purity 97% according to NMR).

Cis/trans: 100:0

M.p.: 72-75°C

Cis:

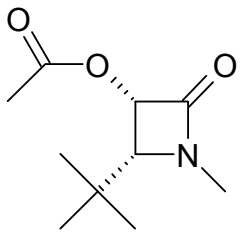
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.04 (s, 9H, N-C(CH_3)$_3$), 1.41 (s, 9H, C(CH_3)$_3$), 2.13 (s, 3H, CH_3COO), 3.75 (d, *J*=5.7Hz, 1H, H-4), 5.91 (d, *J*=5.7Hz, 1H, H-3) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 21.0 (q, CH_3COO), 27.3 (q, C($\underline{C}H_3$)$_3$), 28.8 (q, N-C($\underline{C}H_3$)$_3$), 33.4 (s, $\underline{C}$(CH_3)$_3$), 54.0 (s, N-$\underline{C}$(CH_3)$_3$), 68.1 (d, C-4), 71.7 (d, C-3), 166.9 (s, COO), 169.9 (s, C-2) ppm.

3.2.11 *cis*-3-Acetoxy-4-*tert.*-butyl-1-methyl-azetidin-2-one (2k, $C_{10}H_{17}NO_3$)



2k

Method B: via **GP II**

Imine **1k** (3.45g, 34.8mmol) was reacted with dry triethylamine (10.57g, 104.5mmol) and acetoxyacedic acid chloride (6.18g, 45.3mmol) to give 5.61g, (81%) of **2k**.

Yield: 5.61g, (81%), brown oil (Purity 94% according to NMR).

Cis/trans: 100:0

Cis:

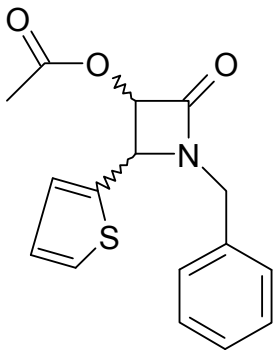
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.02 (s, 9H, $C(CH_3)_3$), 2.14 (s, 3H, CH_3COO), 2.94 (s, 3H, N-CH_3), 3.54 (d, *J*=5.1Hz, 1H, H-4), 5.99 (d, *J*=4.9Hz, 1H, H-3) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ =20.4 (q, CH_3COO), 26.2 (q, $C(CH_3)_3$), 29.2 (q, N-CH_3), 33.2 (s, $C(CH_3)_3$), 67.5 (d, C-4), 74.8 (d, C-3), 165.0 (s, COO), 170.1 (s, C-2) ppm.

3.2.12 3-Acetoxy-1-benzyl-4-thiophen-2-yl-azetidin-2-one (2l, $C_{16}H_{15}NO_3S$)



2l

Method A: via **GP I**

Imine **1l** (4.00g, 19.9mmol) was reacted with dry triethylamine (6.03g, 59.6mmol) and acetoxyacedic acid chloride (4.07g, 29.8mmol) to give 6.48g, (87%) of **2l** as yellow oil after flash chromatography (PE:EtOAc 8:1).

Yield: 6.48g, (87%), yellow oil

Cis/trans: 56:44

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.86 (s, 3H, CH_3), 3.94 (dd, J_1=11.7Hz, J_2=15.1Hz, 1H, CH2-b), 4.79 (dd, J_1=8.0Hz, J_2=15.1Hz, 1H, CH_2-a), 5.01 (d, J=4.5Hz, 1H, H-4), 5.79 (d, J=4.5Hz, 1H, H-3), 6.93-7.05 (m, 2H, H-3' and H-4'), 7.12-7.39 (m, 6H, arom. H and H-5') ppm

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.1 (q, CH_3COO), 44.4 (t, N-CH_2), 56.7 (d, C-4), 77.4 (d, C-3), 126.3 (d, C-4'), 127.0, 127.5 and 128.1 (3d, C-3'', C-4'' and C-5''), 128.5 and 128.9,(2d, C-2' and C-3'), 134.4 and 135.7 (2s, C-1' and C-2''), 164.2 (s, COO), 169.2 (s, C-2) ppm

Trans:

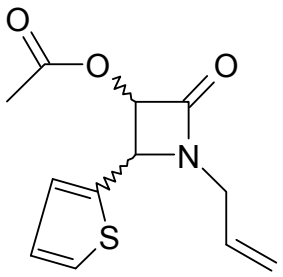
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.86 (s, 3H, CH_3), 3.94 (dd, J_1=11.7Hz, J_2=15.1Hz, 1H, CH_2-b), 4.74 (d, J=1.5Hz, 1H, H-4), 4.79 (dd, J_1=8.0Hz, J_2=15.1Hz, 1H, CH_2-a), 5.46 (d, J=1.6Hz, 1H, H-3), 6.93-7.05 (m, 2H, H-3' and H-4'), 7.12-7.39 (m, 6H, arom. H and H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.4 (q, CH_3COO), 44.4 (t, NCH_2), 58.1 (d, C-4), 82.7 (d, C-3), 126.9 (d, C-4'), 127.0, 128.0 and 128.4 (3d, C-3'', C-4'', and C-5''), 128.6 and 128.9 (2d, C-2' and C-3'), 135.4 and 138.7 (2s, C-1' and C-2''), 164.1 (s, COO), 169.5 (s, C-2) ppm.

3.2.13 3-Acetoxy-1-allyl-4-thiophen-2-yl-azetidin-2-one (2m, $C_{12}H_{13}NO_3S$)



2m

Method A: via **GP I**

Imine **1m** (1.00g, 6.6mmol) was reacted with dry triethylamine (2.00g, 19.8mmol) and acetoxyacedic acid chloride (1.36g, 9.9mmol) to give 1.40g, (85%) of **2m** as yellow oil after flash chromatography (PE:EtOAc 7:1).

Yield: 1.40g, (85%), yellow oil

Cis/trans: 65:35

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.85 (s, 3H, CH_3COO), 3.42-3.62 (m, 1H, -CH_2-b), 4.07-4.26 (m, 1H, -CH_2-a), 5.09-5.24 (m, 3H, CH_2= and H-4), 5.61-5.81 (m, 1H, -CH=), 5.86 (d, *J*=4.5Hz, 1H, H-3), 6.97-7.10 (m, 2H, H-3' and H-4'), 7.30-7.39 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.0 (q, CH_3COO), 43.0 (t, -CH_2-), 57.0 (d, C-4), 77.2 (d, C-3), 119.4 (t, =CH2), 126.7, 127.0 and 128.3 (3d, C-3', C-4' and C-5') 130.4 (d, CH=), 135.9 (s, C-2'), 164.2 (s, COO), 169.1 (s, C-2) ppm.

Trans:

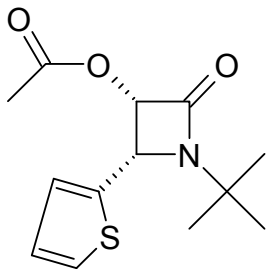
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.85 (s, 3H, CH_3COO), 3.42-3.62 (m, 1H, -CH_2-b), 4.07-4.26 (m, 1H, -CH_2-a), 4.84 (d, *J*=1.4Hz, 1H, H-4), 5.09-5.24 (m, 2H, CH_2=), 5.45 (d, *J*=1.2Hz, 1H, H-3), 5.61-5.81 (m, 1H, -CH=), 6.97-7.10 (m, 2H, H-3' and H-4'), 7.30-7.39 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.4 (q, CH_3COO), 43.0 (t, -CH_2-), 58.4 (d, C-4), 82.7 (d, C-3), 119.4 (t, =CH2), 126.3, 126.8 and 127.4 (3d, C-3', C-4' and C-5') 130.5 (d, CH=), 138.5 (s, C-2'), 164.0 (s, COO), 169.5 (s, C-2) ppm.

3.2.14 *cis*-3-Acetoxy-1-*tert.*-butyl-4-thiophen-2-yl-azetidin-2-one (2n, $C_{13}H_{17}NO_3S$)



2n

Method B: via **GP II**

Imine **1n** (0.50g, 3.0mmol) was reacted with dry triethylamine (0.91g, 9.0mmol) and acetoxyacedic acid chloride (0.53g, 3.9mmol) to give 0.66g, (82%) of **2n**.

Yield: 0.66g, (82%), brown solid (Purity 98% according to NMR).
Cis/trans: 100:0

Method C: via **GP III**

Imine **1n** (0.50g, 3.0mmol) was reacted with dry triethylamine (0.70g, 6.9mmol) and acetoxyacedic acid chloride (0.47g, 3.4mmol) to give 0.54g, (67%) of **2n**.

Yield: 0.54g, (67%), beige solid
Cis/trans: 100:0

M.p.: 94-96°C

Cis:

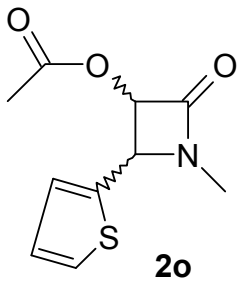
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.32 (s, 9H, $C(CH_3)_3$), 1.84 (s, 3H, CH_3), 5.27 (d, *J*=4.7Hz, 1H, H-4), 5.70 (d, *J*=4.7Hz, 1H, H-3), 6.93-7.09 (m, 2H, H-3' and H-4'), 7.25 -7.36 (m, 1H, H-5') ppm

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.3 (q, CH_3COO), 28.0 (q, $C(CH_3)_3$), 55.1 (s, $C(CH_3)_3$), 56.6 (s, C-4), 75.4 (d, C-3), 126.3 and 126.7 (2d, C-3' and C-4'), 128.0 (d, C-5'), 138.5 (s, C-2'), 164.1 (s, COO), 169.4 (s, C-2) ppm.

3.2.15 3-Acetoxy-1-methyl-4-thiophen-2-yl-azetidin-2-one (2o, $C_{10}H_{11}NO_3S$)



2o

Method A: via **GP I**

Imine **1o** (0.50g, 4.5mmol) was reacted with dry triethylamine (1.35g, 13.5mmol) and acetoxyacedic acid chloride (0.91g, 6.8mmol) to give 0.73g, (73%) of **2o**.

Yield: 0.73g, (73%), brown oil (Purity 93% according to NMR).

Cis/trans: 42:58

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 2.16 (s, 3H, CH_3COO), 2.85 (s, 3H, N-CH_3), 5.16 (d, *J*=4.5Hz, 1H, H-4), 5.85 (d, J=4.3Hz, 1H, H-3), 6.99-7.12 (m, 2H, H-3' and H-4'), 7.31-7.39 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.4 (q, CH_3), 27.0 (q, N-CH_3), 60.3 (d, C-4), 83.1 (d, C-3), 126.3, 126.6 and 127.4, (3d, C-3', C-4' and C-5'), 138.3 (s, C-2'), 164.3 (s, COO), 169.5 (s, C-2) ppm.

Trans:

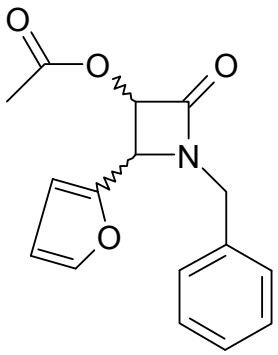
^{1}H-NMR (200MHz, $CDCl_3$):

δ= 2.16 (s, 3H, CH_3COO), 2.85 (s, 3H, N-CH_3), 4.76 (d, *J*=1.2Hz, 1H, H-4), 5.42 (s, 1H, H-3), 6.99-7.12 (m, 2H, H-3' and H-4'), 7.31-7.39 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.0 (q, CH_3), 26.8 (q, N-CH_3), 58.8 (d, C-4), 77.6 (d, C-3), 126.8, 127.1 and 128.1, (3d, C-3', C-4' and C-5'), 135.8 (s, C-2'), 164.6 (s, COO), 169.2 (s, C-2) ppm.

3.2.16 3-Acetoxy-1-benzyl-4-furan-2-yl-azetidin-2-one (2p, $C_{16}H_{15}NO_4$)



2p

Method A: via **GP I**

Imine **1p** (1.00g, 5.4mmol) was reacted with dry triethylamine (1.64g, 16.2mmol) and acetoxyacedic acid chloride (1.11g, 8.1mmol) to give 1.29g, (92%) of **2p** as yellow oil after by flash chromatography (PE:EtOAc 7:1).

Yield: 1.29g, (92%), yellow oil

Cis/trans: 50:50

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.89 (s, 3H, CH_3), 3.94 (dd, J_1=11.7Hz, J_2=15.1Hz, 1H, CH_2-b), 4.71-4.82 (m, 2H, H-4 and CH2-a), 5.78 (d, J=4.5Hz, 1H, H-3), 6.28-6.39 (m, 2H, H-3' and H-4'), 7.12-7.44 (m, 6H, arom. H and H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.5 (q, CH_3COO), 44.8 (t, N-CH_2), 55.7 (d, C-4), 79.3 (d, C-3), 110.4 and 110.7 (2d, H-3'' and H-4''), 127.9 (d, C-4'), 128.0 and 128.5 (2d, C-2' and C-3'), 134.4 (s, C-1'), 143.5 (d, C-5''), 147.0 (s, C-2''), 164,3 (s, COO), 169.3 (s, C-2) ppm

Trans:

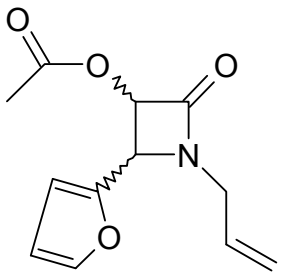
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.89 (s, 3H, CH_3), 3.93 (dd, J_1=14.9Hz, J_2=21.7Hz, 1H, CH_2-b), 4.44 (d, J=1.8Hz, 1H, H-4), 4.79 (dd, J_1=2.3Hz, J_2=15.65Hz, 1H, CH_2-a), 5.71 (d, J=1.6Hz, 1H, H-3), 6.28-6.39 (m, 2H, H-3' and H-4'), 7.12-7.44 (m, 6H, arom. H and H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.5 (q, CH_3COO), 44.8 (t, N-CH_2), 55.7 (d, C-4), 79.3 (d, C-3), 110.7 and 110.8 (2d, H-3'' and H-4''), 127.9 (d, C-4'), 128.5 and 128.8 (2d, C-2' and C-3'), 134.6 (s, C-1'), 143.6 (d, C-5''), 147.9 (s, C-2''), 164.1 (s, COO), 169.5 (s, C-2) ppm

3.2.17 3-Acetoxy-1-allyl-4-furan-2-yl-azetidin-2-one (2q, $C_{12}H_{13}NO_4$)



2q

Method A: via **GP I**

Imine **1q** (1.00g, 7.4mmol) was reacted with dry triethylamine (2.25g, 22.2mmol) and acetoxyacedic acid chloride (1.52g, 11.1mmol) to give 1.30g, (74%) of **2q** as yellow oil after flash chromatography (PE:EtOAc 8:1).

Yield: 1.30g, (74%) yellow solid

Cis/trans: 53:47

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.90 (s, 3H, CH_3COO), 3.40-3.66 (m, 1H, -CH_2-b), 3.99-4.17 (m, 1H, -CH2-a), 4.99 (d, *J*=4.3Hz, 1H, C-4), 5.07-5.22 (m, 2H, CH_2=), 5.59-5.78 (m, 1H, -CH=), 5.82 (d, *J*=4.3Hz, 1H, H-3), 6.35-6.44 (m, 2H, H-3' and H-4'), 7.42-7.47 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 19.9 (q, CH_3COO), 43.0 (t, -CH_2-), 65.0 (d, C-4), 77.6 (d, C-3), 110.2, and 110.6 (2d, C-3' and C-4'), 119.7 (t, =CH_2), 129.7 (d, CH=), 143.6 (d, C-5'), 147.1 (s, C-2'), 164.6 (s, COO), 169.1 (s, C-2) ppm

Trans:

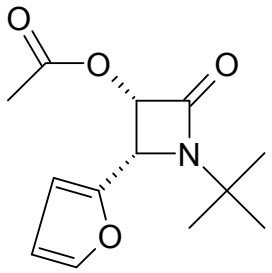
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.90 (s, 3H, CH_3COO), 3.40-3.66 (m, 1H, -CH_2-b), 3.99-4.17 (m, 1H, -CH_2-a), 4.58 (d, *J*=1.8Hz, 1H, C-4), 5.07-5.22 (m, 2H, CH_2=), 5.59-5.78 (m, 2H, -CH= and C-3), 5.69 (d, *J*=1.5Hz, 1H, H-3), 6.35-6.44 (m, 2H, H-3' and H-4'), 7.42-7.47 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 21.4 (q, CH_3COO), 43.2 (t, -CH_2-), 65.9 (d, C-4), 79.0 (d, C-3), 110.2 and 110.6 (2d, C-3' and C-4'), 119.1 (t, =CH_2), 130.0 (d, CH=), 143.6 (d, C-5'), 148.2 (s, C-2'), 164.2 (s, COO), 169.8 (s, C-2) ppm.

3.2.18 *cis*-3-Acetoxy-1-*tert.*-butyl-4-furan-2-yl-azetidin-2-one (2r, $C_{13}H_{17}NO_4$)



2r

Method B: via **GP II**

Imine **1r** (0.50g, 3.3mmol) was reacted with dry triethylamine (1.00g, 9.9mmol) and acetoxyacedic acid chloride (0.59g, 4.3mmol) to give 0.56g, (68%) of **2r**.

Yield: 0.56g, (68%), yellow solid

Cis/trans: 100:0

Method C: via **GP III**

Imine **1r** (0.50g, 3.3mmol) was reacted with dry triethylamine (0.76g, 7.6mmol) and acetoxyacedic acid chloride (0.52g, 3.8mmol) to give 0.57g, (69%) of **2r**.

Yield: 0.57g, (69%), yellow solid

Cis/trans: 100:0

M.p.: 58-60°C

Cis:

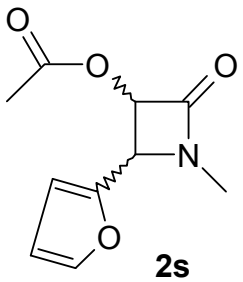
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.29 (s, 9H, $C(CH_3)_3$), 1.89 (s, 3H, CH_3), 5.00 (d, *J*=4.3Hz, 1H, H-4), 5.69 (d, *J*=4.7Hz, 1H, H-3), 6.38 (d, *J*=1.2Hz, 2H, H-3' and H-4'), 7.45 (s, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.0 (q, CH_3COO), 27.7 (q, $C(CH_3)_3$), 53.8 (s, C-4), 54.5 (s, $C(CH_3)_3$), 75.1 (d, C-3), 110.3 and 110.8 (2d, C-3' and C-4'), 142.9 (d, C-5'), 148.7 (s, C-2'), 163.7(s, COO), 169.4 (s, C-2) ppm.

3.2.19 3-Acetoxy-4-furan-2yl-1-methyl-azetidin-2-one (2s, $C_{10}H_{11}NO_4$)



2s

Method A: via **GP I**

Imine **1s** (2.00g, 18.3mmol) was reacted with dry triethylamine (5.56g, 55.0mmol) and acetoxyacedic acid chloride (3.75g, 27.5mmol) to give 3.66g, (95%) of **2s**.

Yield: 3.66g (95%), brown oil (Purity 94% according to NMR).

Cis/trans: 12:88

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 2.17 (s, 3H, CH_3COO), 2.84 (s, 3H, N-CH_3), 4.95 (d, *J*=4.3Hz, 1H, H-4), 5.82 (d, *J*=4.3Hz, 1H, H-3), 6.37-6.48 (m, 2H, H-3' and H-4'), 7.46 (d, J=1.0Hz, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 19.9 (q, $\underline{C}H_3$), 27.1 (q, N-CH_3), 56.7 (d, C-4), 77.4 (d, C-3), 110.5 and 110.7, (2d, C-3' and C-4'), 143.7 (d, C-5'), 148.0 (s, C-2'), 164.6 (s, COO), 169.6 (s, C-2) ppm.

Trans:

^{1}H NMR (200MHz, $CDCl_3$):

δ = 2.14 (s, 3H, CH_3), 2.80 (s, 3H, N-CH_3), 4.53 (d, *J*=1.6Hz, 1H, H-4), 5.66 (d, *J*=1.0Hz, 1H, H-3), 6.37-6.48 (m, 2H, H-3' and H-4'), 7.46 (d, *J*=1.0Hz, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 20.4 (q, CH_3), 27.1 (q, N-CH_3), 57.7 (d, C-4), 79.8 (d, C-3), 110.1 and 110.7, (2d, C-3' and C-4'), 143.7 (d, C-5'), 148.0 (s, C-2'), 164.6 (s, COO), 169.6 (s, C-2) ppm

3.3 Chemical Synthesis of Alcohols

Method A: Hydrolysis of the acetoxy group

GP IV: A 5% solution of the acetyl compound **2a-s** (1equiv.) in THF was prepared and cooled to 0-5°C. This mixture was treated with half volume of 2N KOH so that the temperature stays below 5°C. The reaction was stirred till TLC shows full conversion (usually 3-5h). After addition of the same volume of water, the solution was extracted with EtOAc, the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated.

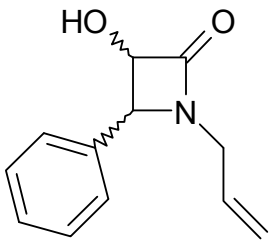
Method B: $NaBH_4$ Reduction

GP V: A 0.1% solution of the keton **3a-3y** in dry ethanol was cooled to 5°C. After addition of to the reaction mixture slowly $NaBH_4$ (1 equiv.) was added. After 1h at 5°C TLC indicated complete conversion, the suspension was hydrolyzed with 2*N* HCl, stirred for another 3h and extracted with ethyl acetate. The combined organic layers were washed with satd. $NaHCO_3$-solution and brine, dried over $MgSO_4$, filtered and evaporated to dryness to give the pure alcohol.

Method C: Biotransformation

GP VIII: According to general procedure (see below)

3.3.1 Allyl-3-hydroxy-4-phenyl-azetidin-2-one (3a, $C_{14}H_{15}NO_3$)



3a

Method A: via **GP IV**

Compound **2a** (1.00g, 4.08mmol) was hydrolyzed with 2N KOH to give 0.80g (97%) of **3a**.

Yield: 0.80g (97%), colorless solid

Cis/trans: 53:47

Method B: via **GP V**

Keton **4a** (25mg, 0.12mmol) was treated with $NaBH_4$ (4.12mg, 0.12mmol) to give **3a**.

Yield: 23.79mg (94%), beige solid

Cis/trans: 100:0

M.p.: 55-56°C (*cis*)

Cis:

Retention time:

RP HPLC: 11.9min

Chiral HPLC: 32.3min/35.0min (SR/RS)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.97 (s, 2H, -CH_2-), 3.22-3.49 (m, 1H, -CH_2-b), 3.96 (dd, J_1= 5.1Hz, J_2= 16.0Hz, 1H, -CH_2-a), 4.36 (d, J=1.4Hz, 1H, H-4), 4.97-5.17 (m, 2H, =CH_2), 5.59-5.82 (m, 1H, -CH=), 4 .77 (d, J=4.7Hz, 1H, H-3), 5.93 (d, J= 7.0Hz, 1H, OH) 7.18-7.45 (m, 5H, arom. H) ppm.

^{13}C-NMR ($CDCl_3$):

δ = 42.1 (t, -CH_2-), 62.1 (d, C-4), 77.7 (d, C-3), 117.7 (t, =CH_2), 127.6 (d, C-4'), 127.9, and 128.2 (2d, C-3', and C-2'), 132.0 (d, CH=), 135.4 (s, C-α), 137.0 (s, C-1'), 168.8 (s, C=O) ppm.

Trans:

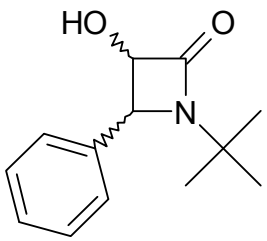
^{1}H-NMR (400MHz, $CDCl_3$):

δ = 1.97 (s, 2H, -CH_2-), 3.22-3.49 (m, 1H, -CH_2-b), 3.96 (dd, J_1= 5.1Hz, J_2= 16.0Hz, 1H, -CH_2-a), 4.40 (d, J=7.8Hz, 1H, H-4), 4.97-5.17 (m, 3H, =CH_2 and H-3), 5.59-5.82 (m, 1H, -CH=), 6.55 (d, J= 8.0Hz, 1H, OH), 7.18-7.45 (m, 5H, arom. H) ppm.

^{13}C-NMR ($CDCl_3$):

δ = 42.2 (t, -CH_2-), 64.8 (d, C-4), 84.0 (d, C-3), 117.7 (t, =CH_2), 127.9 (d, C-4'), 128.2, and 128.7 (2d, C-3', and C-2'), 132.0 (d, CH=), 135.4 (s, C-α), 137.0 (s, C-1'), 168.9 (s, C=O) ppm

3.3.2 1-*tert.*-Butyl-3-hydroxy-4-phenyl-azetidine-2-one (3b, $C_{13}H_{17}NO_2$)



3b

Method A: via **GP IV**

Compound **2b** (4.00g, 7.3mmol) was hydrolyzed with 2N KOH to give 2.94g (88%) of **3b**.

Yield: 2.94g (88%), colorless solid

Cis/trans: 100:0

Method B: via **GP V**

Keton **4b** (25mg, 0.11mmol) was treated with $NaBH_4$ (4.30mg, 0.11mmol) to give **3b**.

Yield: 24.38mg (97%), colorless solid

Cis/trans: 100:0

M.p.: 173-174°C (*cis*)

Combustion analysis:

calculated:	C 71.21%	H 7.81%	N 6.39%
found:	C 70.73%	H 7.59%	N 6.19%

Cis:

Retention time:

RP HPLC: 14.7min

Chiral HPLC: 24.4min/29.2min (SR/RS)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.16 (s, 9H, $C(CH_3)_3$), 3.32 (d, *J*=4.7Hz, 1H, H-4), 4.74-4-86 (m, 2H, H-3), 5.78 (d, *J*=6.5Hz, 1H, OH), 7.29-7.37 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 27.9 (q, $C(CH_3)_3$), 33.3 (d, $C(CH_3)_3$), 61.6 (d, C-4), 75.6 (d, C-3), 127.4 (d, C-4'), 127.7 and 128.3 (2d, C-3' and C-2'), 137.7 (d, C-1'), 168.8 (s, C-2) ppm.

Method C: via **GP VIII**

Trans

Retention time:

RP HPLC: 15.4min

Chiral HPLC: 17.8min/19.3min (SS/RR)

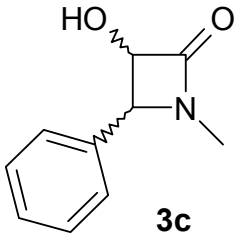
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.17 (s, 9H, $C(CH_3)_3$), 3.48 (d, *J*=4.7Hz, 1H, H-4), 4.74-4-86 (m, 2H, H-3), 6.64 (d, *J*=6.2Hz, 1H, OH), 7.29-7.37 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 27.9 (q, $C(CH_3)_3$), 33.3 (d, $C(CH_3)_3$), 62.7 (d, C-4), 77.1 (d, C-3), 127.4 (d, C-4'), 127.7 and 128.3 (2d, C-3' and C-2'), 137.7 (d, C-1'), 168.8 (s, C-2) ppm.

3.3.3 3-Hydroxy-1-methyl-4-phenyl-azetidin-2-one (3c, $C_{10}H_{11}NO_2$)



3c

Method A: via **GP IV**

Compound **2c** (1.50g, 6.8mmol) was hydrolyzed with 2N KOH to give 0.92g (76%) of **3c**.

Yield: 0.92g (76%), colorless solid

Cis/trans: 100:0

Method B: via **GP V**

Keton **4c** (25mg, 0.14mmol) was treated with $NaBH_4$ (5.40mg, 0.14mmol) to give **3c**.

Yield: 24.85mg (98%), colorless solid

Cis/trans: 100:0

M.p.: 142-144°C (*cis*)

Combustion analysis:

calculated:	C 67.78%	H 6.26%	N 7.90%
found:	C 67.72%	H 5.93%	N 7.58%

Cis:

Retention time:

RP HPLC: 8.02min

Chiral HPLC: -/53.5min (SR/RS)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 2.66 (s, 3H, CH_3), 4.69 (d, 1H, *J*= 4.5Hz, 1H, H-4), 4.90-4.99 (m, 1H, H-3), 5.84 (d, *J*= 6.9Hz, 1H, OH), 7.16-7.43 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 26.1 (q, CH_3), 63.6 (s, C-4), 78.1 (d, C-3), 127.6 (d, C-4'), 128.0 and 128.0 (2d, C-3' and C-2'), 135.4 (s, C-1'), 169.0 (s, C-2) ppm.

Method C: via **GP VIII**

Trans:

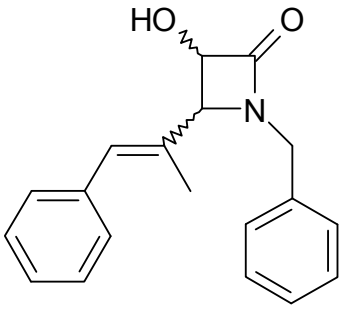
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 2.66 (s, 3H, CH_3), 4.30 (d, 1H, *J*= 1.6Hz, 1H, H-4), 4.90-4.99 (m, 1H, H-3), 6.46 (d, *J*= 8.0Hz, 1H, OH), 7.16-7.43 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 30.4 (q, CH_3), 66.3 (s, C-4), 84.4 (d, C-3), 126.3 (d, C-4'), 128.0 and 128.8 (2d, C-3' and C-2'), 135.4 (s, C-1'), 169.0 (s, C-2) ppm.

3.3.4 1-Benzyl-3-hydroxy-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3d, $C_{19}H_{19}NO_2$)



3d

Method A: via **GP IV**

Compound **2d** (4.02g, 12.0mmol) was hydrolyzed with 2N KOH to give 0.48g (99%) of **3d**.

Yield: 3.48g, (99%), colorless solid

Cis/trans: 100:0

Method B: via **GP V**

Keton **4d** (25mg, 0.09mmol) was treated with $NaBH_4$ (3.20mg, 0.09mmol) to give **3d**.

Yield: 24.27mg (97%), colorless solid

Cis/trans: 100:0

M.p.: 136-138°C (*cis*)

Combustion analysis:

calculated:	C 77.79%	H 6.53%	N 4.77%	O 10.91%
found:	C 77.40%	H 6.45%	N 4.73%	

Cis:

Retention time

RP HPLC: 19.6min

Chiral HPLC: 32.9min/36.2min (SR/RS)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.77 (s, 3H, CH_3), 4.22 (d, *J*=5.1Hz 1H, H-4), 4.24 (d, *J*=15.1Hz, 1H, CH_2-b), 4.59 (d, *J*=15.1Hz, 1H, CH_2-a), 4.97-5.07 (m, 1H, H-3), 6.23 (d, J=7.0Hz, 1H, OH), 6.41 (s, 1H, H-β), 7.26-7.43 (m, 10H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 15.4 (q, CH_3), 44.1 (t, NCH_2), 65.6 (d, C-4), 77.7 (d, C-3), 126.5 (d, C-β), 127.4 and 127.9 (2d, C-4' and C-4''), 128.1, 128.2, 128.5 and 128.7 (4d, C-2', C-2'', C-3' and C-3''),133.5 (s, C-α), 135.6 and 136.1 (2s, C-1' and C-1''), 169.0 (s, C-2) ppm.

Method C: via **GP VIII**

Trans

Retention time:

RP HPLC: 19.7min

Chiral HPLC: 20.9min/22.6min (SS/RR)

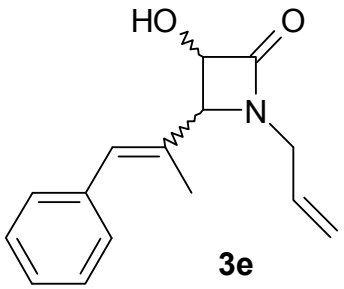
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.79 (s, 3H, CH_3), 4.38 (d, *J*=5.1Hz 1H, H-4), 4.24 (d, *J*=15.1Hz, 1H, CH_2-b), 4.59 (d, *J*=15.1Hz, 1H, CH_2-a), 4.97-5.07 (m, 1H, H-3), 6.52 (d, J=7.2Hz, 1H, OH), 6.41 (s, 1H, H-β), 7.26-7.43 (m, 10H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 15.4 (q, CH_3), 44.1 (t, NCH_2), 67.3 (d, C-4), 78.6 (d, C-3), 126.5 (d, C-β), 127.4 and 127.9 (2d, C-4' and C-4''), 128.1, 128.2, 128.5 and 128.7 (4d, C-2', C-2'', C-3' and C-3''),133.5 (s, C-α), 135.6 and 136.1 (2s, C-1' and C-1''), 169.0 (s, C-2) ppm.

3.3.5 1-Allyl-3-hydroxy-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3e, $C_{15}H_{17}NO_2$)



3e

Method A: via **GP IV**

Compound **2e** (0.90g, 3.4mmol) was hydrolyzed with 2N KOH to give 0.76g (99%) of **3e**.

Yield: 0.76g, (99%), yellow solid

Cis/trans: 91:9

Method B: via **GP V**

Keton **4e** (25mg, 0.10mmol) was treated with $NaBH_4$ (3.90mg, 0.10mmol) to give **3e**.

Yield: 24.61mg (98%), yellow solid

Cis/trans: 100:0

M.p.: 64-66°C (*cis*)

Combustion analysis:

calculated:	C 74.05%	H 7.04%	N 5.76%
found:	C 73.63%	H 6.87%	N 5.68%

Cis:

Retention time:

RP HPLC: 16.5min

Chiral HPLC: 27.6min/29.1min (SR/RS)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.85 (s, 1H, CH_3), 3.65 (dd, J_1=6.5Hz, J_2=15.7Hz, 1H, -CH_2-b), 4.10 (dd, J_1=5.6Hz, J_2=15.9Hz, 1H, -CH_2-a), 4.32 (d, J=4.9Hz, 1H, H-4), 5.02 (dd, J_1=5.1Hz, J_2=7.0Hz, 1H, H-3), 5.14-5.29 (m, 2H, =CH_2), 5.69-5.93 (m, 1H, -CH=), 6.18 (d, J=7.0Hz, 1H, OH), 6.45 (s, 1H, H-β), 7.18-7.44 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = δ = 15.4 (q, CH3), 42.7 (t, -CH_2-), 65.3 (d, H-4), 77.6 (d, H-3), 117.8 (t, =CH2), 126.5 (d, C-β), 127.7 (d, C-4'), 128.1 and 128.8, (2d, C-3' and H-2'), 132.1 (s, C'-1), 134.1 (d, -CH=), 136.6 (s, C-α), 168.9 (s, C-2) ppm.

Trans:

Retention time:

RP HPLC: 16.7min

Chiral HPLC: 21.0min/22.3min (SS/RR)

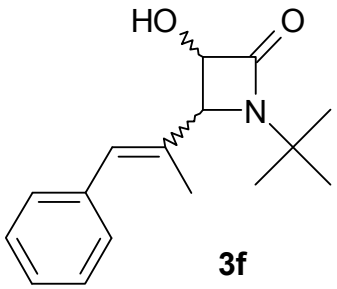
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.91 (s, 1H, CH_3), 3.65 (dd, J_1=6.5Hz, J_2=15.7Hz, 1H, -CH_2-b), 4.10 (dd, J_1=5.6Hz, J_2=15.8Hz, 1H, -CH_2-a), 4.41 (d, J=1.9Hz, 1H, H-4), 5.07 (dd, J_1= 1.8Hz, J_2=5.1Hz, 1H, H-3), 5.14-5.29 (m, 2H, =CH_2), 5.69-5.93 (m, 1H, -CH=), 6.37 (d, J=6.9Hz, 1H, OH), 6.70 (s, 1H, H-β), 7.18-7.44 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 15.4 (q, CH_3), 43.0 (t, -CH_2-), 68.8 (d, H-4), 78.2 (d, H-3), 117.8 (t, =CH_2), 126.5 (d, C-β), 127.7 (d, C-4'), 128.1 and 128.8, (2d, C-3' and H-2'), 132.0 (s, C-1'), 134.1 (d, -CH=), 136.9 (s, C-α), 168.6 (s, C-2) ppm.

3.3.6 1-*tert.*-Butyl-3-hydroxy-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3f, $C_{16}H_{21}NO_2$)



3f

Method A: via **GP IV**

Compound **2f** (0.50g, 1.7mmol) was hydrolyzed with 2N KOH to give 0.39g (91%) of **3f**.

Yield: 0.39g (91%), colorless solid

Cis/trans: 94:6

Method B: via **GP V**

Keton **4f** (25mg, 0.10mmol) was treated with $NaBH_4$ (3.70mg, 0.10mmol) to give **3f**.

Yield: 25.02mg (99%), colorless solid

Cis/trans: 100:0

M.p.: 131-133°C (*cis*)

Combustion analysis:

calculated:	C 74.10%	H 8.16%	N 5.40%
found:	C 73.55%	H 8.00%	N 5.26%

Cis:

Retention time:

RP HPLC: 18.5min

Chiral HPLC: 23.1min/24.9min (SR/RS)

^{1}H-NMR (200MHz, DMSO- d_6):

δ = 1.26 (s, 9H, $C(CH_3)_3$), 1.85 (s, 3H, CH_3), 4.41 (d, *J*=5.1Hz, 1H, H-4), 4.76-4.67 (m, 1H, H-3), 5.97 (d, *J*=6.7Hz, 1H, OH), 6.52 (s, 1H, H-β), 7.16-7.44 (m, 5H, arom. H) ppm

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 15.4 (q, CH_3), 27.5 (q, $C(\underline{C}H)_3$), 53.0 (s, $\underline{C}(CH_3)_3$), 65.2 (d, C-4), 75.6 (d, C-3), 126.5 (s, C-β), 127.2 (d, C-4'), 128.2 and 128.7 (2d, C-3' and C-2'), 136.2 (t, C-1'), 136.6 (d, C-α), 168.4 (s, C-2) ppm.

Trans:

Retention time:

RP HPLC: 18.7min

Chiral HPLC: 16.4min/17.4min (SS/RR)

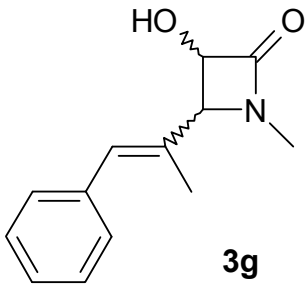
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.34 (s, 9H, $C(CH_3)_3$), 1.91 (s, 3H, CH_3), 4.63 (d, *J*=4.3Hz, 1H, H-4), 4.76-4-67 (m, 1H, H-3), 6.18 (d, *J*=6.7Hz, 1H, OH), 6.61 (s, 1H, H-β), 7.16-7.44 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 15.4 (q, $=CH_3$), 27.0 (q, $C(\underline{C}H_3)_3$), 52.7 (s, $\underline{C}(CH_3)_3$), 65.2 (d, C-4), 76.2 (d, C-3), 126.5 (s, C-β), 127.2 (d, C-4'), 128.2 and 128.5 (2d, C-3' and C-2'), 136.2 (t, C-1'), 137.1 (d, C-α), 168.5 (s, C-2) ppm.

3.3.7 3-Hydroxy-1-methyl-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3g, $C_{13}H_{15}NO_2$)



3g

Method A: via **GP IV**

Compound **2g** (1.50g, 5.8mmol) was hydrolyzed with 2N KOH to give 1.07g (85%) of **3g**.

Yield: 1.07g (85%), beige solid

Cis/trans: 100:0

Method B: via **GP V**

Keton **4g** (25mg, 0.12mmol) was treated with $NaBH_4$ (4.40mg, 0.12mmol) to give **3g**.

Yield: 24.73mg (98%), beige solid

Cis/trans: 100:0

M.p.: 121-124°C (*cis*)

Combustion analysis:

calculated:	C 71.87%	H 6.96%	N 6.45%
found:	C 70.94%	H 6.87%	N 6.27%

Cis:

Retention time:

RP HPLC: 13.6min

Chiral HPLC: 44.8min/- (SR/RS)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.79 (s, 3H, CH_3), 2.73 (s, 3H, N-CH_3), 4.19 (d, *J*= 4.9Hz, 1H, H-4), 4.83-4.94 (m, 1H, H-3), 6.04 (d, *J*=7.0, 1H, OH), 6.38 (s, 1H, H-β), 7.17-7.41 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 15.3 (q, CH_3), 26.7 (q, N-CH_3), 66.6 (d, C-4), 77.9 (d, C-3), 126.4 (d, C-4'), 127.2 (d, C-β), 128.1 and 128.8 (2d, C-3' and C-2'), 134.1 (s, C-1'), 136.9 (s, C-α), 169.0 (s, C-2) ppm.

Method C: via **GP VIII**

Trans

Retention time:

RP HPLC: 14.0min

Chiral HPLC: -min/38.9min (SS/RR)

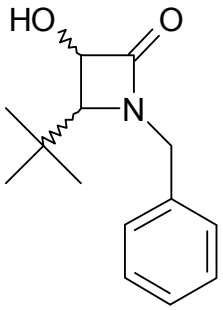
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.81 (s, 3H, CH_3), 2.73 (s, 3H, N-CH_3), 4.25 (d, *J*= 4.9Hz, 1H, H-4), 4.83-4.94 (m, 1H, H-3), 6.43 (d, *J*=7.1, 1H, OH), 6.38 (s, 1H, H-β), 7.17-7.41 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 15.3 (q, CH_3), 26.7 (q, N-CH_3), 67.6 (d, C-4), 78.1 (d, C-3), 126.4 (d, C-4'), 127.2 (d, C-β), 128.1 and 128.8 (2d, C-3' and C-2'), 134.1 (s, C-1'), 136.9 (s, C-α), 169.0 (s, C-2) ppm.

3.3.8 1-Benzyl-4-*tert.*-butyl-3-hydroxy-azetidin-2-one (3h, $C_{14}H_{19}NO_2$)



3h

Method A: via **GP IV**

Compound **2h** (2.00g, 7.3mmol) was hydrolyzed with 2N KOH to give 1.67g (99%) of **3h**.

Yield: 1.67g (99%), colorless solid

Cis/trans: 69:31

Method B: via **GP V**

Keton **4h** (25mg, 0.11mmol) was treated with $NaBH_4$ (4.06mg, 0.11mmol) to give **3h**.

Yield: 25.05mg (99%), colorless solid

Cis/trans: 100:0

M.p.: 118-121°C (*cis*)

Combustion analysis:

calculated:	C 72.07%	H 8.21%	N 6.00%
found:	C 71.94%	H 7.97%	N 5.94%

Cis:

Retention time:

RP HPLC: 15.8min

Chiral HPLC: -min/19.8min (SR/RS)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 0.90 (s, 9H, $C(CH_3)_3$), 3.34 (d, *J*= 5.0Hz, 1H, H-4), 4.18-4.30 (m, 1H, CH_2-b), 4.46-4.76 (m, 1H, CH_2-a), 4.78-4.87 (m, 1H, H-3), 6.11 (d, *J*=6.8Hz, 1H, OH), 7.14-7.37 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 26.5 (q, $C(\underline{C}H_3)_3$), 32.8 (s, $\underline{C}(CH_3)_3$), 44.7 (t, N-CH_2), 66.8 (d, C-4), 76.0 (d, C-3), 127.7 (d, C-4'), 127.8 and 128.5 (2d, C-2' and C-3'), 136.3 (s, C-1'), 170.4 (s, C-2) ppm.

Trans:

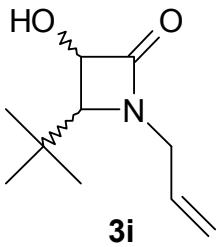
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 0.83 (s, 9H, $C(CH_3)_3$), 3.04 (d, *J*= 1.0Hz, 1H, H-4), 4.18-4.30 (m, 1H, CH_2-b), 4.46-4.76 (m, 2H, CH_2-a and H-3), 4.78-4.87 (m, 1H, H-3), 6.06 (d, *J*=8.4Hz, 1H, OH), 7.14-7.37 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 25.9 (q, $C(\underline{C}H_3)_3$), 31.5 (s, $\underline{C}(CH_3)_3$), 44.7 (t, N-CH_2), 70.9 (d, C-4), 75.9 (d, C-3), 127.2 (d, C-4'), 127.7 and 127.8 (2d, C-2' and C-3'), 136.3 (s, C-1'), 169.9 (s, C-2) ppm.

3.3.9 1-Allyl-4-*tert.*-butyl-3-hydroxy-azetidin-4-one (3i, $C_{10}H_{17}NO_2$)



3i

Method A: via **GP IV**

Compound **2i** (4g, 17.8mmol) was hydrolyzed with 2N KOH to give 2.68g (82%) of **3i**.

Yield: 2.68g (83%), yellow solid.

Cis/trans: 79:21

Method B: via **GP V**

Keton **4i** (25mg, 0.14mmol) was treated with $NaBH_4$ (5.21mg, 0.14mmol) to give **3i**.

Yield: 24.34mg (96%), yellow solid

Cis/trans: 100:0

M.p.: 82-85°C (*cis*)

Combustion analysis:

calculated:	C 65.54%	H 9.35%	N 7.64%
found:	C 65.32%	H 9.09%	N 7.51%

Cis:

Retention time:

RP HPLC: 13.3min

Chiral HPLC: 18.8min/22.9min (SR/RS)

Cis:

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 0.97 (s, 1H, $C(CH_3)_3$), 3.37 (d, J=5.1Hz, 1H, H-4), 3.66 (dd, J_1=6.9Hz, J_2=15.9Hz, 1H, -CH_2-b), 4.10 (dd, J_1=5.1Hz, J_2=16.0Hz, 1H, -CH_2-a), 4.81 (dd, J_1=5.1Hz, J_2=6.7Hz, 1H, H-3), 5.10-5.24 (m, 2H, =CH_2), 5.61-5.83 (m, 1H, -CH=), 6.04 (d, J=6.9Hz, 1H, OH) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 26.5 (q, $C(\underline{C}H_3)_3$), 32.9 (s, $\underline{C}(CH_3)_3$), 43.5 (t, NCH_2), 66.7 (d, H-4), 75.8 (d, H-3), 117.8 (t, =CH_2), 132.6 (d, -CH=), 169.9 (s, C-2) ppm.

Trans:

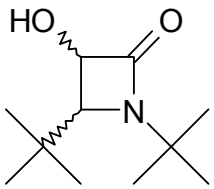
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 0.89 (s, 1H, $C(CH_3)_3$), 3.14 (d, J=1.9Hz, 1H, H-4), 3.66 (dd, J_1=6.9Hz, J_2=15.9Hz, 1H, -CH_2-b), 4.10 (dd, J_1=5.1Hz, J_2=16.0Hz, 1H, -CH_2-a), 5.26 (d, J=1.4Hz, 1H, H-3), 5.10-5.24 (m, 2H, =CH_2), 5.61-5.83 (m, 1H, -CH=), 5.98 (d, J=7.8Hz, 1H, OH) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 25.9 (q, $C(CH_3)_3$), 31.7 (s, $C(CH_3)_3$), 43.5 (t, -CH_2-), 71.0 (d, H-4), 75.8 (d, H-3), 117.8 (t, =CH_2), 132.7 (d, -CH=), 169.4 (s, C-2) ppm.

3.3.10 1,4-Di-*tert.*-butyl-3-hydroxy-azetidine-2-one (3j, $C_{11}H_{21}NO_2$)



3j

Method A: via **GP IV**

Compound **2j** (0.50g, 2.1mmol) was hydrolyzed with 2N KOH to give 0.34g (82%) of **3j**.

Yield: 0.34g (82%), colorless solid.

Cis/trans: 63:38

Method B: via **GP V**

Keton **4j** (25mg, 0.13mmol) was treated with $NaBH_4$ (4.79mg, 0.13mmol) to give **3j**.

Yield: 25.18mg (99%), colorless solid.

Cis/trans: 100:0

M.p.: 213-215°C (*cis*)

Combustion analysis:

calculated:	C 66.29%	H 10.62%	N 7.03%
found:	C 65.57%	H 10.27%	N 6.80%

Cis:

RP HPLC: 14.1min

Chiral HPLC: 15.8min/21.0min (SR/RS)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.01 (s, 9H, $C(CH_3)_3$), 1.41 (s, 9H, N-$C(CH_3)_3$), 3.53 (d, *J*=5.5Hz, 1H, H-4), 4.63 (t, *J*=5.9Hz, 1H, H-3), 5.96 (d, *J*=6.9Hz, 1H, OH) ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 27.1 (q, $C(\underline{C}H_3)_3$), 28.4 (q, N-$C(\underline{C}H_3)_3$), 32.9 (s, $\underline{C}(CH_3)_3$), 49.8 (s, N-$\underline{C}(CH_3)_3$), 67.9 (d, C-4), 73.5 (d, C-3), 170.6 (s, C-2) ppm

Trans:

Retention time:[103]

^{1}H-NMR (200MHz, *DMSO*-d_6):

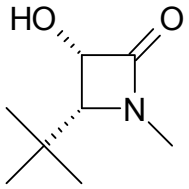
δ = 1.01 (s, 9H, $C(CH_3)_3$), 1.41 (s, 9H, N-$C(CH_3)_3$), 3.67 (d, *J*=5.7Hz, 1H, H-4), 5.40 (t, *J*=5.7Hz, 1H, H-3), 5.96 (d, *J*=6.9Hz, 1H, OH) ppm.

^{13}C-NMR (*DMSO*-d_6):

δ = 27.1 (q, $C(\underline{C}H_3)_3$), 28.4 (q, N-$C(\underline{C}H_3)_3$), 32.9 (s, $\underline{C}(CH_3)_3$), 49.8 (s, N-$\underline{C}(CH_3)_3$), 67.9 (d, C-4), 73.5 (d, C-3), 170.6 (s, C-2) ppm.

[103] The *trans*-product was under the EtOAc-peak after buying a new coloumn. No separation could be achived by changing the measurement methods.

3.3.11 *cis*-4-*tert.*-Butyl-3-hydroxy-1-methyl-azetidine-2-one (3k, $C_8H_{15}NO_2$)



3k

Method A: via **GP IV**

Compound **2k** (0.50g, 2.5mmol) was hydrolyzed with 2N KOH to give 0.27g (67%) of **3k**.

Yield: 0.27g (67%), colorless solid

Cis/trans: 100:0

Method B: via **GP V**

Keton **4k** (25mg, 0.16mmol) was treated with $NaBH_4$ (6.09mg, 0.16mmol) to give **3k**.

Yield: 24.98mg (99%), colorless solid

Cis/trans: 100:0

M.p.: 149-150°C (*cis*)

Combustion analysis:

calculated:	C 61.12%	H 9.62%	N 8.91%
found:	C 60.82%	H 9.82%	N 8.91%

Cis:

Retention time RP HPLC: 9.7min

Chiral HPLC: 25.2min/28.8min (SR/RS)

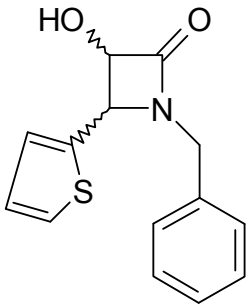
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 0.98 (s, 9H, $C(CH_3)_3$), 2.75 (s, 3H, CH_3), 3.28 (d, 1H, J= 4.9Hz, 1H, H-4), 4.66-4.77 (m, 1H, H-3), 5.99 (d, J= 6.7Hz, 1H, OH) ppm

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 26.4 (q, $C(\underline{C}H_3)_3$), 28.2 (q, N-CH_3), 32.9 (s, $\underline{C}(CH_3)_3$), 68.4 (d, C-4), 76.1 (d, C-3), 169.8 (s, C-2) ppm

3.3.12 1-Benzyl-3-hydroxy-4-thiophen-2-yl-azetidin-2-on (3l, $C_{14}H_{13}NO_2S$)



3l

Method A: via **GP IV**

Compound **2l** (0.49g, 1.6mmol) was hydrolyzed with 2N KOH to give 0.43g (99%) of **3l**.

Yield: 0.43g (99%), colorless solid

Cis/trans: 66:33

Method B: via **GP V**

Keton **4l** (25mg, 0.10mmol) was treated with $NaBH_4$ (3.67mg, 0.10mmol) to give **3l**.

Yield: 24.91mg (99%), colorless solid

Cis/trans: 100:0

M.p.: 81-82°C (*cis*)

Cis:

Retention time:

RP HPLC: 13.6min

Chiral HPLC: 37.0min/44.3min (SS/RR)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 4.28-4.30 (m, 1H, CH_2-b), 4.76 (dd, J_1=9.4Hz, J_2=15.7Hz, 1H, CH_2-a), 5.15 (d, *J*=4.5Hz, 1H, H-4), 5.26 (d, *J*=4.5Hz, 1H, H-3), 7.20-7.29 (m, 2H, H-3' and H-4'), 6.45 (d, J=6.5Hz, 1H, OH), 7.33-7.46 (m, 5H, arom. H), 7.72-7.80 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 43.3 (t, N-CH_2), 58.0 (d, C-4), 77.7 (d, C-3), 126.6 (d, C-4'), 126.7 and 127.4, (2d, C-2' and C-3'), 127.4, 127.8 and 128.5 (3d, C-3'', C-4'' and C-5''), 135.7 and 138.2 (2s, C-1' and C-2''), 168.3 (s, C-2) ppm.

Trans:

Retention time:

RP HPLC: 14.2min

Chiral HPLC: 23.3min/24.1min (SR/RS)

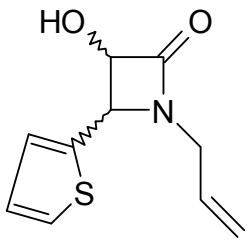
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 4.30-4.28 (m, 2H, CH_2-b and H-4), 4.76 (dd, J_1=9.4Hz, J_2=15.7Hz, 2H, CH_2-a and H-3), 6.90 (d, *J*=7.8Hz, 1H, OH), 7.20-7.29 (m, 2H, H-3' and H-4'), 7.33-7.46 (m, 5H, arom. H), 7.72-7.80 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 43.4 (t, NCH_2), 60.4 (d, C-4), 84.6 (d, C-3), 126.3 (d, C-4'), 126.7 and 127.3, (2d, C-2' and C-3'), 127.6, 127.9 and 128.5 (3d, C-3'', C-4'' and C-5''), 135.4 and 138.7 (2s, C-1' and C-2''), 169.5 (s, C-2) ppm.

3.3.13 1-Allyl-3-hydroxy-4-thiophen-3-yl-azetidin-2-one (3m, $C_{10}H_{11}NO_2S$)



3m

Method A: via **GP IV**

Compound **2m** (1.00g, 4.0mmol) was hydrolyzed with 2N KOH to give 0.82g (98%) of **3m**.

Yield: 0.82g (98%), colorless solid.

Cis/trans: 59:41

Method B: via **GP V**

Keton **4m** (25mg, 0.12mmol) was treated with $NaBH_4$ (4.50mg, 0.12mmol) to give **3m**.

Yield: 24.66mg (98%), colorless solid

Cis/trans: 100:0

M.p.: 56-59°C (*cis*)

Combustion analysis:

calculated:	C 57.40%	H 5.30%	N 6.69%
found:	C 57.50%	H 5.14%	N 6.63%

Cis:

Retention time:

RP HPLC: 9.6min

Chiral HPLC: 35.6min/40.0min (SS/RR)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 3.30-3.51 (m, 1H, -CH_2-b), 3.87-4.05 (m, 1H, -CH_2-a), 5.00-5.20 (m, 4H, =CH_2, H-3 and H-4), 5.58-5.83 (m, 1H, -CH=), 6.12-6.21 (m, 1H, OH), 6.98-7.10 (m, 2H, H-3' and H-4'), 7.47-7.58 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 42.1 (t, -CH_2-), 58.0 (d, H-4), 77.6 (d, H-3), 117.7 (t, CH_2=), 126.5, 126.6 and 127.4 (3d, C-3', C-4' and C-5'), 132.0 (d, -CH=), 138.6 (s, C-2'), 168.4 (s, C-2)ppm.

Trans:

Retention time:

RP HPLC: 10.3min

Chiral HPLC: 20.5min/22.6min (SR/RS)

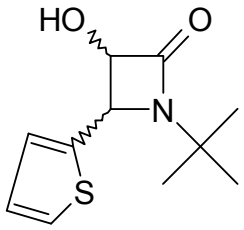
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 3.30-3.51 (m, 1H, -CH_2-b), 3.87-4.05 (m, 1H, -CH_2-a), 4.55 (dd, J_1=1.6, J_2=8.0, 1H, H-3), 4.65 (d, J=1.4, 1H, H-4), 5.00-5.20 (m, 2H, =CH_2), 5.58-5.83 (m, 1H, -CH=), 6.65 (d, J=8.0Hz, 1H, OH), 6.98-7.10 (m, 2H, H-3' and H-4'), 7.47-7.58 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 42.1 (t, -CH_2-), 60.4 (d, H-4), 84.6 (d, H-3), 117.8 (t, CH_2=), 126.1, 126.5 and 126.6 (3d, C-3', C-4' and C-5'), 131.9 (d, -CH=), 140.4 (s, C-2'), 168.3 (s, C-2) ppm.

3.3.14 1-*tert.*-Butyl-3-hydroxy-4-thiophen-2-yl-azetidine-2-one (3n, $C_{11}H_{15}NO_2S$)



3n

Method A: via **GP IV**

Compound **2n** (4.00g, 15.0mmol) was hydrolyzed with 2N KOH to give 2.86g (85%) of **3n**.

Yield: 2.86g (85%), colorless solid.

Cis/trans: 100:0

Method B: via **GP V**

Keton **4n** (25mg, 0.11mmol) was treated with $NaBH_4$ (4.32mg, 0.11mmol) to give **3n**.

Yield: 25.08mg (99%), colorless solid

Cis/trans: 100:0

M.p.: 197-198°C (*cis*)

Combustion analysis:

calculated:	C 58.64%	H 6.71%	N 6.22%
found:	C 58.35%	H 6.68%	N 5.90%

Cis:

Retention time:

RP HPLC: 12.1min

Chiral HPLC: 28.3min/37.9min (SS/RR)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.25 (s, 9H, $C(CH_3)_3$), 4.81-4.91 (m, 1H, H-3), 5.25 (d, *J*=4.7Hz, 1H, H-4), 6.08 (d, *J*=6.5Hz, 1H, OH), 7.01-7.19 (m, 2H, H-3' and H-4'), 7.58 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 27.7 (q, $C(CH_3)_3$), 53.5 (s, $C(CH_3)_3$), 57.2 (d, C-4), 75.5 (d, C-3), 126.2, 126.4 and 127.2 (3d, C-3', C-4' and C-5'), 141.4 (s, C-2'), 162.9 (s, C-2) ppm.

Method C: via **GP VIII**

Trans

Retention time:

RP HPLC: 13.0min

Chiral HPLC: -min/24.5min (SR/RS)

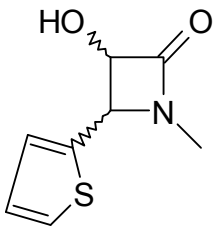
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.29 (s, 9H, $C(CH_3)_3$), 4.81-4.91 (m, 1H, H-3), 5.31 (d, *J*=4.7Hz, 1H, H-4), 6.38 (d, *J*=6.6Hz, 1H, OH), 7.01-7.19 (m, 2H, H-3' and H-4'), 7.58 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 27.7 (q, $C(CH_3)_3$), 53.5 (s, $C(CH_3)_3$), 58.6 (d, C-4), 77.1 (d, C-3), 126.2, 126.4 and 127.2 (3d, C-3', C-4' and C-5'), 141.4 (s, C-2'), 162.9 (s, C-2) ppm.

3.3.15 3-Hydroxy-1-methyl-4-thiophen-2-yl-azetidine-2-one (3o, $C_8H_9NO_2S$)



3o

Method A: via **GP IV**

Compound **2o** (3.04g, 13.5mmol) was hydrolyzed with 2N KOH to give 1.77g (71%) of **3o**.

Yield: 1.77g (71%), orange solid

Cis/trans: 33:66

Method B: via **GP V**

Keton **4o** (25mg, 0.14mmol) was treated with $NaBH_4$ (5.21mg, 0.14mmol) to give **3o**.

Yield: 25.37mg (99%), orange solid

Cis/trans: 100:0

M.p.: 94-96°C (*cis*)

Combustion analysis:

calculated:	C 52.44%	H 4.95%	N 7.64%
found:	C 52.44%	H 4.70%	N 7.64%

Cis:

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 2.72 (s, 3H, CH_3), 4.53 (d, *J*=7.8Hz, 1H, H-4), 4.94-5.04 (m, 1H, H-3), 6.11 (d, *J*=6.3Hz, 1H, OH), 7.01-7.15 (m, 2H, H-3' and H-4'), 7.42-7.50 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ= 25.9 (q, N-CH_3), 59.5 (d, C-4), 78.0 (d, C-3), 126.4, 126.7 and 127.2 (3d, C-3', C-4' and C-5'), 138.6 (s, C-2'), 168.5 (s, C-2) ppm.

Trans:

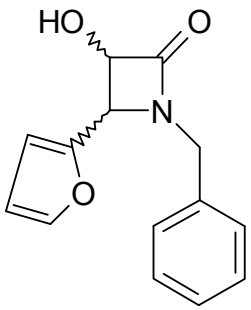
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 2.72 (s, 3H, CH_3), 4.60 (d, *J*=1.2Hz 1H, H-4), 4.94-5.04 (m, 1H, H-3), 6.52 (d, J=7.8Hz, 1H, OH), 7.01-7.15 (m, 2H, H-3' and H-4'), 7.42-7.50 (m, 1H, H-5') ppm

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 26.2 (q, N-CH_3), 61.9 (d, C-4), 85.0 (d, C-3), 126.1, 126.3 and 127.4, (3d, C-3', C-4' and C-5'), 140.5 (s, C-2'), 168.3 (s, C-2) ppm.

3.3.16 1-Benzyl-4-furan-2-yl-3-hydroxy-azetidin-2-one (3p, $C_{14}H_{13}NO_3$)



3p

Method A: via **GP IV**

Compound **2h** (2.00g, 7.mmol) was hydrolyzed with 2N KOH to give 1.66g (97%) of **3h**.

Yield: 1.66g (97%), colorless solid

Cis/trans: 50:50

Method B: via **GP V**

Keton **4p** (25mg, 0.10mmol) was treated with $NaBH_4$ (3.78mg, 0.10mmol) to give **3p**.

Yield: 24.00mg (95%), colorless solid

Cis/trans: 100:0

M.p.: 73-74°C (*cis*)

Cis:

Retention time:

RP HPLC: 13.6min

Chiral HPLC: 36.6min/46.0min (SS/RR)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 3.80-4.08 (m, 2H, CH_2-b and H-4), 4.52 (dd, J_1=4.5Hz, J_2=15.7Hz, 1H, CH_2-a), 4.98-5.08 (m, 1H, H-3), 6.15 (d, *J*=7.8, 1H, OH), 7.08-7.37 (m, 7H, arom. H, H-3' and H-4'), 7.57-7.67 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ =43.6 (t, N-CH_2), 56.2 (d, C-4), 77.8 (d, C-3), 109.6 and 110.5 (2d, H-3'' and H-4''), 127.3 (d, C-4'), 127.7 and 128.4 (2d, C-2' and C-3'), 135.7 (s, C-1'), 143.1 (d, C-5''), 149.3 (s, C-2''), 168.2 (s, C-2) ppm.

Trans:

Retention time:

RP HPLC: 14.2min

Chiral HPLC: 19.1min/23.6min (RS/SR)

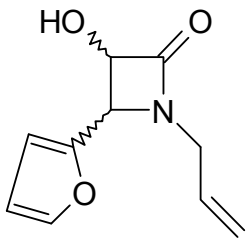
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 3.80-4.08 (m, 1H, CH_2-b), 4.31 (d, *J*=1.4Hz, 1H, H-4), 4.75 (dd, J_1=4.7Hz, J_2=15.7Hz, 1H, CH_2-a), 6.30-6.61 (m, 2H, H-3 and OH), 7.08-7.37 (m, 7H, arom. H, H-3' and H-4'), 7.57-7.67 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 43.7 (t, N-CH_2), 58.1 (d, C-4), 80.8 (d, C-3), 109.7 and 110.7 (2d, H-3'' and H-4''), 127.7 (d, C-4'), 127.8 and 128.4 (2d, C-2' and C-3'), 135.7 (s, C-1'), 143.6 (d, C-5''), 149.4 (s, C-2''), 168.7 (s, C-2) ppm.

3.3.17 1-Allyl-4-furan-3-yl-hydroxy-azetidin-2-one (3q, $C_{10}H_{11}NO_3$)



3q

Method A: via **GP IV**

Compound **2q** (3.00g, 12.8mmol) was hydrolyzed with 2N KOH to give 2.38g (99%) of **3q**.

Yield: 2.38g (99%), yellow oil

Cis/trans: 50:50

Method B: via **GP V**

Keton **4p** (25mg, 0.13mmol) was treated with $NaBH_4$ (4.94mg, 0.13mmol) to give **3p**.

Yield: 24.17mg (96%), yellow oil

Cis/trans: 100:0

Combustion analysis:

calculated:	C 62.17%	H 5.74%	N 7.25%
found:	C 62.15%	H 5.61%	N 7.22%

Cis:

Retention time:

RP HPLC: 11.3min

Chiral HPLC: 36.3min/41.0min (SS/RR)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 3.31-3.56 (m, 1H, -CH_2-b), 3.80-3.99 (m, 1H, -CH_2-a), 4.47-4.81 (m, 1H, H-4), 4.95-5.17 (m, 3H, =CH_2 and H-3), 5.54-5.77 (m, 1H, -CH=), 6.38-6.58 (m, 3H, OH, H-3' and H-4'), 7.60-7.71 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 42.2 (t, -CH_2-), 58.0 (d, H-4), 80.7 (d, H-3), 109.5 and 110.7 (2d, C-3' and C-4'), 117.5 (t, CH_2=), 131.9 (d, C-5'), 143.5 (d, -CH=), 149.7 (s, C-2'), 168.2 (s, C-2) ppm.

Trans:

Retention time:

RP HPLC: 12.2min

Chiral HPLC: 19.0min/22.7min (RS/SR)

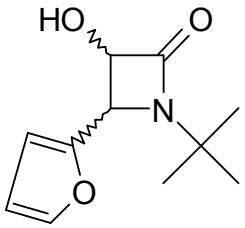
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 3.31-3.52 (m, 1H, -CH_2-b), 3.80-3.97 (m, 1H, -CH_2-a), 4.42 (d, *J*= 1.8Hz, H-3), 4.95-5.17 (m, 3H, =CH_2, and H-4), 5.54-5.77 (m, 1H, -CH=), 6.15 (m, 1H, OH), 6.38-6.58 (m, 2H, H-3' and H-4'), 7.60-7.71 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ =.42.2 (t, -CH_2-), 56.1 (d, H-4), 77.6 (d, H-3), 109.5 and 110.5 (2d, C-3' and C-4'), 117.5 (t, CH_2=), 132.0 (d, C-5'), 143.0 (d, -CH=), 149.6 (s, C-2'), 168.6 (s, COO) ppm.

3.3.18 1-*tert*.-Butyl-4-furan-2-yl-3-hydroxy-azetidin-2-one (3r, $C_{11}H_{15}NO_3$)



3r

Method A: via **GP IV**

Compound **2r** (4.00g, 15.9mmol) was hydrolyzed with 2N KOH to give 3.00g (90%) of **3r**.

Yield: 3.00g (90%), yellow solid

Cis/trans: 100:0

Method B: via **GP V**

Keton **4r** (25mg, 0.12mmol) was treated with $NaBH_4$ (4.56mg, 0.12mmol) to give **3r**.

Yield: 25.04mg (99%), yellow solid

Cis/trans: 100:0

M.p.: 135-137°C (*cis*)

Combustion analysis:

calculated:	C 63.14%	H 7.23%	N 6.69%
found:	C 63.14%	H 7.15%	N 6.62%

Cis:

Retention time:

RP HPLC: 10.5min

Chiral HPLC: 27.6min/36.1min (RS/SR)

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.15 (s, 9H, C(CH_3)$_3$), 4.76-4.83 (m, 1H, H-3), 4.89 (d, *J*=6.9Hz, 1H, H-4), 5.99 (d, *J*=6.9Hz, 1H, OH), 6.43 (d, *J*=1.0, 2H, H-3' and H-4'), 7.64 (s, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 27.4 (q, C($\underline{C}H_3$)$_3$), 52.9 (s, $\underline{C}$(CH_3)$_3$), 54.8 (d, C-4), 75.5 (d, C-3), 109.3, and 110.6 (2d, C-3' and C-4'), 142.6 (d, C-5'), 150.9 (s, C-2'), 168.0 (s, C-2) ppm.

Method C: via **GP VIII**

Trans

Retention time:

RP HPLC: 11.6min

Chiral HPLC: 25.5min/33.1min (SR/RS)

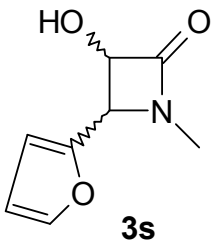
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 1.17 (s, 9H, C(CH_3)$_3$), 4.76-4.83 (m, 1H, H-3), 4.95 (d, *J*=6.9Hz, 1H, H-4), 6.31 (d, *J*=7.2Hz, 1H, OH), 6.43 (d, *J*=1.0, 2H, H-3' and H-4'), 7.64 (s, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 27.4 (q, C($\underline{C}H_3$)$_3$), 52.9 (s, $\underline{C}$(CH_3)$_3$), 55.9 (d, C-4), 76.8 (d, C-3), 109.3, and 110.6 (2d, C-3' and C-4'), 142.6 (d, C-5'), 150.9 (s, C-2'), 168.0 (s, C-2) ppm.

3.3.19 4-Furan-2-yl-3-hydroxy-1-methyl-azetidin-2-one (3s, $C_8H_9NO_3$)



3s

Method A: via **GP IV**

Compound **2s** (0.50g, 2.4mmol) was hydrolyzed with 2N KOH to give 0.29g (74%) of **3s**.

Yield: 0.29g (74%), brown solid (Purity 97% according to NMR).

Cis/trans: 20:80

M.p.: 46-48°C (*cis/trans*-mixture)

Combustion analysis:

calculated:	C 57.48%	H 5.43%	N 8.38%
found:	C 57.27%	H 5.28%	N 8.20%

Cis:

^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 2.79 (s, 3H, CH_3), 4.81 (d, *J*=4.5Hz, 1H, H-4), 5.12 (d, *J*=4.5Hz, 1H, H-3), 6.48-6.52 (m, 3H, OH, H-3' and H-4'), 7.46-7.49 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 26.1 (q, N-CH_3), 57.6 (d, C-4), 78.0 (d, C-3), 109.1, and 110.5, (2d, C-3', and C-4'), 143.1 (d, C-5'), 149.5 (s, C-2'), 168.7 (s, C-2).

Trans:

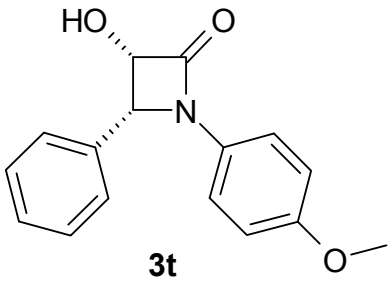
^{1}H-NMR (200MHz, *DMSO*-d_6):

δ = 2.74 (s, 3H, CH_3), 4.48 (d, *J*=1.4Hz, 1H, H-4), 4.95 (d, *J*=1.3Hz 1H, H-3), 5.21-5.79 (bs, 1H, OH), 6.35-6.46 (m, 2H, H-3' and H-4'), 7.39-7.44 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, *DMSO*-d_6):

δ = 26.2 (q, N-CH_3), 59.3 (d, C-4), 81.3 (d, C-3), 109.3 and 110.7, (2d, C-3' and C-4'), 143.6 (d, C-5'), 149.8 (s, C-2'), 168.3 (s, C-2) ppm.

3.3.20 *cis*-3-Hydroxy-1-(4-methoxyphenyl)-4-phenyl-azetidin-2-on (3t, $C_{16}H_{15}NO_3$)



3t

Method B: via **GP V**

Keton **4t** (25mg, 0.09mmol) was treated with $NaBH_4$ (3.54mg, 0.09mmol) to give **3t**.

Yield: 24.08mg (99%), yellow solid

Cis/trans: 100:0

M.p.: 202-205°C (*cis*) (Lit.[55]: 204-208°C)

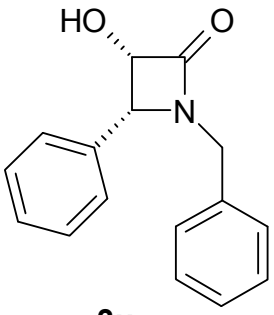
Cis:

Retention time:

RP HPLC: 16.0min

Chiral HPLC: 38.0min/42.1min (SR/RS)

3.3.21 *cis*-3-Hydroxy-4-phenyl-1-(phenylmethyl)-azetidin-2-on (3u, $C_{16}H_{15}NO_2$)



3u

Method B: via **GP V**

Keton **4u** (25mg, 0.10mmol) was treated with $NaBH_4$ (3.76mg, 0.10mmol) to give **3u**.

Yield: 24.61mg (98%), colorless solid

Cis/trans: 100:0

M.p.: 114-118°C (*cis*) (Lit.[55]: 116-120°C)

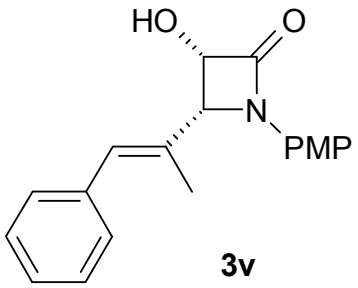
Cis:

Retention time:

RP HPLC: 15.7min

Chiral HPLC: 31.6min/36.3min (SR/RS)

3.3.22 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3v, $C_{19}H_{19}NO_2$)



3v

Method B: via **GP V**

Keton **4v** (25mg, 0.08mmol) was treated with $NaBH_4$ (3.07mg, 0.08mmol) to give **3v**.

Yield: 25.11mg (100%), colorless solid

Cis/trans: 100:0

M.p.: 172-174°C (*cis*) (Lit.[55]: 173-174°C)

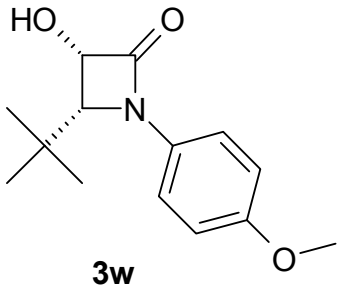
Cis:

Retention time

RP HPLC: 19.7min

Chiral HPLC: 31.9min/34.5 min (SR/RS)

3.3.23 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-tert.-butyl-azetidin-2-one (3w, $C_{14}H_{19}NO_3$)



3w

Method B: via **GP V**

Keton **4w** (25mg, 0.10mmol) was treated with $NaBH_4$ (3.82mg, 0.10mmol) to give **3w**.

Yield: 25.10mg (100%), colorless solid

Cis/trans: 100:0

M.p.: 204-206°C (*cis*) (Lit.[55]: 204-205°C)

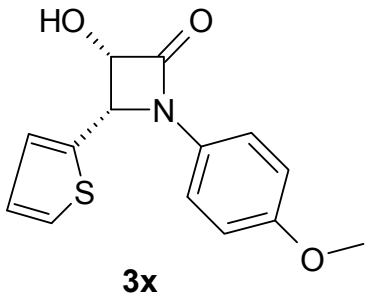
Cis:

Retention time:

RP HPLC: 17.2min

Chiral HPLC: 31.8min/34.9min (SR/RS)

3.3.24 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(2-thienyl)-azetidin-2-one (3x, $C_{14}H_{13}NO_3S$)



3x

Method B: via **GP V**

Keton **4x** (25mg, 0.09mmol) was treated with $NaBH_4$ (3.45mg, 0.09mmol) to give **3x**.

Yield: 24.88mg (99%), colorless solid

Cis/trans: 100:0

M.p.: 176-178°C (*cis*) (Lit.[59]: 178-179)

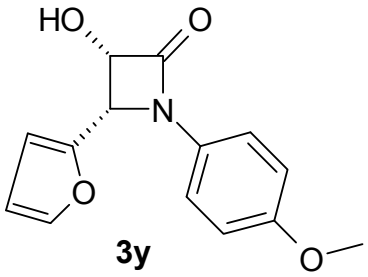
Cis:

Retention time:

RP HPLC: 13.5min

Chiral HPLC: 44.6min/47.8min (SS/RR)

3.3.25 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(2-furyl)-2-azetidinone (3y, $C_{14}H_{13}NO_4$)



3y

Method B: via **GP V**

Keton **4y** (25mg, 0.10mmol) was treated with $NaBH_4$ (3.67mg, 0.10mmol) to give **3y**.

Yield: 24.96mg (99%), yellow solid

Cis/trans: 100:0

M.p.: 156-159°C (*cis*) (Lit.[59]: 158-159°C)

Cis:

Retention time

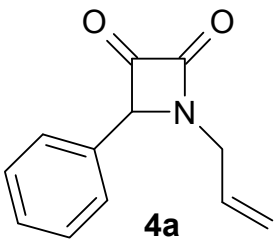
RP HPLC: 12.4min

Chiral HPLC: 45.7min/50.7min (SS/RR)

3.4 Oxidation with Phosphorous Pentoxide

GP V: Phosphorous pentoxide (P_2O_5, 1.5equiv.) was added to dry DMSO (amount calculated for a 5% solution after addition of starting material) under cooling with ice/water and then stirred for 10 min. The corresponding alcohol **1a-r** dissolved in the minimum amount of DMSO was added and the mixture was stirred at room temperature till TLC indicated no further generation of product (it is noteworthy, that consumption of starting material is an insufficient indicator for reaction control and prolonged stirring usually increased the yields). The mixture was hydrolyzed with cooled saturated sodium bicarbonate, extracted with EtOAc, washed three times with brine, dried over sodium sulfate, filtered and evaporated**.** Nearly all products were purified by flash chromatography.

3.4.1 1-Allyl-4-phenyl-azetidin-2,3-dione (4a, $C_{12}H_{11}NO_2$)



4a

Alcohol **3a** (0.50g, 2.5mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.52g, 3.7mmol) to give 0.33g (52%) of **4a** as yellow solid after flash chromatography (PE:EtOAc 7:1).

Yield: 0.33g (52%), yellow solid.

M.p.: 66-68°C

Combustion analysis:

calculated:	C 71.63%	H 5.51%	N 6.96%
found:	C 68.77%	H 5.98%	N 5.61%

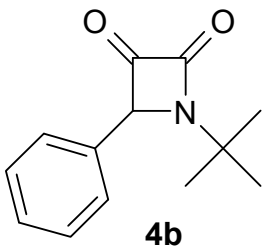
^{1}H-NMR (200MHz, $CDCl_3$):

δ =3.76 (dd, J_1=8.0Hz, J_2=14.7Hz, 1H, CH_2-b), 4.50-4.61 (m, 1H, CH_2-a), 5.15-5.32 (m, 3H, CH_2= and H-4), 5.67-5.89 (m, 1H, CH=), 7.17-7.46 (m, 5H, arom. H) ppm.

^{13}C-NMR ($CDCl_3$):

δ = 44.0 (t, -CH_2-), 73.7 (d, C-4), 120.5 (t, =CH_2), 127.0 (d, C-4'), 128.7, and 129.3 (2d, C-3', and C-2'), 129.5 (d, CH=), 132.1 (s, C-1'), 164.8 (s, COO), 193.4 (s, C=O) ppm.

3.4.2 1-*tert.*-Butyl-4-phenyl-azetidin-2,3-dione (4b, $C_{13}H_{15}NO_2$)



4b

Alcohol **3b** (2.00g, 9.1mmol) was oxidized according to **GP VI** with phosphorous pentoxide (2.1g, 13.7mmol) to give 1.81g (91%) of **4b** after flash chromatography (PE:EtOAc 10:1).

Yield: 1.81g (91%), colorless solid

M.p.: 100-102°C

Combustion analysis:

calculated:	C 71.87%	H 6.96%	N 6.45%
found:	C 71.46%	H 6.76%	N 6.34%

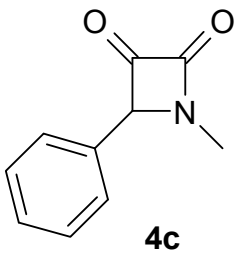
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.42 (s, 9H, $C(CH_3)_3$), 5.16 (s, 1H, H-4), 7.27-7.42 (m, 15H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 25.1 (q, $C(\underline{C}H_3)_3$), 54.6 (s, $\underline{C}(CH_3)_3$), 71.8 (d, C-4), 124.7 (d, C-4'), 126.9, and 127.1 (2d, C-3', C-2'), 132.8 (d, C-1'), 161.4 (s, C-2), 191.8 (s, C-3) ppm.

3.4.3 1-Methyl-4-phenyl-azetidin-2,3-dione (4c, $C_{10}H_9NO_2$)



4c

Alcohol **3c** (0.85g, 4.8mmol) was oxidized according to **GP VI** with phosphorous pentoxide (1.02g, 7.2mmol) to give 0.62g (74%) of **4c** as colorless solid after flash chromatography (PE:EtOAc 6:1).

Yield: 0.62g (74%), colorless solid

M.p.: 80-81°C

Combustion analysis:

calculated:	C 68.56%	H 5.18%	N 8.00%
found:	C 67.94%	H 5.33%	N 7.67%

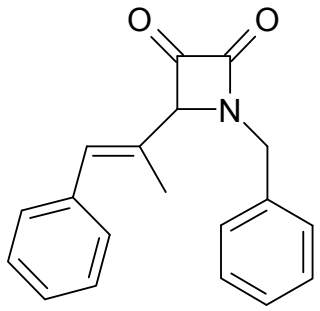
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 3.13 (s, 3H, CH_3), 5.01 (s, 1H, H-4), 7.11-7.40 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 27.9 (q, CH_3), 75.4 (d, C-4), 126.7 (d, C-4'), 129.3, and 129.5, (2d, C-3', and C-2'), 131.9 (s, C-1'), 164.2 (s, C-2), 193.7 (s, C-3) ppm.

3.4.4 1-Benzyl-4-(3-phenylprop-2-en-2-yl)-azetidin-2,3-dione (4d, $C_{19}H_{17}NO_2$)



4d

Alcohol **3d** (2.00g, 6.8mmol) was oxidized according to **GP VI** with phosphorous pentoxide (1.45g, 10.2mmol) to give 1.61g (81%) of **4d** after flash chromatography (PE:EtOAc 6:1).

Yield: 1.61g (81%), colorless solid

M.p.: 112-114°C

Combustion analysis:

calculated:	C 78.33%	H 5.88%	N 4.81%
found:	C 76.92%	H 5.66%	N 4.83%

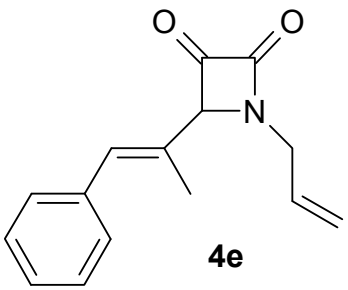
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.73 (s, 3H, CH_3), 4.43 (d, *J*=14.5Hz, 1H, CH_2-b), 4.54 (s, 1H, H-4), 5.13 (d, *J*=14.5Hz, 1H, CH_2-a), 6.44 (s, 1H, H-β), 7.18-7.40 (m, 10H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 14.0 (q, CH_3), 46.2 (t, NCH_2), 76.8 (d, C-4), 127.1 (d, CH=), 127.5 and 127.8 (2d, C-4', and C-4''), 128.3, 128.6, 128.8, and 129.0, (4d, C-3', C-3'', C-2', and C-2''), 132.9 (s, C-β), 135.3 and 135.9 (2s, C-1' and C-1''), 163.6 (s, C-2), 195.2 (s, C-3) ppm.

3.4.5 1-Allyl-4-(3-phenylprop-2-en-2-yl)-azetidin-2,3-dione (4e, $C_{15}H_{15}NO_2$)



4e

Alcohol **3e** (0.50g, 2.0mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.44g, 3.1mmol) to give 0.21g (41%) of **4e**.

Yield: 0.21g (41%), brown oil [104] (Purity 90% according to NMR)

^{1}H-NMR (200MHz, $CDCl_3$):

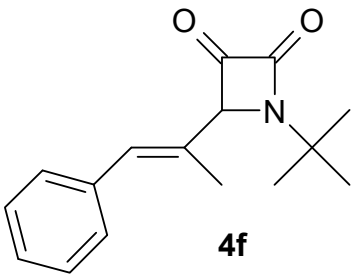
δ = 1.85 (s, 3H, CH_3), 3.97 (dd, J_1=7.2Hz, J_2=15.3Hz, 1H, -CH_2-b), 4.46-4.58 (m, 1H, -CH_2-a), 4.72 (s, 1H, H-4), 5.12- 5.36 (m, 2H, =CH_2), 5.76-5.93 (m, 1H, CH=), 6.54 (s, 1H, H-β), 7.22-7.43 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 15.1 (q, CH_3), 45.5 (t, -CH_2-), 77.9 (d, C-4), 118.6 (t, =CH_2), 130.0 (d, C-4'), 130.5, and 130.8 (2d, C-3', and C-2'), 132.5 (d, C-β), 132.9 (d, -CH=), 136.8 (s, C-α), 139.1 (s, C-1'), 166.7 (s, COO), 196.7 (s, C=O) ppm.

[104] Product **4e** turned out to decompose upon chromatographic purification and was also insufficiently stable for distillation (*Kugelrohr*); consequently, no combustion analysis was conducted for this compound.

3.4.6 1-tert.-Butyl-4-(3-phenylprop-2-en-2-yl)-azetidine-2,3-dione (4f, $C_{16}H_{19}NO_2$)



4f

Alcohol **3f** (1.50g, 5.8mmol) was oxidized according to **GP VI** with phosphorous pentoxide (1.23g, 8.7mmol) to give 1.43g (96%) of **4f** after flash chromatography (PE:EtOAc 8:1).

Yield: 1.43g (96%), yellow/orange solid

M.p.: 81-83°C

Combustion analysis:

calculated:	C 74.68%	H 7.44%	N 5.44%
found:	C 73.81%	H 7.35%	N 5.34%

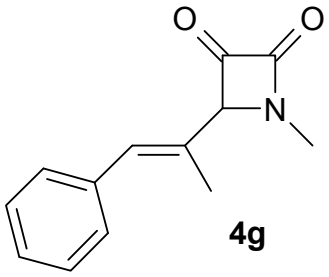
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.42 (s, 9H, $C(CH_3)_3$), 1.77 (s, 3H, CH_3), 4.73 (s, 1H, H-4), 6.53 (s, 1H, H-β), 7.12-7.39 (m, 15H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 11.6 (q, CH_3), 25.6 (q, $C(\underline{C}H_3)_3$), 54.5 (d, $\underline{C}(CH_3)_3$), 76.1 (d, C-4), 125.1 (d, C-4'), 126.0, and 126.6 (2d, C-3', and C-2'), 129.6 (d, C-β), 132.8 (t, C-α), 135.9 (d, C-1'), 160.7 (s, C-2), 194.2 (s, C-3) ppm.

3.4.7 1-Methyl-4-(3-phenylprop-2-en-2-yl)-azetidine-2,3-dione (4g, $C_{13}H_{13}NO_2$)



4g

Alcohol **3g** (0.90g, 4.1mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.88g, 6.2mmol) to give 0.68g (76%) of **4g** as yellow solid after flash chromatography (PE:EtOAc 7:1).

Yield: 0.68g (76%), yellow solid

M.p.: 72-74°C

Combustion analysis:

calculated:	C 72.54%	H 6.09%	N 6.51%
found:	C 70.65%	H 5.96%	N 6.31%

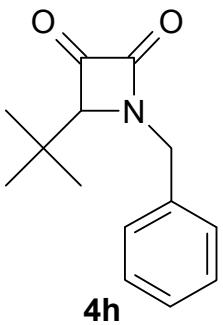
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.85 (s, 3H, CH_3), 3.23 (s, 3H, N-CH_3), 4.97 (s, 1H, H-4), 6.54 (s, 1H, H-β), 7.21-7.43 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 14.1 (q, CH_3), 28.2 (q, N-CH_3), 79.3 (d, C-4), 127.5 (d, C- β), 128.4 (d, C-4'), 129.0, and 129.6, (2d, C-3', and C-2'), 131.5 (s, C-1'), 135.8 (s, C-α), 164.0 (s, C-2), 195.3 (s, C-3) ppm.

3.4.8 1-Benzyl-4-tert.-butyl-azetidin-2,3-dione (4h, $C_{14}H_{17}NO_2$)



4h

Alcohol **3h** (0.50g, 2.1mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.46g, 3.2mmol) to give 0.43g (87%) of **4h** after flash chromatography (PE:EtOAc 6:1).

Yield: 0.43g (87%), yellow solid

M.p.: 90-93°C

Combustion analysis:

calculated:	C 72.70%	H 7.41%	N 6.06%
found:	C 72.51%	H 7.11%	N 6.02

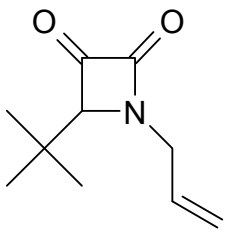
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 0.96 (s, 9H, $C(CH_3)_3$), 3.80 (s, 1H, H-4), 4.39 (d, *J*=15.4Hz, 1H, CH_2-b), 5.27 (d, *J*=15.1Hz, 1H, CH_2-a), 7.25-7.43 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 26.3 (q, $C(\underline{C}H_3)_3$), 33.7 (s, $\underline{C}(CH_3)_3$), 47.2 (t, NCH_2), 79.7 (d, C-4), 128.4 (d, C-4'), 128.5, and 129.1 (2d, C-2' and C-3'), 133.8 (s, C-1'), 164.7 (s, C-2), 197.2 (s, C-3) ppm.

3.4.9 1-Allyl-4-tert.-butyl-azetidin-2,3-dione (4i, $C_{10}H_{15}NO_2$)



4i

Alcohol **3i** (0.40g, 2.2mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.47g, 3.3mmol) to give 0.37g (93%) of **4i**.

Yield: 0.37g (93%), brown oil[105] (Purity 95% according to NMR)

^{1}H-NMR (200MHz, $CDCl_3$):

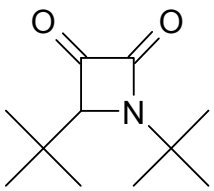
δ = 0.96 (s, 3H, $C(CH_3)_3$), 3.79-3.96 (m, 1H, -CH_2-b), 3.92 (s, 1H, H-4), 4.50-4.60 (m, 1H, -CH_2-a), 5.17-5.32 (m, 2H, =CH_2), 5.61-5.86 (m, 1H, -CH=) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 26.3 (q, $C(\underline{C}H_3)_3$), 33.7 (s, $\underline{C}(CH_3)_3$), 45.8 (t, -CH_2-), 80.0 (d, C-4), 120.5 (t, =CH_2), 130.0 (d, CH=), 163.9 (s, C-2), 197.1 (s, C-3) ppm.

[105] Product **4i** turned out to decompose upon chromatographic purification and was also insufficiently stable for distillation (*Kugelrohr*); consequently, no combustion analysis was conducted for this compound

3.4.10 1,4-Di-tert.-butyl-azetidin-2,3-dione (4j, $C_{11}H_{19}NO_2$)



4j

Alcohol **3j** (1.20g, 6.0mmol) was oxidized according to **GP VI** with phosphorous pentoxide (1.18g, 9.0mmol) to give 1.07g (90%) of **4j** after flash chromatography (PE:EtOAc 6:1).

Yield: 1.07g (90%), yellow solid

M.p.: 99-101°C

Combustion analysis:

calculated:	C 66.97%	H 9.71%	N 7.10%
found:	C 67.02%	H 9.56%	N 7.01%

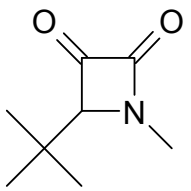
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.00 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, N-$C(CH_3)_3$), 3.98 (s, 1H, H-4) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 26.0 (q, $C(\underline{C}H_3)_3$), 27.8 (q, N-$C(\underline{C}H_3)_3$), 32.6 (s, $\underline{C}(CH_3)_3$), 55.1 (s, N-$\underline{C}(CH_3)_3$), 80.6 (d, C-4), 162.7 (s, C-2), 196.6 (s, C-3) ppm.

3.4.11 4-tert.-Butyl-1-methyl-azetidin-2,3-dione (4k, $C_8H_{13}NO_2$)



4k

Alcohol **3k** (0.20g, 1.3mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.27g, 1.9mmol) to give 0.18g (91%) of **4k**. after flash chromatography (PE:EtOAc 6:1).

Yield: 0.18g (91%), colorless solid

M.p.: 45-46°C

Combustion analysis:

calculated:	C 61.91%	H 8.44%	N 9.03%
found:	C 61.16%	H 8.52%	N 8.74%

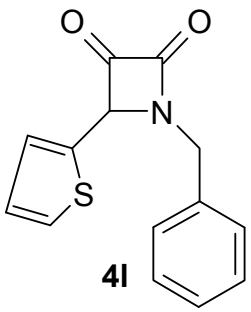
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 0.98 (s, 9H, $C(CH_3)_3$), 3.22 (s, 3H, CH_3), 3.81 (s, 1H, H-4) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 26.1 (q, $C(\underline{C}H_3)_3$), 30.2 (q, N-CH_3), 33.6 (s, $\underline{C}(CH_3)_3$), 81.8 (d, C-4), 164.4 (s, C-2), 197.4 (s, C-3) ppm.

3.4.12 1-Benzyl-4-thiophen-2-yl-azetidin-2,3-dione (4l, $C_{14}H_{11}NO_2S$)



4l

Alcohol **3l** (2.00g, 7.7mmol) was oxidized according to **GP VI** with phosphorous pentoxide (1.65g, 11.6mmol) to give 1.65g (83%) of **4l** after flash chromatography (PE:EtOAc 4:1).

Yield: 1.65g (83%), yellow solid

M.p.: 97-98°C

Combustion analysis:

calculated:	C 65.35%	H 4.31%	N 5.44%
found:	C 63.79%	H 4.34%	N 5.61%

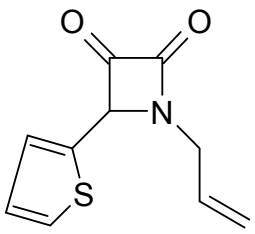
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 4.21 (d, *J*=14.7Hz, 1H, CH_2-b), 5.11-5.22 (m, 2H, H-4 and CH_2-a), 6.93-7.42 (m, 8H, H-3'', H-4'', H-5'' and arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 45.4 (t, NCH_2), 68.9 (d, C-4), 126.9 (d, C-4'), 127.2, and 127.6 (2d, C-2' and C-3'), 127.9, 128.2, and 128.9 (3d, C-3'', C-4'' and C-5''), 133.3 (s, C-1'), 134.8 (s, C-2''), 162.2 (s, C-2), 191.8 (s, C-3) ppm.

3.4.13 1-Allyl-4-thiophen-2-yl-azetidine-2,3-dione (4m, $C_{10}H_9NO_2S$)



4m

Alcohol **3m** (0.50g, 2.4mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.51g, 3.6mmol) to give 0.35g (70%) of **4m**.

Yield: 0.35g (70%), brown oil[106] (Purity 92% according to NMR)

^{1}H-NMR (400MHz, $CDCl_3$):

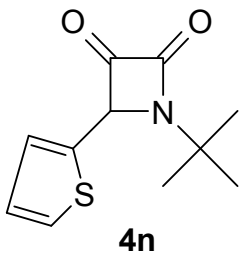
δ = 3.85 (dd, J_1=7.4Hz, J_2=15.3Hz, 1H, -CH_2-b), 4.52 (dd, J_1=4.5Hz, J_2=15.3Hz, 1H, -CH_2-a), 5.19-5.35 (m, 2H, =CH_2), 5.40 (s, 1H, H-4), 5.68-5.91 (m, 1H, -CH=), 7.02-7.11 (m, 2H, H-4' and H-3'), 7.35-7.42 (m, 1H, H-5') ppm.

^{13}C-NMR ($CDCl_3$):

δ = 44.6 (t, -CH_2-), 69.2 (d, C-4), 119.6 (t, =CH_2), 128.1 and 129.4 (2d, C-3', and C-4'), 130.3 (d, CH=), 135.3 (d, C-5'), 136.6 (s, C-2'), 183.2 (s, C-2), 192.2 (s, C-3) ppm.

[106] Product **4m** turned out to decompose upon chromatographic purification and was also insufficiently stable for distillation (*Kugelrohr*); consequently, no combustion analysis was conducted for this compound

3.4.14 1-tert.-Butyl-4-thiophen-2-yl-azetidin-2,3-dione (4n, $C_{11}H_{13}NO_2S$)



4n

Alcohol **3n** (2.00g, 8.9mmol) was oxidized according to **GP VI** with phosphorous pentoxide (1.89g, 13.3mmol) to give 1.76g (89%) of **4n** after flash chromatography (PE:EtOAc 4:1).

Yield: 1.76g (89%), yellow solid

M.p.: 130-133°C

Combustion analysis:

calculated:	C 59.17%	H 5.87%	N 6.27%
found:	C 58.84%	H 5.75%	N 6.18%

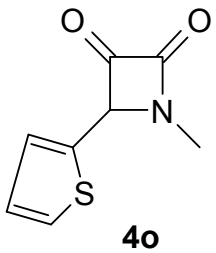
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.45 (s, 9H, $C(CH_3)_3$), 5.45 (s, 1H, H-4), 7.00-7.12 (m, 2H, H-3' and H-4'), 7.23-7.38 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 28.2 (q, $C(\underline{C}H_3)_3$), 57.0 (s, $\underline{C}(CH_3)_3$), 69.4 (d, C-4), 126.9, 127.3, and 127.7 (3d, C-3', C-4' and C-5'), 138.2 (s, C-2'), 162.9 (s, C-2), 193.0 (s, C-3) ppm.

3.4.15 1-Methyl-4-thiophen-2-yl-azetidine-2,3-dione (4o, $C_8H_7NO_2S$)



4o

Alcohol **3o** (0.20g, 1.1mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.23g, 1.6mmol) to give 0.10g (52%) of **4o** after flash chromatography (PE:EtOAc 4:1).

Yield: 0.10g (52%), yellow solid

M.p.: 88-90°C

Combustion analysis:

calculated:	C 53.03%	H 3.89%	N 7.73%
found:	C 53.26%	H 4.00%	N 7.53%

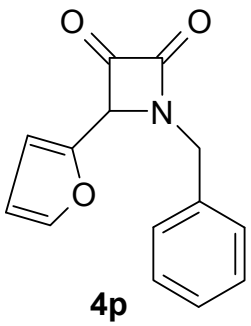
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 3.21 (s, 3H, CH_3), 5.36 (s, 1H, H-4), 7.04-7.12 (m, 2H, H-3' and H-4'), 7.37-7.43 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 26.7 (q, N-CH_3), 70.0 (d, C-4), 126.2, 126.4, and 126.9, (3d, C-3', C-4', and C-5'), 133.5 (s, C-2'), 162.5 (s, C-2), 191.3 (s, C-3) ppm.

3.4.16 1-Benzyl-4-furan-2-yl-azetidin-2,3-dione (4p, $C_{14}H_{11}NO_3$)



4p

Alcohol **3p** (2.00g, 8.2mmol) was oxidized according to **GP VI** with phosphorous pentoxide (1.72g, 12.3mmol) to give 1.29g (65%) of **4p** after flash chromatography (PE:EtOAc 4:1).

Yield: 1.29g (65%), beige solid

M.p.: 95-97°C

Combustion analysis:

calculated:	C 69.70%	H 4.60%	N 5.81%
found:	C 69.67%	H 4.40%	N 5.30%

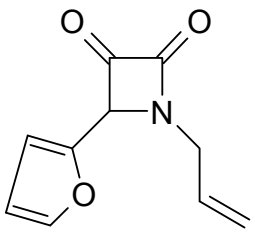
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 4.22 (d, *J*=14.7Hz, 1H, CH_2-b), 4.99 (s, H-4), 5.03 (d, *J*=14.7Hz, 1H, CH_2-a), 6.33-6.41 (m, 3H, H-3'', H-4'', and H-5''), 7.16-7.44 (m, 5H, arom. H) ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 45.3 (t, NCH_2), 68.9 (d, C-4), 127.3, 127.6, and 127.8 (3d, C-3'', C-4'' and C-5''), 128.6 (d, C-4'), 128.8, and 129.2 (2d, C-2', and C-3'), 133.3 (s, C-1'), 134.6 (s, C-2''), 163.0 (s, C-2), 191.9 (s, C-3) ppm.

3.4.17 1-Allyl-4-furan-2-yl-azetidin-2,3-dione (4q, $C_{10}H_9NO_3$)



4q

Alcohol **3q** (0.20g, 1.0mmol) was oxidized according to **GP VI** with phosphorous pentoxide (0.22g, 1.6mmol) to give 0.08g (41%) of **4q**.

Yield: 0.08g (41%), brown oil (Purity 93% according to NMR)

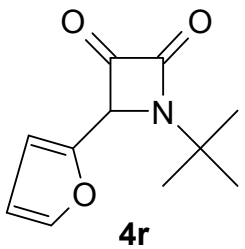
^{1}H-NMR (200MHz, $CDCl_3$):

δ = 3.77 (dd, J_1=7.1Hz, J_2=15.3Hz, 1H, -CH_2-b), 4.32-4.39 (m, 1H, -CH_2-a), 5.05-5.26 (m, 3H, =CH_2 and H-4), 5.56-5.78 (m, 1H, -CH=), 6.31-6.43 (m, 2H, H-4' and H-3'), 7.36-7.43 (m, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 44.9 (t, -CH_2-), 69.2 (d, C-4), 119.6 (t, =CH_2), 128.1 and 129.4 (2d, C-3', and C-4'), 130.3 (d, CH=), 135.3 (d, C-5'), 136.6 (s, C-2'), 183.2 (s, C-2), 192.2 (s, C-3) ppm.

3.4.18 1-tert.-Butyl-4-furan-2-yl-azetidin-2,3-dione (4r, $C_{11}H_{13}NO_3$)



4r

Alcohol **3r** (2.00g, 9.6mmol) was oxidized according to **GP VI** with phosphorous pentoxide (2.04g, 14.3mmol) to give 1.68g (85%) of **4r** after flash chromatography (PE:EtOAc 6:1).

Yield: 1.68g (85%), yellow solid

M.p.: 80-81°C

Combustion analysis:

calculated:	C 63.76%	H 6.32%	N 6.76%
found:	C 63.46%	H 6.23%	N 6.63%

^{1}H-NMR (200MHz, $CDCl_3$):

δ = 1.40 (s, 9H, $C(CH_3)_3$), 5.22 (s, 1H, H-4), 6.37-6.46 (m, 2H, H-3' and H-4'), 7.45 (s, 1H, H-5') ppm.

^{13}C-NMR (50MHz, $CDCl_3$):

δ = 27.8 (q, $C(\underline{C}H_3)_3$), 57.6 (s, $\underline{C}(CH_3)_3$), 66.7 (d, C-4), 111.1, and 111.2 (2d, C-3', C-4'), 143.7 (d, C-5'), 147.2 (s, C-2'), 162.6 (s, C-2), 192.7 (s, C-3) ppm.

3.5 Yeast reduction

GP VII: Screening

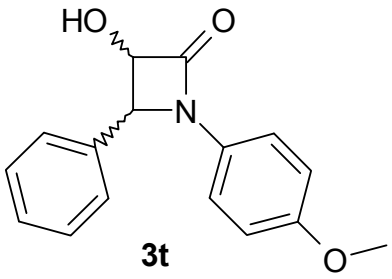
In a 100mL shaking flask commercial yeast (1g, from a local supermarket) was added to a 20% solution of glucose in water (25mL) and incubated for 20 minutes at 30°C. Ketone (10mg in 150µL dioxane) and β-cyclodextrin (1equiv.) were added to this fermentation culture. This mixture was incubated for 48h at 30°C. The biomass was separated by centrifugation (20min at 8600rpm). This solution was saturated with NaCl and extracted with EtOAc. From the dry organic layer HPLC samples were measured.

GP VIII: Preparative Scale

In a 250mL shaking flask commercial yeast (2g, from a local supermarket) was added to a 20% solution of glucose in water (50mL) and incubated for 20 minutes at 30°C. Ketone (50mg in 300µL dioxane) and β-cyclodextrin (1equiv.) were added to this fermentation culture. This mixture was incubated for 48h at 30°C. The biomass was separated by centrifugation (20min at 8600rmp). This solution was saturated with NaCl and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. Pure products were obtained by flash column chromatography.

Assignment of the *cis/trans* products are based on NMR. Both compounds were injected into reverse phase HPLC to obtain reference chromatograms. The peak-assigment on straight phase HPLC was conducted in comparison to the literature[21] whereever possible; elution order of such reference isomers was then also used for tentative assignment of previously unpublished compounds.

3.5.1 3-Hydroxy-1-(4-methoxyphenyl)-4-phenyl-azetidin-2-one (3t, $C_{16}H_{15}NO_3$)



3t

Method A: via **AV VII** using **4t** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans: 31% *Cis*: 69%

Retention time: 16.5min/16.0min (*trans/cis*)

Chiral HPLC:

Trans:	24.7%	*Cis*	75.3%
	SS 1.2%		SR 24.4%
	RR 23.5 %		RS 50.9%
	ee 90		ee 35

Retention time: 28.2min/35.3min/38.0min/42.1min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4t** (50mg, 0.19mmol) to give **3t** after flash chromatography (EtOAc:PE 10:1).

Yield: 24.7mg (49%), colorless solid

Trans: 5.6mg (22.6%) *Cis*: 16.2mg (65.6%) mixture: 2.9mg (11.7%)

M.p.:

Trans: 146-147°C *Cis:* 202-204 °C

Specific rotation:

Trans: *Cis:*

$\alpha_{[25]}$= -80 [*c*=0.02, DMSO] $\alpha_{[25]}$= +8.81[*c*=0.29, DMSO]

Rev. Phase HPLC:

Trans	24%	*Cis*	76%

Chiral HPLC:

Trans	28.7%	*Cis*	71.3%
	SS 7.1%		SR 23.4%
	RR 21.6 %		RS 47.9%
	ee 52		ee 68

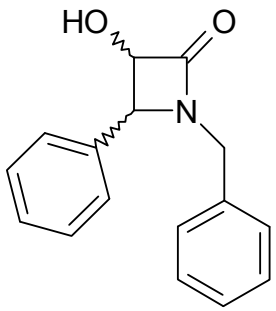
Literature reports[107]:

Chiral HPLC:

Trans:	<39%	*Cis:*	69%
	SS <1%		SR 14%
	RR 38 %		RS 48%
	ee 90		ee 55

[107] Yang, Y.; Kayser, M.M.; Rochon, F.D.; Rodriguez, S.; Stewart, J.D. *J. Mol. Catal. B:Enzym.* **2005**, *32*, 167-174

3.5.2 3-Hydroxy-4-phenyl-1-(phenylmethyl)-azetidin-2-one (<u>3u</u>, $C_{16}H_{15}NO_2$)



3u

Method A: via **AV VII** using **4u** (10mg, 0.05mmol).

Rev. Phase HPLC:

Trans 39.5% *Cis* 60.5%

Retention time: 16.3min/15.7min (*trans/cis*)

Chiral HPLC:

Trans	38.4%	*Cis:*	61.6
	SS 18.6%		SR 36.8%
	RR 19.8%		RS 24.8%
	ee 3		ee 19

Retention time: 19.1min/23.6min/31.6min/36.3min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4u** (50mg, 0.25mmol) to give **<u>3u</u>** after flash chromatography (EtOAc:PE 10:1).

<u>Yield: 21.1mg (42%), colorless solid</u>

Trans: 10.4 mg (49.3%) *Cis:* 10.6mg (50.2%) mixture: 1mg (0.5%)

M.p.:

Trans: 182-183°C *Cis:* 115-118°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -291.25 [*c*=0.02, DMSO]	$\alpha_{[25]}$= +20.66[*c*=0.27, DMSO]

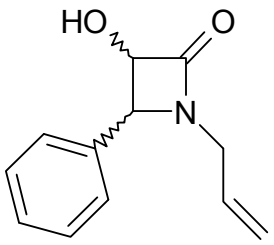
Rev. Phase HPLC:

Trans	49.5%	*Cis*	50.5%

Chiral HPLC:

Trans	39.5%	*Cis:*	60.5%
	SS 17.2%		SR 32.6%
	RR 22.3%		RS 27.9%
	ee 6		ee 16

3.5.3 1-Allyl-3-hydroxy-4-phenyl-azetidin-2-one (3a, $C_{14}H_{15}NO_3$)



3a

Method A: via **AV VII** using **4a** (10mg, 0.05mmol).

Rev. Phase HPLC:

Trans 4.3% *Cis* 95.7%

Retention time: 12.7min/11.9min (*trans/cis*)

Chiral HPLC:

Trans	5.9%	*Cis*	94.1%
	SS 5.9%		SR 49.8%
	RR - %		RS 44.3%
	ee 100		ee 6

Retention time: 19.2min/22.6min/32.3min/35.0min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4a** (50mg, 0.25mmol) to give **3a** after flash chromatography (EtOAc:PE 8:1).

Yield: 32.1mg (64%), colorless solid

Trans: 4.7 mg (14.6%) *Cis:* 27.3mg (85.0%) mixture: 1mg (3.1%)

M.p.:

Trans: -°C *Cis:* 55-56°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -	$\alpha_{[25]}$= +42.08[*c*=0.06, DMSO]

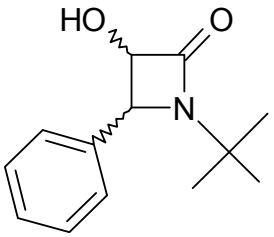
Rev. Phase HPLC:

Trans	14.8%	*Cis*	82.5%

Chiral HPLC:

Trans	18.4%	*Cis*	81.6%
	SS 8.9%		SR 39.3%
	RR 9.5%		RS 42.3%
	ee 14		ee 4

3.5.4 1-tert-Butyl-3-hydroxy-4-phenyl-azetidine-2-one (3b, $C_{13}H_{17}NO_2$)



3b

Method A: via **AV VII** using **4b** (10mg, 0.05mmol).

Rev. Phase HPLC:

Trans 13.1% *Cis* 86.9%

Retention time: 15.4/14.7min (*trans/cis*)

Chiral HPLC:

Trans	28.2%	*Cis*	71.8%
	SS 12.7%		SR 45.8%
	RR 15.5%		RS 26%
	ee 10		ee 28

Retention time: 17.8min/19.3min/24.4min/29.2min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4a** (50mg, 0.23mmol) to give **3b** after flash chromatography (EtOAc:PE 10:1).

Yield: 16mg (32%), colorless solid

Trans: 5.7mg (35.6%) *Cis:* 10.3mg (64.4%)

M.p.:

Trans: 92-93°C *Cis:* 173-175°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -266.67 [*c*=0.03, DMSO]	$\alpha_{[25]}$= +15.24 [*c*=0.32, DMSO]

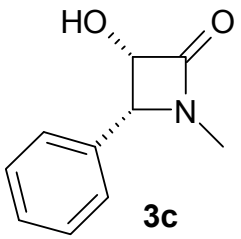
Rev. Phase HPLC:

Trans	27.1%	*Cis*	72.9%

Chiral HPLC:

Trans	27.1%	*Cis*	72.9%
	SS 3.7%		SR 43.8%
	RR 23.4%		RS 29.1%
	ee 85		ee 19

3.5.5 cis-3-Hydroxy-1-methyl-4-phenyl-azetidin-2-one (3c, $C_{10}H_{11}NO_2$)



3c

Method A: via **AV VII** using **4c** (10mg, 0.06mmol).

Rev. Phase HPLC:

Trans - % *Cis* 100 %

Retention time: -/8.0min (*trans/cis*)

Chiral HPLC:

Trans	- %	*Cis*	100 %
	SS - %		SR - %
	RR - %		RS 100%
	ee		ee 100

Retention time: -/-/-/53.5min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4c** (50mg, 0.29mmol) to give **3c** after flash chromatography (EtOAc:PE 10:1).

Yield: 35.3mg (70%), colorless solid

Trans: - *Cis*: 35.3mg (100%)

M.p.:

Trans: - *Cis:* 142-144°C

Specific rotation:

Trans: *Cis:*

$\alpha_{[25]}$= - $\alpha_{[25]}$= +68.0 [*c*=0.03, DMSO]

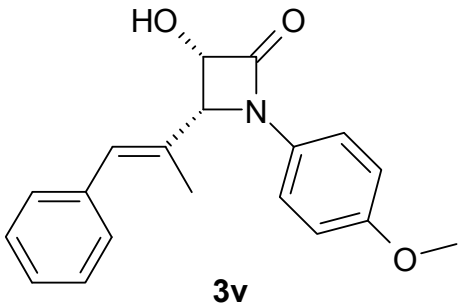
Rev. Phase HPLC:

Trans	- %	*Cis*	100%

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR - %
	RR - %		RS 100%
	ee		ee 100

3.5.6 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3v, $C_{19}H_{19}NO_3$)



3v

Method A: via **AV VII** using **4v** (10mg, 0.03mmol).

Rev. Phase HPLC:

Trans - % *Cis* 100 %

Retention time: - / 19.7min (*trans/cis*)

Chiral HPLC:

Trans	- %	*Cis*	100 %
	SS - %		SR 28.4%
	RR - %		RS 71.6%
	ee		ee 43

Retentiontime: -/-/31.9min/34.5min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4v** (50mg, 0.16mmol) to give **3v** after flash chromatography (EtOAc:PE 10:1).

Yield: 25.2mg (50%), orange solid

Trans: - *Cis*: 25.2mg (100%)

M.p.:

Trans: - *Cis:* 174-175°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -	$\alpha_{[25]}$= +7.61 [*c*=0.67, DMSO]

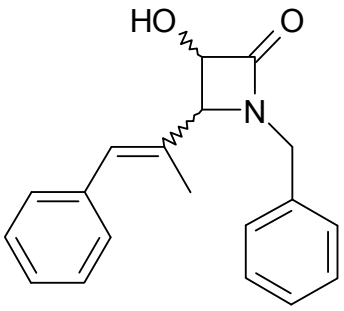
Rev. Phase HPLC:

Trans	- %	*Cis*	100%

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 30%
	RR - %		RS 70%
	ee		ee 40

3.5.7 1-Benzyl-3-hydroxy-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3d, $C_{19}H_{19}NO_2$)



3d

Method A: via **AV VII** using **4d** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans 8.8% *Cis* 91.2%

Retention time: 19.7min/19.6min (trans/cis)

Chiral HPLC:

Trans	7%	*Cis*	93%
	SS 0.8%		SR 72.9%
	RR 6.2%		RS 20.1%
	ee 79		ee 57

Retentiontime: 21.2min/22.2min/33.0min/36.2min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4d** (51mg, 0.18mmol) to give **3d** after flash chromatography (EtOAc:PE 6:1).

Yield: 41.3mg (80%), colorless solid

Trans: 0.7mg (1.3%) *Cis*: 40.6mg (98.7%)

Rev. Phase HPLC:

Trans 0.5% *Cis* 99.5%

M.p.:

Trans:- *Cis:* 134-136°C

Specific rotation:

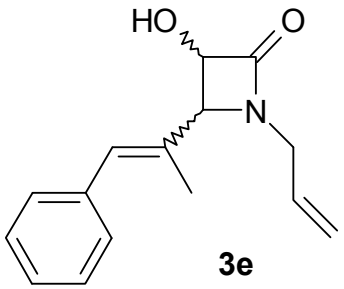
Trans: *Cis:*

$\alpha_{[25]}$= - $\alpha_{[25]}$= +0.74 [*c*=2.13, DMSO]

Chiral HPLC:

Trans	1 %	*Cis*	99%
	SS 0.2%		SR 70.4%
	RR 0.8%		RS 28.6%
	ee 68		ee 17

3.5.8 1-Allyl-3-hydroxy-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3e, $C_{15}H_{17}NO_2$)



3e

Method A: via **AV VII** using **4d** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans 49.5% *Cis* 50.5%

Retention time: 16.7min/16.5min (trans/cis)

Chiral HPLC:

Trans	47.8%	*Cis*	52.2%
	SS 1.7%		SR 30.5%
	RR 46.1%		RS 21.7%
	ee 93		ee 17

Retention time: 19.8/21.1/28.0/29.5min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4d** (50mg, 0.20mmol) to give **3d** after flash chromatography (EtOAc:PE 6:1).

Yield: 20.2mg (40%), colorless solid

Trans: 9.2mg (45.5%) *Cis:* 10.6mg (52.5%) mixture: 0.4mg (2%)

M.p.:

Trans:- *Cis:* 64-66°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -	$\alpha_{[25]}$= +2.86 [*c*=0.14, DMSO]

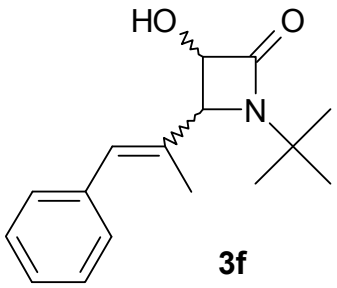
Rev. Phase HPLC:

Trans	46.6%	*Cis*	53.4%

Chiral HPLC:

Trans	52.2%	*Cis*	47.8%
	SS 1.7%		SR 25.3%
	RR 50.5%		RS 22.5%
	ee 95		ee 6

3.5.9 1-tert-Butyl-3-hydroxy-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3f, $C_{16}H_{21}NO_2$)



3f

Method A: via **AV VII** using **4f** (10mg, 0.05mmol).

Rev. Phase HPLC:

Trans - % *Cis* 100 %

Retention time: 18.7min/18.5min (trans/cis)

Chiral HPLC:

Trans	3.9%	*Cis*	96.1%
	SS 3.9%		SR 79.6%
	RR - %		RS 16.5%
	ee 100		ee 66

Retentiontime: 16.4min/17.4min/23.1min/24.9min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4f** (50mg, 0.19mmol) to give **3d** after flash chromatography (EtOAc:PE 8:1).

Yield: 15.5mg (31%), colorless solid

Trans: 0.3mg (2.1%) *Cis:* 15.2mg (97.9%)

M.p.:

Trans: - *Cis:* 130-132°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -	$\alpha_{[25]}$= -1.49 [*c*=1.03, DMSO]

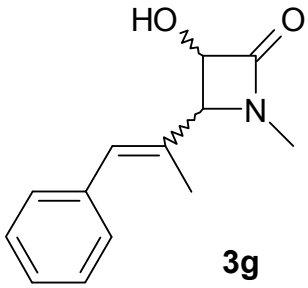
Rev. Phase HPLC:

Trans	1.6%	*Cis*	98.4%

Chiral HPLC:

Trans	2.8%	*Cis*	97.1%
	SS 2.8%		SR 58.5%
	RR - %		RS 38.7%
	ee 100		ee 25

3.5.10 3-Hydroxy-1-methyl-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3g, $C_{13}H_{15}NO_2$)



3g

Method A: via **AV VII** using **4g** (10mg, 0.05mmol).

Rev. Phase HPLC:

Trans 44.8% *Cis* 55.2 %

Retention time: 14.0min/13.6min (trans/cis)

Chiral HPLC:

Trans	35.8%	*Cis*	64.2%
	SS -%		SR 64.2%
	RR 35.8%		RS - %
	ee 100		ee 100

Retention time: -/39.1min/44.8/-min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4g** (50mg, 0.23mmol) to give **3g** after flash chromatography (EtOAc:PE 10:1).

Yield: 29mg (57%), colorless solid

Trans: 7mg (24.1%) *Cis*: 22mg (75.9%)

M.p.:

Trans: 148-150°C *Cis:* 121-124°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -46.67 [*c*=0.02, DMSO]	$\alpha_{[25]}$= +34.84 [*c*=0.41, DMSO]

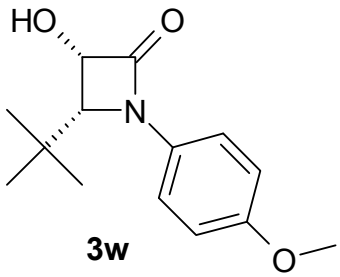
Rev. Phase HPLC:

Trans	41.5%	*Cis*	58.5%

Chiral HPLC:

Trans	28.1%	*Cis*	71.9%
	SS - %		SR 71.9%
	RR 28.1 %		RS - %
	ee 100		ee 100

3.5.11 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-tert-butyl-azetidin-2-one (3w, $C_{14}H_{19}NO_3$)



3w

Method A: via **AV VII** using **4w** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans 37.1% *Cis* 62.9%

Retention time: 18.4/17.2min (trans/cis)

Chiral HPLC:

Trans	28.9%	*Cis*	71.1%
	SS 9.5%		SR 23.3%
	RR 19.4%		RS 47.8%
	ee 34		ee 34

Retentiontime: 21.2/25.4/23.5/28.6min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4w** (50mg, 0.20mmol) to give **3w** after flash chromatography (EtOAc:PE 6:1).

Yield: 26.6mg (73%), colorless solid

Trans: 10mg (37.6) *Cis:* 16.6mg (62.4%)

M.p.:

Trans: - *Cis:* 202-207°C

Specific rotation:

Trans: *Cis:*

$\alpha_{[25]}$= - $\alpha_{[25]}$= -2.45 [*c*=0.36, DMSO]

Rev. Phase HPLC:

Trans	43.8 %	*Cis*	56.2%

Chiral HPLC:

Trans	45.2%	*Cis*	54.8%
	SS - %		SR 34.9%
	RR 45.2%		RS 19.9 %
	ee 100		ee 27

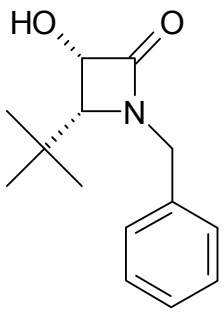
Literature found[108]:

Chiral HPLC:

Trans:	<44%	*Cis*	57%
	SS <1%		SR 2%
	RR 43 %		RS 55%
	ee		ee 93

[108] Yang, Y.; Kayser, M.M.; Rochon, F.D.; Rodriguez, S.; Stewart, J.D. *J. Molecular Catalysis B:Enzymatic* **2005**, *32*, 167-174

3.5.12 cis-1-Benzyl-4-tert-butyl-3-hydroxy-azetidin-2-one (3h, $C_{14}H_{19}NO_2$)



3h

Method A: via **AV VII** using **4h** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans - % *Cis* 100%

Retention time: -/16.90min (trans/cis)

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 55.8%
	RR - %		RS 44.2%
	ee		ee 12

Retentiontime: -/-/18.35min/25.42min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4h** (50mg, 0.22mmol) to give **3h** after flash chromatography (EtOAc:PE 6:1).

Yield: 16.4mg (33%), colorless solid

Trans: - *Cis*: 16.4mg (100%)

M.p.:

Trans: - *Cis:* 116-119°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -	$\alpha_{[25]}$= +9.54 [*c*=0.29, DMSO]

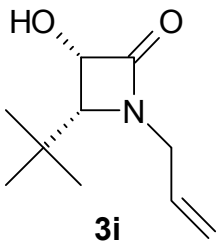
Rev. Phase HPLC:

Trans	- %	*Cis*	100%

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 56%
	RR - %		RS 44 %
	ee		ee 12

3.5.13 cis-1-Allyl-4-tert-butyl-3-hydroxy-azetidin-4-one (4i, $C_{10}H_{17}NO_2$)



3i

Method A: via **AV VII** using **4i** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans - % *Cis* 100%

Retention time: -/13.3min (trans/cis)

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 75.7%
	RR - %		RS 24.3%
	ee		ee 51

Retentiontime: -/-/18.8min/22.9min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4i** (50mg, 0.28mmol) to give **4i** after flash chromatography (EtOAc:PE 6:1).

Yield: 15.2mg (30%), colorless solid

Trans: - *Cis*: 15.2mg (100%)

M.p.:

Trans: - *Cis:* 82-85°C

Specific rotation:

Trans: *Cis:*

$\alpha_{[25]}$= - $\alpha_{[25]}$= +173.33 [*c*=0.03, DMSO]

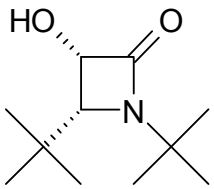
Rev. Phase HPLC:

Trans	- %	*Cis*	100%

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 61%
	RR - %		RS 39%
	ee		ee 21

3.5.14 1,4-di-tert-Butyl-3-hydroxy-azetidine-2-one (3j, $C_{11}H_{21}NO_2$)



3j

Method A: via **AV VII** using **4j** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans	- %	*Cis*	100%

Retention time: -/ 14.117 (*trans/cis*)

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 66.4%
	RR - %		RS 33.6%
	ee		ee 33

Retentiontime: -/-/15.783min/20.983min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4j** (50mg, 0.28mmol) to give **3i** after flash chromatography (EtOAc:PE 6:1).

Yield: 34.4mg (61%), colorless solid

Trans: -	*Cis:* 34.4mg (100%)

M.p.:

Trans: -	*Cis:* 213-215°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -	$\alpha_{[25]}$= -44.0 [*c*=0.03, DMSO]

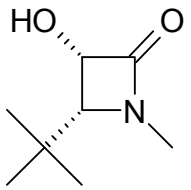
Rev. Phase HPLC:

Trans	%	*Cis*	100%

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 65%
	RR - %		RS 35%
	ee		ee 30

3.5.15 cis-4-tert-Butyl-3-hydroxy-1-methyl-azetidine-2-one (3k, $C_8H_{15}NO_2$)



3k

Method A: via **AV VII** using **4k** (10mg, 0.06mmol).

Rev. Phase HPLC:

Trans - % *Cis* 100%

Retention time: -/9.7min (trans/cis)

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 94.4%
	RR - %		RS 5.6%
	ee		ee 89

Retention time: -/-/25.2min/28.8min (SS/RR/SR/RS)

Method B: via **AV VIII** using **4k** (50mg, 0.28mmol) to give **3k** after flash chromatography (EtOAc:PE 6:1).

Yield: 13.3mg (27%), colorless solid

Trans: - *Cis*: 13.3mg (100%)

M.p.:

Trans: - *Cis:* 148-150°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -	$\alpha_{[25]}$= +15.24 [*c*=0.32, DMSO]

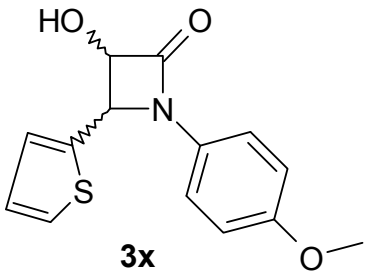
Rev. Phase HPLC:

Trans	- %	*Cis*	100%

Chiral HPLC:

Trans	- %	*Cis*	100%
	SS - %		SR 83.6%
	RR - %		RS 16.4%
	ee		ee 64

3.5.16 3-Hydroxy-1-(4-methoxyphenyl)-4-(2-thienyl)-azetidin-2-one (3x, $C_{14}H_{13}NO_3S$)



3x

Method A: via **AV VII** using **4x** (10mg, 0.03mmol).

Rev. Phase HPLC:

Trans 37.1 % *Cis* 62.9%

Retention time: 14.1min/13.5min (trans/cis)

Chiral HPLC:

Trans	37.2%	*Cis*	62.8%
	SR 1.7%		SS 11.6%
	RS 35.5%		RR 51.2%
	ee 91		ee 63

Retention time: 30.1min/32.08min/44.6min/47.8min (SR/RS/SS/RR)

Method B: via **AV VIII** using **4x** (50mg, 0.17mmol) to give **3x** after flash chromatography (EtOAc:PE 4:1).

Yield: 35.3mg (70%), colorless solid

Trans: 9.6mg (27.2%) *Cis*: 10.9mg (30.9%) mixure: 14.8mg (41.9%)

M.p.:

Trans: 104-105°C *Cis:* 175-177°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -13.14 [*c*=0.18, DMSO]	$\alpha_{[25]}$= +39.76 [*c*=0.21, DMSO]

Rev. Phase HPLC:

Trans	39.1%	*Cis*	60.9%

Chiral HPLC:

Trans	47.3%	*Cis*	52.7%
	SR 10.5%		SS 13.7%
	RS 36.7%		RR 39%
	ee 55		ee 48

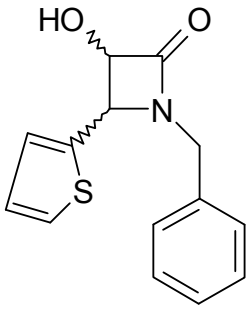
Literature found[109]:

Chiral HPLC:

Trans:	<38%	*Cis:*	63%
	SR <1%		SS 12%
	RS 37 %		RR 51%

[109] Yang, Y.; Kayser, M.M.; Rochon, F.D.; Rodriguez, S.; Stewart, J.D. *J. Molecular Catalysis B:Enzymatic* **2005**, *32*, 167-174

3.5.17 1-Benzyl-3-hydroxy-4-thiophen-2-yl-azetidin-2-one (3l, $C_{14}H_{13}NO_2S$)



3l

Method A: via **AV VII** using **4l** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans	45.8%	*Cis*	54.2%

Retention time: 14.2min/13.6min (trans/cis)

Chiral HPLC:

Trans	38%	*Cis*	62%
	SR 11.1%		SS 50.2%
	RS 26.9%		RR 11.8%
	ee 41		ee 62

Retention time: //37.0min/44.3min(SR/RS/SS/RR)

Method B: via **AV VIII** using **4l** (50mg, 0.22mmol) to give **3l** after flash chromatography (EtOAc:PE 4:1).

Yield: 29.8mg (59%), colorless solid

Trans: 8.4mg (28.2%) *Cis:* 11.2mg (37.6%)

M.p.:

Trans: 98-100°C *Cis:* 80-82°C

Specific rotation:

Trans: *Cis:*

$\alpha_{[25]}$= +19.33 [*c*=0.12, DMSO] $\alpha_{[25]}$= +50.83 [*c*= 0.23, DMSO]

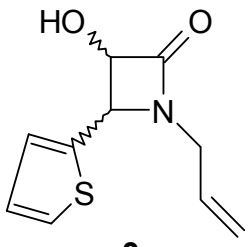
Rev. Phase HPLC:

Trans	43.3%	*Cis*	56.7%

Chiral HPLC:

Trans	41%	*Cis*	59%
	SR 3.8%		SS 39.9%
	RS 37.2%		RR 19.1%
	ee 82		ee 35

3.5.18 1-Allyl-3-hydroxy-4-thiophen-3-yl-azetidin-2-one (3m, $C_{10}H_{11}NO_2S$)



3m

Method A: via **AV VII** using **4m** (10mg, 0.05mmol).

Rev. Phase HPLC:

Trans	40.9%	*Cis*	59.1%

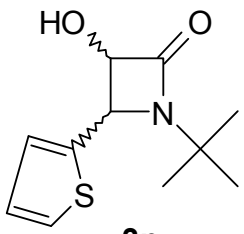
Retention time: 10.3min/9.6min (trans/cis)

Chiral HPLC:

Trans	39.4%	*Cis:*	60.6%
	SR 26.9%		SS 48.1%
	RS 12.5%		RR 12.5%
	ee 36		ee 59

Retention time: 20.5min/22.6min/35.6min/40.0min (SR/RS/SS/RR)

3.5.19 1-tert-Butyl-3-hydroxy-4-thiophen-2-yl-azetidine-2-one (3n, $C_{11}H_{15}NO_2S$)



3n

Method A: via **AV VII** using **4n** (10mg, 0.05mmol).

Rev. Phase HPLC:

Trans 47.4% *Cis* 52.6%

Retention time: 13.0min/12.1min (trans/cis)

Chiral HPLC:

Trans	45.5%	*Cis*	54.5%
	SR - %		SS 49.3%
	RS 45.5%		RR 5.2%
	ee 100		ee 81

Retention time: -/23.4min/28.3min/37.9min(SR/RS/SS/RR)

Method B: via **AV VIII** using **4n** (50mg, 0.22mmol) to give **3x** after flash chromatography (EtOAc:PE 4:1).

Yield: 47.8mg (98%), colorless solid

Trans: 12.4mg (25.9%) *Cis*: 35.4mg (74.1%)

M.p.:

Trans: 105-106°C *Cis:* 195-197°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= -32.20 [*c*=0.30, DMSO]	$\alpha_{[25]}$= +69.09 [*c*=0.33, DMSO]

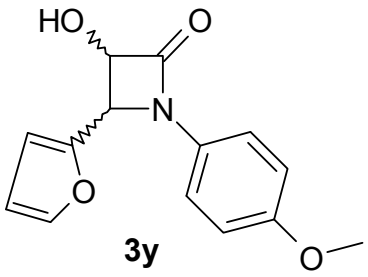
Rev. Phase HPLC:

Trans	25.9%	*Cis*	74.1%

Chiral HPLC:

Trans	25.9%	*Cis*	74.1%
	SR 0.5%		SS 60.9%
	RS 25.4%		RR 13.1%
	ee 96		ee 64

3.5.20 3-Hydroxy-1-(4-methoxyphenyl)-4-(2-furan)-2-azetidinone (3y, $C_{14}H_{13}NO_4$)



3y

Method A: via **AV VII** using **4y** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans 46.8% *Cis* 53.2%

Retention time: 13.0min/12.4min (trans/cis)

Chiral HPLC:

Trans	42.6%	*Cis*	57.4%
	RS 3.1%		SS 12%
	SR 39.5%		RR 45.5%
	ee 85		ee 58

Retentiontime: 31.7min/32.9min/45.7min/50.7min (RS/SR/SS/RR)

Method B: via **AV VIII** using **4y** (50mg, 0.19mmol) to give **3y** after flash chromatography (EtOAc:PE 4:1).

Yield: 33.5mg (66%), colorless solid

Trans: 5.7mg (17%) *Cis:* 19.7mg (58.8%) mixture: 8.1mg (24.2%)

M.p.:

Trans: 94-95°C *Cis:* 156-159°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= +30.56 [*c*=0.16, DMSO]	$\alpha_{[25]}$= +56.62 [*c*=0.36, DMSO]

Rev. Phase HPLC:

Trans	31%	*Cis*	69%

Chiral HPLC:

Trans	49.1%	*Cis*	50.9%
	RS 34.4%		SS 15.6%
	SR 17.1%		RR 35.3%
	ee 37		ee 38

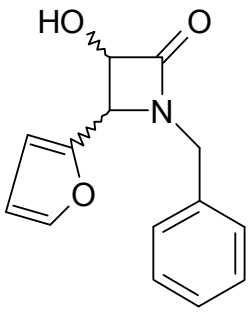
Literature found[110]:

Chiral HPLC:

Trans:	<41%	*Cis:*	59%
	RS 40%		SS 18%
	SR <1%		RR 41%

[110] Yang, Y.; Kayser, M.M.; Rochon, F.D.; Rodriguez, S.; Stewart, J.D. *J. Molecular Catalysis B:Enzymatic* **2005**, *32*, 167-174

3.5.21 1-Benzyl-4-furan-2-yl-3-hydroxy-azetidin-2-on (3p, $C_{14}H_{13}NO_3$)



3p

Method A: via **AV VII** using **4p** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans 46.5% *Cis* 53.5%

Retention time: 14.2min/13.6min (trans/cis)

Chiral HPLC:

Trans	38.1%	*Cis*	61.9%
	RS 35.2%		SS 46.4%
	SR 2.9%		RR 15.5%
	ee 85		ee 50

Retentiontime: 19.1min/23.6min/36.6min/46min (RS/SR/SS/RR)

Method B: via **AV VIII** using **4p** (50mg, 0.20mmol) to give **3p** after flash chromatography (EtOAc:PE 4:1).

Yield: 32.6mg (65%), colorless solid

Trans: 7.1mg (21.8%) Cis: 21.2mg (65.0%) mixture: 4.3mg (13.2%)

M.p.:

Trans: 130-132°C *Cis:* 73-75°C

Specific rotation:

Trans:	*Cis:*
$\alpha_{[25]}$= +44.40 [*c*=0.08, DMSO]	$\alpha_{[25]}$= +47.68 [*c*=0.47, DMSO]

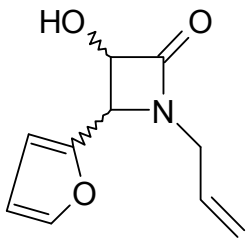
Rev. Phase HPLC:

Trans	31.7%	*Cis*	68.3%

Chiral HPLC:

Trans	23.2%	*Cis*	76.8%
	RS 15.6%		SS 56.1%
	SR 7.6%		RR 20.7%
	ee 35		ee 52

3.5.22 1-Allyl-4-furan-3-yl-hydroxy-azetidin-2-one (3q, $C_{10}H_{11}NO_3$)



3q

Method B: via **AV VIII** using **4q** (50mg, 0.26mmol) to give **3q** after flash chromatography (EtOAc:PE 6:1).

Yield: 34.8mg (69%), colorless solid[111]

Rev. Phase HPLC:

Trans	47.6%	*Cis*	53.4%

Retention time: 12.2min/11.3min (trans/cis)

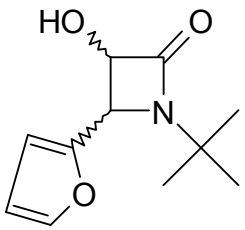
Chiral HPLC:

Trans	53.3%	*Cis*	46.7%
	RS 31.2%		SS 31.4%
	SR 22.1%		RR 15.3%
	ee 34		ee 35

Retention time: 19.0min/22.7min/36.3min/41.0min (RS/SR/SS/RR)

[111] No separation between cis and trans

3.5.23 1-tert-Butyl-4-furan-2-yl-3-hydroxy-azetidin-2-one (3r, $C_{11}H_{15}NO_3$)



3r

Method A: via **AV VII** using **4r** (10mg, 0.04mmol).

Rev. Phase HPLC:

Trans 54.8% *Cis* 45.2%

Retention time: 11.6min/10.5min (*trans/cis*)

Chiral HPLC:

Trans	59.5%	*Cis*	40.5%
	RS 15.8%		SS 33.6%
	SR 43.6%		RR 7%
	ee 47		ee 66

Retention time: 18.7min/20.9min/27.6min/36.1min (RS/SR/SS/RR)

Method B: via **AV VIII** using **4r** (50mg, 0.24mmol) to give **3r** after flash chromatography (EtOAc:PE 6:1).

Yield: 49.2mg (97%), yellow solid

Trans: 13.1mg (26.6%) *Cis*: 29.6mg (60.2%) mixture: 6.5 (13.2%)

M.p.:

Trans: 116-117°C *Cis:* 134-135°C

Specific rotation:

Trans: *Cis:*

$\alpha_{[25]}$= +13.33 [*c*=0.14, DMSO] $\alpha_{[25]}$= +27.03 [*c*=0.15, DMSO]

Rev. Phase HPLC:

Trans	54.8%	*Cis*	45.2%

Chiral HPLC:

Trans	53.6%	*Cis*	46.4%
	RS 20.5%		SS 38.4%
	SR 33.1%		RR 8%
	ee 24		ee 66

3.6 Screening of Overexpressed Yeast Reductases in E. coli (whole cell)

3.6.1 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-phenyl-azetidin-2-on (3t, $C_{16}H_{15}NO_3$)

Expression strain with pIK 2-32 was grown on LB_{kan} agar plates for 15-20 hours at 37°C, then LB_{kan}-media (10mL) was inoculated with a single colony and incubated in a baffled Erlenmeyer flask at 37°C for 1 day in an orbital shaker. These experiments were done in 24 well plates: therefore, to 1mL of the preculture, 20mg educt **4t** dissolved in 150µL dioxane and 4 µL IPTG stock was added. The reaction control was performed by reverse phase HPLC analysis; values are given for 72h conversion times.

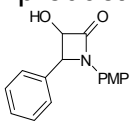
product 	pIK i	conv. HPLC	rev. phase HPLC	
			trans	cis
3t	pIK 3	25.4	56.6	43.4
3t	pIK 4	4.0	32.9	67.1
3t	pIK 5	16.3	46.7	53.3
3t	pIK 6	20.0	73.6	26.4
3t	pIK 7	24.4	38.7	61.3
3t	pIK 8	20.0	42.2	57.8
3t	pIK 9	6.7	-	100
3t	pIK 10	19.7	37.3	62.7
3t	pIK 11	20.9	38.2	61.8
3t	pIK 12	21.1	40.0	60.0
3t	pIK 13	42.8[112]	54.4	45.6
3t	pIK 15	24.1	41.8	58.2
3t	pIK 18	24.5	44.9	55.1
3t	pIK 23	22.3	46	54
3t	pIK 25	27.1	46.5	53.5
3t	pIK 29	30.7	48.2	51.8
3t	pIK 30	22.3	48.5	51.5
3t	pIK 31	18.5	50.1	49.9
3t	pIK 32	22.4	49.3	50.7
3t	pAKS1	21.9	49.8	50.2
3t	pTM3	17.3	33.3	66.7

table 8

[112] The spectra of pIK13 could not be exact integrate, because the peaks were not baseline separated.

3.6.2 cis-3-Hydroxy-1-(4-methoxyphenyl)-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (**3v**, $C_{19}H_{19}NO_3$)

Expression strain with pIK 2-32 was grown on LB_{kan} agar plates for 15-20 hours at 37°C, then LB_{kan}-media (10mL) was inoculated with a single colony and incubated in a baffled Erlenmeyer flask at 37°C for 1 days in an orbital shaker. These experiments were done in 24 well plates: therefore, to 1mL of the preculture, 20mg educt **4v** dissolved in 150µL dioxane and 4 µL IPTG stock was added. The reaction control was performed by reverse phase HPLC analysis; values are given for 72h conversion times.

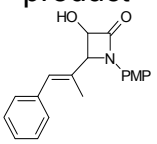
product 	pIK i	conv. HPLC	rev. phase HPLC	
			trans	cis
3v	pIK 3	31.9	38.4	61.6
3v	pIK 4	21.6	44.2	55.8
3v	pIK 5	20.6	44.1	55.9
3v	pIK 6	11.8	62.7	37.3
3v	pIK 7	21.8	49.8	50.2
3v	pIK 8	19.8	46.5	53.5
3v	pIK 9	18.5	48.5	51.5
3v	pIK 10	12.9	44.9	55.1
3v	pIK 11	20.6	48.9	51.1
3v	pIK 12	19.9	48.9	51.1
3v	pIK 13	24.3	45.6	54.4

table 9

3.7 Purification of Yeast reductases overexpressed in *E. Coli*

3.7.1 Biomass production

Fresh plates of engineered *E. coli* strains were streaked from the appropriate frozen stock, and a single colony was used to inoculate 10 mL of LB_{kan} in a 50 mL Erlenmeyer flask. The culture was incubated in a rotary shaker overnight (37°C, 120 r.p.m.). A sample of this culture (2 mL) was used to inoculate 200 mL of fresh LB_{kan} in a 1 L baffled Erlenmeyer flask. The culture was grown under the same conditions for 2.5 h, then 2.7 mL 20% glucose and 40 µL IPTG were added (37°C, 120 r.p.m.). After 2 hours, additional glucose solution (2.7 mL) was added (37°C, 120 r.p.m.). After 8h incubation time, the culture was harvested: cells were separated by centrifugation (4000 r.p.m., 4°C, 20 min), re-suspended in 4 mL PBS-buffer + Triton and stored overnight at 4°C.
According to this procedure, approximately 1.5 g of recombinant cells were collected.

3.7.2 Purification of Yeast Reductases Overexpressed in *E. coli*

After addition of 4 µL of protease solution (1000*Pepstatin A, 1000*Leupeptin, 1000*PMSF), the cells were broken by sonication (Bandelin Sonopuls HP3200: 4°C, 2*2min, energy 70% and 50 cycles). The samples were centrifuged (10000 r.p.m., 4°C, 10 min) and supernatants were filtered (0.2µm) and stored at 4°C. Protein measurements were run according to the general Bradford protocol. It includes 200 µL Bradford reagents, 800 µL dist. water and 5-10 µL sample. The extinction was measured on a photometer (595nm). The indicated value has to be in the range between 0.1-0.5.

The 2 cm column filled with glutathione sepharose was washed with 6 mL of equilibration buffer. Then the supernatant was administered to the column and a slow flow (3 drops /min) was adjusted. Then the column was washed 3 times with 1.5 mL PBS/Triton buffer and dried between each washing. The elution was started with the elution buffer and 500µL fractions were collected. Six fractions were sufficient to

collect the desired enzyme. Protein measurements were run according to the Bradford protocol. The samples containing the largest protein amounts were stored at –20°C after addition of 100 µL glycerol.

Before storage at 4°C, the column was then washed with 1.5 mL buffer R1 and buffer R2 in alternately (5 times), 3 mL EtOH 70% and 3 ml 1*PBS/Triton-Buffer.

Fraction pIK	1	2	3	4	5	6	7
2	8.89	26.97	18.79	8.07			
3		1.81	2.09	0.68			
4 (I)	14.77	37.75	19.71	8.64			
4 (II)	1.877	3.095	4.5914	2.5904	1.3202	1.268	
5	0.35	0.82					
6		0.9648	0.746	0.6938			
7	7.46	1.46	0.7				
8	7.149	7.532	2.59	1.35			
9	15.35	15.50	1.88	2.15			
12	2.3294	9.8114	11.7428	3.3560			
13	3.5996	9.9854	7.0622	4.9568	3.5996	2.7644	
15		0.329	0.262	0.156	0.196	0.162	
29	3.0602	3.4778	3.3734	2.7818	2.8166	2.9558	2.834
30	5.096	5.7398	5.0612	3.5648	2.7122	2.834	2.5034
31	3.0254	2.7818	1.9118	1.6508	1.355		
32	3.4778	5.096	4.8002	3.9128	2.9732	2.8166	

Protein concentrations [mg/2mL] for collected samples according Bradford protocol

The SDS samples were prepared as following, denaturated at 95°C during 5 minutes and stored at 4°C.

piK 2	Total protein	(1:5) 1 µL enzym + 5 µL 2*Loading Buffer
		(1:5) 1 µL enzym + 5 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	2/1	(1:3) 5 µL enzym + 15 µL 2*Loading Buffer
	2/2	(1:10) 5 µL enzym + 50 µL 2*Loading Buffer
	2/3	(1:10) 5 µL enzym + 50 µL 2*Loading Buffer
	2/4	(1:3) 5 µL enzym + 15 µL 2*Loading Buffer
piK 3	Total protein	(1:5) 1 µL enzym + 5 µL 2*Loading Buffer
		(1:5) 1 µL enzym + 5 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	3/1	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	3/2	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	3/3	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer

piK 4	Total protein	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
		(1:5) 1 µL enzym + 5 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	4/1	(1:6) 5 µL enzym + 30 µL 2*Loading Buffer
	4/2	(1:19) 5 µL enzym + 95 µL 2*Loading Buffer
	4/3	(1:9) 5 µL enzym + 45 µL 2*Loading Buffer
	4/4	(1:3) 5 µL enzym + 15 µL 2*Loading Buffer
pIK 5 (1st run)	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	5/1	(3:1) 15 µL enzym + 5 µL 2*Loading Buffer
	5/2	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
pIK 5 (2nd run)	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	5'/1	(3:1) 15 µL enzym + 5 µL 2*Loading Buffer
	5'/2	(3:1) 15 µL enzym +5 µL 2*Loading Buffer
pIK 6	Total protein	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
		(1:5) 1 µL enzym + 5 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	6/1	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	6/2	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	6/3	(3:1) 15 µL enzym + 5 µL 2*Loading Buffer
	6/4	(3:1) 15 µL enzym + 5 µL 2*Loading Buffer
pIK 7	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	7/1	(1:3) 5 µL enzym + 15 µL 2*Loading Buffer
	7/2	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
piK 8	Total protein	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
		(1:5) 1 µL enzym + 5 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	8/1	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	8/2	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	8/3	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	8/4	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	8/5	(3:1) 15 µL enzym + 5 µL 2*Loading Buffer
	8/6	(3:1) 15 µL enzym + 5 µL 2*Loading Buffer
piK 9	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	9/1	(1:9) 1 µL enzym + 9 µL 2*Loading Buffer
	9/2	(1:9) 1 µL enzym + 9 µL 2*Loading Buffer
piK 12	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	12/1	(1:1) 5 µL enzym + 10 µL 2*Loading Buffer
	12/2	(1:4) 5 µL enzym + 20 µL 2*Loading Buffer
	12/3	(1:4) 5 µL enzym + 20 µL 2*Loading Buffer
	12/4	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
piK 13	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	13/1	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
	13/2	(1:3) 5 µL enzym + 15 µL 2*Loading Buffer
	13/3	(1:3) 5 µL enzym + 15 µL 2*Loading Buffer

	13/4	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
	13/5	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
	13/6	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
piK 15	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 5 µL 2*Loading Buffer
	15/2	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
	15/3	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
	15/4	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	15/5	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	15/6	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
piK 29	Total protein	(1:5) 1 µL enzym + 5 µL H2O + 5 µL 2*Loading Buffer
		(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
	29/1	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	29/2	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	29/3	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	29/4	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
piK 30	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 10 µL 2*Loading Buffer
	30/1	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
	30/2	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
	30/3	(1:2) 5 µL enzym + 10 µL 2*Loading Buffer
	30/4	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
piK 31	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 10 µL 2*Loading Buffer
	31/1	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	31/2	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	31/3	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	31/4	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
piK 32	Total protein	(1:4) 1 µL enzym + 4 µL H2O + 5 µL 2*Loading Buffer
		(1:9) 1 µL enzym + 9 µL H2O + 10 µL 2*Loading Buffer
	32/1	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	32/2	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	32/3	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer
	32/4	(1:1) 5 µL enzym + 5 µL 2*Loading Buffer

Table 1: Preparation of the SDS samples for electrophoresis

The electrophoresis was run by using 5 mL SDS-Page samples previously prepared and prestained marker with 1*Elution buffer (dilution of 10*Elution buffer) at 200 V (≅ 70mV). After the migration (from 45min to 75 min), the collecting gel was removed and the elution gel was transferred into the staining solution during 20 min. Afterwards, the gel was transferred to the bleaching solution until the contrast between the protein (in blue) and the background (nearly colorless) was sufficient (Table 2). The bleaching solution was changed every 10 min.

3.7.3 Ketone Reduction with Purified GST-fusion Proteins

GP X:
The reaction mixture were implemented in 24 well plates containing β-lactam substrate (10mM), purified GST-fusion protein (20-200µL, including 10-100µg of protein), NADP (0.40µmoles, 0.30mg), glucose-6-phosphate (0.28µmoles, 8.6mg), glucose-6-phosphat dehydrogenase (10µg) in 2.0mL of 100mM KP_i, pH 7.0 (1M K_2HPO_4 + 1M KH_2PO_4). Reactions were incubated at 30°C for 48h.
700µL of the reaction solution were extracted with 700µL EtOAc, dried with Na_2SO_4 and sampled for HPLC.

For better handling Stock solutions of the different substrates were used. Every experiment was “double-made” to overcome failures.
NADP solution (solid):

0.30mg x 35 slots=10.5mg

10.5mg diluted in 800 µL

0.30mg → 22.86 µL / multi-well

glucose-6-phosphat solution (solid):

8.6mg x 35 slots= 301mg

301mg diluted in 800 µL

8.6mg → 22.86 µL/ multi-well

glucose-6-phosphat dehydrogenase solution (solution: 1ml=14.3mg):

10µg = 0.010mg x 35 slots= 0.35mg

1ml = 14.3mg

0.0245ml=24.5 µL → 0.35mg

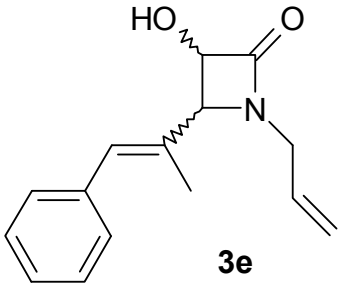
24.5µL diluted in 800 µL

0.7 µL → 22.86 µL/ multi-well

GST-fusion protein:

pIK i	**µL**	**µg**
pIK 2/2	2 µL	48.95 µg
pIK 3/3	20 µL	34.82 µg
pIK 4/3 (I)	2 µL	39.71µg
pIK 4/3 (II)	10 µL	45.91µg
pIK 5/2 (I)	50 µL	40.00µg
pIK 5/2 (II)	100 µL	39.02µg
pIK 6/2	40 µL	38.59µg
pIK 7/1	5 µL	37.30µg
pIK 8/2	5 µL	37.66µg
pIK 9/2	3 µL	46.5µg
pIK 12/3	3 µL	35.22µg
pIK 13/2	4 µL	39.92µg
pIK 15/2	130 µL	42.77µg
pIK 29/1	12 µL	41.73µg
pIK 30/2	7 µL	40.18µg
pIK 31/2	12 µL	36.30µg
pIK 32/2	8 µL	40.77µg

3.7.3.1 1-Allyl-3-hydroxy-4-(3-phenylprop-2-en-2-yl)-azetidin-2-one (3e, $C_{15}H_{17}NO_2$)

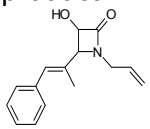


3e

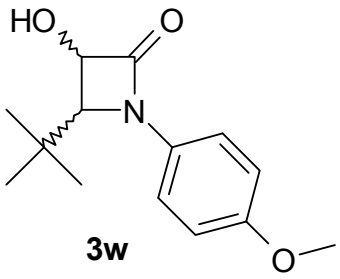
For better handling (the scale is small), also the educts were diluted in dioxane:

2.49mg (20µL stock solution in pK_i-buffer) per multi-well (2mL reaction volume)

In 2ml PK_i buffer, precursor **4e** (20 µL, 2.49mg) were reduced with the different pIK i according to **GP X**.

product 	pIK i	conv. HPLC	rev. phase HPLC	
			trans	cis
3e	pIK 2	5.9	11.5	88.5
3e	pIK 3	5	21	79
3e	pIK 4	11	53.6	46.4
3e	pIK 5	6.3	13.1	86.9
3e	pIK 6	6.3	16.1	83.9
3e	pIK 7	5.8	10.9	89.1
3e	pIK 8	6.5	10.1	89.9
3e	pIK 9	5.2	13	87
3e	pIK 12	5.7	9.9	90.1
3e	pIK 13	4.5	8.6	91.4
3e	pIK 15	4.8	10.1	89.9
3e	pIK 29	5	11.8	88.2
3e	pIK 30	17.4	79.4	20.6
3e	pIK 31	4.7	17.8	82.2
3e	pIK 32	4.8	11.3	88.7

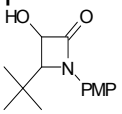
3.7.3.2 3-Hydroxy-1-(4-methoxyphenyl)-4-tert-butyl-azetidin-2-one (**3w**,$C_{14}H_{19}NO_3$)

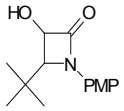


3w

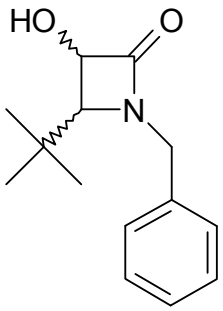
For better handling (the scale is small), also the educts were diluted in dioxane:
2.47mg (20µL stock solution in pK_i-buffer) per multi-well (2mL reaction volume)

In 2ml PK_i buffer, precursor **4w** (20 µL, 2.47mg) were reduced with the different pIK I according to **GP X**.

product 	pIK i	rev. Phase HPLC		
		conv. HPLC	trans	cis
3w	pIK 2	73.2	52.4	47.6
3w	pIK 3	9.4	28	72
3w	pIK 4	9.1	33.6	66.4
3w	pIK 5	8.1	31.5	68.5
3w	pIK 6	9.7	28.2	71.8
3w	pIK 7	10.1	30.5	69.5
3w	pIK 8	76.5	77.7	22.3
3w	pIK 9	9.5	31.8	68.2
3w	pIK 12	8.3	31.4	68.6
3w	pIK 13	10.3	26.3	73.7
3w	pIK 15	9.6	31	69
3w	pIK 29	9.6	30	70
3w	pIK 30	14.6	48.8	51.2
3w	pIK 31	11.9	30	70
3w	pIK 32	11.9	30.7	69.3

str. Phase HPLC									
Product 	pIK i	trans 3R,4R	trans 3S, 4S	ee	TRANS	cis 3S,4R	cis 3R,4S	ee	CIS
3w	pIK 2	56.4	-	100	56.4	24.7	18.9	13	43.6
3w	pIK 8	-	73.6	100	73.6	1.3	25.1	90	26.4

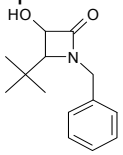
3.7.3.3 1-Benzyl-4-tert-butyl-3-hydroxy-azetidin-2-one (3h, $C_{14}H_{19}NO_2$)

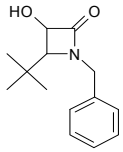


3h

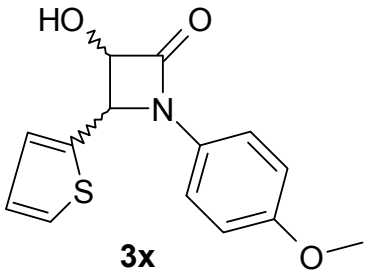
For better handling (the scale is small), also the educts were diluted in dioxane:
2.31mg (20µL stock solution in pK_i-buffer) per multi-well (2mL reaction volume)

In 2ml PK_i buffer, precursor **4h** (20 µL, 2.31mg) were reduced with the different pIK i according to **GP X**.

product 	pIK i	rev. Phase HPLC		
		conv. HPLC	TRANS	CIS
3h	pIK 2	94.7	51.8	48.2
3h	pIK 3	2.9	-	100
3h	pIK 4	1.2	-	100
3h	pIK 5	1.3	-	100
3h	pIK 6	1.5	-	100
3h	pIK 7	1.2	-	100
3h	pIK 8	95.3	51.7	48.3
3h	pIK 9	1.5	-	100
3h	pIK 12	4.61	-	100
3h	pIK 13	18.52	4.8	95.2
3h	pIK 15	1.7	-	100
3h	pIK 29	0.8	33.2	66.8
3h	pIK 30	5.5	46.4	53.6
3h	pIK 31	1.7	41	59
3h	pIK 32	1.9	37.1	62.9

str. Phase HPLC									
Product 	pIK i	trans RR	trans SS	ee	TRANS	cis SR	cis RS	ee	CIS
3h	pIK 2	51.5	-	100	51.5	48.5	-	100	48.5
3h	pIK 8	52.9	-	100	52.9	47.1	-	100	47.1
3h	pIK 13	6.3	-	100	6.3	93.7	-	100	93.7

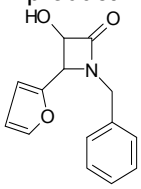
3.7.3.4 3-Hydroxy-1-(4-methoxyphenyl)-4-(2-thienyl)-azetidin-2-on (3x,$C_{14}H_{13}NO_3S$)



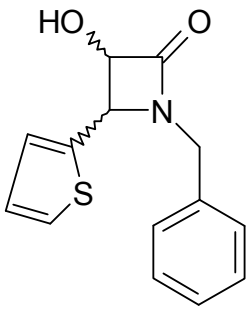
3x

For better handling (the scale is small), also the educts were diluted in dioxane:
2.89mg (20µL stock solution in pK_i-buffer) per multi-well (2mL reaction volume)

In 2mL PKi buffer, precursor **4x** (20 µL, 2.89mg) were reduced with the different pIK i according to GP X.

product 		rev. Phase HPLC			str. Phase HPLC							
	pIK i	conv. HPLC	trans	cis	trans SR	trans RS	ee	TRANS	cis SS	cis RR	ee	CIS
3x	pIK 2	100	37.2	62.8	36.8	8.0	64	44.8	4.6	50.6	83	55.2
3x	pIK 3	100	88.1	11.9	46.7	39.2	9	85.9	-	14.1	100	14.1
3x	pIK 4	100	22.1	77.9	3.2	30.1	81	33.3	4.3	62.4	9	66.7
3x	pIK 5	94.1	83.2	16.8	27.2	60.8	38	88	12	-	100	12
3x	pIK 6	94.5	80.4	19.6	54.5	28.6	31	83.1	16.9	-	100	16.9
3x	pIK 7	85.3	92.1	7.9	32.4	57.3	26	89.7	10.3	-	100	10.3
3x	pIK 8	99.8	50	50	56.9	-	100	56.9	-	43.1	100	43.1
3x	pIK 9	96.6	98.2	1.8	37.4	54.3	18	91.7	-	8.3	100	8.3
3x	pIK 12	99.7	46	54	2.5	44.2	89	46.7	49.3	4	85	53.3
3x	pIK 13	99.8	53.1	46.9	3.3	52.1	93	55.4	42.7	1.9	95	44.6
3x	pIK 15	94.6	90.6	9.4	21.4	65.5	51	86.9	-	13.1	100	13.1
3x	pIK 29	94.4	94.9	5.1	55.4	36.1	21	91.5	-	8.5	100	8.5
3x	pIK 30	96.7	65.3	34.7	14.3	54.1	58	68.4	16.7	14.9	5	31.6
3x	pIK 31	93.4	95.1	4.9	45.8	41.1	5	86.9	13.1	-	100	13.1
3x	pIK 32	92.1	92.7	7.3	61	35.6	26	96.6	-	3.4	100	3.4

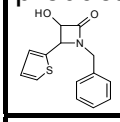
3.7.3.5 1-Benzyl-3-hydroxy-4-thiophen-2-yl-azetidin-2-on (3l , $C_{14}H_{13}NO_2S$)



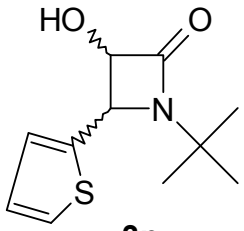
3l

For better handling (the scale is small), also the educts were diluted in dioxane:
2.57mg (20µL stock solution in pK_i-buffer) per multi-well (2mL reaction volume)

In 2ml PK_i buffer, precursor **4l** (20 µL, 2.57mg) were reduced with the different pIK i according to **GP X**.

product 	pIK i	rev. Phase HPLC			str. Phase HPLC							
		conv. HPLC	trans	cis	trans SR	trans RS	ee	TRANS	cis SS	cis RR	ee	CIS
3l	pIK 2	94.4	32.1	67.9	8.4	31.1	58	39.5	42.6	17.9	41	60.5
3l	pIK 3	89.3	51.5	48.5	7.5	39	68	46.5	38.7	14.8	45	53.5
3l	pIK 4	100	28.3	71.7	7.9	30.5	60	38.4	40.6	21	31	61.6
3l	pIK 5	87.9	59.9	40.1	7.9	43.2	69	51.1	45.3	3.6	85	48.9
3l	pIK 6	89.6	51.5	48.5	29.8	19.3	22	49.1	45.6	4.4	81	50.9
3l	pIK 7	87.7	59	41	8.2	52.5	73	60.7	33.4	4.9	67	39.3
3l	pIK 8	98.9	33.7	66.3	4.7	35.9	68	40.6	35.5	23.9	17	59.4
3l	pIK 9	88.2	60.6	39.4	6.2	64	83	70.2	24.0	5.8	58	29.8
3l	pIK 12	99	57.3	42.7	7.7	54.1	75	61.8	37.8	0.4	98	38.2
3l	pIK 13	99.4	55.5	44.5	0.7	49.4	97	50.1	49.7	0.2	99	49.9
3l	pIK 15	88	61.3	38.7	8.3	57	75	65.3	34.1	0.6	96	34.7
3l	pIK 29	88.6	61.9	38.1	11	40.1	57	51.1	48.3	0.6	97	48.9
3l	pIK 30	95	70.7	29.3	7.8	53.3	74	61.1	38.2	0.7	93	38.9
3l	pIK 31	86.3	51.8	48.2	7.5	53.1	75	60.6	37.9	1.5	92	39.4
3l	pIK 32	85.4	58.9	41.1	8.2	52.6	73	60.8	38.9	0.3	98	39.2

3.7.3.6 1-tert-Butyl-3-hydroxy-4-thiophen-2-yl -azetidine-2-one (3n, $C_{11}H_{15}NO_2S$)

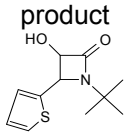


3n

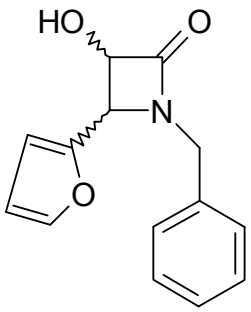
For better handling (the scale is small), also the educts were diluted in dioxane:

2.23mg (20µL stock solution in pK_i-buffer) per multi-well (2mL reaction volume)

In 2ml PK_i buffer, precursor **4n** (20 µL, 2.23mg) were reduced with the different pIK i according to **GP X**.

product 	pIK	rev. Phase HPLC			str. Phase HPLC							
		conv. HPLC	TRANS	CIS	trans SR	trans RS	ee	TRANS	cis SS	cis RR	ee	CIS
3n	pIK 2	100	55.1	44.9	50.9	0.4	98	51.3	46.8	1.9	92	48.7
3n	pIK 4	100	53.9	46.1	50.7	0.3	99	51.0	37.0	12.0	51	49.0
3n	pIK 8	100	55.6	44.4	51.4	-	100	51.4	45.6	3.0	87	48.6
3n	pIK 12	100	55.2	44.8	55.1	-	100	55.1	3.5	41.4	85	44.9
3n	pIK 13	100	55.2	44.8	56.5	-	100	56.5	2.1	41.4	90	43.5

3.7.3.7 1-Benzyl-4-furan-2-yl-3-hydroxy-azetidin-2-on (<u>3p</u>, $C_{14}H_{13}NO_3$)

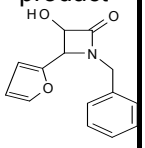


3p

For better handling (the scale is small), also the educts were diluted in dioxane:

2.41mg (20µL stock solution in pK_i-buffer) per multi-well (2mL reaction volume)

In 2ml PK_i buffer, precursor **4p** (20 µL, 2.41mg) were reduced with the different pIK i according to **GP X**.

product 		rev. Phase HPLC			str. Phase HPLC							
	pIK i	conv. HPLC	TRANS	CIS	trans RS	trans SR	ee	TRANS	cis SS	cis RR	ee	CIS
<u>3p</u>	pIK 2	100	36.6	63.4	40.2	-	100	40.2	59.8	-	100	59.8
<u>3p</u>	pIK 3	97.1	22.8	77.2	22.9	-	100	22.9	76.4	0.7	98	77.1
<u>3p</u>	pIK 4	100	56	44	60.3	-	100	60.3	8.3	31.4	58	39.7
<u>3p</u>	pIK 5	97.6	23.8	76.2	24.3	-	100	24.3	-	75.7	100	75.7
<u>3p</u>	pIK 6	100	19.7	80.3	24.5	-	100	24.5	12.4	63.1	67	75.5
<u>3p</u>	pIK 7	100	22.5	77.5	30.4	-	100	30.4	12.3	57.3	65	69.6
<u>3p</u>	pIK 8	100	42.7	57.3	9.8	31.3	52	41.1		58.9	100	58.9
<u>3p</u>	pIK 9	97.3	25.2	74.8	-	25.3	100	25.3	44.4	30.3	19	74.7
<u>3p</u>	pIK 12	100	53.1	46.9	53.9	-	100	53.9	-	46.1	100	46.1
<u>3p</u>	pIK 13	100	51.3	48.7	55.2	-	100	55.2	44.8	-	100	44.8
<u>3p</u>	pIK 15	100	25	75	14	13.6	1	27.6		72.4	100	72.4
<u>3p</u>	pIK 29	100	27.4	72.6	20.9	-	100	20.9	4.7	74.4	63	79.1
<u>3p</u>	pIK 30	93.1	66.1	33.9	32.4	34.4	3	66.8	16.7	16.5	1	33.2
<u>3p</u>	pIK 31	80.2	15.2	84.8	18.4	-	100	18.4	2.2	79.4	95	81.6
<u>3p</u>	pIK 32	75.9	15.3	84.7	21.7	-	100	21.7	18.6	59.7	53	78.3

Acknowledgements

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[105] Product **4e** turned out to decompose upon chromatographic purification and was also insufficiently stable for distillation (*Kugelrohr*); consequently, no combustion analysis was conducted for this compound.

[106] Product **4i** turned out to decompose upon chromatographic purification and was also insufficiently stable for distillation (*Kugelrohr*); consequently, no combustion analysis was conducted for this compound

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[112] The spectra of pIK13 could not be exact integrate, because the peaks were not baseline separated.

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