DISSERTATION

Synthesis and Biooxidation of Polycyclic and Linear Ketones

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

A.o. Univ. Prof. Dipl.-Ing. Dr. techn.
Marko D. Mihovilovic

E163

Institut für Angewandte Synthesechemie

eingereicht an der Technischen Universität Wien
Fakultät für Technische Chemie

von

Mag. Alenka Lengar

Matr. Nr. 9613363
Friesgasse 9/3a
1150 Wien

Wien, am 27. Oktober 2008

First of all I would like to thank my supervisor Prof. Marko D. Mihovilovic, who gave me the opportunity to join his research group, for the interesting and challenging topic of my thesis, for his excellent supervising, for his advice and support in several fields, for giving me the opportunity to visit international conferences and organizing a scientific stay at the Eötvös Loránd University in Budapest, Hungary. I am deeply grateful for everything.

Further I would like to thank Prof. Peter Stanetty for showing interest in my Thesis and his comments and advices especially in the Group seminar.

For the very pleasant three years at the Technical University of Vienna I have to thank all members of our research group, who made the workday life much more colorful. I thank the "desperate-housewives–team" Radka Snajdrova, Petra Kapitanova and Daniela Rial for professional advice and also a lot of fun in the lab. Further I thank Dario Bianchi, Alexander Winninger, Naseem Iqbal, Michael Schön and Michael Ghobrial for a good atmosphere in our lab. I also thank the colleagues from the "big lab" Ather Khan, Farooq Zia, Johanna Hämmerle, Michael Schnürch, Marko Susnik, Markus Holzweber, Abraham Garcia and Michael Fink and the colleagues from the "small lab" Diana Orski-Ritchie, Thomas Fischer, Florian Rudroff, Maria Sahar, Saima Feroz and Markus Spina for their help and the good work climate. Special thanks goes to my colleagues Moumita Koley and Rado Flasik for a lot of fun in the daily routine making the long evenings at work shorter.

I would like to thank Prof. Zoltan Novak for welcoming me in his research group at the Eötvös Loránd University in Budapest, Hungary and for his supervision there.

I also thank Dr. Christian Hametner for structure elucidations and recording of NMR-spectra on the 400MHz machine. I thank Florian Untersteiner for helping me with all "computer problems" and for interesting discussions.

I deeply thank my dear sister Irena who was always interested in my work, for her love and support.

My very special and biggest thank goes to my beloved boyfriend Alexander Faschinger for his support, his interest in my work, professional discussions and encouragement during all phases of my work. Thank you my dear Medo.

Financial support for this project by the Austrian Research Fund FWF (project no.P18945) is gratefully acknowledged.

KURZFASSUNG DER DISSERTATION

Chirale Laktone und Ester sind wichtige Intermediate bei der Synthese von biologisch aktiven Verbindungen.
In der Forschungsgruppe von Prof. Marko Mihovilovic wurden in den vergangenen Jahren bereits viele Studien über die Anwendung von Baeyer-Villiger Monooxigenasen (BVMO) in der synthetischen organischen Chemie durchgeführt. Die bestehende Substratbibliothek enthält monozyklische- bizyklische- und überbrückte bizyklische- Ketone, welche sich als gute Substrate für einige bekannte BVMO- Expressionssysteme erwiesen, deren Anwendungen in der synthetischen Chemie bis zu einem gewissen Grad etabliert sind.

Die Aufgabe dieser Dissertation was es, den Anwendungsbereich und Grenzen der Substratakzeptanz dieser Enzyme zu untersuchen und dafür die Substratbibliothek zu erweitern, indem man neue trizyklische überbrückte Ketone und funktionalisierte lineare Ketone herstellte und deren Substratakzeptanz mit den verfügbaren BVMOs prüfte.

Die gewünschten polyzyklischen Substrate wurden mittels [4+3]-Zykloaddition hergestellt. Die gewünschten bizyklischen Substrate wurden mittels Olefin Metathese aus den polyzyklischen Substraten dargestellt. Die gewünschten linearen β-Hydroxyketone wurden mittels Aldolkondensation aus Aceton und den entsprechenden Aldehyden hergestellt. Die gewünschten funktionalisierten linearen Ketone wurden einerseits aus den linearen β-Hydroxyketonen durch Derivatisierung und andererseits aus den entsprechenden β-ungesättigeten Ketonen mittels Michael-Addition hergestellt. Mit diesen Substraten wurde Biotransformation mit etlichen Baeyer-Villiger Monooxygenase (BVMO) expremierenden rekombinanten *E. coli* Stämmen durchgeführt. Die Beobachtung der Biooxidierungen wurde im Allgemeinen in *Screening*-experimenten in 1 mL oder 2 mL Maßstab in einer 24- oder 12-well Platte durchgeführt. Diese Methode erlaubt schnelles und effektives paralleles Ausführen der Reaktionen. Im größeren Maßstab wurden die Biotrasformationen in Schüttelkolben durchgeführt.

Dabei wurden Desymmetrisation der prochiralen zyklischen Ketone und kinetische Resolution der chiralen linearen Ketone ausgeführt um optisch reine Produkte zu erhalten.

SUMMARY

Chiral lactones and esters are important intermediates in the synthesis of biologically active compounds.
In the research group of Prof. Marko Mihovilovic, many studies about application of several recombinant *E. coli* strains overexpressing different Baeyer-Villiger monooxygenases (BVMO) in the synthetic organic chemistry and preparation of chiral lactones were conducted within the past years. The existing substrate library contains monocyclic-, bicyclic- and bridged bicyclic ketones, which turned out to be quite good substrates for some of the known BVMO overexpression systems and whose applications in synthetic chemistry are to some extend established.

The task of this thesis was to explore the scope and limitations of the substrate acceptance of the BVMOs and therefore extend the substrate library in synthesizing tricyclic bridged ketones and acyclic functionalized ketones and examine the acceptance with the available BVMOs.

The desired polycyclic substrates for biooxidation were synthesized via [4+3] cycloaddition and by subsequent olefin metathesis bridged bicyclic ketones were obtained. Linear β-hydroxyketones were synthesized via Aldol Addition reaction. Functionalized linear ketones were synthesized by derivatisation of β-hydroxyketones or via Michael-Addition from the corresponding 3-en-2-ones. With these substrates biotransformation with several Baeyer-Villiger monooxygenases (BVMO) overexpressing recombinant *E. coli* strains was conducted. Monitoring of biooxidations was in general preformed in screening experiments in 1 mL or 2 mL scale in a 24- or 12-well plate. This method allows fast and effective parallel performing of reactions in contrast to shaking flask experiments. The proceeding of the reactions was monitored and analyzed by GC/MS and/or chiral phase GC. Scale up of the reactions was performed in shaking flasks.

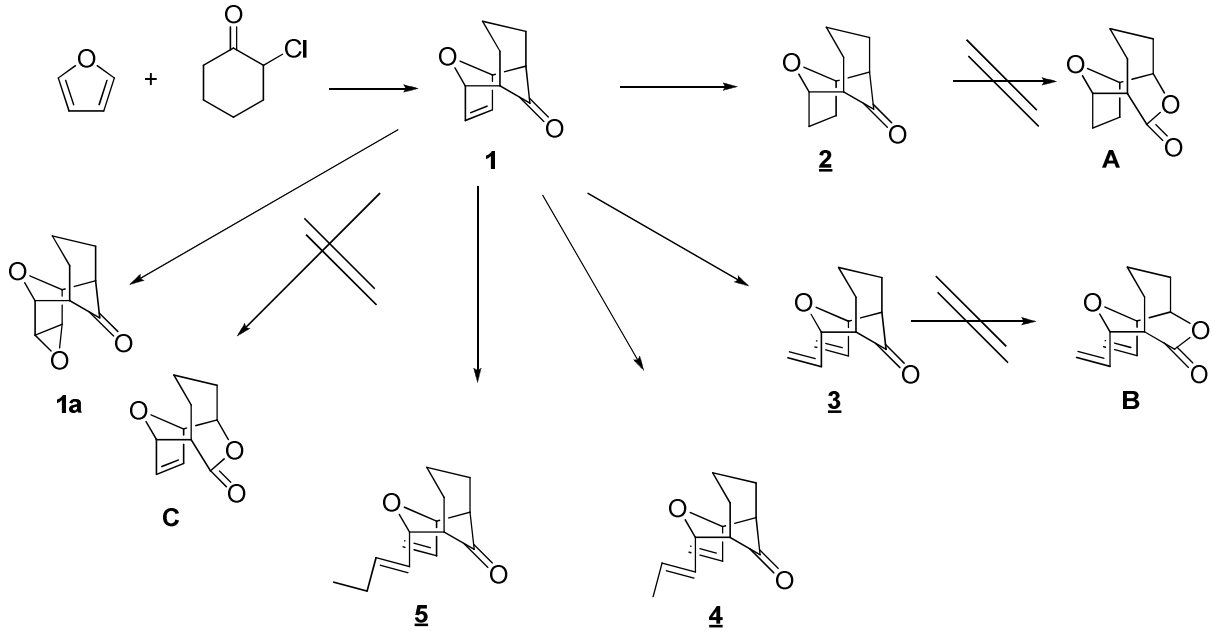
Beside conversion to the desired products also desymmetrisation of the prochiral cyclic ketones and kinetic resolution of the chiral linear ketones in particular was executed to obtain optically pure products.

LEGEND

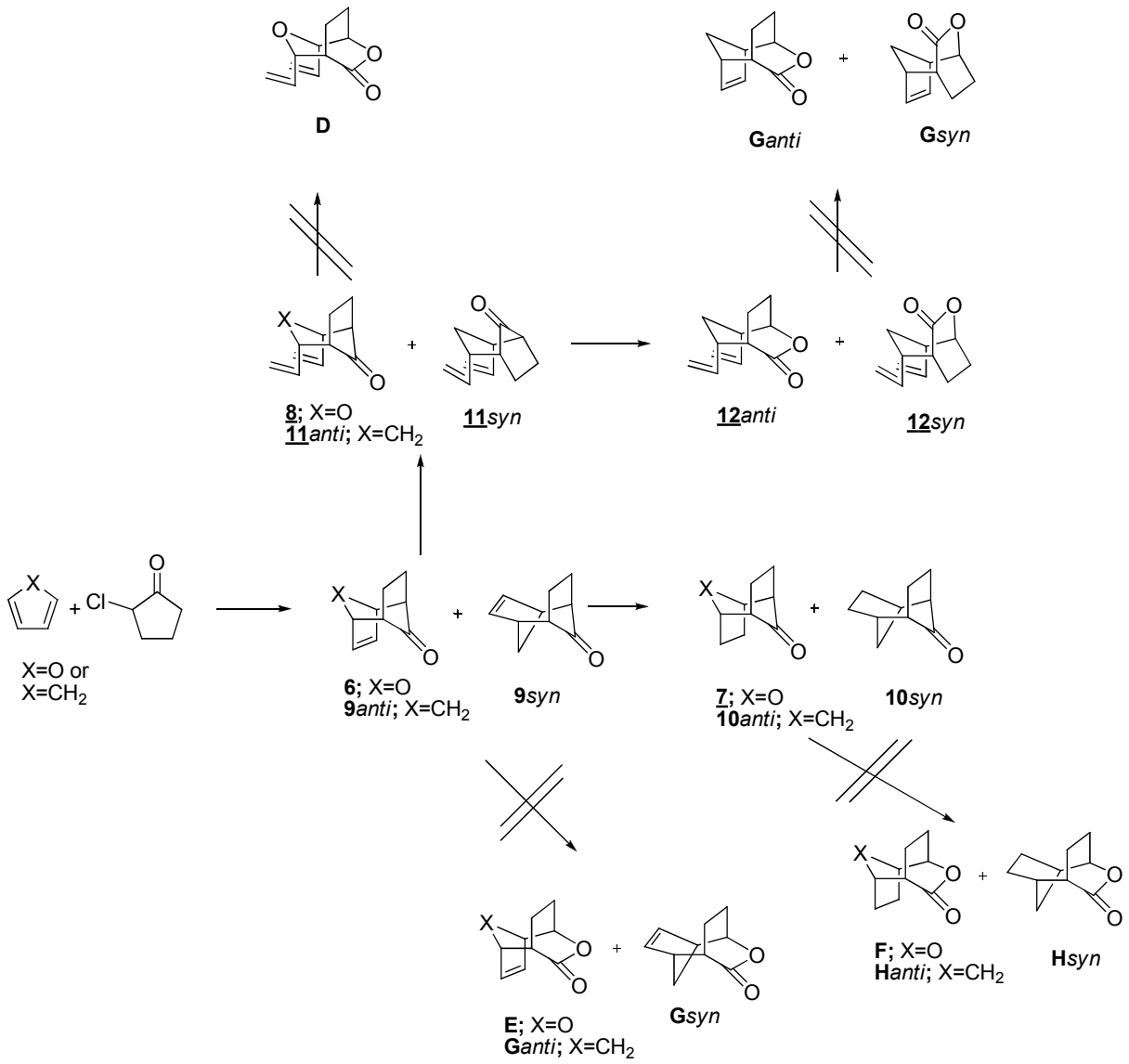
All compounds prepared or used as starting material in this thesis are numbered in bold Arabic numbers. Compounds unknown to the literature are additionally underlined. Literature citations are indicated by superscript Arabic numbers.

General Schemes and Substrate Library

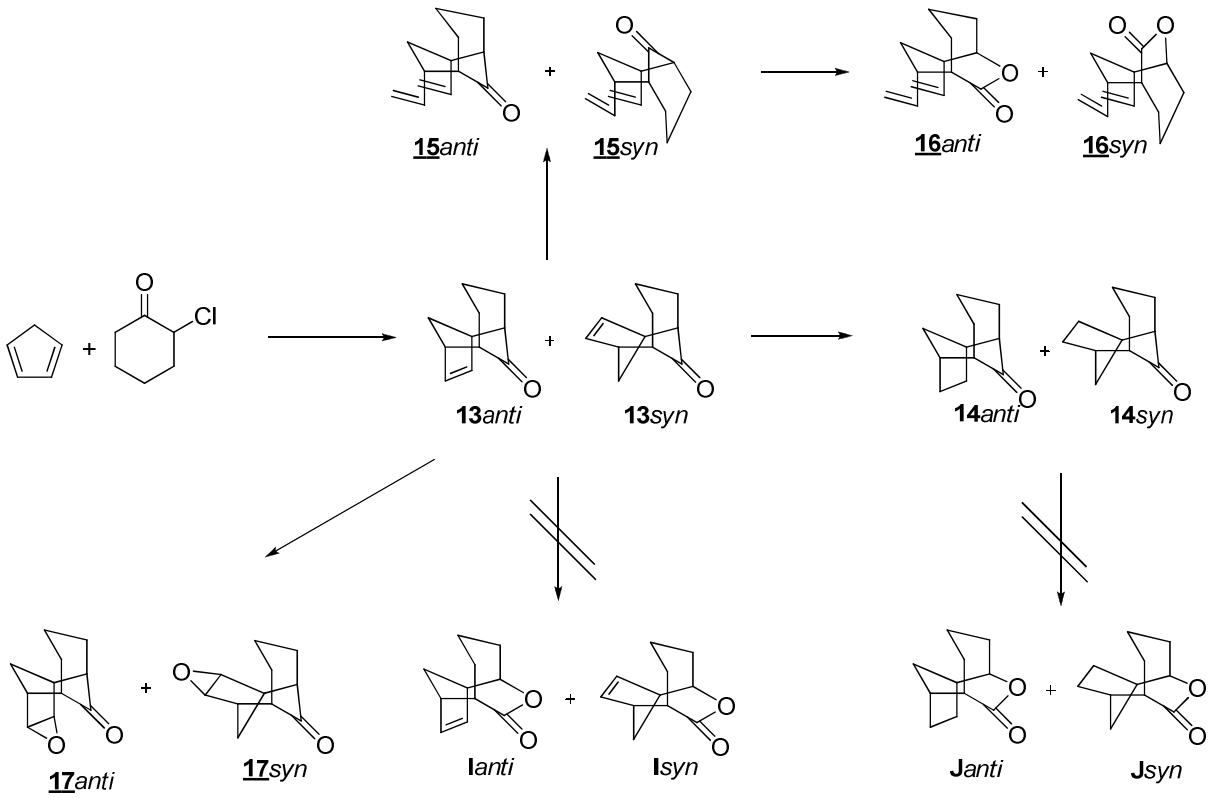
Scheme I



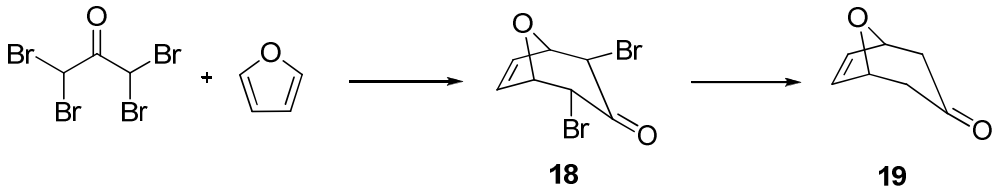
Scheme II

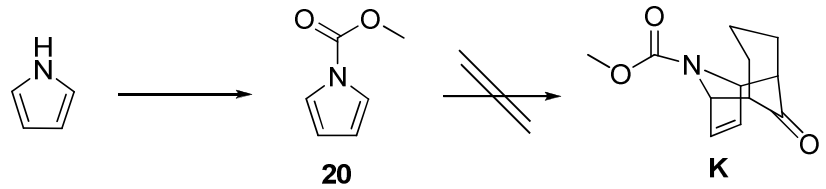

D
Ganti
Gsyn
8; X=O
11anti; X=CH2
11syn
12anti
12syn
X=O or
X=CH2
6; X=O
9anti; X=CH2
9syn
7; X=O
10anti; X=CH2
10syn
E; X=O
Ganti; X=CH2
Gsyn
F; X=O
Hanti; X=CH2
Hsyn

Scheme III

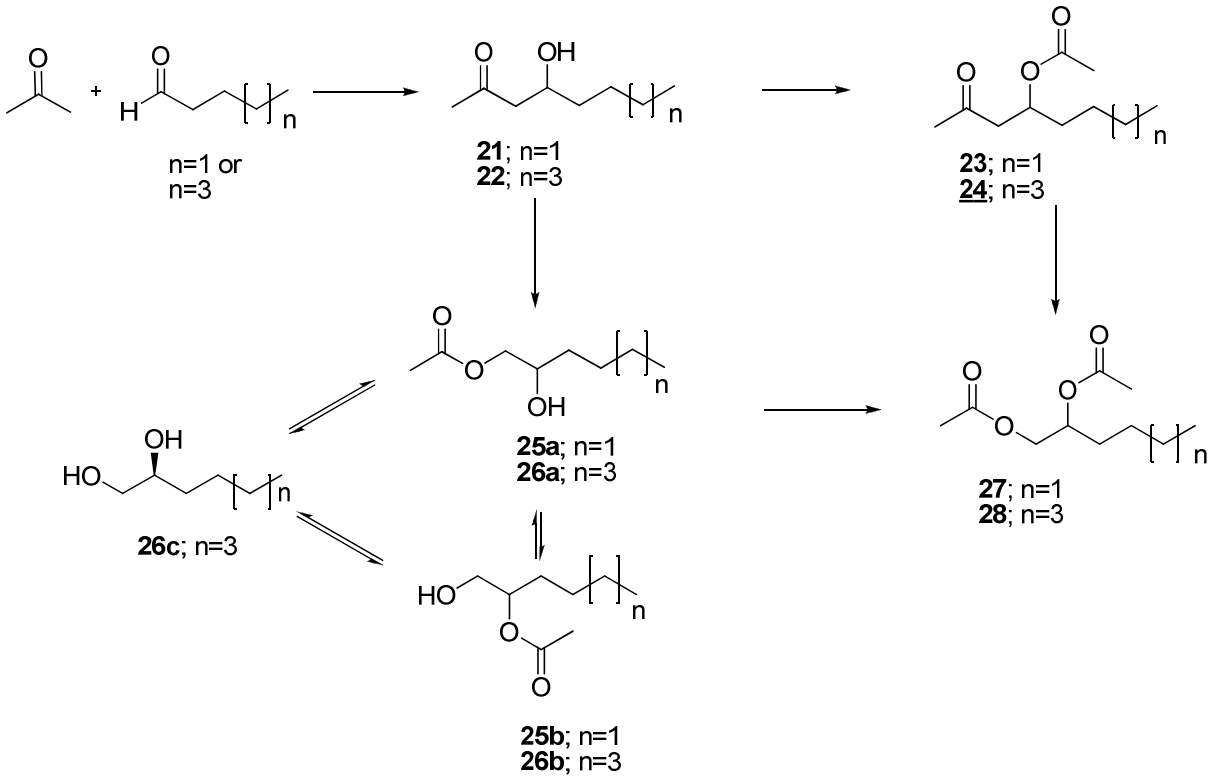

15anti
15syn
16anti
16syn
Cl
13anti
13syn
14anti
14syn
17anti
17syn
Ianti
Isyn
Janti
Jsyn

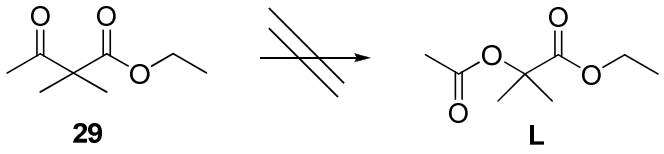
Scheme IV



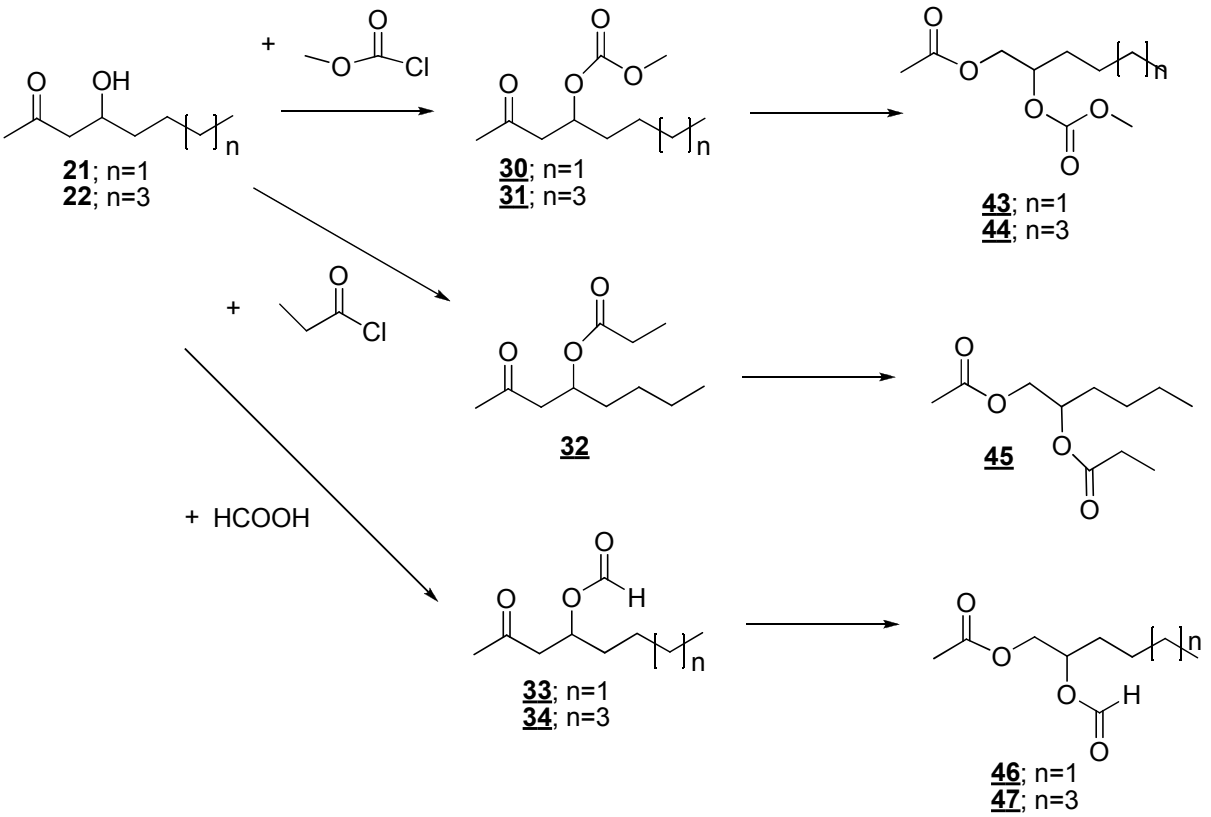


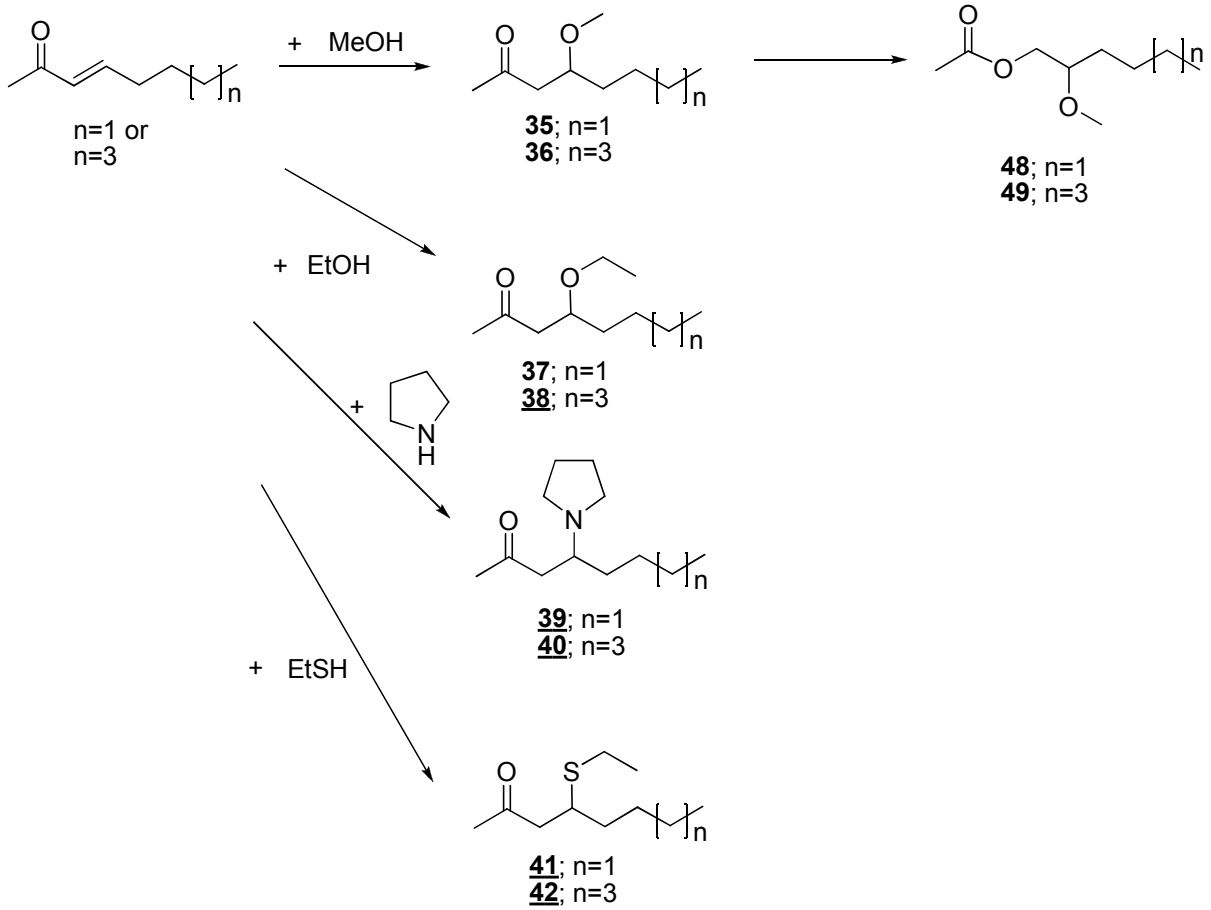
Scheme V



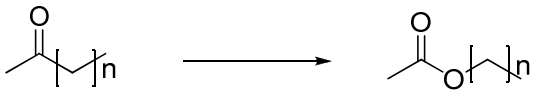


Scheme VI


21; n=1
22; n=3
30; n=1
31; n=3
43; n=1
44; n=3
32
45
+ HCOOH
33; n=1
34; n=3
46; n=1
47; n=3


n=1 or
n=3
+ MeOH
35; n=1
36; n=3
48; n=1
49; n=3
+ EtOH
37; n=1
38; n=3
39; n=1
40; n=3
+ EtSH
41; n=1
42; n=3

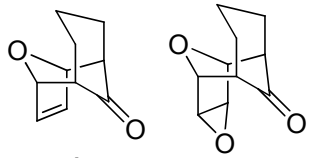
Scheme VII



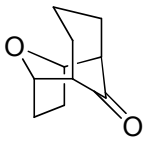
50; n=6
51; n=7
52; n=8

53; n=6
54; n=7
55; n=8

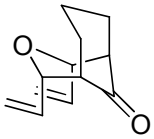
Substrate Library



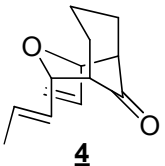
1 **1a**



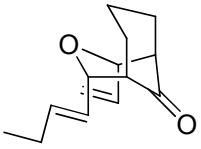
2



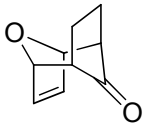
3



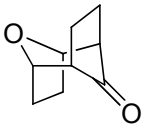
4



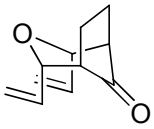
5



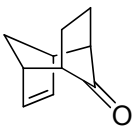
6



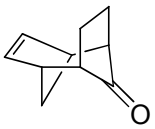
7



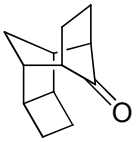
8



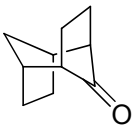
9*anti*



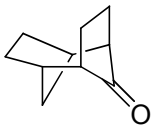
9*syn*



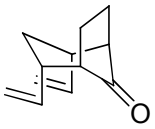
9a



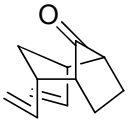
10*anti*



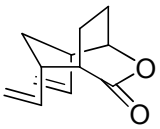
10*syn*



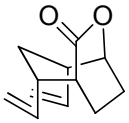
11*anti*



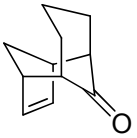
11*syn*



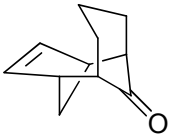
12*anti*



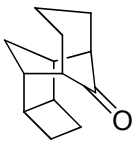
12*syn*



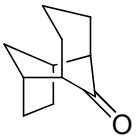
13*anti*



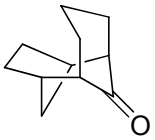
13*syn*



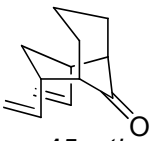
13a



14*anti*

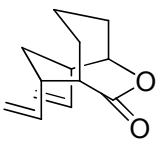


14*syn*

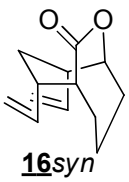


15*anti*

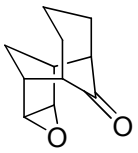
15*syn*



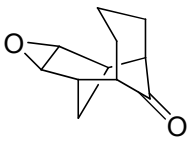
16*anti*



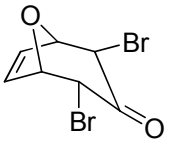
16*syn*



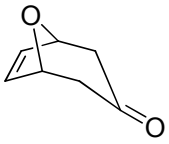
17*anti*



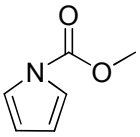
17*syn*



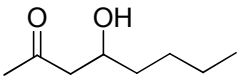
18



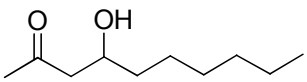
19



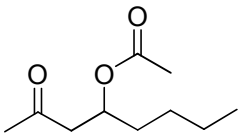
20



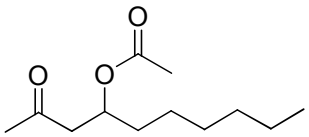
21



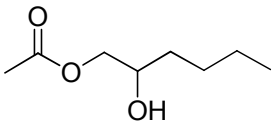
22



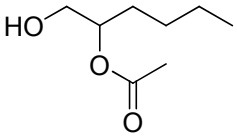
23



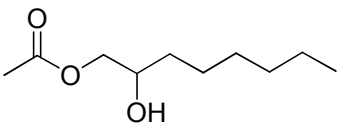
24



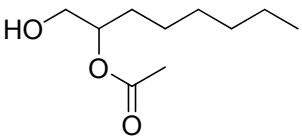
25a



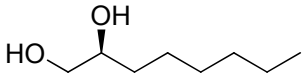
25b



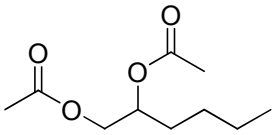
26a



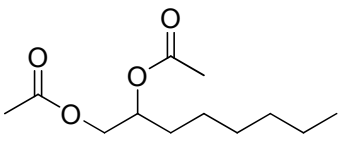
26b



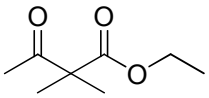
26c



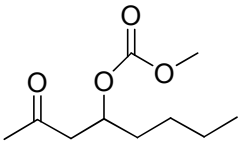
27



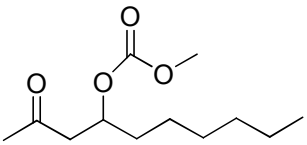
28



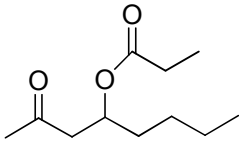
29



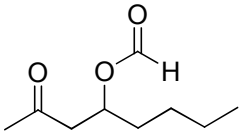
30



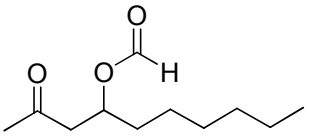
31



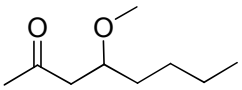
32



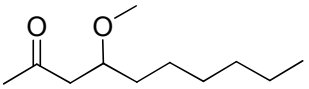
33



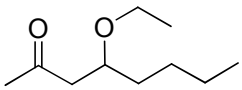
34



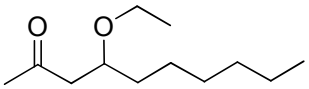
35



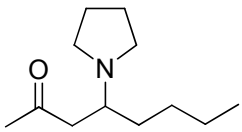
36



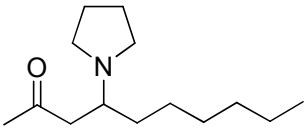
37



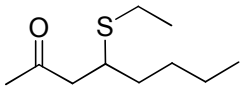
38



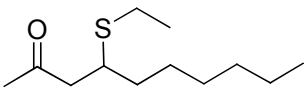
39



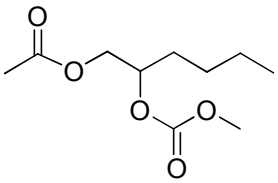
40



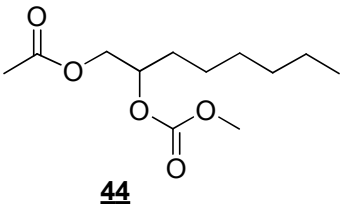
41



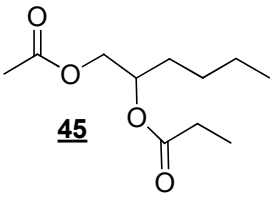
42



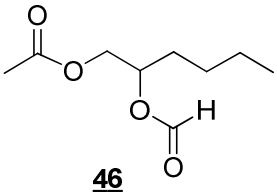
43



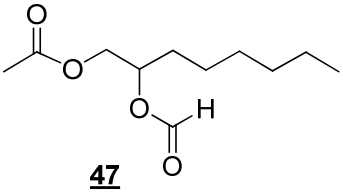
44



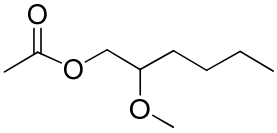
45



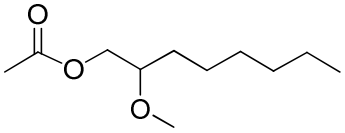
46



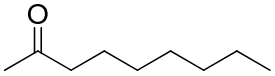
47



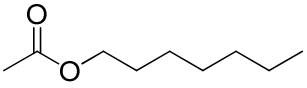
48



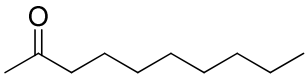
49



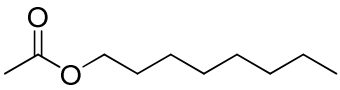
50



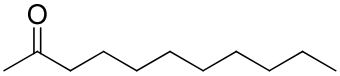
53



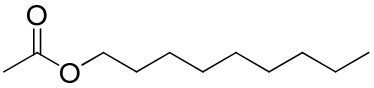
51



54



52



55

1 INTRODUCTION

This thesis was dedicated to the investigation of microbial Baeyer-Villiger oxidations of polycyclic as well as linear ketones, as these substrates were so far hardly considered in previous work. In addition to the work in the area of biocatalysis, two fields in organic synthesis were further developed due to their relevance for the preparation of the required biotransformation substrates: [4+3] cycloaddition and olefin metathesis. In the introduction these fields will be described on a general basis and a more detailed description will be given in the discussion of results.

1.1 Baeyer-Villiger Oxidation

The Baeyer-Villiger oxidation[1], a transformation of ketones into esters or cyclic ketones into lactones, was discovered in 1899 by the German chemists Adolf von Baeyer and Victor Villiger[2]. Initially, the oxidations of menthon and tetrahydrocarvon to the corresponding lactones was carried out with Caro`s reagent (2 $KHSO_4$ + H_2SO_5)[3], later organic peracids were found to be suitable reagents[4].

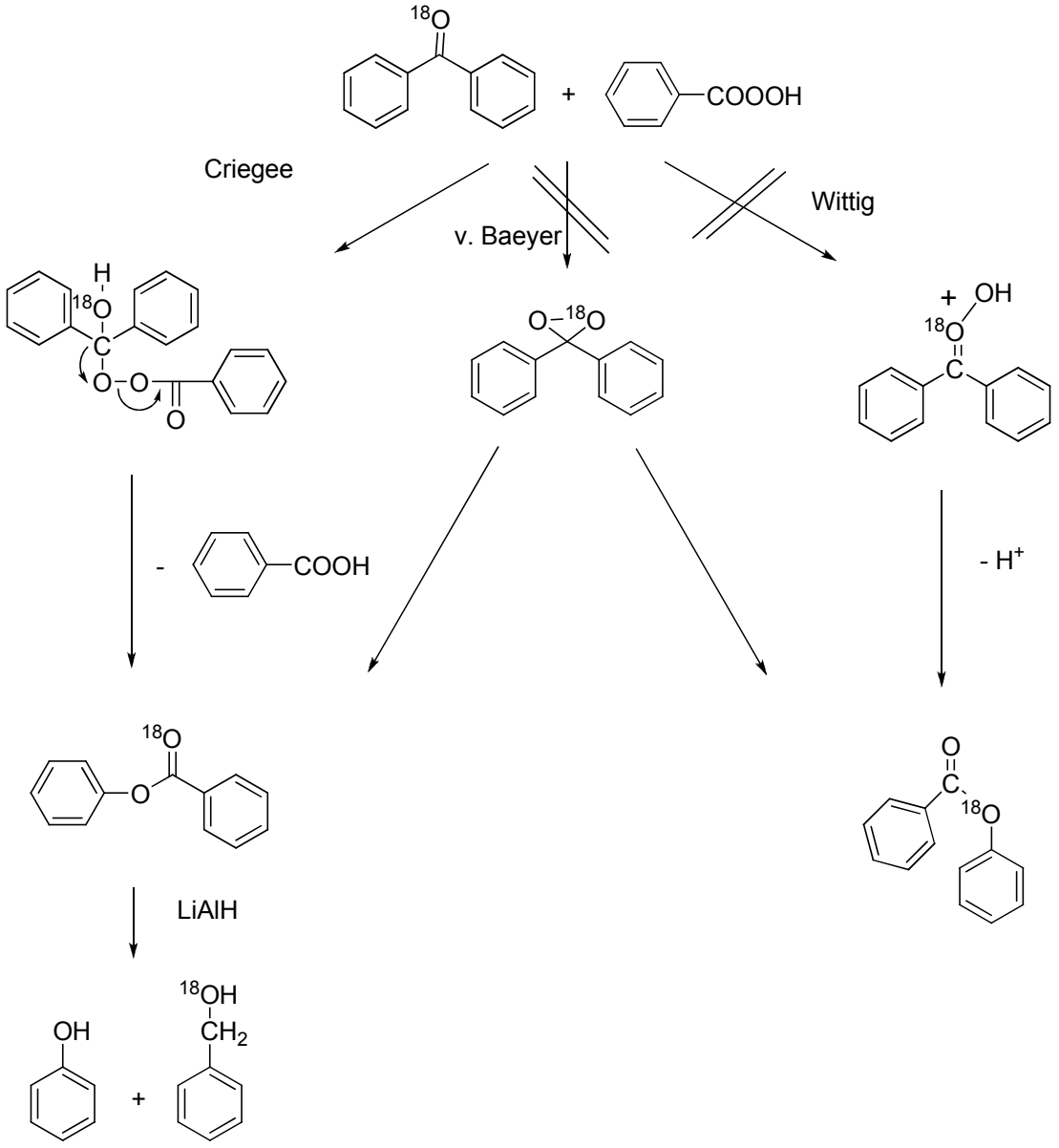
With the Baeyer-Villiger reaction a variety of different carbonyl groups can be oxidized: ketones are converted into esters, cyclic ketones into lactones, and aldehydes into carboxylic acids. It is compatible with some functional groups, even in the presence of double bonds mostly the carbonyl group will be oxidized, except if the double bonds are conjugated.

1.1.1 Mechanism

There were some attempts to elucidate the mechanism of the Baeyer-Villiger reaction, and a first rational was proposed already by Baeyer and Villiger[2] with a dioxirane as intermediate. The second mechanism was suggested by Wittig[5] with a carbonyl oxide intermediate,

[1] Krow, G. R. *Org. React.* **1993**, *43*, 251.
[2] Baeyer, A.; Villiger, V. *Ber. Dtsch. Chem. Ges.* **1899**, *32*, 3623.
[3] Caro, H. *Angew. Chem.* **1898**, 845.
[4] Baeyer, A.; Villiger, V. *Ber. Dtsch. Chem. Ges.* **1900**, *33*, 1569.
[5] Wittig, G.; Pieper, G. *Ber. Dtsch. Chem. Ges.* **1940**, 73, 295.

followed by work from Criegee proposing a tetrahedral intermediate[6]. A large variety of tentative intermediates was postulated[7] and finally the Criegee mechanism could be verified with a labeling experiment[8], where the position of the inserted oxygen could be defined. At the same time the mechanism proposals by Wittig and Baeyer were invalidated: The reaction was carried out with ^{18}O labeled benzophenone (Scheme 1). This was oxidized with perbenzoic acid, which resulted in phenylbenzoate bearing the ^{18}O-label at the carbonyl group. The position of ^{18}O was shown by reduction with lithium aluminum hydride to benzyl alcohol containing excess ^{18}O and phenol having the normal abundance. This particular isotope distribution confirms the mechanism as proposed by Criegee.



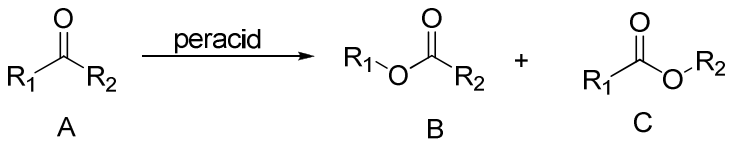
Scheme 1

[6] Criegee, R. *Justus Liebegs Ann. Chem.* **1948**, *560*, 127.
[7] Doering, W. v. E; Speers, L. *J. Am. Chem. Soc.* **1950**, *72*, 5515.
[8] Doering, W. v. E ; Dorfman, E. *J. Am. Chem. Soc.* **1953**, *75*, 55.

The reaction proceeds in two steps, in the first step a nucleophilic attack at the carbonyl-C atom occurs by the peracid, forming the so called Criegee intermediate. In the second step, one alkyl group migrates to the peroxide and at the same time the carboxylate-anion is released. The second step is the rate determining one and crucial for the stereoselectivity of the reaction. Migration and release of the carboxylate-anion proceed in a concerted fashion[9] leading to retention of the chirality at the migrating chiral C-atom.
Electron-withdrawing groups on the peracid accelerate the reaction rate by increasing the stability of the conjugated base and converting it into a better leaving group (*m*-chlorperbenzoic acid and trifluoroacetic acid are better oxidants than perbenzoic acid or peracetic acid). Electron-donating substituents on the migrating group also facilitate the rearrangement.

1.1.2 Stereochemistry of the Baeyer-Villiger Oxidation

One of the important characteristics of the Baeyer-Villiger oxidation and an important asepct for asymmetric synthesis is the retention of the stereochemistry of the migrating group. This could be shown for the first time in the oxidation of *cis*- and *trans*-2-methylcyclohexyl acetate with perbenzoic acid, which occurred with retention of configuration at the migrating centers.[10]
The second interesting aspect is the competitive migration of substituents in unsymmetrical ketones. In general secondary and tertiary alkyl groups migrate more readily than primary groups.[7] In the context of the suggested hypothesis that groups which bear positive charge on the migrating carbon atom will rearrange more rapidly[7], a series of experiments was performed: asymmetric substituted benzophenones were oxidized with peracetic acid and the migration behavior of the substituents was observed (Scheme 2, Table 1[7]):



Scheme 2

[9] Berson, J. A.; Suzuki, S. *J. Am. Chem. Soc.* **1959**, *95*, 4088.
[10] Turner, R. B. *J. Am. Chem. Soc*. **1950**, *72*, 878.

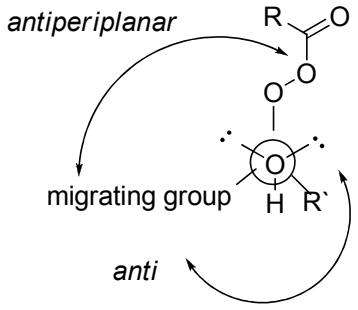
Table 1 [7]

	R_1	R_2	Conv.[%]	Product distribution B:C [%]
1	p-CH_3O-Ar	Ph	96	100:0
2	p-CH_3-Ar	Ph	61	100:0
3	p-Cl-Ar	Ph	26	9:91
4	p-NO_2-Ph	Ph	100	0:100

The migration of substituents with electron-donating groups (CH_3O-, CH_3-) in the *para*-position was preferred over the phenyl migration. In contrast, electron-withdrawing groups (NO_2-, Cl-) in the *para*-position migrate slower or not at all in competition with the phenyl group.

However, migration preferences can not be predicted in this simplified way for every reaction, since other factors like the nucleophilic attack of the substituents on the aromatic moiety the carbonyl bond also play a role.
Also the nature of peracid used in the reaction can affect the product distribution:[11] difference or more than 10% in distribution of the two possible esters from the same asymmetric ketone have been described in the literature depending on the peracid used.

Two conditions have to be fulfilled for successful alkyl-migration and carboxylic acid ejection, regarding the stereoelectronic effects: the migrating –C bond has to be in an antiperiplanar position with respect to the peroxy bond and electron release from the hydroxyl oxygen atom to the originally attached migrating group is essential for the alkyl shift and a lone pair in the *anti* position at the oxygen atom is essential[12] (Scheme 3).

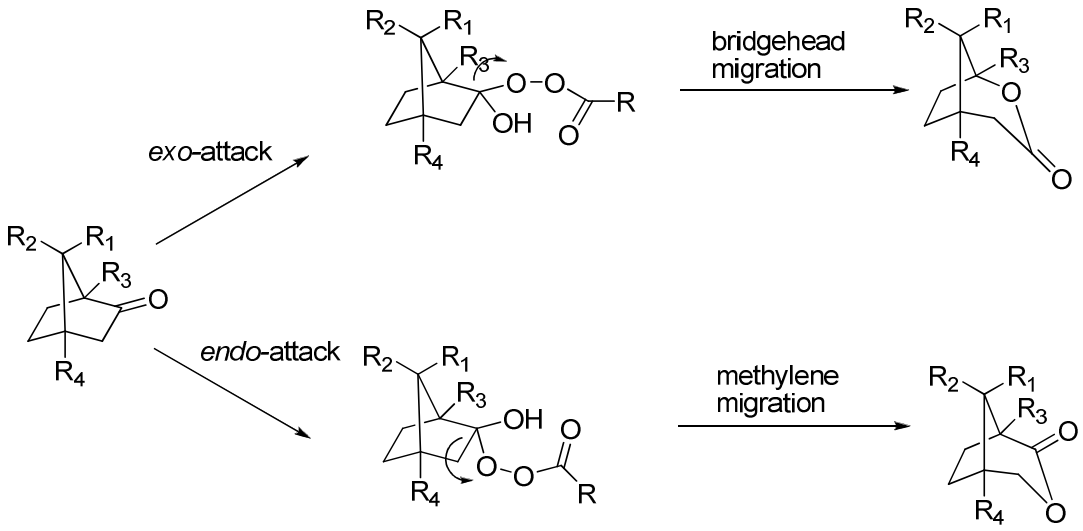


Scheme 3

In the conversion of bicyclic ketones to lactons, the competition takes place between the migration of the bridgehead- (*exo*-attack) or the methylene- (*endo*-attack) moiety. After the

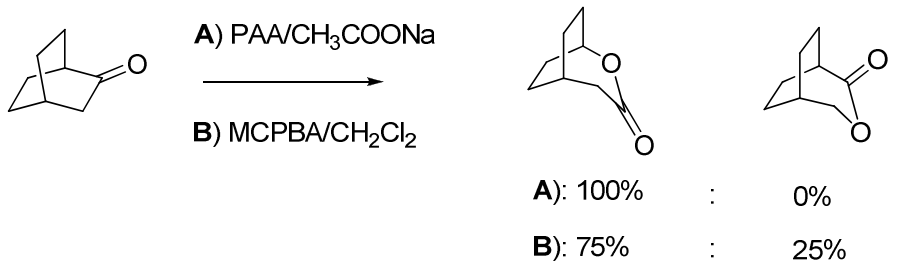
[11] Hawthorne, M. F.; W. D. Emmons, W. D.; McCallum, K. S. *J. Am. Chem. Soc*. **1958**, *80*, 6393.
[12] Noyori, R.; Sato, T.; Kobayashi, H. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2661.

expected alkyl migratory aptitude preference, tertiary > primary, the bridgehead-moiety should migrate. In this case the substituents on the bridgehead (R_1, R_2) and others (R_3, R_4) play a crucial role, they can stericaly hinder the *exo*- attack and have influence on the product distribution as demonstrated on the example of the bicyclo [2.2.1] heptanone in Scheme 4.[13]



Scheme 4

The method of oxidation influences the product distribution as well: bicyclo[2.2.2]octanone gave 100% of the bridgehead migrated lactone upon oxidation with sodium acetate buffered solution of peracetic acid [14], while oxidation with *m*-chlorperbenzoic acid gives 75% of the bridgehead migrated product, but also 25% of the methylene migrated lactone[13] (Scheme 5). Since here no steric hindrance appears it can be concluded, that in bridged bicyclic systems an interplay between electronic and steric/torsional strain factors define the regioselectivty of the oxygen insertions.



Scheme 5

Several similar examples are known and were summarized by Krow[13].

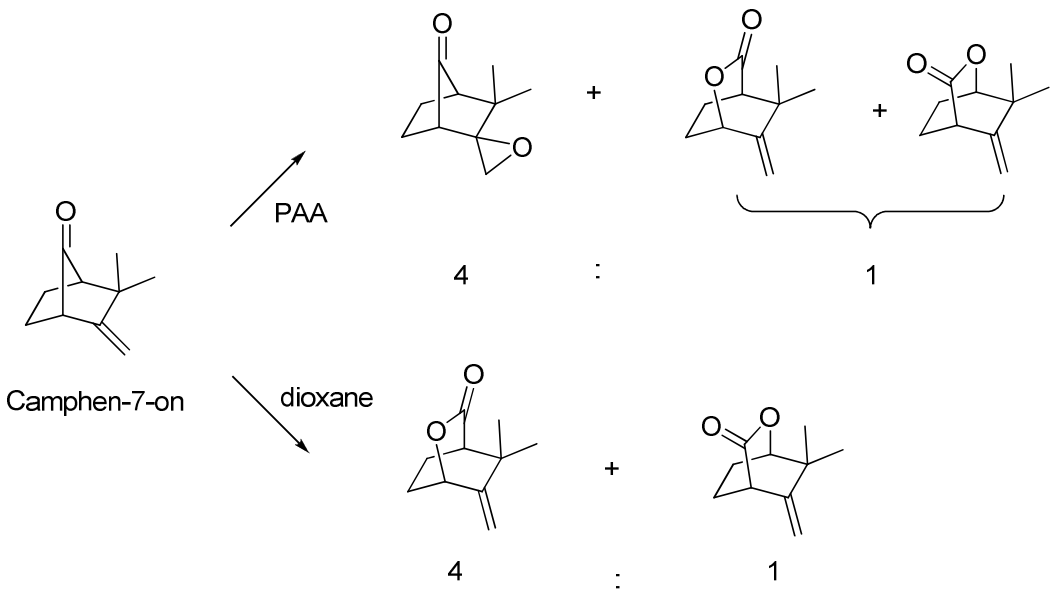
[13] Krow, G. R. *Tetrahedron*, **1981**, *37*, 2697.
[14] Meinwald, J.; Frauenglass, E. *J. Am. Chem. Soc*. **1960**, *82*, 5235.

1.1.3 Different Methods for Baeyer-Villiger Oxidation

The Baeyer-Villiger oxidation can be performed by employing different methods. Apart of the already mentioned oxidation with peracids, also H_2O_2 can be used for the oxidation. This system has advantages and disadvantages in comparison to oxidation with peracids. The biggest advantage is the environmentally friendly water as byproduct, in contrast to the peracid-reaction, where the byproduct is an acid, which has to be disposed (or recycled with H_2O_2). But the formation of water represents also the main disadvantage, since many of the esters formed can be hydrolyzed. This can be partly avoided by using water insoluble solvents and creating a two phase system, if the esters stay in the organic phase.
Another disadvantage of peracids is their explosive character and shock sensitivity and the high price, which limits applicability on big scale industrial processes. One possibility to reduce the hazard is the formation of peracids in situ from carboxylic acids and H_2O_2. Also the high oxidative character of peracids can be a problem, if other functional groups are in the molecule, which have to be protected prior to use.
Both in oxidation with peracids and oxidation with H_2O_2 catalyzed by transition metals, the migratory aptitude is as follows: *t*-alkyl> cyclohexyl> *s*-alkyl> benzyl> phenyl> *n*-alkyl> cyclopentyl> methyl.[15]
The activity of the oxidizing agents decreases as follows: trifluoroperacetic acid > monopermaleic acid > monoperphthalic acid > 3,5-dinitroperbenzoic acid > *p*-nitrobenzoic acid > *m*-chloroperbenzoic acid ≈ performic acid > perbenzoic acid > peracetic acid >> H_2O_2.

In unsaturated (bicyclic) ketones different methods of oxidation strongly influence the outcome of the reaction. The classical oxidation of camphen-7-on (Scheme 6) with peracetic acid gives a 4:1 mixture of epoxide and both possible lactones, while mildly acidic conditions in dioxane, contaminated with its hydroperoxides give only both possible lactones in ratio 4:1.[13]

[15] Stukul, G. *Angew. Chem. Int. Ed.* **1998**, *37*, 1198.



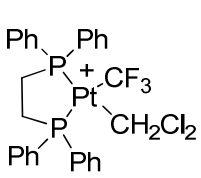
Scheme 6

1.1.4 Transition-metal Catalyzed Baeyer-Villiger Oxidation

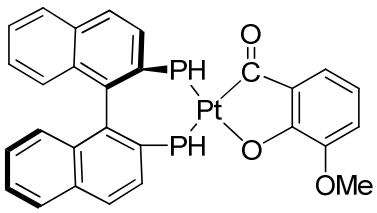
Baeyer-Villiger oxidation could be performed successfully with 35% H_2O_2 with platinum-complex **a** (Scheme 7) as catalyst using cyclic ketones to give the corresponding lactones, but failed with acyclic ketones[16]. In the reaction of unsaturated ketones the main product was the epoxide (oxidation of the double bond was preferred to the BVO).

With chiral platinum (II) catalyst **b** (Scheme 7) asymmetric Baeyer-Villiger oxidation of a variety of racemic mixtures of simple chiral ketones could be performed and resulted in a kinetic resolution leading to chiral lactones with moderate *ee* values[17]. With the platinum-complex **c** (Scheme 7), also acyclic ketones could be oxidized[18]. Other transition metal catalytic systems to catalyze the Baeyer-Villiger oxidation with hydrogen peroxide exist with rhenium ($MeReO_3$),[19] titanium (titaniumsilicates),[20] nickel-, iron- and cobalt-complexes[21] and also chiral copper-complexes have been described to for asymmetric induction.[22]

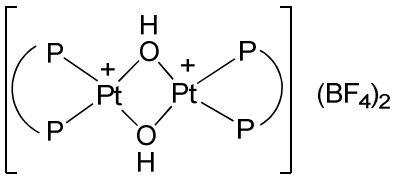
[16] Del Todesco Frisone, M.; Pinna, F.; Stukul, G. *Organometallics* **1993**, *12*, 148.
[17] Gusso, A.; Baccin, C.; Pinna, F. ; Stukul, G. *Organometallics* **1994**, *13*, 3442.
[18] Gavagnin, R.; Cataldo, M.; Pinna, F.; Stukul, G. *Organometallics* **1998**, *17*, 661.
[19] Hermann, W. A.; Fischer, R. W.; Scherer, W.; Correia, J. D. G. *J. Mol. Catal*. **1994**, *94*, 213.
[20] Notari, B. *Adv. Catal*, **1996**, *41*, 253.
[21] Giannandrea, R.; Mastrorilli, P.; Nobile, C. F.; Suranna, G.P. *J. Mol. Catal*. **1994**, *94*, 27.
[22] Bolm, C.; Schlingloff, G.; Weikhardt, K. *Angew. Chem. Int. Ed*. **1994**, *33*, 1848.



a

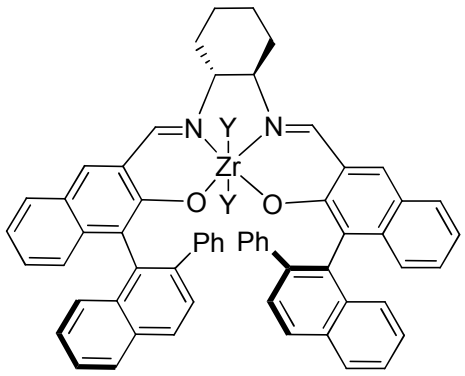


b



P-P=bis(diphenylphosphino)butane

c



Y = OPh

d

Scheme 7

Before 2001, the optical purities of Baeyer-Villiger products, prepared with microbial BVO, were appreciably higher than those, prepared with chiral metal catalysts. In the last years some improvements with different chiral metal compexes have been achieved, culminating in the application of zirconium complex **d** (Scheme 7), which gives a high enantioselectivity of 87% *ee* for the Baeyer–Villiger reaction of 3-phenylcyclobutanone[23]. More recent achievements in metal-catalyzed asymmetric Baeyer-Villiger reactions were reviewed by Katsuki and Mihovilovic in 2004.[24]

1.1.5 Acid Versus Base Catalysis

From the beginning, the Baeyer-Villiger oxidation was considered to be accelerated by acids, as already outlined by Baeyer and Villiger in their seminal first article when using Caro`s acid. There are many examples, where this favorable effect has been confirmed. The reaction

[23] Watanabe, A.; Uchida, T.; Ito, K.; Katsuki, T. *Tetrahedron Lett.* **2002**, *43*, 4481.
[24] a)Katsuki, T. *Russ. Chem. Bull., Int. Ed.* **2004**, *53*, 1859. b)Mihovilovic, M. D.; Rudroff, F.; Groetzl, B. *Curr. Org. Chem.* **2004**, *8*, 1057.

of meta- and para-substituted acetophenones with perbenzoic acid to the corresponding esters was reported by Friess and Soloway[25] demonstrating catalysis by benzoic and acetic acids. Since the by-product from peracid reduction is acid, it could also be observed, that the reaction of ketones with trifluoroperacetic acid was accelerated by the trifluoroacetic acid accumulated during the reaction as required for an autocatalytic process.[26] Corresponding with these results, a decelerating effect was reported when lowering the acidity of the reaction medium by adding Na_2HPO_4 in a reaction of trifluoroperacetic acid with ketones.[27] Also an experiment where the reaction time could be shortened from eight days in pure acetic acid to half an hour with 13 vol% of sulfuric acid added to the reaction mixture is a strong indication for catalysis by acids,[7] but no simple connection between acidity and reaction progress can be established.

On the other hand there are reported cases, where Baeyer-Villiger reactions can be accelerated in a basic environment by addition of heterogeneous $NaHCO_3$. In the reaction of bicyclic ketones with *m*-chloroperbenzoic acid in dichloromethane addition of $NaHCO_3$ doubled the reaction rate[28], probably due to the removal of the proton in the Ciregee intermediate, facilitating the rearrangement process (Scheme 8).

Scheme 8

1.1.6 Biooxidation

As an alternative to transition metal catalyzed systems, microbial or enzyme catalysis can be employed. This is possible in both classical and asymmetric synthesis. Optically pure esters and lactones are important intermediates within the synthesis of natural products and bioactive compounds. Since biological processes in the nature require and produce optically

[25] Friess, S. L.; Soloway, A. L. *J. Am. Chem. Soc*. **1951**, *73*, 3968.
[26] Hawthorne, M. F.; Emmons, W. D. *J. Am. Chem. Soc*. **1958**, *80*, 6398.
[27] Rassant, A.; Ourisson, G. *Bull. Soc. Chim. Fr*. **1959**, 1133.
[28] Whitesell, J. K.; Mathews, R. S.; Helbling, A. M. *J. Org. Chem*. **1978**, *43*, 784.

pure products, the idea was closeby that employing these systems in organic synthesis could be a new option for the production of optically pure compounds.
The challenge was and still is, to convert also substrates, which are not natural targets of the available enzymes. With directed evolution, new and modified enzymes are developed, which can have a broad substrate profile and can therefore be employed in preparative organic chemistry.

1.1.7 Microbial Baeyer-Villiger Oxidation

Several reviews about Microbial Baeyer-Villiger Oxidation have been published in the recent years.[29]

The first asymmetric enzymatic Baeyer-Villiger oxidation was reported in 1966,[30] as a racemic mixture of 2-heptylcyclopentanone was incubated with a whole cell system of *Pseudomonas oleovorans* NCIMB 6576 and the corresponding optically active lactone was generated. At that time the enzymes responsible for the reaction were not identified.

Oxygenases are enzymes, which catalyze the insertion of one (monooxygenases) or two (dioxygenases) atoms of molecular oxygen into a substrate.

Among the Baeyer-Villiger monooxygenases a very broad substrate profile has been found for cyclohexanone monooxygenase (CHMO) from *Acinenetobacter sp.* NCIB 9871. It has been used for oxidation of bicyclic ketones to lactones in particular, but also represents a very versatile catalyst for the oxygenation of heteroatoms, e.g. disulfides to thiosulfinates,[31]cyclic sulfites to sulfates,[32] sulfides to sulfoxides,[33] tertiary amines to amine N-oxides[34] and selenides to selenoxides.[35]
The enzyme is NADPH and oxygen dependent and contains flavin adenine dinucleothide (FAD) as prosthetic group.

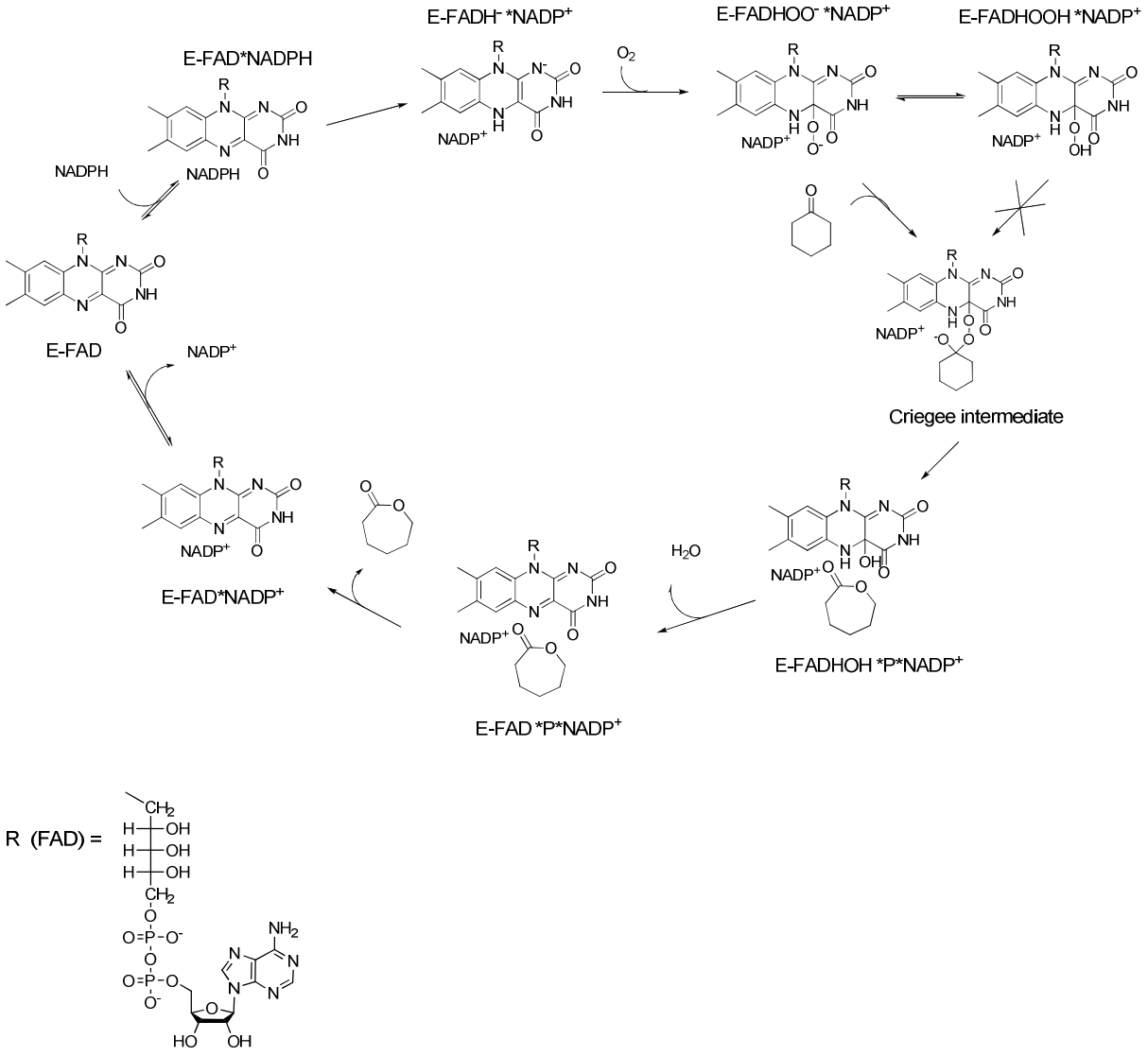
[29] a) Mihovilovic, M. D. *Curr. Org. Chem.* **2006**, *11*, 1265. b) Mihovilovic, M. D.; Muller, B.; Stanetty, P. *Eur. J. Org. Chem.* **2002**, *22*, 3711. c) Roberts, S. M.; Wan, P. W. H. *J. Mol. Catal. B: Enzym.* **1998**, *4*, 111. d) Kamerbeek, N. M.; Janssen, D. B.; van Berkel, W. J. H.; Fraaije, M. W. *Adv. Synth. Catal.* **2003**, *345*, 667. e) Alphand, V.; Carrea, G.; Wohlgemuth, R.; Furstoss, R.; Woodley, J. M. *Trends Biotechnol.* **2003**, *21*, 318.
[30] Shaw, R. *Nature* **1966**, *209*, 1369.
[31] Colonna, S.; Del Sordo, S.; Gaggero, N.; Carrea, G.; Pasta, P. *Heteroatom Chem.* **2002**, *13*, 467.
[32] Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P.; *Chem. Comm.* **1998**, 415.
[33] Kelly, D. R.; Knowles, C. J.; Mahdi, J. G.; Taylor, I. N.; Wright, M. A. *Tetrahedron Asymmetry* **1996**, *7,* 365.
[34] Ottolina, G.; Bianchi, S.; Belloni, B.; Carrea, G.; Danieli, B.*Tetrahedron Lett.* **1999**, *40*, 8483-8486.
[35] Branchaud, B. P.; Walsh, C. *J. Am. Chem. Soc*. **1985**, *107*, 2153.

Also the mechanism of BVMOs was studied on cyclohexanone monooxygenase (CHMO) from *Acinenetobacter sp.* NCIB 9871 for the conversion of cyclohexanone to ε-caprolactone. A first account was given by Walsh[36] subsequently refined by Massey et al.[37] (Scheme 9).

1.1.7.1 Mechanism of Baeyer-Villiger Monooxygenases (BVMOs)

The enzyme reacts with NADPH to form an E-FADH$^-$ *NADP$^+$ complex. This complex reacts very rapidly with molecular oxygen to form the presumed flavin C4a-peroxyanion (E-FADHOO$^-$ * NADP+). In the absence of the substrate this species is in equilibrium with the presumed flavin C4a-hydroperoxide (E-FADHOOH * NADP+). The protonated species is unreactive with cyclohexanone, but the peroxyanion acts as a nuclephile towards the ketone to yield an oxidized enzyme species, presumably a complex with NADP and the ε -caprolactone product still bound (E-FAD*P*NADP+). In analogy to the chemical Baeyer-Villiger reaction, also here a Criegee adduct is formed. Then dehydration, release of the ε-caprolactone product and the slow release of NADP take place. The peroxyanion species is also capable to initiate a Michael-type addition in activated C=C double bonds.
On the other hand, the electrophilic protonated species (E-FADHOOH* NADP$^+$) seems to be responsible for the heteroatom oxidations of BVMOs.

[36] Ryerson, C. C.; Ballou, D. P.; Walsh, C. *Biochem.* **1982**, *21*, 2644.
[37] Sheng, D.; Ballou, D. P.; Massey, V. *Biochem.* **2001**, *40*, 11156.



Scheme 9

This mechanism was esablished on CHMO, but it can be assigned as a model for other BVMOs without essential changes.

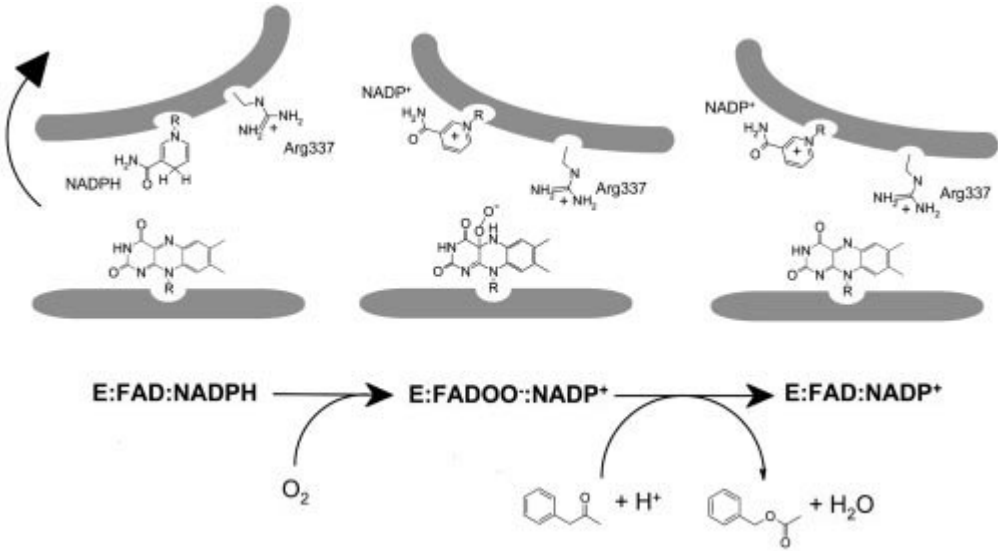
All known BVMOs are NAD(P)H dependent flavoproteins. They can be devided into two groups: Type I BVMOs contain flavin adenine dinucleotide (FAD) as cofactor, use NADPH as source for electrons and consist of identical subunits. Type II BVMOs contain flavin mononucleotide (FMN) as cofactor, use NADH as electron donor and are composed of $\alpha_2\beta$ trimers[38]. So far, all BVMOs that have been cloned could be classified as Type I enzymes while no Type II BVMO sequence is known.

[38] Willetts A. *Trends Biotechnol*. **1997**, *15*, 55.

1.1.7.2 Structure of BVMO

In 2004 Malito et al. could establish the crystal structure of phenylacetone monooxygenase from *Thermobifida fusca* ($PAMO_{Thermo}$), which allowed new insights into the arrangement and function of BVMO enzymes[39]. In contrast to other BVMOs, the stability of this enzyme in purified form made the structural analysis possible, as the originating organism is moderately thermophilic.
The enzyme shows a two-domain architecture: the active site is located in the cleft at the domain interface. Both domains are linked by a region of high specificity, a "fingerprint" sequence (FxGxxxHxxxW) for the family of the BVMO enzymes.

An arginine residue, Arg-377 plays a central role, as its exchange (through mutation) inactivates the enzyme completely. Arg-337 is positioned above the flavin ring and is tentatively capable to stabilize the flavin peroxide (E-FADHOO$^-$ *$NADP^+$) and the "Criegee" intermediate formed during the reaction. It is also involved in the transmission of NADPH to the flavin, which is then reduced (Scheme 10).



Scheme 10

In nature BVMOs are incorporated in degradative metabolic pathways, e.g. $CHMO_{Acineto1}$ from *Acinenetobacter sp.* NCIB 9871 is responsible for the insertion of oxygen during degradation of cyclohexanol.
Several overexpression systems could be developed in the last years.

[39] Malito, E.; Alfieri, A.; Fraaije, M. W.; Mattevi, A. *Proc. Nat. Acad. Sci. USA* **2004**, *101*, 13157.

1.1.7.3 Directed Evolution of Biocatalysts

Initially, the appropriate enzymes for the specified task have to be found. Often, no suitable wild-type enzymes are available. One of the possibilities to solve the problem is to design enzyme variants which are efficient in catalyzing the specified reaction. This method is called "directed evolution" or "Darwinian evolution in the test tube".
This approach was started only about twenty years ago and is inspired by natural evolution.[40] The concept is to optimize existing biomolecules or create new ones by incorporating mutations into existing genes and selecting the resulting biomolecules which have improvements in comparison to starting ones; the selection criteria are usually defined by the aimed for process (better conversion, stereoselectivity, enzyme stability, solvens tolerance etc.). Since the generation of improvement of the phenotype by mutation is very rare, many mutations have to be executed and fast and effective selection procedures have to be at hand. Several techniques to generate diversity of mutant enzymes are known:

i) Site-directed mutagenesis is one of the most important tools of designing proteins. In this technique, a mutation is created at a specific site of the gene encoding the required biocatalyst. As a consequence, the corresponding amino acid at this position in the resulting protein is substituted by another one. Most site-directed mutants are nowadays made by one of the simplest and most rapid methods for site-directed mutagenesis, the QuickChangeTM non-PCR protocol originally developed by Stratagene (La Jolla, CA, USA).
However, it is difficult to predict the change of activity or acceptance of a substrate after changing a specific amino acid in an enzyme. To improve the efficiency, *saturation mutagenesis* was developed. It is based upon a site-directed mutagenesis protocol, adapted to the use of degenerated oligonucleotides, introducing any of the 20 amino acids at a specific position in the protein, to get full diversity. As example for applicability, this technique was used to improve thermostability of a bacterial phytase.[41] Several different protocols for this method where developed over the last years.[42]
ii) Random Mutagenesis Using Error-Prone PCR:[43] to accelerate the creation of libraries, random mutagenesis is the suitable technique. Different methods for initiation of random mutagenesis are known, chemical mutagenesis, UV or mutator bacterial strains, but today the

[40] a) Liao, H.; McKenzie, T.; Hageman, R. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 576. b) Chen, K.; Arnold, F. H. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5618.
[41] Garrett, J. B.; Kretz, K. A.; O'Donoghue, E.; Kerovuo, J.; Kim, W.; Barton, N. R.; Hazlewood, G. P.; Short, J. M.; Robertson, D. E.; Gray, K. A. *Appl. Environ. Microbiol.* **2004**, *70*, 3041.
[42] a) Olins, P. O.; Bauer, S. C.; Braford-Goldberg, S.; Sterbenz, K.; Polazzi, J. O.; Caparon, M. H.; Klein, B. K.; Easton, A. M.; Paik, K.; Klover, J. A.; et al. *J. Biol. Chem.* **1995**, *270*, 23754; b) Chiang, L. W. *Methods Mol. Biol.* **1996**, *57*, 311; c) Kegler-Ebo, D. M.; Polack, G. W.; DiMaio, D. *Methods Mol. Biol.* **1996**, *57*, 297.
[43] a) Leung, D.; Chen, E.; Goeddel, D. *Technique* **1989**, *1*, 11. b) Cirino, P. C.; Mayer, K. M.; Umeno, D. *Methods Mol. Biol.* **2003**, *231*, 3.

most common method is the error-prone PCR.[44] The limitation of this technique is the far higher feasibility of producing certain mutations above others. There is a very low probability of two or three substitutions occurring next to each other. About two thirds of all amino acids in a certain position will not be accessible using random mutagenesis.

The first attempts to improve enzymes with error-prone PCR where conducted by Chen and Arnold [40b] and since then several parameters of various biocatalysts such as thermostability, catalytic activity, enantioselectivity, activity in organic media, specifity etc. could be improved with this method.[45]

iii) In Gene shuffling several techniques are employed, where the recombination of homologous genes originating from nature is implemented. The advantage of this method is the pre-selection of the utilized parental genes by nature and their functionality[46].

iv) With Generation of Diversity by de novo Gene Synthesis costume-tailored diversity can be generated by introducing structural information or natural polymorphismus identified from databases.[47]

v) Massive mutagenesis was first described in 1999 – 2000 and combines the best properties of random and site-directed mutagenesis, thus allowing the generation of large, custom, combinatorial genetic libraries.[48]. Any desired mutation can be accommodated, including all substitutions, deletions as well as insertions, just by slightly modifying the oligonucleotide design. Large library sizes (up to 10^9 variants) are also accessible.

1.1.7.4 Isolation of Positive Mutants, Screening

After obtaining a library with numerous mutants, the next challenge is to identify and isolate those, bearing a positive mutation. This is only a very small minority, but a large number has to be tested. An effective selection of the library for the desired catalytic activity is needed since the typical library size is many orders of magnitude larger then the number of protein variants that can be screened.

---

[44] a) Leung, D.; Chen, E.; Goeddel, D. *Technique* **1989**, *1*, 11. b) Cirino, P. C.; Mayer, K. M.; Umeno, D. *Methods Mol. Biol.* **2003**, *231*, 3.

[45] a) Kim, P.; Yoon, S. H.; Seo, M. J.; Oh, D. K.; Choi, J. H. *Biotechnol. Appl. Biochem.* **2001**, *34*, 99. b) Moore, J. C.; Arnold, F. H. *Nat. Biotechnol.* **1996**, *14*, 458. c) Zhao, H.; Arnold, F. H. *Protein Eng.* **1999**, *12*, 47. d) Lingen, B.; Kolter-Jung, D.; Dunkelmann, P.; Feldmann,R.; Grotzinger, J.; Pohl, M.; Muller, M. *ChemBioChem* **2003**, *4*, 721. e) Axarli, I.; Prigipaki, A.; Labrou, N. E. *Biomol. Eng* **2005**, *22*, 81. f) Henke, E.; Bornscheuer, U. T. *Biol. Chem.* **1999**, *380*, 1029.

[46] a) Sylvestre, J; Chautard, H.; Cedrone, F.; Delcourt, M. *Org. Process Res. Develop.* **2006**, *10*, 562; b) Stemmer, W. P. *Nature* **1994**, *370*, 389. c) Zhao, H.; Giver, L.; Shao, Z.; Affholter, J. A.; Arnold, F. H. *Nat. Biotechnol.* **1998**, *16*, 258. d) Neylon, C. *Nucleic Acids Res.* **2004**, *32*, 1448. e) Streit, W. R.; Daniel, R.; Jaeger, K. E. *Curr. Opin. Biotechnol.* **2004**, *15*, 285.

[47] Zha, D.; Eipper, A.; Reetz, M. T. *ChemBioChem* **2003**, *4*, 34.

[48] a) Delcourt M.; Blesa S. Method for massive directed mutagenesis. US patent application 20040048268 (**2004**); FR patent application 20000010962 (**2000**). b) Sylvestre, J.; Blesa, S.; Delcourt, M. *DrugPlus Int.* **2005**.

Two approaches can be applied: screening and selection.
The difference between both is that screening is performed on individual genes or clones and requires some spatial organization of the screened variants on agar plates, microtiter plates, arrays, or chips, whereas selections act simultaneously on the entire pool of genes.
Both, screening and selection should test directly for the property of interest[49] and the assay should be sensitive over the desired dynamic range.

In libraries of high quality, the frequency of positive mutants should exceed $1/10^4$. Individual screening provides quantitative and precise data. This requires appropriate small volume assays and automation of the process.[50] Multi-well plates with 96- to up to 1536 wells can be used. Currently high-throughput screening usually employs fluorescent or coloured substrates or products to identify the positive mutants. With this method only a limited amount of information can be obtained and for more detailed information a second round of screening with the positive mutants is necessary. The second round screening in not conducted on high-throughput as only a limited number of clones needs to be tested. Other analytical techniques such as (chiral) liquid- or gas chromatography, mass spectrometry, NMR or capillary electrophoresis can be used. Many colorimetric and fluorimetric assays were developed specifically for certain biocatalysts. For those, where no specific substrates for activity determination are available, indirect assays can be used, which take advantage of a specific (color) reaction with a typical by-product of the main biotranformation or quantify cofactor (e.g. NADH) increase spectrophotometrically after a cascade of reactions.[51]

Selection techniques allow the direct isolation of positive mutants from pools and hence circumvent the time-consuming individual high- throughput screening. These techniques are based on the establishment of a link between each genotype (an easy-to-amplify nucleic acid sequence) and the phenotype it encodes (carried by the protein of interest).
Selection techniques can be divided into display technologies[52] and direct selection by compartmentalization.[53, 46a]

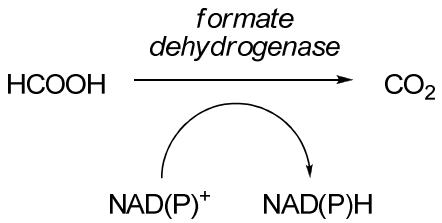
[49] Schmidt-Dannert, C.; Arnold, F. H. *Trends Biotechnol.* **1999**, *17*, 135.
[50] Goddard, J. P.; Reymond, J. L. *Curr. Opin. Biotechnol.* **2004**, *15*, 314.
[51] a) Wahler, D.; Reymond, J.-L. *Curr.Opin. Biotechnol.* **2001**, *12*, 535; b) Reetz, M. T. *Angew. Chem. Int. Ed.* **2002**, *41*, 1335; c) Goddard, J. P.; Reymond, J.-L. *Trends Biotechnol.* **2004**, *22*, 363; d) Bornscheuer, U. T. *Biocat. Biotrans.* **2004**, *19*, 84.
[52] a) Hoogenboom, H. R. *Methods Mol. Biol.* **2002**, *178*, 1; b) Fernandez-Gacio, A.; Uguen, M.; Fastrez, J. *Trends Biotechnol*. **2003**, *21*, 408; c) Lee, S. Y.; Choi, J. H.; Xu, Z. *Trends Biotechnol*. **2003**, *21*, 45.
[53] Aharoni, A.; Griffiths, A. D.; Tawfik, D. S. *Curr. Opin. Chem. Biol.* **2005**, *9*, 210.

1.1.8 Methods for Biocatalysis in Synthesis (Isolated Enzymes vs. Whole Cells)

Monooxygenases require cofactors such as NADH or NADPH for their functional efficiency as the flavin peroxyanion has to be continuously formed for each catalytic cycle. The cofactors (NAD(P)H) are quite costly and therefore they can not be used in equimolar amounts for synthesis[54]. Hence, the challenge is the recycling of the cofactor $NAD(P)^+$, which has to be reduced back to NAD(P)H before entering a new catalytic cycle.
Two approaches are possible:

Isolated Enzymes

One possible approach is working with isolated enzymes and the cofactor is provided by introducing an additional enzyme – substrate system with the auxiliary substrate acting as the hydride donor. The interplay of the two enzymes and substrates has to be established *in situ*, unwanted interactions, formation of side products and enzyme inactivation can result. The reaction environment must be suitable for both enzymes.
The most frequently used enzyme to recover NADH is formate dehydrogenase (FDH)[55], which is commercially available. It oxidizes formate to CO_2 and usually does not show undesired interactions with the other reaction partners. Since CO_2 is gaseous the reaction equilibrium moves far towards formation of CO_2 (Scheme 11).



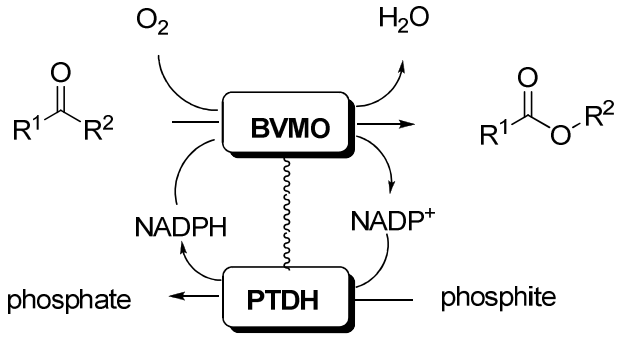
Scheme 11

Regeneration of NADPH, is more complicated, since the wild-type FDH does not accept the phosphorylated cofactor. However, recently a mutant FDH became available also capable to recycle NADPH. A new formate dehydrogenase FDH with $NADP^+$ as cofactor was designed by multipoint site-directed mutagenesis from the bacterium *Pseudomonas* sp. 101.[56] This

[54] Hummel, W., *Trends Biotechnol.* **1999**, *17*, 487.
[55] Wichmann, R.; Wandrey, C.; Bückmann, A.F.; Kula, M.-R. *Biotechnol. Bioeng.* **1981**, *23*, 2789-2802.
[56] a) Seelbach, K.; Riebel, B.; Hummel, W.; Kula, M.-R.; Tishkov, V. I.; Egorov, H. M.; Wandrey, C.; Kragl, U. *Tetrahedron Lett.* **1996**, *37*, 1377; b) Tishkov, V. I.; Galkin, A. G.; Marchenko, G. N.; Tsyganokov, Y. D.; Egorov, H. M. *Biotechnol. Appl. Biochem.* **1993**, *18*, 201.

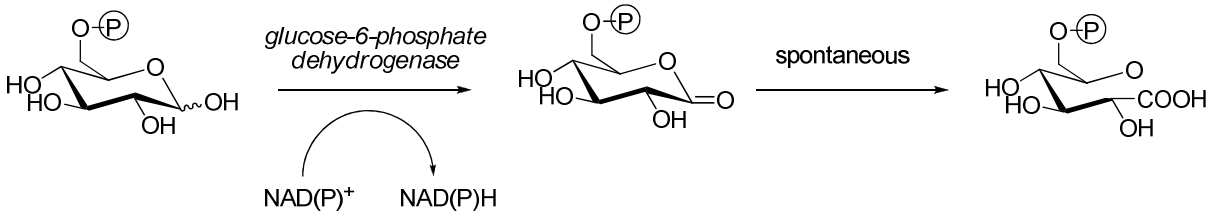
system offers all advantages of wild-type FDH cofactor recycling system and was successfully applied in combination with $CHMO_{Acineto\ 1}$.[57]

Another possibility of NADPH regeneration offers the phosphite dehydrogenase (PTDH) system. The favorable thermodynamic equilibrium constant makes the oxidation of phosphite a nearly irreversible process.[58] The exquisite selectivity of PTDH for phosphite also precludes any side reactions, such as those that can occur, for example, when an alcohol dehydrogenase is used. These characteristics make PTDH an ideal candidate for use as a coenzyme regenerating enzyme (CRE) in combination with BVMOs or other NAD(P)H-dependent enzymes, in particular as fusion proteins (Scheme 12)[59] .



Scheme 12

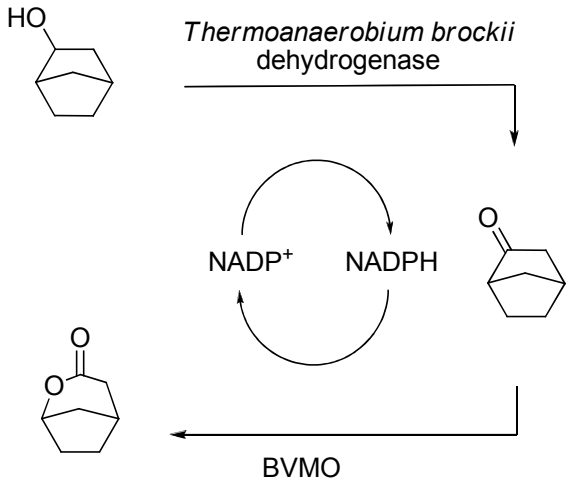
Systems based on glucose-6-phosphate dehydrogenase (G6PDH) were used as an alternative, where glucose-6-phosphate G6P has to be added as auxiliary substrate.[60] G6P is spontaneously hydrolyzed to gluconate (Scheme 13), which represents another hurdle since it may cause difficulties in separation from the reaction product. Furthermore G6P and G6PDH are quite costly.



Scheme 13

[57] a) Rissom, S.; Schwarz-Linek, U.; Vogel, M.; Tishkov, V. I.; Kragl, U. *Tetrahedron Asymm.* **1997**, *8*, 2523; b) Schwarz- Linek, U.; Krödel, A.; Ludwig, F.-A.; Schulze, A.; Rissom, S.; Kragl, U.; Tishkov, V. I.; Vogel, M. *Synthesis* **2001**, 947.
[58] Woodyer, R. D.; van der Donk, W. A.; Zhao, H. *Biochemistry* **2003**, *42*, 11604.
[59] Torres Pazmino D. E.; Snajdrova, R.; Baas, J. B.; Ghobrial, M.; Mihovilovic, M. D.; Fraaije, M. W. *Angew. Chem. Int. Ed.* **2008**, *47*, 2275.
[60] Wong, C.-H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 4890.

Another alternative is the closed-loop system using the dehydrogenase from *Thermoanaerobium brockii*[61] for regeneration of NADPH, where various secondary alcohols can be used as hydride donors, which can be coupled to a purified BVMO. The dehydrogenase converts the substrate alcohol to a ketone and reduces $NADP^+$ to NADPH. Subsequently, the obtained ketone is converted to the corresponding lactone by the BVMO and NADPH to $NADP^+$ (Scheme 14).



Scheme 14

A further improvement for this system represents the application of PEG-NADPH in a membrane reactor.[62] Initital studies using this system in industrial scale fermentations, where cheap secondary alcohols such as 2-propanol are used as hydride donors, show the applicability of this method for big scale production of the desired products.[63] This system was already optimized in laboratory scale.[64]

Whole Cells

The second possibility is to employ whole-cell systems, where the cofactor is regenerated by the operational cell metabolism. This is the case as long as the organisms are intact. Here, another problem arises: the cell as a living organism has a complex system of enzymatic cascades and this can lead to unwanted side reactions and degradation of the desired Baeyer-

[61] a) Willetts, A. J.; Knowles, C. J.; Levitt, M. S.; Roberts, S. M.; Sandey, H.; Shipston, N. F. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1608; b) Grogan, G.; Roberts, S.; Willetts, A. J. *Biotechnol. Lett.* **1992**, *14*, 1125.
[62] Secundo, F.; Carrea, G.; Riva, S.; Battistel, E.; Bianchi, D. *Biotechnol. Lett.* **1993**, *15*, 865.
[63] Hogan, M. C.; Woodley, J. M. *Chem. Eng. Sci.* **2000**, *55*, 2001.
[64] Zambianchi, F.; Pasta, P.; Carrea, G.; Colonna, S.; Gaggero, N; Woodley, J. M. *Biotechnol. Bioeng.* **2002**, *78*, 489.

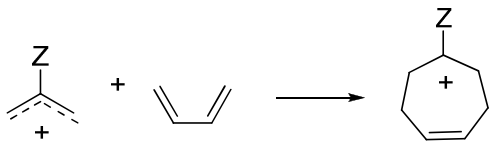
Villiger product respectively, since in nature the oxygenation by BVMOs is followed by hydrolysis of the lactone by a hydrolase. Also, low expression rates are often the limiting factor for an effective reaction. To overcome these difficulties, several new genetically manipulated overexpression systems were created, in which the desired enzyme can be overexpressed and at the same time the competing reactions are suppressed. In this way the degradation of the product can be reduced to a minimum. The problem still to be solved is substrate- and product toxicity vis-a-vis the whole cells, resulting in low concentration biotransformations in large reaction volumes. One method to circumvent this limitation was outlined by Walton and Stewart. For the conversion of cyclohexanone to ε-caprolactone employing $CHMO_{Acineto1}$ they were able to increase volumetric productivity by one order of magnitude under non growing conditions (simultaneously using a solid-phase work-up protocol to absorb the product lactone on a polymer) and could produce up to 20g/l of lactones[65]. Further scale-up attempts are based on the immediate application of a solid-phase reservoire in situ in order to decrease toxic concentrations of substrate and product (SFPR – substrate feed, product removal). Large-scale synthetic applications of BVMOs have been reviewed by Alphand et al.[66] and recently the asymmetric microbial Baeyer-Villiger oxidation of racemic bicyclo [3.2.0]hept-2-en-6-one could be performed in kilogram scale in a 50L reactor[67] and three different ketones (4-methylcyclohexanone, *rac*-3-methylcyclohexanone, and 8-oxabicyclo[3.2.1]oct-6-en-3-one) were converted in 5-15 g/L scale in a conventional bioreactor, with a volumetric productivity of up to 1 g L^{-1} h^{-1} in good to excellent yield and enantiomeric purity.[68]

[65] Walton, A. Z.; Steward, J. D. *Biotechnol. Prog.* **2002**, *18*, 262.
[66] Alphand, V.; Carrea, G.; Wolgemuth, R.; Furstoss, R. *Trends Biotechnol.* **2003**, *21*, 318.
[67] Holker, I.; Wolgemuth, R.; Alphand, V.; Furstoss, R. *Biotechnol. Bioeng.* **2005**, *92*, 207.
[68] Rudroff, F.; Alphand, V.; Furstoss, R.; Mihovilovic, M. D. *Org, Process Res. Develop.* **2006,** *10,* 599.

1.2 [4+3] Cycloaddition

1.2.1 Introduction

Different types of cycloadditions are a most important class of reactions to form cyclic structures. These reactions are being classified by the number of the carbon atoms involved in the cyclization reaction. There are several cycloaddition reactions, giving rise to five and six-membered rings, which are typically constructed by the [3+2] dipolar and [4+2] Diels-Alder cycloaddition reactions. The construction of seven-membered rings was a problem for a long time, until Fort reported the generation of oxyallyl cations and their subsequent trapping with furan in 1962 for the first time.[69] Since then, [4+3] cycloaddition reactions have been further developed and optimized, so that a large number of functionalized seven-membered ring systems are accessible today.
This transformation is a very powerful tool for the relatively simple preparation of complex (eventually additionally bridged and/or fused) seven-membered rings from simple starting materials. The product formed from the reaction depends on the nature of the "Z" group (Scheme 15) in the 2 position of the allylic cation and also on other reaction conditions like catalyst, solvent, etc. This group is in most cases an oxygen-bearing functionality.

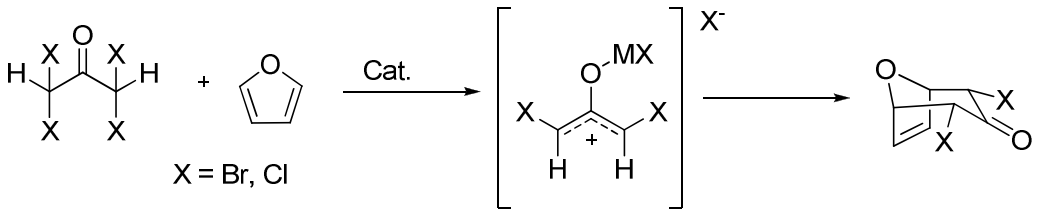


Scheme 15

The seven-membered rings, obtained from allylcations and 1,3-dienes have turned out to be very interesting starting materials for the synthesis of natural products. Many different functionalities are compatible with this reaction and the mostly concerted process displays a certain control of the relative stereochemistry. In reactions with asymmetric substituted reactants, a high regioselectivity could be observed. For the development and application of the asymmetric [4+3] cycloaddition and the synthesis of chiral building blocks see a recent review by Hartung and Hoffmann.[70]

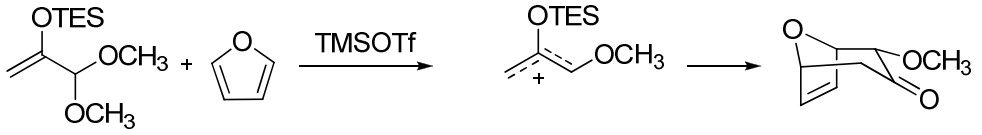
[69] Fort, A. W. *J. Am. Chem. Soc.* **1962**, *84*, 4979.
[70] Hartung, I. H.; Hoffmann, H. M. R. *Angew. Chem. Int. Ed.* **2004,** *43*, 1934.

In the beginning, mainly α,α'-perhalogenated ketones were used, which have been transformed into the reactive oxoallyl cations under Lewis acid catalysis (Scheme 16):



Scheme 16

After reductive dehalogenation of the cycloadducts, seven-membered rings and also bi- and tricyclic compounds were obtained.
Later, oxoallyl cations were generated also from non-halogen precursors[71] (Scheme 17):



Scheme 17

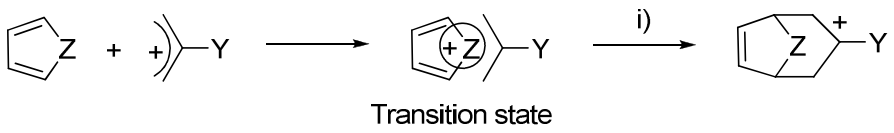
In intramolecular [4+3] cycloadditions even better control of the stereochemistry was observed. Usually, the stereoselectivity is less dependent on the reaction conditions.

1.2.2 Mechanism

The reactions of allylic cations with 1,3-dienes have been classified in three types[72]:

[71] Albizati, K. F.; Murray, D. H. *Tetrahedron Lett.* **1990**, *31*, 4109.
[72] Hoffmann, H. M. R. *Angew. Chem.* **1984**, *96*, 29.

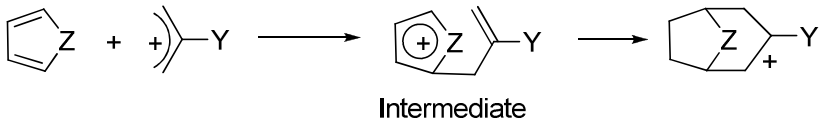
Type A: Concerted Bond Formation (Scheme 18):



Scheme 18

Products: Bridged seven-membered rings.
The transition state can be "compact" or more "stretched", which leads to endo- or exo products, respectively.

Type B: Stepwise Bond Formation (Scheme 19):

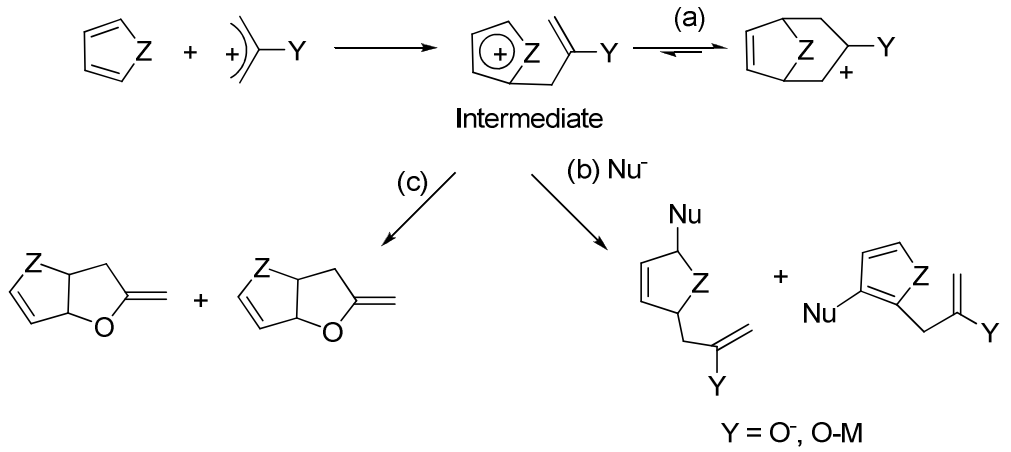


Scheme 19

Products: Bridged seven-membered rings.
Stereochemistry: wether the allylic cation retains or looses the configuration depends on the life-time of the carbocation (intermediate).

Type C: Electrophilic Addition, followed by:

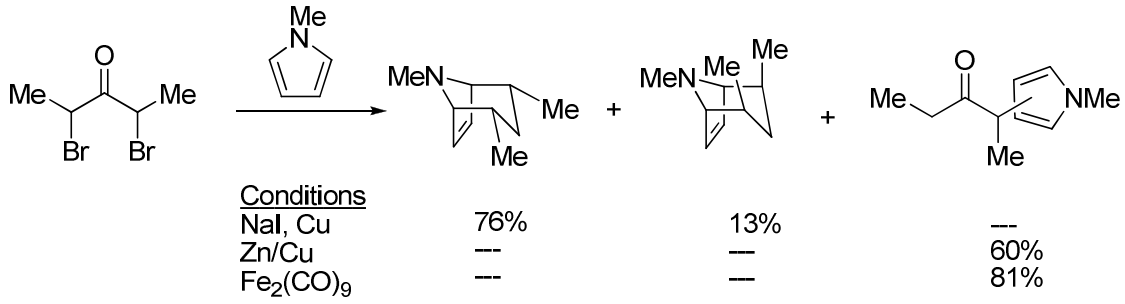
Path (a): loss of proton (Electrophilic substitution)
Path (b): intermolecular nucleophilic attack on the carbocationic intermediate
Path (c): intramolecular nucleophilic attack on the carbocationic intermediate (Scheme 20):



Scheme 20

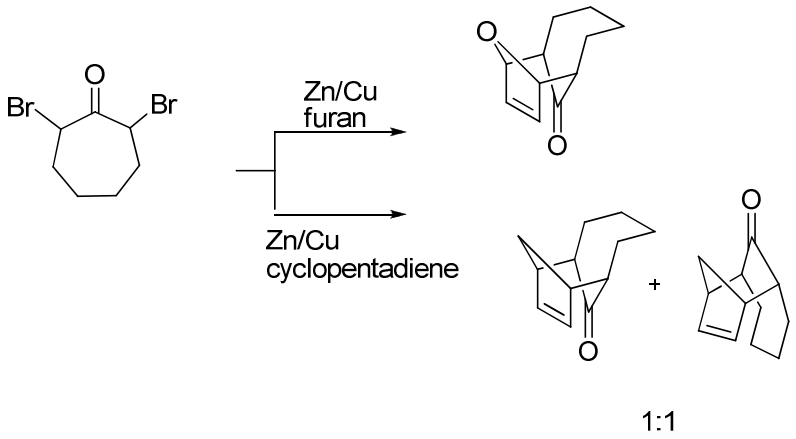
Predictable consequences for products: five-membered carbocycles and heterocycles, acyclic 1:1-adducts, if only one σ-bond is formed.
The distinction between types B and C depends on the rate constants of the reaction step i) (Scheme 18) and the corresponding reverse reaction, which on its part is determined by the stability of the intermediate.
The stereochemical result depends on the life-time of carbocationic intermediate (type B).

An example with the nucleophilic pyrrole shows the effect of the diene (Scheme 21):



Scheme 21

The reaction profile for the reaction of the oxyallyl cation derived from 2,7-dibromocycloheptanone is typical, reacting with either furan or cyclopentadiene. In the reaction with furan, the cyclic cations prefer to react via the compact transition state, and afford the anti-product, while with cyclopentadiene no selectivity is observed and both anti- and syn-products are formed in ratio 1:1 (Scheme 22).



Scheme 22

For more examples see the original article by Hoffmann[72] and related articles.[73,74]

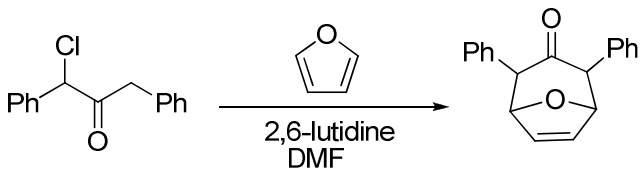
1.2.3 Generation of Oxoallyl Cations

[4+3] Cycloaddition reactions can be performed in an inter- and intramolecular fashion. The best established procedures for the intermolecular reactions are described below:

1.2.3.1 Thermal methods

Intermolecular [4+3] cycloaddition reactions:

Fort first reported the generation of oxoallyl cations and their trapping with furan[69] (Scheme 23):

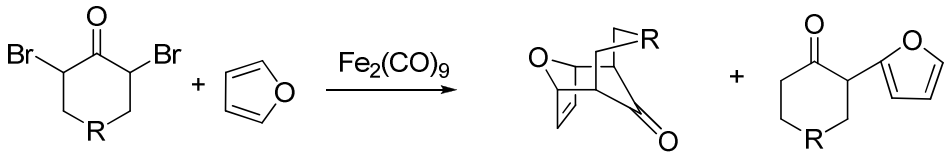


Scheme 23

[73] Noyori, R.; Hayakawa, H.; Takaya, H.; Murai, S.; Kobayashi, R.; Sonoda, N. *J. Am. Chem. Soc.* **1978,** *100,* 1759.
[74] a) Ishizu, T.; Mori, M.; Kanemazsu, K. *J. Org. Chem.* **1981**, *46,* 526; b) Paquette L. A.; Kravetz, T. M. *J. Org. Chem.* **1985**, *50* ,3781.

The Noyori conditions[75,73,74] using $Fe_2(CO)_9$ under N_2 in benzene (or only excess of furan) are suitable for reactions between perbromoketones and unsaturated substrates. They are more suitable for open-chain dibromoketones, which give higher yields (up to 96%) with both, cyclic and acyclic dienes. Also the first intramolecular [4+3] cycloadditions are reported using this method. However, toxicity of metal carbonyl compounds and elaborate purification protocols represent a significant disadvantage of this methodology

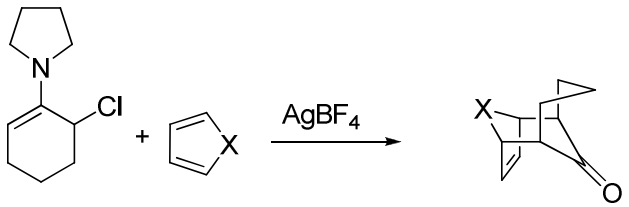
With cyclic dibromoketones, lower yields (35% - 54%)[76, 77] and more side products were obtained (Scheme 24):



Scheme 24

Hoffmann developed several protocols for this reaction[78] (Table 2). The [4+3] cycloaddition of cyclic dibromoketones in the presence of NaI/Cu gives yields from 10% to 35%, however much better results are obtained with acyclic α,α-dibromoketones (yields up to 82%):

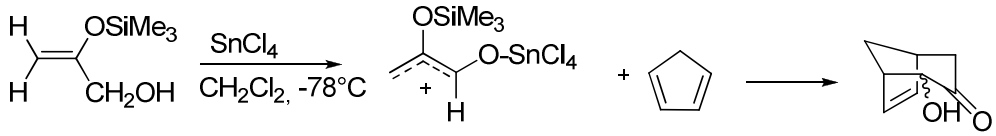
Schmid[79] improved the yield considerably by using α-chloroenamine as precursor for the allylcation (68% - 76%), however, stoichiometric amounts of $AgBF_4$ are required (Scheme 25):



Scheme 25

[75] Noyori, R.; Nishizawa, M.; Shimizu, F.; Hayakawa, Y.; Maruoka, K.; Hashimoto, S.; Yamamoto, H.; Nozaki, H. *J. Am. Chem. Soc.* **1979**, *101*, 220.
[76] Siemionko, R. K.; Berson, J. A. *J. Am. Chem. Soc.* **1980,** *102,* 3870.
[77] Noyori, R.; Baba, Y.; Makino, S.; Takaya, H. *Tetrahedron Lett.* **1973,** 1741.
[78] a) Vinter, J. G.; Hoffmann, H. M. R. *J. Am. Chem. Soc.* **1973,** *95,* 3051; b) Hofmann, H. M. R. *Angew. Chem.* **1972**, *11*, 324; c) Greenwood, G.; Hoffmann, H. M. R. *J. Org. Chem.* **1972**, *37,* 611; d) Hoffmann, H. M. R.; Weber, A.; Giguere, R. J. *Chem. Ber.* **1984**, *117*, 3325.
[79] Schmid, R.; Schmid, H. *Helv. Chim. Acta* **1974,** *57,* 1883.

Föhlisch developed a new procedure,[80] involving cyclic α-monohaloketones in the presence of Et_3N or CF_3CH_2ONa in trifluoroethanol and improved the yields up to 84%.
In contrast to the methods known before, Föhlisch uses chlorinated ketones instead of brominated substrates in most cases, which are more facile to handle.
Another method was reported by Föhlisch when stirring an ethereal solution of the starting material with excess of $LiClO_4$ and triethylamine at room temperature. This protocol gives good yields (84%) in both intermolecular[81] and intramolecular[82] reactions.
Sasaki found, that $SnCl_4$, added in equimolar concentration, catalyses the [4+3] cycloaddition of 2-silyloxyacrolein and cyclopentadiene or furan[83] (Scheme 26).



Scheme 26

Montana developed a procedure using Cu/Zn couple in acetonitrile,[84] sonicating the reaction mixture under mild conditions, which strongly shortened the reaction times (in some cases to 15 min). A summary of the methods described above and other methods is given in the Table 2:

[80] Fohlisch, B.; Gehrlach, E.; Herter, R. *Angew. Chem. Int. Ed.* **1982**, *21*, 137.
[81] Herter, R.; Föhlisch, B. *Synthesis* **1982**, 976.
[82] Kaiser, R.; Föhlisch, B. *Helv. Chim. Acta* **1990**, *73,* 1504.
[83] Sasaki, T.; Ishibashi, Y.; Masatomi, O. *Tetrahedron Lett.* **1982**, *23*, 1693.
[84] Montana, A. M.; Grima, P. M. *Tetrahedron Lett.* **2001**, *42*, 7809.

Table 2

Method	Reaction conditions	Precursor for allyl cation [yield]
Fort [69]	2,6-lutidine, DMF, R.T.	α-chloro-dibenzylketone [18%]
Noyori [73, 85]	$Fe_2(CO)_9$ N_2 in benzene (or only excess of furan), 60-120°C	Open-chained α,α-dibromoketones [96%]
		cyclic α,α-dibromoketones [54%]
Hoffmann[78a, 86]	NaI/Cu N_2, MeCN, R.T. to 78°C	Cyclic α,α-dihaloketones [9.5% - 36%]
		open chained α,α-dihaloketones [77-82%]
Hoffmann[72, 87]	$B(OEt)_3$/Zn N_2, 10°C, sonication	Cyclic dihaloketones [35%]
		open chained α,α-polyhaloketones [35%]
Hoffmann [78b,d] [88, 89]	Zn/Ag, Cu/Zn (Me_3SiCl accelerates the reaction) Dimethoxyethane or THF or DMF or DMA, -10°C to R.T.	α,α-dibromoketones [65%]
Hoffmann [78c, 90]	$AgOCOCF_3$, Na_2CO_3 Benzene/pentane	α-halo-methoxyprop-(1)-enes [60%]
Schmid [79 , 91]	$AgBF_4$ Et_3N CF_3CH_2OH,	α-chloroenamine [76%]
Föhlisch [80, 92]	Et_3N oder CF_3CH_2ONa CF_3CH_2OH R.T.	α-monohalo ketones, di- und α,α-polyhaloketone (particularly Cl) [84%]
Föhlisch[81, 93]	$LiClO_4$, Et_3N Et_2O, RT	α-monohalo ketones [81%]
Föhlisch[94]	Ag_2O furan, CH_2Cl_2 or ether, R.T.	γ-bromo-β-oxonitriles, aryl substituted α-bromo-β-oxonitriles, but not aliphatic substituted α-bromo-β-oxonitriles [85%]
Sasaki [83]	$SnCl_4$, CH_2Cl_2 Furan or cyclopentadiene, -78°C	2-silyloxyacrolein [43-72%]

[85] Noyori, R.; Souchi, T.; Hayakawa, Y. *J. Org. Chem.* **1975**, *40,* 2681.
[86] a) Hoffmann, H. M. R.; Wagner, D.; Wartchow, R. *Chem. Ber.* **1990**, *123,* 2131; b) Schottelius, T.; Hoffmann, H. M. R. *Chem. Ber.* **1991,** *124*, 1673; c) Busch, A.; Hoffmann, H. M. R. *Tetrahedron. Lett.* **1976**, 2379.
[87] Hoffmann, H. M. R.; Iqbal, M. N. *Tetrahedron Lett*. **1975**, *50*, 4487.
[88] Hoffmann, H. M. R.; Clemens, E. K.; Smithers, R. H. *J. Am. Chem. Soc.* **1972**, *94*, 3940.
[89] Sato, T.; Noyori, R. *Bull. Chem. Soc. Jpn.* **1978**, *51,* 2745.
[90] A. E. Hill, G. Greenwood, H. M. R. Hoffmann, *J. Am. Chem. Soc.,* **1973**, *95*, 1338
[91] Ramos Tombo, G. M.; Pfund, R. A.; Ganter, C. *Helv. Chim. Acta* **1981**, *64,* 813.
[92] a) Fohlisch, B.; Joachimi, R. *Chem. Ber.* **1987**, *120*, 1951; b) Fohlisch, B.; Joachimi, R.; Reiner, S. *J. Chem. Res. (S)* **1993**, 253.
[93] Harmata, M.; Elahmad, S. *Tetrahedron Lett.* **1993**, *34*, 789.
[94] a) Föhlisch, B.; Herter, R.; Wolf, E.; Stezowski, J. J.; Eckle, E. *Chem. Ber.* **1982**, *115*, 355; b) Föhlisch, B.; Wolf, E. *J. Chem. Res. (S)* **1983**, *7*, 166.

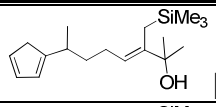
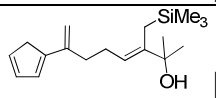
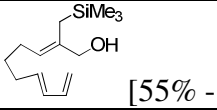
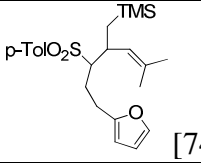
Method	Reaction conditions	Precursor for allyl cation [yield]
Mann[95]	Et_3N Furan (excess) and MeOH or CF_3CH_2OH, -20°C to R.T.	α-haloketones, polyhaloketones [75-85%]
Mann[96]	$NaBF_4$ or $AgBF_4$, Et_3N MeCN or CH_2Cl_2, R.T., furan	α-bromoketones EtCOCHBrMe [80%]
Shimizu[97]	$AgClO_4$ CH_3NO_2 furan	2-(trimethylsiloxy) allyl chlorides The stereochemistry of the cycloadducts strongly depends on the solvent [66-92%]
Hosomi[98]	$ZnCl_2$/ether CH_2Cl_2	2-(trimethylsiloxy) allyl chlorides [60-97%]
Montana[84]	Cu/Zn, NaI MeCN, Sonication, or stirring -44°C – 0°C	Open-chained and cyclic α,α – diiodoketones [90%]
Mihovilovic[99]	Zn or Cu/Zn, MeCN, Sonication, RT	tetrabromoacetone [62%]

[95] Mann, J.; Wilde, P. D.; Finch, M. W. *Chem. Commun.* **1985**, 1543.
[96] Mann, J.; Usmani, A. A. *Chem. Commun.* **1980**, 1119.
[97] Shimizu, N.; Tanaka, M.; Tsuno, Y. *J. Am. Chem. Soc.* **1982,** *104,* 1330.
[98] Sakurai, H.; Shirahata, A.; Hosomi, A. *Angew. Chem. Int. Ed.* **1979**, *18*, 163.
[99] Mihovilovc, M. D.; Grötzl, B.; Kandioller, W.; Snajdrova, R.; Muskotal, A.; Bianchi, D.; Stanetty, P. *Adv. Synth. Catal.* **2006**, *348*, 463.

Intramolecular [4+3] cycloaddition reactions:

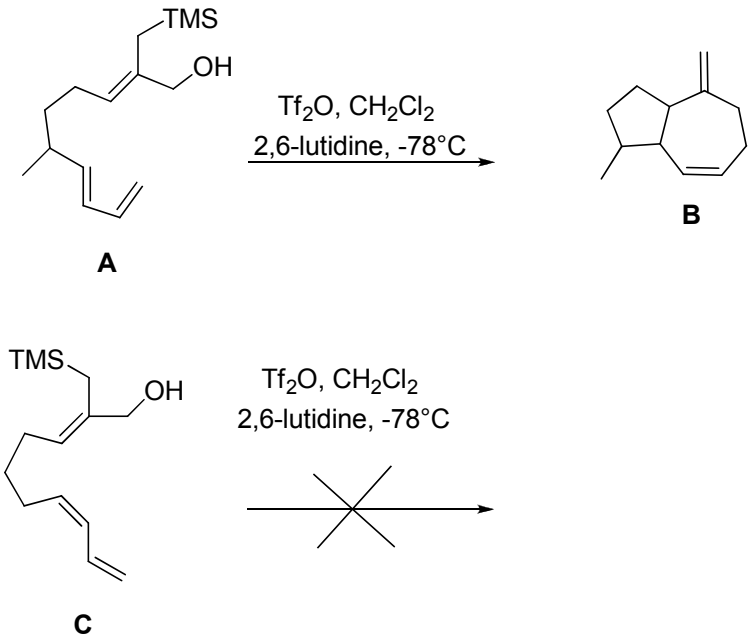
Products of intramolecular reactions possess complex carbocyclic architectures. Beside the procedures described above, other protocols have been applied in these reactions[100] (Table 3).

Table 3

Method	Reaction condition	Substrate [Yield]
Hoffmann[101]	$(CF_3CO_2)_2O$, $ZnCl_2$ on alumina CH_2Cl_2, -70°C, EtN-*i*-Pr_2	 [16%]
Hoffmann[101]	$TiCl_4$/PhNHMe, CH_2Cl_2, -78°C	 [20%]
Giguere[102]	Tf_2O, CH_2Cl_2, -78°C 2,6-lutidine	 [55% - 82%]
Harmata[103]	$TiCl_4$ or $AlMe_3$, CH_2Cl_2, -78°C	 [74%]

The geometry and the substituents on the precursor molecules (in this example the geometry of the diene) are very important in these cases and have a big impact on the reaction outcome. For example, product **B** is obtained in 82% yield starting from compound **A**, but no product can be observed under identical reaction conditions from **C**, only electrophilic addition and elimination products were observed[104] (Scheme 27):

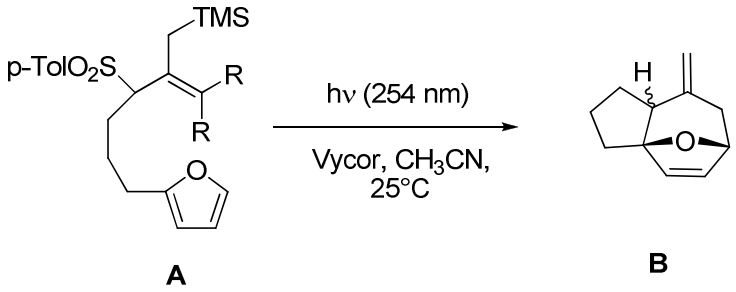
[100] a) Harmata, M. *Tetrahedron* **1997**, *53,* 6235; b) Harmata, M. *Acc. Chem. Res.* **2001**, *34*, 595.
[101] Hoffmann, H. M. R.; Eggert, U.; Gibbels, U.; Giesel, K.; Koch, O.; Lies, R.; Rabe, J. *Tetrahedron* **1988**, *44,* 3899.
[102] a) Giguere, R. J.; Duncan, S. M.; Bean, J. M.; Purvis, L. *Tetrahedron Lett.* **1988**, *29,* 6071; b) Giguere, R. J.; Tassely, S. M.; Rose, M. I.; Krishnamurthy, V. V. *Tetrahedron Lett.* **1990**, *31,* 4577.
[103] a) Harmata, M.; Gamlath, C. B. *J. Org. Chem.* **1988**, *53,* 6154; b) Harmata, M.; Herron, B. F. *J. Org. Chem.* **1993**, *58,* 7393; c) Harmata, M.; Herron, B. F. *Synthesis* **1993**, 202.
[104] Kuja, E.; Giguere, R. J. *Synth. Commun.* **1995,** *25*, 2105.



Scheme 27

1.2.3.2 Photochemical Methods

Photochemical generation of acyclic and cyclic allylic cations is possible, though the generation of the later species is more facile.
Photolysis of an acetonitrile solution of **A** (254 nm, Vycor filter) resulted in the formation of cycloadduct **B** in 56% yield[105] (Scheme 28):

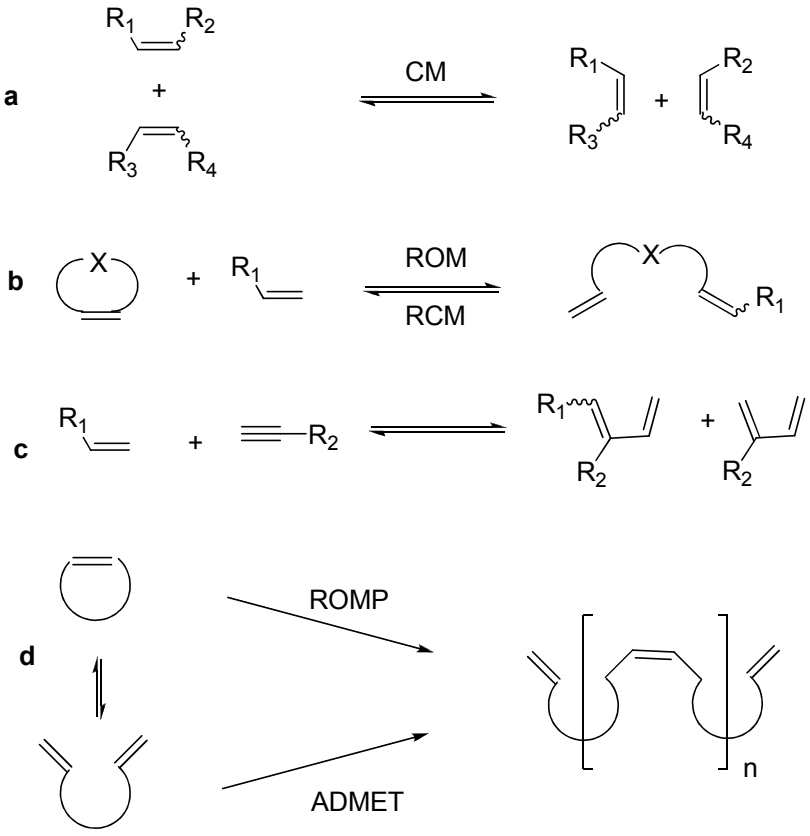


Scheme 28

[105] Harmata, M.; Herron, B. F. *Tetrahedron Lett.* **1993**, *34*, 5381.

1.3 Olefin Metathesis

Olefin metathesis is mutual alkylidene exchange reaction of alkenes, where one C-C bond is cleaved and another is formed.[106] Five main versions are known: a) cross metathesis (CM), b) ring opening cross-metathesis (ROM) and ring closing metathesis (RCM) – reverse ROM, c) intermolecular enyne metathesis and d) ring opening polymerization (ROMP) and acyclic diene metathesis polymerization (ADMET) (Scheme 29).



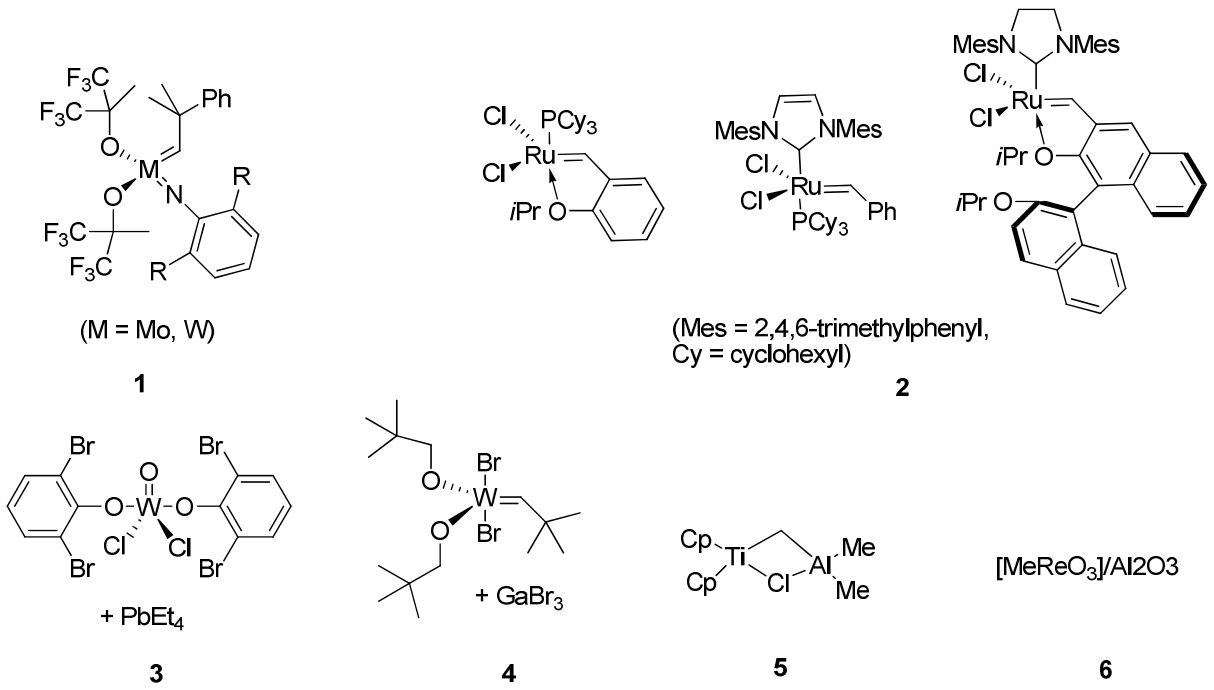
Scheme 29

Olefin metathesis is a powerful C-C bond forming tool and proceeds under mild conditions. In general, only 1-5 mol% of catalyst are required and a wide range of functional groups are tolerated.

Olefin metathesis was discovered in the 1950s and after elucidation of the reaction mechanism (Chauvin mechanism)[107] the door was open to a new generation of transition-metal catalysts, which in situ convert into metal alkylidenes. Most popular systems are

[106] a) Connon, S. J.; Blechert, S. *Angew. Chem. Int. Ed.* **2003**, *42*, 1900; b) Fürstner, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 3012; c) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem. Int. Ed.* **2005**, *44*, 4490.
[107] Hérisson, J.-L.; Chauvin, Y. *Makromol. Chem.* **1970**, *141*, 161.

tungsten and molybdenum based catalysts (**1**) developed by Schrock et al.[108] and ruthenium based catalysts (**2**), introduced by Grubbs et al.[109], where the later ones display the advantage to be less sensitive to oxygen as well as moisture and, consequently, which are easier to handle. In Scheme 30 some typical metathesis catalysts are summerized:



Scheme 30

In Table 4, functional group tolerance of different catalysts based on different transition metals is compiled:

Table 4

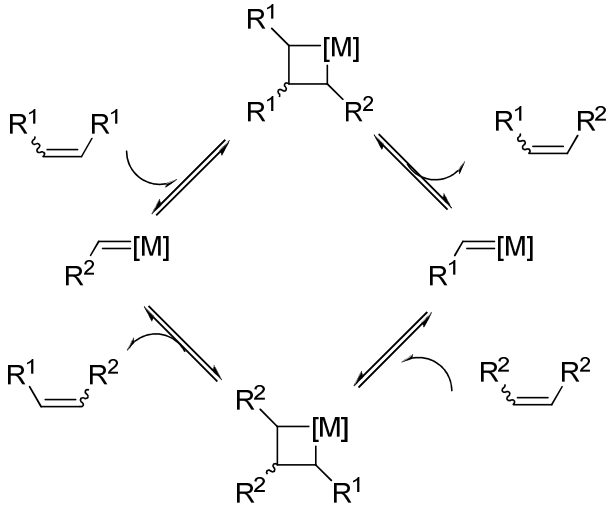
Ti	W	Mo	Ru
Acids	Acids	Acids	**Olefins**
Alcohols, Water	Alcohols, Water	Alcohols, Water	Acids
Aldehydes	Aldehydes	Aldehydes	Alcohols, Water
Ketones	Ketones	**Olefins**	Aldehydes
Esters, Amides	**Olefins**	Ketones	Ketones
Olefins	Esters, Amides	Esters, Amides	Esters, Amides

Increasing reactivity

[108] a) Schrock, R. R.; Murdzek, J. S.; Bazan, G. C.; Robbins, J.; DiMare, M.; O`Regan, M. *J. Am. Chem. Soc.* **1990**, *112*, 3875; b) Oskam, J. H.; Fox, H. H.; Yap, K. B.; McConville, D. H.; O`Dell, R.; Lichtenstein, B. J.; Schrock, R. R. *J. Organomet. Chem.* **1993**, *459*, 185; c) Feldman, J.; Murdzek, J. S.; Davis, W. M.; Schrock, R. R. *Organometallics* **1989**, *8*, 2260.

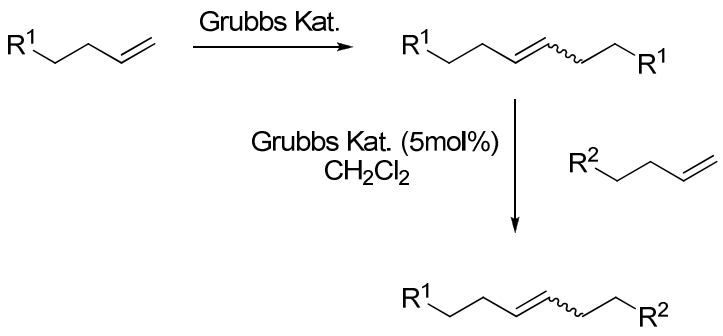
[109] a) Nguyen, S. T.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 9858; b) Wu, Z.; Nguyen, S. T.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 5503; c) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100; d) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **1995**, *34*, 2039.

The mechanism of olefin metathesis is a sequence of reversible steps which are formal [2+2] cycloadditions involving alkenes, carbenes, and methallcyclobutanone intermediates and results in an equilibrium mixture of olefins (Scheme 31).



Scheme 31

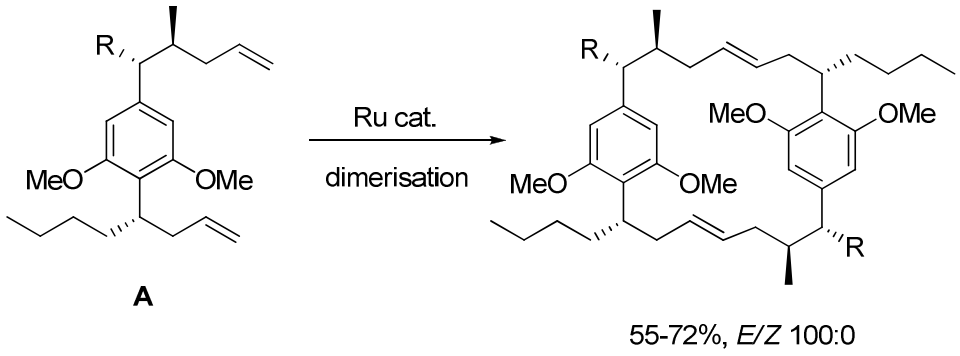
In cross metathesis (CM) it is important to avoid self-metathesis. Some attempts were made and a new two-step method was elaborated, where first a homodimer of a terminal olefin is formed in a cross- metathesis reaction and then is allowed to react with a second terminal olefin in presence of Grubbs catalyst (Scheme 32).[110] Although not always applicable it was shown that in many cases this strategy was preferable to straightforward cross-metathesis coupling of two terminal olefins. Unreacted homodimers could be recovered for further use.
The good selectivity of this reaction was explained in terms of the preferential formation (due to the presence of excess homodimer) of a more stable, substituted ruthenium alkylidene (rather than methylidene) intermediate during the catalytic cycle[111].



Scheme 32

[110] Grubbs, R. H.; O`Leary, D. J.; Blackwell, H. E.; Washenfelder, R. A. *Tetrahedron Lett.* **1998**, *39*, 7427.
[111] Grubbs, R.H.; Ulman, M. *Organometalics* **1998**, *17*, 1484.

The *E/Z* selectivity is another challenge in this reaction. In some cases the steric nature of the substrates allows specific formation of only a single isomer.[112] In most other cases, the reversibility of the reaction makes *E/Z* rations higher than 1:1 possible, since the reaction partners react towards the thermodynamically most stable product. This was shown by Smith et al. with the synthesis of (-)-cylindrocyclophanes A and F by dimerisation of **A**,[113] where only one product was generated out of all eight possible products (head-to-head, head-to-tail and *E/Z* isomers), which turned out to be the most thermostable (Scheme 33):



A

55-72%, *E/Z* 100:0

Scheme 33

The ring opening cross metathesis (ROM-CM) is the more efficient, the more strained the olefin ring is. Cyclobutenes, norbornenes and oxanorbornenes are very good substrates, since the reverse reaction – the ring closing metathesis (RCM) – is unlikely from the energetically point of view.

In the last decade, asymmetric metathesis catalysts were developed. Asymmetric ROM-CM was performed with enantiopure molybdenum catalyst[114] and a one-pot sequential asymmetric ROM-CM reaction could be performed, where the ring-opened and arylated product was obtained in 51% yield with > 98% *ee.*

[112] Roy, R.; Das, S. K.; Dominique, R.; Trono, M. C.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *Pure Appl. Chem.* **1999**, *71*, 565.

[113] a) Smith III, A. B.; Kozmin, S. A.; Adams, C. M.; Paone, D. V. *J. Am. Chem. Soc.* **2000**, *122*, 4984; b) Smith III, A. B.; Adams, C. M.; Kozmin, S. A. *J. Am. Chem. Soc.* **2001**, *123*, 990; c) Smith III, A. B.; Adams, C. M.; Kozmin, S. A.; Paone, D. V. *J. Am. Chem. Soc.* **2001**, *123*, 5925.

[114] a) La, D. S.; Ford, J. G.; Sattely, E. S.; Bonitatebus, P. J.; Schrock, R. R.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1999**, *121*, 11603; b) La, D. S.; Sattely, E. S.; Ford, J. G.; Schrock, R. R.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2001**, *123*, 7767.

2 SCOPE OF THIS THESIS

Chiral lactones and esters are important intermediates in the synthesis of biologically active compounds.

In the research group of Prof. Marko Mihovilovic, many studies about application of Baeyer-Villiger monooxigenases (BVMO) in the synthetic organic chemistry and preparation of chiral lactones were conducted within the past years. The existing substrate library contains monocyclic-, bicyclic- and bridged bicyclic ketones, which turned out to be quite good substrates for some of the known BVMO overexpression systems and whose applications in synthetic chemistry are to some extend established.

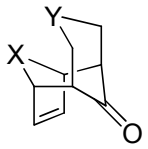
The task of this thesis was to explore the limitations of the substrate acceptance of the BVMOs and therefore extend the substrate library in synthesizing tricyclic bridged ketones and acyclic functionalized ketones and examine the acceptance with the available BVMOs.
The desired polycyclic substrates for biooxidation were synthesized via [4+3] cycloaddition and subsequent olefin metathesis.

Beside conversion to the desired products also desymmetrisation and kinetic resolution of linear ketone substrates in particular was executed to obtain optically pure products.

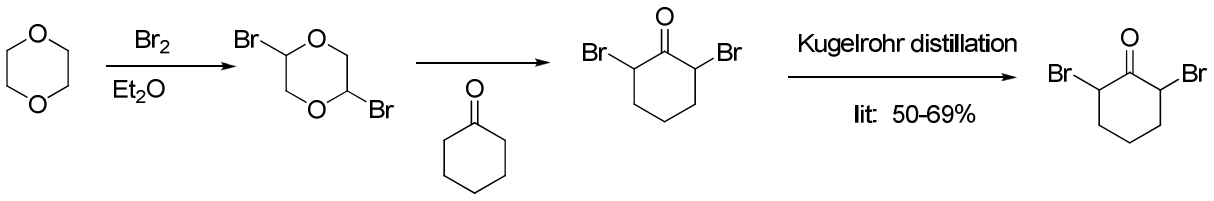
3 RESULTS AND DISCUSSION

3.1 Synthesis of Tricyclic Ketones

The synthesis of prochiral ketones is an important step in production of optically pure lactones which have a big potential as precursor in production of different naturally active compounds.[115]



Several different methods are known to prepare tricyclic ketones. In the literature two main strategies are followed, starting from α-haloketones [119,116] and from α,α-dihaloketones[117] via [4+3] cycloaddition.
The second strategy is described by Harmata et al. and has the disadvantage of the rather poor yield, the overall yield in this reaction is less than 20% over two steps. An alternative method for the synthesis of the 1,6-dibromocyclohexanone was demanded. A protocol from literature[118] which promised a yield of 50-69% was followed (Scheme 34):



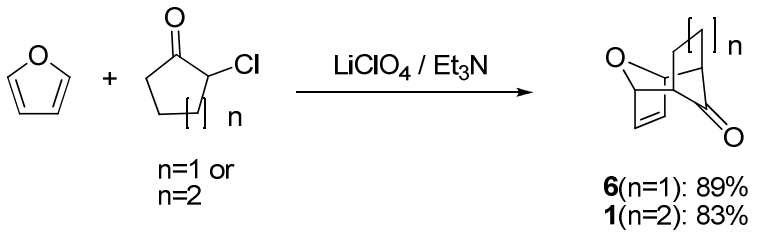
Scheme 34

Unfortunately, in our hands the experiment ended with decomposition during *Kugelrohr* distillation.

[115] Bode, J.W.; Doyle, M.P.; Protopopova, M.N.; Zhou, Q.-L. *J. Org.Chem.* **1996**, *61*, 9146-9155.
[116] Jin, S.J.; Choi, J.R.; Oh, J.; Lee, D.; Cha, J.K. *J. Am. Chem. Soc.* **1995**, *117,* 10914.
[117] Harmata, M.; Shao, L.; Kürti, L. Abeywardane,A. *Tetrahedron Lett.* **1999**, *40*, 1075.
[118] Machinskaia, I.V.; Podberezina, A.S. *J. Gen. Chem. USSR* **1958**, 1550.

No further attempts were made to follow this synthetic strategy, since the synthesis from α-haloketones gives far better yields.

3.1.1 Synthesis of Oxotricyclic Ketones



Scheme 35

Tricyclic ketones **1** and **6** were prepared according to the literature protocol.[119] To a dispersion of lithium perchlorate in diethyl ether consecutively triethylamine and excess of furan were added. Then, slowly the appropriate α-haloketone was added and the reaction mixture was stirred at room temperature. After all α-haloketone was consumed, the reaction mixture was quenched with water and extracted with ether. The products **6** and **1** were obtained in excellent yield of 89% or 83% respectively in 95% purity (area GC/MS), so no further purification was necessary before using as substrates for the subsequent reaction. In both cases only the *anti* diastereomer was obtained (**6**[120], **1,**[121] assignment by comparing with data from the literature).

Sonochemical Conditions

Because the [4+3] cycoaddition was performed very successfully in our research group in case of the bicyclic compounds under sonochemical conditions,[122] the method was attempted also for the synthesis of the tricyclic substrates. Three experiments were carried out, where furan and 2-chlorocyclohexanone in presence of copper-zinc couple[123] were sonicated at room temperature or under reflux using acetonitrile or diethyl ether as solvents. No conversion to the desired product could be observed, only decomposition of 2-chlorocyclohexanone. Sonochemical and conventional experiments and yields of **1** and **6** are summarized in Table 5.

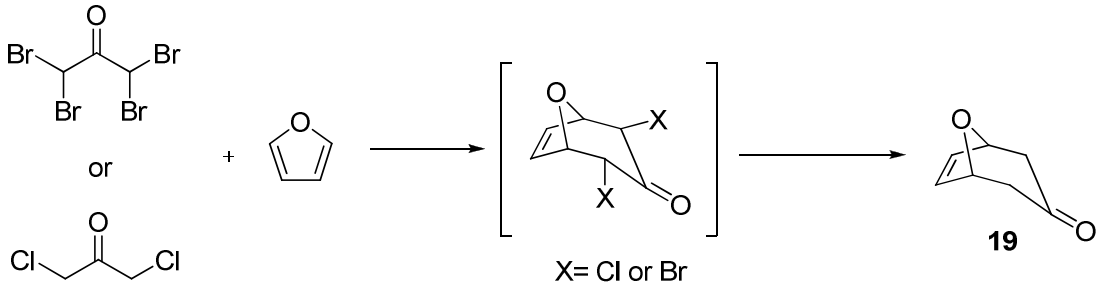
[119] Herter, R.; Föhlisch, B. *Synthesis* **1982**, 976.
[120] Föhlisch, B.; Joachimi, R. *Chem. Ber.* **1987**, *120,* 1951.
[121] Schmid, R.; Schmid, H. *Helv. Chim. Acta* **1974,** *57***,** 1883.
[122] Mihovilovic, M. D.; Groetzl, B.; Kandioller, W.; Snajdrova, R.; Muskotal, A.; Bianchi, D. A.; Stanetty, P. *Adv. Synth. Catal.* **2006**, *348(4 + 5),* 463.
[123] Kim, H.; Hoffmann, H. M. R. *Eur. J. Org. Chem.* **2000,** 2195.

Table 5

	Product	Reactants	Solvent	Time, Temp.	Conversion [%]	Yield [%]
1	**1**	$LiClO_4$,TEA	Et_2O	45h, R.T.	100	83
2	**1**	Cu/Zn, US*	MeCN	6h, R.T.	Decomp.	*n. c.*
3	**1**	Cu/Zn, US*	Et_2O	3h, R.T.	Decomp.	*n. c.*
4	**1**	Cu/Zn, US*	Et_2O	8h , reflux	Decomp.	*n. c.*
5	**6**	$LiClO_4$,TEA	Et_2O	4h, R.T.	100	89

*US = ultrasound

***Synthesis of 8-Oxabicyclo [3.2.1]oct-6-en-3-one* 19 *and anti-11(N-methoxycarbonyl) azatricyclo[4.3.1.1^{2,5}]undec-3-en-10-one* K**



Scheme 36

As mentioned above, a sonochemical protocol for synthesis of bicyclic ketones via [4+3] cycloaddition is well established in our research group. After reproducing the synthesis of **19** in this way, we tried to transfer the method from Föhlisch[119] which turned out to be very successful in synthesis of tricyclic ketones to this bicyclic ketone. Although both substrates show structural similarities, the attempts under Föhlisch conditions were fruitless. Also the change of tetrabromoacetone to 1,2-dichloroacetone [124] did not give any improvement.
A summary of these experiments is given in the Table 6.

[124] Föhlisch, B.; Korfant, M.; Meining, H.; Frey, W. *Eur. J. Org. Chem.* **2000,** 1335.

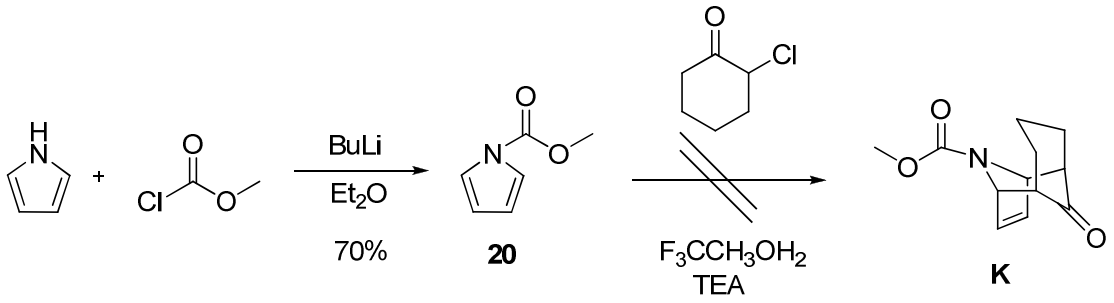
Table 6

	Reagent	Reactants	Solvent	Time, Temp.	Conversion [%]	Yield [%]
1	*TBA**	1.) Cu/Zn, US*** 2.)NH_4Cl, Cu/Zn	MeCN	1.)25°C, 30min 2.)-78°C, 75min	100	56
2	*TBA**	$LiClO_4$,TEA	Et_2O	10 min-21h, R.T.	0	*n. c.*
3	*DCA***	$LiClO_4$,TEA	Et_2O	30 min, R.T.	Decomp.	*n. c.*

*TBA = tetrabromoacetone; **DCA = 1,2-dichloroacetone

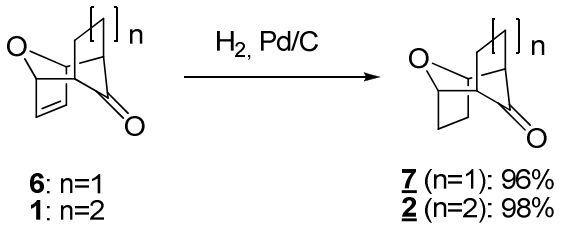
***US = ultrasound

Pyrrolo-compound **20** was synthesized as described in the literature[125] in 70% yield (Scheme 37) and converted with 2-chlorocyclohexanone under Föhlisch conditions, too. Also in this case no conversion could be observed.



Scheme 37

Catalytic Hydrogenation



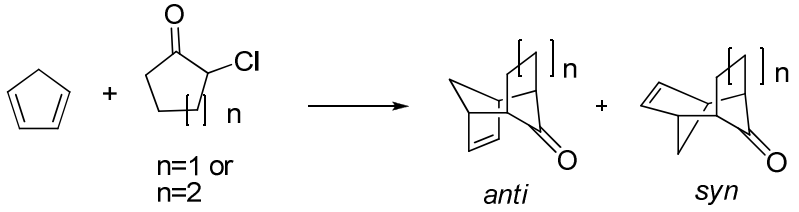
Scheme 38

[125] Hodge, P.; Rickards, R. W. *J. Chem .Soc.* **1963,** 2543.

The catalytic hydrogenation of the double bond in tricyclic ketones was carried out in a Paar apparatus.[126] A dispersion of the substrate and palladium on charcoal in ethyl acetate or ethanol was treated with H_2 under ca. 50 PSI pressure. Complete conversion could be observed after 2h in average. The reactions proceeded quantitatively and the catalyst was removed by filtration. The crude products **2** and **7** were purified by column chromatography. Since conversions were started from *anti*-starting materials, only *anti*-products in 96% (**7**) or 98% (**2**) yield, respectively, were obtained.

3.1.2 Synthesis of Carbotricyclic Ketones

In the synthesis of carbotricyclic ketones different methods were applied. It also turned out, that the substrates 2-chloro cyclopentanone and 2-chloro cyclohexanone require different reaction conditions for successful cyclizations. A main difference between the oxocyclic- and carbocyclic series was observed in the stereoselectivity of the cyclization process: while only *anti*-diastereomers were observed in the oxocyclic series, conversion in the carbocyclic series led to formation of two stereoisomers, *anti*- and *syn*- regarding the relative position of the one-membered bridge;[121] the isomers were formed in different ratios with the *anti* product always prevailing (Scheme 39). The ratio *anti: syn* was determined by area in GC/MS. The assignment of the single products as *anti* or *syn* isomers was conducted by comparing the NMR spectra with references from the literature. For **13*anti***,[116] for **13*syn*** [121] and for **9*anti***[127] references could be found. For **9*syn***, no literature reference could be found, but it was assigned by comparison to spectra of the structural analogs and by 2D NMR spectroscopy (see Appendix).



Scheme 39

[126] Lee, J.; Oh, J.; Choi, J.-R.; Atwood, J. L.; Cha, J. K. *J. Org. Chem* .**1994,** *59*, 6955.
[127] Zimmerman, H. E.; Linder, L. W., *J. Org. Chem.* **1985,** *50,* 1637.

Although the reaction conditions were successfuly applied in synthesis of oxotricyclic ketones, full conversion could not be reached in the synthesis of carbotricyclic ketones and reaction conditions were modified. The optimization of the reactions is summarized in Table 7. Both products were to some extent subliming during evaporation of the solvent under reduced pressure which is the reason that the conversions and isolated yields do not match. Good to excellent yields could be reached in both cases, **9** and **13**.

Table 7

	Product	Reagents	Solvent	Time, Temp.	Conversion [%]	*anti :syn*	Yield [%]
1	**13**[119]	$LiClO_4$,TEA	Et_2O	41h, R.T.	46	85 : 15	*n.d.*
2	**13**[116]	TEA	TFE*	4.5d, R.T.	100	86 : 14	62
3	**13**	TEA (2 eq.)	TFE*	3d, R.T.	90	88 : 12	83
4	**9**[92a]	TFENa	TFE*	30 min, 0°C	100	85 : 15	90
5	**9**	TFENa	TFE*	20 min, 0°C	100	94 : 6	78

**TFE = trifluoroethanol;*

With both products the ratio between *anti* and *syn* diasteromer was not always the same and sometimes changed during the reaction; however, with increasing reaction time the equilibrium moved closer to the ratio 88:12 in favor of the *anti* isomer. The ratio of isomers in the literature[92a] for product **9** is 77% to 23% in favor of the *anti* isomer.

Catalytic Hydrogenation

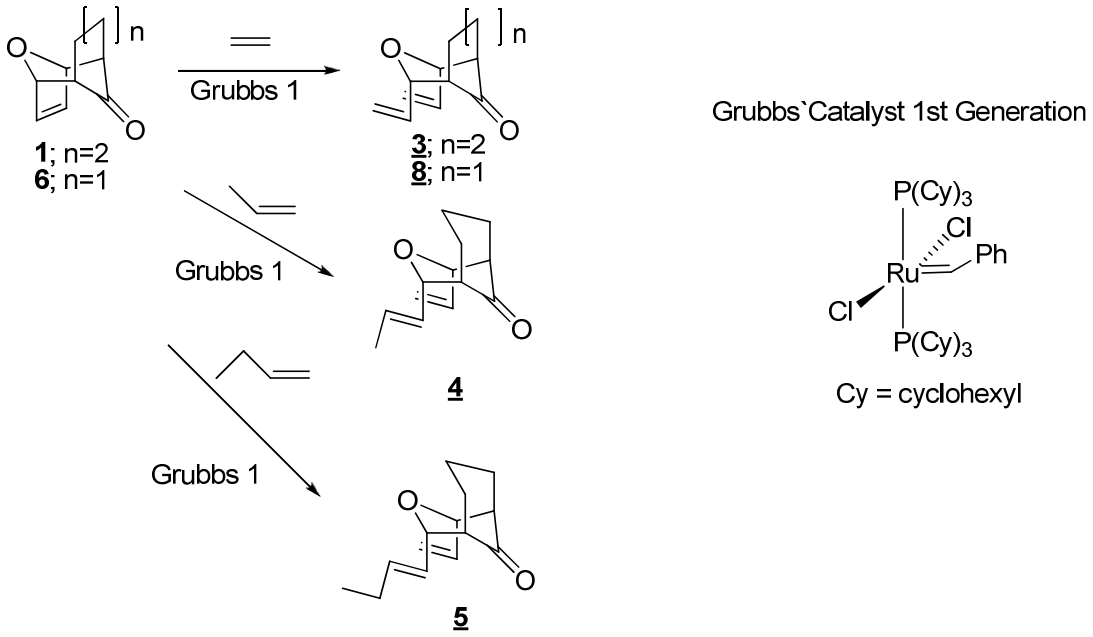
The catalytic hydrogenation was carried out in ethyl acetate in the Paar apparatus under approximately 50 PSI H_2 pressure, analogous to the hydrogenation of oxotricyclic ketones (see above) and excellent yields of 93% **10** or 97% **14** respectively could be reached. Although in the synthesis of **10** (Table 8, entry 2) the *anti: syn* ratio of the starting material was 94:6, the ratio of the hydrogenated product was 86:14 (the ratio of isomers changed during the hydrogenation).

Table 8

	Product	Reagents	Time, Temp.	Conversion [%]	*anti :syn*	Yield [%]
1	**14**	Pd/C, H_2	7h, R.T.	100	83 : 17	97
2	**10**	Pd/C, H_2	4.5d, R.T.	100	86 : 14	93

3.2 Olefin metathesis

The obtained tricyclic ketones **1**, **6**, **9** and **13** were converted into bicyclic ketones via ring opening cross metathesis (ROCM) using gaseous olefins.
The oxocyclic series was started from pure *anti* diasteromere and gave only *anti* products. The metathesis was carried out with Grubbs catalyst, 1st generation (Scheme 40) in dichloromethane at room temperature[128] and ethene, propene and butene were used as reaction partners (Scheme 40).



Scheme 40

In none of the reactions full conversion could be reached, but the transformation stopped at an equilibrium (in most cases in favor of the product). The optimization of the reactions is summarized in Table 9.

[128] Mihovilovic, M. D.; Grötzl, B.; Kandioller, W.; Snajdrova, R.; Muskotal, A.; Bianchi, D. Stanetty, P. *Adv. Synth. Catal.* **2006**, *348*, 463.

Table 9

	Product	S M	Reagent	Grubbs1[%]	Time	Conversion [%]	Yield [%]
1	**3**	**1**	ethene	1	23h	84	59
2	**3**	**1**	ethene	2	28h	81	59
3	**3**	**1**	ethene	2	20h	79	61
4	**8**	**6**	ethene	1	24h	29	*n. d.*
5	**8**	**6**	ethene	3	48h	92	*n. d.*
6	**8**	**6**	ethene	3	6d	*n. d.*	69
7	**4** [b]	**1**	propene	2	2h	*n. d.*[a]	75
8	**5** [c]	**1**	butene	2.5	2h	*n. d.*[a]	85

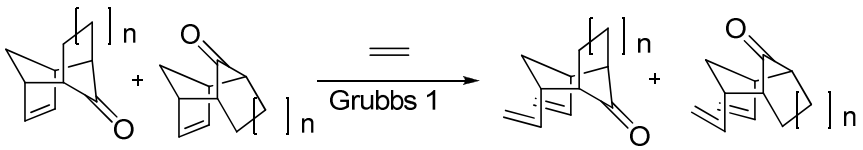
[a] since GC/MS was not available for these experiments, the conversion was determined only by TLC; with progressive time the conversion seemed to be constant.

[b] *E/Z* ratio ca. 90:10

[c] *E/Z* ratio ca. 95:5

In synthesis of **3** no improvement in yield (59%) could be observed, when the amount of the catalyst was doubled from 1% to 2% (Table 9, Entries 1 and 2). Shorter reaction time improved the yield slightly, but not significantly (Table 9, entry 3). Better yields could be achieved with propene (75%) and butene (85%) (Table 9, entries 7 and 8).
In the synthesis of **8** longer reaction time and the threefold amount of catalyst improved the yield (Table 9, entries 4-6). In synthesis of **4** (ethene-gas as reaction partner) the *E/Z* ratio of the products was approximately 90:10, in synthesis of **5** (butene-gas as reaction partner) the *E/Z* ratio of the products was approximately 95:5.

The metathesis of the carbocyclic series was started from a defined mixture of *anti* and *syn* diastereomeres of the starting material and resulted in an *anti* and *syn* mixture of the product (Scheme 41). All reactions have been carried out in dichloromethane at room temperature. With carbotricycles **9** and **13** only ethene was used as reaction partner.



9; n=1
13; n=2

11; n=1
15; n=2

Scheme 41

Table 10

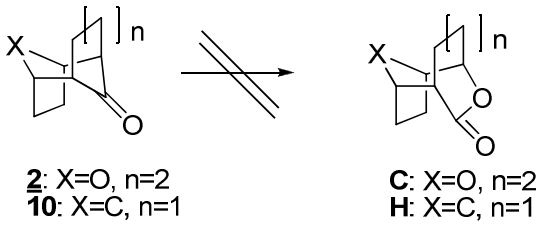
	Product	SM	Grubbs1[%]	Time	Conversion [%]	*anti:syn* (SM)	*anti:syn* (Prod.)	Yield [%]
1	**15**	**13**	1	16h	100	88 : 12	69 : 31	50
2	**15**	**13**	1	17h	100	98 : 2	83 : 17	59
3	**15**	**13**	1	17h	100	87 : 13	91 : 9	74
4	**15**	**13**	2	36h	100	100 : 0	100 : 0	76
5	**15**	**13**	1	36h	10	0 : 100	0 : 100	47
6	**11**	**9**	1	18h	96	90 : 10	*n. d.*	51
7	**11**	**9**	1	15h	75	100 : 0	*n. d.*	51
8	**11**	**9**	2.5	18h	100	100 : 0	100 : 0	76
9	**11**	**9**	2	15h	66	0 : 100	*n. d.*	23
10	**11**	**9**	2.5	23h	86	0 : 100	*n. d.*	70

The diastereomers of the tricyclic ketones **13** and **9** were in some cases separated or enriched by column chromatography, before using as starting material for metathesis (Table 10, entries 2, 4 and 5).

In some cases the *anti*: *syn* ratio of the starting material differed from the *anti*: *syn* ratio of the product **15**. In the hexacyclic series, when pure *anti* or pure *syn* isomers were used as starting material, also the product gave only one diastereomer (Table 10, entries 4 and 5). In the pentacyclic series, the ratio of isomers of the metathesis-product **11** could not be determined since the peaks could not be separated by GC/MS. Detailed analysis of the product **11** showed that the steric properties remain the same after metathesis (Table 10, entry 8). Full conversion could be reached by adding 2.5% of Grubbs1 catalyst starting from the *anti* isomer, but not starting from the *syn* isomer (Table 10, entry 10). In contrast to the *syn* isomer, the *anti* isomer gave full conversion and better yields within shorter reaction times. **11*anti*** was identified by 2D NMR spectroscopy (see Appendix).

3.3 Chemical Baeyer-Villiger Oxidation

The saturated oxotricyclic ketone **2** and carbotricyclic ketone **10** (Scheme 42, Table 11) were intended to be converted into the corresponding lactones by chemical Baeyer-Villiger oxidation to obtain racemic reference material for the subsequently performed biooxidations. Several attempts were carried out and different methods were implemented, but no lactone could be obtained.



2: X=O, n=2
10: X=C, n=1

C: X=O, n=2
H: X=C, n=1

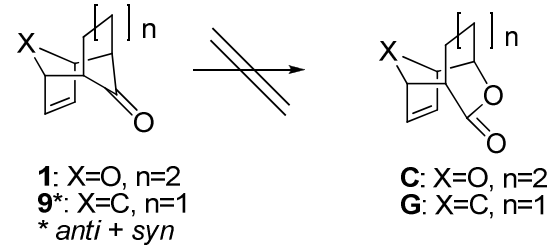
Scheme 42

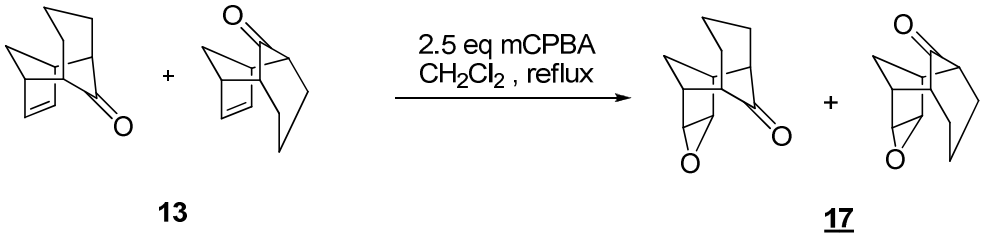
Table 11

	Substrate	React. Cond. [equiv.]	Solvent	Time, Temp.	Conversion [%]
1	**2**	mCPBA [1.5][129]	CH_2Cl_2	Overnight - 6d, R.T.	*n. c.*
2	**2**	mCPBA[2]	$CHCl_3$	17h – 4d, reflux	decomposition
3	**2**	mCPBA[10], K_2CO_3[0.23]	$CHCl_3$	4d – 5d, reflux	decomposition
4	**2**	mCPBA [1.5], K_2CO_3 [0.23]	CH_2Cl_2	Overnight, reflux	decomposition
5	**2**	mCPBA [10 – 25], K_2CO_3[130][0.23]	CH_2Cl_2	Overnight – 2d, reflux	decomposition
6	**2**	H_2O_2 [2.33 – 12], NaOH [1- 6][131]	MeOH	2h – 6d R.T.	*n. c.*
7	**2**	AcONa [10] 30%H_2O_2 [10][132]	90% aq. AcOH	Overnight - 3d R.T.	decomposition
8	**10**	mCPBA [10], K_2CO_3[0.23]	CH_2Cl_2	Overnight - 7d R.T. - reflux	*n. c.*

[129] Harmata, M.; Elahmad, S.; Barnes, C. L. *J. Org. Chem* .**1994,** *59*, 1335.
[130] Kandioller, W. *Diploma Thesis*, 70.
[131] Trost, B. M. ; Bogdanowicz, M. J. *J. Amer. Chem. Soc.* **1973,** *95*, 5321.
[132] Mehta, G.; Kumaran, S. , *Tetrahedron Lett*. **2001**, *42*, 8097.

Chemical Baeyer-Villiger oxidation of unsaturated tricyclic ketones was not successful either, only in one case the epoxidation of the double bond could be observed (Scheme 43, Table 12). The peroxide **17** could be obtained in 39% yield and the 94:6 *anti:syn* ratio of the starting material **13** was shifted to 70:30 for the isolated product **17**. The position of the epoxy-ring could not be defined doubtlessly.



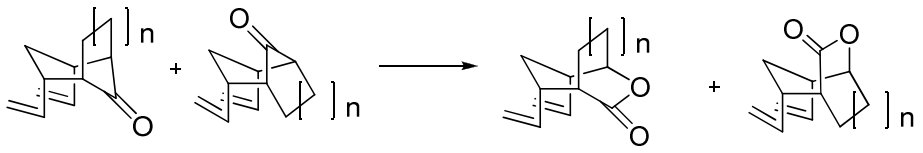


Scheme 43

Table 12

	Substrate	React. Cond. [equiv.]	Solvent	Time, Temp.	Conversion [%]	Yield [%]
1	**1**	H_2O_2, [2.5] NaOH [1][131]	MeOH	3h - 5d, R.T.	*n. c.*	*n. c.*
2	**9**	H_2O_2 [10], NaOH [1]	MeOH	18h – 3d, R. T.	*n. c.*	*n. c.*
3	**13→17**	m-CPBA [2.5]	CH_2Cl_2	20h, reflux	100	39

After several experiments with the carbobicyclic compounds **9** and **13** one lactone could be isolated in 71% yield and characterized (Scheme 44, Table 13). In addition to the desired lactone **12**, three by-products could be observed in this reaction (together 18% area GC/MS), which are assumed to be the three possible epoxide-products; no further analysis of these fractions was conducted.



11: n=1
15:n=2

12: n=1
16:n=2

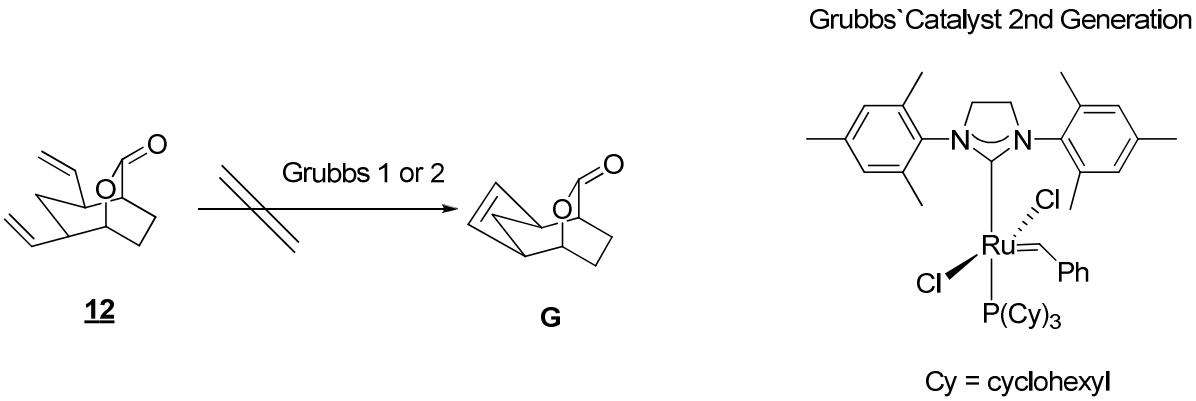
Scheme 44

Table 13

	Substrate	React. Cond. [equiv.]	Solvent	Time, Temp.	Conversion [%]	Yield [%]
1	**15**	H_2O_2 [2.5], NaOH [1][131]	MeOH	3h, R.T.	*n. c.*	*n. c.*
2	**11**	mCPBA [3]	CH_2Cl_2	22h, R.T.	*n. c.*	*n. c.*
3	**11**	H_2O_2 [2.33], NaOH [1]	MeOH	22h, R.T.	8	*n. d.*
4	**11**	mCPBA [2] $NaHCO_3$ [3]	CH_2Cl_2	19h, R.T.	100	71

3.4 Ring Closing Metathesis

The attempt to perform a ring closing metathesis reaction (RCM) to obtain a tricyclic lactone was not successful despite of applying Grubbs`catalyst 1st generation in high concentration as well as Grubbs` catalyst 2nd generation; probably, this is due to the small ring-size and ring strain of the target compound. All reactions were carried out in CH_2Cl_2 at room temperature. No conversion could be observed, after longer reaction times the starting material started to decompose (Scheme 45, Table 14).



Scheme 45

Table 14

	Substrate	Catalyst[%]	Time	Conversion
1	**12**	Grubbs 1 25%	1d – 6d	*n. c.* → decomposition
2	**12**	Grubbs 2 10%	20h – 15d	*n. c.* → decomposition

3.5 Biotransformations

3.5.1 General

As an alternative to the known metal-based catalysts[15] biocatalysis is a "green" and sustainable method for stereoselective oxidation processes.

Desymmetrisation is the most important synthetic application of the microbial BVO. Lactones with theoretical yield of 100% of the optically pure product can be obtained from prochiral cyclic ketones. Several cyclic ketones have been converted with different BVMOs and good yields and high *ee*-s could be obtained. One of the main challenges of biocatalysis is enantiodivergence which means to synthesize both enantiomers of the desired product. The approach used in classical catalysis, where the chirality of the inducing ligand field can be inverted to give the opposite enantiomer, is not applicable to biocatalysis, since the enzymes consist of L-amino acids. The identification and characterisation of enzymes with overlapping substrate acceptance that yield antipodal enantiomers of the product are essential for establishing biocatalysis in synthetic chemistry.
A phylogenetic tree for many different monooxygenases with biomolecular interpretation has been reported before[133] and desymmetrisation of prochiral ketones with eight BVMOs was investigated.[134] A clustering into two groups was proposed based on substrate acceptance and stereopreference: the CHMO type group yielded antipodal products in most cases compared to the CPMO type group with good to excellent *ee* values. The prototype of the first group is cyclohexanone monooxygenase from *Acinetobacter* sp. NCIMB 9871 ($CHMO_{Acineto}$)[135] and contains further the cyclohexanon monooxygenase from *Brevibacterium* DPR# 14 ($CHMO_{Brevi1}$)[136], from *Rhodococcus* DPR#455 ($CHMO_{Rhodo1}$) and *Rhodococcus* DPR#460 ($CHMO_{Rhodo2}$)[137], from *Arthrobacter* DPR#453 ($CHMO_{Arthro}$)[137] and from *Brachimonas* DPR#192 ($CHMO_{Brachy}$). [138] The prototype of the second group is cyclopentanone

[133] Fraaije, M. W.; Kamerbeek, N. M.; van Berkel, W. J. H.; Janssen, D. B., *FEBS Lett.* **2002**, *518*, 43.
[134] Mihovilovic, M. D.; Rudroff, F.; Grötzl, B.; Kapitan, P; Snajdrova,R.; Rydz, J.; Mach, R. *Angew. Chem. Int. Ed.* **2005**, *44*, 3609.
[135] a) Donoghue, N, A.; Norris, D. B.; Trudgill, P.W. *Eur. J. Biochem.***1976**, *63*, 175. b) Stewart, J.D. *Curr. Org. Chem.* **1998**, *2*, 195.
[136] Brzostowicz, P.; Gibson, K. L.; Thomas, S. M.; Blasko, M. S.; Rouviere, P. E. *J. Bacteriol.* **2000**, *182*, 4241.
[137] Brzostowicz, P.; Walters, D. M.; Thomas, S. M.; Nagarajan, V.; Rouviere, P. E. *Appl. En*Viron. *Microbiol.* **2003**, *69*, 334.
[138] a) Bramucci, M. G.; Brzostowicz, P. C.; Kostichka, K. N.; Nagarajan, V.; Rouviere, P. E.; Thomas, S. M. E. I. DuPont de Nemours and Co., U.S.A. Identification, cloning and sequences of bacterial Baeyer- Villiger monooxygenases and encoding genes and application to the conversion of ketones to the corresponding esters.

monooxygenase from *Comamonas* sp. NCIMB 9872 ($CPMO_{Coma}$)[139] and contains also cyclohexanone monooxygenase from *Brevibacterium* DPR#399 ($CHMO_{Brevi2}$).[136]

Xanthobacter sp. ZL5 was first cloned in 2003[140] and classified as CHMO type enzyme.[141] The BVMO from *P. fluorescens* DSM 50106 was hardly efficient in converting cyclic ketones[142], but very good in transformations of acyclic (hydroxyl) ketones.[143]

Another phylogenetic tree shows the relationships within different BVMOs, incorporating the named enzymes and furthermore PAMO from *Thermobifida fusca*[144], HAPMO from *Pseudomonas fluorescens*[145] ACB, CPDMO, CDMO from *Rhodococcus ruber* and other BVMOs (Figure 1)[146]. The red marked enzyme groups were investigated in this thesis.

International Patent, WO 2003020890, 2003. b) Bramucci, M. G.; Brzostowicz, P. C.; Kostichka, K. N.; Nagarajan, V.; Rouviere, P. E.; Thomas, S. M. *Chem. Abstr.* **2003**, *138*, 233997.

[139] a) Griffin, M.; Trudgill, P. W. *Eur. J. Biochem.* **1976**, *63*, 199. b) Iwaki, H.; Hasegawa, Y.; Wang, S.; Kayser, M. M.; Lau, P. C. K. *Appl. EnViron. Microbiol.* **2002**, *68*, 5671.

[140] van Beilen, J. B.; Mourlane, F.; Seeger, M. A.; Kovac, J.; Li, Z.; Smits, T. H. M.; Fritsche, U.; Witholt, B., *Environ. Microbiol.* **2003**, *5*, 5174.

[141] Rial, D. V., Bianchi, D. A., Kapitanova, P., Lengar, A., van Beilen, J. B., Mihovilovic, M. D. *Eur. J. Org. Chem.,* **2008**, 1203.

[142] Kirschner, A.; Altenbuchner, J.; Bornscheuer, U. T., *Appl. Microbiol. Biotechnol.* **2006**, *73*, 1065.

[143] Kirschner, A.; Bornscheuer, U. T., . *Angew. Chem. Int. Ed.* **2006**, *45*, 7004.

[144] a) Fraaije, M. W.; Wu, J.; Heuts, D. P. H. M.; van Hellemond, E. W.; Spelberg, L. J. H.; Janssen, D. B., *Appl. Microbiol. Biotechnol.* **2005**, *66*, 393; b) Torres, Pazmino, D. E.; Snajdrova, R.; Rial, D. V.; Mihovilovic, M. D.; Fraaije, M. W., *Adv. Synth. Catal.* **2007**, *349,* 1361.

[145] Kamerbeek, M. N.; Moonen, M. J. H.; van der Ven, J. G. M.; van Berkel, W. J. H.; Fraaije, M. W.; Janssen, D. B., *Eur. J. Biochem.* **2001**, *268*, 2547.

[146] Rial, D. V.; Cernuchova, P.; van Beilen, J. B.; Mihovilovic, M. D., *J. Mol. Catal. B: Enzym.* **2008**, *50*, 61.

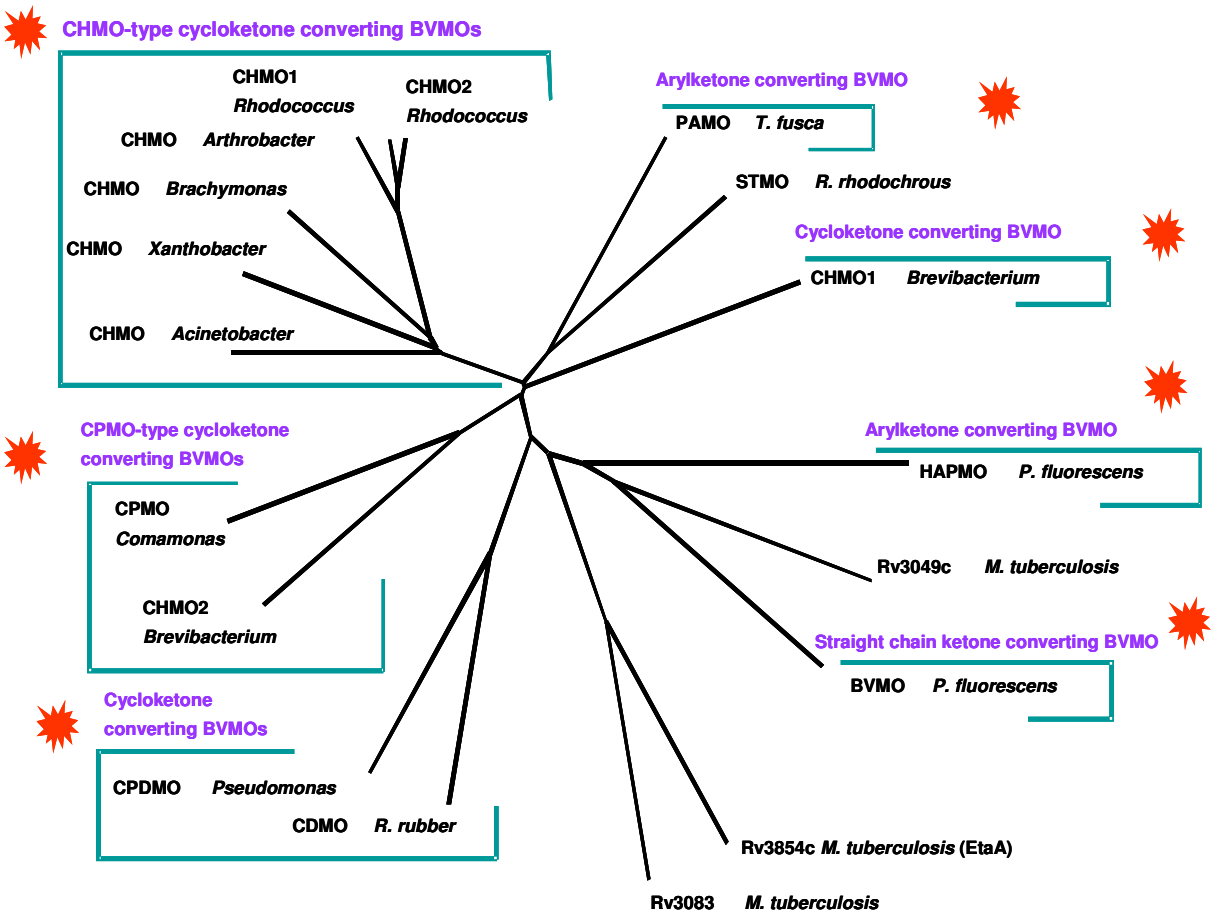


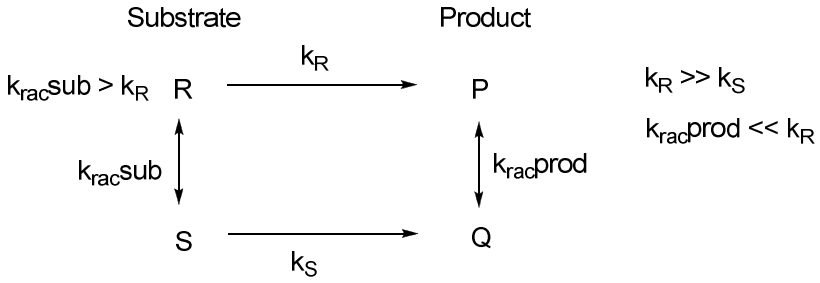
Figure 1

Abbreviations and GenBank accession numbers of protein sequences:

CHMO *Acinetobacter*: CHMO *Acinetobacter* sp. NCIMB 9871: BAA86293; CHMO2 *Brevibacterium*: CHMO 2 *Brevibacterium* sp. HCU: AAG01290; CHMO*Arthrobacter*:CHMO*Arthrobacter* sp. BP2: AAN37479; CHMO*Brachymonas*:CHMO*Brachymonas petroleovorans*: AAR99068; CHMO1 *Brevibacterium*: CHMO1 *Brevibacterium* sp. HCU: AAG01289; CHMO1 *Rhodococcus*:CHMO*Rhodococcus* sp. Phi1: AAN37494; CHMO2 *Rhodococcus*:CHMO*Rhodococcus* sp. Phi2: AAN37491; CHMO*Xanthobacter*:BVMO*Xanthobacter* sp. ZL5: CAD10801; CPMO *Comamonas*: cyclopentanone monooxygenase *Comamonas* sp. NCIMB 9872: BAC22652; PAMO *T. fusca*: phenylacetone monooxygenase *Thermobifida fusca*: 1W4X A; HAPMO *P. fluorescens*: 4-hydroxyacetophenone monooxygenase *Pseudomonas fluorescens*: AAK54073; STMO *R. rhodochrous*: steroid monooxygenase *Rhodococcus rhodochrous*: BAA24454; CDMO *R. rubber*: cyclododecanone monooxygenase *Rhodococcus rubber*: AAL14233; Rv3854c *M. tuberculosis*: ETaA *Mycobacterium tuberculosis* H37Rv: CAB06212; CPDMO *Pseudomonas*: cyclopentadecanone monooxygenase *Pseudomonas* sp. HI-70: BAE93346; Rv3049c *M. tuberculosis:* BVMOfrom *Mycobacterium tuberculosis* H37Rv: CAA16134; Rv3083 *M. tuberculosis:* BVMO from *Mycobacterium tuberculosis* H37Rv: CAA16141; BVMO *P. fluorescens*: BVMO from *Pseudomonas fluorescens*: AAC36351.

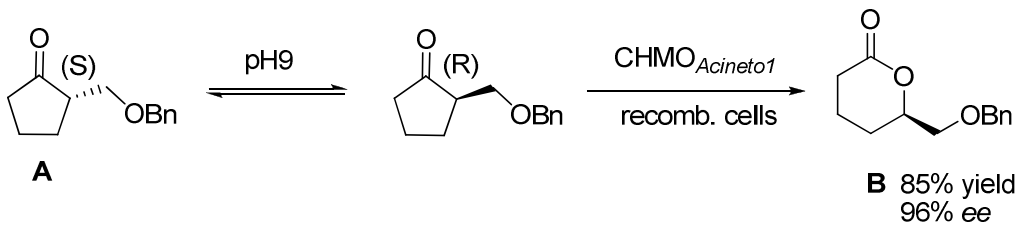
Kinetic resolution occurs, when starting from chiral racemic ketones, one enantiomer of the substrate is converted exclusively or preferentially by the enzyme. In the ideal case, only one

enantiomer is converted to the ester or lactone and gives 50 % yield, while the other enantiomer remains unchanged, but optically pure as well.
In dynamic kinetic resolutions the enantiomers of the substrate are in racemic equilibrium and after one of the enantiomers is consumed faster or exclusively and removed from the equilibrium, racemisation of the substrate occurs. If the racemisation of the product is slower than the transformation, in the ideal case optically pure product can be obtained in 100% yield (Scheme 46). The efficacy of the process is largely determined by the velocity of racemization, which has to be in the range of the biotransformation rate of the preferred substrate at least.



Scheme 46

Dynamic kinetic resolution was optimized for 2-(benzoxymethyl)cyclopentanone **A**[147] (Scheme 47). Conventional biooxidation using recombinant whole cells at pH 7 gave lactone **B** in excellent *ee* and almost 50% yield, while the remaining substrate displayed rather low stereoselectivity (43% *ee*). This data was interpreted by the increased acidity of the 2-proton in ketone **A**. Increasing the pH to 9 resulted in sufficient acceleration of substrate racemization and complete conversion to (*R*)-**B** was achieved in 85% yield and excellent stereoselectivity (96% *ee*).



Scheme 47

[147] Berezina, N.; Alphand, V.; Furstoss, R. *Tetrahedron Asymm.* **2002**, *13*, 1953.

3.5.2 Screening

Monitoring of biooxidations was in general preformed in screening experiments in 1 mL or 2 mL scale in a 24- or 12-well plate. This method allows fast and effective parallel performing of reactions in contrast to shaking flask experiments. The whole-cell experiments were conducted following a standardised procedure (Figure 2).

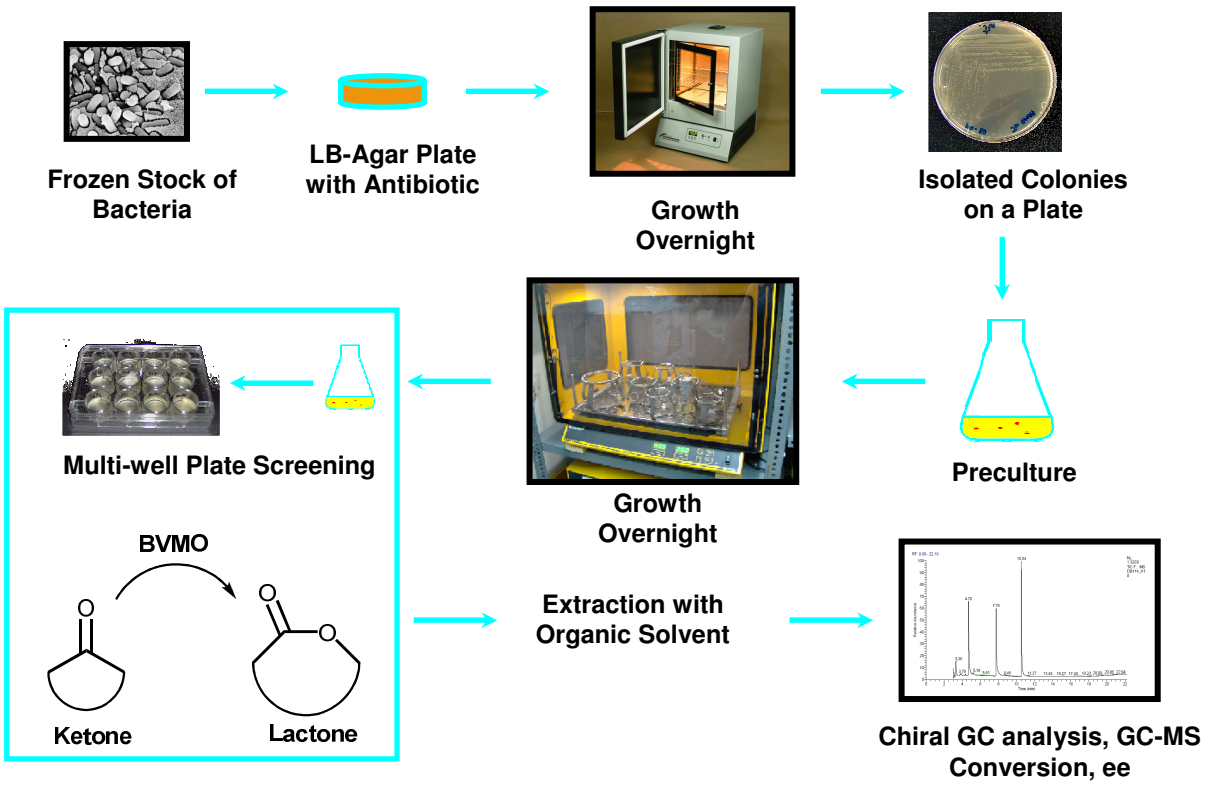


Figure 2

From a frozen stock bacteria were streaked on a LB-agar plate with antibiotic and grown overnight at 37°C. The plate was then stored at 4°C (for up to two weeks). In the evening an isolated colony of bacteria from the plate was transferred into 10 mL of $LB_{antibiotic}$ in a shaking flask and the preculture was grown overnight at a specified temperature, depending on the *E. coli* strain. On the next day, each well of the multi-well plate was charged with $LB_{antibiotic}$ (1 mL or 2 mL) and inoculated with 10 µL or 20 µL (1%) of the overnight preculture of the appropriate recombinant *E. coli* strain; afterwards, incubation at 37°C or 30°C, respectively, at 120 rpm on an orbital shaker was continued until the culture reached an OD_{600} of 0.5; IPTG or *L*-arabinose was added (final concentration of 0.166 mM in general, but some strains require other concentrations) together with substrate (1 mg, dissolved in 1,4-dioxane). The multi well-dishes were then incubated at 24°C for 24 hours. The samples were centrifuged

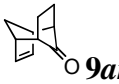
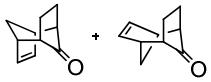
(removal of the biomass), the aqueous layer extracted was with ethyl acetate, dried over Na_2SO_4, and analyzed by GC/MS and/or chiral phase GC.

For preparative-scale experiments the procedure was the same except the fermentation was run in an appropriate shaking flask (250mL – 2000mL) and the conversion was monitored during the reaction by GC/MS. The reaction times could be therefore adapted.

The synthesized ketones **2, 14, 7, 10, 1, 13, 6,** and **9** were screened for conversion to lactones via microbial Baeyer-Villiger oxidation.

We started with several experiments in 30mg substrate scale in shaking flasks to avoid excessive evaporation because of the long reaction times. The substrates **2, 14, 7, 10, 3, 13, 6,** and **9** were tested for biooxidation with $CHMO_{Acineto}$ and $CPMO_{Coma}$ (Table 15). All experiments were performed at 24°C, 1 equivalent of β-cyclodextrine was added.

Table 15

	Substrate	Strain	Time	Conversion [%]
1	 **2**	$CHMO_{Acineto}$	24h	*n. c.*
2		$CPMO_{Coma}$	24h	*n. c.*
3	 **14**	$CHMO_{Acineto}$	24h - 3d	*n. c.*
4		$CPMO_{Coma}$	24h - 4d	*n. c.*
5	 **7**	$CHMO_{Acineto}$	24h - 4d	*n. c.*
6		$CPMO_{Coma}$	24h - 4d,	*n. c.*
7	 **10**	$CHMO_{Acineto}$	24h - 4d	*n. c.*
8		$CPMO_{Coma}$	24h - 4d	*n. c.*
9	 **1**	$CHMO_{Acineto}$	24h - 4d	*n. c.*
10		$CPMO_{Coma}$	24h - 4d	*n. c.*
11	 **13**	$CHMO_{Acineto}$	24h - 4d	*n. c.*
12		$CPMO_{Coma}$	24h - 4d	*n. c.*
13	 **6**	$CHMO_{Acineto}$	24h - 4d	*n. c.*
14		$CPMO_{Coma}$	24h - 4d	*n. c.*
15	 ***9anti***	$CHMO_{Acineto}$	22h - 5d	*n. c.*
16		$CPMO_{Coma}$	22h - 5d	*n. c.*
17	 ***9anti*+9*syn***	$CHMO_{Acineto}$	3d	*n. c.*
18		$CPMO_{Coma}$	3d	*n. c.*

No conversion could be observed in any case.

Mutant enzyme strains were designed from the wild-type CHMO from *Acinetobacter* sp. NCIMB 9871 ($CHMO_{Acineto}$) by directed evolution.[148] With directed evolution the catalytic performance of enzymes was modified by point mutations, which were introduced into the gene of the biocatalyst in a random or knowledge-based fashion. This influences the substrate acceptance and the enantiodivergence of the enzyme. The promising mutants were then chosen by a selection process.

In the literature[148] the enantioselectivity of CHMO mutants was in some cases significantly improved in comparison with the wild-type enzyme and antipodal enantiomers could be obtained. In a recent study, our group could also demonstrate that the substrate specificity of mutant enzymes can be expanded relative to their wild-type parents.[149] In our case we hoped to obtain conversion to the desired lactones in contrast to the wild-type enzyme. In the present mutant strains, one, two, three or four amino acids of the wild-type enzyme were replaced with other amino acids. Table 16 shows the amino acid modifications of $CHMO_{Acineto1}$ in the mutants, obtained by error-prone PCR as the mutagenesis method.

Table 16

	Mutant	**Amino Acid Modifications***
1	1-K6-G2	K78E, F432S
2	1-E12-B5	F432I
3	1-F1-F5	L143F
4	1-F4-B9	D41N, F505Y
5	2-A10-B6	F432I, V82E
6	1-H3-C9	L220Q, P428S, T433A
7	1-H7-F4	L426P, A541V
8	1-C2-B7	F432A, K500R
9	1-K2-F5	F432S
10	2-D19-E6	L143F, E292G, L435Q, T464A

*Abbreviations of amino acids: G: Glycine; A: alanine; V: valine; L: leucine; I: isoleucine; M: methionine; F: phenylalanine; W: tryptophan; P: proline; S: serine; T: threonine; C: cysteine; Y: tyrosine; N: asparagine; Q: glutamine; D: aspartic acid; E: glutamic acid; K: lysine; R: arginine; H histidine;

[148] Reetz, M. T.; Brunner, B.; Schneider, T.; Schulz, F.; Clouthier, C. M.; Kayser, M. M. *Angew. Chem.* **2004**, *116*, 4167.
[149] Mihovilovic, M. D.; Rudroff, F.; Winninger, A.; Schneider, T.; Schulz, F.; Reetz, M. T. *Org. Lett.* **2006**, *8*, 1221.

We used these CHMO mutant strains[150] for biotransformation of the ketones **1** and **9** (Table 17 - Table 18) in the hope to observe conversion to the desired lactones. All experiments were performed at 24°C in 1 mL scale (screening), the reaction time was 24h.

Table 17

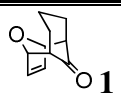
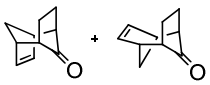
	Substrate	Strain	Conversion [%]
1	 **1**	1-K6-G2	traces of epoxide
2		1-E12-B5	*n. c.*
3		1-F1-F5	*n. c.*
4		1-F4-B9	*n. c.*
5		2-A10-B6	*n. c.*
6		1-H3-C9	traces of epoxide
7		1-H7-F4	*n. c.*
8		1-C2-B7	*n. c.*
9		1-K2-F5	traces of epoxide
10		2-D19-E6	*n. c.*

Table 18

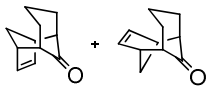
	Substrate	Strain	Conversion [%]
1	 ***9anti+9syn***	1-K6-G2	traces of epoxide
2		1-E12-B5	*n. c.*
3		1-F1-F5	*n. c.*
4		1-F4-B9	*n. c.*
5		2-A10-B6	*n. c.*
6		1-H3-C9	traces of epoxide
7		1-H7-F4	*n. c.*
8		1-C2-B7	*n. c.*
9		1-K2-F5	traces of epoxide
10		2-D19-E6	*n. c.*
11		1-K6-G2, no IPTG	5% of epoxide
12		1-H3-C9, no IPTG	5% of epoxide
13		1-K2-F5, no IPTG	5% of epoxide

[150] The mutant strains were provided by Prof. Dr. M. T. Reetz, Max-Planck-Institut für Kohlenforschung Mülheim/Ruhr (Germany)

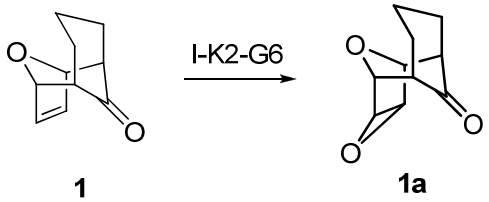
Only the corresponding epoxide could be observed in some cases as trace product. In three experiments (Table 18, entries 11-13) no IPTG was added to the reaction medium, so the overexpression of the enzymes did not take place. That means that under these conditions the conversion of substrate **9** to the epoxide is not catalyzed by the enzymes but rather the result of aerial oxidation. The epoxide was identified by comparing of retention times and mass spectrum in GC/MS with a reference, obtained by the same experiment with the strain 1-K6-G2 in preparative scale.

Due to the completeness, the tricyclic ketones **13, 6, 2** and **1** were also incubated with three of the CHMO mutant strains (I-K6-G2, I-H3-C9, I-K2-F5) and also these experiments yielded only the corresponding epoxides, the conversion was even higher, if no IPTG was added to the reaction mixture (Table 19, entries 13-15).

Table 19

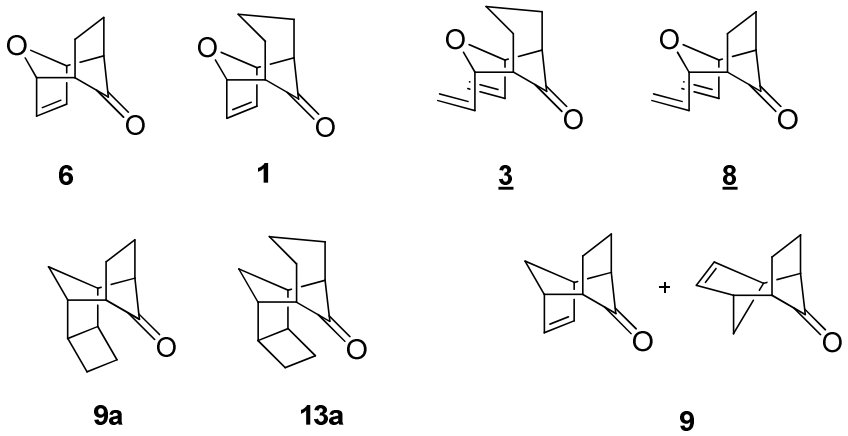
	Substrate	Strain	Conversion [%]
1	 **13*anti*+13*syn***	1-K6-G2	*n. c.*
2		1-H3-C9	*n. c.*
3		1-K2-F5	*n. c.*
4	 **6**	1-K6-G2	*n. c.*
5		1-H3-C9	*n. c.*
6		1-K2-F5	*n. c.*
7	 **2**	1-K6-G2	*n. c.*
8		1-H3-C9	*n. c.*
9		1-K2-F5	*n. c.*
10	 **1**	1-K6-G2	3% to epoxide
11		1-H3-C9	1% to epoxide
12		1-K2-F5	2% to epoxide
13		1-K6-G2, no IPTG	8% to epoxide
14		1-H3-C9, no IPTG	8% to epoxide
15		1-K2-F5, no IPTG	9% to epoxide

To verify the structure, the epoxide **1a** was prepared in preparative scale under the same conditions with the mutant strain I-K6-G2. The conversion was 9%, product **1a** was isolated, purified and analyzed (Scheme 48). The position of the epoxy-ring was not defined doubtlessly.



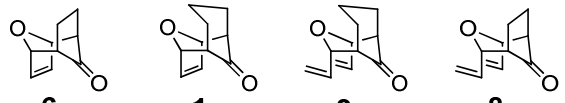
Scheme 48

The next screening experiments were performed with the DuPont strains (provided by DuPont de Nemours & Co., USA) $CHMO_{Acineto}$, $CPMO_{Coma}$, $CHMO_{Brevi1}$, $CHMO_{Brevi2}$, $CHMO_{Rhodo1}$, $CHMO_{Rhodo2}$, $CHMO_{Brachy}$ and $CHMO_{Arthro}$. The reaction time was 24h at 24°C. None of the bicyclic-, tricyclic- and tetracyclic ketones **6, 1, 3, 8, 1a, 13a** and **9** gave any conversion[151]. (Scheme 49, Table 20).



Scheme 49

Table 20

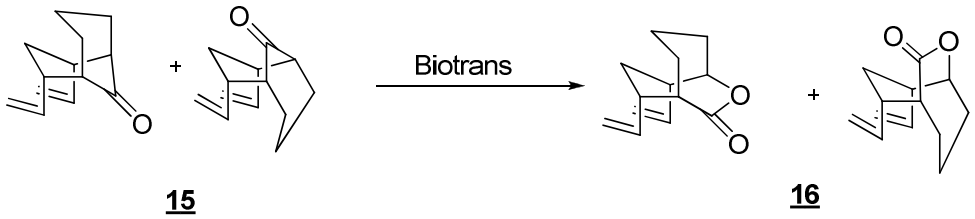
	Substrate	Strain	Conversion [%]
1		$CHMO_{Acineto}$	*n. c.*
2		$CPMO_{Coma}$	*n. c.*
3		$CHMO_{Brevi1}$	*n. c.*
4		$CHMO_{Brevi2}$	*n. c.*
5		$CHMO_{Rhodo1}$	*n. c.*
6		$CHMO_{Rhodo2}$	*n. c.*
7		$CHMO_{Brachy}$	*n. c.*
8		$CHMO_{Arthro}$	*n. c.*

[151] Substrates **9a** and **13a** (Tetracycles) were provided by Prof. Dr. Thorsten Bach, Technical University of Munich, Germany

3.5.3 Synthesis of Carbobicyclic Lactones **12** *and* **16**

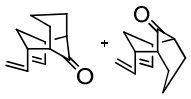
3.5.3.1 Lactone **16**

Screening with the DuPont strains $CHMO_{Acineto}$, $CPMO_{Coma}$, $CHMO_{Brevi1}$, $CHMO_{Brevi2}$, $CHMO_{Rhodo1}$, $CHMO_{Rhodo2}$, $CHMO_{Brachy}$ and $CHMO_{Arthro}$ was performed starting from carbobicyclic ketone **15**. The reaction time was 24h at 24°C. Conversion could be observed with the strains $CHMO_{Rhodo1}$ (12%), $CHMO_{Rhodo2}$ (11%), $CHMO_{Brachy}$ (24%) and $CHMO_{Arthro}$ (19%) (Scheme 50, Table 21, entries 5-8).



Scheme 50

Table 21

	Substrate	Strain	Conversion [%]
1	 **15*anti+syn***	$CHMO_{Acineto}$	*n. c.*
2		$CPMO_{Coma}$	*n. c.*
3		$CHMO_{Brevi1}$	*n. c.*
4		$CHMO_{Brevi2}$	*n. c.*
5		$CHMO_{Rhodo1}$	12
6		$CHMO_{Rhodo2}$	11
7		$CHMO_{Brachy}$	24
8		$CHMO_{Arthro}$	19

To verify the structure of the product **16**, the biotransformation was performed in preparative scale with $CHMO_{Brachy}$, starting from a mixture of **15*anti*** and **15*syn*** (diastereomeric ratio 70%:30%). The biotransformation was carrid out for 48 hours to diplay 36% conversion; after work-up 34% isolated yield could be obtained. Only one lactone diastereomer **16*anti*** or ***syn*** could be observed and isolated (a spectral analysis (C-H hsqc, C-H hmbc, H-H cosy and H-H noesy) of the isolated lactone **16** showed that only one diastereomer is formed, but could not

give a doubtless determination of the steric structure of the molecule (either *anti* or *syn*), see Appendix).

Additional experiments in preparative scale were performed with $CHMO_{Brachy}$ and $CHMO_{Xantho}$ to investigate preferences in the *anti/syn* diastereomer consumption of the enzymes (Table 22). All experiments were performed at 24°C. Since only one peak of the product-lactone is visible in GC/MS, two possibilities exist: a) overlap of the peaks of two formed diastereomers of the lactone in GC/MS; b) only one diastereomer is formed during the biotransformation. Consumption of each individual isomer of the ketones was monitored by GC/MS (the conversion was calculated from the areas of the product and the area of the starting material, without standard) (Table 22).

Table 22

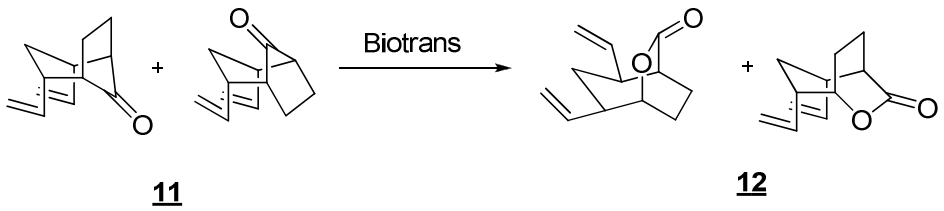
	Scale*, V**	Strain	Ratio *anti/syn* in SM at t = 0 [%]	Time	Conversion [%]	Ratio *anti/syn* of unconsumed SM [%]	Yield [%]
1	38mg, 250mL	$CHMO_{Brachy}$	70 : 30	24h 48h	15 36	80 : 20 88 : 12	34
2	10mg, 50mL	$CHMO_{Brachy}$	81 : 19	5h 23h 3d	traces 12 18	81 : 19 91 : 9 89:11	*n. d.*
3	120mg, 500mL	$CHMO_{Brachy}$	91 : 9	24h 3d	3 5	94 : 6 97 : 3	3
4	80mg, 500mL	$CHMO_{Brachy}$	91 : 9	24h 48h	3 3.6	97 : 3 98 : 2	*n. d.*
5	20mg 350 mL	$CHMO_{Brachy}$	0 : 100	20h 25h 47h	30 39 50	0 : 100 0 : 100 0 : 100	23
6	30mg, 150mL	$CHMO_{Xantho}$	91 : 9	20h 44h	6.6 6.4	100 : 0 100 : 0	*n. d.*

*Scale = amount of starting material; ** V = volume of biotransformation medium, where the reaction was performed

The highest yield (34%) of the lactones could be achieved in the experiment, where the *anti/syn* start-ratio of ketones was 70% : 30% (Table 22, entry 1). A trend can be recognized, that the *syn* ketone-isomer is consumed preferably by both enzymes, $CHMO_{Brachy}$ and $CHMO_{Xantho}$. If started from pure *syn* ketone, the conversion was 50% with $CHMO_{Brachy}$ after 47 hours (entry 5). With $CHMO_{Xantho}$ the *syn* ketone was completely consumed already after

20h (entry 6). Unfortunately, the *syn* isomer was the minor product in the synthesis of all ketones. These findings also suggest that the metabolite isolated has a *syn*-structural scaffold.

3.5.3.2 Lactone 12



11 **12**

Scheme 51

The carbobicyclic ketone **11** gave conversion in screening after incubation with the DuPont strains $CHMO_{Acineto}$, $CHMO_{Brevi1}$, $CHMO_{Rhodo1}$, $CHMO_{Rhodo2}$, $CHMO_{Brachy}$ and $CHMO_{Arthro}$. The reaction time was 24h at 24°C (Table 23, entries 1, 3, 5-8). The reactions were stared from 93:7 *anti/syn* diastereomeric ratio of **11**.

Table 23

	Strain	Conversion [%]	Product ratio *anti/syn*
1	$CHMO_{Acineto}$	33	58 : 42
2	$CPMO_{Coma}$	*n. c.*	--
3	$CHMO_{Brevi1}$	15	0 : 100
4	$CHMO_{Brevi2}$	*n. c.*	--
5	$CHMO_{Rhodo1}$	50	76 : 24
6	$CHMO_{Rhodo2}$	52	77 : 23
7	$CHMO_{Brachy}$	55	78 : 22
8	$CHMO_{Arthro}$	90	87 : 13
9	$CHMO_{Xantho}$	100	99 : 1

The fact, that the strains $CPMO_{Coma}$ and $CHMO_{Brevi2}$ did not give any conversion corresponds to a deviation in substrate acceptance of the enzymes as described above in the phylogenetic tree. In all cases, the diastereomer **12*anti*** (identification by 2D NMR spectroscopy, see Appendix) of the lactone was the major product with one excpetion: $CHMO_{Brevi1}$ yielded exclusively the diastereomer **12*syn***. The best result could be achieved with $CHMO_{Xantho}$ which gave 100% conversion. The diastereomeric ratio *anti/syn* was 99 : 1.

Preparative scale

After synthesizing lactone **12** in preparative scale, the structure of the product could be verified by detailed NMR analysis and the major product could be identified as lactone **12*anti***. With $CHMO_{Xantho}$ 92% isolated yield could be achieved (Table 24, entry 4). The *anti/syn* diastereomeric ratio was 98:2. $[\alpha_D]$ value of +35.5 could be measured, what indicates enantiomeric excess of one enantiomer. However, all attempts to separate the enantiomers on chiral phase GC, failed. Unlike $CHMO_{Arthro}$ and $CHMO_{Xantho}$, which gave the *anti* diastereomer as the major product, $CHMO_{Brevi1}$ yielded the corresponding diastereomer *syn* as the only product, however, in very low isolated yields.

Table 24

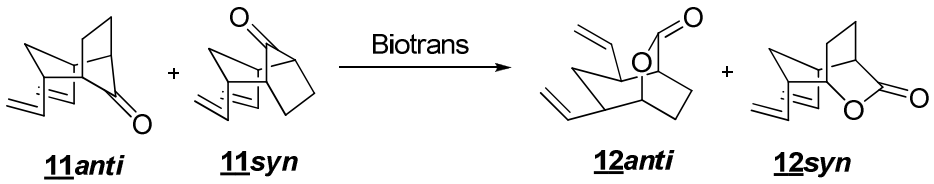
	Scale*, V**	Strain	Time	Conversion [%]	Product ratio *anti/syn* [%]	Yield [%]
1	80mg, 300mL	$CHMO_{Arthro}$	29h 48h 3d	40 45 53	80 : 20 84 : 16 91 : 9	30
2	150mg, 500mL	$CHMO_{Brevi1}$	24h 3d	8 10	0 : 100 0 : 100	3
3	120mg, 500mL	$CHMO_{Arthro}$	29h 48h 3d	14 25 32	86 : 14 92 : 8 92 : 8	30
4	70mg, 250mL	$CHMO_{Xantho}$	20h	100	98 : 2	92

*Scale = amount of starting material; ** V of biotransformation medium, where the reaction was performed

3.6 Anti/syn Equilibrium

After obtaining the above results, we became interested in the steric processes accompanying the oxidation and the apparent equilibrium that seems to be formed between both diastereomers *anti* and *syn*. To explore this phenomenon, the crucial screening experiments were repeated, starting from either pure *anti* or pure *syn* diastereomer or the mixture of both of the ketone **15**. The pure diastereomers of ketones **11** and **15** were obtained by separation of the tricyclic precursors **9** and **13** by column chromatography and the subsequent metathesis of diasteromerically pure **9** and **13**. Both starting ketones **9** and **13** keep their steric properties during the metathesis to yield **11** and **15** (*anti*→*anti*, *syn*→*syn*) which was verified by GC/MS and NMR analysis (see above).
An additional problem was the fact that the diastereomers of ketone **11** and the diastereomers of the lactone **16** could not be separated in GC/MS. The estimated approximated ratio of both diastereomers of starting **11** was *anti/syn* 93:7. This was a limitation to the monitoring of the diastereomeric behavior of the substrates during the reaction.

Consequently, in screening experiments (performed at 24°C, 24h) with substrate **11** the formation and *anti/syn* ratio of the lactones **12** could be monitored (Scheme 52, Table 25).



Scheme 52

Table 25

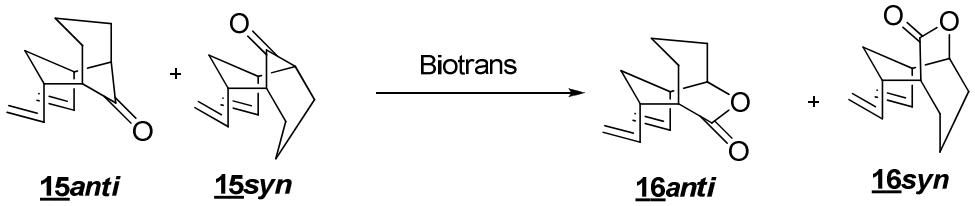
	Strain	S. M.	Conversion [%]	Product ratio *anti/syn* [%]
1	$CHMO_{Arthro}$	*anti*	29	52 : 48
2	$CHMO_{Brevi1}$	*anti*	7	0 : 100
3	$CHMO_{Arthro}$	*syn*	96	0 : 100
4	$CHMO_{Brevi1}$	*syn*	90	0 : 100
5	$CHMO_{Arthro}$	*anti*+*syn**	40	38 : 62
6	$CHMO_{Brevi1}$	*anti*+*syn**	38	0 : 100

*Approximate ratio *anti/syn* 93:7

These screening experiments show, that an *anti/syn* equilibrium is formed during the reaction, the steric structure of a certain portion of the substrate is inverted. So from pure *anti* ketone a nearly 1:1 mixture of *anti/syn* lactone is formed with $CHMO_{Arthro}$ (Table 25, entry 1) and only the *syn* lactone is formed with $CHMO_{Brevi1}$ (Table 25, entry 2). From the *anti/syn* mixture of the ketones $CHMO_{Brevi1}$ again gives only the *syn* lactone and *anti/syn* mixture of lactones is formed with $CHMO_{Arthro}$.
The explanation for this behavior could be that in the reaction mixture a certain part of the ketone inverts its steric structure and is then accepted by $CHMO_{Brevi1}$as the only diastereomer. $CHMO_{Arthro}$ accepts both diasteromers, but *syn* seems to be the better substrate. It is then removed by oxidation from the *anti/syn* equilibrium and more *anti* ketone is inverted to *syn*. The results from $CHMO_{Arthro}$ do not match completely with the results of the previous screening (Table 23, entry 8), where conversion of 90% could be achieved and consequently the *anti/syn* ratio was shifted more in direction of the *anti* diastereomer. This might be due to the fine tuning of the experiment.

Interestingly, $CHMO_{Xantho}$ yields almost exclusively the lactone **12*anti*** from the same ketone **11** in a preparative scale experiment in 92% yield (*anti/syn* ratio is 98:2, Table 24, entry 4).

With substrate **15** the conversion to the lactone and the *anti/syn* ratio of the remaining starting material **15** was monitored (Scheme 53, Table 26). Screening experiments (performed at 24°C, 24h) with $CHMO_{Brachy}$ and $CHMO_{Arthro}$ were performed, since in the previous experiment these strains gave the best conversion. Additionally only substrates without cells were incubated for the same time in LB_{amp} medium.



Scheme 53

Table 26

	Strain	Substrate	Conversion [%]	Recovered S. M. ratio *anti/syn* [%]*
1	$CHMO_{Brachy}$	*anti*	5	98 : 2
2	$CHMO_{Arthro}$	*anti*	*n. c.*	100 : 0
3	$CHMO_{Brachy}$	*syn*	84	44 : 56
4	$CHMO_{Arthro}$	*syn*	25	67 : 33
5	$CHMO_{Brachy}$	*anti+syn***	13	95 : 5
6	$CHMO_{Arthro}$	*anti+syn***	7	92 : 8
7	--	*anti*	--	96 : 4
8	--	*syn*	--	60 : 40
9	--	*anti+syn***	--	92 : 8

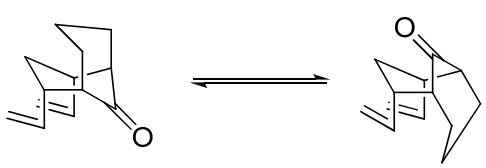
* Only *anti/syn* ratio in the recovered starting material could be determined, the *anti/syn* diastereomers of the product could not be resolved in GC/MS.

** *anti/syn* ratio of the starting material was 92:8

The diasteromer **15*syn*** is accepted more readily by both strains. Starting from pure diastereomer **15*anti***, conversion with $CHMO_{Brachy}$ is only 5% and no conversion can be observed with $CHMO_{Arthro}$. Starting from pure diasteromer **15*syn***, conversion with $CHMO_{Brachy}$ is 84% and with $CHMO_{Arthro}$ 25%. In the recovered starting material always (except entry 2, Table 26) both diasteromers can be found. In the experiments without cells (Table 26, entries 7-9), an equilibrium between both diastereomers is formed. The results are comparable to the results of the substrate **12**.

The fact that *syn* diastereomers were always formed as the minor product, but were accepted better by the enzymes, was unsatisfactory. For this reason we decided to explore, if the equilibrium can be influenced by factors such as reaction time or pH-value of the medium, but the changes of the reaction conditions should stay in a range, where the cultivating conditions for the *E. coli* cells are still intact.

The ketone **15*syn***, the ketone **15*anti*** and a mixture of both diastereomers with *anti/syn* ratio 92 : 8 were incubated in formate buffer (pH=4), phosphate buffer (pH=6), TRIS buffer (pH=8), and carbonate buffer (pH=10) and the diasteromeric ratio was monitored by GC/MS (Scheme 54, Table 27 - Table 29).



15*anti* **15*syn***

Scheme 54

1.) Starting from **15*anti***)

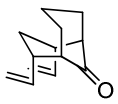


Table 27

pH	12h		24h		36h	
isomer	*anti* [%]	*syn* [%]	*anti* [%]	*syn* [%]	*anti* [%]	*syn* [%]
4	99.8	0.2	98	2	94	6
6	99	1	98	2	96	4
8	98	2	97	3	91	9
10	97	3	97	3	94	6

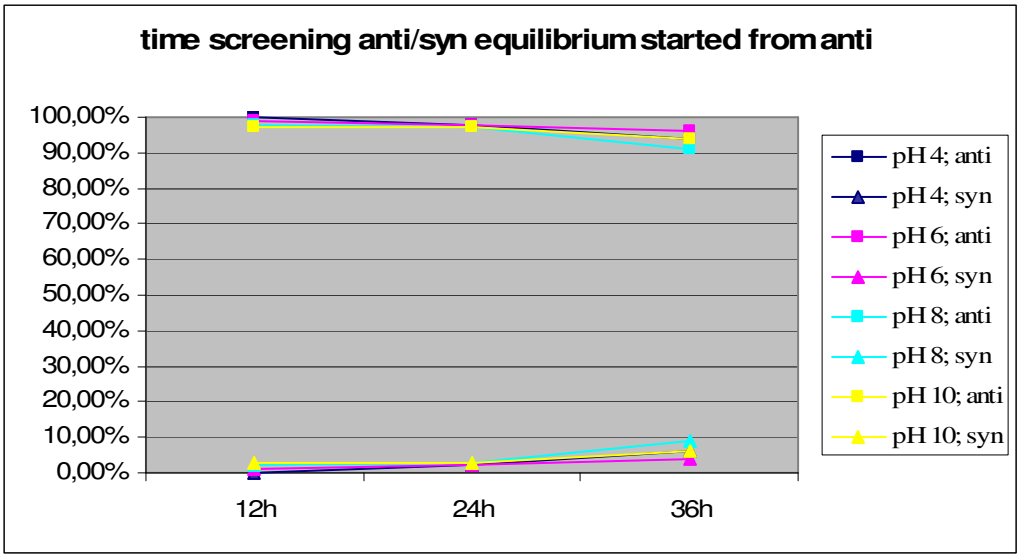


Figure 3

Starting from the diastereomer **15*anti***, slowly the diastereomer **15*syn*** was formed under all conditions, no significant differences could be observed in dependence to the pH. The biggest shift of the equilibrium was observed after 36h at pH = 8 (*anti/syn* = 91 : 9).

2.) Starting from **15*syn***

Table 28

pH	12h		24h		36h	
isomer	*anti* [%]	*syn* [%]	*anti* [%]	*syn* [%]	*anti* [%]	*syn* [%]
4	8	92	6	94	33	67
6	11	89	7	93	32	68
8	11	89	11	89	28	72
10	9	91	13	87	26	74

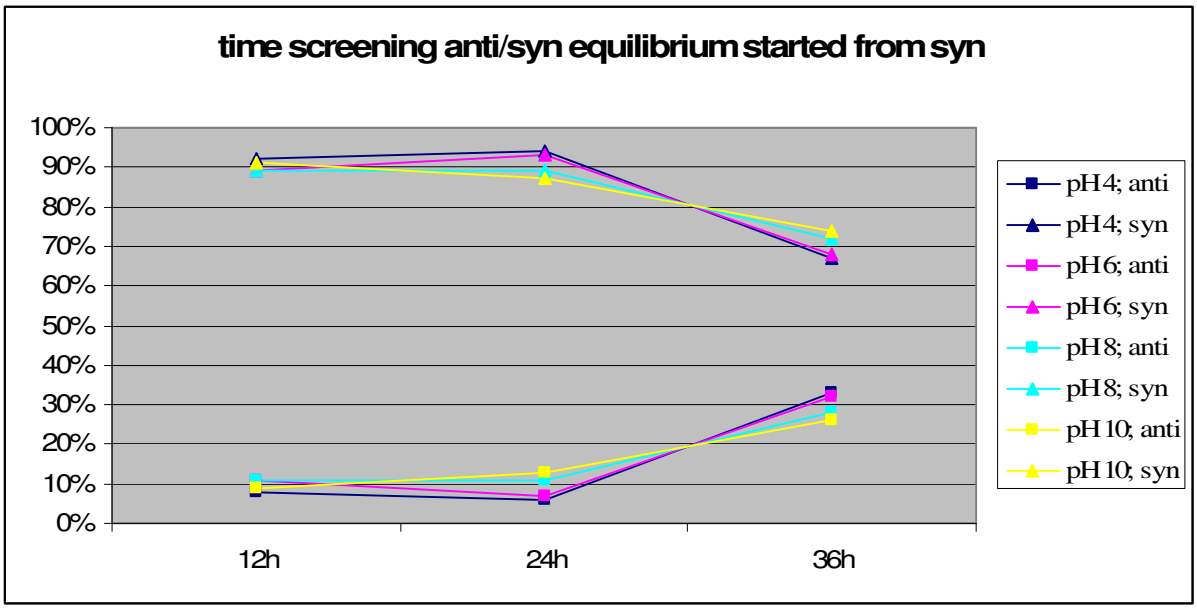
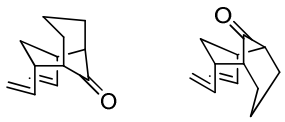


Figure 4

Started from the diastereomer **15*syn***, under all conditions the diastereomer **15*anti*** was formed faster than vice versa. No significant differences could be observed in dependence to the pH. The biggest shift of the equilibrium was observed after 36h at pH = 4 (*anti/syn* = 33 : 67) (Table 28, Figure 4).

3.) Starting from **15** *anti/syn* 92 : 8



92 : 8

Table 29

pH	12h		24h		36h	
isomer	*anti* [%]	*syn* [%]	*anti* [%]	*syn* [%]	*anti* [%]	*syn* [%]
4	92	8	92	8	92	8
6	92	8	92	8	92	8
8	91	9	92	8	92	8
10	92	8	94	6	92	8

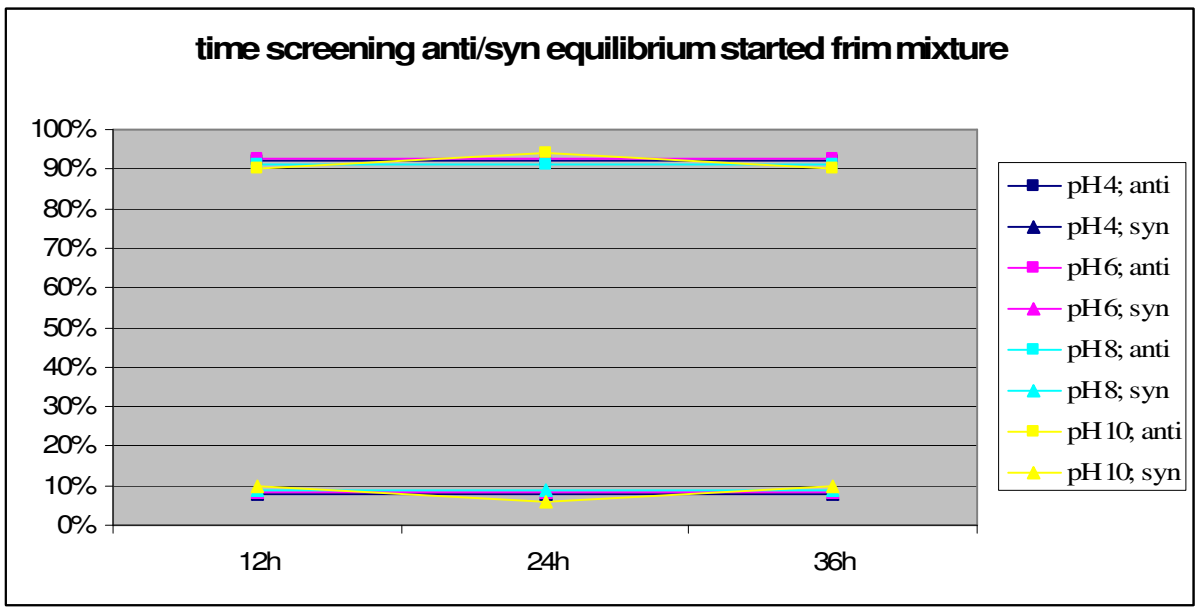


Figure 5

Starting from the diastereomeric mixture *anti/syn* 92 : 8, no changes could be observed in any pH. It seems that this ratio is the equilibrium all diastereomeric mixtures are heading to (Table 29, Figure 5).

No change in the diastereomeric equilibrium-ratio could be induced by changing the pH of the reaction media and consequently the conversion of the ketones **11** and **15** to the corresponding lactones with different *E. coli* strains could not be improved.

In a literature survey several examples for *anti* - *syn* interconversion could be found; several examples of diastereomeric interconversions of organic lignands bound to metals or forming chelate-complexes,[152] photoswitches/chiroptical switches,[153] equilibrium switch of two conformers depending on solvent polarity,[154] temperature-controlled interconversion,[155] pressure-dependent *anti/syn* interconversion,[156] diastereomeric dynamic equilibrium where the R/S ratio depends on the side chains of the substrate,[157] solvent-dependent *anti/syn* equilibrium of diastereomeric antropisomers[158] and slow isomerisation of *endo* ozonides to *exo* ozonides until an equilibrium, characteristic for the corresponding compound was reached.[159] Mechanistic aspects are discussed, but are not transferable to the isomerisation observed on these substrates.

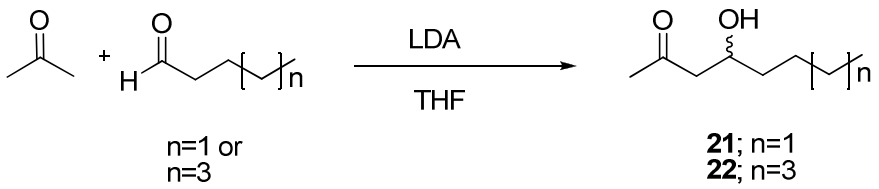
Since the rigid cyclic compounds so far investigated for their acceptance by available enzymes gave rather disappointing results, we decided to switch to another compound species: acyclic β-functionalized ketones.

[152] a) Oskam, J. H.; Schrock, R., *J. Am. Chem. Soc.* **1992**, *114*, 7588; b) Attar, S.; Nelson, H., *Organometallics* **1995**, *14*, 4559; c) Keane, J. M.; Ding,F.; Sabat, M; Harman, W. D., *J. Am. Chem. Soc.* **2004**, *126*, 785; d) Feast, W. J.; Gibson, V. C.; Ivin, K. J.; Kenwright, A. M.; Khosravi, E., *J. Chem. Soc. Chem. Commun.* **1994**, 1399.
[153] Feringa, B. L.; van Delden, R. A.; Koumura, N.; Geertsema, E. M., *Chem. Rev.* **2000**, *100*, 1789.
[154] Bhalla, V.; Kumar, M.; Kabuto, C.; Hattori, T.; Sotaro, M., *Chem. Lett.* **2004**, *33*, 184.
[155] Tumabac, G. E.; Mei, X.; Wolf, C., *Eur. J. Org. Chem.* **2004**, 3850.
[156] Klärner, F.-G.; Wurche, F.; Doering, W. von E.; Yang, J., *J. Am. Chem. Soc.* **2005**, *127*, 18107.
[157] Rix, U.; Zheng, J.; Remsing Rix, L. L.; Greenwell, L.; Yang, K.; Rohr, J., *J. Am. Chem. Soc.* **2004**, *126*, 4496.
[158] Clayden, J.; Vallverdu, L; Clayton, J.; Helliwell, M., *Chem. Comm.* **2008**, 561.
[159] Miaura, M; Ikegami, A.; Nojima, M.; Kusabayashi, S.; McCollough, K. J.; Nagase, S., *J. Am. Chem. Soc.* **1983**, *105*, 2414.

3.7 Linear Ketones

3.7.1 Synthesis of Linear Ketones

Two 4-hydroxy-2-ketones **21** and **22** were synthesized via a low temperature aldol condensation[160] according to the literature protocol (Scheme 55).[161]



Scheme 55

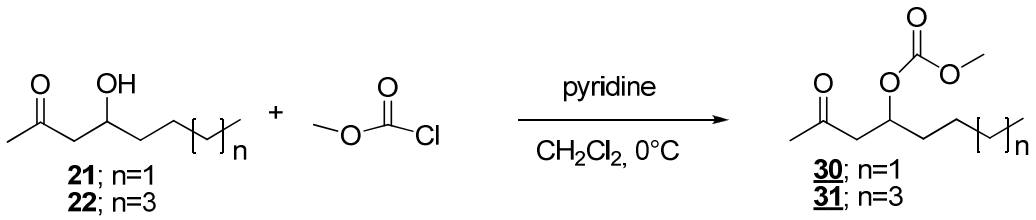
A solution of lithium diisopropylamide in THF was prepared from diisopropylamine and buthyllithium in *n*-hexane at -78°C. Subsequently, acetone and the appropriate aldehyde were added to this solution and then quenched with a saturated NH_4Cl solution. The product was extracted with diethylether, washed and dried and purified by flash column chromatography. During the column chromatography on silicagel the product apparently decomposed, a similar side product could be observed after NMR analysis. Another purification step was implemented and after *Kugelrohr* distillation the pure products were obtained in 81% (**21**) and 71% yield (**22**) respectively.
These substrates are chiral and were obtained as a racemic mixture of both enantiomers. Kinetic resolution to the resulting hydroxyalkylacetates was aspired within BVMO-catalyzed oxidation.

3.7.1.1 Synthesis of Functionalized Linear Ketones

Functionalized linear ketones were synthesized either by functionalization from 4-hydroxy-2-ketones **21** and **22** or by Michael-addition from *trans*-3-octen-2-on and *trans*-3-decen-2-on.

[160] Stork, G.; Kraus, G. A.; Garcia, G. A., *J. Org. Chem.* **1974**, *39*, 3459.
[161] Smith, A. B.; Levenberg, P. A., *Synthesis*, **1981**, 567.

Synthesis of Methyl-Carbonates



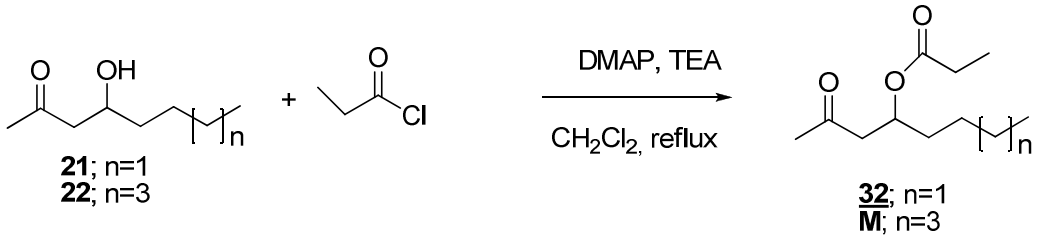
Scheme 56

Substrates **30** and **31** were synthesized from β-hydroxyketones by reacting with methyl chloroformate in dichloromethane at 0°C in presence of pyridine (Scheme 56).[162] After all starting material was consumed (GC/MS), the reaction mixture was quenched with water, extracted with diethylether, washed and dried and purified by flash column chromatography. During the column chromatography on silicagel or alumina the products apparently decomposed. Also during purification with *Kugelrohr* distillation decomposition (elimination) was observed. In further experiments no column chromatography or other purification techniques were performed and the temperature during workup was kept at 0°C. The crude products were obtained in excellent yields in 92-95% purity (GC/MS). The results are summarized in Table 30.

Table 30

	Product	React. Cond.	Solvent	Time, Temp.	Conversion [%]	Yield [%] (%GC/MS)
1	**30**	pyridine, ClCOOMe	CH_2Cl_2	1^{15}, 0°C	100	100 (95)
2	**31**	pyridine, ClCOOMe	CH_2Cl_2	3^{20}, 0°C	100	97 (92)

Synthesis of Propionates



Scheme 57

[162] Ito, H.; Kawakami, C.; Sawamura, M. *J. Am. Chem. Soc.* **2005**, *127*, 16034.

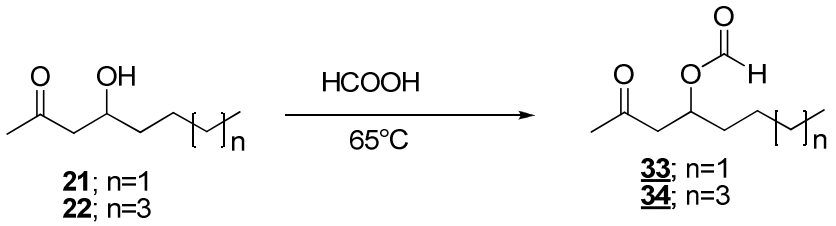
Substrate **32** was synthesized from **21** by reacting with propionyl chloride (Scheme 57).[163] The ice cold solution of the starting material propionyl chloride in dichloromethane was added dropwise in presence of triethylamine and DMAP. Then the solution was heated to reflux. After no increase in conversion could be observed anymore (GC/MS), the reaction mixture was washed with 2M HCl and dried and purified by flash column chromatography. During the column chromatography on silicagel the product **32** apparently decomposed. Product **M** decomposed (eliminated) already during workup. In further experiments no column chromatography or other purification techniques were performed and the temperature during workup was kept at 0°C. During workup the product **32** was partly converted back to the starting material. The crude product **32** was obtained in 60% purity (GC/MS) together with 40% of the starting material. No product **M** could be isolated. The results are summarized in Table 31.

Table 31

	Product	React. Cond.	Solvent	Time, Temp.	Conversion [%]	Yield [%] (%GC/MS)
1	**32**	PAC*, DMAP, TEA	CH_2Cl_2	2h, reflux	85	79 (60)
2	**M**	PAC*, DMAP, TEA	CH_2Cl_2	2.5h, reflux	91	traces

* PAC=propionyl chloride

Synthesis of Formates



Scheme 58

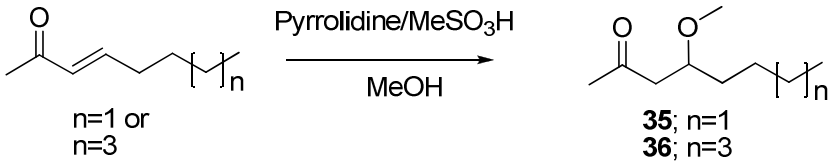
Substrates **33** and **34** were synthesized from β-hydroxyketones **21** and **22** by heating them at 65°C in formic acid (Scheme 58). During the ice-cold workup the products **33** and **34** were partly decomposed (eliminated). The crude products were obtained in 50% (**33**) and 87% (**34**) purity (GC/MS) together with the corresponding enone. The results are summarized in Table 32.

[163] Jones, A. D.; Knight, D. W.; Hibbs, D. E. *J. Chem. Soc., Perkin Trans. 1*, **2001**, 1182.

Table 32

	Product	Solvent	Time, Temp.	Conversion [%]	Yield [%] (%GC/MS)
1	**33**	HCOOH	2h, 65°C	100	100 (50)
2	**34**	HCOOH	2h, 65°C	100	100 (87)

Synthesis of Methoxyketones



Scheme 59

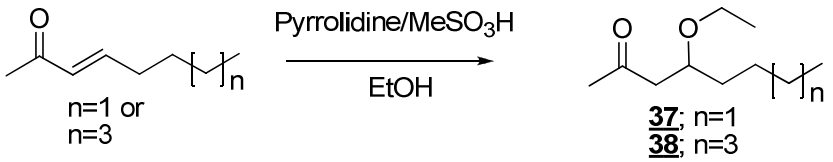
Substrates **35** and **36** were synthesized from the corresponding 3-en-2-ones by adding a mixture of pyrrolidine/methanesulfonic acid to a methanolic solution of the enon (Scheme 59).[164] After no increase of the conversion was observed anymore, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution, extracted with dichloromethane, dried and concentrated in *vacuo*. No purification of the products was performed and the temperature at the workup was kept low. The crude products were obtained in 80% (**35)** and 85% (**36**) purity (GC/MS) together with the corresponding enone. The results are summarized in Table 33.

Table 33

	Product	React. Cond.	Solvent	Time, Temp.	Conversion [%]	Yield [%] (%GC/MS)
1	**35**	Pyrrolidine, $MeSO_3H$	MeOH	3d, R.T.	81	92 (80)
2	**36**	Pyrrolidine, $MeSO_3H$	MeOH	24h, R.T.	84	51 (85)

[164] Ramachary, D. B. ; Modal, R. *Tetrahedron Lett.* **2006**, *47*, 7689.

Synthesis of Ethoxyketones



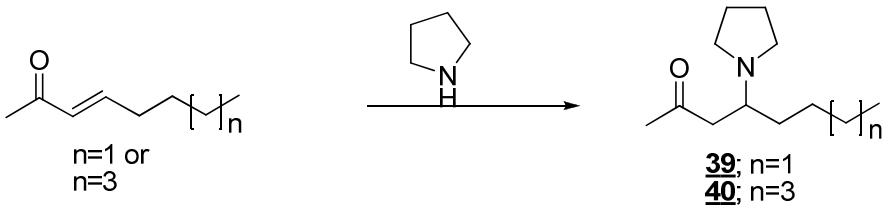
Scheme 60

Substrates **37** and **38** were synthesized from the corresponding 3-en-2-ones by adding a mixture of pyrrolidine/methanesulfonic acid to a ethanolic solution of the enon (Scheme 60).[164] After no increase of the conversion was observed anymore, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution, extracted with dichloromethane, dried and concentrated in *vacuo*. No purification of the products was performed and the temperature at the workup was kept low. During the ice-cold workup the products **37** and **38** were partly converted back to the starting material (elimination). The crude products were obtained in 65% (**37**) and 70% (**38**) purity (GC/MS) together with the corresponding enone. The results are summarized in Table 34.

Table 34

	Product	React. Cond.	Solvent	Time, Temp.	Conversion [%]	Yield [%] (%GC/MS)
1	**37**	Pyrrolidine, $MeSO_3H$	MeOH	3d, R.T.	70	82 (65)
2	**38**	Pyrrolidine, $MeSO_3H$	MeOH	18d, R.T.	100	70 (70)

Synthesis of Pyrrolidyl-Ketones



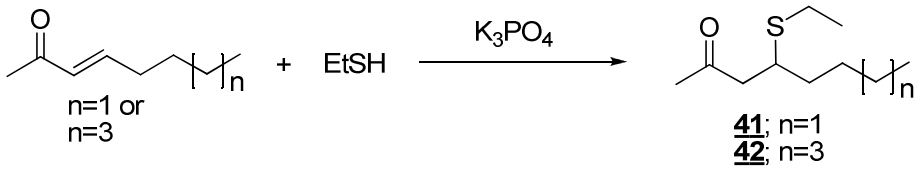
Scheme 61

Substrates **39** and **40** were synthesized from the corresponding 3-en-2-ones by stirring the appropriate enone with pyrrolidine at room temperature (Scheme 61).[165] In synthesis of **39**, almost full conversion could be reached already after one hour, in synthesis of **40** after 53h only 64% conversion could be observed. Workup was done by simply evaporate the excess pyrrolidine (cold rotary evaporator bath). The crude products were obtained in 95% (**39**) and 64% (**40**) purity (GC/MS) together with the corresponding enone. The results are summarized in Table 35.

Table 35

	Product	React. Cond.	Solvent	Time, Temp.	Conversion [%]	Yield [%] (%GC/MS)
1	**39**	Pyrrolidine	neat	1h, R.T.	95	97 (95)
2	**40**	Pyrrolidine	neat	53, R.T.	64	96 (64)

Synthesis of Thioethers



Scheme 62

Substrates **41** and **42** were synthesized from the corresponding 3-en-2-ones by stirring with excess ethanethiol in presence of 25 mol% K_3PO_4 at room temperature (Scheme 62).[166] After full conversion was observed, chloroform was added to the reaction mixture and K_3PO_4 was removed by filtration. Chloroform and excess ethanethiol were removed in *vacuo*. No further purification was conducted. The products **41** and **42** were obtained in excellent yield in very good purity (>95% GC/MS). The results are summarized in Table 36.

Table 36

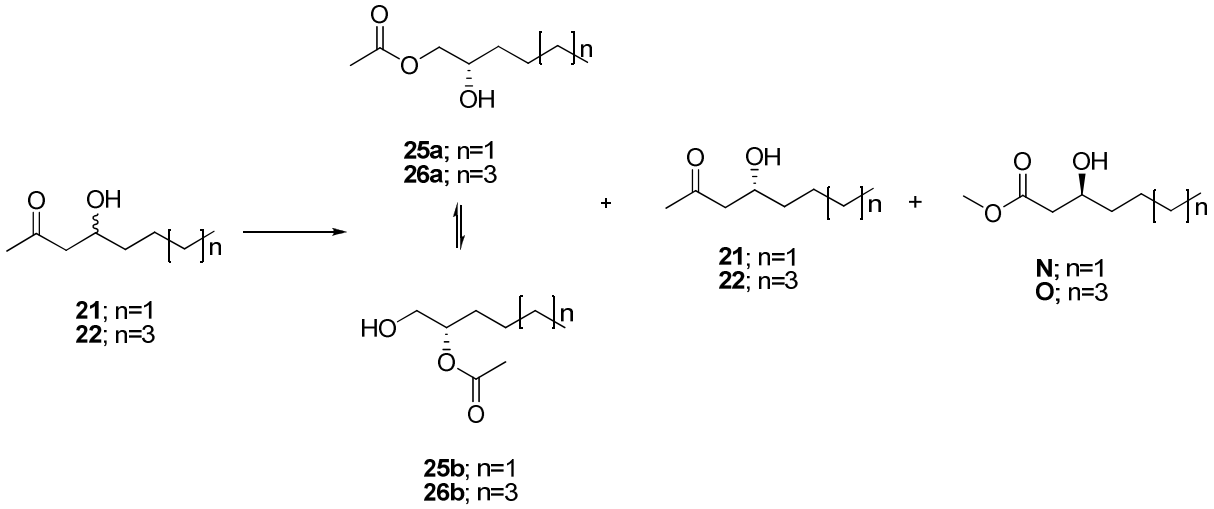
	Product	React. Cond.	Solvent	Time, Temp.	Conversion [%]	Yield [%] (%GC/MS)
1	**41**	EtSH, K_3PO_4	neat	4d, R.T.	96	95 (>95)
2	**42**	EtSH, K_3PO_4	neat	24h, R.T.	100	100 (>99)

[165] Ranu, B. C.; Dey, S. S.; Hajra, A. *Arkivoc* **2002,** *vii*, 76.
[166] Pore, D. M.; Desai, U. V.; Thopate, T. S.; Wadagaonkar, P. P. *Tetrahedron Lett.* **2006**, *47*, 9325.

3.7.2 Biotransformations

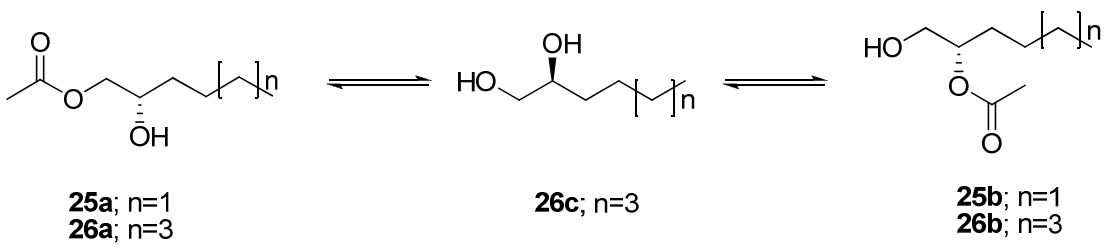
The biooxidation of compounds **21** and **22** with the BVMO from *P. fluorescens* DSM 50106 ($BVMO_{Pseudo}$) was previously reported in literature.[143] The biotransformation was carried out with resting cells. The appealing strategy behind this biotransformation is the generation of dihydroxy-compounds (in part protected) at a terminal position in a longer hydrocarbon chain; this represents a formal hydroxylation of a terminal olefin, but established methods (Sharpless, Jacobsen) usually fail to give good stereoselectivities in the absence of polar anchor groups.

In contrast to the reported protocol our experiments were performed under growing conditions (Scheme 63).



Scheme 63

Migration of the acyl-functionality from the primary to the secondary hydroxy group had been reported in the literature[143, 167](Scheme 63), the reported ratio between both species varies from 7:3 to 4:1 mixtures. No formation of the other possible oxidation product, the hydroxyacid methylester **N** or **O** could be observed. But a third product could be identified in the reaction mixture in preparative biooxidations of **22**, which was assigned as the "deprotected" dihydroxy compound (**26c**, Scheme 64).



[167] Park, P; Kozikowski, A. P., *Tetrahedron Lett.* **1988**, *29*, 6703.

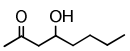
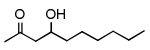
Scheme 64

We carried out screening with 13 *E. coli* strains, including all DuPont strains, $CHMO_{Xantho}$, HAPMO, CDMO, PAMO and $BVMO_{Pseudo}$ as reference. The conversion was monitored by GC/MS.

3.7.2.1 Screening of 21 and 22

In all experiments the substrates **21** and **22** were incubated with the appropriate *E. coli* strain under growing conditions at 24°C for 24h, except with PAMO, which was incubated at 37°C (Table 37).

Table 37

	Strain	Conversion/Yield [%] 	Conversion/Yield [%] 
1	$CHMO_{Acineto}$	17	26
2	$CPMO_{Coma}$	93/66	100/38
3	$CHMO_{Brevi1}$	2	*n. c.*
4	$CHMO_{Brevi2}$	92	100
5	$CHMO_{Rhodo1}$	76	76
6	$CHMO_{Rhodo2}$	79	87
7	$CHMO_{Brachy}$	82	78
8	$CHMO_{Arthro}$	90	79
9	$CHMO_{Xantho}$	72	87
10	HAPMO	66	93
11	CDMO	94	100
12	$BVMO_{Pseudo}$	22	75
13	PAMO	6	6

The substrate **22** gave better results than the substrate **21**, according to literature. The conversion of **21** even after 24h was lower than after 4h in resting cells-conditions with $BVMO_{Pseudo}$.[143] The highest conversions could be achieved with $CPMO_{Coma}$, $CHMO_{Brevi2}$ and CDMO, which gave almost complete conversion with **21** (Table 37, entries 2, 4 and 11) and complete conversion with **22** (Table 37, entries 15, 17 and 24).

Preparative Biotransformations of ß-Hydroxyketones

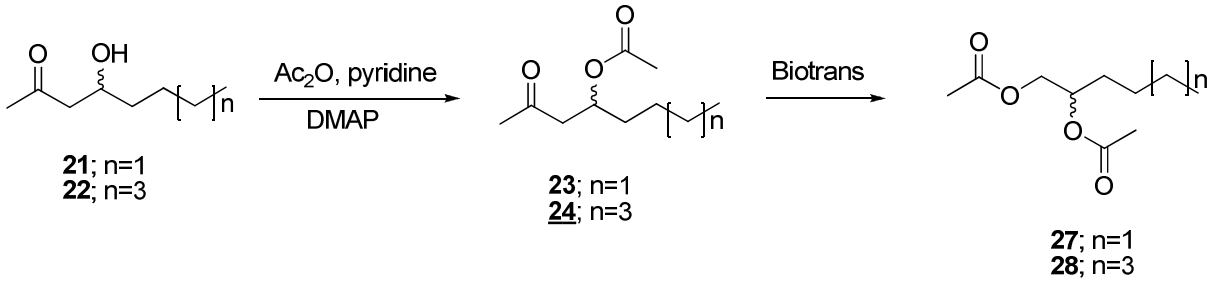
In preparative scale biotransformations the products **25a** and **25b** could be prepared and analyzed by incubation of **21** with $CPMO_{Coma}$. After 21h full conversion was observed, the reaction mixture centrifuged, the supernatant extracted with ethyl acetate, the crude product was purified with flash column chromatography and the products **25a** and **25b** were obtained in 66% yield in ratio ca. 2.5 : 1.

The compounds **26a**, **26b** and **26c** were prepared and analyzed by incubating substrate **22** with $CPMO_{Coma}$. After 44h the reaction mixture was centrifuged, the products were extracted with ethyl acetate and purified by flash column chromatography. Products **26a** and **26b** were obtained as a mixture in ratio ca. 2.5:1, the diol **26c** could be separated during the chromatography. The yield of all three products was 38% (but probably increasable, since according to GC/MS before workup approximately half of the product decomposed).

Except the migration of the acyl functionality we faced another problem: with the available equipment we were not able to separate the enantiomers on the chiral GC-column and were consequently not able to determine the *ee*-values of the products.

3.7.2.2 Screening of 23 and 24

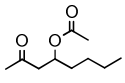
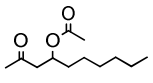
To circumvent these problems (three different biotransformation products and the separation) we decided to acylate the substrates before biotransformation, which can then give only one product **27** or **28** respectively (Scheme 65).



Scheme 65

Screening was performed with the acylated substrates **27** and **28** with thirteen different BVMO overexpression systems (Table 38).

Table 38

	Strain	Conversion [%](*ee* R or S)  **23**	Conversion [%](*ee* R or S)  **24**
1	$CHMO_{Acineto}$	4	1
2	$CPMO_{Coma}$	10 (95 R)	38 (100 R)
3	$CHMO_{Brevi1}$	1	2
4	$CHMO_{Brevi2}$	10 (91 R)	27 (100 R)
5	$CHMO_{Rhodo1}$	2	2
6	$CHMO_{Rhodo2}$	3	1
7	$CHMO_{Brachy}$	12 (66 S)	2
8	$CHMO_{Arthro}$	14 (79 S)	2
9	$CHMO_{Xantho}$	1	7
10	HAPMO	38 (15 S)	3
11	CDMO	19 (0)	96 (0)
12	$BVMO_{Pseudo}$	0	0
13	PAMO	Conversion, Decomp.	Conversion, Decomp.

The interesting feature in this screening is, that the *R*-enantiomer was in excess when incubated with $CPMO_{Coma}$ and $CHMO_{Brevi2}$, but the *S*-enantiomer was in excess in the reactions with $CHMO_{Brachy}$, $CHMO_{Arthro}$ and HAPMO for substrate **27**. In all experiments starting from the not-acylated substrates **21** or **22** respectively, the S-enantiomer was in excess (see below). The conversions in these screenings were much lower than with the non-acylated substrates **21** and **22**. In screening with PAMO, some conversion could be observed, but mainly decomposition occurred, probably due to the higher incubation temperature (37°C) in comparison to the other strains (24°C).

In addition time screening of **28** was performed with three strains: $CPMO_{Coma}$ (Table 39), $CHMO_{Brevi2}$ (Table 40) and CDMO (Table 41).

Table 39

	Strain	Time [h]	Conversion [%]	*ee* Prod [%]	*ee* SM [%]	E
1	$CPMO_{Coma}$	3	*n.c.*	-----	-----	-----
2	$CPMO_{Coma}$	7	traces	100 (R)	2	>200
3	$CPMO_{Coma}$	19	20	100 (R)	39	>200
4	$CPMO_{Coma}$	24	25	100 (R)	45	>200
5	$CPMO_{Coma}$	30	26	100 (R)	56	>200
6	$CPMO_{Coma}$	45	27	95 (R)	57	69

Very good enantiomeric ratio could be obtained in all cases. The limitation in this system is the low conversion, which reaches only 27% after 45h.

Table 40

	Strain	Time [h]	Conversion [%]	*ee* Prod [%]	*ee* SM [%]	E
1	$CHMO_{Brevi2}$	4	*n.c.*	-----	-----	-----
2	$CHMO_{Brevi2}$	8	3	100 (R)	8	>200
3	$CHMO_{Brevi2}$	13	11	100 (R)	22	>200
4	$CHMO_{Brevi2}$	20	23	91 (R)	41	31
5	$CHMO_{Brevi2}$	25	27	88 (R)	33	21
6	$CHMO_{Brevi2}$	37	decomp.	-----	-----	-----

Very good enantiomeric ratio could be obtained at conversions lower than 25%. At higher conversions the enantiomeric ratio drops. With longer reaction times decomposition was observed.

Table 41

	Strain	Time [h]	Conversion [%]	*ee* Prod [%]	*ee* SM [%]	E
1	CDMO	3	*n.c.*	-----	-----	-----
2	CDMO	6	8	24 (S)	2	1.7
3	CDMO	9	30	19 (S)	8	1.6
4	CDMO	17	62	17 (S)	11	1.6
5	CDMO	24	82	2 (S)	10	1.1
6	CDMO	30	90	0	10	1.1

As expected, CDMO shows good acceptance, but low enantioselectivity towards the substrate **28**. At reaction time of 17h and more, partly decomposition of the material was observed. The results of all three time screenings are summarized in Figure 6:

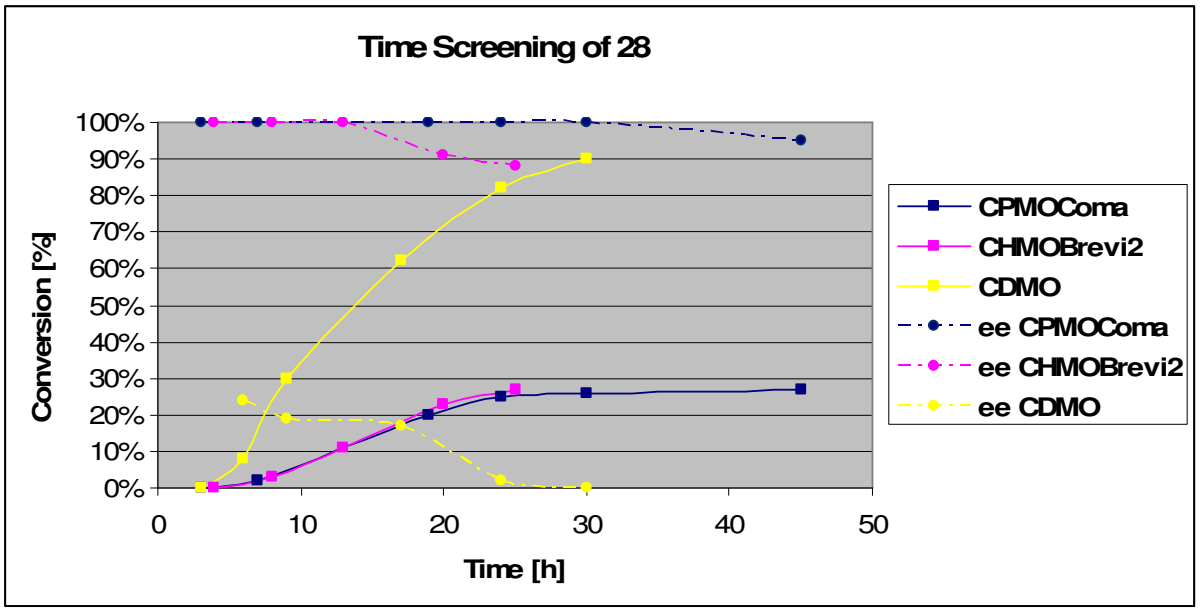
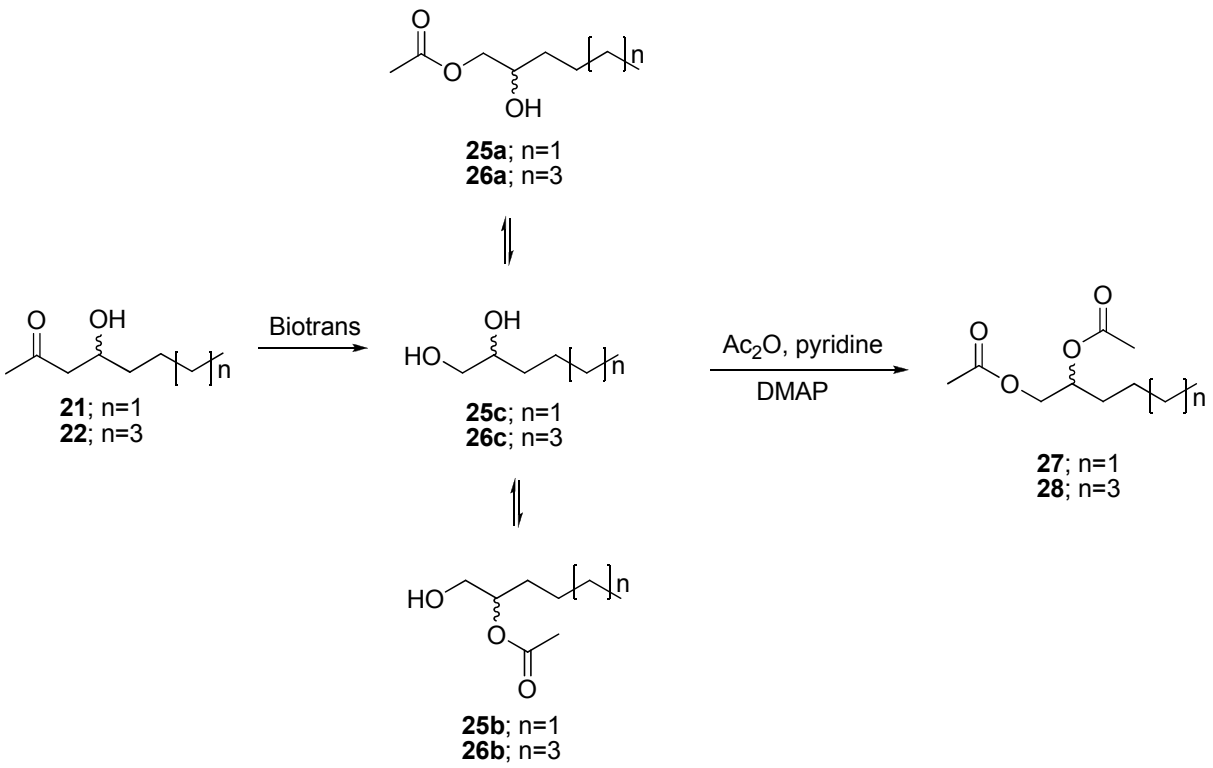


Figure 6

After obtaining these results we concluded that the best option in this series would be the acetylation of the substrates **25** or **26** after the biooxidation; this may solve the analytical problems in *ee* determination, leads to only one product, and allows the biotransformation with the β-hydroxy-ketones to give better conversions (Scheme 66), see page 104.

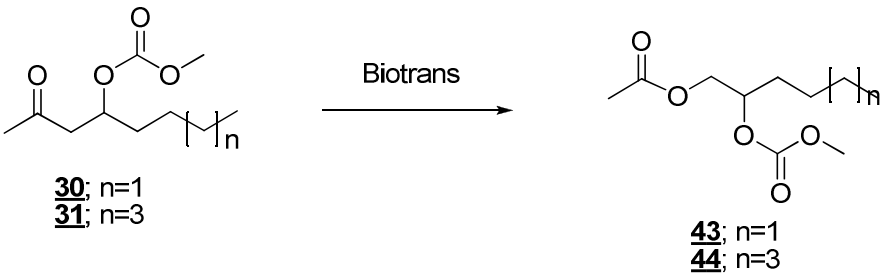


Scheme 66

All other β-functionalized linear ketones were screened with 12 different BVMO overexpression systems, $CHMO_{Acineto}$, $CPMO_{Coma}$, $CHMO_{Brevi1}$, $CHMO_{Brevi2}$, $CHMO_{Rhodo1}$, $CHMO_{Rhodo2}$, $CHMO_{Brachy}$, $CHMO_{Arthro}$, $CHMO_{Xantho}$, CDMO, $BVMO_{Pseudo}$ and HAPMO. No experiments in preparative scale were made with these substrates, the products (esters) were identified in GC/MS.

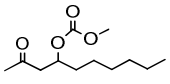
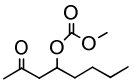
3.7.2.3 Screening of Methyl-Carbonates 30 and 31

Screening was performed with the substrates **30** and **31** with twelve different BVMO overexpression systems (Scheme 67, Table 42).



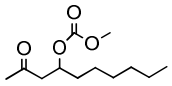
Scheme 67

Table 42

	Strain	Conversion [%](*ee*)	
		 **31**	 **30**
1	$CHMO_{Acineto}$	*n.c.*	*n.c.*
2	$CPMO_{Coma}$	*n.c.*	*n.c.*
3	$CHMO_{Brevi1}$	*n.c.*	3
4	$CHMO_{Brevi2}$	*n.c.*	traces
5	$CHMO_{Rhodo1}$	*n.c.*	traces
6	$CHMO_{Rhodo2}$	*n.c.*	traces
7	$CHMO_{Brachy}$	*n.c*	2
8	$CHMO_{Arthro}$	*n.c.*	4
9	$CHMO_{Xantho}$	*n.c.*	16 (66)
10	CDMO	*n.c.*	3
11	$BVMO_{Pseudo}$	41	*n.c.*
12	HAPMO	*n.c.*	19 (65)

With **30** rather poor conversions were obtained and only strains expressing $CHMO_{Xantho}$ and HAPMO displayed moderate biooxygenations and product *ee*s. No further attempts were made with this substrate.

With **31**, conversion could be observed only with the strain HAPMO. Since the conversion was lower, but close to 50%, time screening was carried out and *ee* values were calculated (on chiral phase GC only the starting material, but not the product could be resolved, *ee* of the product was calculated from *ee* of the starting material and conversion (Table 43). The products (esters) were identified in GC/MS.



31

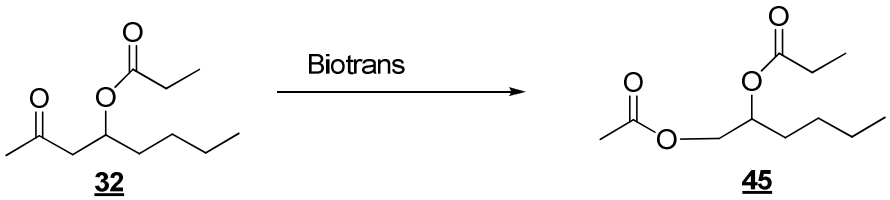
Table 43

	Strain	Time [h]	Conversion [%]	*ee* Prod [%]	*ee* SM [%]	E
1	HAPMO	5.5	12	91	12	23
2	HAPMO	19	39	85	55	21
3	HAPMO	24	50	68	68	10
4	HAPMO	31	53	63	71	9.1
5	HAPMO	44	66	52	100	21

Conversion, close to 50% was observed after 24 to 31 hours, but the enantiomeric ratio (E) values were only moderate.

3.7.2.4 Screening of Propionate 32

Screening was performed with the substrate **32** with twelve different BVMO overexpression systems (Scheme 68, Table 44).



Scheme 68

Table 44

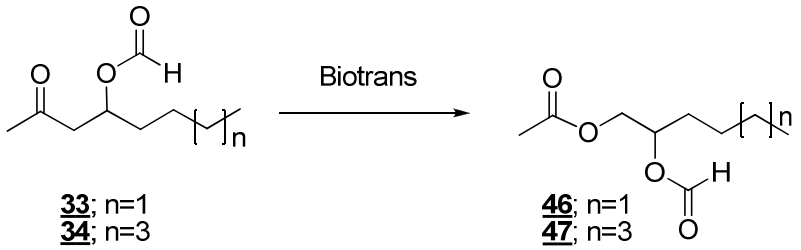
	Strain	Conversion [%]	*ee* Prod [%]*	*ee* SM [%]	E
1	$CHMO_{Acineto}$	*n.c.*	---	---	---
2	$CPMO_{Coma}$	*n.c.*	**---**	**---**	**---**
3	$CHMO_{Brevi1}$	*n.c.*	---	---	---
4	$CHMO_{Brevi2}$	*n.c.*	---	---	---
5	$CHMO_{Rhodo1}$	*n.c.*	---	---	---
6	$CHMO_{Rhodo2}$	10	**---**	**---**	**---**
7	$CHMO_{Brachy}$	8	---	---	---
8	$CHMO_{Arthro}$	7	31 (A)	1	1.9
9	$CHMO_{Xantho}$	33	21 (B)	13	1.7
10	CDMO	84	2 (B)	21	1.2
11	$BVMO_{Pseudo}$	5	0	0	0
12	HAPMO	5	87 (B)	3	>200

* **A** and **B** are enantiomers, assignment of absulute configuration is pending;

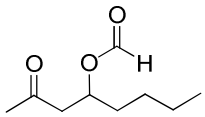
With **32** moderate conversion was obtained with $CHMO_{Xantho}$ and good conversion was obtained with CDMO. In both experiments the E-values are low (1.7 or 1.2 respectively). With HAPMO E >200 was obtained, but since the conversion is only 5%, the result is of no practical value. The product was identified in GC/MS.

3.7.2.5 Screening of Formates 33 and 34

Screening was performed with the substrates **33** and **34** with twelve different BVMO overexpression systems (Scheme 69, Table 45, Table 46).



Scheme 69



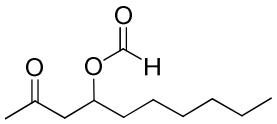
33

Table 45

	Strain	Conversion [%]	*ee* Prod [%]*	*ee* SM [%]	E
1	$CHMO_{Acineto}$	42	33 (S)	25 (R)	2.5
2	$CPMO_{Coma}$	59	99 (R)	99.9 (S)	>200
3	$CHMO_{Brevi1}$	7	99 (R)	0.4 (S)	>200
4	$CHMO_{Brevi2}$	28	99.9 (R)	27 (S)	>200
5	$CHMO_{Rhodo1}$	10	31 (S)	12 (R)	2.1
6	$CHMO_{Rhodo2}$	15	31 (S)	11 (R)	2.1
7	$CHMO_{Brachy}$	47	67 (S)	68 (R)	10
8	$CHMO_{Arthro}$	39	61 (S)	49 (R)	6.6
9	$CHMO_{Xantho}$	57	66 (S)	73 (R)	10
10	CDMO	61	63 (R)	29 (S)	5.8
11	$BVMO_{Pseudo}$	3	90 (R)	1 (S)	19
12	HAPMO	62	85 (R)	75 (S)	27

* **R** and **S** enantiomers were assigned by comparison with

Assignment of the enantiomers for **33** and **46** was conducted by comparison of chiral GC-chromatogramms of **33**/**46** and **23/27** after an preparative-scale experiment partly converting **33** with $CHMO_{Arthro}$ to **46**, splitting up the functional group of **33**/**46** and subsequent acetylation.



34

Table 46

	Strain	Conversion [%]	*ee* Prod [%]*	*ee* SM [%]	E
1	$CHMO_{Acineto}$	*n.c.*[#]	------	-----	-----
2	$CPMO_{Coma}$	67	60(A)	100	27
3	$CHMO_{Brevi1}$	4	100(A)	1	>200
4	$CHMO_{Brevi2}$	55[#]	100(A)	100	>200
5	$CHMO_{Rhodo1}$	*n.c.*	-----	-----	-----
6	$CHMO_{Rhodo2}$	*n.c.*	-----	-----	-----
7	$CHMO_{Brachy}$	5	5(B)	6	1.2
8	$CHMO_{Arthro}$	7[#]	100(B)	9	>200
9	$CHMO_{Xantho}$	6[#]	100(B)	10	>200
10	CDMO	50	41(A)	85	6
11	$BVMO_{Pseudo}$	traces[#]	------	------	-----
12	HAPMO	59[#]	47(A)	96	9.9

* **A** and **B** are enantiomers, assignment of absulute configuration is pending;

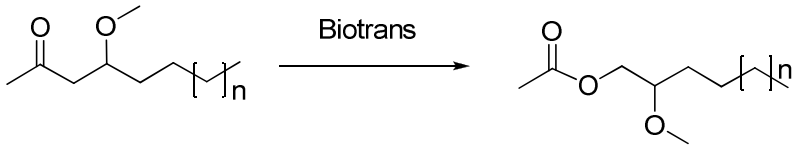
[#] considerable part (>50%) of SM was converted back to the hydroxyketone **22**.

In screening of **33** very good *ee* and E values where obtained with $CPMO_{Coma}$ and $CHMO_{Brevi2}$. They also yield the opposite enantiomer compared to the rest of the DuPont strains. These results are in accordance with the BVMO phylogenetic tree, where these two enzymes are closely related. With $CHMO_{Brevi1}$ E >200 was obtained, but since the conversion is only 7%, the result is of no practical value. CDMO and HAPMO preferably yield the same enantiomer as the CPMO-group. The product was identified in GC/MS.

The conversions of **34** were in general lower then with **33**. Two enantiomers are labeled as A and B, assignment of the absolute configuration is pending. Since the trend is the same as with substrate **33**, we can assume, that A=R and B=S. In this reaction ca. 50% loss of material was observed after 24h reaction time. The substrate **34** was partly converted back to the hydroxyketone **22** (which was then also biooxidized). The conversions in the Table 46 correspond to the areas of **34** and the corresponding ester **47**, without taking into account the decomposed material. Like in previous experiments, good *ee* and E values where obtained with $CPMO_{Coma}$ and $CHMO_{Brevi2}$ and the opposite enantiomer was obtained with the rest of the DuPont strains (except $CHMO_{Brevi1}$). E values >200, obtained with $CHMO_{Arthro}$ and $CHMO_{Xantho}$ are of no practical value since the conversions are very low. The product was identified in GC/MS.

3.7.2.6 Screening of Methoxyketones 35 and 36

Screening was performed with the substrates **35** and **36** with twelve different BVMO overexpression systems (Scheme 70).

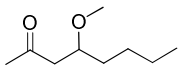


35; n=1
36; n=3

48; n=1
49; n=3

Scheme 70

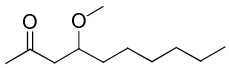
Substrate **35** and product **48** could not be resolved on the chiral phase GC, only conversion to the corresponding ester was determined, the determination of *ee* is subject of further investigations (Table 47). Substrate **36** could be resolved on the chiral phase GC, the results are summarized in Table 48.



35

Table 47

	Strain	Conversion [%]
1	$CHMO_{Acineto}$	40
2	$CPMO_{Coma}$	37
3	$CHMO_{Brevi1}$	14
4	$CHMO_{Brevi2}$	38
5	$CHMO_{Rhodo1}$	50
6	$CHMO_{Rhodo2}$	51
7	$CHMO_{Brachy}$	60
8	$CHMO_{Arthro}$	61
9	$CHMO_{Xantho}$	80
10	CDMO	58
11	$BVMO_{Pseudo}$	18
12	HAPMO	22



36

Table 48

	Strain	Conversion [%]	*ee* Prod [%]*	*ee* SM [%]	E
1	$CHMO_{Acineto}$	8	75 (B)	6(A)	7.4
2	$CPMO_{Coma}$	89	11 (A)	88(B)	2.8
3	$CHMO_{Brevi1}$	20	60 (A)	14(B)	4.6
4	$CHMO_{Brevi2}$	44	99 (A)	87(B)	>200
5	$CHMO_{Rhodo1}$	8	68 (B)	6(A)	5.6
6	$CHMO_{Rhodo2}$	11	45 (B)	6(A)	2.8
7	$CHMO_{Brachy}$	24	89 (B)	28(A)	22
8	$CHMO_{Arthro}$	26	86 (B)	28(A)	17
9	$CHMO_{Xantho}$	4	0	0	0
10	CDMO	57	38 (A)	50(B)	3.5
11	$BVMO_{Pseudo}$	5	0	0	0
12	HAPMO	45	5 (A)	7(B)	1.2

* **A** and **B** are enantiomers, assignment of absulute configuration is pending;

In screening with **35**, moderate to good conversions were obtained with all BVMO strains. Interesting results were obtained especially with $CHMO_{Rhodo1}$, $CHMO_{Rhodo}$ and CDMO, where the conversion stopped close to 50%. At the first glance these are promising results and good enantioselectivity can be expected with these strains. Unfortunately, neither the substrate **35** nor the product **48** could be resolved on the chiral phase GC. The product was identified in GC/MS.

In screening of **36** moderate to good conversions were obtained with five out of twelve BVMO strains. The best result was obtained with $CPMO_{Coma}$ with E>200. $CPMO_{Coma}$, $CHMO_{Brevi1}$, $CHMO_{Brevi2}$, CDMO and HAPMO yield preferably the opposite enantiomer then the rest of the DuPont strains. Also here the trend of both enantiomers is the same as with substrate **33**, so we can assume, that A=R and B=S. The product was identified in GC/MS.

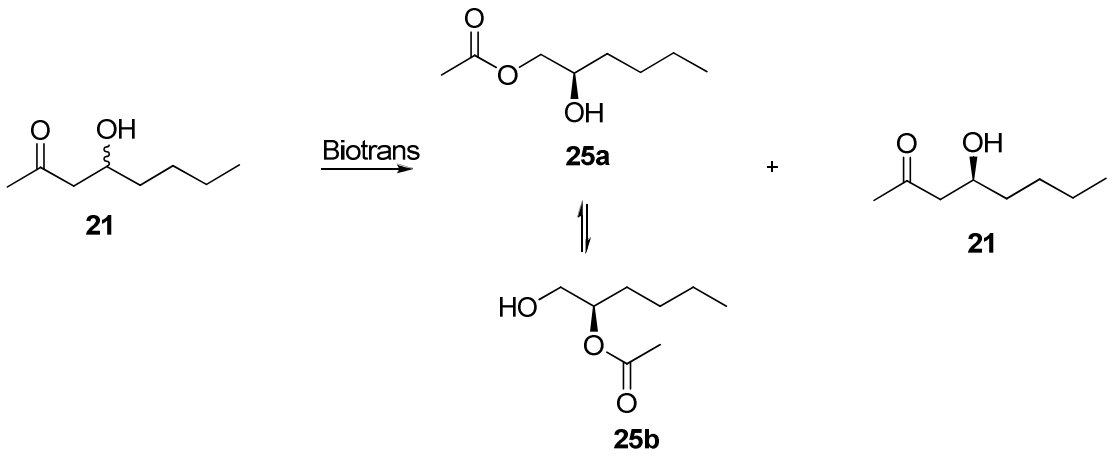
With ethoxyketones **37**, **38** and **41**, **42** screening was done as well, but since neither the ketones nor the esters could be resolved on chiral phase GC, the results are subject of further investigations and not included in this thesis.

3.7.3 Time Screening and Kinetic Resolution

To obtain optically pure product from chiral substrate, kinetic resolution must occur. Enzymes which convert one enantiomer of the substrate exclusively or much faster than the other are desired. An indication for this behavior can be recognized when the conversion stops at approximately 50% or becomes much slower after reaching 50%. For this reason time screening was performed with 11 different *E. coli* strains ($CHMO_{Brevi2}$ and PAMO were not used, since they gave almost no conversion in the first screening) to monitor the time-course of the conversion and estimate the time of 50% conversion with the single *E. coli* strains.

3.7.3.1 Time screening of 21

Time screening of **21** was performed with 11 different *E. coli* strains (Scheme 71, Table 49, Figure 7, Figure 8). Characterization of compounds was conducted after acetylation (see above). For clarity the graphs of conversions are divided on two figures.



Scheme 71

Table 49

Conversion [%]							
	Strain	**1h** [%]	**3h** [%]	**6h** [%]	**9h** [%]	**11h** [%]	**24h** [%]
1	$CHMO_{Acineto}$	traces	28	54	60	63	72
2	$CPMO_{Coma}$	15	58	97	100	100	100
3	CDMO	4	18	53	71	78	96
4	$CHMO_{Rhodo1}$	1	10	28	46	57	77
5	$CHMO_{Brevi2}$	traces	9	39	69	82	100
6	$CHMO_{Rhodo2}$	traces	5	23	29	42	76
7	$CHMO_{Brachy}$	traces	38	61	67	67	80
8	$CHMO_{Arthro}$	2	9	27	68	73	91
9	HAPMO	0	traces	5	16	21	77
10	$BVMO_{Pseudo}$	0	0	traces	7	12	33

		2h [%]	**5h** [%]	**8h** [%]	**10h** [%]	**23h** [%]
11	$CHMO_{Xantho}$	0	3	50	64	86

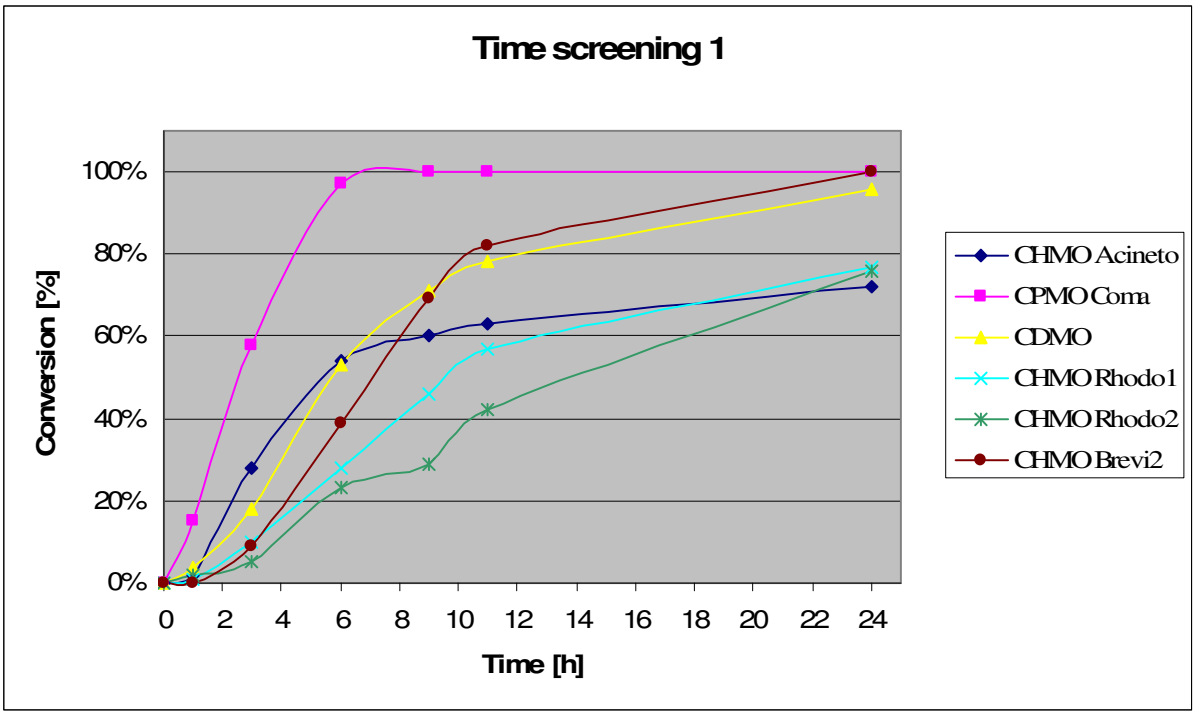


Figure 7

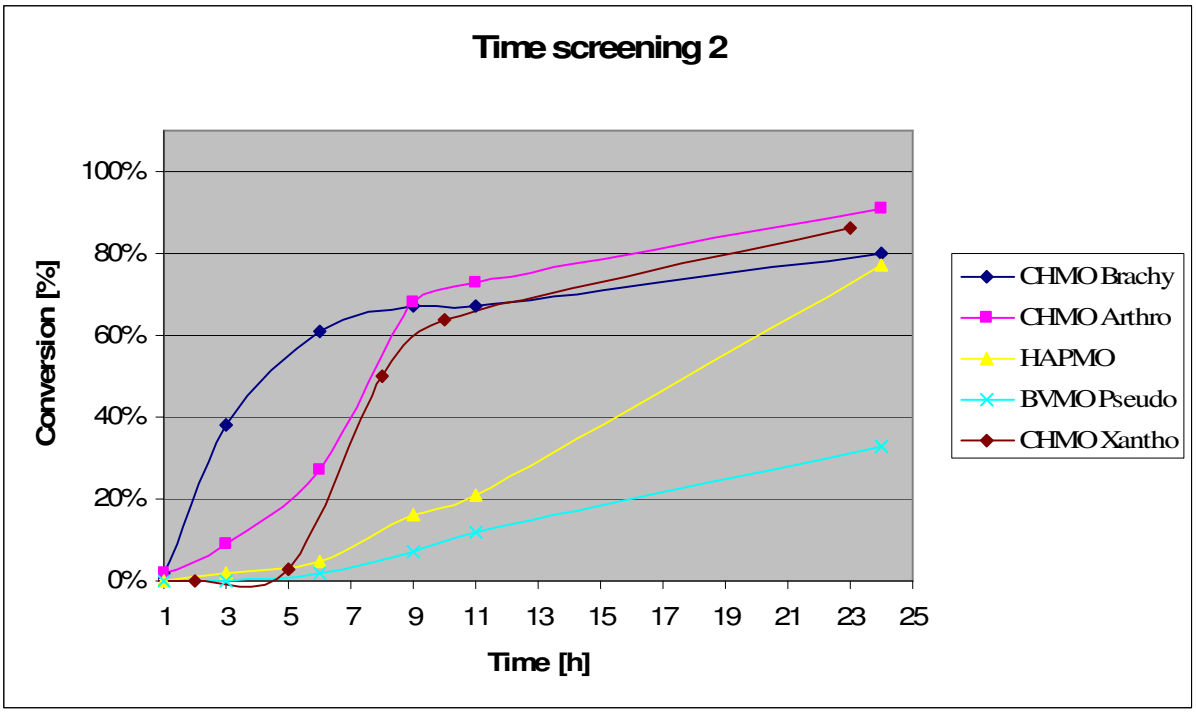
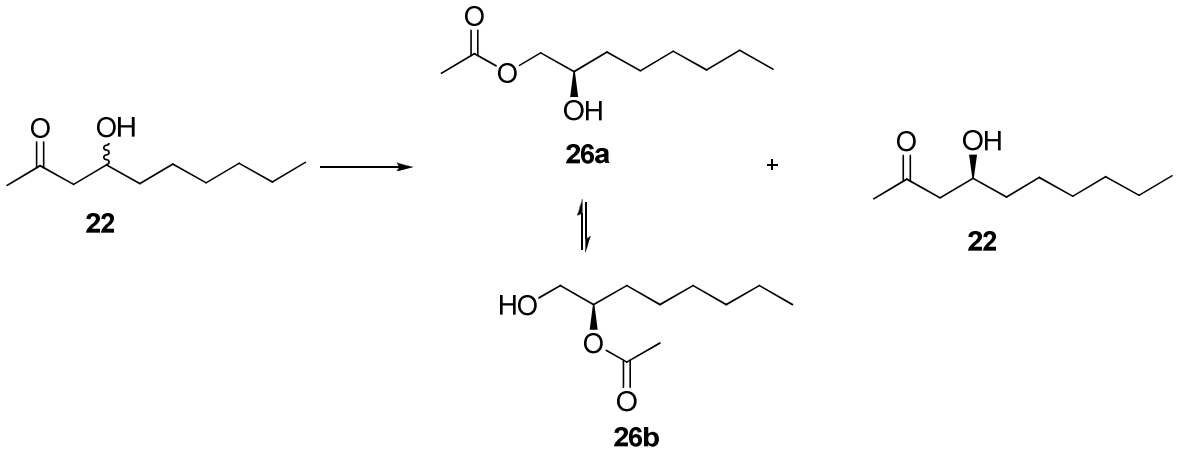


Figure 8

From the time-screening results, depicted in Figure 7 and Figure 8, the time courses of strains $CHMO_{Acinteo}$, $CHMO_{Rhodo1}$, $CHMO_{Brachy}$, $CHMO_{Xantho}$, and CDMO look promising, since they show a bend in the curve after the conversion has reached ca. 50%. This allows the assumption, that after one enantiomer was fully consumed, the oxidation of the second enantiomer proceeds slower. On the other hand $CPMO_{Coma}$ seems to be quite unselective.
To verify this assumption, in a later experiment the reaction was stopped at 50% conversion and the *ee* of the product and remaining starting material was determined (see 110).

3.7.3.2 Time Screening of 22

Time screening of **22** was performed with 11 different *E. coli* strains (Scheme 72, Table 50, Figure 9, Figure 10). For clarity the graphs of conversions are divided on two figures.



Scheme 72

Table 50

	Strain	**1h** [%]	**3h** [%]	**6h** [%]	**9h** [%]	**11h** [%]	**24h** [%]
1	$CHMO_{Acineto}$	2	30	41	47	41	52
2	$CPMO_{Coma}$	17	74	92	100	100	100
3	CDMO	16	34	88	91	83	90
4	$CHMO_{Rhodo1}$	1	5	15	23	46	74
5	$CHMO_{Brevi2}$	4	36	90	100	100	100
6	$CHMO_{Rhodo2}$	0	0	6	13	29	74
7	$CHMO_{Brachy}$	4	29	59	68	74	95
8	$CHMO_{Arthro}$	0	0	13	26	31	77
9	HAPMO	0	traces	16	43	38	91
10	$BVMO_{Pseudo}$	0	traces	36	63	63	74

		2h [%]	**5h** [%]	**8h** [%]	**10h** [%]	**23h** [%]
11	$CHMO_{Xantho}$	0	4	54	65	85

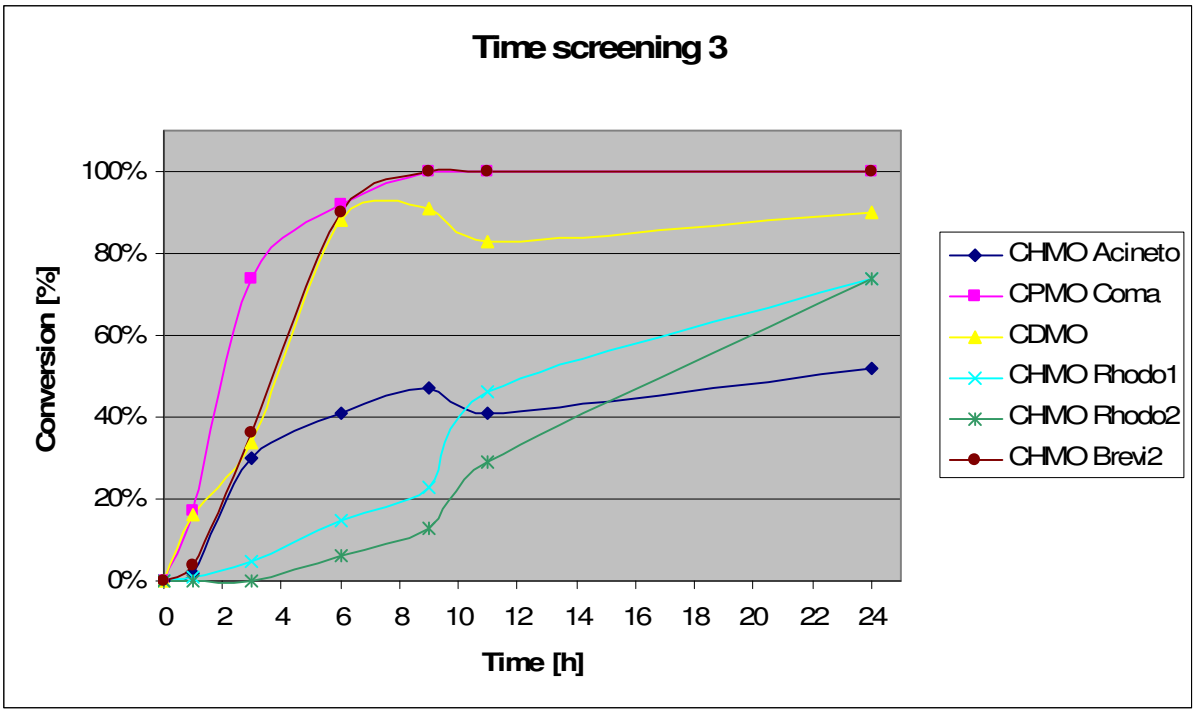


Figure 9

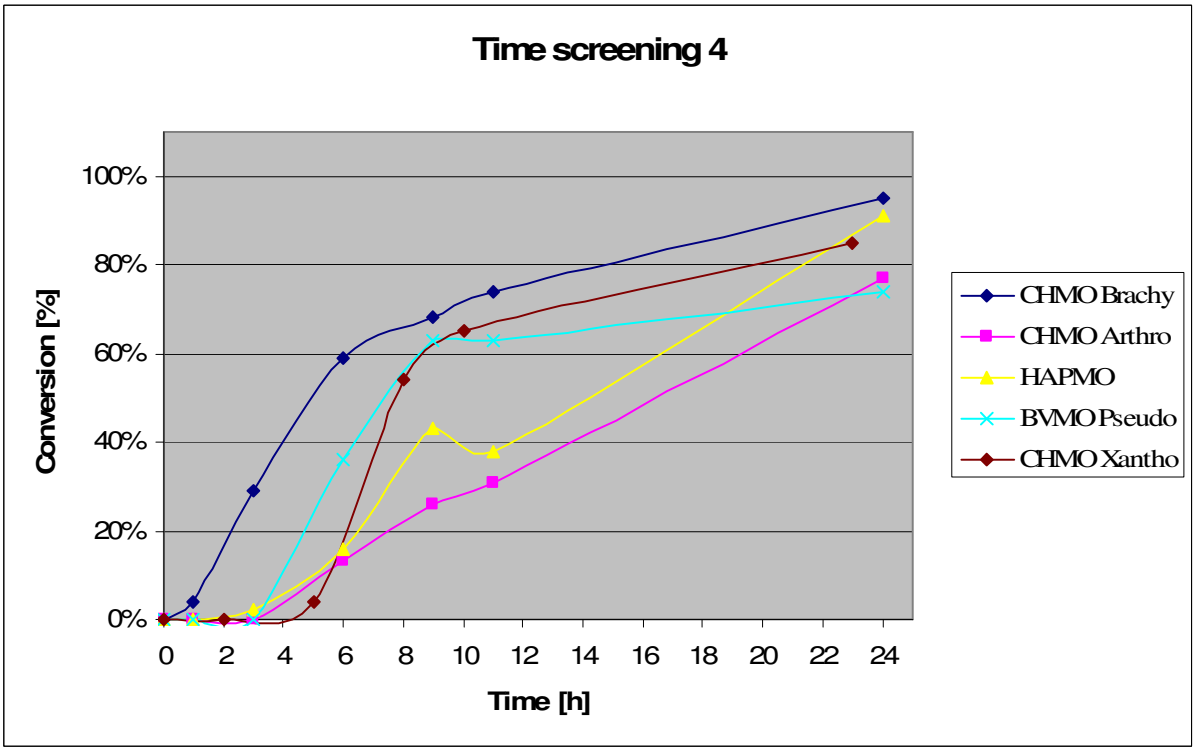


Figure 10

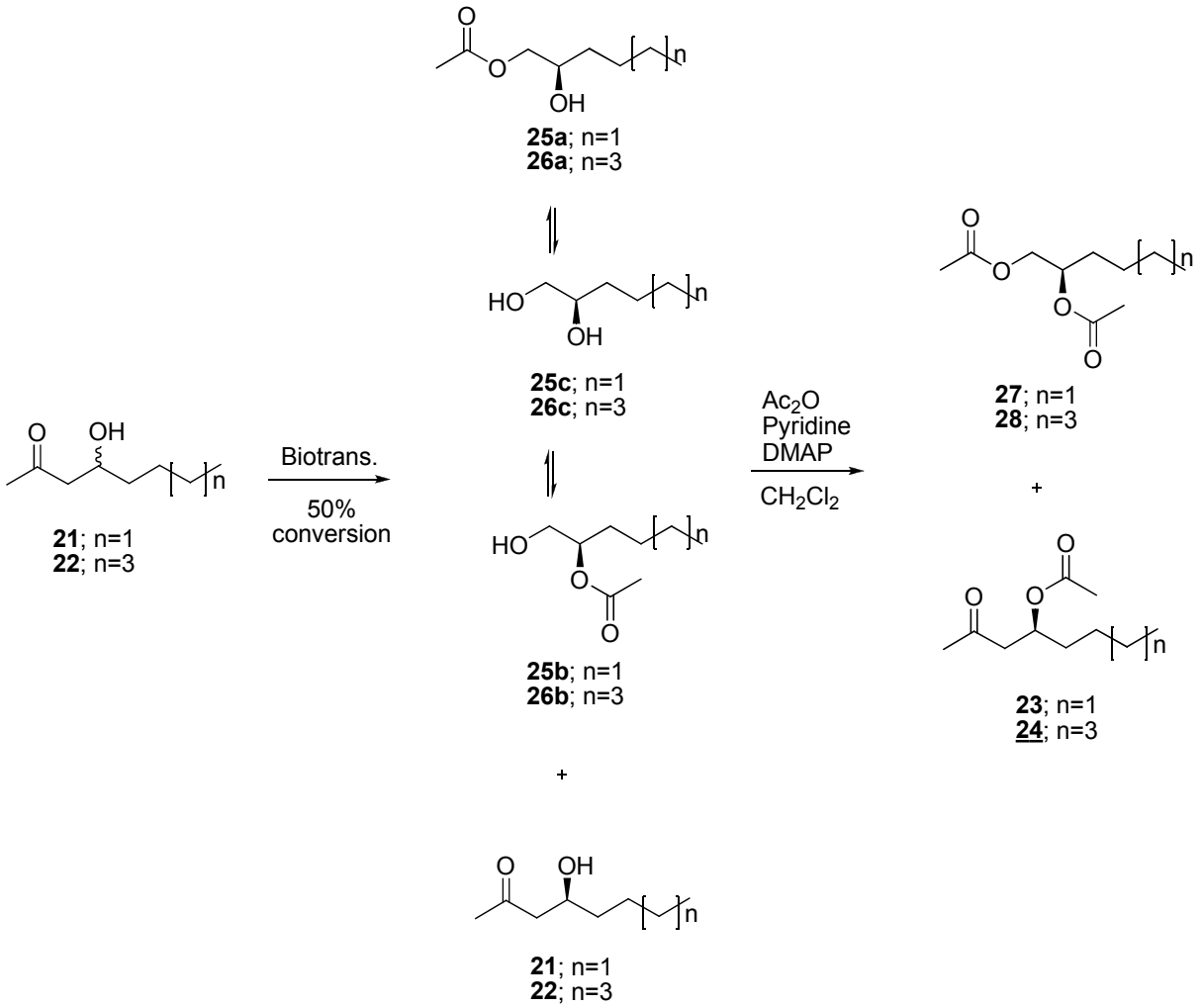
From the time-screening results, depicted in Figure 9 and Figure 10, the time course of the strains $CHMO_{Acinteo}$, $CHMO_{Rhodo1}$, $CHMO_{Brachy}$, $CHMO_{Xantho}$, and $BVMO_{Pseudo}$ looks

promising, since they show a bend in the curve after the conversion has reached ca. 50%. This allows the assumption, that after one enantiomer was fully consumed, the oxidation of the second enantiomer proceeds slower. On the other hand $CPMO_{Coma}$ and $CHMO_{Brevi2}$ seem to be quite unselective. To verify this assumption, in a later experiment the reaction was stopped at 50% conversion and the *ee* of the product and remaining starting material was determined (see p. 110).

3.7.3.3 Kinetic Resolution with Subsequent Acetylation

After having the time course of substrates **21** and **22**, the same experiments were preformed in preparative scale (12 mg substrate **21** or **22** in 50 mL LB_{amp}) and the reaction was stopped as the conversion was close to 50% (monitored by GC/MS).

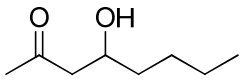
The reaction mixture was centrifuged, the product and remaining substrate were extracted with ethyl acetate, the solvent was evaporated and the acetylation was conducted without further purification: the crude reaction mixture was dissolved in dichloromethane at 0°C, acetic anhydride, pyridine and DMAP were added and the solution was stirred overnight at room temperature. After dilution with ether, quenching with brine and extraction, the ratio of the acetylated substrate and product and the *ee* of both were determined by measuring the sample on chiral phase GC-column (Scheme 73).



Scheme 73

***Biooxidation and Acetylation of* 21**

The biooxidation and subsequent acetylation of **21** was carried out with 11 different strains of *E. coli* (Scheme 73, Table 51, Figure 11).



21

Table 51

	Strain	Conversion1 GC/MS [%]*	Conversion2 measured/calc. [%]**		*ee* Prod	*ee* SM	E	Reaction time (h:min)***
1	$CHMO_{Acineto}$	46	35	31	93(S)	41(R)	41	4:50
2	$CPMO_{Coma}$	60	61	34	57(S)	30(R)	4.9	2:35
3	CDMO	65	66	34	37(S)	19(R)	2.6	6:50
4	$CHMO_{Brevi2}$	48	36	34	35(S)	18(R)	2.5	5:25
5	$CHMO_{Rhodo1}$	47	39	35	79(S)	43(R)	12	8:45
6	$CHMO_{Rhodo2}$	46	40	39	77(S)	49(R)	12	9:00
7	$CHMO_{Brachy}$	42	31	29	94(S)	38(R)	46	3:10
8	$CHMO_{Arthro}$	47	33	32	71(S)	33(R)	8.1	4:10
9	HAPMO	49	53	42	14(S)	10(R)	1.5	14:30
10	$BVMO_{Pseudo}$	20	21	19	96(S)	22(R)	60	32:30
11	$CHMO_{Xantho}$	58	54	51	84(S)	88(R)	33	5:20

*at this conversion the biooxidation was stopped

**conversion after acetylation 1) measured by GC; 2) calculated

***time of biooxidation until the corresponding conversion

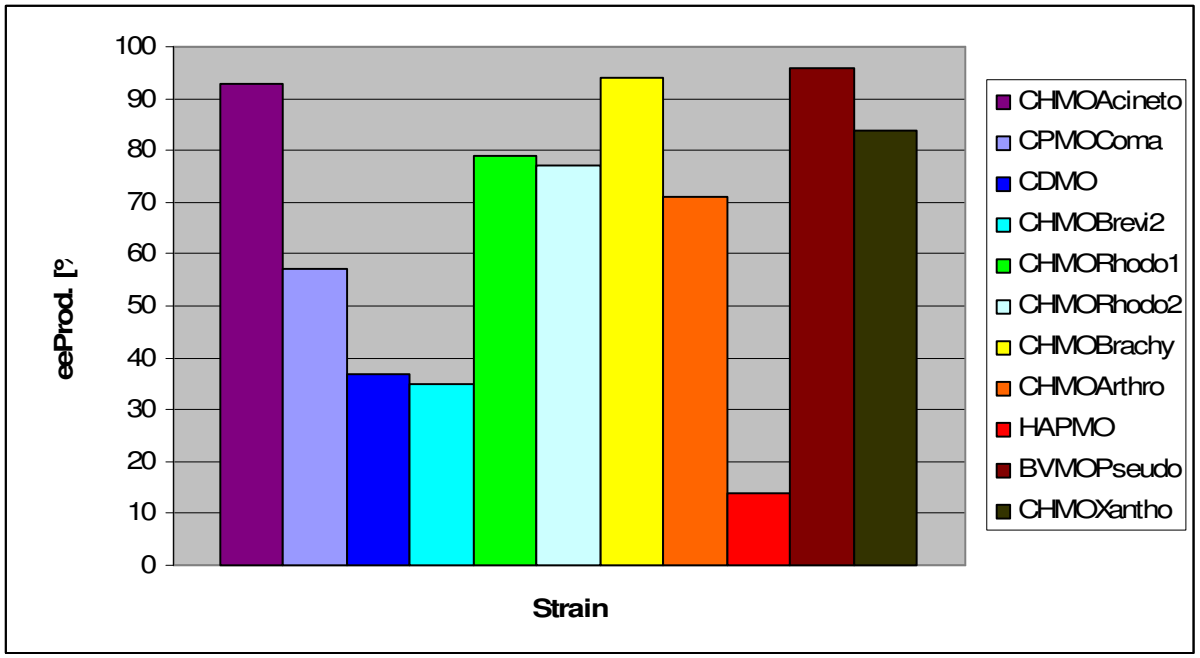


Figure 11

"Conversion 1" in Table 51 shows the conversion calculated from areas of product and remaining starting material in GC/MS at the time as the biooxidation was stopped.
"Conversion 2 measured" in Table 51 shows the ratio between product and starting material after acetylation and workup, determined by chiral phase GC from the ratio between the areas of product and starting material.
"Conversion 2 calculated" in Table 51 shows the conversion, fitted in the mathematic equation relating the conversion of the reaction with the enantiomeric excess of the remaining starting material and product (obtained by chiral phase GC), using the *Selectivity* software.[168] Enantiomeric Ratio E was calculated with the same software. E is a quantitative description for enantioselectivity of an enzyme which corresponds to the ratio of the relative second-order rate constants of the individual substrate enantiomers and remains constant throughout the reaction.[169] E should be higher than 30, if reasonable kinetic resolutions are expected in synthetic transformations.

In Table 51 "Conversion 1" is in all cases higher than "Conversion 2". One reason for it could be the loss of the product during workup (for example the product (ester) is better water soluble than the starting material (ketone)). Another reason could be that the difference in detector sensitivity between GC/MS and chiral phase GC or relative difference in sensitivity of both detectors between the not-acetylated starting material and product and the acetylated starting material and product.

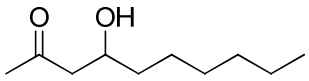
[168] Faber, K.; Hönig, H.; Kleewein, A., Free shareware programs (Selectivity-1.0) for calculation of the Enantiomeric Ratio, available from the author's website: http://borgc185.kfunigraz.ac.at./.
[169] a) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294; b) Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237; c) Gredig, G.; Fajans, K. *Chem. Ber.* **1908**, *41*, 752.

Since the *ee* values were determined by chiral phase GC and this data was used also for the calculation of E, we decided to rely on these values and regard the "Conversion 1" values only as a tool for monitoring the reaction course.
Except of that, the measured and the calculated "Conversion 2" values fit well in most of the cases.

The best product *ee*-s were obtained with $CHMO_{Acineto}$ (93%), $CHMO_{Brachy}$ (94%) and $BVMO_{Pseudo}$ (96%).
In all of these reactions the S-enantiomer was in excess. In contrast to these results, excess of the R-enanriomer was obtained, when the sequence of the reaction steps was inverted (first acetylation with subsequent biooxidation, see above). The allocation of the enantiomers was made by comparison of our results with published ones.[143]
In the entries 2 and 3, Table 51, the conversion was significantly higher than 50%, the reactions were proceeding quite fast. The theoretical optical purity of the resulting product is reduced, but at the same time the theoretical optical purity of the remaining starting material rises. In both cases the *ee* values of the remaining starting material were moderate, so we concluded that both reactions did not proceed with very high enantioselectivty and were therefore not repeated.

Biooxidation and Acetylation of 22

The biooxidation and subsequent acetylation of **22** was carried out with 11 different strains of *E. coli* (Scheme 73, Table 52, Figure 12).



22

Table 52

	Strain	Conversion1 GC/MS [%]*	Conversion2 measured/calcul. [%]**		*ee* Prod	*ee* SM	E	Reaction time (h:min)***
1	$CHMO_{Acineto}$	64	52	52	89(S)	96(R)	67	5:15
2	$CPMO_{Coma}$	59	62	53	25(S)	28(R)	2.1	1:45
3	CDMO	66	68	33	4(S)	2(R)	1.1	3:55
4	$CHMO_{Brevi2}$	51	35	29	35(S)	14(R)	2.4	2:45
5	$CHMO_{Rhodo1}$	40	31	34	79(S)	40(R)	12	10:00
6	$CHMO_{Rhodo2}$	46	41	40	75(S)	50(R)	11	12:00
7	$CHMO_{Brachy}$	8	6	6	97(S)	6(R)	69	2:40
8	$CHMO_{Arthro}$	42	24	25	86(S)	28(R)	17	7:30
9	HAPMO	56	37	35	99(S)	53(R)	>200	5:40
10	$BVMO_{Pseudo}$	46	38	36	99(S)	56(R)	>200	5:05
11	$CHMO_{Xantho}$	59	51	51	83(S)	85(R)	28	5:20

*at this conversion the biooxidation was stopped; see above.

**conversion after acetylation 1) measured by GC; 2) calculated; see above.

***time of biooxidation until the corresponding conversion

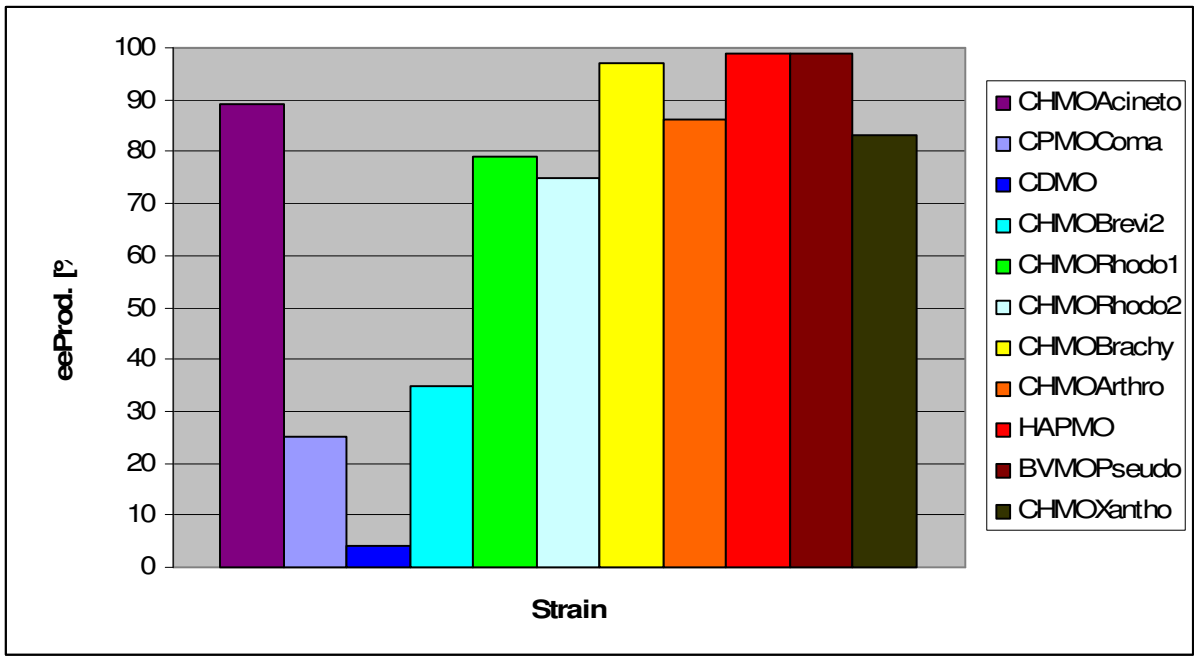
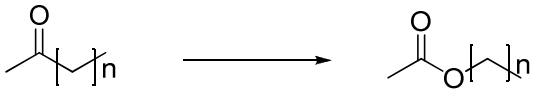


Figure 12

The best *ee* values were obtained with $CHMO_{Acineto}$ (89%), $CHMO_{Brachy}$ (97%), HAPMO (99%) and $BVMO_{Pseudo}$ (99%) which gave also reasonable E values (>30).

3.7.4 Screening of 2-Ketones

Screening with commercially available straight chain 2-ketones, 2-nonanone **50**, 2-decanone **51** and 2-undecanone **52** was carried out with 13 BVMO strains (Scheme 74). The ketones turned out to be too volatile to perform a normal screening experiment, so the screening was carried out with 10mg of substrate in 25mL of medium in tight closed shaking flasks (Table 53).



50; n=6
51; n=7
52; n=8

53; n=6
54; n=7
55; n=8

Scheme 74

Table 53

	Strain	Conversion [%] 		Conversion [%] 		Conversion [%] 
1	$CHMO_{Acineto}$	*n. c.*	14	*n. c.*	27	45
2	$CPMO_{Coma}$	*n. c.*	15	100	28	100
3	$CHMO_{Brevi1}$	*n. c.*	16	4	29	6
4	$CHMO_{Brevi2}$	*n. c.*	17	100	30	100
5	$CHMO_{Rhodo1}$	*n. c.*	18	98	31	100
6	$CHMO_{Rhodo2}$	*n. c.*	19	100	32	100
7	$CHMO_{Brachy}$	*n. c.*	20	100	33	100
8	$CHMO_{Arthro}$	*n. c.*	21	100	34	88
9	$CHMO_{Xantho}$	*n. c.*	22	16	35	99
10	HAPMO	*n. c.*	23	100	36	100
11	CDMO	*n. c.*	24	5	37	100
12	$BVMO_{Pseudo}$	*n. c.*	25	3	38	100
13	PAMO	*n. c.*	26	*n. c.*	39	5

No conversion could be observed with 2-nonanone **50**. With ketones **51** and **52** good conversions were observed, again $CHMO_{Acineto}$ and $CHMO_{Brevi1}$ gave low conversion in

contrast to the other DuPont strains, which gave in most cases very good conversions. PAMO gives very poor or no conversion in all experiments. Interesting are the results with $CHMO_{Xantho}$ which gives 99% conversion with 2-undecanone **52**, but only 16% with 2-decanone **51**. Similar results are given also by CDMO and $BVMO_{Pseudo}$ which give full conversion with 2-undecanone **52**, but only 5 or 3% respectively with 2-decanone **51**.

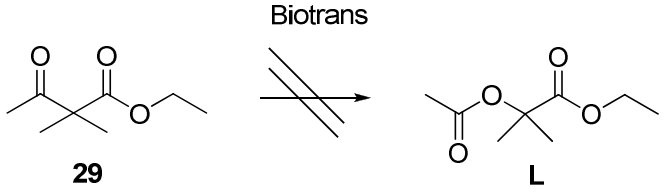
These experiments demonstrated, that often no predictions about the substrate profile of an enzyme can be made, since these three very similar substrates show partly a completely different behavior with the same overexpressing system.

Preparative Biotransformation of 2-Decanone and 2-Undecanone

In preparative scale the esters **57** and **58** could be prepared and analyzed by incubating the corresponding ketone with $CPMO_{Coma}$ for 21h (**54**; full conversion) or 24h (**55**; 94% conversion). After centrifugation, extraction and purification by *Kugelrohr* distillation 72% (**57**) yield or 69% yield (**58**) respectively could be obtained.

3.7.5 Screening with 29

With the available 13 *E. coli* strains screening of **29**[170] was performed (Scheme 75, Table 54). This compound represents a non-enolizable carbonyl compound; it had been shown in previous studies, that enolizable carbonyl groups are not converted by the enzymes of the available BVMO collection



Scheme 75

[170] Compound **29** was provided by Andras Kotschy, associate Professor, Institute of Chemistry, Eötvös Lorand Universtiy, Budapest, Hungary

Table 54

	Strain	Conversion [%]
1	$CHMO_{Acineto}$	7
2	$CPMO_{Coma}$	15
3	$CHMO_{Brevi1}$	8
4	$CHMO_{Brevi2}$	15
5	$CHMO_{Rhodo1}$	3
6	$CHMO_{Rhodo2}$	3
7	$CHMO_{Brachy}$	3
8	$CHMO_{Arthro}$	2
9	$CHMO_{Xantho}$	*n. c.*
10	HAPMO	*n. c.*
11	CDMO	5
12	$BVMO_{Pseudo}$	*n. c.*
13	PAMO	*n. c.*

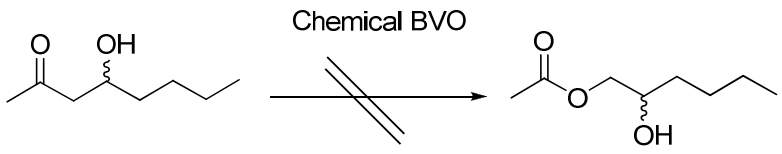
The best results could be observed with $CPMO_{Coma}$ and $CHMO_{Brevi2}$ which gave 15% conversion. No preparative experiments were carried out with this substrate due to the very moderate efficiency and the product **L** was not isolated.

3.7.6 Chemical Baeyer-Villiger Oxidation and Acetylation

Reference compounds were prepared by chemical Baeyer-Villiger oxidation.

3.7.6.1 Chemical Baeyer-Villiger oxidation of 21

All attempts to obtain a reasonable conversion and yield of the desired ester failed with compound **21** (Scheme 76, Table 55).



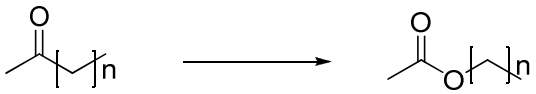
Scheme 76

Table 55

	React. Cond. [equiv.]	Solvent	Time, Temp.	Conversion [%]
1	m-CPBA [1.5]	CH_2Cl_2	21h, R. T.	*n. c.*
2	m-CPBA [3], K_2CO_3[cat.]	CH_2Cl_2	40h, R. T.	*n. c.*
3	m-CPBA [3], K_2CO_3[cat.]	$CHCl_3$	18h, reflux	traces
4	m-CPBA [3], K_2CO_3[cat.]	$CHCl_3$	2d, reflux	20 + decomposion
5	m-CPBA [3], $NaHCO_3$ [3]	CH_2Cl_2	Overnight., R. T	*n. c.*

3.7.6.2 Chemical Baeyer-Villiger oxidation of acyclic ketones 50 - 53

The acyclic ketone **50** gave in chemical Baeyer-Villiger reaction far lower conversion than **51** and **52** (Scheme 77, Table 56).

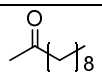


50; n=6
51; n=7
52; n=8

53; n=6
54; n=7
55; n=8

Scheme 77

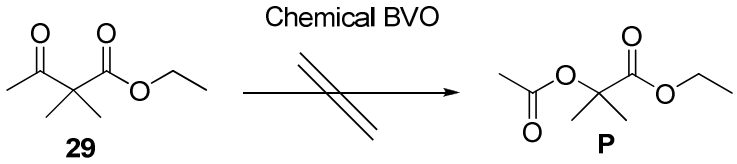
Table 56

	Substrate	React. Cond. [equiv.]	solvent	Time, Temp.	Conversion [%]
1	 **50**	m-CPBA [1.5]	CH_2Cl_2	21h, R. T.	10
2		m-CPBA [3], K_2CO_3[cat.]	CH_2Cl_2	40h, R. T.	24
3	 **51**	m-CPBA [3], K_2CO_3[cat.]	$CHCl_3$	2d, reflux	88
4		m-CPBA [2], $NaHCO_3$ [3]	CH_2Cl_2	18h, R. T.	12
5	 **52**	m-CPBA [3], K_2CO_3[cat.]	$CHCl_3$	93h, reflux	76

The obtained esters were not isolated, since the reference compounds were isolated and analyzed by microbial Baeyer-Villiger reaction before.

3.7.6.3 Chemical Baeyer-Villiger oxidation of (29)

Just as with biotransformations, the adequate 2-acetoxy-2-methyl-propionic acid ethyl ester could not be prepared by chemical Baeyer-Villiger reaction either (Scheme 78, Table 57), only starting material could be recovered.



Scheme 78

Table 57

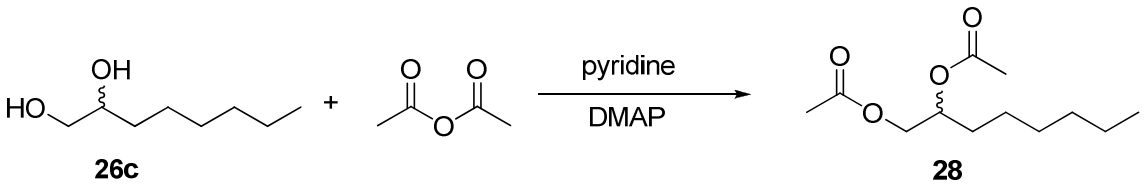
	React. Cond. [equiv.]	Solvent	Time, Temp.	Conversion
1	m-CPBA [2], $NaHCO_3$ [3]	CH_2Cl_2	2d, R. T.	*n. c.*
2	m-CPBA [2]	CH_2Cl_2	4d, R. T.	*n. c.*

3.7.6.4 Acetylation of Substrates 26c, 26a+b, 21 and 22

The preparative scale preparation of the substrates **28**, **23** and **24** was uncomplicated and gave full conversion and moderate to excellent yields in all cases. The non-acylated substrates **26c**, **26a+b**, **21** and **22** were dissolved in a minimum amount of dichloromethane, three equivalents of acetic anhydride, 10 equivalents of pyridine and a catalytic amount of DMAP were added per free hydroxyl-group. All reactions were started at 0°C and then carried out at room temperature overnight.

***Acetylation of* 26c**

To obtain a reference acetylated product **28**, the reaction was performed with the "deprotected" product of preparative microbial Baeyer-Villiger oxidation of **26c** with $CPMO_{Coma}$ after separation of the other reaction products **26a** and **26b** by flash column chromatography (Scheme 79, Table 58). Full conversion and quantitative yield could be achieved.



26c **28**

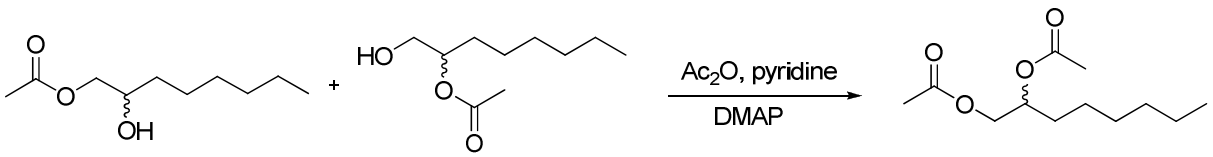
Scheme 79

Table 58

	React. Cond. [equiv.]	Solvent	Time,Temp	Conversion [%]	Yield[%]
1	Ac_2O [6] Pyridine [20], DMAP [cat.]	CH_2Cl_2	0°C / R. T., overnight	100	100

***Acetylation of* 26a+b**

The reaction was performed with the products of preparative microbial Baeyer-Villiger oxidation of **22** with $CPMO_{Coma}$ after separation of the product **26c** from **26a** and **26b** by flash column chromatography. The two different substrates were not separated since they gave the same product **28** after the acetylation in 85% yield (Scheme 80, Table 59).



26a **26b** **28**

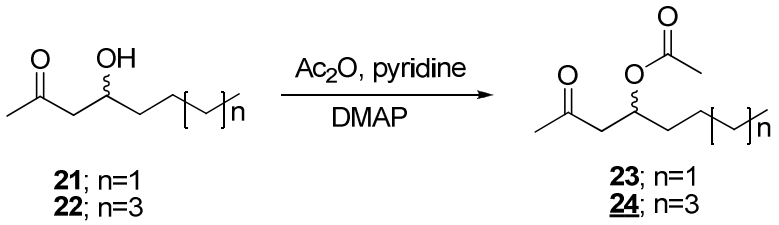
Scheme 80

Table 59

	React. Cond. [equiv.]	Solvent	Time, Temp.	Conversion [%]	Yield[%]
1	Ac_2O [3] Pyridine [10], DMAP [cat.]	CH_2Cl_2	0°C / R. T., overnight	100	85

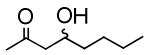
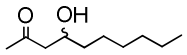
***Acetylation of* 21 *and* 22**

The reactions were performed with the substrates **21** or **22** before the biotransformation. The obtained acetylated products **23** (66% yield) and **24** (59% yield) were used for biotransformations with several BVMO strains (see above) (Scheme 81,Table 60).



Scheme 81

Table 60

	Substrate	React. Cond. [equiv.]	Solvent	Time, Temp.	Conversion [%]	Yield[%]
1	 **21**	Ac_2O [3] Pyridine [10], DMAP [cat.]	CH_2Cl_2	0°C / R. T., overnight	100	66
2	 **22**	Ac_2O [3] Pyridine [10], DMAP [cat.]	CH_2Cl_2	0°C / R. T., overnight	100	59

All attempts to synthesize the substrates **23** and **24** by chemical Baeyer-Villiger oxidation, failed.

4 EXPERIMENTAL PART

4.1 General

Unless otherwise noted, chemicals and microbial growth media were purchased from commercial suppliers and used without further purification. All solvents were distilled prior to use.

Flash column chromatography was performed on silica gel 60 from Merck (40-63 μm).
Melting points were determined using a Kofler-type Leica Galen III micro hot stage microscope and are uncorrected. Sonochemical transformations have been performed in a Bandelin Sonorex super RK102H ultrasound bath or utilizing a Bandelin Sonoplus HD3200 sonificator.
Elemental analyses were carried out in the Microanalytical Laboratory, Institute of Chemistry, University of Vienna.
NMR-spectra were recorded from $CDCl_3$ solutions on a Bruker AC 200 (200 MHz) or Bruker Advance UltraShield 400 (400 MHz) spectrometer and chemical shifts are reported in ppm using TMS as internal standard. Peak assignment is based on correlation experiments.
Specific rotation $[\alpha]_D^{20}$ was determined using Perkin Elmer Polarimeter 241 by the following equation: $[\alpha]_D^{20} = 100^* \alpha/(c^*1)$; c [e/mL], 1[dm]
Centrifugations for removing of cell debris (screening) were realized on (bench) centrifuge: Sigma 1-13, Sigma 3K30 (rotor 19777), Sigma 6K15.
Bacterial cultures were incubated in shakers (thermoshake, Gerhardt) or incubator (Heidolph Titramax 1000).
Enantiomeric excess was determined *via* GC using a BGB 175 column (30 m x 0.25 mm ID, 0.25 μm film) on ThermoQuest Trace GC 2000 with FID detector (240 °C) or BGB 173 column (30 m x 0.25 mm ID, 0.25 μm film) on Thermo Finnigan Focus GC with FID detector (240 °C). General conversion control and examination of purified products were performed with a standard capillary column DB5 (30 m x 0.32 mm ID, 1.0 μm film) on GC/MS Voyager 8000 Top.

Dip reagent for TLC: 13.2 g conc. sulfuric acid
0.8 g cerium (IV)- ammonium nitrate
10.0 g phosphor molybdate
150 mL ethanol

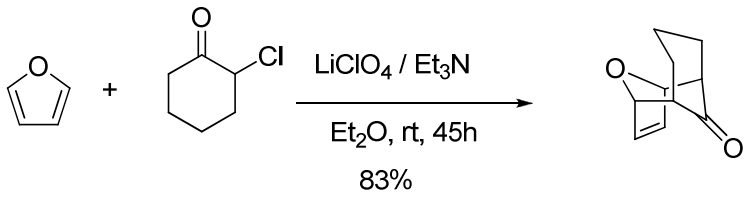
Abbreviations:

AcOH.....acetic acid
b.p……..boiling point
BVMO.....aeyer-Villiger monooxygenase
BVO.....Baeyer-Villiger oxidation
DCA……. 1,2-dichloroacetone
ee.....enantiomeric excess
Et_2O.....diethylether
EtOH.....ethanol
GC.....gas chromatography
h.....hours
IPTG ……isopropyl β-D-1-thiogalactopyranoside
L-Ara …….....L-arabinose
mCPBA.....*meta*-chloroperbenzoic acid
MeOH.....methanol
min.....minute
m.p.....melting point
n. c. .....no conversion
n. d. .....not determined
OD_{600} .....optical density at 600 nm
Prod. .....product
RCM.....ring closing metathesis
ROCM……..… ring opening cross metathesis
R.T.....room temperature
SM .....starting material
TBA …… tetrabromoacetone
Temp..... .....temperature
TFE……. .trifluoroethanol
THF.....tetrahydrofuran
TLC.....thin layer chromatography
US …….....ultrasound

4.2 Synthetic Part

4.2.1 Oxotricyclic Ketones

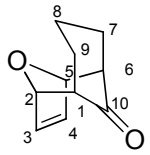
4.2.1.1 *anti*-11-Oxatricyclo[4.3.1.1^{2,5}]undec-3-en-10-one [1]



$LiClO_4$, (4.26, 40 mmol)
Furan 54 mL
Triethylamine (4.05g, 40 mmol)
Diethylether (36 mL)
2-Chlorocyclohexanone (2.65 g, 20 mmol)

Procedure: To a suspension of lithium perchlorate (4.26g, 40 mmol) in diethylether (36 mL), triethylamine (4.05 g, 40mmol) and furan (54 mL) were added subsequently under stirring at room temperature. 2-Chlorocyclohexanone (2.65 g, 20 mmol) was dissolved in 10mL of a furan/ether mixture (6:4 /v:v) and slowly added to the reaction mixture *via* dropping funnel. After stirring for 45h at room temperature, all 2-chlorocyclohexanone was consumed (GC/MS control) and the mixture was quenched with water (200 mL). The aqueous phase was separated and extracted with ether (5x 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate and the solvent and excess of furan were removed in *vacuo*. Product **1** was obtained as yellow solid in 95 % purity (area GC/MS).
Column chromatography on silicagel (saturated with triethylamine) was carried out with eluent hexane/ethyl acetate 4:1 and the product was obtained as colorless crystals. The *anti* isomer was obtained exclusively.

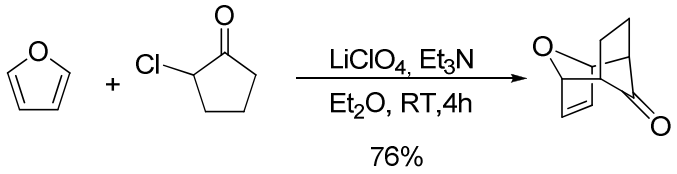
Yield: 2.71g (83%) (lit.[81] 81%), colorless crystals
MW: 164.20g/mol; $C_{10}H_{12}O_2$
m.p.: 42-45°C (lit.[81] 45-47°C)



^{1}H-NMR ($CDCl_3$): 1.45-1.55 (m, 1H, $H8_a$), 1.96-2.15 (m, 2H, H1/H6), 2.22-2.33 (m, 4H, H7/H9), 2.49-2.68 (m, 1H, $H8_b$), 4.93 (d, J=1.5 Hz, 2H, H2/H5), 6.35 (s, 2H, H3/H4)

^{13}C-NMR ($CDCl_3$): 20.8 (t, C8), 31.0 (t, C7/C9), 53.1 (d, C1/C6), 83.5 (d, C2/C5), 135.4 (d, C3/C4), 214.9 (s, C10)

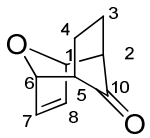
4.2.1.2 *anti*-9-Oxatricyclo[4.2.1.1^{2,5}]dec-7-en-10-one [6]



$LiClO_4$, (2.13 g, 20 mmol)
Furan 27 mL
Triethylamine (2.03 g, 20 mmol)
Diethylether (18 mL)
2-Chlorocyclopentanone (1.19 g, 10 mmol)

Procedure: To a suspension of lithium perchlorate (2.13 g, 20 mmol) in diethyl ether (18 mL), subsequently triethylamine (2.03 g, 20mmol) and furan (27 mL) were added under stirring at room temperature. 2-Chlorocyclopentanone (1.19g, 10mmol) was dissolved in 5mL of a furan/ether mixture (6:4 /v:v) and slowly added to the reaction mixture *via* dropping funnel. After stirring 4h at room temperature, all 2-chlorocyclopentanone was consumed (GC/MS control) and the mixture was quenched with water (100 mL). The aqueous phase was separated and extracted with ether (5x 25 mL). The combined organic layers were washed with brine, dried over sodium sulfate and the solvent and excess of furan were removed in *vacuo*. Product **6** was purified by column chromatography on silicagel, saturated with triethylamine, with eluent hexane/ethyl acetate 3:1 and obtained as colorless crystals. The *anti* isomer was obtained exclusively.

Yield: 1.32g (76%) (lit.[81]64%), colorless crystals
MW: 150.17g/mol; $C_9H_{10}O_2$
m.p.: 93-94°C (lit.[120] 92-94°C)



^{1}H-NMR ($CDCl_3$): 1.72-2.01 (m, 4H, H3/H4), 2.36-2.41 (m, 2H, H2/H5), 4.73 (d, J=3.7Hz, 2H, H1/H6,), 6.33 (s, 2H, H7/H8)
^{13}C-NMR ($CDCl_3$): 21.2 (t, C3/C4), 51.1 (d, C2/C5), 82.8 (d, C1/C6), 132.8 (d, C7/C8), 210.1 (s, C10)

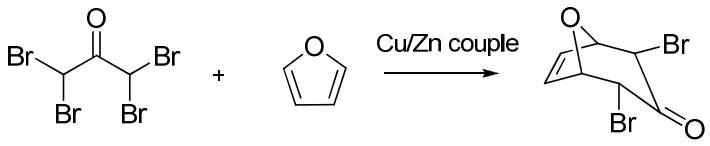
4.2.1.3 Copper-Zinc Couple [123, 171]

Copper acetate monohydrate ($Cu(CH_3COO)_2 \cdot H_2O$), (7.26 g, 0.036 mol)
Zinc powder (180 g, 2.75 mol)
Glacial acetic acid (300 mL)

Procedure: Copper acetate monohydrate (7.26 g, 0.036 mol) was dissolved in 300 mL of glacial acetic acid and heated to reflux under vigorous stirring (KPG stirrer). Zinc (180 g, 2.75 mol) was added slowly and the mixture was refluxed for 5-10 minutes. After cooling to room temperature using an ice bath, the precipitate was collected by filtration and washed with diethylether until acetic acid was removed and dried in *vacuo*.

[171] LeGoff, E., *J. Org. Chem*. **1964**, *29*, 2048.

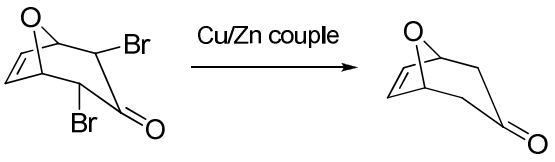
4.2.1.4 Dibromo-8-Oxabicyclo [3.2.1]oct-6-en-3-one [19a]



Tetrabromoacetone (6 g, 0.101 mol)
Furan (100 mL, 1.38 mol)
Copper-zinc-couple (20.6 g, 320 mmol, 3 equiv.)
Dibromoethane (few drops)
Dry acetonitrile (130 mL)

Procedure: A dispersion of copper-zinc-couple (20.6 g, 100.6 mmol) was prepared under N_2 atmosphere in dry acetonitrile (130 mL) and furan (100 mL, 1.38 mol) with a few drops of dibromoethane. Slowly a solution of tetrabromoacetone (6 g, 0.1006 mol) in 50 mL of acetonitrile was added over 35 min., while the solution was sonicated. The temperature was kept around 25°C and was not allowed to rise above 31°C (an ice bath was used if necessary). After all tetrabromoacetone was added, the dispersion was sonicated for 10 min more, and full conversion was determined (GC/MS control). The crude reaction mixture was used in the next step without further purification.

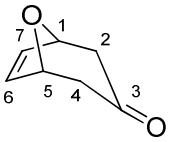
4.2.1.5 Debromination to 8-Oxabicyclo [3.2.1]oct-6-en-3-one [19]



Ammonium chloride (26 g, 0.486 mol)
Copper-zinc-couple (75 g, 1.147 mol)
Ethanol (300 mL)
Acetonitrile (230 mL)

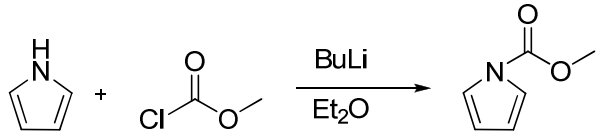
Procedure: In a three neck flask a mixture of ammonium chloride (26 g, 0.486 mol) and copper-zinc couple in 300 mL of ethanol was prepared and cooled to -78°C. Approximately 20% of the crude reaction mixture of **19a**, dissolved in 230 mL of acetonitrile was added to the flask and this mixture was stirred for 15 min. at -78°C. Then, the MeOH/N_2 bath was removed and the rest of the crude reaction mixture was added slowly through a dropping funnel and the temperature was allowed to rise to -10°C. The reaction mixture was stirred for 1h at -10°C to 0°C (ice bath). After full conversion was determined (GC/MS control), the reaction mixture was filtered through a pad of celite and a saturated solution of sodium bicarbonate was added to the solution until pH ≈7 was reached. The reaction mixture was filtered again, the organic layers were separated and the water phase was extracted with dichloromethane. The combined organic layers were dried ($NaSO_4$) and the solvents were removed in *vacuo*.

Yield: 6.98 g, 56% over two steps, brown oil (lit.[122] 60%)
MW: 124.14 g/mol; $C_7H_8O_2$



^{1}H-NMR ($CDCl_3$): 2.35 (d, J=16.4 Hz, 2H, $H2_a/H4_a$), 2.79 (dd, J=5.1 Hz, 16.9 Hz, 2H, $H2_b/H4_b$), 5.03 (d, J= 5.0 Hz, 2H, H1/H5,), 6.25 (s, 2H, H6/H7)
^{13}C-NMR ($CDCl_3$): 46.5 (t, C2/C4), 77.1 (d, C1/C5), 133.3 (d, C6/C7), 205.3 (s, C3)

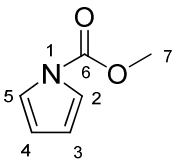
4.2.1.6 Pyrrole-1-carboxylic Acid Methyl Ester [20]



Pyrrole, (2.23 g, 33.3 mmol)
Dry diethylether (40 mL)
n-Buthyllithium, 2.15 M/hexane (15.5 mL, 33.3 mmol, 1 equiv.)
Methyl chloroformate $ClCOOCH_3$ (3.15 g, 33.3 mmol, 1 equiv.)

n-Buthyllithium, 2.15 M/hexane (15.5 mL, 33.3 mmol, 1 equiv.) was added slowly under stirring to a solution of pyrrole (2.23 g, 33.3 mmol) in 40 mL dry diethylether under N_2 atmosphere at -40°C. The mixture was warmed slowly to 0°C and methyl chloroformate (3.15 g, 33.3 mmol, 1 equiv.) dissolved in 10 mL of dry diethylether was added. The suspension was refluxed for 3h. The lithium salts were filtered and the filtrate was washed with saturated $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 filtered and the solvent removed in *vacuo*. The product was purified by distillation and obtained as colorless oil.

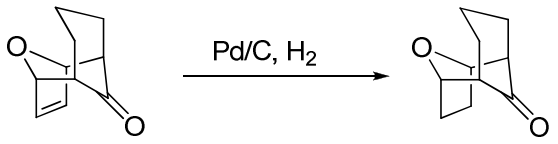
Yield: 2.92 g, 70%, colorless oil (lit.[125] 48%)
MW: 125.13 g/mol; $C_6H_7NO_2$



^{1}H-NMR ($CDCl_3$): 3.95 (s, 3H, H7), 6.24 (t, J= 2.3 Hz, 2H, H3/H4), 7.26 (t, J= 2.3 Hz, 2H, H2/H5)

^{13}C-NMR ($CDCl_3$): 55.4 (q, C7), 111.9 (d, C3/C4), 119.5 (d, C2/C5), 150.5 (s, C6)

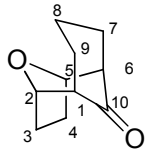
4.2.1.7 *anti*-11-Oxatricyclo[4.3.1.1^{2,5}]undec-10-one [2]



Substrate **1** (0.5 g, 3.05 mmol)
Pd/C (10%) (162 mg, 0.153 mmol Pd) 5 mol%)
Ethyl acetate (60 mL)
H_2 under pressure

Procedure: Palladium on charcoal (324 mg, 5 mol% Pd) was added to a solution of substrate **1** (0.5 g, 3.05 mmol) in ethyl acetate (60 mL). The dispersion was shaken in a Parr apparatus under hydrogen atmosphere (approximately 50 PSI). After 2h full conversion was determined by GC/MS. The reaction mixture was filtered through celite to remove the solids and the solvent was removed in *vacuo*. The crude product was purified by chromatography on silicagel (saturated with triethylamine) with eluent hexane/ethyl acetate 3:1, and product **2** as obtained as colorless oil (in the freezer colorless crystals) in excellent yield. As starting material was the *anti*-**1**, only the *anti*-**2** was obtained.[79]

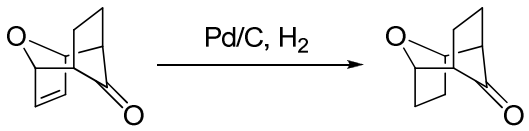
Yield: 495mg (98%), colorless oil
MW: 166.2g/mol; $C_{10}H_{14}O_2$



^{1}H-NMR ($CDCl_3$): 1.31-1.48 (m, 1H, $H8_a$), 1.61-1.93 (m, 4H, H3/H4) 1.99-2.12 (m, 2H, H1/H6), 2.20-2.36 (m, 4H, H7/H9) 2.67-2.43 (m, 1H, $H8_b$), 4.61 (m, 2H, H2/H5)

^{13}C-NMR ($CDCl_3$): 20.2 (t, C8), 27.9 (t, C7/C9), 33.3 (t, C3/C4), 55.4 (d, C1/C6), 81.8 (d, C2/C5), 217.0 (s, C10)

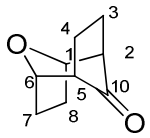
4.2.1.8 *anti*-9-Oxatricyclo[4.2.1.1^{2,5}]dec-10-one [7]



Substrate **6** (0.4 g, 2.66 mmol)
Pd/C (5%) (254 mg, 0.166 mmol, Pd 5 mol%)
Ethyl acetate (60 mL)
H_2 under pressure

Procedure: Palladium on charcoal (254 mg, 5 mol% Pd) was added to a solution of substrate **6** (0.4 g, 2.66 mmol) in ethyl acetate (60 mL). The dispersion was shaken in a Parr apparatus under hydrogen atmosphere (approximately 50 PSI). After 2.3h full conversion was determined by GC/MS. The reaction mixture was filtered through celite to remove the solids and the solvent was removed in *vacuo*. The crude product **7** was obtained as yellow oil in 100% purity (GC/MS area), in excellent yield, so no further purification was necessary. As starting material was the *anti*-**6**, only the *anti*-**7** was obtained.[92a]

Yield: 389mg (96%), yellow oil
MW: 152.17g/mol; $C_9H_{12}O_2$

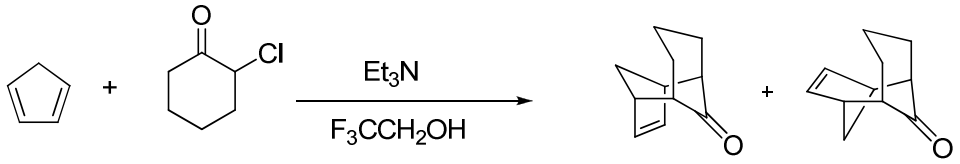


^{1}H-NMR ($CDCl_3$): 1.63-1.72 (m, 2H, $H3_a$/$H4_a$), 1.86-1.98 (m, 4H, H7/H8), 2.01-2.09 (q, J =7.3 Hz, 2H, H2/H5,), 2.15-2.20 (m, 2H, $H3_b$/$H4_b$), 4.38 (q, J=2.4 Hz 2H, H1/H6)

^{13}C-NMR ($CDCl_3$): 21.0 (t, C3/C4), 28.3 (t, C7/C8), 52.6 (d, C2/C5), 82.3 (d, C1/C6), 213.4 (s, C10)

4.2.2 Carbotricyclic ketones

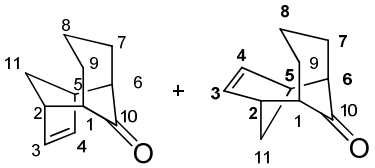
4.2.2.1 *anti-* and *syn*-Tricyclo[4.3.1.1^{2,5}]undec-3-en-10-one [13]



Triethylamine (3.03 g, 30 mmol)
Cyclopentadiene (5.00 g, 75 mmol, 5 equiv.)
2,2,2-Trifluoroethanol (225 mL)
2-Chlorocyclohexanone (1.98 g, 15 mmol)

Procedure: Triethylamine (3.03 g, 30 mmol, 2 equiv.) was added dropwise with vigorous stirring to a solution of 2-chlorocyclohexanone (1.98 g, 15 mmol) and 5 equiv. of freshly distilled cyclopentadiene (5.00 g, 75 mmol) in 2,2,2-trifluoroethanol (225 mL) at room temperature. After stirring 4 days at room temperature, all 2-chlorocyclohexanone was consumed (GC/MS control) and the mixture was quenched with water (250 mL). The aqueous phase was separated and extracted with ether (5x 60 mL). The combined organic layers were washed with brine, dried over sodium sulfate and the solvent removed in *vacuo*. The product **13** was obtained in two diasteromers **13*anti*** and **13*syn*** in ratio 88% to 12% and purified by column chromatography on silicagel with eluent hexane/ethyl acetate 20:1. Under these conditions the single diasteromers could be separated. (From 7.12 g of the crude product (yellow oil), 1.5 g (21%) were taken for separation of single diastereomers. 25 mg of pure **13*syn*** (5%) and 60 mg of pure **13*anti*** (12%) could be obtained). **13*anti*** and **13*syn*** were obtained as colorless crystals.

Yield (total)**: 2.02g (83%)** (lit.[116] 51%), colorless crystals
Yield (**13*syn*): 25 mg (5%)**
Yield (13*anti*): 60 mg (12%)
MW: 162.23 6/mol; $C_{11}H_{14}O$
m.p.: **13*anti*** : 83-85°C, **13*syn***: 222-228 °C (lit.[79] **13*anti*** : 86.1-86.8°C, **13*syn***: 228-230 °C)



13anti **13syn**

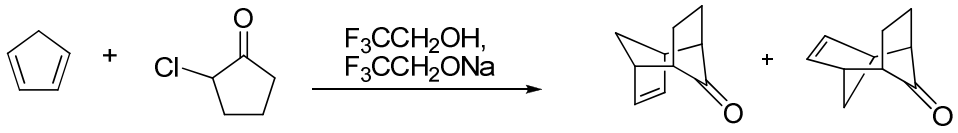
^{1}H-NMR **13*anti*** [79] ($CDCl_3$):1.46-1.87 (m, 3H, H8/H11$_b$), 2.00–2.12 (m, 4H, H7/H9), 2.38-2.42 (m, 2H, H1/H6), 2.71-2.77 (m, 3H, H2/H5/ H11$_a$), 6.10 (s, 2H, H3/H4)

^{1}H-NMR **13*syn*** [79] ($CDCl_3$):1.19-1.24 (m, 1H, H8$_a$, 1.50-1.70 (m, 2H, H11), 1.95-2.12 (m, 5H, H7/H9/H8$_b$), 2.57-2.61(m, 2H, H1/H6), 2.91-2.95 (m, 2H, H2/H5), 6.44-6.46 (m, 2H, H3/H4).

^{13}C-NMR **13*anti*** [116] ($CDCl_3$): 19.3 (t, C8), 29.0 (t, C7/C9), 34.9 (t, C11), 46.1 (d, C2/C5), 48.8 (d, C1/C6), 137.6 (d, C3/C4), 219.4 (s, C10)

^{13}C-NMR **13*syn*** ($CDCl_3$): 23.2 (t, C8), 31.7 (t, C7/C9), 34.2 (t, C11), 42.3 (d, C2/C5), 49.3 (d, C1/C6), 138.3 (d, C3/C4), 208.5(s, C10)

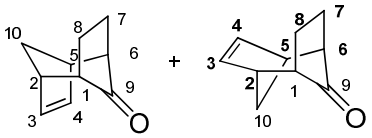
4.2.2.2 *anti*- and *syn*-Tricyclo[4.2.1.1^{2,5}] dec-3-en-9-one [9]



2-Chlorocyclopentanone (948 mg, 8.0 mmol)
Cyclopentadiene (6.40 g, 97 mmol, 12 equiv.)
2,2,2-Trifluoroethanol (4 mL)
Sodium 2,2,2-trifluoroethanolate (2M in 2,2,2-Trifluoroethanol, 4 mL)
Diethylether, 200 mL

Procedure: Under vigorous stirring 4 mL of 2M sodium 2,2,2-trifluoroethanolate in 2,2,2-trifluoroethanol were added drop wise to a solution of 2-chlorocyclopentanone (948 mg, 8.0 mmol) and 8 mL of freshly distilled cyclopentadiene (6.40 g, 97 mmol, 12 equiv.) in 2,2,2-trifluoroethanol (4 mL) cooled to 0°C. After stirring 25 min at 0°C all 2-chlorocyclopentanone was consumed (GC/MS control) and the mixture was diluted with diethylether (200 mL) to precipitate NaCl. The mixture was filtered through Celite and the solvent removed was in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 7:1. The product **9** was obtained in two diasteromers **9*anti*** and **9*syn*** in ratio 85% to 15% (lit[92a] ratio 77% to 23%) as colorless crystals. (The crude reaction mixture (1.72g, yellow oil) was put on a column of 110g silicagel, saturated with Et_3N and eluent PE/EE 7:1; no pressure was performed on the column. The first three fractions with product contained pure **9*syn*,** 121 mg, 10%. The following fractions contained a mixture of both diastereomers. The last seven fractions contained pure **9*anti***, 419 mg, 35%).

Yield (total)**: 1.138 g (96%)** (lit.[92a] 79%), colorless crystals
Yield (9*syn*): 121 mg (10%)
Yield (9*anti*): 419 mg (35%)
MW: 148.20g/mol; $C_{10}H_{12}O$
m.p.: not determined (mixture of isomers)(lit.[127] for the anti isomer 178-180°C)



9anti **9syn**

^{1}H-NMR ***9anti*** [127] ($CDCl_3$): 1.48 (dt, J=11 Hz, J=3.6Hz, 1H, $H10_b$), 1.61-1.84 (m, 4H, H7/H8), 1.99 (d, 1H, J=11 Hz, $H10_a$) 2.24-2.30 (m, 2H, H1/H6), 2.75-2.79 (m, 2H, H2/H5), 6.13 (s, 2H, H3/H4)

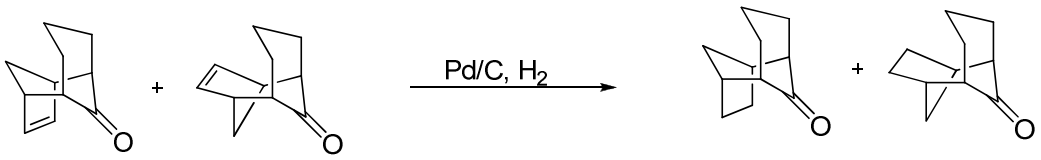
^{1}H-NMR ***9syn*** ($CDCl_3$): 1.36-1.58 (m, 6H, H7/H8/H10), 2.45-2.50 (m, 2H, H1/H6), 2.93-2.99 (m, 2H, H2/H5), 6.41 (s, 2H, H3/H4).

^{13}C-NMR ***9anti***[127] ($CDCl_3$): 22.7 (t, C7/C8), 37.0 (t, C10), 45.4 (d, C2/C5), 47.9 (d, C1/C6), 135.6 (d, C3/C4), 213.3 (s, C9)

^{13}C-NMR ***9syn*** ($CDCl_3$): 21.0 (t, C7/C8), 39.1 (t, C10), 42.7 (d, C2/C5), 48.9 (d, C1/C6), 138.4 (d, C3/C4), 217.6(s, C9)

For 2D NMR spectra of ***9syn*** see Appendix.

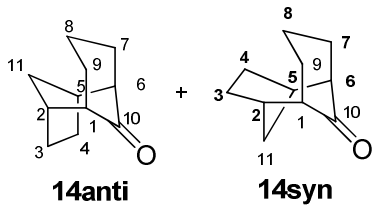
4.2.2.3 anti- and syn-Tricyclo[4.3.1.12,5]undecan-10-one [14]



Substrate **13*anti+syn*** (200 mg, 1.23 mmol)
Pd/C (10%) (132 mg, 0.062 mmol Pd, 5 mol%)
Ethyl acetate (60 mL)
H_2 under pressure

Procedure: Palladium on charcoal (132 mg, 5 mol% Pd) was added to a solution of substrate **13*anti+syn*** (87:13) (200 mg, 1.23 mmol) in ethyl acetate (60 mL). The suspension was shaken in a Parr apparatus under hydrogen atmosphere (approximately 50 PSI). After 2h full conversion was determined by GC/MS. The reaction mixture was filtered through Celite to remove the solids and the solvent was removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 5:1. Product **14** was obtained as a mixture of two diasteromers **14*anti*** and **14*syn*** in ratio 83% to 17% as colorless crystals in excellent yield.

Yield: 196mg (97%), (lit.[126] 93%), colorless crystals
MW: 164.24 g/mol; $C_{11}H_{16}O$
m.p.: not determined (mixture of isomers) (lit. **14*anti*** [126] 56-58°C)

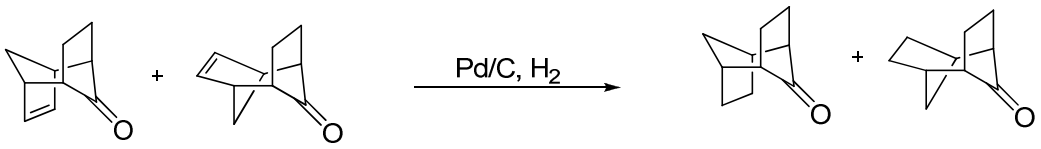


^{1}H-NMR **14*anti*** + **14*syn***[126] ($CDCl_3$):1.15-1.24 (m, 2H, $H8_a$/$H11_a$), 1.31-1.44 (m, 2H, $H3_a$/$H4_a$), 1.49-1.74 (m, 4H, H7/H9), 2.02-2.16 (m, 4H, $H3_b$/$H4_b$H/H2/H5), 2.27 (s, 1H, $H8_b$), 2.37 (s, 2H, H1/H6), 2.52-2.58 (m, 1H, $H11_b$)

^{13}C-NMR **14*anti***[126] ($CDCl_3$): 18.6 (t, C8), 26.9 (t, C7/C9), 29.0 (t, C11), 30.7 (t, C3/C4), 43.2 (d, C2/C5), 52.3 (d, C1/C6), 221.7 (s, C10)

^{13}C-NMR **14*syn*** ($CDCl_3$): 17.2 (t, C8), 26.6 (t, C7/C9), 32.1 (t, C3/C4), 38.0 (d, C2/C5), 38.4 (t, C11), 49.9 (d, C1/C6), 221.8 (s, C10)

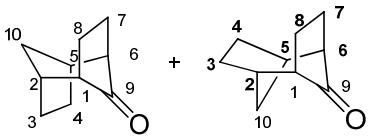
4.2.2.4 *anti*- and *syn*-Tricyclo[4.2.1.12,5]decan-9-one [10]



Substrate **9*anti* + *syn*** (200 mg, 1.35 mmol)
Pd/C (10%) (144 mg, 0.067 mmol Pd, 5 mol%)
Ethyl acetate (60 mL)
H_2 under pressure

Procedure: Palladium on charcoal (144 mg, 5 mol% Pd) was added to a solution of substrate **9*anti* + *syn*** (94:6) (200 mg, 1.35 mmol) in ethyl acetate (60 mL). The suspension was shaken in a Parr apparatus under hydrogen atmosphere (approximately 50 PSI). After 2h full conversion was determined by GC/MS. The reaction mixture was filtered through Celite to remove the solids and the solvent was removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 3:1. The product **10** was obtained as a mixture of two diasteromers **10*anti*** and **10*syn*** in ratio 81% to 19% as colorless crystals in excellent yield.

Yield: 189mg (93%), (lit. for the anti isomere: 98%)
MW: 150.22 g/mol; $C_{10}H_{14}O$
m.p.: not determined (mixture of isomers) (lit.[127] for **10*anti*** 120°C-125°C)



10anti **10syn**

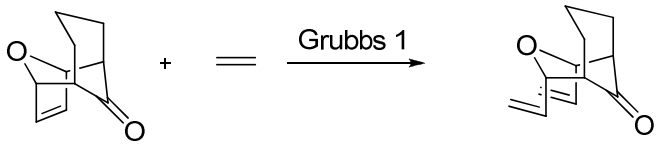
^{1}H-NMR **10*anti*** [127] + **syn** ($CDCl_3$): 1.20-2.00 (m, 10H, H3/H4/H7/H8/H10), 2.08-2.11 (m, 2H, (H2/H5), 2.32-2.34 (m, 2H, H1/H6)

^{13}C-NMR **10*anti*** [127] ($CDCl_3$): 22.2 (t, C7/C8), 28.1 (t, C3/C4), 30.2 (t, C10), 42.8 (d, C2/C5), 49.7 (d, C1/C6), 217.0 (s, C9)

^{13}C-NMR **10*syn*** ($CDCl_3$): 19.0 (t, C7/C8), 24.8 (t, C3/C4), 33.7 (t, C10), 37.8 (d, C2/C5), 44.8 (d, C1/C6), 217.5 (s, C9)

4.2.3 Olefin metathesis

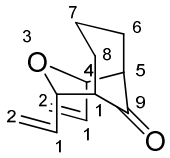
4.2.3.1 *anti*- 2,4-Divinyl-3-oxa-bicyclo[3.3.1]nonan-9-one [3]



Substrate **1** (100 mg, 0.61 mmol)
Ethylene (balloon)
Grubbs catalyst, 1st generation (5.0 mg, 1 mol%)
Dry dichloromethane (20 mL)
Ethylvinylether (5 mL)

Procedure: Ethylene-gas was bubbled through a solution of **1** (100 mg, 0.61 mmol) in 20 mL of dichloromethane at room temperature until the solution was saturated with ethylene. Then 1 mol% of the Grubbs catalyst, 1st generation (5.0 mg), dissolved in 2 mL of dichloromethane was added in one shot through a septum. The solution was kept under ethylene atmosphere and every 3 hours ethylene was bubbled through. The conversion was controlled by GC/MS. The conversion reached 84% after 23h and dropped to 81% after 28h. The reaction was stopped by adding 5 mL of ethylvinylether and stirring was continued for 30 more minutes. Silicagel (1 g) was added to the reaction mixture and the solvent was removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 5:1. The product **3** was obtained as orange oil.

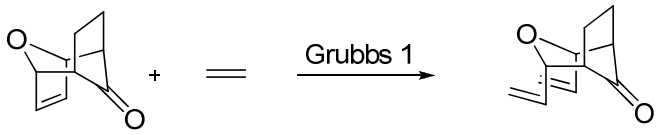
Yield: 72 mg (61%), orange oil
MW: 192.25g/mol; $C_{12}H_{16}O_2$



^{1}H-NMR ($CDCl_3$): 1.58-1.66 (m, 1H, $H7_b$), 1.91-2.19 (m, 4H, H6/H8), 2.32-2.48 (m, 1H, $H7_a$), 2.53 (d, J=2 Hz, 2H, H1/H5), 4.63 (d, J=6.7 Hz, 2H, H2/H4), 5.09-5.26 (m, 4H, vinyl H2), 5.84-6.01 (m, 2H, vinyl H1)

^{13}C-NMR ($CDCl_3$): 17.5 (t, C7), 34.0 (t, C6/C8), 51.9 (d, C1/C5), 82.6 (d, C2/C4), 115.93 (t, vinyl C2), 138.7 (d, vinyl C1), 213.4 (s, C9)

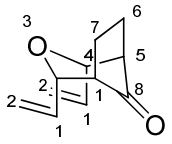
4.2.3.2 *anti*-2,4-Divinyl-3-oxa-bicyclo[3.2.1]octan-8-one [8]



Substrate **6** (100 mg, 0.66 mmol)
Ethylene (balloon)
Grubbs catalyst, 1st generation (16.3 mg, 3 mol%; 2+1)
Dry dichloromethane (20 mL)
Ethylvinylether (5 mL)

Procedure: Ethylene-gas was bubbled through a solution of **6** (100 mg, 0.66 mmol) in 20 mL of dichloromethane at room temperature until the solution was saturated with ethylene. Then 2 mol% of the Grubbs catalyst, 1st generation (11 mg), dissolved in 2 mL of dichloromethane were added in one shot through a septum and after 24h 5.3 mg (1 mol%), dissolved in 2 mL dichloromethane more were added (in total 3 mol%). The solution was kept under ethylene atmosphere and every 3 hours ethylene was bubbled through. The conversion was controlled by GC/MS. The conversion reached 92% after 48h. The reaction was stopped by adding 5 mL of ethylvinylether and stirring was continued for 30 more minutes. Silicagel (1g) was added to the reaction mixture and the solvent was removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 5:1. The product **8** was obtained as yellow-brown oil.

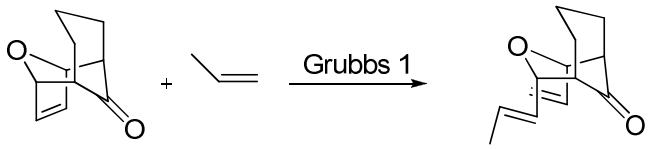
Yield: 81 mg (69%), yellow oil
MW: 178.23g/mol; $C_{11}H_{14}O_2$



^{1}H-NMR ($CDCl_3$): 2.00-2.18 (m, 4H, H6/H7), 2.35-2.39 (m, 2H, H1/H5), 4.56 (d, J=6.5 Hz, 2H, H2/H4), 5.12-5.28 (m, 4H, vinyl H2), 5.92-6.09 (m, 2H, vinyl H1)

^{13}C-NMR ($CDCl_3$): 22.4 (C6/C7), 49.61 (C1/C5), 88.5 (C2/C4), 117.6 (vinyl C2), 137.8 (vinyl C1), 213.7 (C8)

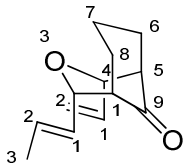
4.2.3.3 *E* and *Z anti*-2-Propenyl-4-vinyl-3oxa-bicyclo[3.3.1]nonan-9-one [4]



Substrate **1** (350 mg, 2.13 mmol)
Propene (balloon)
Grubbs catalyst, 1st generation (35.0 mg, 2 mol%)
Dry dichloromethane (80 mL)
Ethylvinylether (10 mL)

Procedure: Propene-gas was bubbled through a solution of **1** (350 mg, 2.13 mmol) in 80 mL of dichloromethane, at room temperature until the solution was saturated with propene. Then 2 mol% of the Grubbs catalyst, 1st generation (35.0 mg), dissolved in 2 mL of dichloromethane were added in one shot through a septum. The solution was kept under propene atmosphere (the gas was three times bubbled through the solution). The conversion was controlled by TLC. After 1.5h, most of the starting material was consumed and a strong new spot appeared. After 1.5h more the TLC showed still the same appearance and the reaction was stopped by adding 10 mL of ethylvinylether and stirring was continued for 30 more minutes. Silicagel (1 g) was added to the reaction mixture and the solvent was removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 8:1. The product **4** was obtained as colorless oil as a mixture of stereoisomers *Z* and *E* in ratio of ca. 10:90, where *E* is assumed to be the major product. Due to the small quantity of the *Z* isomer and overlaps, no ^{1}H-NMR spectral data are available.

Yield: 328 mg (77%), colorless oil
MW: 206.28g/mol; $C_{13}H_{18}O_2$

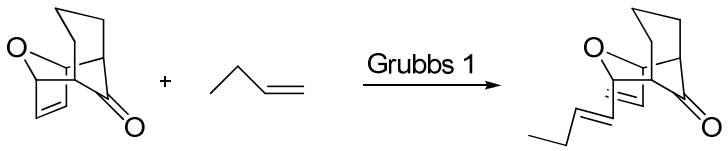


^{1}H-NMR **4** ($CDCl_3$): 1.56-1.71 (m, 1H, $H7_a$) 1.66 (d, J=5.5 Hz, 3H, propenyl H3), 1.92-2.15 (m, 4H, H6/H8), 2.31-2.40 (m, 1H, $H7_b$), 2.45-2.51 (m, 2H, H1/H5), 4.49-4.64 (m, 2H, H2/H4), 5.07-5.24 (m, 2H, vinyl H2), 5.48-5.73 (m, 2H, propenyl H1/H2), 5.82-5.99 (m, 1H, vinyl H1)

^{13}C-NMR **4** ***E*** ($CDCl_3$): 17.1 (t, C7), 17.3 (q, propenyl C3), 33.5 (t, C6), 33.6 (t, C8), 51.7 (d, C5), 52.3 (d, C1), 82.1 (d, C2), 82.3 (d, C4), 115.4 (t, vinyl C2), 127.7 (d, propenyl C2), 131.8 (d, propenyl C1), 138.7 (d, vinyl C1), 213.3 (s, C9)

^{13}C-NMR **4** ***Z*** ($CDCl_3$): 17.0 (t, C7), 33.4 (t, C6), 33.8 (t, C8), 51.6 (d, C5), 52.3 (d, C1), 53.2 (q, propenyl C3), 82.0 (d, C2), 82.4 (d, C4), 115.6 (t, vinyl C2), 127.6 (d, propenyl C2), 131.9 (d, propenyl C1), 138.6 (d, vinyl C1), 213.7 (s, C9)

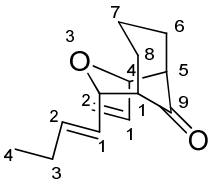
4.2.3.4 *E* and *Z anti*-2-(But-1-enyl)-4-vinyl-3oxa-bicyclo[3.3.1]nonan-9-one [5]



Substrate **1** (350 mg, 2.13 mmol)
1-Butene (balloon)
Grubbs catalyst, 1st generation (44.0 mg, 2.5 mol%)
Dry dichloromethane (80 mL)
Ethylvinylether (10 mL)

Procedure: Through a solution of **1** (350 mg, 2.13 mmol) in 80 mL of dichloromethane, at room temperature 1-butene-gas was bubbled until the solution was saturated with 1-butene. Then, 2.5 mol% of the Grubbs catalyst, 1st generation (44.0 mg), dissolved in 2 mL of dichloromethane were added in one shot through a septum. The solution was kept under 1-butene atmosphere (the gas was three times bubbled through the solution). The conversion was controlled by TLC. After 1.5h most of the starting material was consumed and a strong new spot appeared. After 1.5h more the TLC showed still the same appearance and the reaction was stopped by adding 10 mL of ethylvinylether and stirring was continued for 30 more minutes. Silicagel (1g) was added to the reaction mixture and the solvent was removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 8:1. The product **5** was obtained as colorless oil as a mixture of stereoisomers *Z* and *E* in ratio of ca. 5 : 95, where *E* is assumed to be the major product. Due to the small quantity of the *Z* isomer, no spectral data are available.

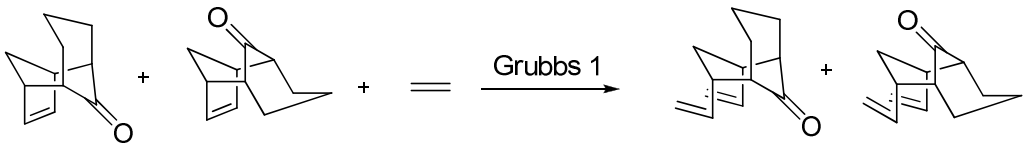
Yield: 398 mg (85%), colorless oil
MW: 220.31g/mol; $C_{14}H_{20}O_2$



^{1}H-NMR **5** ***E*** ($CDCl_3$): 0.90 (t, J=7.5 Hz, 3H, butenyl H4), 1.57-1.64 (m, 1H, $H7_a$), 1.94-2.15 (m, 6H, H6/H8/ butenyl H3), 2.29-2.41 (m, 1H, $H7_b$), 2.48-2.52 (m, 2H, H1/H5), 4.54-4.60 (m, 2H, H2/H4), 5.01-5.24 (m, 2H, vinyl H2), 5.45-5.77 (m, 2H, butenyl H1/butenyl H2), 5.82-5.99 (m, 1H, vinyl H1).

^{13}C-NMR **5** ***E*** ($CDCl_3$): 12.8 (q, butenyl C4), 17.3 (t, butenyl C3), 24.9 (t, C7), 33.7 (t, C8), 33.8 (t, C6), 51.8 (d, C5), 52.6 (d, C1), 82.3 (d, C2), 82.6 (d, C4), 115.6 (t, vinyl C2), 129.6 (d, butenyl C2), 134.5 (d, butenyl C1), 138.8 (d, vinyl C1), 213.6 (s, C9)

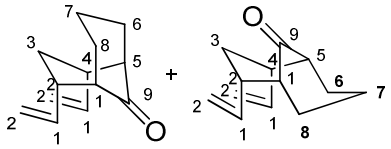
4.2.3.5 *anti*- and *syn*-2,4-Divinyl-bicyclo[3.3.1]nonan-9-one [15]



Substrate **13*anti* + 13*syn***, ratio 87%:13% (700 mg, 4.32 mmol)
Ethylene (balloon)
Grubbs catalyst, 1st generation (35.6 mg, 1 mol%)
Dry dichloromethane (120 mL)
Ethylvinylether (20 mL)

Procedure: Ethylene-gas was bubbled through a solution of **13*anti* + *syn*** (700 mg, 4.32 mmol) in 120 mL of dichloromethane, at room temperature until the solution was saturated with ethylene. Then 1 mol% of the Grubbs catalyst, 1st generation (35.6 mg), dissolved in 2 mL of dichloromethane was added in one shot through a septum. The solution was kept under ethylene atmosphere and every 3 hours ethylene was bubbled through. The conversion was controlled by GC/MS. Full conversion could be observed after 17h. The reaction was stopped by adding 20 mL of ethylvinylether and stirring was continued for 30 more minutes. Silicagel (1g) was added to the reaction mixture and the solvent was removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 15:1. The product **15*anti* + 15*syn***, ratio 91%:9%, was obtained as orange oil. Pure diastereomers of **15** could be prepared by starting the reaction from diastereomerically pure **13**, for **15*syn*** started from 57 mg **13*syn*** and for **15*anti*** started from 208 mg **13*anti***.

Yield: 607 mg (74%), orange oil
Yield (15*anti*): 184 mg (76%)
Yield (15*syn*): 31 mg (47%)
MW: 190.28g/mol; $C_{13}H_{18}O$



15anti **15syn**

^{1}H-NMR **15*anti*** ($CDCl_3$): 0.96-1.15 (m, 1H, $H7_b$), 1.48-1.59 (m, 1H, $H3_a$) 1.70-2.06 (m, 6H, $H3_b$/H6/H8/$H7_a$), 2.26 (s, 2H, H1/H5), 2.60-2.73 (m, 2H, H2/H4), 4.89-5.18 (m, 4H, vinyl H2), 5.65-5.94 (m, 2H, vinyl H1)

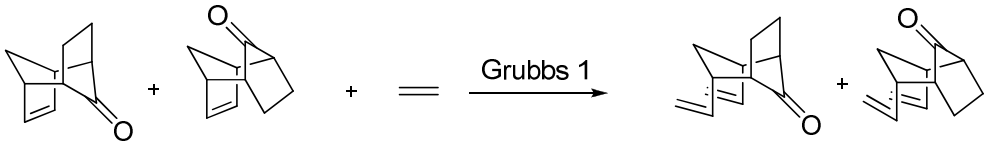
^{1}H-NMR **15*syn*** ($CDCl_3$): 0.99-1.18 (m, 1H, $H7_b$), 1.50-2.06 (m, 7H, H3/H6/H8/$H7_a$), 2.40 (s, 2H, H1/H5), 2.60-2.73 (m, 2H, H2/H4), 4.89-5.18 (m, 4H, vinyl H2), 5.65-5.94 (m, 2H, vinyl H1)

^{13}C-NMR **15*anti*** ($CDCl_3$): 16.4 (t, C7), 33.6 (t, C3), 34.4 (t, C6/C8), 44.9 (d, C2/C4), 50.9 (d, C1/C5), 112.9 (t, vinyl C2), 142.1 (d, vinyl C1), 218.9 (s, C9)

^{13}C-NMR **15*syn*** ($CDCl_3$): 20.7 (t, C7), 30.0 (t, C3), 28.2 (t, C6/C8), 45.0 (d, C2/C4), 50.2 (d, C1/C5), 115.0 (t, vinyl C2), 138.9 (d, vinyl C1), 219.0 (s, C9)

$C_{13}H_{18}O$: calcd.: C 82.06; H 9.53; found: C 81.97; H 9.47.

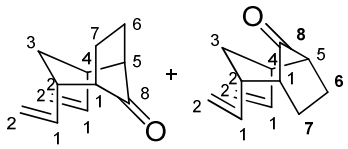
4.2.3.6 *anti*- and *syn*-2,4-Divinyl-bicyclo[3.2.1]octan-8-one [11]



Substrate **9*anti* + 9*syn***, ratio 82%:18% (1g, 6.76 mmol)
Ethylene (balloon)
Grubbs catalyst, 1st generation (56 mg, 1 mol%)
Dry dichloromethane (80 mL)
Ethylvinylether (50 mL)

Procedure: Ethylene-gas was bubbled through a solution of **9*anti* + *syn*** (1 g, 6.76 mmol) in 80 mL of dichloromethane, at room temperature until the solution was saturated with ethylene. Then 1 mol% of the Grubbs catalyst, 1st generation (56 mg), dissolved in 2 mL of dichloromethane was added in one shot through a septum. The solution was kept under ethylene atmosphere and every 3 hours ethylene was bubbled through. The conversion was monitored by TLC. After 48h the conversion did not increase anymore. The reaction was stopped by adding 50 mL of ethylvinylether and stirring was continued for 30 more minutes. Silicagel (1g) was added to the reaction mixture and the solvent was removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate 12:1. The product was obtained as **11*anti* + 11*syn***, ratio 80%:20% as colorless oil. The ratio of isomers was determined by the height of the peaks in C^{13}NMR, because of the overlap of peaks in GC/MS.
Pure diastereomers of **11** could be prepared by starting the reaction from diastereomerically pure **9**, for **11*syn*** started from 213 mg **9*syn*** and for **11*anti*** started from 300 mg **9*anti***.
If the reaction was started from pure **11*anti*** or pure **11*syn*** respectively, slightly higher yields were obtained.

Yield: 761 mg (64%), colorless oil
Yield (11*anti*): 76%
Yield (11*syn*): 176 mg (70%)
MW: 176.25g/mol; $C_{12}H_{16}O$



11anti **11syn**

^{1}H-NMR **11*anti*** ($CDCl_3$): 1.57 (d, J=14.6 Hz, 1H, $H3_a$), 1.82-1.86 (m, 2H, $H6_a/H7_a$), 2.01-2.08 (m, 2H, $H6_b/H7_b$), 2.24-2.27 (m, 2H, H1/H5), 2.33-2.47 (m, 1H, J=7.2 Hz, $H3_b$), 2.77-2.80(m, 2H, H2/H4), 4.91-5.07 (m, 4H, vinyl H2), 5.78-5.93 (m, 2H, vinyl H1)

^{1}H-NMR **11*syn*** ($CDCl_3$): 1.52-1.76 (m, 6H, H3/H6/H7), 2.21 (d, J=2.3 Hz, 2H, H1/H5), 2.70-2.76 (m, 2H, H2/H4), 5.01-5.09 (m, 4H, vinyl H2), 5.63-5.81 (m, 2H, vinyl H1)

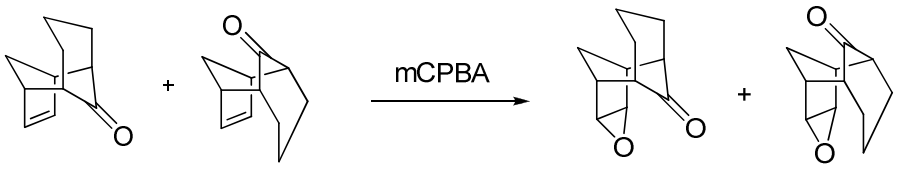
C^{13}-NMR **11*anti*** ($CDCl_3$): 22.9 (t, C6/C7), 28.8(t, C3), 48.5 (d, C1/C5), 51.6 (d, C2/C4), 114.9 (t, vinyl C2), 140.8 (d, vinyl C1), 218.8 (s, C8)

C^{13}-NMR **11*syn*** ($CDCl_3$): 18.5 (t, C6/C7), 27.7 (t, C3), 46.7 (d, C1/C5), 48.8 (d, C2/C4) 114.8 (t, vinyl C2), 138.9 (d, vinyl C1), 220.3 (s, C8)

For 2D NMR spectra of **11*anti*** see Appendix.

4.2.4 Chemical Baeyer-Villiger Oxidation

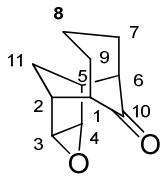
4.2.4.1 *anti-* and *syn-* Tetracyclo[4.2.1.1.1.^{2,5}]dodecan -3-oxa-10-one [**17**]



Substrate **13*anti* + 13*syn*,** ratio 94%:6% (30 mg, 0.203 mmol)
m-CPBA (70%) (125 mg, 0.50 mmol, 2.5 equiv.)
Dry dichloromethane (10 mL)
Triethylamine

Procedure: m-Chloroperbenzoic acid (m-CPBA) (125 mg, 0.50 mmol, 2.5 equiv.) was added to a solution of **13*anti* + *syn*** (30 mg, 0.203 mmol) in dry dichloromethane (10 mL) and stirred at room temperature. After 20 h full conversion was determined by GC/MS.
Excess of triethylamine was added and the mixture was stirred for 15 min. The mixture was quenched with water (10 mL). The aqueous phase was separated and extracted with dichloromethane (2x 15 mL). The combined organic layers were washed with brine, dried over sodium sulfate and the solvent was removed in *vacuo*. The product **17** was purified by column chromatography on silicagel, with eluent hexane/ethyl acetate 3:1 and obtained as colorless oil as a mixture of isomers *syn* and *anti* in ratio of ca. 30:70. Due to the small quantity of the sample (13 mg) and overlaps, no ^{13}C-NMR spectral data are available.

Yield: 13 mg (39%), colorless oil
MW: 164.08g/mol; $C_{10}H_{12}O_2$

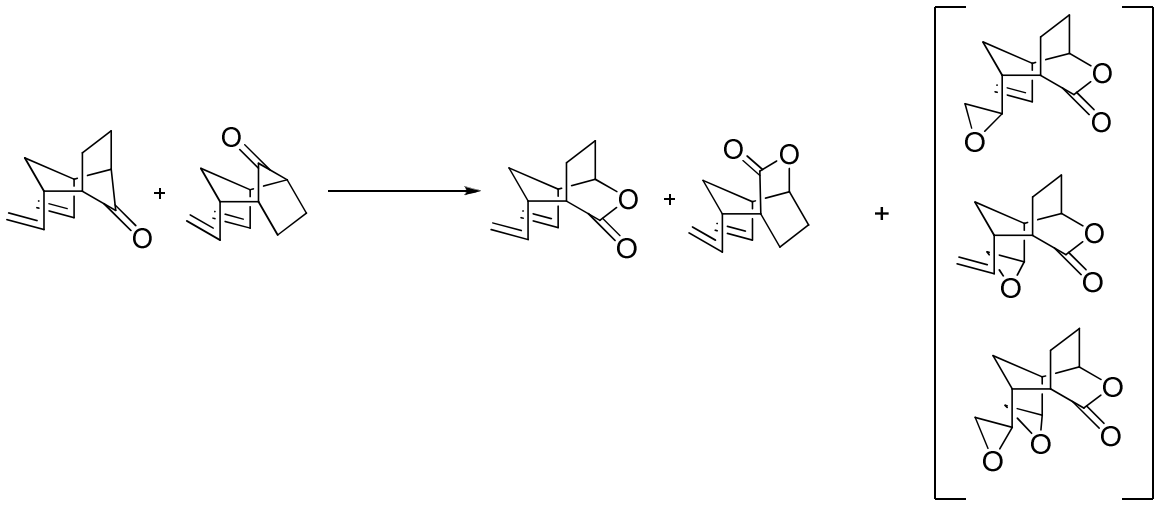


^{1}H-NMR **<u>17</u>*anti*** ($CDCl_3$):1.21–1.46 (m, 2H, H10), 1.71-1.81 (m, 4H, H7/H8), 2.25-2.30 (m, 2H, H2/H5), 2.47-2.51 (m, 2H, H1/H6), 3.40 (s, 2H, H3/H4)

^{1}H-NMR **<u>17</u>*syn*** ($CDCl_3$):1.25–1.38 (m, 2H, H10), 1.63-1.68 (m, 2H, $H7_a$/$H8_a$), 2.00-2.05 (m, 2H, $H7_b$/$H8_b$), 2.35-2.38 (m, 2H, H2/H5), 2.60-2.65 (m, 2H, H1/H6), 3.72 (s, 2H, H3/H4)

^{13}C-NMR **<u>17</u>*anti*** ($CDCl_3$): 19.0 (t, C10), 23.0 (t, C7/C8), 40.7 (d, C2/C5), 46.6 (d, C1/C6), 52.9 (d, C3/C4), 121.7 (s, C9)

4.2.4.2 *anti*- and *syn*-2,4-Divinyl-6-oxa-bicyclo[3.2.2]nonan-7-one [12]



Chemical Baeyer-Villiger Oxidation

Substrate **11*anti* + *syn*,** diastereomeric ratio 82%:18% (20 mg, 0.114 mmol)
m-CPBA (70%) (56 mg, 0.223 mmol, 2 equiv.)
Dry dichloromethane (10 mL)
Sodium bicarbonate $NaHCO_3$ (30 mg, 0.342 mmol, 3 equiv.)
Sodium bisulphite $NaHSO_3$ aq. solution (≈ 40%) (11.9 mg, 0.114 mmol, 1 equiv.)
KOH 5% solution

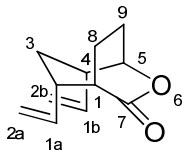
Procedure: m-CPBA (56 mg, 0.223 mmol, 2 equiv.) and $NaHCO_3$ (30 mg, 0.342 mmol, 3 equiv.) were added to a solution of **11*anti* + *syn*** (20 mg, 0.114 mmol) in dry dichloromethane (10 mL) and stirred at room temperature. After 19 h full conversion was determined by GC/MS. $NaHSO_3$ aq. solution (40%) (11.9 mg, 0.114 mmol, 1 equiv.) was added and the mixture was stirred for 30 min. The mixture was washed with $NaHCO_3$ (saturated solution) and KOH (5% solution). The organic layer was dried over sodium sulfate and the solvent was removed in *vacuo*.
The product **12*anti* + 12*syn*** (diastereomeric ratio ≈ 84%:16%) was purified by column chromatography on silicagel, with eluent hexane/ethyl acetate 8:1 and obtained as colorless oil.
In addition to the desired lactone **12**, three byproducts could be observed in this reaction (totaling 18% area according to GC/MS), which are assumed to be the three possible epoxide-products (see the scheme above).

Microbial Baeyer-Villiger Oxidation

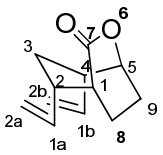
Substrate **11*anti* + *syn*,** diastereomeric ratio 82%:18%, 70 mg (0.40 mmol)

Procedure: Fresh LB_{amp} medium (250 mL) were inoculated with 2.5 mL (1%) of the $CHMO_{Xantho}$ overnight preculture of recombinant *E. coli* in a 1000 mL baffled Erlenmeyer flask. The culture was incubated at 120 rpm at 30°C on an orbital shaker for 3 hours (OD_{600}= 0.60), then, IPTG stock solution (c=50 mg/mL in deion. water) was added (50 µL, final concentration 0.083mM). The substrate Substrate **11*anti* + *syn*** (70 mg, 0.40 mmol) and β-cyclodextrin (1 equivalent) were added and the culture was incubated at 24°C. Progress of the reaction was monitored by GC/MS. After 20h full conversion could be observed. The reaction was stopped, the biomass removed by centrifugation. The aqueous layer was saturated with NaCl, extracted with ethyl acetate, the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and the solvent was removed in *vacuo*. The crude product and recovered starting material were separated by column chromatography on silicagel with eluent hexane/ethyl acetate 8:1. The product **12*anti* + *syn*** was obtained as colorless oil in diastereomeric ratio 98%:2%.

Yield: 16 mg (71%) (chemical BVO) (diastereomeric ratio ≈ 84%:16%)
70 mg (92%) (microbial BVO) (diastereomeric ratio ≈ 98%:2%), colorless oil
$[\alpha_D]^{26}$ = +35.5 (c=1.91; $CHCl_3$
MW: 192.25g/mol; $C_{12}H_{16}O_2$



12anti



12syn

^{1}H-NMR **12*anti*** ($CDCl_3$): 1.28-1.32 (m, 1H, $H3_a$), 1.72-1.77 (m, 1H, $H3_b$), 1.79-1.81 (m, 1H, $H9_a$), 1.91-1.96 (m, 1H, $H8_a$), 2.07-2.14 (m, 2H, $H8_b$/$H9_b$), 2.25-2.30 (m, 1H, H2), 2.32-2.38 (m, 1H, H4), 2.77 (d, J=5.8, 1H, H1), 4.56 (d, J=5.9 Hz, 1H, H5), 4.95-5.07 (m, 4H, vinyl H2), 5.77-5.91 (m, 2H, vinyl H1)

^{1}H-NMR **12*syn*** ($CDCl_3$): 1.28-1.32 (m, 1H, $H3_a$), 1.72-1.77 (m, 1H, $H3_b$), 1.79-1.81 (m, 1H, $H9_a$), 1.91-1.96 (m, 1H, $H8_a$), 2.07-2.14 (m, 2H, $H8_b$/$H9_b$), 2.25-2.30 (m, 1H, H2), 2.32-2.38 (m, 1H, H4), 2.73-2.77 (m, 1H, H1), 4.47 (d,

J=5.6 Hz, 1H, H5), 4.87-4.90 (m, 4H, vinyl H2), 5.77-5.99 (m, 2H, vinyl H1)

^{13}C-NMR **12*anti*** ($CDCl_3$): 24.3 (t, C8), 25.9 (t, C9), 32.9 (t, C3), 45.0 (d, C1), 46.1 (d, C2), 46.8 (d, C4), 80.0 (d, C5), 114.0 (t, vinyl $C2_a$), 114.4 (t, vinyl $C2_b$), 140.7 (d, vinyl $C1_a$), 141.0 (d, vinyl $C1_b$), 173.2 (s, C7)

^{13}C-NMR **12*syn*** ($CDCl_3$): 24.5 (t, C8), 25.8 (t, C9), 32.9 (t, C3), 44.9 (d, C1), 45.7 (d, C2), 46.6 (d, C4), 79.8 (d, C5), 114.3 (t, vinyl $C2_a$), 114.8 (t, vinyl $C2_b$), 140.3 (d, vinyl $C1_a$), 140.5 (d, vinyl $C1_b$), 173.0 (s, C7)

GC/MS :
m/z : 192 (4, M^+), 177 (8), 164 (14), 148 (22), 15 (28), 107 (26), 91 (45), 79 (100), 67 (40), 53 (35).
$C_{12}H_{16}O_2$.: calcd.: C 74.97; H 8.39; found: C 75.17; H 8.42.

For 2DNMR spectra of **12*anti*** see Appendix.

4.3 Biotransformations

4.3.1 General Protocol for Biotransformations on Preparative Scale

Fresh LB_{amp} medium was inoculated with 1% v/v of an overnight preculture of recombinant *E. coli* strain overexpressing the appropriate BVMO in a baffled Erlenmeyer shaking flask (volume of the medium is equivalent to 20-25% of the flask volume). The culture was incubated at 120 rpm at 37°C on an orbital shaker for 2-3 hours. After reaching an optical density OD_{600} between 0.5 and 0.7, IPTG or L-arabinose (L-Ara) stock solution was added to a final concentration adequate to the used strain (Table 61) to induce expression of the corresponding monooxygenase. The substrate (3-6 mM) was added neat along with β-cyclodextrin (1 equiv.). The culture was incubated at 24°C until no increase in conversion could be monitored anymore (GC/MS control).
The biomass was separated by centrifugation (10 min, 7000 x g), the supernatant was saturated with NaCl and extracted with ethyl acetate (5x). The combined organic layers were washed with brine, filtered through a pad of celite (if necessary), dried over Na_2SO_4 and the solvent was removed in *vacuo*. The crude biotransformation product was purified by column chromatography on silicagel, with eluent hexane/ethyl acetate in appropriate ratio.

4.3.2 General Protocol for Screening Experiments in Plates

Plates with either 12 or 24 wells were used. Each well was charged with 2 mL LB_{amp} medium (12 well plaes) or 1 mL LB_{amp} medium (24 well plates) and inoculated with 1 % v/v of the overnight preculture of recombinant *E. coli* strain overexpressing the appropriate BVMO. The plate was incubated at 37°C or 30°C at 120 rpm on an orbital shaker for 2-3 hours (depending on the strain). IPTG or arabinose (L-Ara) was added to a final concentration adequate for the used strain (Table 61) together with the substrate (dissolved in dioxane), final concentration 3-6 mM. The plate was incubated at 24°C or 37°C at 120 rpm on an orbital shaker for the defined time. The biotransformation was stopped by taking the samples (700 µL), centrifugation to sepatate the biomass, extraction with ethyl acetate (800 µL, supplemented by internal standard methyl benzoate/final concentration 1 mM), drying over Na_2SO_4 and analysis of the samples on GC/MS or chiral GC.

4.3.3 Preparation of Buffers, Media and Stock Sollutions

Buffers:

pH 4:

Formate buffer; for 50 mL of buffer:
Formic acid HCOOH, 115 mg (2.5 mmol), adjust with NaOH to pH = 4

pH 6:

Kaliumdihydrogenphosphat buffer; for 50 mL buffer:
KH_2PO_4, 340 mg (2.5 mmol), adjust with NaOH to pH = 6

pH 8:

TRIS buffer; for 50 mL of buffer:
tris(hydroxymethyl)aminomethane, $(HOCH_2)_3CNH_2$, 303 mg (2.5 mmol), adjust with HCl to pH = 8

pH 10:

Carbonate buffer; for 50 mL of buffer:
$NaHCO_3$, 210 mg (2.5 mmol), adjust with NaOH to pH = 10

Medium:

LB_{amp}
5.0 g peptone
2.5 g yeast extract
5.0 g sodium chloride
500 mL distilled water
sterilized at 121 °C for 20 min, then added 2 mL ampiciline stock sollution

Stock Sollutions:

Ampiciline -stock solution
50 mg/mL ampiciline (M = 349.41 g/mol) dissolved in deion. water, sterilization by filtration (0.2 μm) (c = 0.14M).

IPTG-stock solution
200 mg/mL IPTG (M = 238.30 g/mol) dissolved in deion. water, sterilization by filtration (0.2 μm) (c = 0.84M).

L-Arabinose-stock solution
L-Arabinose (M = 150.13), 20%, dissolved in deion. water, sterilization by filtration (0.2 μm) (c = 1.33M).

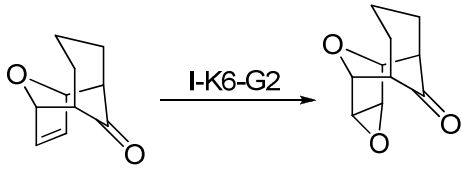
Cultivation conditions for *E. coli* overexpressing wild-type BVMOs and mutant strains[150] are summarized in Table 61.

Table 61

	Strain	Growing T [°C]	Incubation T [°C]	Inductor (final Konc.) [mmol/l]
1	$CHMO_{Acineto}$	37	24	IPTG (0.166)
2	$CPMO_{Coma}$	37	24	IPTG (0.166)
3	$CHMO_{Brevi1}$	37	24	IPTG (0.166)
4	$CHMO_{Brevi2}$	37	24	IPTG (0.166)
5	$CHMO_{Rhodo1}$	37	24	IPTG (0.166)
6	$CHMO_{Rhodo2}$	37	24	IPTG (0.166)
7	$CHMO_{Brachy}$	37	24	IPTG (0.166)
8	$CHMO_{Arthro}$	37	24	IPTG (0.166)
9	$CHMO_{Xantho}$	30	24	IPTG (0.083)
10	HAPMO	37	24	*l*-Ara (2.66)
11	CDMO	37	24	IPTG (0.083)
12	$BVMO_{Pseudo}$	37	24	IPTG (0.083)
13	PAMO	37	37	*l*-Ara (1.33)
14	All Mutant strains*	37	24	IPTG (0.166)

*1-K6-G2, 1-E12-B5, 1-F1-F5, 1-F4-B9, 2-A10-B6, 1-H3-C9, 1-H7-F4, 1-C2-B7, 1-K2-F5, 2-D19-E6

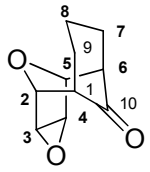
4.3.3.1 *anti*-11,12-Dioxatetrayclo[4.3.1.1.1^{2,5}]dodec-10-one [1a]



Substrate **1** (100 mg, 0.61 mmol)
500 mL of fresh LB_{amp} medium
Strain: I-K6-G2

Procedure: The reaction was carried out according to the general protocol for biotransformations on preparative scale.
The proceeding of the reaction was monitored by GC/MS. After 24h 4.5% conversion and after 48 hours, 9% conversion could be observed. The reaction was stopped after 48h. The product **1a** was obtained as colorless oil.

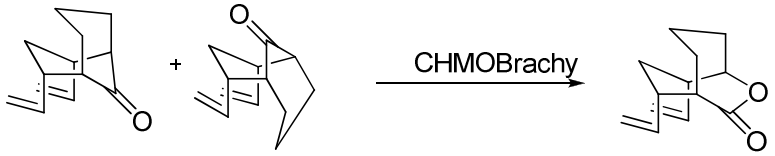
Yield: 8 mg (7%), colorless oil
MW: 180.20g/mol; $C_{10}H_{12}O_3$



^{1}H-NMR ($CDCl_3$): 1.39-1.54 (m, 1H, $H8_a$) 2.03-2.19 (m, 2H, H1/ H6), 2.30-2.41 (m, 4H, H7/H9), 2.48-2.63 (m, 1H, $H8_b$), 3.53 (s, 2H, H3/H4), 4.43 (d, J=2Hz, 2H, H2/H5)

^{13}C-NMR ($CDCl_3$): 20.2 (t, C8), 32.8 (t, C7/C9), 50.1 (d, C1/C6), 52.9 (d, C3/C4), 77.3 (d, C2/C5), 214.1 (s, C10)

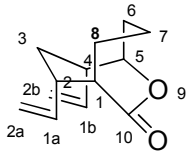
4.3.3.2 2,4-Divinyl-9-oxabicyclo[3.3.2]decan-10-one[16]



Substrate **15*anti + syn*** (diastereomeric ratio 70%:30%) (38 mg, 0.2 mmol)
250 mL of fresh LB_{amp} medium
Strain: $CHMO_{Brachy}$

Procedure: The reaction was carried out according to the general protocol for biotransformations on preparative scale.
After 24h 15% conversion and after 48 hours, 36% conversion could be observed (GC/MS). The reaction was stopped after 48h.
A spectral analysis (C-H hsqc, C-H hmbc, H-H cosy and H-H noesy) of the isolated lactone **16** showed that only one diastereomer is formed, but could not give a doubtless determination of the steric structure of the molecule (either anti or syn) (see Appendix).The product **16** was obtained as colorless oil.

Yield: 14 mg (34%), colorless oil
MW: 206.28g/mol; $C_{13}H_{18}O_2$



^{1}H-NMR ($CDCl_3$): 1.60-1.63 (m, 1H, $H7_a$), 1.65-1.67 (m, 1H, $H6_a$), 1.78-1.81 (m, 1H, $H3_a$), 1.85-1.86 (m, 1H, $H8_a$), 1.88-1.90 (m, 1H, $H6_b$), 1.92-1.93 (m, 1H, $H7_b$), 1.95-1.97 (m, 1H, $H3_b$), 2.08-2.10 (m, 1H, $H8_b$), 2.66-2.69 (m, 1H, H4), 2.78-2.80 (m, 1H, H2), 3,18 (d, J=7.7 Hz, 1H, H5), 4.52 (d, J=8.0 Hz, 1H, H1), 5.06-5.20 (m, 4H, vinyl$H2_a$/vinyl $H2_b$), 5.64-5.88 (m, 2H, vinyl$H1_a$/vinyl $H1_b$)

^{13}C-NMR: ($CDCl_3$): 20.7 (t, C6), 22.1 (t, C7), 25.5 (t, C8), 30.0 (t, C3), 40.4 (d, C4), 46.1 (d, C2), 49.1 (d, C5), 78.3 (d, C1), 115.0 (t, vinyl $C2_b$), 116.3 (t, vinyl $C2_a$), 138.6 (d, vinyl $C1_a$), 140.4 (d, vinyl $C1_b$), 176.2 (s, C10)

For 2D NMR Spectra of **16** see Appendix.

4.3.4 Acyclic Ketones

General Protocol for Preparation of β-Hydroxy Ketones

A solution of diisopropylamine (1.1 equiv.) in dry THF was cooled to -78 °C under nitrogen atmosphere and *n*-butyllithium (3.3M in hexane, 1.1 equiv.) was added through a septum. The solution was stirred for 30 min, then dry acetone (1 equiv.) dissolved in dry THF (0.2 M solution) was added. The solution was stirred at -78 °C for 50 min. Freshly distilled pentanal (1.1 equiv.) was added and the solution was stirred for 5 min before it was quenched with saturated NH_4Cl solution. The reaction mixture was extracted with diethylether, the organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed in *vacuo*.
The crude reaction product was purified by *Kugelrohr* distillation (in previous attemts to purify the product by column chromatography on silicagel, decomposition of the product could be observed).

General Protocol for Acetylation

Acetic anhydride (3 equiv.), pyridine (10 equiv.) and a catalitic amount of DMAP were added subsequently under stirring to the substrate dissolved in minimum amount of dichloromethane at 0 °C. The ice-bath was removed and the reaction mixture was allowed to come to room temperature and stirred overnight at room temperature. The reaction mixture was then diluted with diethylether and quenched with brine. The aqueous layer was extracted with diethylether, the combined organic layers were dried over Na_2SO_4, filtered and the solvent and excess acetic anhydride were removed in *vacuo*. The crude product was purified by column chromatography on silicagel with eluent hexane/ethyl acetate.

General Protocol for Synthesis of Methylcarbonates

Methyl chloroformate (2.3 equiv.) was added drop wise to a solution of β-hydroxyketone and pyridine (3.6 equiv.) in minimal amount of dichloromethane at 0°C. The conversion was monitored by GC/MS. After no increase of conversion was observed, the reaction mixture

was quenched with water, the aqueous layer was extracted repeatedly with diethylether, the organic layer washed with brine, dried over Na_2SO_4 and concentrated in *vacuo*.

General Protocol for Synthesis of Propionates

Propionylchloride (1.1 equiv.) was added drop wise to a solution of β-hydroxyketone, triethylamine (1 equiv.) and DMAP (cat. amount) in minimal amount of dichloromethane at 0°C. The reaction mixture was heated to reflux. The conversion was monitored by GC/MS. After no increase of conversion was observed, the reaction mixture was cooled to room temperature and washed with 2M HCl. The organic layer was dried over Na_2SO_4 and concentrated in *vacuo*.

General Protocol for Synthesis of Formates

A mixture of β-hydroxyketone and formic acid was stirred at 65°C for 2h. After cooling the reaction mixture to room temperature, diethylether was added and the mixture was washed with ice-cold aqueous $NaHCO_3$ sat., extracted with diethylether, dried over Na_2SO_4 and concentrated in *vacuo*.

General Protocol for Synthesis of Alkoxyketones

Pyrrolidine (0.3 equiv.) and methanesulfonic acid (0.3 equiv.) were stirred at room temperature for 10 min. Alcohol and α,β-unsaturated ketone (1 equiv., 1M in the appropriate alcohol) were added and the reaction mixture was stirred at room temperature. The conversion was monitored by GC/MS. After no increase of conversion was observed, the reaction mixture was quenched with aqueous $NaHCO_3$ sat., extracted with dichloromethane, dried over Na_2SO_4 and concentrated in *vacuo*.

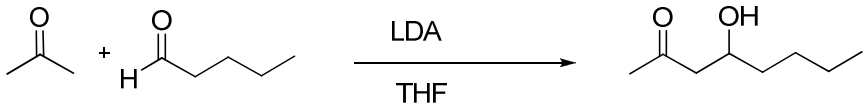
General Protocol for Synthesis of Pyrrolidyl-Ketones

A mixture of pyrrolidine (1.2 equiv.) and α,β-unsaturated ketone was stirred neat at room temperature. The conversion was monitored by GC/MS. After no increase of conversion was observed, the excess pyrrolidine was removed under reduced pressure.

General Protocol for Synthesis of Thioethers

A mixture of α,β-unsaturated ketone, ethanethiol (1 equiv.) and K_3PO_4 (0.25 equiv.) was stirred neat at room temperature. The conversion was monitored by GC/MS. After no increase of conversion was observed, chloroform was added to the reaction mixture and K_3PO_4 was removed by filtration. Chloroform and excess ethanethiol were removed in *vacuo*.

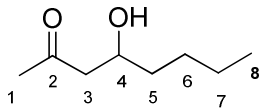
4.3.4.1 4-Hydroxyoctane-2-one [21]



Pentanal (1.447 g, 16.6 mmol, 1.1 equiv.), freshly distilled
Dry Acetone (0.888 g, 15.3 mmol)
Diisopropylamine (1.68 g, 16.6 mmol, 1.1 equiv.)
n-Butyllithium, 3.3M in hexane (5.3 mL, 16.6 mmol, 1.1 equiv.)
dry THF
NH_4Cl, saturated solution (7.5 mL)

Procedure: Product **21** was prepared according to the general protocol for preparation of β-hydroxy ketones. The crude reaction product was obtained as yellow oil and purified by *Kugelrohr* distillation to give a colorless oil.

Yield: 1.787 g (81%), colorless oil (lit.[172]: 80%)
MW: 144.12g/mol; $C_8H_{16}O_2$
b.p.: 60-65°C/0.1mbar (lit.[172]: 88-92°C/1.5 mmHg)

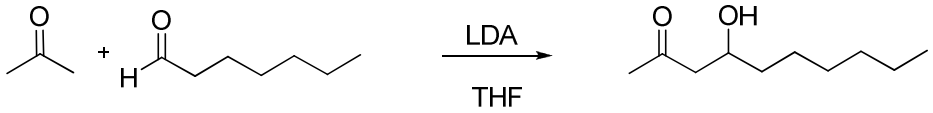


^{1}H-NMR ($CDCl_3$)[172]: 0.81 (t, J=7.2 Hz, 3H, H8) 1.18-1.28 (m, 6H, H5/H6/H7), 2.05 (s, 3H, H1), 2.45-2.48 (m, 2H, H3), 3.20 (s, 1H, OH), 3.89-3.92 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.7 (q, C8), 22.3 (t, C7), 27.4 (t, C6), 30.5 (q, C1), 36.0 (t, C5), 49.9 (t, C3), 67.3 (d, C4), 209.6 (s, C2)

[172] Kourouli, T.; Kefalas, P.; Ragoussis, N.; Ragoussis, V. *J. Org. Chem.* **2002**, *67*, 4615.

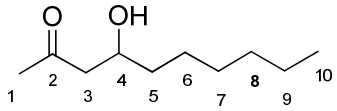
4.3.4.2 4-Hydroxydecane-2-one [22]



Heptanal (3.785 g, 33.2 mmol, 1.1 equiv.), freshly distilled
Dry Acetone (1.75 g, 30.2 mmol)
Diisopropylamine 83.36 g, 33.2 mmol, 1.1 equiv.)
n-Butyllithium (2.5 M in hexane, 13.3 mL, 33.2 mmol, 1.1 equiv.)
dry THF
NH_4Cl, saturated solution (15 mL)

Procedure: Product **22** was prepared according to the general protocol for preparation of β-hydroxy ketones. The crude reaction product was obtained as yellow oil and purified by *Kugelrohr* distillation to give a colorless oil.

Yield: 3.694 g (71%), colorless oil (lit.[172]: 72%)
MW: 144.12g/mol; $C_8H_{16}O_2$
b.p.: 64-68°C/0.07mbar (lit.[173]: 88-92 °C/1.5 mmHg)

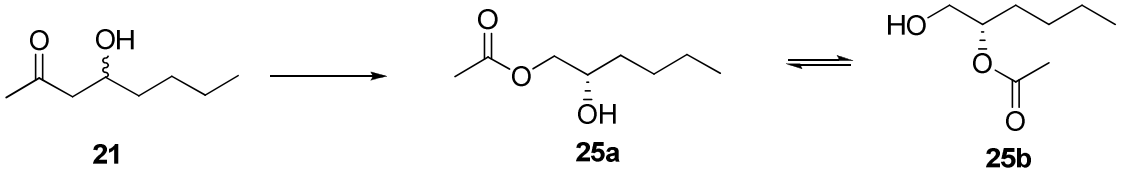


^{1}H-NMR($CDCl_3$)[172]: 0.83 (t, J=7.3 Hz, 3H, H10) 1.15-1.39 (m, 10H, H5/H6/H7/H8/H9), 2.13 (s, 3H, H1), 2.41-2.64 (m, 2H, H3), 3.10 (s, 1H, OH), 3.99 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 14.1 (q, C10), 22.6 (t, C9), 25.4 (t, C8), 29.2 (t, C7), 30.8 (q, C1), 31.8 (t, C6), 36.4 (t, C5), 49.9 (t, C3), 67.5 (d, C4), 208.9 (s, C2)

[173] Inokuchi, T.; Kusumoto M.; Torii, S. *J. Org. Chem.* **1990**, *55*, 1548.

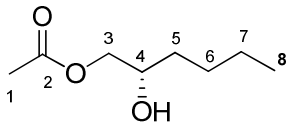
4.3.4.3 1-Acetoxy-2-hexanol and 2-Acetoxy-1-hexanol [25]



4-Hydroxyoctane-2-one **21** (120 mg, 0.83 mmol)
250 mL of fresh LB_{amp} medium
Strain: $CPMO_{Coma}$
IPTG (50 µL, final concentration 0.166mM)

Procedure: Product **25** was prepared according to the General protocol for biotransformations on preparative scale.
The crude products **25a** and **25b** were obtained as mixture as yellow oil and were purified by column chromatography on silicagel with eluent pentane/diethylether 4:1→ pure diethylether. The products **25a** and **25b** were obtained as mixture in ratio ca. 2:1. (could not be separated during the column chromatography and gave also only one peak in GC/MS) as yellow oil.

Yield: 87 mg (66%), yellow oil
MW: 160.21g/mol; $C_8H_{16}O_3$

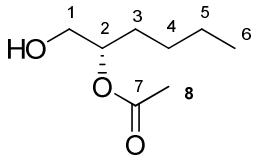


25a

^{1}H-NMR **25a** ($CDCl_3$)[174]: 0.80-0.87 (m, 3H, H8), 1.24-1.52 (m, 6H, H5/H6/H7), 2.02 (s, 3H, H1), 2.70 (s, 1H, OH), 3.71-3.80 (m, 1H, H4), 3.84-3.90 (m, 1H, $H3_a$), 4.02-4.09 (m, 1H, $H3_b$)

[174] Bonini, C.; Righi, C.; Sotgiu, G. *J. Org. Chem.* **1991**, *56*, 6206.

^{13}C-NMR **25a** ($CDCl_3$)[174]: 13.8 (q, C8), 20.7 (q, C1), 22.5 (t, C7), 27.4 (t, C6), 32.9 (t, C5), 68.6 (t, C3), 69.5 (d, C4), 171.2 (s, C2)



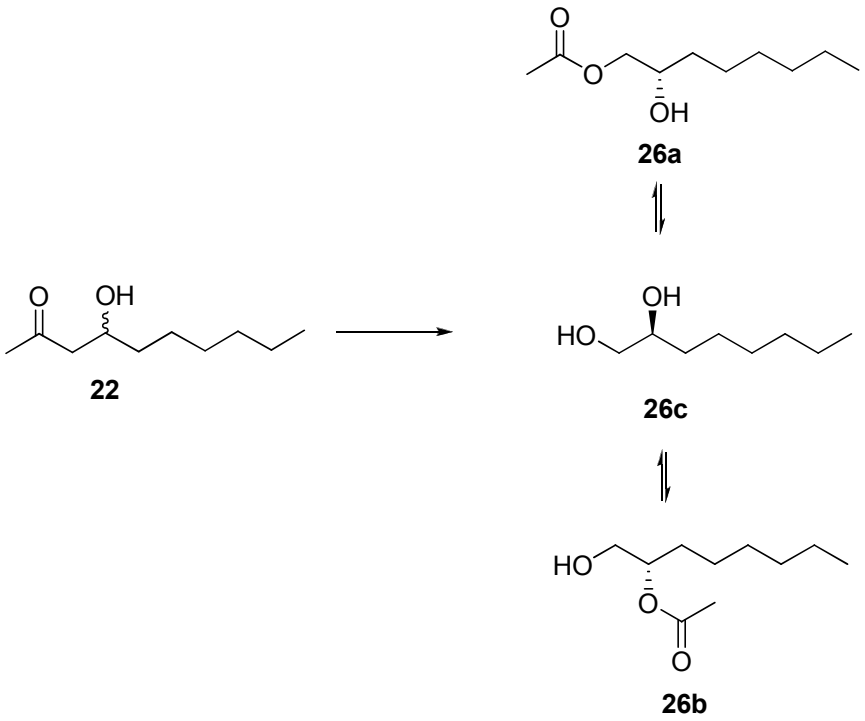
25b

^{1}H-NMR **25b**[175] ($CDCl_3$): 0.80-0.87 (m, 3H, H6), 1.24-1.52 (m, 6H, H3/H4/H5), 2.01 (s, 3H, H8), 2.70 (s 1H, OH), 3.53-3.60 (m, 2H, H1), 4.77-4.89 (m, 1H, H2)

^{13}C-NMR **25b** ($CDCl_3$): 13.5 (q, C6), 21.0 (q, C8), 22.4 (t, C5), 27.3 (t, C4), 30.1 (t, C3), 64.2 (t, C1), 75.4 (d, C2), 171.4 (s, C7)

[175] Ogawa, H.; Ide, Y.; Honda, R.; Chihara, T. *J. Phys. Org. Chem.* **2003**, *16*, 355.

4.3.4.4 1-Acetoxy-2-octanol [26a], 2-Acetoxy-1-octanol [26b] and octane-1,2-diol [26c]

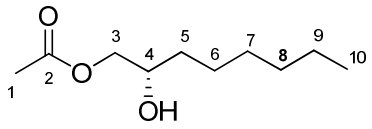


4-Hydroxydecane-2-one **22** (100 mg, 0.58 mmol)
250 mL of fresh LB_{amp} medium
Strain: $CPMO_{Coma}$
IPTG (50 µL, final concentration 0.166mM)

Procedure: Products **26a, 26b** and **26c** was prepared according to the General protocol for biotransformations on preparative scale.
The crude products **26a, 26b** and **26c** were obtained as mixture as yellow oil and were purified by column chromatography on silicagel with eluent pentane/diethylether 4:1→ pure diethylether. The products **26a, 26b** were obtained as mixture in ratio ca. 3:1. (could not be separated during the column chromatography and gave also only one peak in GC/MS) as yellow oil in 15% yield. Diol **26c** could be separated and was obtained as colorless oil in 23% yield.
The yield can probably be increased since after stopping the reaction the crude reaction mixture was stored at 4°C and the workup was done after 5 days. In this time, according to GC/MS, approximately half of the product decomposed.

Yield 26a + 26b[176]: 17 mg (15%), yellow oil
MW: 188.26g/mol; $C_{10}H_{20}O_3$

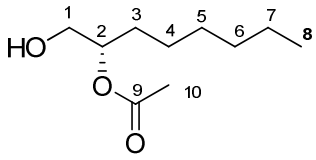
Yield 26c[176]: 25 mg (23%), colorless oil
MW: 146.23g/mol; $C_8H_{18}O_2$



26a

^{1}H-NMR **26a** ($CDCl_3$): 0.84-0.90 (m, 3H, H10), 1.23-1.49 (m, 10H, H5/H6/H7/H8/H9), 2.09 (s, 3H, H1), 3.81-3.85 (m, 1H, H4), 3.89-3.99 (m, 1H, $H3_a$), 4.11-4.17 (m, 1H, $H3_b$)

^{13}C-NMR **26a** ($CDCl_3$): 14.0 (q, C10), 20.9 (q, C1), 22.5 (t, C9), 25.3 (t, C8) 29.2 (t, C7), 31.7 (t, C6), 33.3 (t, C5), 68.8 (t, C3), 69.9 (d, C4), 171.2 (s, C2)

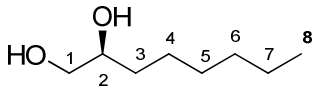


26b

^{1}H-NMR **26b** ($CDCl_3$): 0.84-0.90 (m, 3H, H10), 1.23-1.49 (m, 10H, H3/H4/H5/H6/H7), 2.08 (s, 3H, H10), 3.62-3.69 (m, 2H, H1), 4.84-4.96 (m, 1H, H2)

^{13}C-NMR **26b** ($CDCl_3$): 14.0 (q, C8), 21.2 (q, C10), 22.4 (t, C7), 25.2 (t, C6), 29.1 (t, C5), 30.5 (t, C4), 31.6 (t, C3), 64.8 (t, C1), 75.7 (d, C2), 171.5 (s, C9)

[176] Moghadam, M.; Tangestaninejad, S.; Mirkhani, V.; Shaibani, R. *Tetrahedron* **2004**, *60*, 6105.

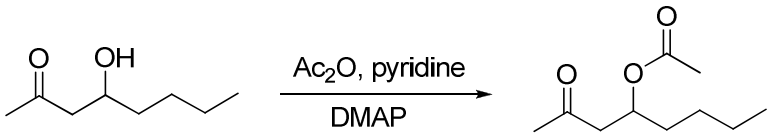


26c

^{1}H-NMR **26c** ($CDCl_3$): 0.84-0.90 (m, 3H, H8), 1.25-1.41 (m, 10H, H3/H4/H5/H6/H7), 3.07 (s, 2H, 2x OH), 3.35-3.44 (m, 1H, H2), 3.58-3.69 (m, 2H, H1)

^{13}C-NMR **26c** ($CDCl_3$): 14.0 (q, C8), 22.6 (t, C7), 25.5 (t, C6), 29.3 (t, C5), 31.7 (t, C4), 33.1 (t, C3), 66.8 (t, C1), 72.3 (d, C2)

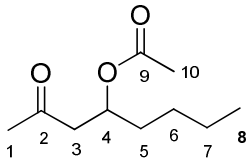
4.3.4.5 4-Acetoxy-2-octanone [23]



4-Hydroxyoctane-2-one **21** (230 mg, 1.59 mmol)
Acetic anhydride (487 mg, 4.77 mmol, 3 equiv.)
Pyridine (1.26 g, 15.9 mmol, 10 equiv.)
4-(Dimethylamino)-pyridine (DMAP) (catalitic amount)
Dichloromethane
Diethylether

Procedure:[177] Product **23** was prepared according to the general protocol for acetylation. The product **23** was obtained as colorless oil.

Yield 23[178]**: 197 mg (66%)**, colorless oil
MW: 186.25g/mol; $C_{10}H_{18}O_3$

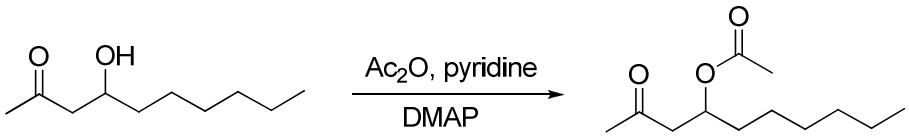


^{1}H-NMR ($CDCl_3$): 0.81 (t, J=6.6 Hz, 3H, H8), 1.20-1.26 (m, 4H, H6/H7), 1.45-1.49 (m, 2H, H5), 1.93 (s, 3H, H10), 2.07 (s, 3H, H1), 2.54-2.61 (m, 2H, H3), 5.10-5.16 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.5 (q, C8), 20.6 (q, C10), 22.0 (t, C7), 26.9 (t, C6), 29.9 (q, C1), 33.4 (t, C5), 47.4 (t, C3), 69.8 (d, C4), 169.9 (s, C9), 205.2 (s, C2)

[177] McGarth, M. J.; Fletcher, M. T.; König, W. A.; Moore, C. J.; Cribb, B. W.; Allsopp, P. G.; Kitching, W., *J. Org. Chem.* **2003**, *68*, 3739.
[178] Mandai, t.; Nokami, J.; Yano, T.; Yoshinaga, Y.; Otera, J. ., *J. Org. Chem.* **1984**, *49*, 172.

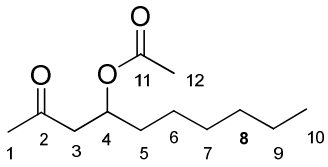
4.3.4.6 4-Acetoxy-2-decanone [24]



4-Hydroxydecane-2-one **22** (300 mg, 1.74 mmol)
Acetic anhydride (532 mg, 5.22 mmol, 3 equiv.)
Pyridine (1.375 g , 17.4 mmol, 10 equiv.)
4-(Dimethylamino)-pyridine (DMAP) (catalitic amount)
Dichloromethane
Diethylether

Procedure:[177] Product **24** was prepared according to the general protocol for acetylation. The product **24** was obtained as colorless oil.

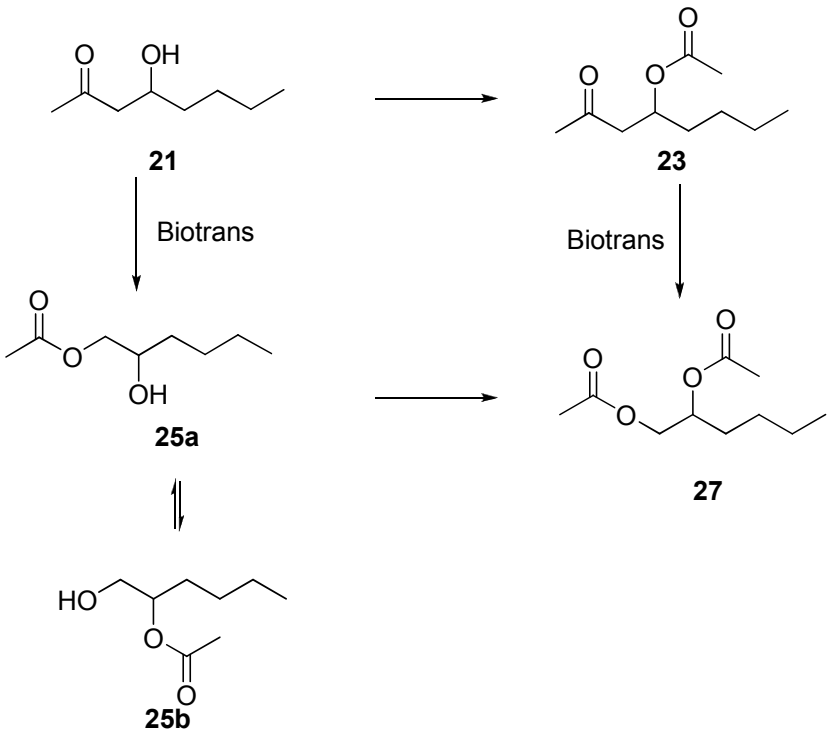
Yield: 221 mg (59%), colorless oil
MW: 214.30 g/mol; $C_{12}H_{22}O_3$



^{1}H-NMR ($CDCl_3$): 0.76-0.87 (m, 3H, H10), 1.14-1.26 (m, 8H, H6/H7/H8/H9), 1.47-1.53 (m, 2H, H5), 1.94 (s, 3H, H12), 2.07 (s, 3H, H1), 2.56-2.64 (m, 2H, H3), 5.12-5.18 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.6 (q, C10), 20.6 (q, C12), 22.1 (t, C9), 24.7 (t, C8), 28.6 (t, C7) 29.9 (q, C1), 31.3 (t, C6), 33.7 (t, C5), 47.5 (t, C3), 69.9 (d, C4), 170.0 (s, C11), 205.3 (s, C2)

4.3.4.7 Hexane-1,2-diyl diacetate [27]



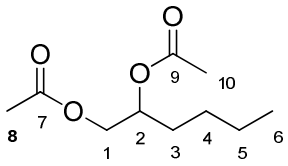
Compound **27** was prepared starting from different substrates; always starting from β-hydroxyketone **21** either first biooxidation with subsequent acetylation was carried out or vice versa.

Substrates **25a** + **25b** (121 mg, 0.76 mmol)
Acetic anhydride (233 mg, 2.28 mmol, 3 equiv.)
Pyridine (600 mg, 7.6 mmol, 10 equiv.)
4-(Dimethylamino)-pyridine (DMAP) (catalitic amount)
Dichloromethane
Diethylether

Procedure: Product **27** was prepared according to the general protocol for acetylation.
The product **27** was obtained as yellow oil.

Yield 27[179]**: 98 mg (64%)**, yellow oil
MW: 202.30 g/mol; $C_{10}H_{18}O_4$

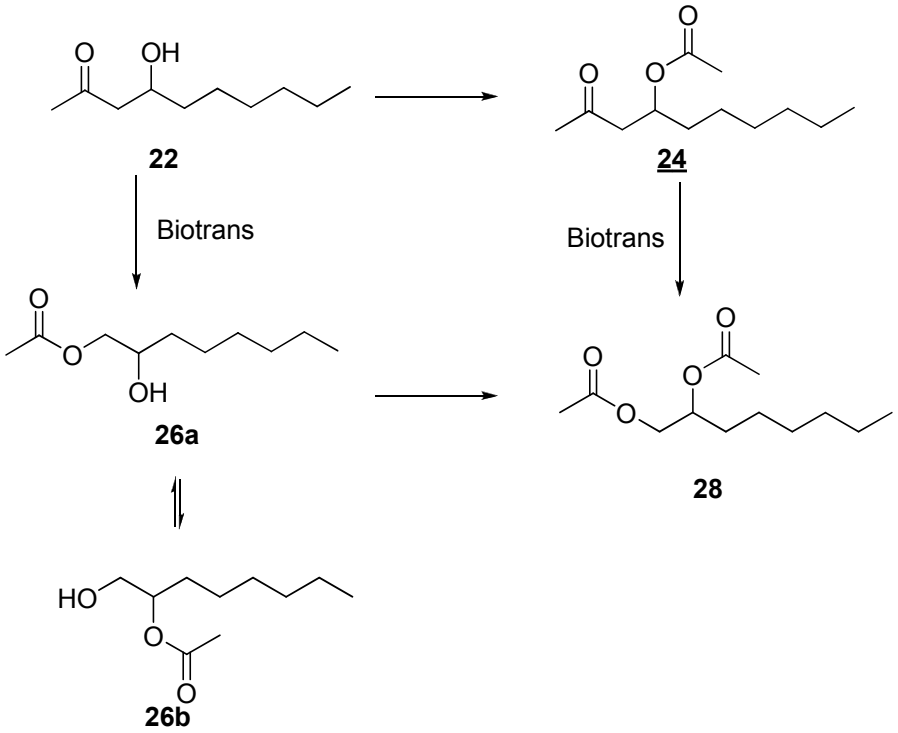
[179] Branytska, O.; Neumann, R. *Synlett* **2005,** *16*, 2525.



^{1}H-NMR ($CDCl_3$): 0.84 (t, J=5.9 Hz, 3H, H6), 1.23-1.27 (m, 4H, H6/H7), 1.50-1.53 (m, 2H, H3), 2.00 (s, 3H, H10), 1.99 (s, 3H, H8), 3.97 (dd, J=6.6 Hz, 1H, $H1_a$), 4.17 (dd, J=8.6 Hz, 1H, $H1_b$), 4.96-5.06 (m, 1H, H2)

^{13}C-NMR ($CDCl_3$): 13.7 (q, C6), 20.6 (q, C8), 20.9 (q, C10), 22.3 (t, C5), 27.1 (t, C4), 30.2 (t, C3), 65.0 (t, C1), 71.4 (d, C2), 170.4 (s, C7), 170.6 (s, C9)

4.3.4.8 1,2-Diacetoxyoctane [28]

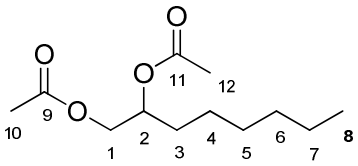


Compound **28** was prepared starting from different substrates; always starting from β-hydroxyketone **22** either first biooxidation with subsequent acetylation was carried out or vice versa.

Substrates **26a** + **26b** (21 mg, 0.11 mmol)
Acetic anhydride (34 mg, 0.34 mmol, 3 equiv.)
Pyridine (87 mg, 1.1 mmol, 10 equiv.)
4-(Dimethylamino)-pyridine (DMAP) (catalitic amount)
Dichloromethane
Diethylether

Procedure: Product **28** was prepared according to the general protocol for acetylation.
The product **28** was obtained as yellow oil.

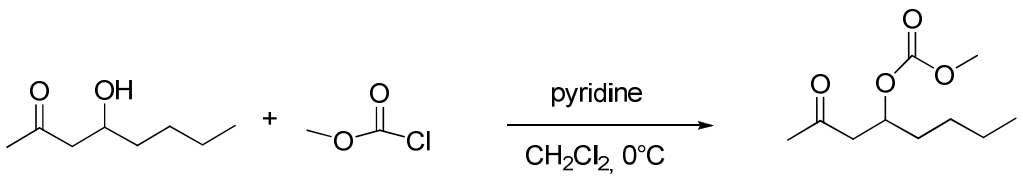
Yield 28[179]**: 22 mg (85%)**, yellow oil
MW: 230.30 g/mol; $C_{10}H_{18}O_4$



^{1}H-NMR ($CDCl_3$): 0.86 (t, J=6.8 Hz, 3H, H8), 1.26 (m, 8H, H4/H5/H6/H7), 1.51-1.57 (m, 2H, H3), 2.04 (s, 3H, H10), 2.05 (s, 3H, H12), 4.02 (dd, J=6.6 Hz, 1H, $H1_a$), 4.23 (dd, J=11.9 Hz, 1H, $H1_b$), 5.05 (m, 1H, H2)

^{13}C-NMR ($CDCl_3$): 14.0 (q, C8), 20.7 (q, C10), 21.0 (q, C12), 22.5 (t, C7), 25.0 (t, C6), 29.0 (t, C5), 30.7 (t, C4), 31.6 (t, C3), 65.1 (t, C1), 71.6 (d, C2), 170.6 (s, C9), 170.8 (s, C11)

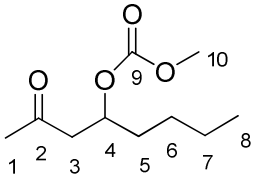
4.3.4.9 Carbonic acid methyl ester 1-(2-oxo-propyl)-pentyl ester [30]



4-Hydroxyoctane-2-one **21** (800 mg, 5.55 mmol)
Pyridine (1.58 g, 20 mmol, 3.6 equiv.)
Methyl chloroformate (1.680 g, 17.8 mmol, 2.3 equiv.)
CH_2Cl_2

Procedure: Product **30** was prepared according to the general protocol for synthesis of methylcarbonates.
The crude product **30** was obtained as yellow oil in 100% yield in 95% purity (GC/MS) and was not purified.

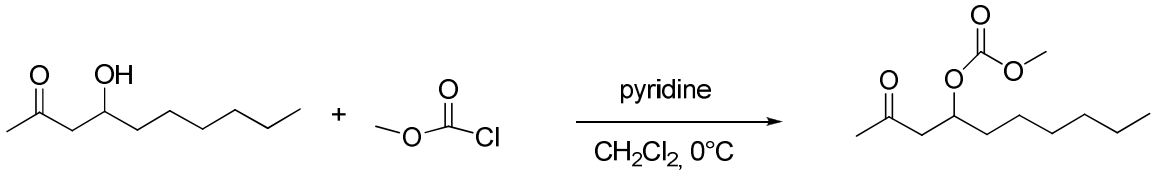
Yield: 1.20 g (100%) 95% purity (GC/MS), yellow oil
MW: 202.25 g/mol; $C_{10}H_{18}O_4$



^{1}H-NMR ($CDCl_3$): 0.82-0.85 (m, 3H, H8), 1.24-1.28 (m, 4H, H6/H7), 1.53-1.56 (m, 2H, H5), 2.09 (s, 3H, H1), 2.53 (dd, J=16.8 Hz, J= 5.4 Hz, 1H, $H3_a$), 2.72 (dd, J=16.8 Hz, J=7.2 Hz, 1H, $H3_b$), 3.70 (s, 3H, H10), 5.04 (quint, J=6.5 Hz, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.8 (q, C8), 22.5 (t, C7), 27.0 (t, C6), 30.4 (q, C1) 33.7 (t, C5), 47.5 (t, C3), 54.6 (q, C10), 74.3 (d, C4), 155.1 (s, C9), 205.2 (s, C2)

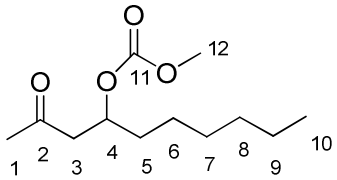
4.3.4.10 Carbonic acid methyl ester 1-(2-oxo-propyl)-heptyl ester [31]



4-Hydroxydecane-2-one **22** (300 mg, 1.74 mmol)
Pyridine (470 mg, 6.3 mmol, 3.6 equiv.)
Methyl chloroformate (526 mg, 5.57 mmol, 2.3 equiv.)
CH_2Cl_2

Procedure: Product **31** was prepared according to the General protocol for synthesis of methylcarbonates.
The crude product **31** was obtained as yellow oil in 97% yield in 92% purity (GC/MS) and was not purified.

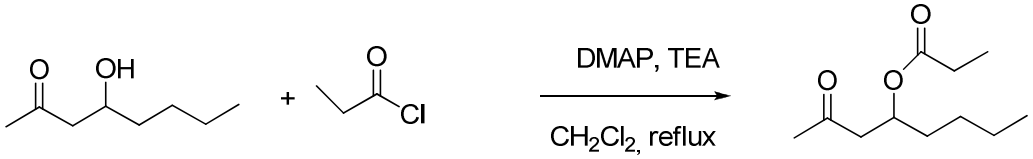
Yield: 390 mg (97%) 92% purity (GC/MS), yellow oil
MW: 230.30 g/mol; $C_{12}H_{22}O_4$



^{1}H-NMR ($CDCl_3$): 0.85-0.88 (m, 3H, H10), 1.24-1.28 (m, 8H, H6/H7/H8/H9), 1.58-2.00 (m, 2H, H5), 2.17 (s, 3H, H1), 2.63 (dd, J=16.8 Hz, J= 5.4 Hz, 1H, $H3_a$), 2.83 (dd, J=16.8 Hz, J=7.3 Hz, 1H, $H3_b$), 3.80 (s, 3H, H12), 5.11 (quint, J=6.3 Hz, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.6 (q, C10), 22.2 (t, C9), 24.5 (t, C8), 28.6 (t, C7), 30.2 (q, C1) 31.3 (t, C6), 36.3 (t, C5), 47.1 (t, C3), 54.1 (q, C12), 73.9 (d, C4), 154.8 (s, C11), 204.8 (s, C2)

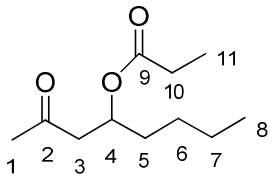
4.3.4.11 Propionic acid 1-(2-oxo-propyl)-pentyl ester [32]



4-Hydroxyoctane-2-one **21** (300 mg, 2.1 mmol)
Propionylchloride (212 mg, 2.3 mmol, 1.1 equiv.)
Triethylamine (210 mg, 2.1 mmol, 1 equiv.)
DMAP cat. amount
CH_2Cl_2

Procedure: Product **32** was prepared according to the general protocol for synthesis of propionates.
The crude product **32** was obtained as yellow oil in 79% yield in 60% purity (GC/MS) and was not purified.

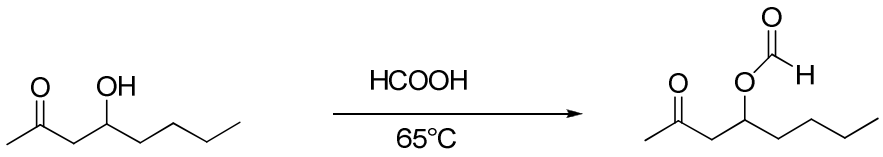
Yield: 329 mg (79%) 60% purity (GC/MS), yellow oil
MW: 200.27 g/mol; $C_{11}H_{20}O_3$



^{1}H-NMR ($CDCl_3$): 0.82-0.86 (m, 3H, H8), 1.06 (t, J=7.6 Hz, 3H, H11), 1.20-1.28 (m, 4H, H6/H7), 1.50-1.53 (m, 2H, H5), 2.10 (s, 3H, H1), 2.25 (q, J= 7.6 Hz, 2H, H10), 2.53-2.65 (m, 2H, H3), 5.18 (quint, J=6.9 Hz, 1H, H4)

^{13}C-NMR ($CDCl_3$): 9.0 (q, C11), 13.8 (q, C8), 22.3 (t, C7), 27.0 (t, C6), 27.6 (t, C10), 30.2 (q, C1) 33.8 (t, C5), 47.9 (t, C3), 70.0 (d, C4), 73.9 (d, C4), 173.8 (s, C9), 205.7 (s, C2)

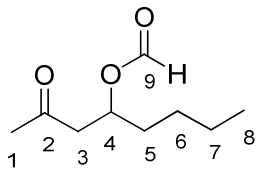
4.3.4.12 Formic acid 1-(2-oxo-propyl)-pentyl ester [33]



4-Hydroxyoctane-2-one **21** (300 mg, 2.1 mmol)
Formic acid (15 ml)

Procedure: Product **33** was prepared according to the general protocol for synthesis of formates.
The crude product **33** was obtained as yellow oil in 100% yield in 50% purity (GC/MS) and was not purified.

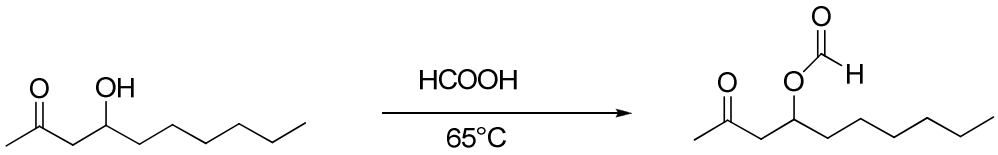
Yield: 353 mg (100%) 50% purity (GC/MS), yellow oil
MW: 172.22 g/mol; $C_9H_{16}O_3$



^{1}H-NMR ($CDCl_3$): 0.83-0.88 (m, 3H, H8), 1.17-1.35 (m, 6H, H5/H6/H7), 2.10 (s, 3H, H1), 2.58 (dd, J=16.8 Hz, J= 5.2 Hz, 1H, $H3_a$), 2.75 (dd, J=16.8 Hz, J=7.4 Hz, 1H, $H3_b$), 5.27 (quint, J=6.2 Hz, 1H, H4), 7.98 (s, 1H, H9)

^{13}C-NMR ($CDCl_3$): 13.6 (q, C8), 22.2 (t, C7), 26.6 (q, C1), 27.0 (t, C6), 33.8 (t, C5), 47.5 (t, C3), 70.2 (d, C4), 160.5 (d, C9), 205.2 (s, C2)

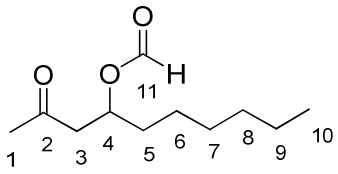
4.3.4.13 Formic acid 1-(2-oxo-propyl)-heptyl ester [34]



4-Hydroxydecane-2-one **22** (500 mg, 2.9 mmol)
Formic acid (15 ml)

Procedure: Product **34** was prepared according to the General protocol for synthesis of formates.
The crude product **34** was obtained as brown oil in 100% yield in 87% purity (GC/MS) and was not purified.

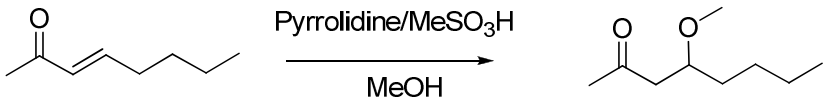
Yield: 590 mg (100%) 87% purity (GC/MS), brown oil
MW: 200.14 g/mol; $C_{11}H_{20}O_3$



^{1}H-NMR ($CDCl_3$): 0.80-0.84 (m, 3H, H10), 1.18-1.28 (m, 8H, H6/H7/H8/H9), 1.39-1.45 (m, 2H, H5) 2.10 (s, 3H, H1), 2.60 (dd, J=17.1 Hz, J= 5.1 Hz, 1H, $H3_a$), 2.78 (dd, J=16.8 Hz, J=7.4 Hz, 1H, $H3_b$), 5.33 (quint, J=6.2 Hz, 1H, H4), 7.98 (s, 1H, H9)

^{13}C-NMR ($CDCl_3$): 14.0 (q, C10), 25.0 (t, C9), 28.9 (t, C8), 30.4 (q, C1) 31.6 (t, C7), 32.5 (t, C6), 34.0 (t, C5), 47.6 (t, C3), 70.4 (d, C4), 160.9 (s, C11), 205.8 (s, C2)

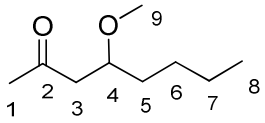
4.3.4.14 4-Methoxy-octan-2-one [35]



3-Octen-2-one (630 mg, 5 mmol)
Pyrrolidine (108 mg, 1.5 mmol, 0.3 equiv)
Methanesulfonic acid (144 mg, 1.5 mmol, 0.3 equiv.)
MeOH, 5 ml

Procedure: Product **35** was prepared according to the general protocol for synthesis of alkoxyketones.
The crude product **35** was obtained as yellow oil in 92% yield in 80% purity (GC/MS) and was not purified.

Yield 35[180]**: 727 mg (92%)** 80% purity (GC/MS), yellow oil
MW: 158.24 g/mol; $C_9H_{18}O_2$

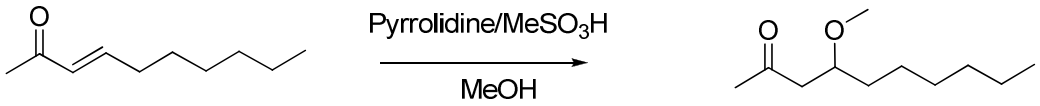


^{1}H-NMR ($CDCl_3$): 0.80-0.84 (m, 3H, H8), 1.23-1.42 (m, 6H, H5/H6/H7), 2.11 (s, 3H, H1), 2.39 (dd, J=15.8 Hz, J=4.9 Hz, 1H, $H3_a$), 2.61 (dd, J=15.8 Hz, J=7.4 Hz, 1H, $H3_b$), 3.25 (s, 3H, H9), 3.56-3.66 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.8 (q, C8), 22.6 (t, C7), 27.1 (t, C6), 30.9 (q, C1) 33.3 (t, C5), 48.1 (t, C3), 56.7 (q, C9), 77.1 (d, C4), 207.6 (s, C2)

[180] a) Ramachary, D. B.; Mondal, R. *Tetrahedron Lett.* **2006**, *47*, 7689. b) Matsuda, I.; Hasegawa, Y.; Makino, T.; Itoh, K. *Tetrahedron Lett.* **2000**, *41*, 1405.

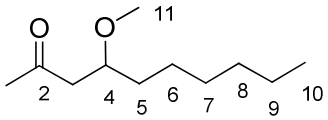
4.3.4.15 4-Methoxy-decan-2-one [36]



3-Decen-2-one (462 mg, 3 mmol)
Pyrrolidine (65 mg, 0.9 mmol, 0.3 equiv)
Methanesulfonic acid (87 mg, 0.9 mmol, 0.3 equiv.)
MeOH, 3 ml

Procedure: Product **36** was prepared according to the general protocol for synthesis of alkoxyketones.
The crude product **36** was obtained as yellow oil in 51% yield in 85% purity (GC/MS) and was not purified.

Yield 36[181]**: 283 mg (51%)** 85% purity (GC/MS), yellow oil
MW: 186.29 g/mol; $C_{11}H_{22}O_2$

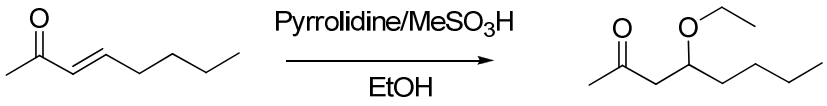


^{1}H-NMR ($CDCl_3$): 0.66-0.68 (m, 3H, H10), 1.00-1.12 (m, 8H, H6/H7/H8/H9), 1.25-1.27 (m, 2H, H5), 1.93 (s, 3H, H1), 2.20-2.25 (m, 2H, H3), 3.01 (s, 3H, H11), 3.41-3.46 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.7 (q, C10), 22.1 (t, C9), 24.6 (t, C8), 28.9 (t, C7), 30.9 (q, C1), 31.2 (t, C6), 33.3 (t, C5), 47.6 (t, C3), 56.2 (q, C11), 76.7 (d, C4), 206.8 (s, C2)

[181] Dodda, R.; Zhao, C.-G. *Synthesis* **2006**, *19*, 3238.

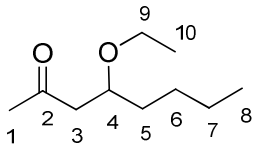
4.3.4.16 4-Ethoxy-octan-2-one [37]



3-Octen-2-one (630 mg, 5 mmol)
Pyrrolidine (108 mg, 1.5 mmol, 0.3 equiv)
Methanesulfonic acid (144 mg, 1.5 mmol, 0.3 equiv.)
EtOH, 5 ml

Procedure: Product **37** was prepared according to the general protocol for synthesis of alkoxyketones.
The crude product **37** was obtained as yellow oil in 82% yield in 65% purity (GC/MS) and was not purified.

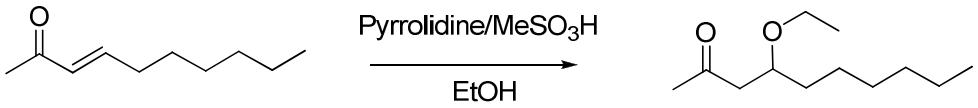
Yield 37[180a]**: 709 mg (82%)** 65% purity (GC/MS), yellow oil
MW: 172.26 g/mol; $C_{10}H_{20}O_2$



^{1}H-NMR ($CDCl_3$): 0.80-0.86 (m, 3H, H8), 1.06 (t, J=7.0 Hz, 3H, H10), 1.20-1.40 (m, 6H, H5/H6/H7), 2.09 (s, 3H, H1), 2.36 (dd, J=15.6 Hz, J= 4.9 Hz, 1H, $H3_a$), 2.72 (dd, J=15.6 Hz, J=7.5 Hz, 1H, $H3_b$), 3.34-3.46 (m, 2H, H9), 3.60-3.71 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.8 (q, C8), 15.3 (q, C10), 22.6 (t, C7), 27.2 (t, C6), 30.0 (q, C1), 34.0 (t, C5), 48.5 (t, C3), 64.4 (t, C9), 75.5 (d, C4), 207.8 (s, C2)

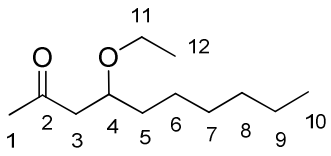
4.3.4.17 4-Ethoxy-decan-2-one [38]



3-Decen-2-one (770 mg, 5 mmol)
Pyrrolidine (108 mg, 1.5 mmol, 0.3 equiv)
Methanesulfonic acid (144 mg, 1.5 mmol, 0.3 equiv.)
EtOH, 5 ml

Procedure: Product **38** was prepared according to the general protocol for synthesis of alkoxyketones.
The crude product **38** was obtained as yellow oil in 70% yield in 70% purity (GC/MS) and was not purified.

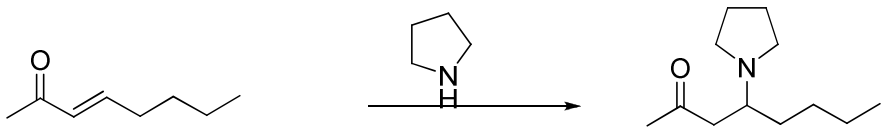
Yield: 700 mg (70%) 70% purity (GC/MS), yellow oil
MW: 200.32 g/mol; $C_{12}H_{24}O_2$



^{1}H-NMR ($CDCl_3$): 0.78-0.81 (m, 3H, H10), 1.08 (t, J=7.0 Hz, 3H, H12), 1.20-1.40 (m, 10H, H5/H6/H7/H8/H9), 2.10 (s, 3H, H1), 2.37 (dd, J=15.6 Hz, J= 4.9 Hz, 1H, $H3_a$), 2.60 (dd, J=15.6 Hz, J=7.4 Hz, 1H, $H3_b$), 3.35-3.45 (m, 2H, H11), 3.61-3.70 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.9 (q, C10), 15,4 (q, C12), 22.4 (t, C9), 25.6 (t, C8), 29.2 (t, C7), 31.0 (q, C1), 31.6 (t, C6), 34.3 (t, C5), 48.5 (t, C3), 64.5 (t, C11), 75.6 (d, C4), 207.8 (s, C2)

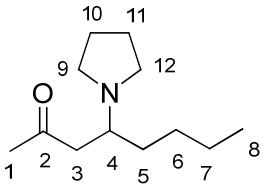
4.3.4.18 4-Pyrrolidin-1-yl-octan-2-one [39]



Pyrrolidine (852 mg, 12 mmol, 1.2 equiv.)
3-Octen-2-one (1260 mg, 10 mmol)

Procedure: Product **39** was prepared according to the General protocol for synthesis of pyrrolidyl-ketones.
The crude product **39** was obtained as yellow oil in 97% yield in 95% purity (GC/MS) and was not purified.

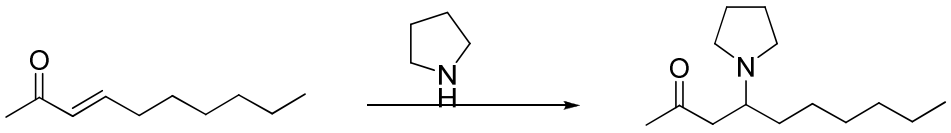
Yield: 1920 mg (97%) 95% purity (GC/MS), yellow oil
MW: 197.32 g/mol; $C_{12}H_{23}NO$



^{1}H-NMR ($CDCl_3$): 0.78 (t, J=6.5 Hz, 3H, H8), 1.17-1.27 (m, 6H, H5/H6/H7), 1.57-1.64 (m, 4H, H10/H11), 2.05 (s, 3H, H1), 2.31-2.53 (m, 6H, H3/H9/H12), 2.71-2.78 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.8 (q, C8), 22.7 (t, C7), 23.3 (t, C10/C11), 27.9 (t, C6), 30.1 (q, C1), 31.9 (t, C5), 45.9 (t, C3), 49.3 (t, C9/C12), 57.4 (d, C4), 208.2 (s, C2)

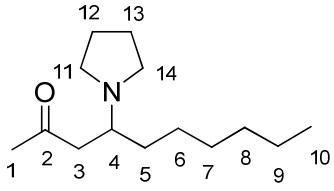
4.3.4.19 4-Pyrrolidin-1-yl-decan-2-one [40]



Pyrrolidine (852 mg, 12 mmol, 1.2 equiv.)
3-Decen-2-one (1540 mg, 10 mmol)

Procedure: Product **40** was prepared according to the General protocol for synthesis of pyrrolidyl-ketones.
The crude product **40** was obtained as yellow oil in 96% yield in 64% purity (GC/MS) and was not purified.

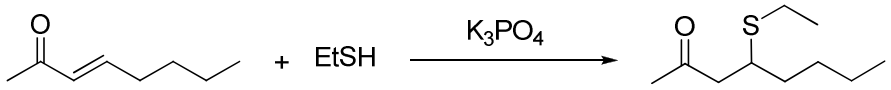
Yield: 2150 mg (97%) 95% purity (GC/MS), yellow oil
MW: 225.37 g/mol; $C_{14}H_{27}NO$



^{1}H-NMR ($CDCl_3$): 0.78-0.84 (m, 3H, H10), 1.17-1.27 (m, 10H, H5/H6/H7/H8/H9), 1.62-1.70 (m, 4H, H12/H13), 2.11 (s, 3H, H1), 2.37-2.56 (m, 6H, H3/H11/H14), 2.78-2.88 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.9 (q, C10), 22.5 (t, C9), 23.4 (t, C12/C13), 25.3 (t, C8), 25.8 (t, C7), 29.5 (t, C6), 30.3 (q, C1), 31.7 (t, C5), 46.7 (t, C3), 49.4 (t, C11/C14), 57,6 (d, C4), 208.4 (s, C2)

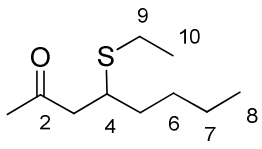
4.3.4.20 4-Ethylsulfanyl-octan-2-one [41]



3-Octen-2-one (504 mg, 4 mmol)
Ethanthiol (248 mg, 4 mmol, 1 equiv.)
K_3PO_4 (338 mg, 1 mmol, 0.25 equiv.)

Procedure: Product **41** was prepared according to the General protocol for synthesis of thioethers.
The crude product **41** was obtained as yellow oil in 95% yield in >95% purity (GC/MS) and was not purified.

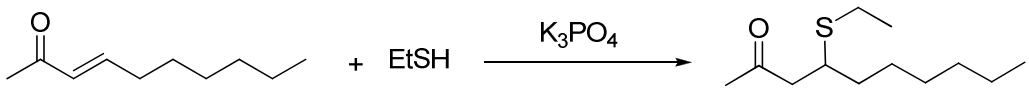
Yield: 711 mg (95%) >95% purity (GC/MS), yellow oil
MW: 188.33 g/mol; $C_{10}H_{20}OS$



^{1}H-NMR ($CDCl_3$): 0.86 (t, J=6.5 Hz, 3H, H8), 1.20 (t, J=7.5 Hz, 3H, H10) 1.28-1.54 (m, 6H, H5/H6/H7), 2.14 (s, 3H, H1), 2.48 (q, J=7.4 Hz, 2H, H9), 2.61-2.67 (m, 2H, H3), 3.00-3.13 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.9 (q, C8), 14.7 (q, C10), 22.5 (t, C7), 24.8 (t, C9), 28.9 (t, C6), 30.7 (q, C1), 35.0 (t, C5), 40.2 (d, C4), 49.6 (t, C3), 207.0 (s, C2)

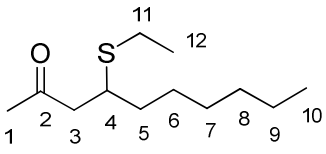
4.3.4.21 4-Ethylsulfanyl-decan-2-one [42]



3-Decen-2-one (616 mg, 4 mmol)
Ethanthiol (744 mg, 12 mmol, 3 equiv.)
K_3PO_4 (338 mg, 1 mmol, 0.25 equiv.)

Procedure: Product **42** was prepared according to the General protocol for synthesis of thioethers.
The crude product **42** was obtained as yellow oil in 95% yield in >99% purity (GC/MS) and was not purified.

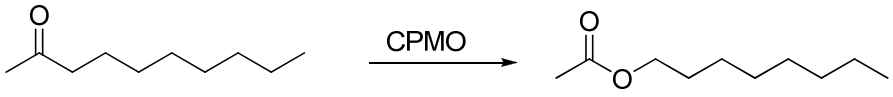
Yield: 864 mg (100%) >99% purity (GC/MS), yellow oil
MW: 216.38 g/mol; $C_{12}H_{24}OS$



^{1}H-NMR ($CDCl_3$): 0.83 (t, J=6.8 Hz, 3H, H10), 1.15 (t, J=7.4 Hz, 3H, H12) 1.22-1.50 (m, 10H, H5/H6/H7/H8/H9), 2.13 (s, 3H, H1), 2.47 (q, J=7.4 Hz, 2H, H11), 2.60-2.67 (m, 2H, H3), 3.00-3.13 (m, 1H, H4)

^{13}C-NMR ($CDCl_3$): 13.9 (q, C10), 14.7 (q, C12), 22.4 (t, C11), 24.7 (t, C9), 26.6 (t, C8), 29.0 (t, C7), 30.6 (q, C1), 31.6 (t, 6), 35.2 (t, C5), 40.2 (d, C4), 49.6 (t, C3), 206.9 (s, C2)

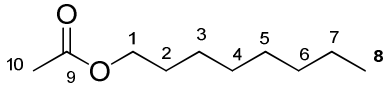
4.3.4.22 Acetic Acid Octyl Ester [54]



2-Decanone **51** (100 mg, 0.64 mmol)
250 mL of fresh LB_{amp} medium
Strain: $CPMO_{Coma}$
IPTG (50 µL, final concentration 0.166mM)

Procedure: Product **54** was prepared according to the general protocol for biotransformations on preparative scale.
The crude product **54** was obtained as yellow oil and was purified by *Kugelrohr* distillation. The product **54** was obtained as colorless oil in 72% yield.

Yield 54[182]**: 79 mg (72%)**, colorless oil
MW: 172.26 g/mol; $C_{10}H_{20}O_2$



^{1}H-NMR ($CDCl_3$): 0.84 (t, J=6.9 Hz, 3H, H8), 1.26 (m, 10H, H3/H4/H5/H6/H7), 1.58 (t, J=6.9 Hz, 2H, H2), 2.00 (s, 3H, H10), 4.01 (t, J=6.7 Hz, 2H, H1)

^{13}C-NMR ($CDCl_3$): 14.0 (q, C8), 20.9 (q, C10), 22.5 (t, C7), 25.8 (t, C6), 28.5 (t, C5), 29.1 (2 x t, C4/C3), 31.7 (t, C2), 64.5 (t, C1), 171.1 (s, C9)

[182] Available at Sigma-Aldrich, CAS Number 112-14-1

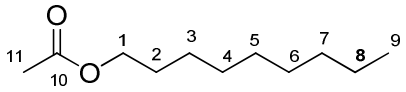
4.3.4.23 Acetic Acid Nonyl Ester [55]

2-Undecanone **52** (100 mg, 0.60 mmol)
250 mL of fresh LB_{amp} medium
Strain: $CPMO_{Coma}$
IPTG (50 µL, final concentration 0.166mM)

Procedure: Product **55** was prepared according to the General protocol for biotransformations on preparative scale.

The crude product **55** was obtained as yellow oil and was purified by *Kugelrohr* distillation. The product **55** was obtained colorless oil in 69% yield.

Yield 55[183]**: 77 mg (69%)**, colorless oil
MW: 186.29 g/mol; $C_{11}H_{22}O_2$



^{1}H-NMR ($CDCl_3$): 0.85 (t, J=6.9 Hz, 3H, H9), 1.18-1.34 (m, 12H, H3/H4/H5/H6/H7/H8), 1.59 (t, J=6.6 Hz, 2H, H2), 2.00 (s, 3H, H11), 4.02 (t, J=6.8 Hz, 2H, H1)

^{13}C-NMR ($CDCl_3$): 14.0 (q, C9), 20.9 (q, C11), 22.6 (t, C8), 25.8 (t, C7), 28.5 (t, C6), 29.1 (t, C5), 29.2 (t, C4), 29.4 (t, C3), 31.8 (t, C2), 64.5 (t, C1), 171.1 (s, C10)

[183] Available at Sigma-Aldrich, CAS Number 143-13-5

5 REFERENCES

1) Krow, G. R. *Org. React.* **1993**, *43*, 251.
2) Baeyer, A.; Villiger, V. *Ber. Dtsch. Chem. Ges*. **1899**, *32*, 3623.
3) Caro, H. *Angew. Chem*. **1898**, 845.
4) Baeyer, A.; Villiger, V. *Ber. Dtsch. Chem. Ges*. **1900**, *33*, 1569.
5) Wittig, G.; Pieper, G. *Ber. Dtsch. Chem. Ges*. **1940**, 73, 295.
6) Criegee, R. *Justus Liebegs Ann. Chem*. **1948**, *560*, 127.
7) Doering, W. v. E; Speers, L. *J. Am. Chem. Soc.* **1950**, *72*, 5515.
8) Doering, W. v. E ; Dorfman, E. *J. Am. Chem. Soc.* **1953**, *75*, 55.
9) Berson, J. A.; Suzuki, S. *J. Am. Chem. Soc.* **1959**, *95*, 4088.
10) Turner, R. B. *J. Am. Chem. Soc*. **1950**, *72*, 878.
11Hawthorne, M. F.; W. D. Emmons, W. D.; McCallum, K. S. *J. Am. Chem. Soc*. **1958**, *80*, 6393.
12) Noyori, R.; Sato, T.; Kobayashi, H. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2661.
13) Krow, G. R. *Tetrahedron*, **1981**, *37*, 2697.
14) Meinwald, J.; Frauenglass, E. *J. Am. Chem. Soc*. **1960**, *82*, 5235.
15) Stukul, G. *Angew. Chem. Int. Ed*. **1998**, *37*, 1198.
16) Del Todesco Frisone, M.; Pinna, F.; Stukul, G. *Organometallics* **1993**, *12*, 148.
17) Gusso, A.; Baccin, C.; Pinna, F. ; Stukul, G. *Organometallics* **1994**, *13*, 3442.
18) Gavagnin, R.; Cataldo, M.; Pinna, F.; Stukul, G. *Organometallics* **1998**, *17*, 661.
19) Hermann, W. A.; Fischer, R. W.; Scherer, W.; Correia, J. D. G. *J. Mol. Catal*. **1994**, *94*, 213.
20) Notari, B. *Adv. Catal*, **1996**, *41*, 253.
21) Giannandrea, R.; Mastrorilli, P.; Nobile, C. F.; Suranna, G.P. *J. Mol. Catal*. **1994**, *94*, 27.
22) Bolm, C.; Schlingloff, G.; Weikhardt, K. *Angew. Chem. Int. Ed*. **1994**, *33*, 1848.
23) Watanabe, A.; Uchida, T.; Ito, K.; Katsuki, T. *Tetrahedron Lett*. **2002**, *43*, 4481.
24) a)Katsuki, T. *Russ. Chem. Bull., Int. Ed*. **2004**, *53*, 1859. b)Mihovilovic, M. D.; Rudroff, F.; Groetzl, B. *Curr. Org. Chem.* **2004**, *8*, 1057.
25) Friess, S. L.; Soloway, A. L. *J. Am. Chem. Soc*. **1951**, *73*, 3968.
26) Hawthorne, M. F.; Emmons, W. D. *J. Am. Chem. Soc*. **1958**, *80*, 6398.
27) Rassant, A.; Ourisson, G. *Bull. Soc. Chim. Fr*. **1959**, 1133.
28) Whitesell, J. K.; Mathews, R. S.; Helbling, A. M. *J. Org. Chem*. **1978**, *43*, 784.
29) a) Mihovilovic, M. D. *Curr. Org. Chem.* **2006**, *11*, 1265. b) Mihovilovic, M. D.; Muller, B.; Stanetty, P. *Eur. J. Org. Chem.* **2002**, *22*, 3711. c) Roberts, S. M.; Wan, P. W. H. *J. Mol. Catal. B: Enzym.* **1998**, *4*, 111. d) Kamerbeek, N. M.; Janssen, D. B.; van Berkel, W. J. H.;

Fraaije, M. W. *Adv. Synth. Catal.* **2003**, *345*, 667. e) Alphand, V.; Carrea, G.; Wohlgemuth, R.; Furstoss, R.; Woodley, J. M. *Trends Biotechnol.* **2003**, *21*, 318.
30) Shaw, R. *Nature* **1966**, *209*, 1369.
31) Colonna, S.; Del Sordo, S.; Gaggero, N.; Carrea, G.; Pasta, P. *Heteroatom Chem.* **2002**, *13*, 467.
32) Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P.; *Chem. Comm.* **1998**, 415.
33) Kelly, D. R.; Knowles, C. J.; Mahdi, J. G.; Taylor, I. N.; Wright, M. A. *Tetrahedron Asymmetry* **1996**, *7,* 365.
34) Ottolina, G.; Bianchi, S.; Belloni, B.; Carrea, G.; Danieli, B.*Tetrahedron Lett.* **1999**, *40*, 8483-8486.
35) Branchaud, B. P.; Walsh, C. *J. Am. Chem. Soc*. **1985**, *107*, 2153.
36) Ryerson, C. C.; Ballou, D. P.; Walsh, C. *Biochem.* **1982**, *21*, 2644.
37) Sheng, D.; Ballou, D. P.; Massey, V. *Biochem.* **2001**, *40*, 11156.
38) Willetts A. *Trends Biotechnol*. **1997**, *15*, 55.
39) Malito, E.; Alfieri, A.; Fraaije, M. W.; Mattevi, A. *Proc. Nat. Acad. Sci. USA* **2004**, *101*, 13157.
40) a) Liao, H.; McKenzie, T.; Hageman, R. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 576. b) Chen, K.; Arnold, F. H. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5618.
41) Garrett, J. B.; Kretz, K. A.; O'Donoghue, E.; Kerovuo, J.; Kim, W.; Barton, N. R.; Hazlewood, G. P.; Short, J. M.; Robertson, D. E.; Gray, K. A. *Appl. Environ. Microbiol.* **2004**, *70*, 3041.
42) a) Olins, P. O.; Bauer, S. C.; Braford-Goldberg, S.; Sterbenz, K.; Polazzi, J. O.; Caparon, M. H.; Klein, B. K.; Easton, A. M.; Paik, K.; Klover, J. A.; et al. *J. Biol. Chem.* **1995**, *270*, 23754; b) Chiang, L. W. *Methods Mol. Biol.* **1996**, *57*, 311; c) Kegler-Ebo, D. M.; Polack, G. W.; DiMaio, D. *Methods Mol. Biol.* **1996**, *57*, 297.
43) a) Leung, D.; Chen, E.; Goeddel, D. *Technique* **1989**, *1*, 11. b) Cirino, P. C.; Mayer, K. M.; Umeno, D. *Methods Mol. Biol.* **2003**, *231*, 3.
44) a) Leung, D.; Chen, E.; Goeddel, D. *Technique* **1989**, *1*, 11. b) Cirino, P. C.; Mayer, K. M.; Umeno, D. *Methods Mol. Biol.* **2003**, *231*, 3.
45) a) Kim, P.; Yoon, S. H.; Seo, M. J.; Oh, D. K.; Choi, J. H. *Biotechnol. Appl. Biochem.* **2001**, *34*, 99. b) Moore, J. C.; Arnold, F. H. *Nat. Biotechnol.* **1996**, *14*, 458. c) Zhao, H.; Arnold, F. H. *Protein Eng.* **1999**, *12*, 47. d) Lingen, B.; Kolter-Jung, D.; Dunkelmann, P.; Feldmann,R.; Grotzinger, J.; Pohl, M.; Muller, M. *ChemBioChem* **2003**, *4*, 721. e) Axarli, I.; Prigipaki, A.; Labrou, N. E. *Biomol. Eng* **2005**, *22*, 81. f) Henke, E.; Bornscheuer, U. T. *Biol. Chem.* **1999**, *380*, 1029.
46) a) Sylvestre, J; Chautard, H.; Cedrone, F.; Delcourt, M. *Org. Process Res. Develop.* **2006**, *10*, 562; b) Stemmer, W. P. *Nature* **1994**, *370*, 389. c) Zhao, H.; Giver, L.; Shao, Z.; Affholter, J. A.; Arnold, F. H. *Nat. Biotechnol.* **1998**, *16*, 258. d) Neylon, C. *Nucleic Acids Res.* **2004**, *32*, 1448. e) Streit, W. R.; Daniel, R.; Jaeger, K. E. *Curr. Opin. Biotechnol.* **2004**, *15*, 285.

47) Zha, D.; Eipper, A.; Reetz, M. T. *ChemBioChem* **2003**, *4*, 34.
48) a) Delcourt M.; Blesa S. Method for massive directed mutagenesis. US patent application 20040048268 (**2004**); FR patent application 20000010962 (**2000**). b) Sylvestre, J.; Blesa, S.; Delcourt, M. *DrugPlus Int.* **2005**.
49) Schmidt-Dannert, C.; Arnold, F. H. *Trends Biotechnol.* **1999**, *17*, 135.
50) Goddard, J. P.; Reymond, J. L. *Curr. Opin. Biotechnol.* **2004**, *15*, 314.
51) a) Wahler, D.; Reymond, J.-L. *Curr.Opin. Biotechnol.* **2001**, *12*, 535; b) Reetz, M. T. *Angew. Chem. Int. Ed.* **2002**, *41*, 1335; c) Goddard, J. P.; Reymond, J.-L. *Trends Biotechnol.* **2004**, *22*, 363; d) Bornscheuer, U. T. *Biocat. Biotrans.* **2004**, *19*, 84.
52) a) Hoogenboom, H. R. *Methods Mol. Biol.* **2002**, *178*, 1; b) Fernandez-Gacio, A.; Uguen, M.; Fastrez, J. *Trends Biotechnol*. **2003**, *21*, 408; c) Lee, S. Y.; Choi, J. H.; Xu, Z. *Trends Biotechnol*. **2003**, *21*, 45.
53) Aharoni, A.; Griffiths, A. D.; Tawfik, D. S. *Curr. Opin. Chem. Biol.* **2005**, *9*, 210.
54) Hummel, W., *Trends Biotechnol.* **1999**, *17*, 487.
55) Wichmann, R.; Wandrey, C.; Bückmann, A.F.; Kula, M.-R. *Biotechnol. Bioeng.* **1981**, *23*, 2789-2802.
56) a) Seelbach, K.; Riebel, B.; Hummel, W.; Kula, M.-R.; Tishkov, V. I.; Egorov, H. M.; Wandrey, C.; Kragl, U. *Tetrahedron Lett.* **1996**, *37*, 1377; b) Tishkov, V. I.; Galkin, A. G.; Marchenko, G. N.; Tsyganokov, Y. D.; Egorov, H. M. *Biotechnol. Appl. Biochem.* **1993**, *18*, 201.
57) a) Rissom, S.; Schwarz-Linek, U.; Vogel, M.; Tishkov, V. I.; Kragl, U. *Tetrahedron Asymm.* **1997**, *8*, 2523; b) Schwarz- Linek, U.; Krödel, A.; Ludwig, F.-A.; Schulze, A.; Rissom, S.; Kragl, U.; Tishkov, V. I.; Vogel, M. *Synthesis* **2001**, 947.
58) Woodyer, R. D.; van der Donk, W. A.; Zhao, H. *Biochemistry* **2003**, *42*, 11604.
59) Torres Pazmino D. E.; Snajdrova, R.; Baas, J. B.; Ghobrial, M.; Mihovilovic, M. D.; Fraaije, M. W. *Angew. Chem. Int. Ed.* **2008**, *47*, 2275.
60) Wong, C.-H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 4890.
61) a) Willetts, A. J.; Knowles, C. J.; Levitt, M. S.; Roberts, S. M.; Sandey, H.; Shipston, N. F. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1608; b) Grogan, G.; Roberts, S.; Willetts, A. J. *Biotechnol. Lett.* **1992**, *14*, 1125.
62) Secundo, F.; Carrea, G.; Riva, S.; Battistel, E.; Bianchi, D. *Biotechnol. Lett.* **1993**, *15*, 865.
63) Hogan, M. C.; Woodley, J. M. *Chem. Eng. Sci.* **2000**, *55*, 2001.
64) Zambianchi, F.; Pasta, P.; Carrea, G.; Colonna, S.; Gaggero, N; Woodley, J. M. *Biotechnol. Bioeng.* **2002**, *78*, 489.
65) Walton, A. Z.; Steward, J. D. *Biotechnol. Prog.* **2002**, *18*, 262.
66) Alphand, V.; Carrea, G.; Wolgemuth, R.; Furstoss, R. *Trends Biotechnol.* **2003**, *21*, 318.
67) Holker, I.; Wolgemuth, R.; Alphand, V.; Furstoss, R. *Biotechnol. Bioeng.* **2005**, *92*, 207.
68) Rudroff, F.; Alphand, V.; Furstoss, R.; Mihovilovic, M. D. *Org, Process Res. Develop.* **2006,** *10,* 599.

69) Fort, A. W. *J. Am. Chem. Soc.* **1962**, *84*, 4979.
70) Hartung, I. H.; Hoffmann, H. M. R. *Angew. Chem. Int. Ed.* **2004,** *43*, 1934.
71) Albizati, K. F.; Murray, D. H. *Tetrahedron Lett.* **1990**, *31*, 4109.
72) Hoffmann, H. M. R. *Angew. Chem.* **1984**, *96*, 29.
73) Noyori, R.; Hayakawa, H.; Takaya, H.; Murai, S.; Kobayashi, R.; Sonoda, N. *J. Am. Chem. Soc.* **1978,** *100,* 1759.
74) a) Ishizu, T.; Mori, M.; Kanemazsu, K. *J. Org. Chem.* **1981**, *46,* 526; b) Paquette L. A.; Kravetz, T. M. *J. Org. Chem.* **1985**, *50* ,3781.
75) Noyori, R.; Nishizawa, M.; Shimizu, F.; Hayakawa, Y.; Maruoka, K.; Hashimoto, S.; Yamamoto, H.; Nozaki, H. *J. Am. Chem. Soc.* **1979**, *101*, 220.
76) Siemionko, R. K.; Berson, J. A. *J. Am. Chem. Soc.* **1980,** *102,* 3870.
77) Noyori, R.; Baba, Y.; Makino, S.; Takaya, H. *Tetrahedron Lett*. **1973,** 1741.
78) a) Vinter, J. G.; Hoffmann, H. M. R. *J. Am. Chem. Soc.* **1973,** *95,* 3051; b) Hofmann, H. M. R. *Angew. Chem.* **1972**, *11*, 324; c) Greenwood, G.; Hoffmann, H. M. R. *J. Org. Chem.* **1972**, *37,* 611; d) Hoffmann, H. M. R.; Weber, A.; Giguere, R. J. *Chem. Ber.* **1984**, *117*, 3325.
79) Schmid, R.; Schmid, H. *Helv. Chim. Acta* **1974,** *57,* 1883.
80) Fohlisch, B.; Gehrlach, E.; Herter, R. *Angew. Chem. Int. Ed.* **1982**, *21*, 137.
81) Herter, R.; Föhlisch, B. *Synthesis* **1982**, 976.
82) Kaiser, R.; Föhlisch, B. *Helv. Chim. Acta* **1990**, *73,* 1504.
83) Sasaki, T.; Ishibashi, Y.; Masatomi, O. *Tetrahedron Lett.* **1982**, *23*, 1693.
84) Montana, A. M.; Grima, P. M. *Tetrahedron Lett.* **2001**, *42*, 7809.
85) Noyori, R.; Souchi, T.; Hayakawa, Y. *J. Org. Chem.* **1975**, *40,* 2681.
86) a) Hoffmann, H. M. R.; Wagner, D.; Wartchow, R. *Chem. Ber.* **1990**, *123,* 2131; b) Schottelius, T.; Hoffmann, H. M. R. *Chem. Ber.* **1991,** *124*, 1673; c) Busch, A.; Hoffmann, H. M. R. *Tetrahedron. Lett.* **1976**, 2379.
87) Hoffmann, H. M. R.; Iqbal, M. N. *Tetrahedron Lett*. **1975**, *50*, 4487.
88) Hoffmann, H. M. R.; Clemens, E. K.; Smithers, R. H. *J. Am. Chem. Soc.* **1972**, *94*, 3940.
89) Sato, T.; Noyori, R. *Bull. Chem. Soc. Jpn.* **1978**, *51,* 2745.
90) A. E. Hill, G. Greenwood, H. M. R. Hoffmann, *J. Am. Chem. Soc.,* **1973**, *95*, 1338
91) Ramos Tombo, G. M.; Pfund, R. A.; Ganter, C. *Helv. Chim. Acta* **1981**, *64,* 813.
92) a) Fohlisch, B.; Joachimi, R. *Chem. Ber.* **1987**, *120*, 1951; b) Fohlisch, B.; Joachimi, R.; Reiner, S. *J. Chem. Res. (S)* **1993**, 253.
93) Harmata, M.; Elahmad, S. *Tetrahedron Lett.* **1993**, *34*, 789.
94) a) Föhlisch, B.; Herter, R.; Wolf, E.; Stezowski, J. J.; Eckle, E. *Chem. Ber.* **1982**, *115*, 355; b) Föhlisch, B.; Wolf, E. *J. Chem. Res. (S)* **1983**, *7*, 166.
95) Mann, J.; Wilde, P. D.; Finch, M. W. *Chem. Commun.* **1985**, 1543.
96) Mann, J.; Usmani, A. A. *Chem. Commun.* **1980**, 1119.
97) Shimizu, N.; Tanaka, M.; Tsuno, Y. *J. Am. Chem. Soc.* **1982,** *104,* 1330.
98) Sakurai, H.; Shirahata, A.; Hosomi, A. *Angew. Chem. Int. Ed.* **1979**, *18*, 163.

99) Mihovilovc, M. D.; Grötzl, B.; Kandioller, W.; Snajdrova, R.; Muskotal, A.; Bianchi, D.; Stanetty, P. *Adv. Synth. Catal.* **2006**, *348*, 463.

100) a) Harmata, M. *Tetrahedron* **1997**, *53,* 6235; b) Harmata, M. *Acc. Chem. Res.* **2001**, *34*, 595.

101) Hoffmann, H. M. R.; Eggert, U.; Gibbels, U.; Giesel, K.; Koch, O.; Lies, R.; Rabe, J. *Tetrahedron* **1988**, *44,* 3899.

102) a) Giguere, R. J.; Duncan, S. M.; Bean, J. M.; Purvis, L. *Tetrahedron Lett.* **1988**, *29,* 6071; b) Giguere, R. J.; Tassely, S. M.; Rose, M. I.; Krishnamurthy, V. V. *Tetrahedron Lett.* **1990**, *31,* 4577.

103) a) Harmata, M.; Gamlath, C. B. *J. Org. Chem.* **1988**, *53,* 6154; b) Harmata, M.; Herron, B. F. *J. Org. Chem.* **1993**, *58,* 7393; c) Harmata, M.; Herron, B. F. *Synthesis* **1993**, 202.

104) Kuja, E.; Giguere, R. J. *Synth. Commun.* **1995,** *25*, 2105.

105) Harmata, M.; Herron, B. F. *Tetrahedron Lett.* **1993**, *34*, 5381.

106) a) Connon, S. J.; Blechert, S. *Angew. Chem. Int. Ed.* **2003**, *42*, 1900; b) Fürstner, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 3012; c) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem. Int. Ed.* **2005**, *44*, 4490.

107) Hérisson, J.-L.; Chauvin, Y. *Makromol. Chem.* **1970**, *141*, 161.

108) a) Schrock, R. R.; Murdzek, J. S.; Bazan, G. C.; Robbins, J.; DiMare, M.; O`Regan, M. *J. Am. Chem. Soc.* **1990**, *112*, 3875; b) Oskam, J. H.; Fox, H. H.; Yap, K. B.; McConville, D. H.; O`Dell, R.; Lichtenstein, B. J.; Schrock, R. R. *J. Organomet. Chem.* **1993**, *459*, 185; c) Feldman, J.; Murdzek, J. S.; Davis, W. M.; Schrock, R. R. *Organometallics* **1989**, *8*, 2260.

109) a) Nguyen, S. T.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 9858; b) Wu, Z.; Nguyen, S. T.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 5503; c) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100; d) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **1995**, *34*, 2039.

110) Grubbs, R. H.; O`Leary, D. J.; Blackwell, H. E.; Washenfelder, R. A. *Tetrahedron Lett.* **1998**, *39*, 7427.

111) Grubbs, R.H.; Ulman, M. *Organometalics* **1998**, *17*, 1484.

112) Roy, R.; Das, S. K.; Dominique, R.; Trono, M. C.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *Pure Appl. Chem.* **1999**, *71*, 565.

113) a) Smith III, A. B.; Kozmin, S. A.; Adams, C. M.; Paone, D. V. *J. Am. Chem. Soc.* **2000**, *122*, 4984; b) Smith III, A. B.; Adams, C. M.; Kozmin, S. A. *J. Am. Chem. Soc.* **2001**, *123*, 990; c) Smith III, A. B.; Adams, C. M.; Kozmin, S. A.; Paone, D. V. *J. Am. Chem. Soc.* **2001**, *123*, 5925.

114) a) La, D. S.; Ford, J. G.; Sattely, E. S.; Bonitatebus, P. J.; Schrock, R. R.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1999**, *121*, 11603; b) La, D. S.; Sattely, E. S.; Ford, J. G.; Schrock, R. R.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2001**, *123*, 7767.

115) Bode, J.W.; Doyle, M.P.; Protopopova, M.N.; Zhou, Q.-L. *J. Org.Chem.* **1996**, *61*, 9146-9155.

116) Jin, S.J.; Choi, J.R.; Oh, J.; Lee, D.; Cha, J.K. *J. Am. Chem. Soc.* **1995**, *117,* 10914.

117) Harmata, M.; Shao, L.; Kürti, L. Abeywardane,A. *Tetrahedron Lett.* **1999**, *40*, 1075.
118) Machinskaia, I.V.; Podberezina, A.S. *J. Gen. Chem. USSR* **1958**, 1550.
119) Herter, R.; Föhlisch, B. *Synthesis* **1982**, 976.
120) Föhlisch, B.; Joachimi, R. *Chem. Ber.* **1987**, *120,* 1951.
121) Schmid, R.; Schmid, H. *Helv. Chim. Acta* **1974,** *57***,** 1883.
122) Mihovilovic, M. D.; Groetzl, B.; Kandioller, W.; Snajdrova, R.; Muskotal, A.; Bianchi, D. A.; Stanetty, P. *Adv. Synth. Catal.* **2006**, *348(4 + 5)*, 463.
123) Kim, H.; Hoffmann, H. M. R. *Eur. J. Org. Chem.* **2000,** 2195.
124) Föhlisch, B.; Korfant, M.; Meining, H.; Frey, W. *Eur. J. Org. Chem.* **2000,** 1335.
125) Hodge, P.; Rickards, R. W. *J. Chem .Soc.* **1963,** 2543.
126) Lee, J.; Oh, J.; Choi, J.-R.; Atwood, J. L.; Cha, J. K. *J. Org. Chem* .**1994,** *59*, 6955.
127) Zimmerman, H. E.; Linder, L. W., *J. Org. Chem.* **1985,** *50,* 1637.
128) Mihovilovic, M. D.; Grötzl, B.; Kandioller, W.; Snajdrova, R.; Muskotal, A.; Bianchi, D. Stanetty, P. *Adv. Synth. Catal.* **2006**, *348*, 463.
129) Harmata, M.; Elahmad, S.; Barnes, C. L. *J. Org. Chem* .**1994,** *59*, 1335.
130) Kandioller, W. *Diploma Thesis*, 70.
131) Trost, B. M. ; Bogdanowicz, M. J. *J. Amer. Chem. Soc.* **1973,** *95*, 5321.
132) Mehta, G.; Kumaran, S. , *Tetrahedron Lett*. **2001**, *42*, 8097.
133) Fraaije, M. W.; Kamerbeek, N. M.; van Berkel, W. J. H.; Janssen, D. B., *FEBS Lett.* **2002**, *518*, 43.
134) Mihovilovic, M. D.; Rudroff, F.; Grötzl, B.; Kapitan, P; Snajdrova,R.; Rydz, J.; Mach, R. *Angew. Chem. Int. Ed.* **2005**, *44*, 3609.
135) a) Donoghue, N, A.; Norris, D. B.; Trudgill, P.W. *Eur. J. Biochem.***1976**, *63*, 175. b) Stewart, J.D. *Curr. Org. Chem.* **1998**, *2*, 195.
136) Brzostowicz, P.; Gibson, K. L.; Thomas, S. M.; Blasko, M. S.; Rouviere, P. E. *J. Bacteriol.* **2000**, *182*, 4241.
137) Brzostowicz, P.; Walters, D. M.; Thomas, S. M.; Nagarajan, V.; Rouviere, P. E. *Appl. En*V*iron. Microbiol.* **2003**, *69*, 334.
138) a) Bramucci, M. G.; Brzostowicz, P. C.; Kostichka, K. N.; Nagarajan, V.; Rouviere, P. E.; Thomas, S. M. E. I. DuPont de Nemours and Co., U.S.A. Identification, cloning and sequences of bacterial Baeyer- Villiger monooxygenases and encoding genes and application to the conversion of ketones to the corresponding esters. International Patent, WO 2003020890, 2003. b) Bramucci, M. G.; Brzostowicz, P. C.; Kostichka, K. N.; Nagarajan, V.; Rouviere, P. E.; Thomas, S. M. *Chem. Abstr.* **2003**, *138*, 233997.
139) a) Griffin, M.; Trudgill, P. W. *Eur. J. Biochem.* **1976**, *63*, 199. b) Iwaki, H.; Hasegawa, Y.; Wang, S.; Kayser, M. M.; Lau, P. C. K. *Appl. En*V*iron. Microbiol.* **2002**, *68*, 5671.
140) van Beilen, J. B.; Mourlane, F.; Seeger, M. A.; Kovac, J.; Li, Z.; Smits, T. H. M.; Fritsche, U.; Witholt, B., *Environ. Microbiol.* **2003**, *5*, 5174.
141) Rial, D. V., Bianchi, D. A., Kapitanova, P., Lengar, A., van Beilen, J. B., Mihovilovic, M. D. *Eur. J. Org. Chem.,* **2008**, 1203.

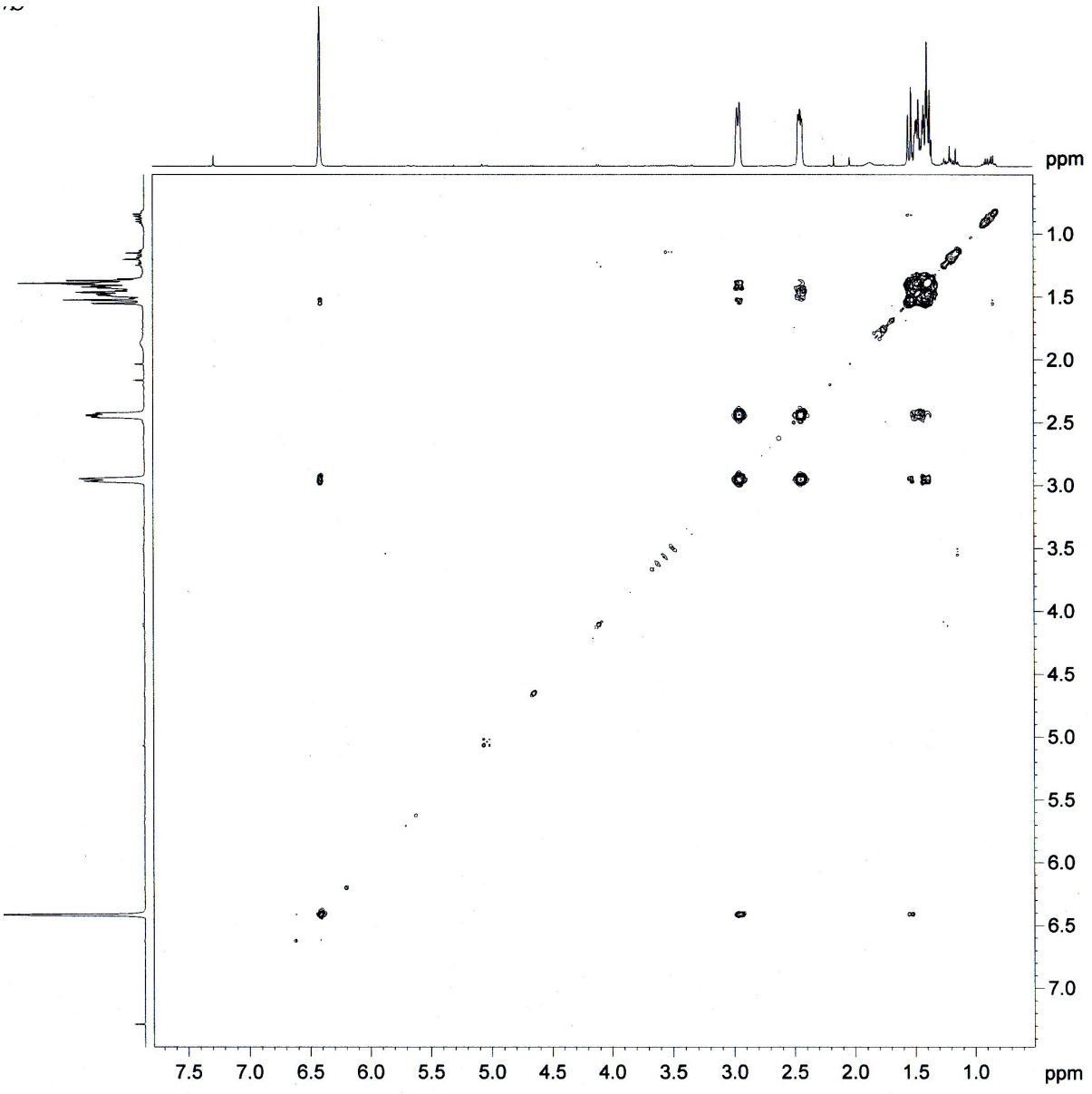
142) Kirschner, A.; Altenbuchner, J.; Bornscheuer, U. T., *Appl. Microbiol. Biotechnol.* **2006**, *73*, 1065.
143) Kirschner, A.; Bornscheuer, U. T., . *Angew. Chem. Int. Ed.* **2006**, *45*, 7004.
144) a) Fraaije, M. W.; Wu, J.; Heuts, D. P. H. M.; van Hellemond, E. W.; Spelberg, L. J. H.; Janssen, D. B., *Appl. Microbiol. Biotechnol.* **2005**, *66*, 393; b) Torres, Pazmino, D. E.; Snajdrova, R.; Rial, D. V.; Mihovilovic, M. D.; Fraaije, M. W., *Adv. Synth. Catal.* **2007**, *349,* 1361.
145) Kamerbeek, M. N.; Moonen, M. J. H.; van der Ven, J. G. M.; van Berkel, W. J. H.; Fraaije, M. W.; Janssen, D. B., *Eur. J. Biochem.* **2001**, *268*, 2547.
146) Rial, D. V.; Cernuchova, P.; van Beilen, J. B.; Mihovilovic, M. D., *J. Mol. Catal. B: Enzym.* **2008**, *50*, 61.
147) Berezina, N.; Alphand, V.; Furstoss, R. *Tetrahedron Asymm.* **2002**, *13*, 1953.
148) Reetz, M. T.; Brunner, B.; Schneider, T.; Schulz, F.; Clouthier, C. M.; Kayser, M. M. *Angew. Chem.* **2004**, *116*, 4167.
149) Mihovilovic, M. D.; Rudroff, F.; Winninger, A.; Schneider, T.; Schulz, F.; Reetz, M. T. *Org. Lett.* **2006**, *8*, 1221.
150) The mutant strains were provided by Prof. Dr. M. T. Reetz, Max-Planck-Institut für Kohlenforschung Mülheim/Ruhr (Germany)
151) Substrates **9a** and **13a** (Tetracycles) were provided by Prof. Dr. Thorsten Bach, Technical University of Munich, Germany
152) a) Oskam, J. H.; Schrock, R., *J. Am. Chem. Soc.* **1992**, *114*, 7588; b) Attar, S.; Nelson, H., *Organometallics* **1995**, *14*, 4559; c) Keane, J. M.; Ding,F.; Sabat, M; Harman, W. D., *J. Am. Chem. Soc.* **2004**, *126*, 785; d) Feast, W. J.; Gibson, V. C.; Ivin, K. J.; Kenwright, A. M.; Khosravi, E., *J. Chem. Soc. Chem. Commun.* **1994**, 1399.
153) Feringa, B. L.; van Delden, R. A.; Koumura, N.; Geertsema, E. M., *Chem. Rev.* **2000**, *100*, 1789.
154) Bhalla, V.; Kumar, M.; Kabuto, C.; Hattori, T.; Sotaro, M., *Chem. Lett.* **2004**, *33*, 184.
155) Tumabac, G. E.; Mei, X.; Wolf, C., *Eur. J. Org. Chem.* **2004**, 3850.
156) Klärner, F.-G.; Wurche, F.; Doering, W. von E.; Yang, J., *J. Am. Chem. Soc.* **2005**, *127*, 18107.
157) Rix, U.; Zheng, J.; Remsing Rix, L. L.; Greenwell, L.; Yang, K.; Rohr, J., *J. Am. Chem. Soc.* **2004**, *126*, 4496.
158) Clayden, J.; Vallverdu, L; Clayton, J.; Helliwell, M., *Chem. Comm.* **2008**, 561.
159) Miaura, M; Ikegami, A.; Nojima, M.; Kusabayashi, S.; McCollough, K. J.; Nagase, S., *J. Am. Chem. Soc.* **1983**, *105*, 2414.
160) Stork, G.; Kraus, G. A.; Garcia, G. A., *J. Org. Chem.* **1974**, *39*, 3459.
161) Smith, A. B.; Levenberg, P. A., *Synthesis*, **1981**, 567.
162),Ito, H.; Kawakami, C.; Sawamura, M. *J. Am. Chem. Soc.* **2005**, *127*, 16034.
163) Jones, A. D.; Knight, D. W.; Hibbs, D. E. *J. Chem. Soc., Perkin Trans. 1*, **2001**, 1182.
164) Ramachary, D. B. ; Modal, R. *Tetrahedron Lett.* **2006**, *47*, 7689.

165) Ranu, B. C.; Dey, S. S.; Hajra, A. *Arkivoc* **2002,** *vii*, 76.
166) Pore, D. M.; Desai, U. V.; Thopate, T. S.; Wadagaonkar, P. P. *Tetrahedron Lett.* **2006**, *47*, 9325.
167) Park, P; Kozikowski, A. P., *Tetrahedron Lett.* **1988***, 29*, 6703.
168) Faber, K.; Hönig, H.; Kleewein, A., Free shareware programs (Selectivity-1.0) for calculation of the Enantiomeric Ratio, available from the author's website: http://borgc185.kfunigraz.ac.at./.
169) a) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294; b) Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237; c) Gredig, G.; Fajans, K. *Chem. Ber.* **1908**, *41*, 752.
170) Compound **29** was provided by Andras Kotschy, associate Professor, Institute of Chemistry, Eötvös Lorand Universtiy, Budapest, Hungary
171) LeGoff, E., *J. Org. Chem*. **1964**, *29*, 2048.
172) Kourouli, T.; Kefalas, P.; Ragoussis, N.; Ragoussis, V. *J. Org. Chem.* **2002**, *67*, 4615.
173) Inokuchi, T.; Kusumoto M.; Torii, S. *J. Org. Chem.* **1990***, 55*, 1548.
174) Bonini, C.; Righi, C.; Sotgiu, G. *J. Org. Chem.* **1991***, 56*, 6206.
175) Ogawa, H.; Ide, Y.; Honda, R.; Chihara, T. *J. Phys. Org. Chem.* **2003**, *16*, 355.
176) Moghadam, M.; Tangestaninejad, S.; Mirkhani, V.; Shaibani, R. *Tetrahedron* **2004**, *60*, 6105.
177) McGarth, M. J.; Fletcher, M. T.; König, W. A.; Moore, C. J.; Cribb, B. W.; Allsopp, P. G.; Kitching, W., *J. Org. Chem.* **2003**, *68*, 3739.
178) Mandai, t.; Nokami, J.; Yano, T.; Yoshinaga, Y.; Otera, J. ., *J. Org. Chem.* **1984**, *49*, 172.
179) Branytska, O.; Neumann, R. *Synlett* **2005,** *16*, 2525.
180) a) Ramachary, D. B.; Mondal, R. *Tetrahedron Lett.* **2006**, *47*, 7689. b) Matsuda, I.; Hasegawa, Y.; Makino, T.; Itoh, K. *Tetrahedron Lett.* **2000**, *41*, 1405.
181) Dodda, R.; Zhao, C.-G. *Synthesis* **2006**, *19*, 3238.
182) Available at Sigma-Aldrich, CAS Number 112-14-1
183) Available at Sigma-Aldrich, CAS Number 143-13-5

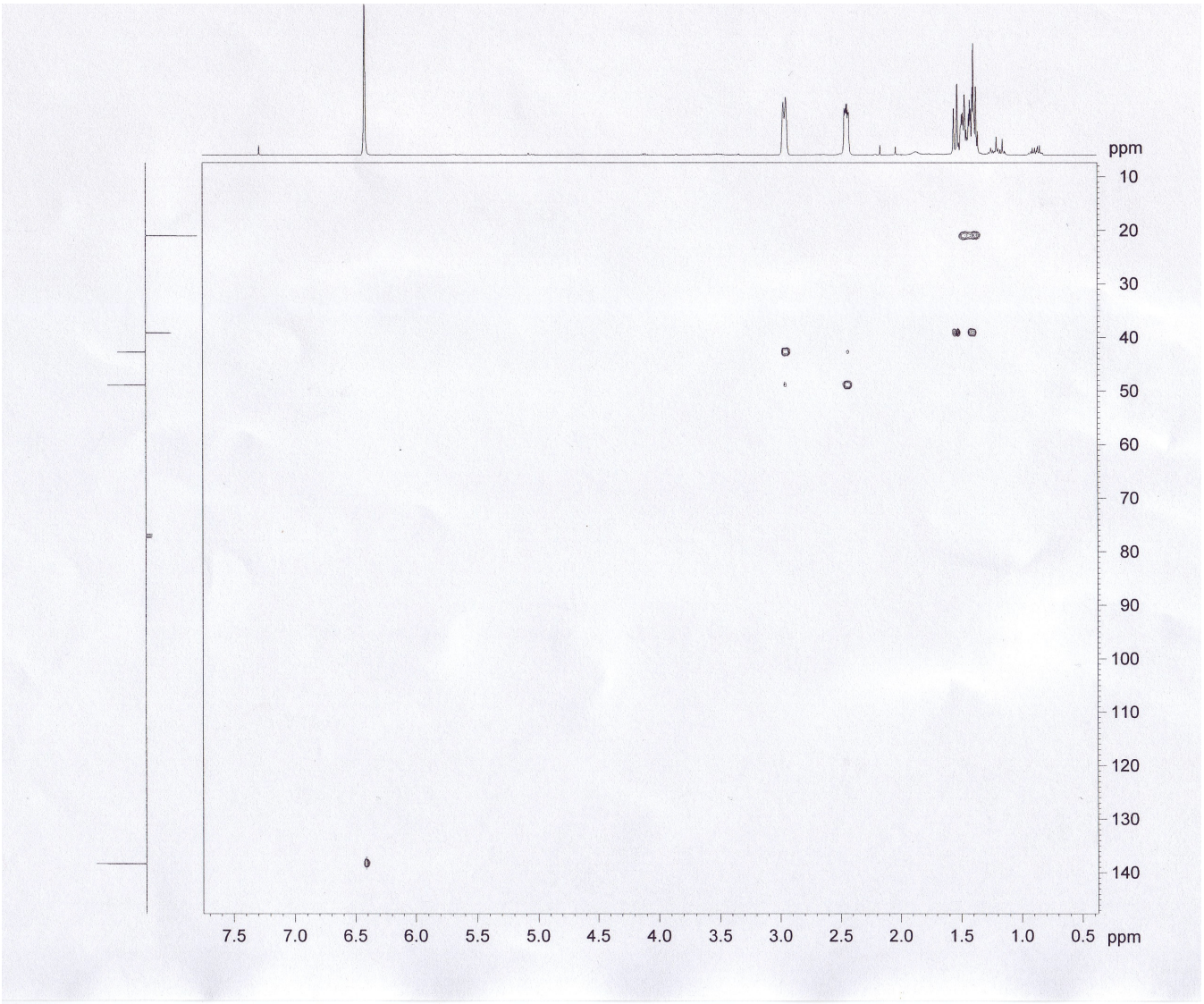
6 APPENDIX

6.1 2D NMR Spectra of *syn*-Tricyclo[4.2.1.1^{2,5}] dec-3-en-9-one [9*syn*]

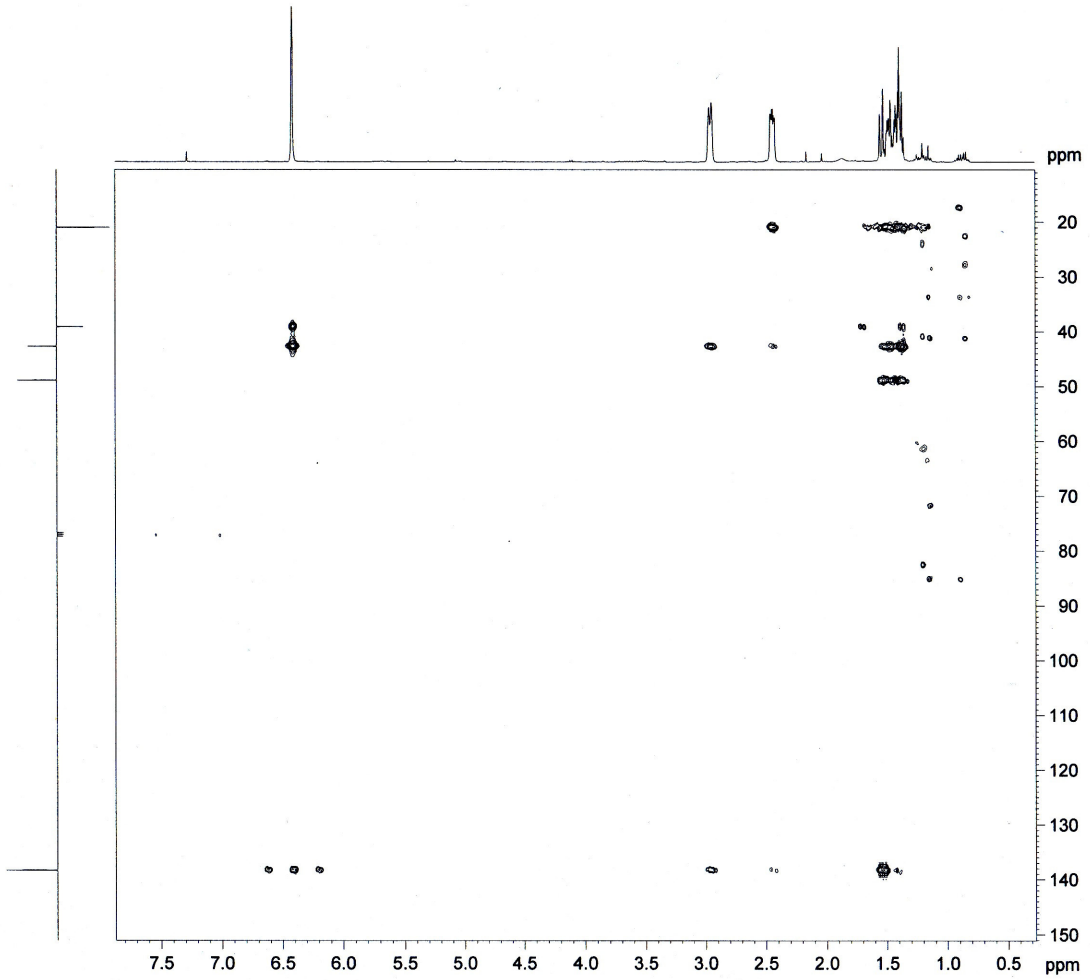
COSY 9syn



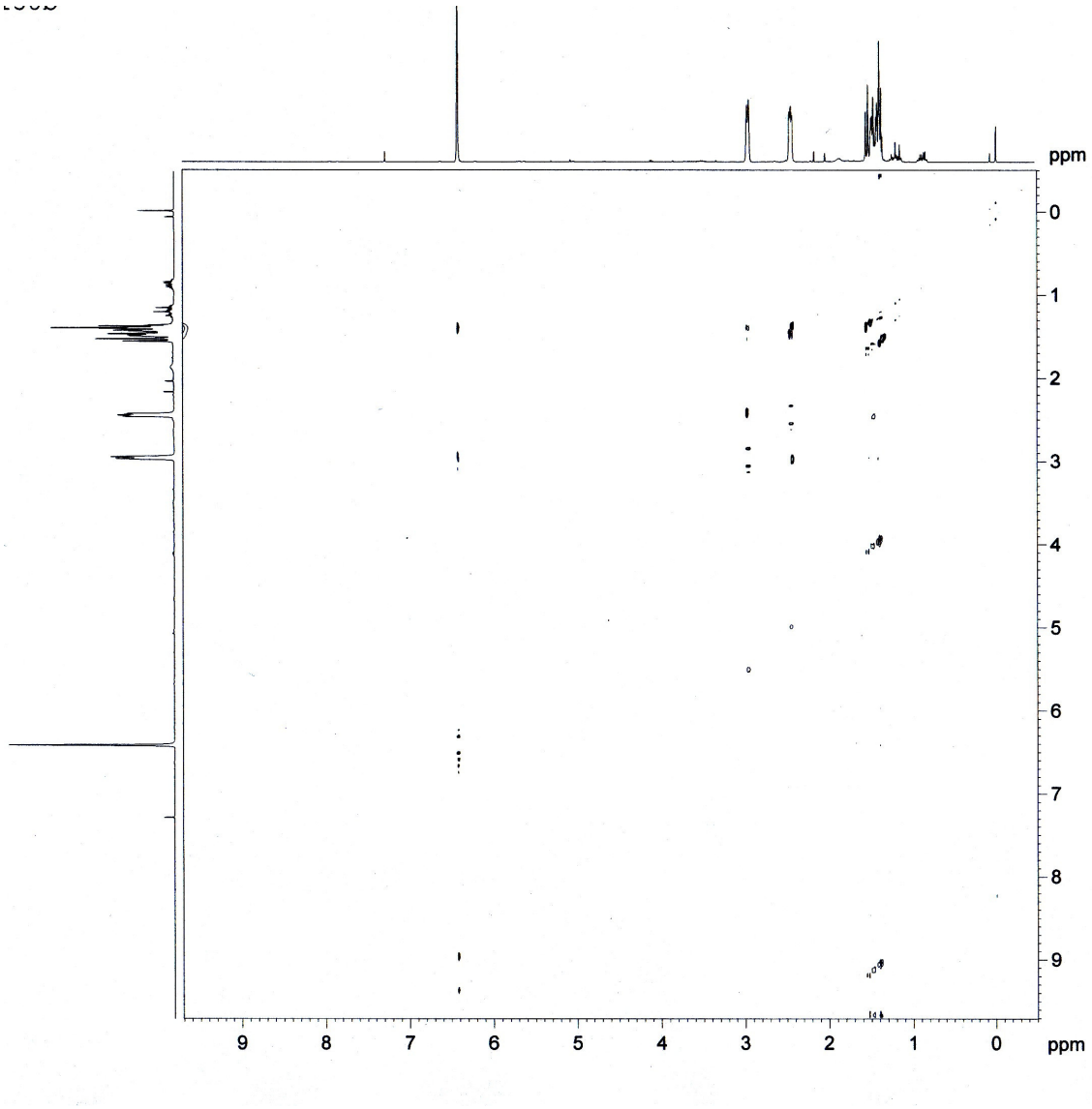
HSQC 9syn


ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

HMBC 9syn

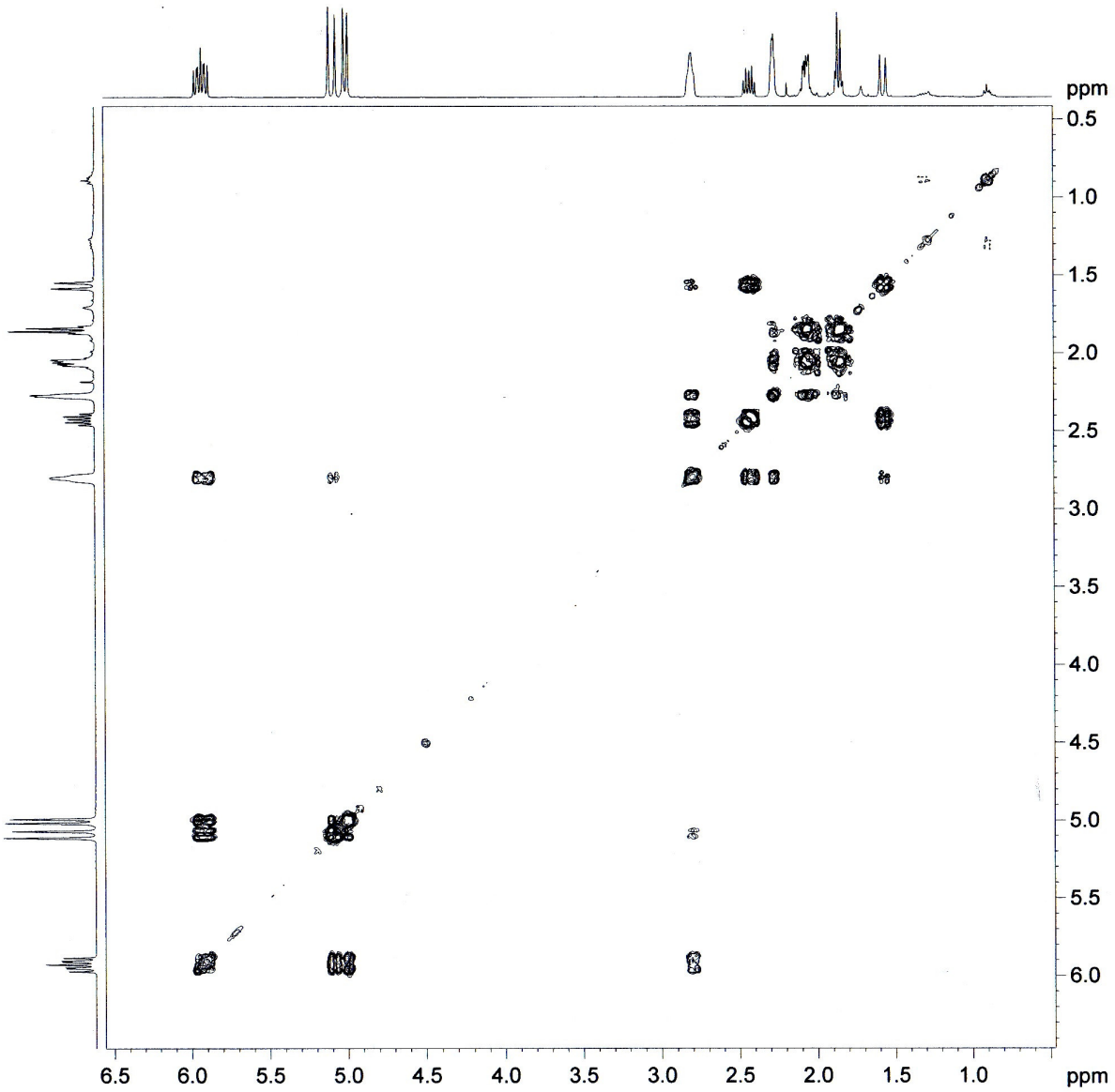

ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
150
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

NOESY 9syn

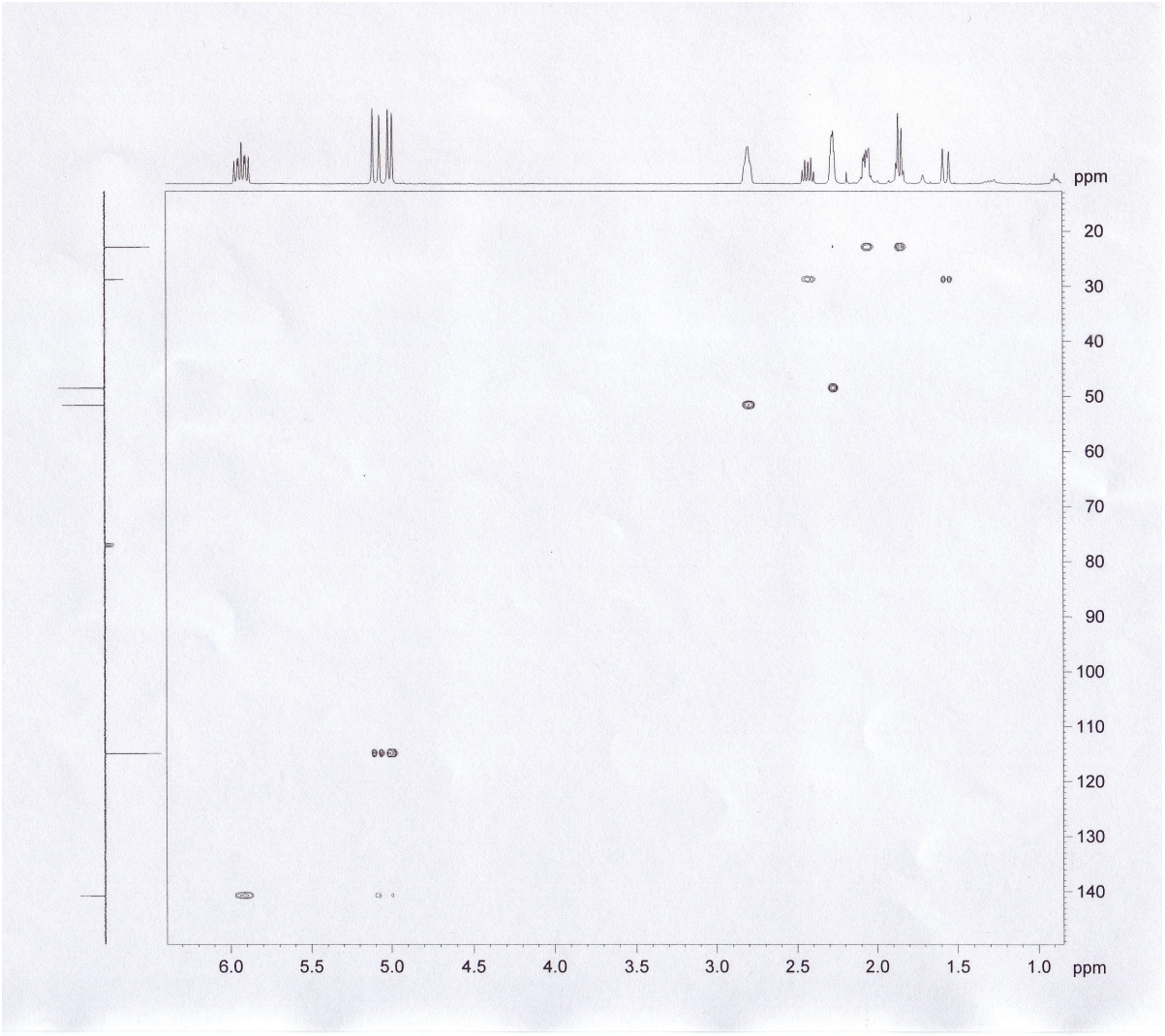


6.2 2D NMR Spectra of *anti*-2,4-Divinyl-bicyclo[3.2.1]octan-8-one [11*anti*]

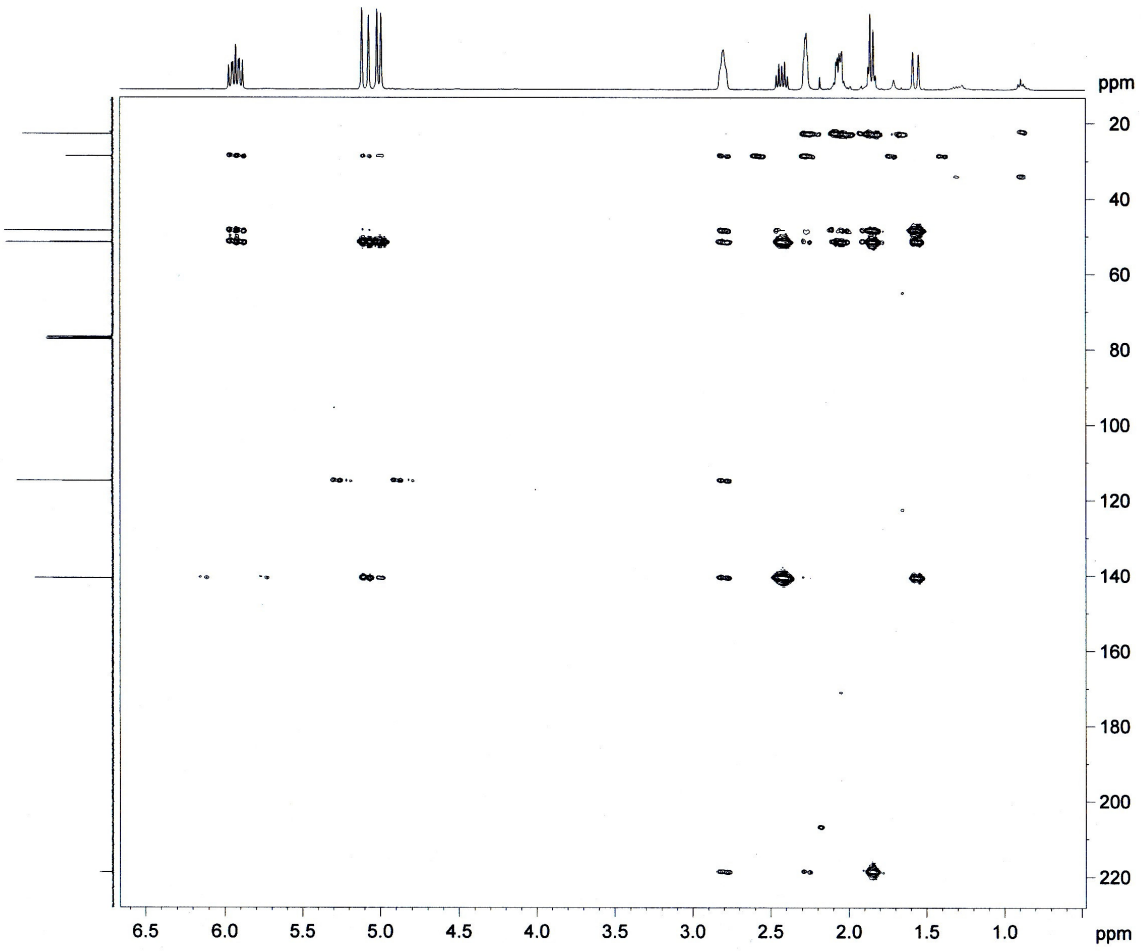
COSY 11anti



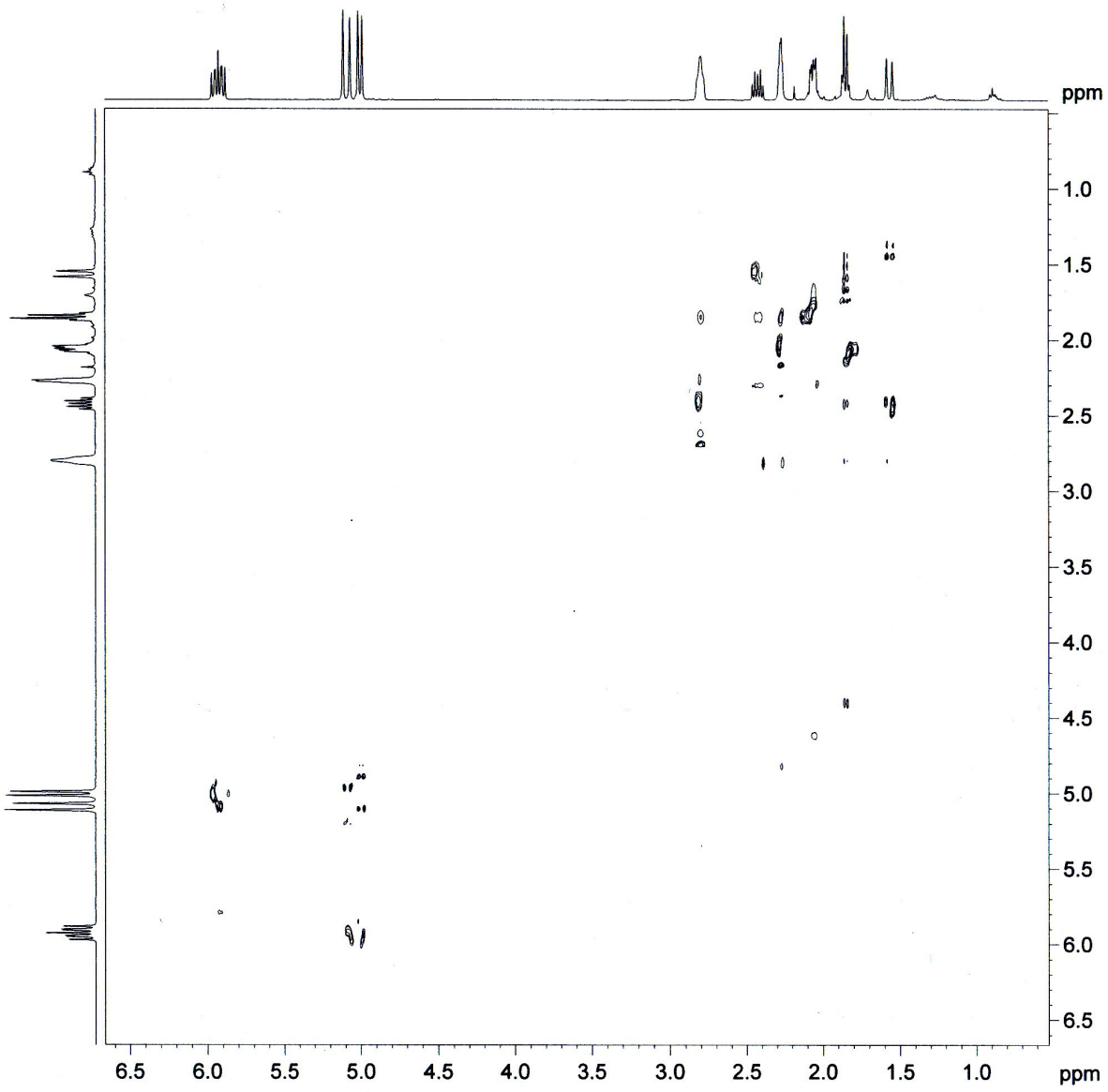
HSQC 11anti


ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

HMBC 11anti

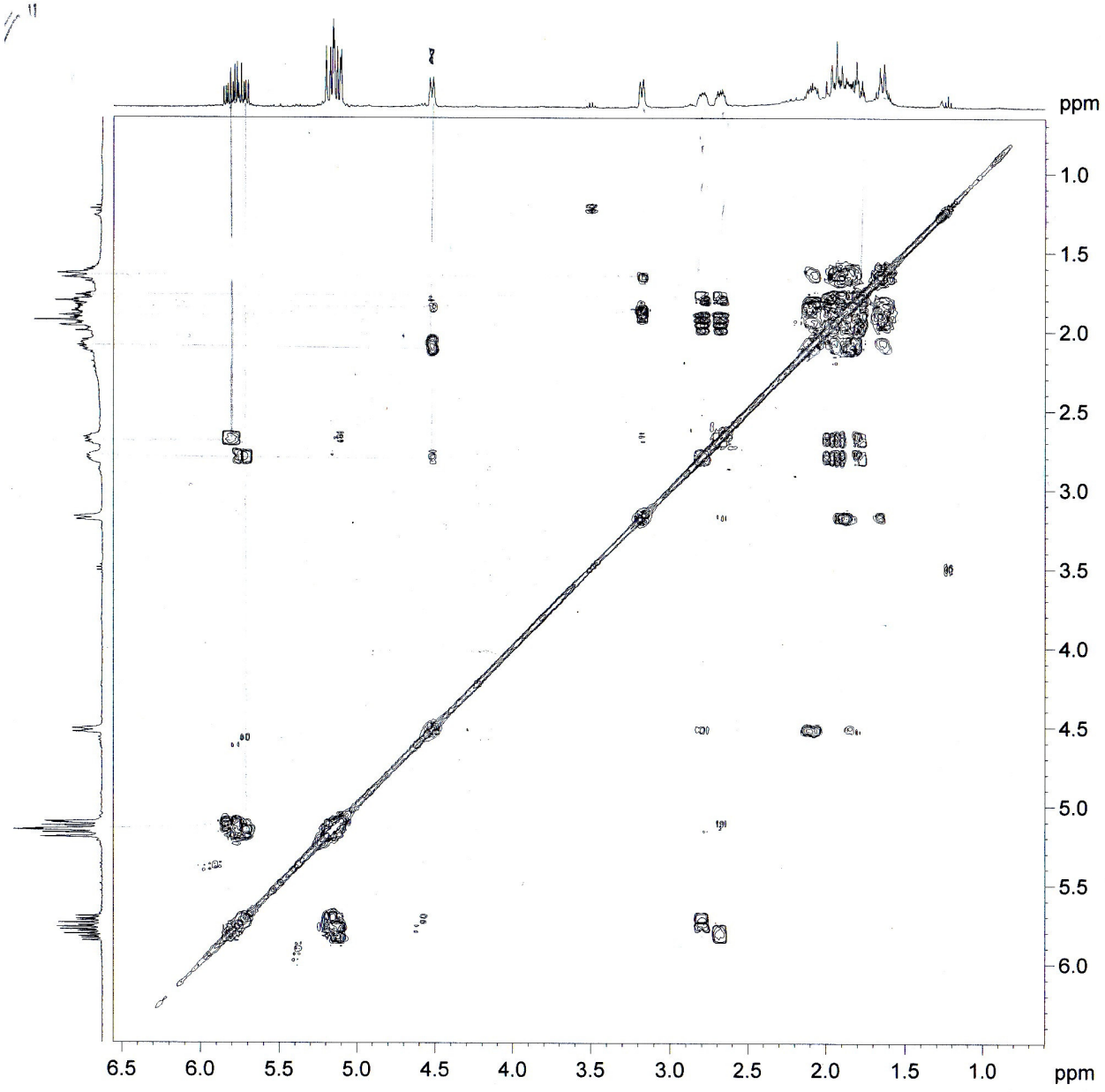

ppm
20
40
60
80
100
120
140
160
180
200
220
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

NOESY 11anti

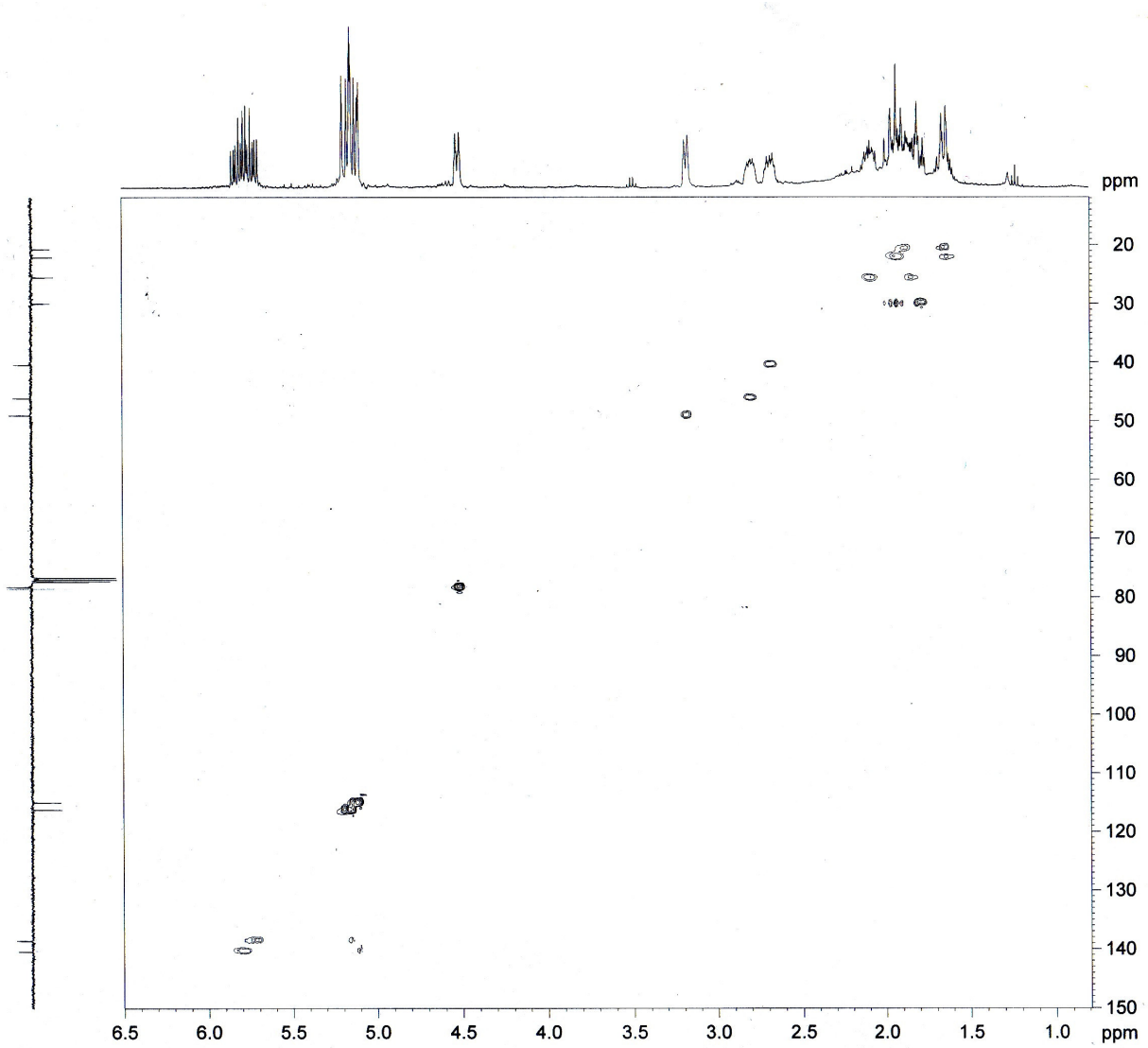


6.3 2D NMR Spectra of 2,4-Divinyl-9-oxabicyclo[3.3.2]decan-10-one[16]

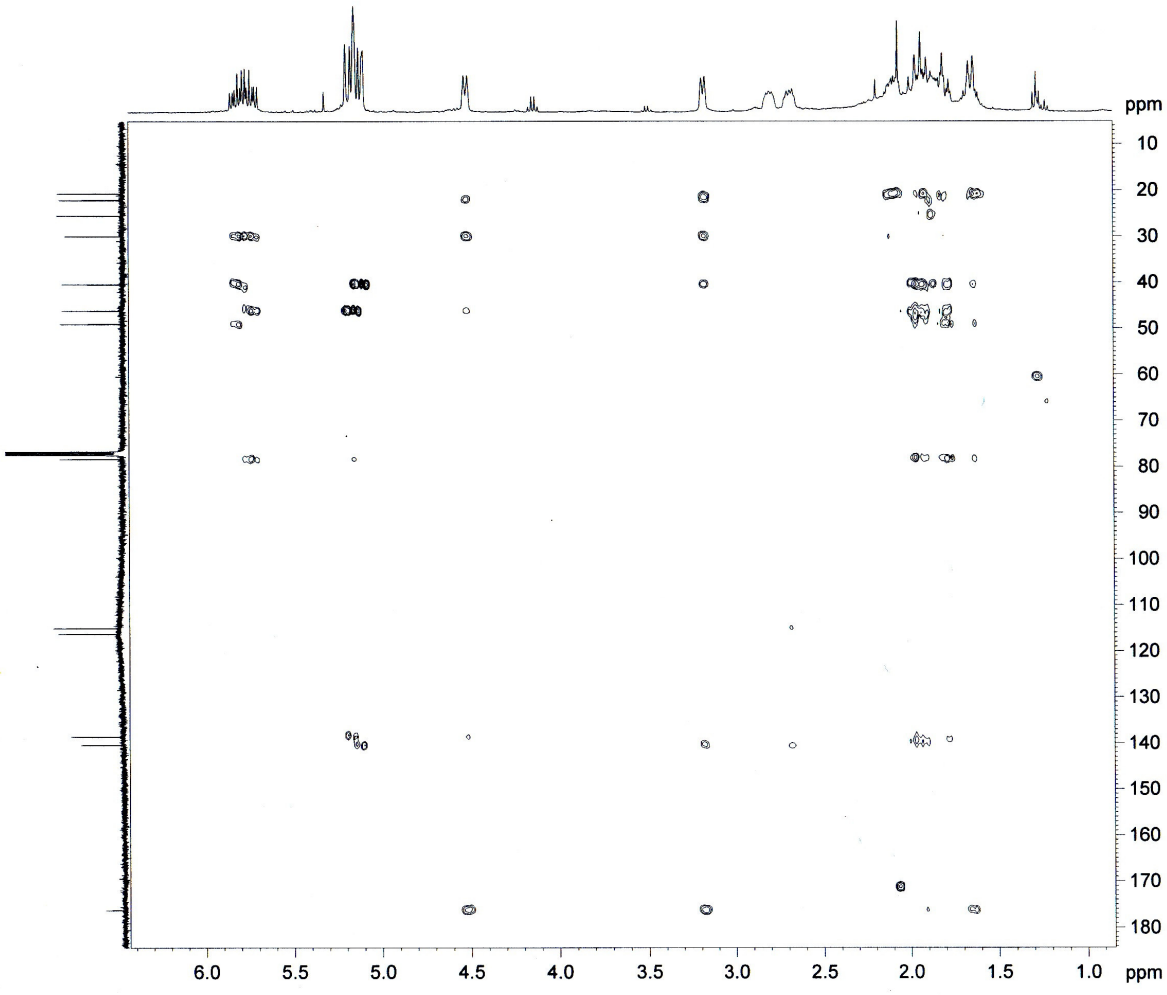
COSY 16



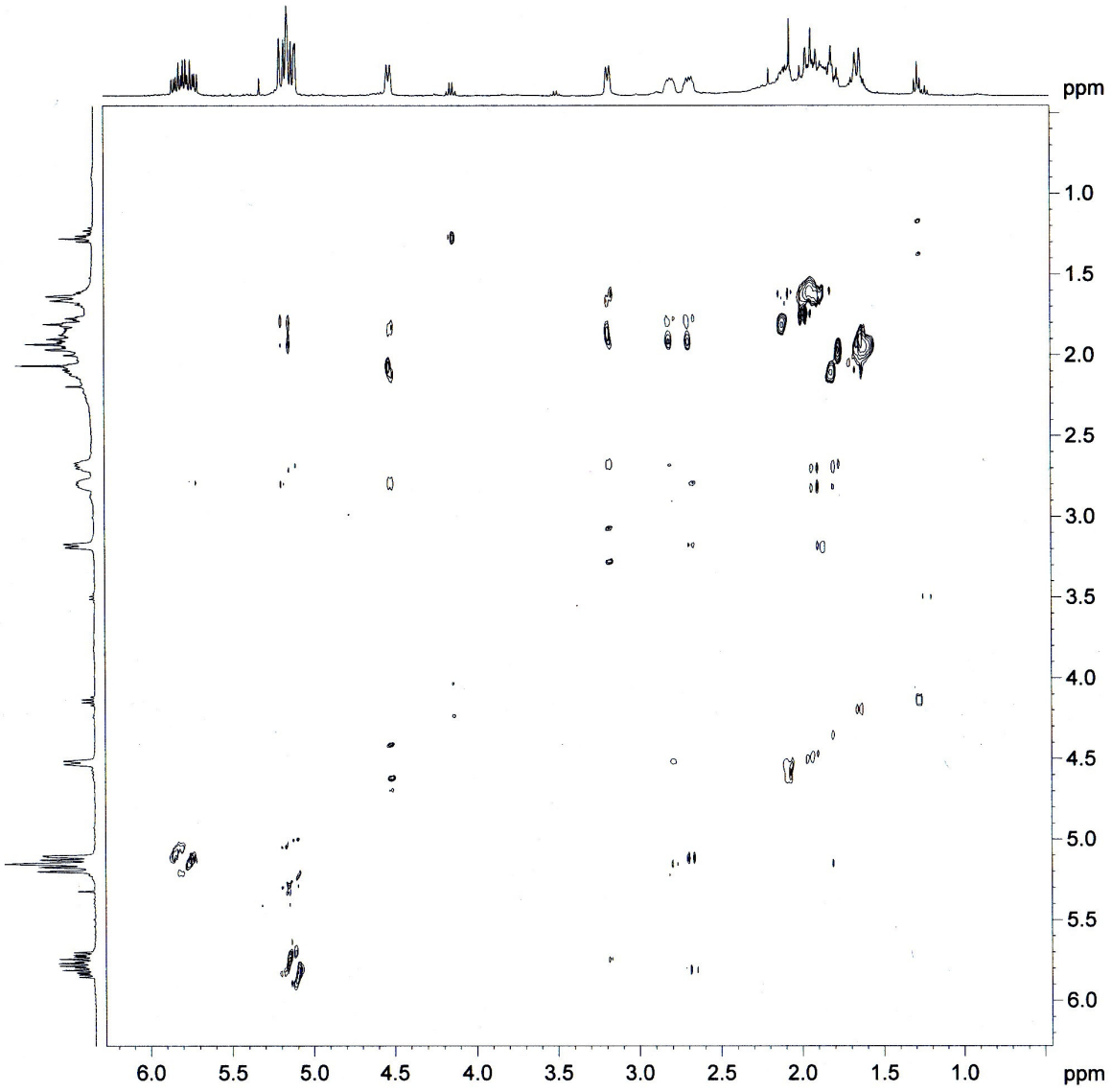
HSQC 16


ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
150
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

HMBC 16

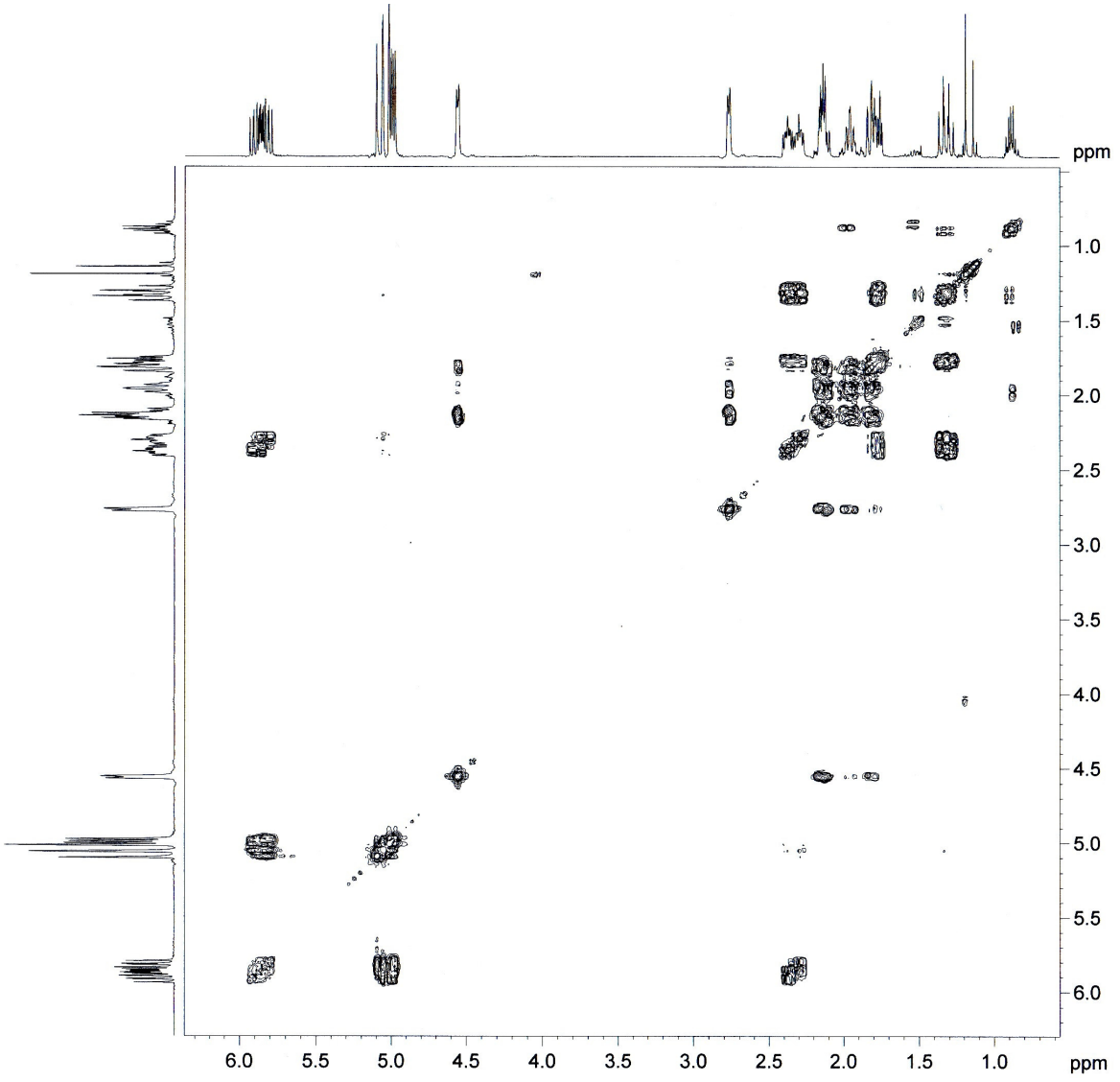

ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

NOESY 16

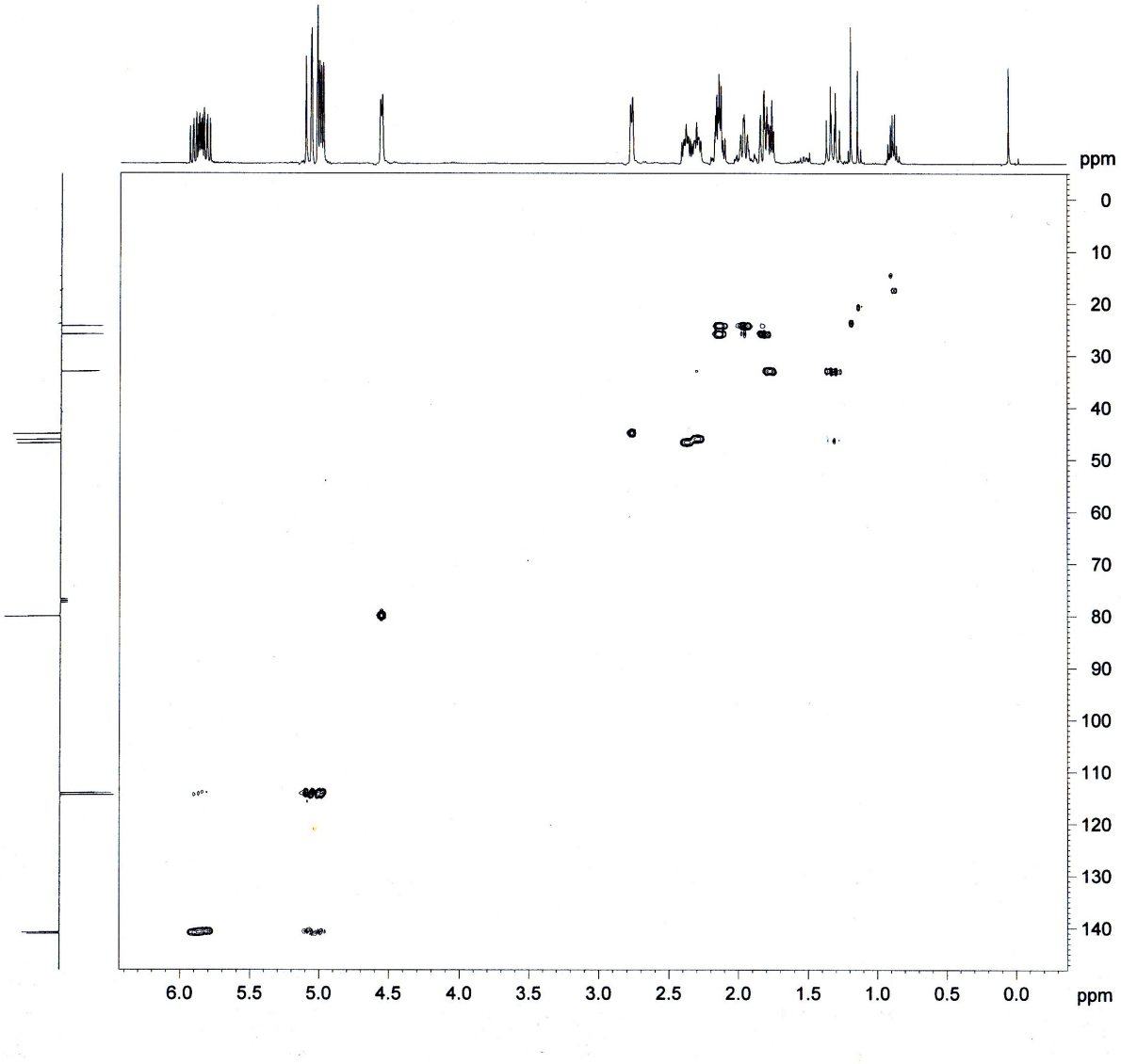

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

6.4 2D NMR Spectra of *anti*-2,4-Divinyl-6-oxa-bicyclo[3.2.2]nonan-7-one [12*anti*]

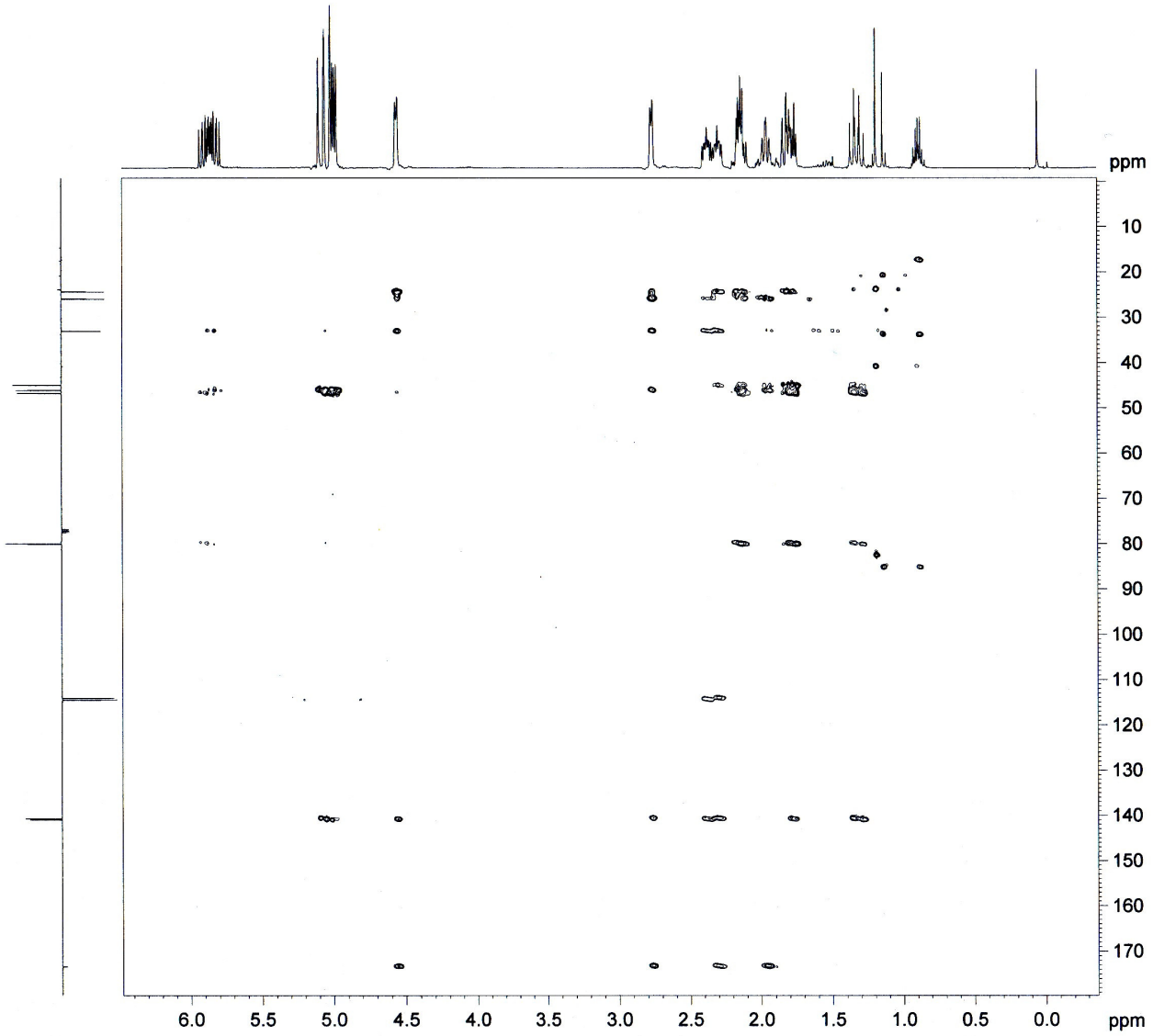
COSY 12anti



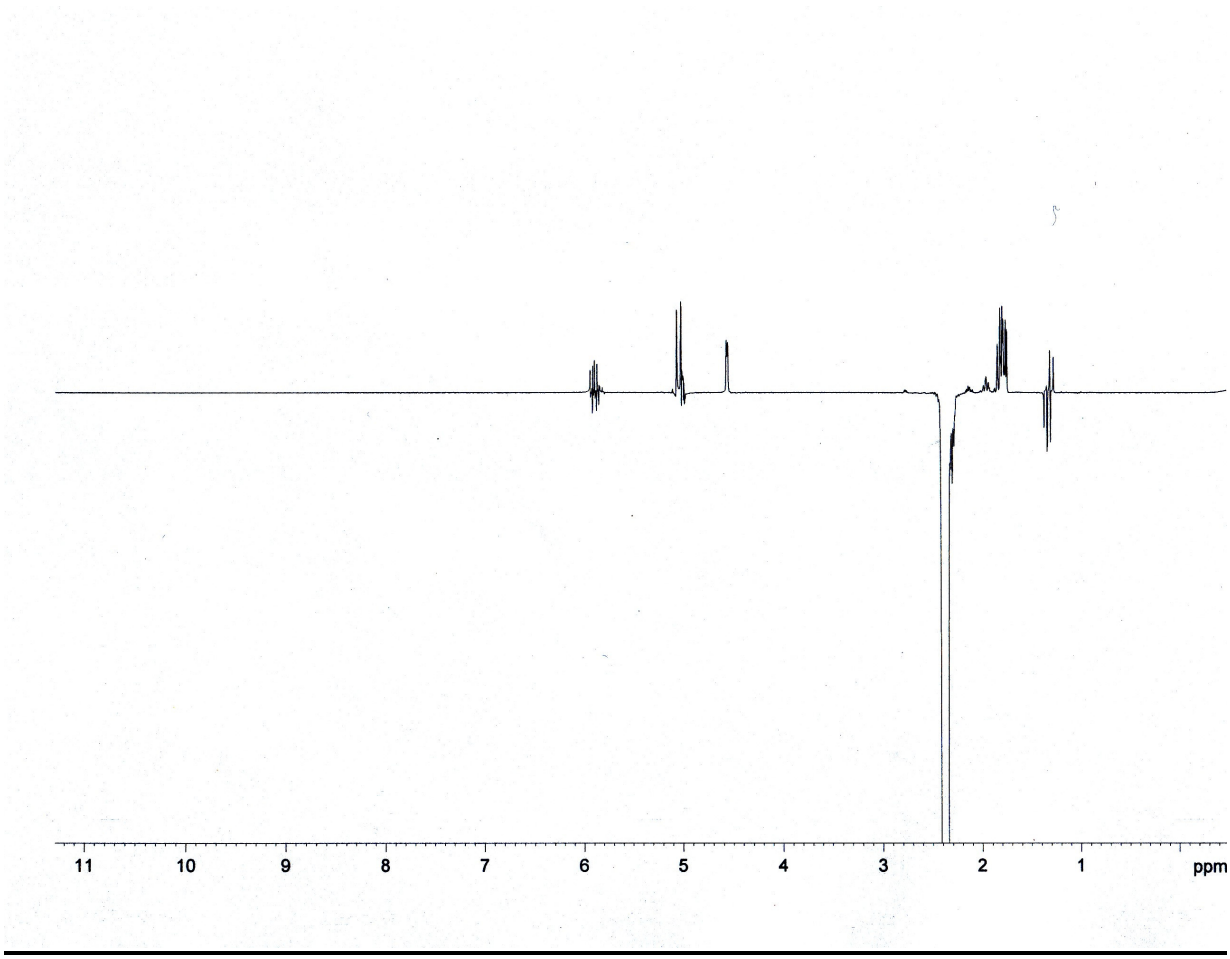
HSQC 12anti


ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

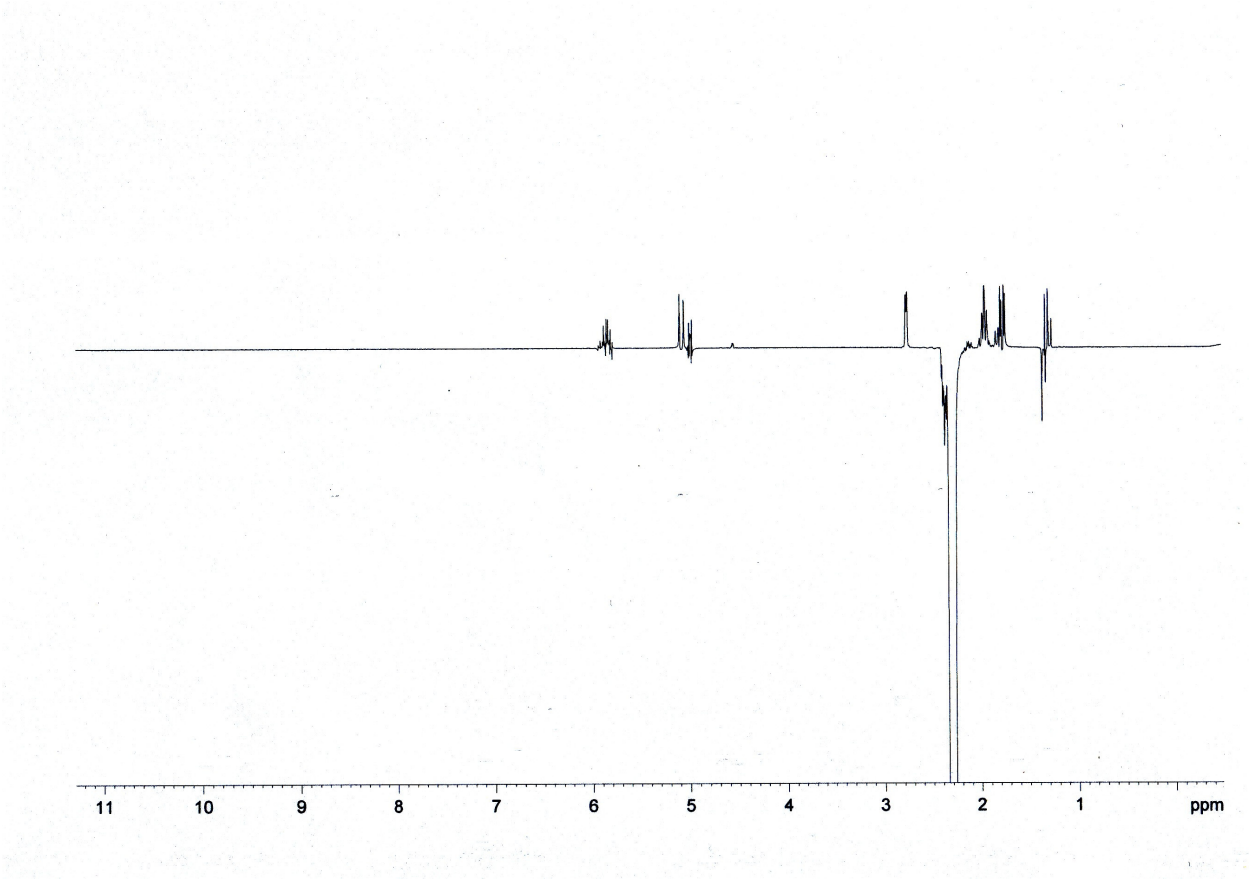
HMBC 12anti


ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

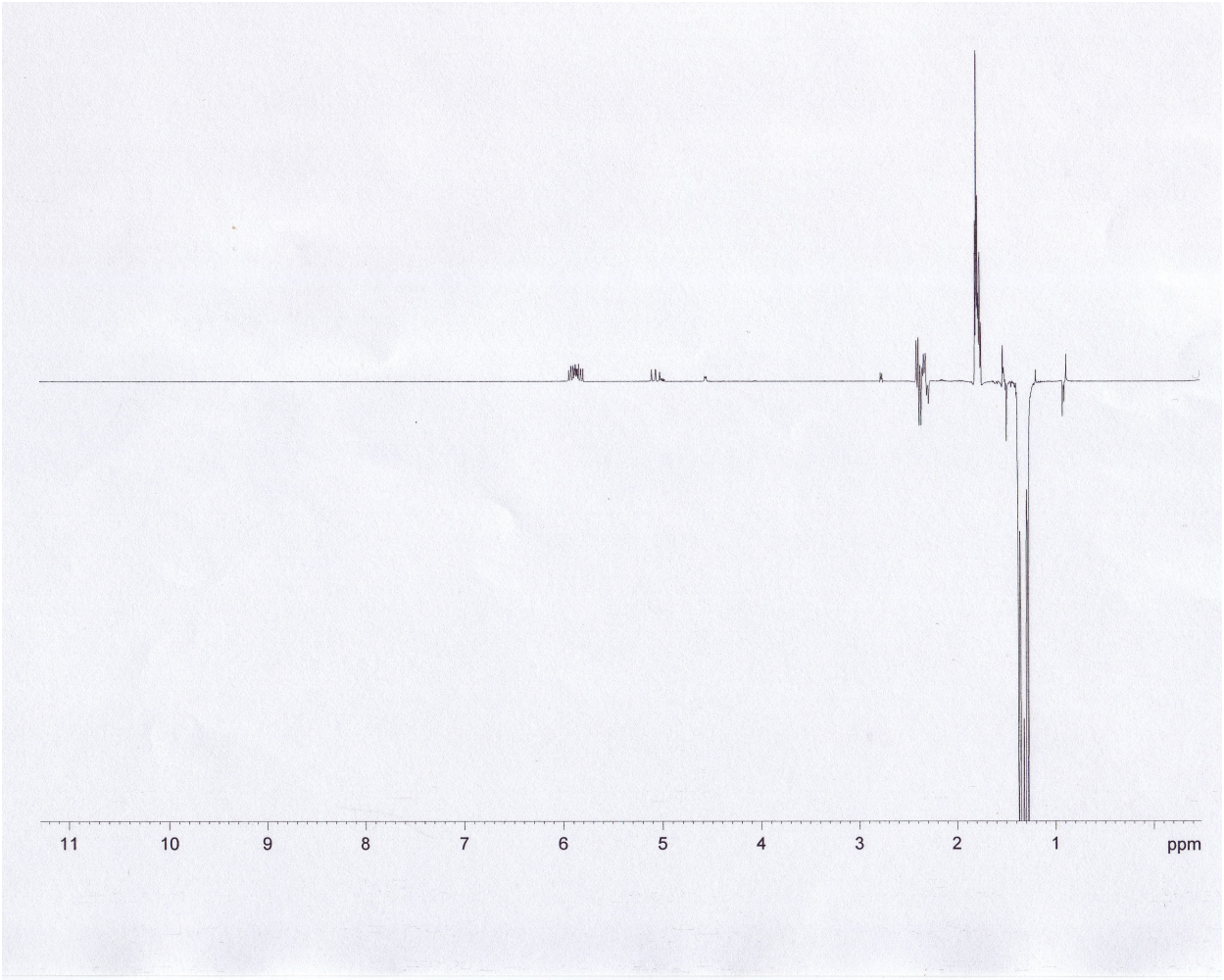
NOE difference 1 12anti



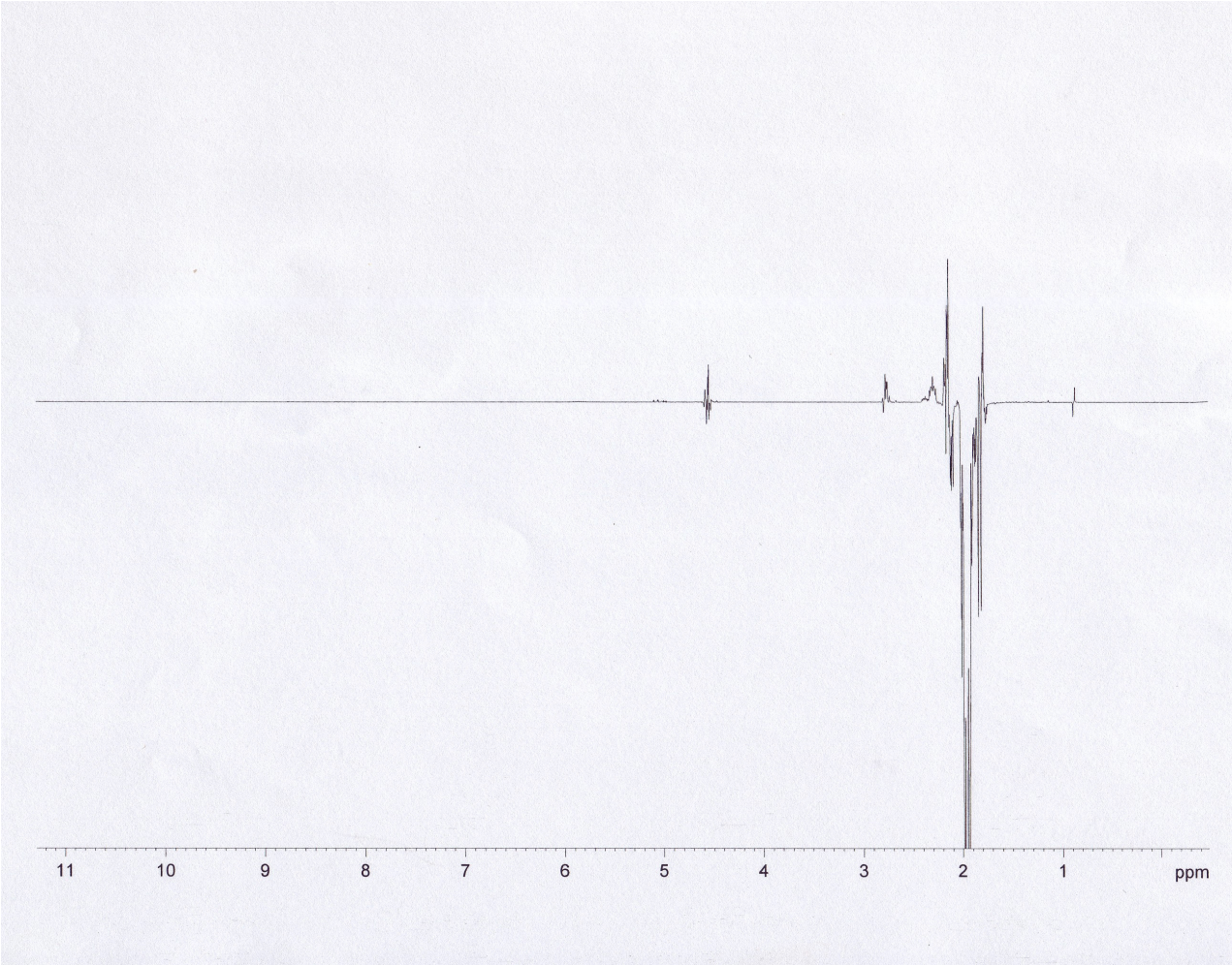
NOE difference 2 12anti



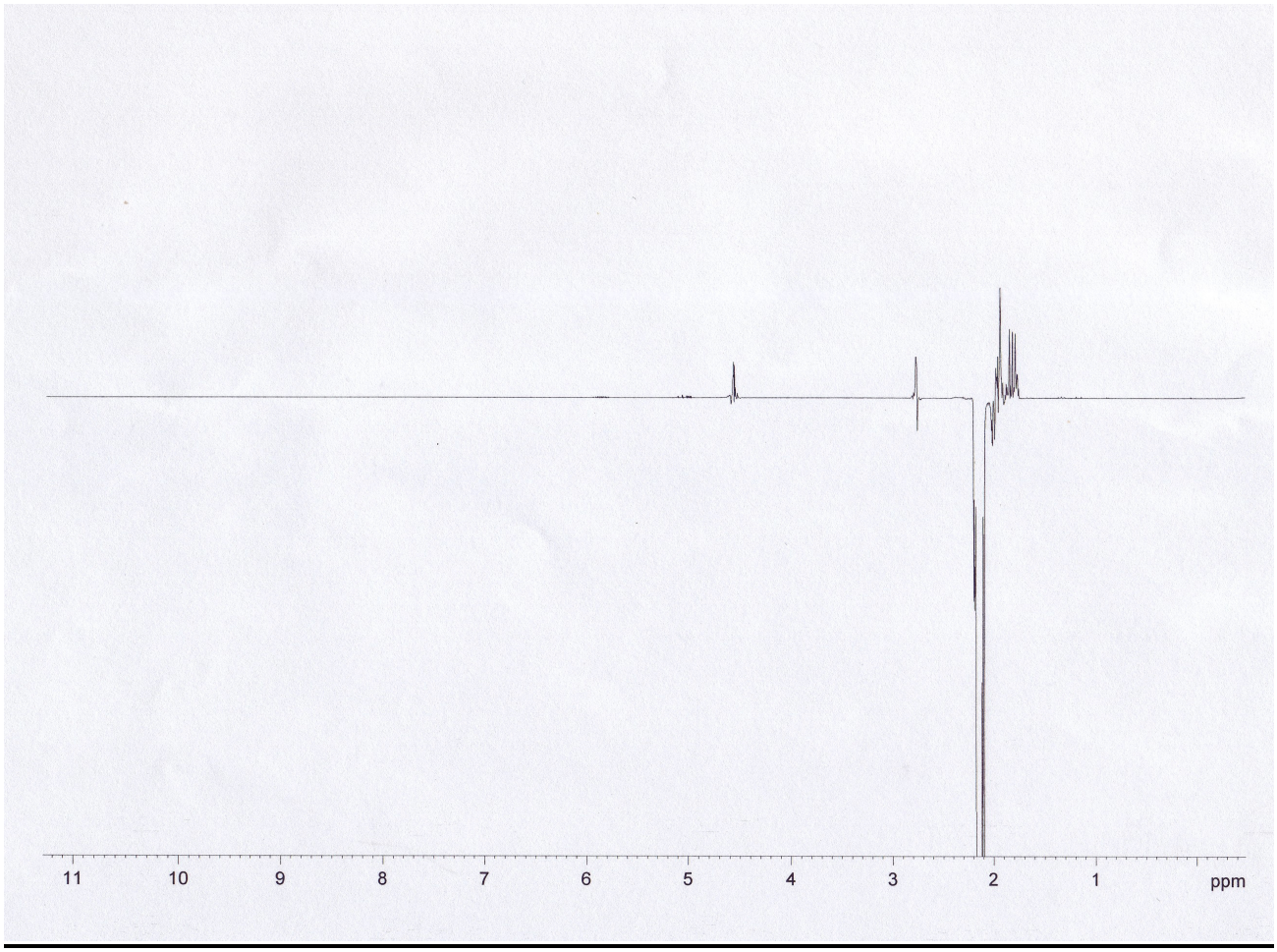
NOE difference 3 12anti



NOE difference 4 12anti



NOE difference 5 12anti



6.5 Curriculum Vitae

Mag. Alenka Lengar

Date of birth: May 3rd 1973
Place of birth: Maribor, Slovenia
Nationality: Slovenian
Institution: IAS (Institute of Applied Synthetic Chemistry), Vienna University of Technology, A-1060 Vienna, Getreidemarkt 9/ 163-OC Phone: +43-1-58801-15451, Mobile: +43-650-3333577
Email: alengar@ioc.tuwien.ac.at, alenkalengar@hotmail.com

Education:

Since October 2005:
PhD thesis and research assistant (FWF project) in the research group of Prof. Dr. Marko D. Mihovilovic, Vienna University of Technology, Institute of Applied Synthetic Chemistry
Subject: Synthesis and biooxidations of polycyclic and acyclic ketones by recombinant whole-cells
PhD defense expected in November 2008

February 2008:
Research fellow in the research group of András Kotschy, Eötvös Loránd University, Budapest, Hungary
Subject: Sonogashira couplings

December 2004 – September 2005:
University assistant at the Institute of Organic Chemistry, University of Vienna in the research group of Prof. Dr. Udo H. Brinker in the field of physical organic chemistry and structural chemistry
Subject: Synthesis of hemicarcerands, complexation and incarceration

September 2004:
Master of Science at the Karl-Franzens-University Graz, Austria, Institute of Chemistry

May 2003 – December 2003:
Diploma thesis in the research group of Prof. Dr. C. Oliver Kappe, Institute of Chemistry, Karl-Franzens University Graz, Austria
Subject: Microwave assisted palladium catalyzed cross-coupling reactions

October 1996 – September 2004:
Study of chemistry at the Karl-Franzens-University Graz, Austria, Institute of Chemistry

October 1991 – October 1995:
Study of medicine at the Faculty of Medicine, University of Ljubljana, Slovenia

September 1987 – June 1991:
Grammar School of Natural Sciences, Maribor, Slovenia

Skills and characteristics:

- Bioorganic chemistry, biocatalysis, biotransformations using recombinant whole-cells
- Heterocyclic chemistry, "Green chemistry" (solvent-free reactions, microwave assisted organic synthesis)
- Methodology in organic synthesis
- Structure elucidation by NMR, MS, IR, UV spectral methods
- Analytical and purification techniques: chromatography, GC, HPLC
- User of special software for organic chemists (ChemOffice, WinNMR, MestReC, Beilstein Commander, SciFinder, HyperChem)
- Flexible, interested in several fields of chemistry, open for new challenges

Teaching experience:

Laboratory practice, teaching assistant at the University of Vienna, Institute of Organic Chemistry

Publications:

Stereoselective desymmetrizations by recombinant whole cells expressing the Baeyer-Villiger monooxygenase from Xanthobacter sp. ZL5: a new biocatalyst accepting structurally demanding substrates.
Rial, Daniela V.; Bianchi, Dario A.; Kapitanova, Petra; Lengar, Alenka; van Beilen, Jan B.; Mihovilovic, Marko D. *Eur. J. Org. Chem.* **2008**, *7*, 1203-1213.

Tunable carbon-carbon and carbon-sulfur cross-coupling of boronic acids with 3,4-dihydropyrimidine-2-thiones.
Lengar, A.; Kappe, C.O. *Org. Lett.* **2004**, *6*, 771-774.

Poster presentation:

Stereoselective Baeyer-Villiger Biooxidation of β-Functionalized Linear Ketones Towards Optically Pure Esters
Alenka Lengar and Marko D. Mihovilovic*, 2nd EuCheMS Chemistry Congress, Torino, Italy; 16.9.2008 – 20.9.2008

Synthesis of Carbo- and Oxotricyclic Ketones and Subsequent Ring-Opening Olefin Metathesis
Alenka Lengar and Marko D. Mihovilovic*, 12th Blue Danube Symposium on Heterocyclic Chemistry (BDSHC-12), Tihany, Hungary; 10.06.2007 - 13.06.2007

Synthesis of Hemicarcerands, Complexation and Incarceration
Lengar, A., Brinker, U.H., 11th Austrian Chemistry Days, University of Leoben, Leoben, Austria; 19.09.2005 - 22.09.2005

Language skills:

Slovenian – mother tongue
German – excellent speaking and writing
English – excellent speaking and writing
Croatian – excellent speaking and writing
French – intermediate
Spanish – basic

Other interests:
Sport (ballet and modern dance (professional dancer 1990 – 2004), skiing, hiking,...), music (good pianist and saxophone player), traveling to far destinations (Africa and Asia)