Monooxygenase-Mediated Baeyer–Villiger Oxidations

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Enzyme-mediated Baeyer–Villiger oxidations offer a "green chemistry" approach for the production of chiral lactones. Several organisms have been found to catalyze this reaction in the course of their metabolic pathways. A number of flavin-dependent monooxygenases have been characterized, and acceptance of a multitude of non-natural substrates has been found. Such biocatalysts are used in synthetic chemistry either as isolated enzymes in combination with appropriate cofactor recycling systems or as living whole cells, in native or recombinant form, for the production of valuable intermediates. This review gives an overview of the most widely utilized enzymes and the corresponding substrate profiles, together with applications in natural product and bioactive compound synthesis.

1. Introduction

The Baeyer–Villiger oxidation of ketones (Scheme 1) represents a powerful methodology in synthesis for breaking carbon–carbon bonds in an oxygen-insertion process. Since the discovery of the reaction by Adolf Baeyer and Victor Villiger back in 1899,[1] substantial progress has been made in understanding the mechanism, in predicting migratory preferences, and in application of this conversion in preparative chemistry.[2,3]

The mechanism is generally accepted to proceed by a two-step process and was initially proposed by Criegee[4]

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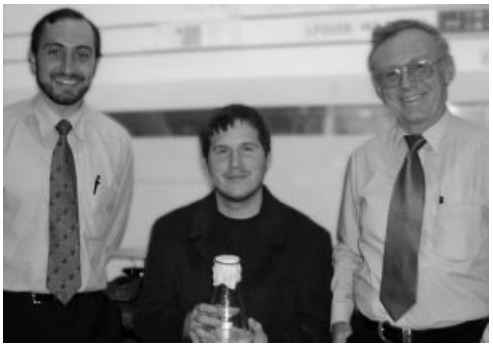
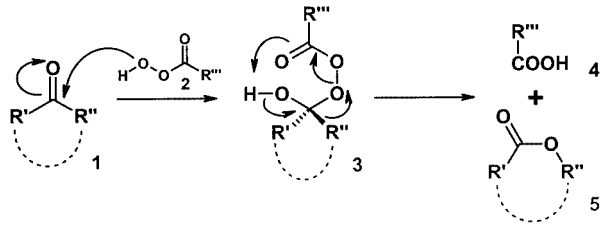


Dr. Marko D. Mihovilovic (left) graduated in organic chemistry at the Vienna University of Technology (VUT) in 1993. He carried out his Ph.D. thesis under the supervision of Prof. Peter Stanetty and received his Ph.D. in 1996. In 1997 he moved to the University of New Brunswick, Saint John, Canada, for a postdoctoral stay to join the group of Prof. Margaret M. Kayser as Schroedinger Fellow of the Austrian Science Fund (FWF). This first encounter with biocatalysis was followed by a subsequent postdoctoral stay within the group of Prof. Jon D. Stewart at the University of Florida, Gainesville, in 1998, focussing on the development of recombinant biocatalysts. After his return to the VUT, Dr. Mihovilovic started a biocatalysis group at the Institute of Organic Chemistry, now the Institute of Applied Synthetic Chemistry. His major research targets and the topic of his "Habilitation" are the characterization and application of recombinant whole-cell systems for the production of chiral intermediates in bioactive compound synthesis. Dr. Mihovilovic is currently coordinator of the Marie-Curie Training Site GEMCAT (Genetically Engineered Microorganisms as Whole-Cell Biocatalysts).

Dipl.-Ing. Bernhard Müller (center) studied chemistry at the Vienna University of Technology and graduated in general chemistry in March 2002. For his Diploma thesis he performed substrate-profiling studies for two recombinant Baeyer–Villiger monooxygenase expression systems and applied the whole cells to produce key intermediates for the synthesis of indole alkaloids. Dipl.-Ing. Müller is currently working on his Ph.D. thesis at the Institute of Applied Synthetic Chemistry.

Prof. Dr. Peter Stanetty (right) graduated in organic chemistry in 1969 and received his Ph.D. from the VUT in 1971. He finished his "Habilitation" in 1981 and was appointed Assistant Professor at the Institute of Organic Chemistry in 1983. In 1988 he became Associate Professor, and in 1993 University Professor. His major research interests are heterocyclic and organometallic chemistry aimed at the synthesis of bioactive compounds for pharmaceutical and agrochemical applications.

MICROREVIEWS: This feature introduces the readers to the author's research through a concise overview of the selected topic. Reference to important work from others in the field is included.



Scheme 1. Baeyer−Villiger oxidation by peracids

(Scheme 1); nucleophilic attack of a carbonyl group by a peroxo species **2** results in the formation of a tetrahedral "Criegee" intermediate **3**, which undergoes rearrangement to the corresponding ester **5**. Widely used oxidants for Baeyer−Villiger reactions are *m*-chloroperoxybenzoic acid, trifluoroperoxyacetic acid, peroxyacetic acid, and hydrogen peroxide. In the case of α-substitution, the more nucleophilic group displays preferred migration towards the oxygen atom, with substantial influence from conformational, steric, and electronic factors.

Chiral Baeyer−Villiger oxidation of cyclic ketones allows rapid access to asymmetric lactones, valuable intermediates in organic chemistry and frequently encountered precursors in enantioselective synthesis. While approaches utilizing organometallic reagents have been continuously improved over recent years,[5] the efficient and highly selective preparation of this class of compounds is still the domain of biocatalytic methods and is considered a "green chemistry" alternative.

The biocatalytic equivalent to the above peracids is represented by monooxygenases, which are a sub-class of the oxygenase enzyme family.[6] While dioxygenases catalyze the incorporation of both oxygen atoms from molecular oxygen into an organic substrate, monooxygenases insert only one, the second atom being reduced at the expense of a donor to form water. Monooxygenases are able to catalyze a variety of oxidation reactions, ranging from hydroxylation of aliphatic and aromatic alcohols and epoxidation of alkenes to heteroatom oxidations and Baeyer−Villiger transformations. The type of oxidation is generally dependent on the prosthetic groups present within the enzyme; hydroxylations and epoxidations are catalyzed by metal-dependent monooxygenases of the cytochrome P-450 type,[7] heteroatom and Baeyer−Villiger oxidations are mediated by flavin-dependent enzymes.[8] Thanks to the versatile nature of the cofactor, flavoproteins are capable of performing a variety of catalytic reactions.[9,10] Baeyer−Villiger monooxygenases (BVMOs) have been found to perform *S*-oxidation[11] of thiols,[12] dithioketals,[13] and sulfites,[14] *N*-oxide formation,[15] boron oxidation,[16] selenium oxidation,[17] and, very recently, epoxidation of alkenylphosphonates,[18] as well as the biotransformation from which they take their name.

Previous articles have covered biocatalytic aspects of BVMOs in general.[19−24] This review covers relevant literature relating to monooxygenase-mediated Baeyer−Villiger oxidations until early 2002.

2. Enzymatic Baeyer−Villiger Oxidations

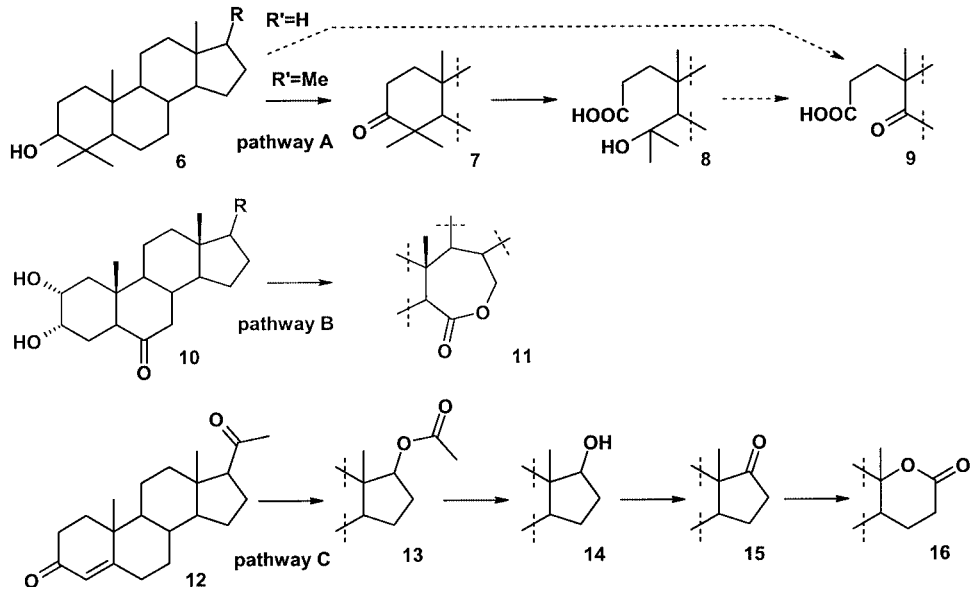
The first indication for the involvement of a biocatalytic Baeyer−Villiger process in the degradation of the steroid A-ring was found by Turfitt in 1948. He observed the formation of Windaus' oxo acid **9** in minute quantities upon fermentation of cholestanone with *Proactinomyces erythropolis*, and an oxidative degradation by pathway A in Scheme 2 can be assumed.[25] Degradation of eburicoic acid (**6**) by *Glomerella fusarioides* produces carboxylic acid **8** through a similar process, and ring-cleavage was postulated to proceed by way of a Baeyer−Villiger oxidation of the intermediate ketone **7**.[26] Recently, B-ring expansion of the steroid system **10** by tomato cell suspension cultures has been observed to produce 24-epibassinolide (**11**) (pathway B).[27]

In 1953, two groups reported Baeyer−Villiger oxidations of progesterone and testosterone to testololactone by *Penicillium*, *Cylindrocarpon*, *Mucor*, and *Aspergillus* sp.[28,29] Peterson and co-workers were able to demonstrate that the biotransformation proceeds via the intermediate **15**.[29] These results indicated a biocatalytic double Baeyer−Villiger process, as outlined in pathway C, with an initial side chain scission to form acetate **13**. This mechanism was supported by the isolation of this intermediate upon fermentation of progesterone with *Cladosporium*,[30] biotransformations with partially purified enzymes from *C. radicicola*,[31] and inhibition studies.[32] Hydrolysis and oxidation gives 4-androstene-3,17-dione (**15**), which is finally converted, in a second Baeyer−Villiger reaction, into D-ring lactone **16**. The BVMO responsible was dependent on molecular oxygen, as was demonstrated for a partially purified enzyme preparation.[33]

Initial structure acceptance studies revealed significantly different substrate profiles for steroid monooxygenases.[34] A BVMO from *C. radicicola* was purified by affinity chromatography and oxidatively cleaved the side chains of several steroidal structures.[35,36] However, a similar enzyme[37] originating from *Rhodococcus rhodochrous* displayed very high selectivity for progesterone and 11-hydroxyprogesterone.

Microbial Baeyer−Villiger reactions are not restricted to steroidal systems. In the degradation of isoprenoid ketones, oxygen insertion was observed in fungal[38] and bacterial species.[39] Baeyer−Villiger steps are frequently encountered in the biosynthesis of several natural products, such as aflatoxins in fungi,[40] shellfish toxins,[41] and iridoids in plants,[42] while a flavin-containing monooxygenase oxidizing salicylaldehyde to pyrocatechol and formate has been isolated from pig liver microsomes.[43]

The above Baeyer−Villiger reactions were mainly studied in order to elucidate metabolic pathways and to characterize the enzymes involved. Substrate profiling was performed predominantly on natural targets, without large structural



Scheme 2. Baeyer−Villiger oxidations of the steroid system

and functional diversity. Because of the lack of predictability of the catalytic repertoire, these enzymes are of limited value for synthetic chemists to date.

However, a number of different monooxygenases have been found to oxidize a remarkably large range of non-natural substrates. Broad substrate acceptance profiles are a key prerequisite for a biocatalytic system and to enable successful application in preparative chemistry. In addition, these enzymes display high chemoselectivity for the Baeyer−Villiger-type oxygen insertion. Several of the following enzymes are enantio-complementary, hence allowing access to both optical antipodes of a required product lactone.

3. Mechanism of BVMOs

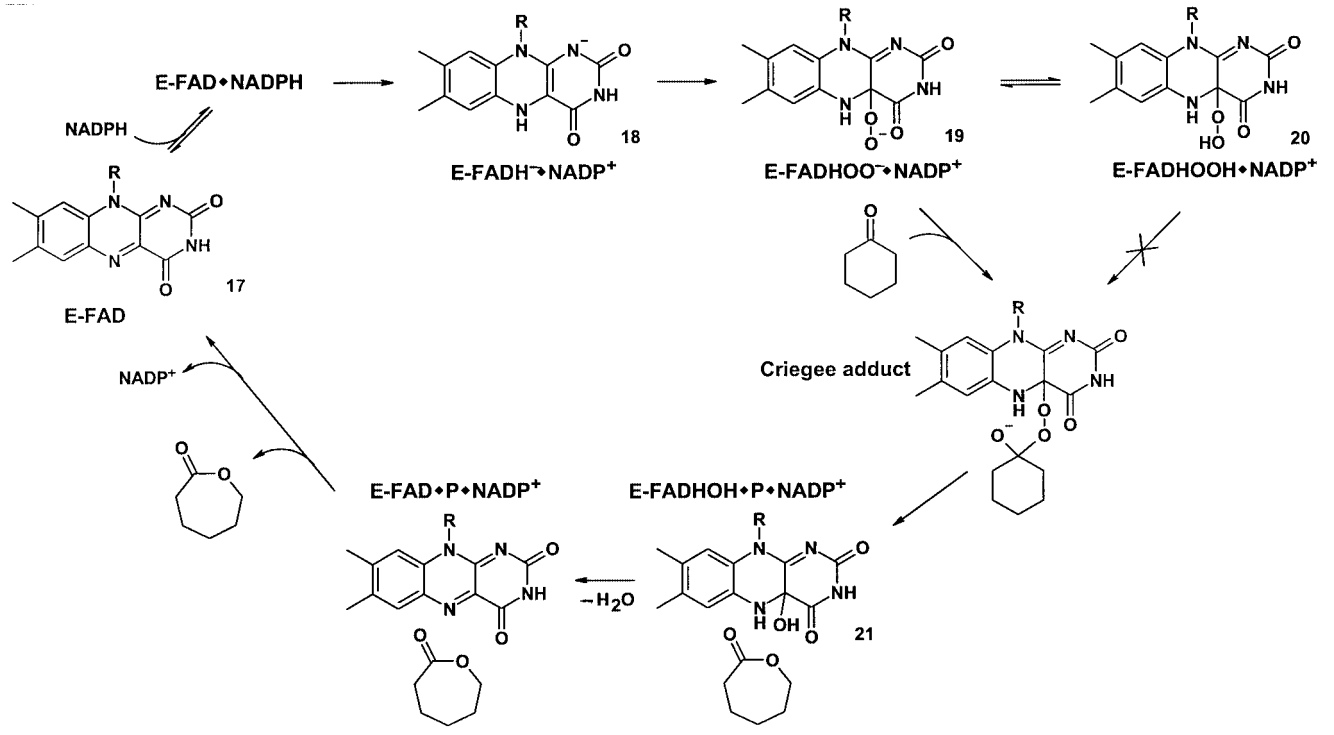
The generally accepted mechanism for the enzymatic Baeyer−Villiger oxidation is based on results obtained with cyclohexanone monooxygenase (CHMO) isolated from *Acinetobacter calcoaceticus* NCIMB 9871.[44] The enzyme possesses flavin adenine dinucleotide (FAD) as a prosthetic group and was found to be NADPH- and oxygen-dependent. Walsh and co-workers proposed several steps for the oxidation and determined a number of rate constants.[45]

The biocatalytic process is initiated by reduction of the tightly bound FAD (**17**) by NADPH (Scheme 3) and subsequent rapid oxidation by molecular oxygen to produce the flavin 4a-peroxide anion **19**. This intermediate constitutes the oxygenating species in the subsequent Baeyer−Villiger reaction. Massey and co-workers, in a detailed spectroscopic survey, demonstrated that the anion is formed initially and, in the absence of a substrate, exists in equilibrium with the 4a-hydroperoxide **20** as a stabilized form.[46]

The peroxide acts as a nucleophile, attacking the substrate carbonyl group to give the "classical" tetrahedral Criegee intermediate. Rearrangement of this species results in formation of the product lactone and 4a-hydroxyflavin (**21**). The catalytic cycle is closed by elimination of water to form FAD and release of product and cofactor.

The rearrangement is believed to be governed by the same stereoelectronic effects as in the non-enzymatic Baeyer−Villiger oxidation.[47,48] It has been demonstrated that two prerequisites must be satisfied for successful alkyl migration and carboxylic acid ejection: i) the migrating C−C bond has to be in an antiperiplanar position with respect to the peroxy bond, and ii) electron release from the hydroxy oxygen atom to the originally attached migrating entity is essential for the alkyl shift and requires a lone pair in the *anti* position at the oxygen atom (Figure 1).[49] Schwab and co-workers[50] used isotopically labeled substrates to confirm that the fragmentation of the tetrahedral intermediate proceeds with retention of configuration at the migrating center, in analogy to the chemical oxidation.[51] Hence, CHMO can catalyze regio- and enantioselective oxidations by permitting only one C−C bond to be antiperiplanar to the O−O bond in the Criegee-type intermediate. Since the FAD is tightly bound to the enzyme, interaction of the substrate with amino acids in the active site influences this alignment and the selection of the migratory group.

This mechanism for CHMO and the directing effects involved generally serve as a model for other BVMOs with some differences; both NADH and NADPH are found as cofactors for monooxygenases, and the prosthetic group FAD can be replaced by FMN with no essential changes in the mechanism as outlined above.



Scheme 3. Mechanism of flavin-dependent Baeyer−Villiger monooxygenases

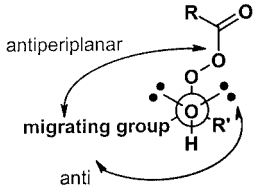


Figure 1. Stereoelectronic requirements for migration

4. Cofactor Recycling − Isolated Enzyme vs. Whole-Cell Systems

Monooxygenases are dependent on cofactors, which complicates their utilization in organic chemistry and has hampered their widespread use among the synthetic community. The flavin prosthetic group is usually tightly bound to the enzyme and is regenerated in the catalytic cycle. NAD(P)H, however, has to be provided in equimolar amounts to facilitate the conversion from substrate to product. Because of the price of these compounds, the cofactor has to be recycled to enable a cost-efficient biotransformation on a preparative scale.

Two general strategies have been developed to solve this problem: (i) implementation of a second enzymatic reaction, and (ii) utilization of whole-cell techniques. Both approaches have their benefits and disadvantages.

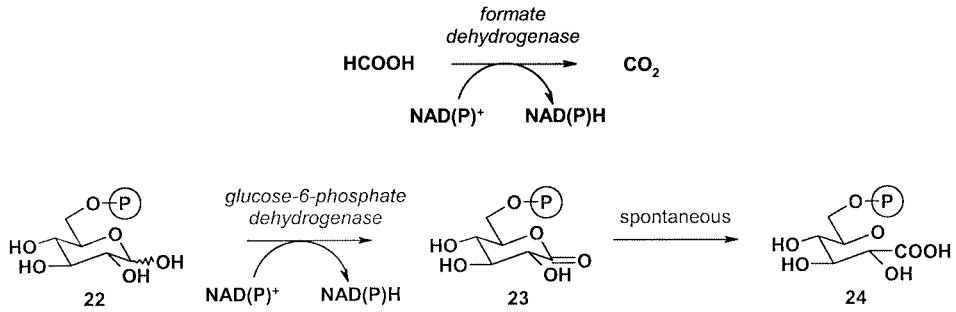
4.1 Isolated Enzymes

When isolated enzymes are used in biocatalysis, a regeneration cycle has to be established. The cofactor $NAD(P)^+$ is reduced by an additional enzyme at the expense of an appropriate auxiliary substrate acting as hydride donor. Hence, a two-enzyme system has to be constructed in situ, with conditions compatible for both catalytic entities. This to some extent complicates the application of such systems for synthetic chemists.

The best and most widely used method for NADH recycling utilizes formate dehydrogenase (FDH), which catalyzes the oxidation of formate to carbon dioxide (Scheme 4).[52] Neither the auxiliary substrate nor the co-product cause problems such as inactivation or inhibition, and they can easily be removed from the reaction mixture. The equilibrium is in favor of the formation of volatile CO_2, hence driving the recycling reaction in the desired direction. FDH is commercially available and can be stabilized by immobilization.

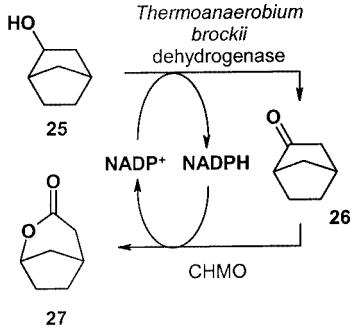
Until recently, the most efficient method for NADPH regeneration used the glucose-6-phosphate (G6P)/glucose-6-phosphate dehydrogenase (G6PDH) system. The activated sugar **22** is enzymatically oxidized to 6-phosphoglucolactone (**23**), which spontaneously hydrolyzes to the corresponding gluconate **24** (Scheme 4). G6PDH from *Leuconostoc mesenteroides* is cheap, stable, and accepts both NAD^+ and $NADP^+$.[53] A major disadvantage of this system is the high cost of G6P.

This approach was improved by the designing of a mutant formate dehydrogenase from *Pseudomonas* sp. 110,[54] in which the active site of the enzyme was engineered by multipoint site-directed mutagenesis to meet the additional spatial requirements for $NADP^+$.[55] Recently, this system was successfully used for biotransformations of isolated CHMO.[56,57]



Scheme 4. Cofactor recycling

An elegant solution to the cofactor problem for Baeyer–Villiger oxidations was developed by Willetts and co-workers, by the generation of a closed-loop system.[58][59] A purified BVMO was coupled with an alcohol dehydrogenase from *Thermoanaerobium brockii*. The dehydrogenase converts the substrate alcohol **25** into the ketone **26** upon reduction of $NADP^+$ to NADPH. In a subsequent enzymatic step, the BVMO transforms **26** into lactone **27** and regenerates $NADP^+$ (Scheme 5).



Scheme 5. Substrate-coupled cofactor recycling

This methodology was further optimized by use of the macromolecular coenzyme polyethylene glycol-NADPH (PEG-NADPH) in a membrane reactor.[60] Instead of a substrate hydroxy precursor, an additional alcohol (e.g., 2-propanol) can be employed for cofactor recycling, and such linked systems were modeled to bring costs within economic limits for large-scale fermentations.[61]

4.2 Whole-Cell Systems

Whole-cell fermentations offer a different approach to overcoming the cofactor obstacle: living organisms provide natural recycling systems for all factors required. The tedious process of protein purification can be avoided, and applications are not limited by possible enzyme instability. In many cases, cells can be easily cultivated and stored, hence representing a regrowable source of the catalytic entity.

In contrast with the two artificial enzyme recycling methods above, however, the whole-cell approach involves a multitude of additional enzymes, increasing the complexity of the system. The potential for unwanted side-reactions is increased, since both substrate and product can be accepted by other enzymes present in the cell (enzyme B and enzyme C in Figure 2) with overlapping acceptance profiles. Such competitive processes decrease the yield of the desired biotransformation. This is often encountered in whole-cell biotransformations with native strains, since the subsequent step in the metabolic degradation pathway is represented by lactone hydrolysis by a corresponding hydrolase. To circumvent this obstacle, addition of a suitable enzyme inhibitor, such as tetraethyl pyrophosphate, is required.[62]

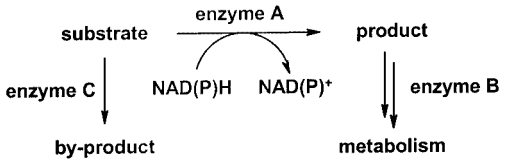


Figure 2. Competitive biotransformations in whole-cell fermentations

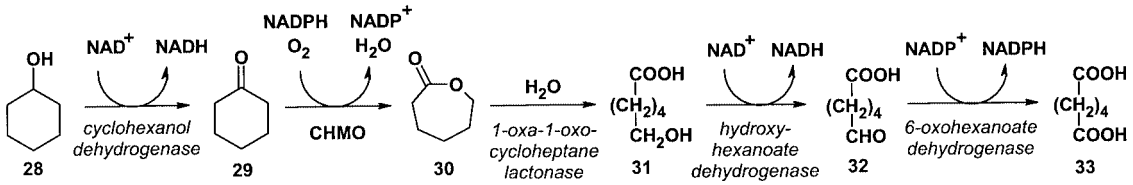
In another approach, we have solved such unwanted side-reaction problems by using recombinant overexpression systems in place of native strains.[63] Production of the required protein at high levels can often be accomplished by use of a strong promoter. As a consequence, the amount of required enzyme is substantially increased and becomes a dominant fraction in the biocatalytic proteome of the organism. In most cases, unwanted biotransformations are minimized and become negligible due to the minute concentrations of competing enzymes relative to that of the overexpressed biocatalyst. This approach was successfully demonstrated for *Acinetobacter* CHMO in recombinant strains based on *Saccharomyces cerevisiae*[64] and *Escherichia coli*.[65]

Cultivation of pathogenic organisms, which requires special laboratory equipment and substantial microbiological expertise, can be avoided by choosing appropriate host organisms. Easy-to-use catalytic systems can be provided to preparative chemists for subsequent applications in organic synthesis.

5. Synthetic Applications of BVMOs

5.1 Cyclohexanone Monooxygenase (CHMO) from *Acinetobacter*

Since the purification and characterization of CHMO from *Acinetobacter* sp. NCIMB 9871 (E.C. 1.14.13.22) by



Scheme 6. Metabolic role of CHMO in native *Acinetobacter*

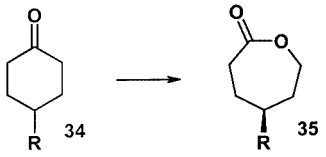
Trudgill and co-workers in 1976, this enzyme has become the best-studied Baeyer−Villigerase to date.[44] CHMO is a 61 kDa monomeric flavoprotein consisting of 542 amino acids encoded by a 1.6kbp open reading frame. The enzyme is responsible for the oxygen insertion in the biodegradation of cyclohexanol (**28**) to adipate (**33**) (Scheme 6).[66−68] Apart from its role in nature, it oxidizes over 100 non-natural substrates and generally performs desymmetrization of prochiral ketones as well as kinetic resolution of racemic precursors with good enantioselectivity.[69] Structural analysis by X-ray crystallography has yet to succeed, and so only indirect models of the active site, based on substrate acceptance experiments, are available. Data from profiling studies enable the spatial and polar requirements of the active site to be estimated and allow predictions to be made.

Production of CHMO in native *Acinetobacter* was originally stimulated by elective culture with cyclohexanol as sole carbon source.[66] The gene for *Acinetobacter* CHMO was first cloned and sequenced in 1988.[70] The native strain NCIMB 9871 produces a lactone hydrolase, and successful production of lactones require addition of appropriate inhibitors.[62] Furstoss and co-workers found another way to circumvent this problem by using *Acinetobacter* TD 63, a strain lacking the hydrolase, in fermentations.[62,71] However, the organism is a class II pathogen, and expression systems in *S. cerevisiae* ("designer yeast")[64] and *E. coli*[65,72] hosts are available for whole-cell biotransformations. Fermentation[73] and workup conditions using adsorbent resins[74] were optimized for recombinant *E. coli* strains. Such biocatalysts are extremely simple and safe to use and can be even utilized in undergraduate laboratories.[75]

Original enzyme purification procedures reported by Trudgill et al.[44] have recently been improved for large-scale production of CHMO from the native organism.[76] Immobilized protein has been used in biotransformations.[77] Facile purification of recombinant enzyme was achieved by incorporation of a His_6-tag at the C-terminus of CHMO.[78] Recombinant protein from overexpression systems was characterized by ESI- and MALDI-MS[79] and used in mechanistic studies.[46] Application of purified CHMO from a recombinant strain to Baeyer−Villiger and sulfide oxidations was recently reported, and several aspects from cofactor recycling, stabilization by immobilization, to scale-up in fermentors have been studied.[80]

The mechanism was established on the basis of valuable contributions by Walsh and co-workers,[45][81] and cyclic thiol ester substrates were identified as mechanism-based inhibitors.[82][83] In this context, Walsh et al. also discovered the capability of CHMO to oxidize heteroatoms.

The enzyme displays high selectivity for the formation of (*S*)-lactones **35** from prochiral 4-substituted cyclohexanone precursors **34** (Scheme 7, Table 1). Straight chains of up to C_3 can be accommodated by the active site, larger chains (butyl) cause inversion of the stereoselectivity with moderate *ees*.[84][85] Both isolated enzyme and recombinant whole cells give comparable results with respect to yield and enantioselectivity in most cases.[86] The enzyme is sensitive to additional steric hindrance imposed by branched substituents, and a *tert*-butyl group seems to reach the spatial limits, with significantly decreased yields.



Scheme 7. Biocatalytic oxidation of prochiral 4-substituted cyclohexanones

In contradiction of earlier reports, a phenyl substituent is accepted as substrate (**34i**), but addition of an organic solvent and prolonged biotransformation times are required. While 10% ethanol gave 95% *ee* with conversion stopping at 14%, addition of 5% ethylene glycol gave improved conversion at the cost of selectivity.[87]

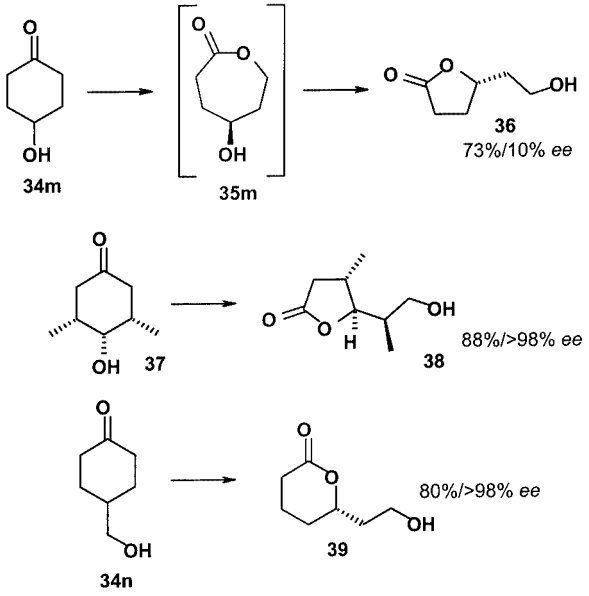
CHMO is highly selective for the Baeyer−Villiger oxidation and tolerates functional groups such as double bonds in substituents (**34h**).[88] Recently, we utilized an *E. coli* expression system for the production of methoxy- (**35j**) and halolactones (**35k/l**) from mesomeric precursors as valuable chiral precursors.[89]

Different behavior by the isolated enzyme was observed by Taschner et al. for the conversion of 4-hydroxycyclohexanone (**34m**) (Scheme 8);[85] the expected ring-expanded lactone **35m** was not observed, but rather underwent an intramolecular attack by the hydroxy group to give the five-membered system **36** in 73% yield and with a poor 10% *ee*. We have observed the same rearrangement with recombinant whole cells.[89] In contrast, 4-(hydroxymethyl)cyclohexanone (**34n**) and the trisubstituted substrate **37** both gave excellent enantioselectivities for the rearranged lactones **38** and **39**.

In the natural metabolic pathway, CHMO is responsible for the oxidation of achiral cyclohexanone.[66] Nevertheless, the enzyme is capable of performing highly enantioselective

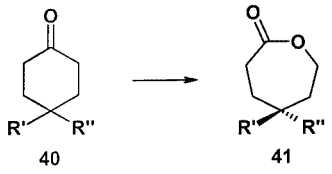
Table 1. Biocatalytic oxidation of prochiral 4-substituted cyclohexanones

	R	Config.	Isol. enzyme	Recomb. cells
35a	H	(*S*)	n.r.[44]	79%/n.a. (yeast)[86]
35b	Me	(*S*)	80%/> 98% *ee*[84]	83%/> 98 % *ee* (yeast)[86][88] 61%/> 98 % *ee* (*E. coli*)[89]
35c	Et	(*S*)	83%/> 98% *ee*[85]	74%/> 98% *ee* (yeast)[86][88] 91%/97% *ee* (*E. coli*)[89]
35d	Pr	(*S*)	80%/> 98% *ee*[85]	63%/92% *ee* (yeast)[86][88]
35e	Bu	(*R*)	70%/52% *ee*[85]	
35f	*i*Pr	(*S*)	60%/> 98% *ee*[85]	60%/> 98% *ee* (yeast)[86][88]
35g	*t*Bu	(*S*)	17%/> 98% *ee*[85]	
35h	allyl	(*S*)		62%/95% *ee* (yeast)[86][88]
35i	Ph	(*S*)	14%/95% *ee* (EtOH)[87] 80%/60% *ee* (glycol)[87]	
35j	OMe	(*S*)	76%/75% *ee*[84]	84%/78% *ee* (*E.coli*)[89]
35k	Br	(*S*)		63%/97% *ee* (*E.coli*)[89]
35l	I	(*S*)		60%/97% *ee* (*E.coli*)[89]



Scheme 8. Ring-contraction of lactones with free hydroxy groups

oxidations of mesomeric substrates. Investigating the origin of the enantioselectivity of CHMO, we compared the microbial Baeyer–Villiger oxidation of some 4-mono- and 4,4-disubstituted ketones **40** by our recombinant whole-cell system (Scheme 9, Table 2).[89] We found that disubstitution with methyl and ethyl groups obviously does not interfere with the spatial properties of the catalytic cavity. Spiro systems of up to five atoms (**40g**)[90] can also be accommodated by this region of the active site. The mixed system **40d**



Scheme 9. Biocatalytic oxidation of 4-mono- and 4,4-disubstituted cyclohexanones

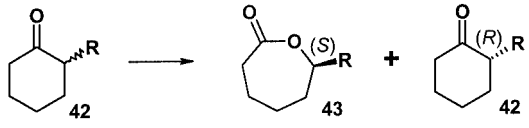
Table 2. Biocatalytic oxidation of 4-mono- and 4,4-disubstituted cyclohexanones

	R′	R′′	Yield	ee
41a	Me	H	61%[89]	≥ 98%
41b	Me	Me	61%[89]	n.a.
41c	Et	H	91%[89]	97%
41d	Et	Me	91%[89]	75%
41e	Et	Et	60%[89]	n.a.
41f	*cyclo*-CH_2CH_2		74%[89]	n.a.
41g	*cyclo*-OCH_2CH_2O		40%[90]	n.a.

(R′ = Et; R′′ = Me) displayed an optical purity significantly lower than those found for the monosubstituted substrates.

In monosubstituted systems the 4-hydrogen atom adopts the thermodynamically favored *axial* position, with the alkyl group *equatorial*, and a pronounced energy difference exists relative to the opposite conformer. In the case of nonidentical alkyl groups R′ and R′′, the energy difference is decreased, due to the closer similarity of the substituents. Hence, the energy difference in the possible Criegee intermediates is blurred and both forms can be adopted. The observed *ee* of 75% for **41d** corresponds to a difference of 1.2 kcal/mol between the free energies of the two transition states, supporting this interpretation.

The same preference for generation of (*S*)-lactones **43** is observed for racemic 2-substituted cyclohexanones **42** (Scheme 10, Table 3). (Note that branched groups and substituents with unsaturation or heavy atoms may require a change in priority numbering, giving rise to a reversal of the (*R*)/(*S*) assignment; in all cases the sense of chirality



Scheme 10. Kinetic resolution of racemic 2-substituted cyclohexanones

Table 3. Kinetic resolution of racemic 2-substituted cyclohexanones (yield and *ee* of recovered ketones in italics)

	R	Isol. enzyme	Native cells	Recombinant cells
43a	Me	n.r./n.r.[44] n.r./ca. 30% *ee*[50]	35%/61% *ee*[91] *52%/35% ee* (E = 6)	50%/49% *ee* (yeast)[88][92] (E = 10)
43b	Et		60%/38% *ee*[91] *10%/> 98% ee* (E = 58)	79%/95% *ee* (yeast)[88][92] *69%/>98% ee* (E > 200)
43c	Pr			54%/97% *ee* (yeast)[88][92] *66%/92% ee* (E > 200)
43d	*i*Pr			41%/> 98% *ee* (yeast)[88][92] *46%/96% ee* (E > 200)
43e	allyl			59%/> 98% *ee* (yeast)[88][92] *58%/> 98% ee* (E > 200)
43f	Bu		6%/> 98% *ee*[91] *37%/10% ee* (E > 100)	59%/> 98% *ee* (yeast)[88][92] *64%/98% ee* (E > 200)
43g	C_6H_{13}		23%/98% *ee*[91] *25%/35% ee* (E > 100)	
43h	C_9H_{19}		26%/85% *ee*[91] *32%/42% ee* (E = 20)	
43i	Ph		40%/> 98% *ee*[91] *48%/86% ee* (E > 100)	
43j	Bn		22%/> 96% *ee*[91] *28%/78% ee* (E > 100)	
43k	CH_2COOEt	39%/> 99% *ee*[57] *60%/64% ee*		
43l	CH_2CH_2OAc	34%/> 99% *ee*[57] *66%/76% ee*		

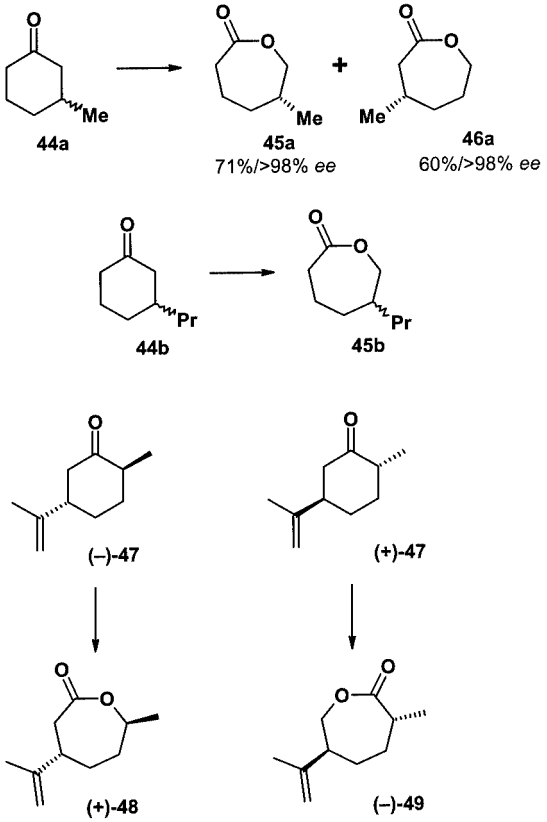
remained the same.) Here, the enzyme selectivity recognizes the preexisting chiral center and converts only (*S*)-ketones, with the opposite enantiomers remaining unchanged. Such a kinetic resolution gives a maximum yield of 50% under optimum conditions. In elaborate studies, the groups of Furstoss and Willetts[91] and of Stewart and Kayser[88,92] have investigated several substrates, using both native and recombinant organisms. They generally noticed a beneficial effect of chain size on the optical purities both of unchanged ketone and of lactone produced. Vogel et al. showed that ester groups are tolerated by the enzyme.[57]

The enantioselectivity of the enzyme for a specific substrate can be quantitatively described by the enantiomeric ratio *E*,[93] which corresponds to the ratio of the relative second-order rate constants of the individual substrate enantiomers and remains constant throughout the transformation. For synthetically useful kinetic resolutions, *E* needs to be > 30.

The outcome of BVMO-mediated oxidation of 3-substituted cyclohexanones depends heavily on chain size (Scheme 11). In the cases of methyl (**44a**) and ethyl, the two enantiomeric substrate ketones are oxidized to alternate lactone regioisomers **45a** and **46a**. Substrates with three or more carbon chains at position 3 (**44b**) are oxidized to one regioisomer (**45b**) with poor enantioselectivity.

Similar enantiodivergent behavior of the enzyme was observed with dihydrocarvone. While (−)-dihydrocarvone **47** gave the expected optically pure lactone (+)-**48** (80%/95%, depending on native strain), biotransformation of (+)-**47** afforded (−)-**49** through preferred migration of the less substituted center.[94]

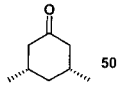
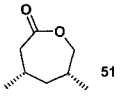
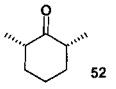
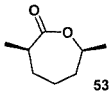
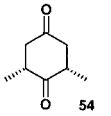
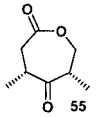
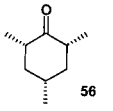
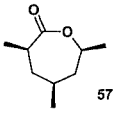
Mesomeric disubstituted cyclohexanones generally give good enantioselectivity, but yields depend on the positions



Scheme 11. Microbial Baeyer−Villiger oxidation of 3-substituted cyclohexanones

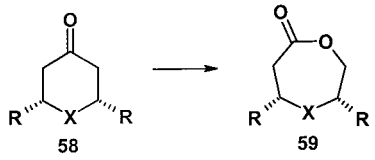
of side chains (Table 4); while 3,5-dimethyl-substituted **50** is efficiently converted, α-substitution (**52**) results in decreased yields.[84] Hence, diketone **54** is regioselectively converted

Table 4. Mesomeric di- and trisubstituted cyclohexanones

R	R	Isol. enzyme
 50	 51	73%/> 98% *ee*[84]
 52	 53	27%/> 98% *ee*[84]
 54	 55	25%/> 98% *ee*[84]
 56	 57	n.r./48% *ee*[57]

into lactone **55**. A drop in enantioselectivity was reported for trimethyl lactone **57**.[57]

The first six-membered heterocyclic ketones containing sulfur as substrates for CHMO were investigated by Walsh and co-workers.[82] We recently expanded the substrate profile to 6-membered heterocycles **58**, bearing both free amine base and protected nitrogen functionalities (Scheme 12, Table 5).[95] Recombinant *E. coli* cells displayed high yields and enantioselectivity for oxygen-containing systems (**59e/f**).[96]



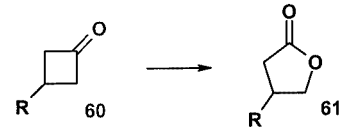
Scheme 12. Microbial oxidation of heterocyclic ketones

Table 5. Microbial oxidation of heterocyclic ketones (yields in parenthesis based on consumed starting material)

	X	R	Recomb. cells
59a	S[82]	H	48%[95]
59b	NMe	H	50%[95]
59c	NCOMe	H	39% (59%)[95]
59d	NCOOMe	H	40% (67%)[95]
59e	O	H	79%[95]
59f	O	Me	79%/> 99% *ee*[96]

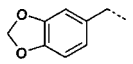
CHMO is also able to oxidize ring systems of different sizes. Kinetic resolution of 2-alkylcyclopentanones was first reported by Furstoss and co-workers, using native cells.[62] A detailed survey by the groups of Kayser and Stewart, utilizing recombinant "designer yeast", gave results similar to those obtained in the cyclohexanone series.[97] Enantiopreference for the (*S*) configuration tends to be lower, which was attributed to the smaller energy differences between substituents occupying the pseudo-*axial* or -*equatorial* position relative to the situation with six-membered rings. Biotransformation of 3-substituted cyclopentanones gave mixtures of both regioisomers in moderate optical purity.[97]

A series of prochiral cyclobutanones **60** was converted by *Acinetobacter* whole cells into the corresponding (*S*)-lactones **61** with moderate to good enantioselectivities (Scheme 13, Table 6).[98–100]

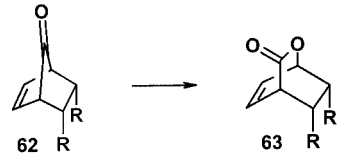


Scheme 13. Biotransformation of prochiral cyclobutanones

Table 6. Biotransformation of prochiral cyclobutanones

	R	*Acinetobacter*	*Cunninghamella*
61a	Bu	68%/17% *ee* (*S*)[98]	
61b	*i*Bu	56%/84% *ee* (*S*)[98]	
61c	Ph	70%/43% *ee* (*R*)[99]	65%/98% *ee* (*R*)[99]
61d	4-FC_6H_4	89%/19% *ee* (n.d.)[99]	80%/> 98% *ee* (n.d.)[99]
61e	4-ClC_6H_4	88%/85% *ee* (*S*)[100]	30%/> 98% *ee* (*R*)[99]
61f	4-MeC_6H_4	73%/91% *ee* (*S*)[99]	4%/n.d. *ee* (n.d.)[99]
61g	Bn	57%/82% *ee* (*S*)[98]	
61h	3-$MeOC_6H_4CH_2$	83%/96% *ee* (*S*)[99]	68%/77% *ee* (*S*)[99]
61i		83%/95% *ee* (*S*)[98]	68%/91% *ee* (*S*)[99]
61j	$PhCH_2OCH_2$	90%/55% *ee* (*S*)[98]	74%/98% *ee* (*R*)[99]
61k	*t*$BuOCH_2$	43%/89% *ee* (n.d.)[99]	25%/> 98% *ee* (n.d.)[99]

Taschner et al. used CHMO to convert prochiral bicyclic substrates **62** (Scheme 14, Table 7).[101] The enzyme is able to differentiate the olefinic and aliphatic bridge and tolerates a multitude of cyclic *endo* substituents.

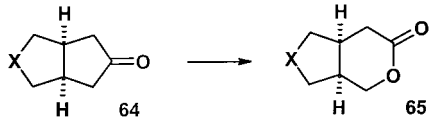


Scheme 14. Enzymatic Baeyer–Villiger oxidation of bicyclic ketones with CHMO

Table 7. Enzymatic Baeyer–Villiger oxidation of bicyclic ketones with CHMO

	R	Isol. enzyme
63a	H	62%/80% *ee*[101]
63b	Me	70%/> 98% *ee*[101]
63c	Et	83%/93% *ee*[101]
63d	$-CH_2OCH_2-$	74%/> 98% *ee*[101]
63e	$-C_3H_6-$	80%/97% *ee*[101]
63f	$-C_4H_8-$	78%/> 98% *ee*[101]
63g	$-C_5H_{10}-$	57%/> 98% *ee*[101]
63h	$-C_6H_{12}-$	55%/87% *ee*[101]

In a further expansion of the substrate profile of our recombinant *E. coli* expression system for CHMO, we became interested in fused systems of type **64** as interesting probes with which to study the influence of ring size and polarity of functional groups at the bicyclic core. We found that rigidity in the fused system is essential for high enantioselectivity; bicyclo[4.3.0] ketones **64a/b** were biotransformed with hardly any stereo-preference, while the corresponding bicyclo[3.3.0] system **64c** displayed high optical selectivity for the product lactone **65c** (Scheme 15, Table 8).[102]



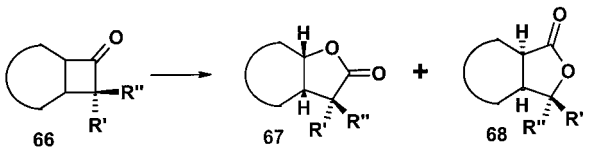
Scheme 15. Biotransformation of functionalized fused systems with CHMO- and CPMO-expressing recombinant cells

Table 8. Biotransformation of functionalized fused systems with CHMO- and CPMO-expressing recombinant cells (yields in parentheses based on consumed starting material)

	X	*E. coli* [CHMO]	*E. coli* [CPMO]
65a	−CH=CH−	33% (85%)/5% *ee*[102] (4a*S*,8a*S*)	76%/> 99% *ee*[102] (4a*R*,8a*R*)
65b	$-CH_2CH_2-$	21% (65%)/3% *ee*[102] (4a*S*,8a*S*)	83%/99% *ee*[102] (4a*R*,8a*R*)
65c	CH_2	50%/89% *ee*[103] (4a*S*)	85%/9% *ee*[132] (4a*R*)
65d	*endo*-CHOMe	24% (71%)/9% *ee*[103] [4a*S*-(4aα,6β,7aα)]	81%/34% *ee*[132] [4a*R*-(4aα,6β,7aα)]
65e	*exo*-CHOMe	40% (81%)/96% *ee*[103] [4a*S*-(4aα,6α,7aα)]	
65f	*endo*-CHCl	75% (98%)/80% *ee*[103] [4a*R*-(4aα,6β,7aα)]	
65g	*exo*-CHCl	78% (97%)/> 99% *ee*[103] [4a*R*-(4aα,6α,7aα)]	92%/> 99% *ee*[132] [4a*S*-(4aα,6α,7aα)]

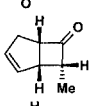
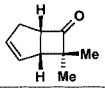
The recombinant expression system gave better yields with less polar chloro-substituted ketones **64f/g** than with methoxylated precursors **64d/e**.[103] Insight into the steric requirements of the enzyme's active site was gained by comparing the bioconversion of *exo* (**65e/g**) and *endo* substrates (**65d/f**) with respect to enantioselectivity. The stereochemistry at the 6-position plays a significant role in the recognition and introduction of chirality by the biocatalyst. An *exo* substitution pattern with a "stretched" geometry had a beneficial effect on the enantioselectivity, while *endo*-lactones ("angled" configuration) displayed significantly decreased optical purity.

The rigidity of fused systems is further reinforced when a cyclobutanone moiety is incorporated, hence improving the enantioselectivity of CHMO. Biotransformation of such racemic substrates **66** proceeds in a regiodivergent manner (Scheme 16, Table 9); while the enantiomeric (*S*,*S*)-ketone (R′ = R″ = H) generates the expected lactone **67** with migration of the more substituted center ("normal" product), the opposite (*R*,*R*) enantiomer produces lactone **68**,



Scheme 16. Regiodivergent oxidation of racemic fused systems

Table 9. Regiodivergent oxidation of fused systems by CHMO

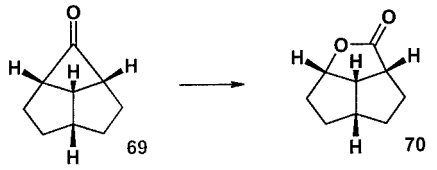
Substrate ketone	"Normal" lactone **67**		"Abnormal" lactone **68**
	44%/> 95% *ee* (1*S*,5*R*)[104]	**a**	42%/> 95% *ee* (1*R*,5*S*)[104]
	36%/> 95% *ee* (1*S*,5*S*)[104]	**b**	31%/> 95% *ee* (1*R*,5*S*)[104]
	43%/> 95% *ee* (1*R*,6*S*)[104]	**c**	37%/> 95% *ee* (1*S*,6*R*)[104]
	41%/86% *ee* (1*S*,6*S*)[104]	**d**	36%/> 95% *ee* (1*S*,6*R*)[104]
	52%/60% *ee* (1*S*,6*S*)[104]	**e**	28%/> 95% *ee* (1*S*,6*R*)[104]
	35%/90% *ee* (1*R*,5*S*)[105]	**f**	32%/> 98% *ee* (1*R*,5*S*)[105]
	35%/97% *ee* (1*R*,5*R*)[105]	**g**	35%/> 98% *ee* (1*S*,5*R*)[105]
	33%/> 98% *ee* (1*S*,5*S*)[105]	**h**	41%/> 98% *ee* (1*R*,5*S*)[105]
	33%/70% *ee* (1*R*,6*S*)[105]	**i**	33%/> 98% *ee* (1*R*,6*S*)[105]
	60%/33% *ee* (1*S*,6*S*)[105]	**j**	18%/> 98% *ee* (1*S*,6*R*)[105]
	55% combined yield > 96% *ee*[106,107] R′ = Me; R″ = H	**k**	55% combined yield > 96% *ee*[106,107] R′ = Me; R″ = H
	0%[106,107] R′ = Me; R″ = Me	**l**	63%/29% *ee*[106,107] R′ = Me; R″ = Me

with oxygen insertion at the less substituted site ("abnormal" product).[104,105]

The study of substituted fused systems revealed regiodivergent behavior of CHMO in the case of one α-methyl group in the *endo* position (**66k**), while double substitution decreased the optical purity of the single (1*R*,5*S*)-lactone **68l** substantially.[106,107]

The origin of the regiodivergent behavior of CHMO was an open question for a considerable time; the proposed existence of isozymes was ruled out by inhibitory studies in Furstoss' group.[83]

Prompted by the biocatalytic profile of the enzyme, initial active site, models were developed by Taschner[108] and Furstoss.[94] In further investigations of the origin of regiodivergence, Kelly and co-workers demonstrated that both enantiomeric substrates are accommodated within the active site of the enzyme. They successfully converted the tricyclic ketone **69**, as a superimposition of both substrate



Scheme 17. Enzymatic oxidation of tricyclic ketone **69**

enantiomers **66**, into lactone **70** with > 98% *ee* (Scheme 17), elegantly confirming prior evidence for a single BVMO, bearing a single active site.[109,110] Hence, the reactivity of the active site was explained by the ability of a bond to occupy the antiperiplanar position in the Criegee intermediate (Figure 3).

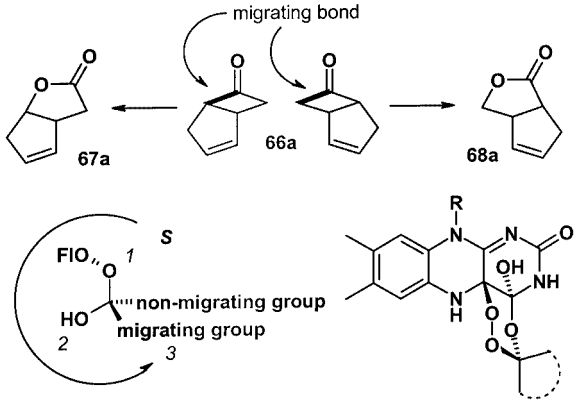


Figure 3. Origin of the regiodivergent behavior of CHMO

By application of the results obtained with the tricyclic substrate, it is possible to deduce the nature of the Criegee intermediate. By using the preference rules expanded for migrating group > non-migrating group, an (*S*) configuration can be assigned for the intermediate, and *si* migration occurs. This orientation was observed for *Type 1* (NADPH/FAD-dependent) BVMOs, while *Type 2* (NADH/FMN-dependent) enzymes such as diketocamphane-MOs (DKCMOs) from *Pseudomonas* displayed formation of *ent*-lactones, and hence antipodal migration.[23,111] (Willetts proposed a classification of BVMOs into two groups depending on the nature of cofactors required by the enzyme: *Type 1* proteins are NADPH- and FAD-dependent, while *Type 2* BVMOs possess NADH as cofactor and FMN as prosthetic group.[21])

This concept is consistent with Taschner's model, from which he concluded that the peroxide is formed on the *re* face of the flavin and will occupy an equatorial position in the Criegee intermediate.[108]

Kelly further suggested additional fixation of the Criegee intermediate by ring-closure at C-4 of the pyrimidine core of FAD (Figure 3). The resulting moiety has all the requisite bonding and non-bonding orbitals optimally aligned for the Baeyer−Villiger rearrangement under stereoelectronic control, with abstraction of the C-4 hydroxy proton initiating rearrangement.[112] While this concept offers an intriguing solution to the question of entropy in the rearrangement process, no experimental evidence for such a cyclized species has yet been found.

Ottolina and co-workers further developed and expanded the cubic space model into a predictive theory, also for the normal/abnormal oxidations of fused ketones **66**.[113,114] Recently, substrate concentration has been found to influence the oxidation of substrate **66a**, with substantial substrate inhibition. Carrea et al., working on the basis of the cubic space model, developed the hypothesis of a second binding site with lower affinity and published an interpretation to explain this effect.[115]

Stewart developed the above interpretations into a more simplistic "diamond-lattice" model. Estimations of substrate acceptance of − for example − cyclohexanones are possible by assignation of "allowed" and "forbidden" sites of substitution.[88,89]

Taken together, the above observations suggest that CHMO imposes only small steric constraints resulting in stereoselectivity on the substrates. Rather, diastereofacial selectivity in the peroxide addition plays a dominant role, establishing the configuration in the Criegee intermediate, while stereoelectronic effects are responsible for migratory preference.

5.2 Other Microbial BVMOs that Oxidize Alicyclic Ketones

A number of other enzymes and organisms capable of converting cyclohexanone have been described in the literature. Two electrophoretically distinct forms of CHMO from *Nocardia globerula* CL1 have been isolated and characterized.[116] A native strain was found to convert cycloheptanone to pimelic acid, while also accepting C_5 to C_8 cycloketones as substrates.[117]

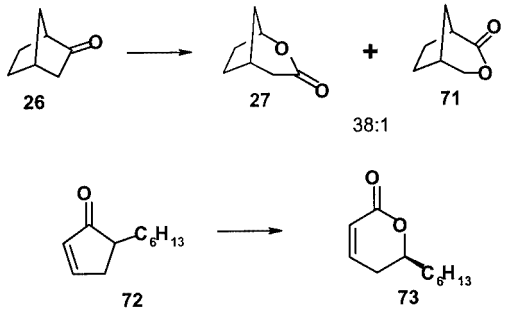
The biodegradation of cyclohexylacetate to adipate by *Arthrobacter* CA1[118] and of cyclohexylamine by *Brevibacterium oxydans*[119] was demonstrated to proceed through Baeyer−Villiger oxidation of cyclohexanone. Both NADPH- and NADH-dependent BVMOs were reported to be present in *Xanthobacter*.[120] Purification of an enzyme, induced by elective culture on cyclohexane, established FMN as a prosthetic group.[121] Whole cells of *Xanthobacter* NCIMB 10811 display regiodivergent oxidation of bicyclic ketone **66** in favor of "normal" lactone **67**, although with only moderate optical purity (Scheme 16, R′ = R″ = H).[122]

Rhodococcus erythropolis DCL14 contains a BVMO that is dependent on NADPH and plays a role in the degradation of limonene.[39,123] In contrast to camphor degradation in *Pseudomonas*, in which three enzymes are involved, *Rhodococcus* seems to use only one biocatalyst in three different pathways. This enzyme MMKMO (monocyclic monoterpene ketone MO) has been isolated and the substrate profile includes several monoterpene ketones, together with substituted cyclohexanones.[124]

A BVMO with substrate specificity for larger-ring ketones was found in another *Rhodococcus* species (*R. ruber* CD4); the activity of the FAD-containing NADPH-dependent enzyme was insignificant for the oxidation of cyclic

C_6 or C_8 ketones, whereas C_{12} and C_{15} substrates were efficiently converted into the corresponding lactones.[125]

Elective growth of *Pseudomonas* NCIMB 9872 on cyclopentanol for determination of the metabolic pathway resulted in the discovery of another enzyme accepting alicyclic substrate ketones.[126] Cyclopentanone monooxygenase (CPMO, EC 1.14.13.16) was isolated and identified as an FAD-containing enzyme dependent on NADPH.[127] Initial substrate specificity tests showed acceptance for cyclic C_4 to C_8 ketones and norbornanone. The first preparative-scale biotransformations were performed by Willetts et al., who used washed-cell suspensions of the *Pseudomonas* strain for the conversion of norbornanone. The quantitative enzyme-catalyzed conversion showed a 38:1 selectivity in favor of lactone **27**, with some accompanying by-product **71** (Scheme 18).[128] CPMO demonstrates an enantioselectivity similar to that of CHMO towards the formation of (*S*)-lactones from α-substituted cyclopenta- and -hexanones, but lower optical purities are obtained. Incorporation of functionalized groups into the α-chains produces an improvement of enantiomeric excess for the product lactones, as reported recently.[129] It is noteworthy that conversion of enone **72** was reported to produce lactone **73** regioselectively (Scheme 18).[130]



Scheme 18. Baeyer−Villiger oxidations by CPMO-producing cells

A recombinant expression system for CPMO for an *E. coli* host was recently constructed.[131] We utilized the engineered strain for the conversion of prochiral fused systems **64a/b** and found a preference of CPMO for the formation of the opposite enantiomer to that obtained with CHMO. The enzyme produced optically pure (+)-lactones **65a/b** in excellent yield from six/five-fused ketone precursors,[102] while the five/five-fused substrate **64c** gave an almost racemic product (**65c**). Introduction of functional groups into such five/five-fused ketones improves the enantioselectivity of the biocatalyst (**65d,g**). The optical purity of the products is, as in CHMO, dependent on conformational aspects, with *exo* substitution exhibiting a beneficial effect (Scheme 15, Table 8).[132]

5.3 Camphane-Metabolizing Monooxygenases

Microbial degradation of camphor[133] involves several BVMOs, and the pathway for *Pseudomonas putida* has been identified (Scheme 19). (+)-Camphor **74** is oxidized by an enzyme complex containing cytochrome P450 to 5-*exo*-hydroxycamphor (**75**), followed by a dehydrogenase-mediated oxidation to 2,5-diketocamphane (**76**). This compound serves as a substrate for 2,5-diketocamphane monooxygenase (2,5-DKCMO), an NADH-dependent enzyme containing FMN bound in a non-covalent manner.[134,135] It forms a loose complex with an NADH dehydrogenase, enabling a coupled electron transfer to take place.[136,137]

The resulting lactone **77** undergoes elimination to provide the enone **78**. In this context it is interesting to note that camphor-grown *P. putida* cells do not possess lactone hydrolase activity towards any of the lactone products. Conversion into the coenzyme A derivative by a CoA-synthetase generates the substrate for the second BVMO, 2-oxo-Δ^3-4,5,5-trimethylcyclopentylacetyl coenzyme A monooxygenase (MO2). (A mixture of 2,5-DKCMO and 3,6-DKCMO was originally named "MO1".) This enzyme possesses FAD as a prosthetic group and requires NADPH and oxygen for biocatalytic activity.[138] The corresponding genes for the BVMOs are located as a cluster on a transmissible plasmid.[139]

While oxidation by 2,5-DKCMO is only observed in the bioconversion of (+)-camphor, (−)-camphor is metabolized by the isofunctional enzyme 3,6-DKCMO.[140] (In this case the numbering system for diketocamphane *ent*-**76** was changed to identify corresponding centers in the substrate molecules.) Both 2,5- and 3,6-DKCMO are able to oxidize sulfur functionalities, and a cubic space active site model, based on ample data, has been developed.[141]

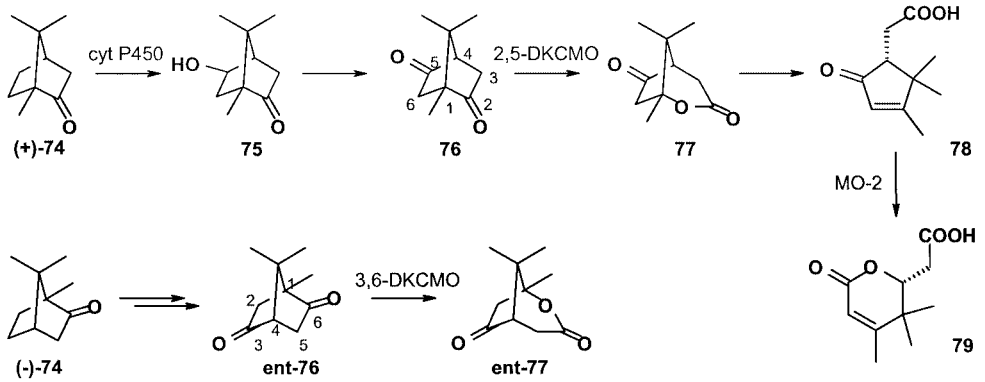
Both pathways can be induced by elective growth on the corresponding camphor enantiomer. Such behavior is also observed for *Corynebacterium*.[142]

Preparative biotransformations were initially carried out with whole cells of *Pseudomonas putida* NCIMB 10007 or with enzyme preparations. 2,5-DKCMO demonstrated enantioselectivity opposite to that of MO2 (and of CHMO from *Acinetobacter*), consistently with Kelly's general active site concept for FAD- and FMN-dependent enzymes.[110]

Conversion of the racemic ketone **66a** by 2,5-DKCMO gave regiodivergent oxidation to the "normal" lactone (1*R*,5*S*)-**67a** (89% *ee*) and the "abnormal" product (1*S*,5*R*)-**68a** (99% *ee*), while biotransformation with MO2 produced enantiomeric products with 35% *ee* and 95% *ee*, respectively.[143] The performance of isolated 2,5- and 3,6-DKCMO displayed similar enantiopreference for the conversion of this substrate type, with lower selectivity of the latter enzyme.[144] Whole-cell transformations furnished 86% total yield of (1*R*,5*S*)-**67a** (50% *ee*) and (1*S*,5*R*)-**68a** (> 95% *ee*).[145]

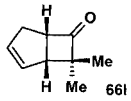
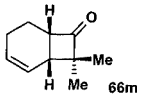
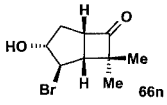
Regiopreference similar to that of CHMO was observed for the Baeyer−Villiger oxidation of α,α-disubstituted bicyclic ketones **66l−n**, preferentially affording lactone **68**. However, the enantioselectivity tends to be low unless additional substituents impose a directing effect (Scheme 16, Table 10).[146]

Biotransformation of norbornanone **26** with preparations of (+)-camphor-grown cells resulted in the formation of



Scheme 19. Camphor degradation through Baeyer−Villiger oxidation

Table 10. Baeyer−Villiger oxidation of α,α-disubstituted fused systems by *Pseudomonas putida* AS1

Substrate ketone	Lactone **67**	Lactone **68**
 **66l**	1%/n.d.[146]	58%/15% *ee*[146]
 **66m**	11%/n.d.[146]	59%/10% *ee*[146]
 **66n**	0%/n.d.[146]	11%/92% *ee*[146]

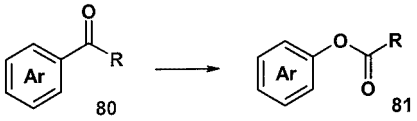
(1*R*,5*R*)-lactone **27** (53% *ee*) and recovery of optically active substrate ketone (1*S*,4*R*)-**26** (70% *ee*, Scheme 18).[145]

Both 2,5-DKCMO and MO2 perform kinetic resolutions of a variety of racemic 2-alkylcyclopentanones to produce (*S*)-lactones.[143] Biotransformations using cell suspensions or partially purified BVMOs from *P. putida* also displayed conversions of cyclopentenones into the corresponding α,β-unsaturated δ-alkyllactones. Mixtures of products were obtained, however, due to additional reduction of the C−C double bond by a tentatively proposed enoyl reductase.[147]

Application of isolated DKCMOs was improved by utilizing PEG-NADH in a membrane reactor.[148] The regioselectivity of norbornane oxidation with whole cells in biphasic systems and organic solvents was studied recently.[149]

5.4 Acetophenone Monooxygenases (APMOs)

Microbial degradation of acetophenone and derivatives is reported to be initiated by a Baeyer−Villiger oxidation in *Arthrobacter*,[150] *Nocardia*,[151] *Alcaligenes*,[152] and *Pseudomonas*[152] species. The acetophenone MOs involved are able to convert aromatic oxo groups, which are generally less reactive than aliphatic carbonyl functionalities (Scheme 20). In early studies, production of the enzymes was induced by elective growth on acetophenone-supplemented media. Cell suspensions and cell extracts were



Scheme 20. Biocatalytic Baeyer−Villiger oxidation of aromatic ketones

studied for oxygen take-up upon feeding of substrates with halogen substitution in the phenyl system and the side chain. Isolation of the corresponding esters required inhibition of a highly active hydrolase that is generally present for subsequent saponification of the BVMO products.[152]

Two enzymes have been isolated from *Pseudomonas putida* JD1[153] and *P. flourescens* ACB[154] and characterized as NADPH-dependent flavoproteins. A recombinant expression system has been created for the latter biocatalyst for large-scale production of protein (hydroxy-APMO−HAPMO).

Table 11 gives an overview of substrate profiles for APMOs and their origins. The data compiled are in most cases based on indirect evidence (oxygen take-up of cultures etc.). Product characterization[152][153] and kinetic parameters[154] are provided in a minority of examples.

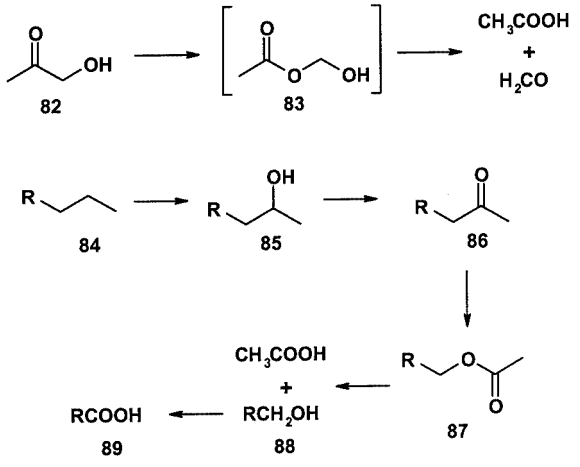
Identification of several metabolites in the degradative pathway of fluorene in *Arthrobacter* suggests the microbial Baeyer−Villiger oxidation of 1-indanone to dihydrocoumarin.[155] Taken together with the above data, this family of enzymes seems to have potential to become synthetically useful catalysts for chemo- and regioselective biotransformations, with further investigations promising interesting results.

5.5 Aliphatic Straight-Chain Ketone Oxidation

A monooxygenase from *Mycobacterium* Py1 is responsible for the conversion of acetol **82** into acetate and formaldehyde.[156] Aliphatic methyl ketones **86** are converted by BVMOs from *Pseudomonas*[157−159] and *Nocardia*[160] species. Labeling experiments confirmed the Baeyer−Villiger mechanism,[160] and these biotransformations represent one step in a general sequence for the subterminal oxidation pathway of long-chain alkanes **84** to carboxylic acids **89** and acetate (Scheme 21).[161]

Table 11. Biocatalytic Baeyer−Villiger oxidation of aromatic ketones

Ar	R	*Alcaligenes*	*Arthrobacter*	*Pseudomonas*
Ph	CH_3	[152]	[150,151]	[152−154]
4-HOC_6H_4	H			[154]
4-HOC_6H_4	CH_3			[153,154]
4-$MeOC_6H_4$	CH_3			[153,154]
4-HO-3-MeC_6H_3	CH_3			[154]
4-$H_2NC_6H_4$	CH_3			[154]
4-MeC_6H_4	CH_3			[154]
2-FC_6H_4	CH_3	[152]		[152]
3-FC_6H_4	CH_3	[152]		[152]
4-FC_6H_4	CH_3	[152]		[152,154]
2-ClC_6H_4	CH_3	[152]	[150]	[152]
3-ClC_6H_4	CH_3	[152]	[150]	[152]
4-ClC_6H_4	CH_3	[152]	[150]	[152]
2,4-$Cl_2C_6H_3$	CH_3	[152]	[150]	[152]
2,5-$Cl_2C_6H_3$	CH_3	[152]		[152]
2,6-$Cl_2C_6H_3$	CH_3	[152]		[152]
3,4-$Cl_2C_6H_3$	CH_3	[152]		[152]
2,3,4-$Cl_3C_6H_2$	CH_3		[150]	
2,4,5-$Cl_3C_6H_2$	CH_3	[150]	[150]	[150]
2-BrC_6H_4	CH_3	[152]		[152]
3-BrC_6H_4	CH_3	[152]		[152]
4-BrC_6H_4	CH_3	[152]		[152]
2-IC_6H_4	CH_3	[152]		[152]
4-IC_6H_4	CH_3	[152]		[152]
2,4-$Cl_2C_6H_3$	CH_2Cl	[150]	[150]	
Ph	CH_2Cl	[150]	[150]	
4-HOC_6H_4	CH_2CH_3			[153,154]



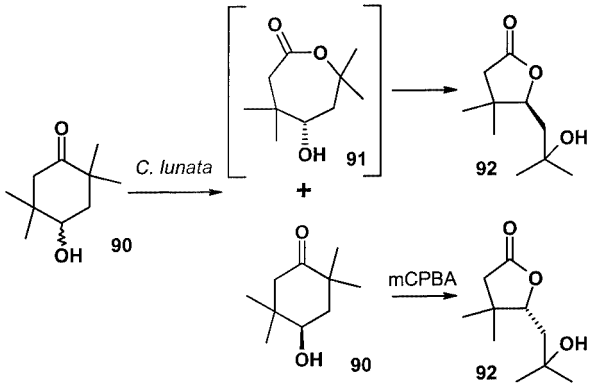
Scheme 21. Acyl chain degradation

Britton and co-workers purified a monooxygenase from *Pseudomonas cepacia* grown on 2-tridecanone.[162] The enzyme was characterized as consisting of two identical subunits, possesses FAD as cofactor, and is dependent on NADPH and oxygen. Substrate specificity tests showed formation of acetate esters **87** from methyl ketones **86** for C-7 to C-14. Additionally, ketones with the carbonyl group in positions 3 to 7 were oxidized. Specific activity determination of cyclic substrates showed high selectivity for cyclopentanone and a significantly decreased acceptance for cyclohexanone.

Similar pathways for methyl ketone degradation through acetate cleavage have also been encountered in some fungal species, such as *Cunninghamella*[163] and *Beauveria*.[164]

5.6 Fungal BVMOs Applied in Synthetic Chemistry

Synthetic applications of fungi for the production of optically pure lactones were first reported by Azerad and coworkers. In an optimized fermentation procedure, *Curvularia lunata* was used for kinetic resolution of racemic ketone **90** to obtain the (*S*)-lactone **92** (63%) and unchanged (*R*)-substrate (83%, 97% *ee*). Both biotransformation and chemical oxidation proceed via an unstable intermediate **91**, which rearranges to give compound **92** (Scheme 22).[165]



Scheme 22. Fungal oxidation by *Curvularia lunata*

Willetts' group, on the basis of these initial results, carried out a detailed study of BVMO activity in a survey including 29 fungal strains. They used racemic samples of fused ketone **66a** as probe and identified several *Curvularia* and *Drechslera* species as potent biocatalysts.[166] Some *Curvularia* strains displayed regiodivergent biotransformations with acceptable optical purities, but significant deviation was observed among members of the same genera.[167] Similar biocatalytic activity was reported for *Cylindrocarpon destructans*.[168]

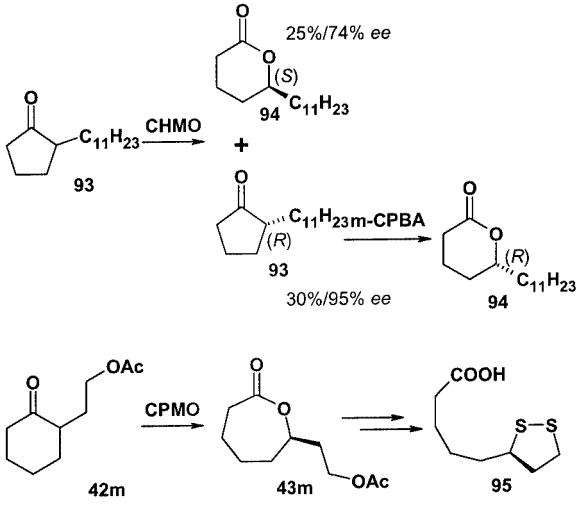
In contrast, *Cunninghamella echinulata* NRRL 3655 was reported to produce the "abnormal" (1*R*,5*S*)-lactone **68a** (30−35%, > 95% *ee*) exclusively, hence avoiding cumbersome chromatographic separation from the isomeric lactone.[169] In a comparative study on an array of mesomeric cyclobutanones, this fungus gave superior or antipodal results in some cases compared to CHMO from *Acinetobacter* (Scheme 13, Table 6),[99] and fermentation procedures for this organism have been optimized.[170]

Protein purification from eukaryotic organisms is more troublesome than from bacteria, so biotransformations utilizing fungal BVMOs have been carried out predominantly with whole cells. Recently, a flavo-protein was isolated from *Exophiala jeanselmei*. Substrate specificity was determined and significant conversion was reported for alicyclic C_4 to C_{10} ketones.[171]

6. Compounds of Interest

While much scientific effort has been dedicated to elucidation of the mechanistic basis of enzymatic Baeyer−Villiger oxidations and to evaluation of substrate profiles, a key issue has always been to demonstrate the applicability of this "green chemistry" approach for the production of chiral compounds as crucial intermediates in the synthesis of natural products or bioactive compounds. A remarkably broad tolerance of non-natural substrates, together with high enantioselectivity, are prerequisites for a powerful catalytic system for chiral synthesis.

Kinetic resolution of 2-undecylcyclopentanone (**93**) with the aid of hydrolase-deficient *Acinetobacter* TD63 furnished the (*S*)-lactone **94**, a pheromone isolated from the oriental hornet *Vespa orientalis*, in acceptable optical purity. Chemical oxidation of the recovered (*R*)-ketone afforded the antipodal product, which is also isolated from the natural source (Scheme 23).[62]

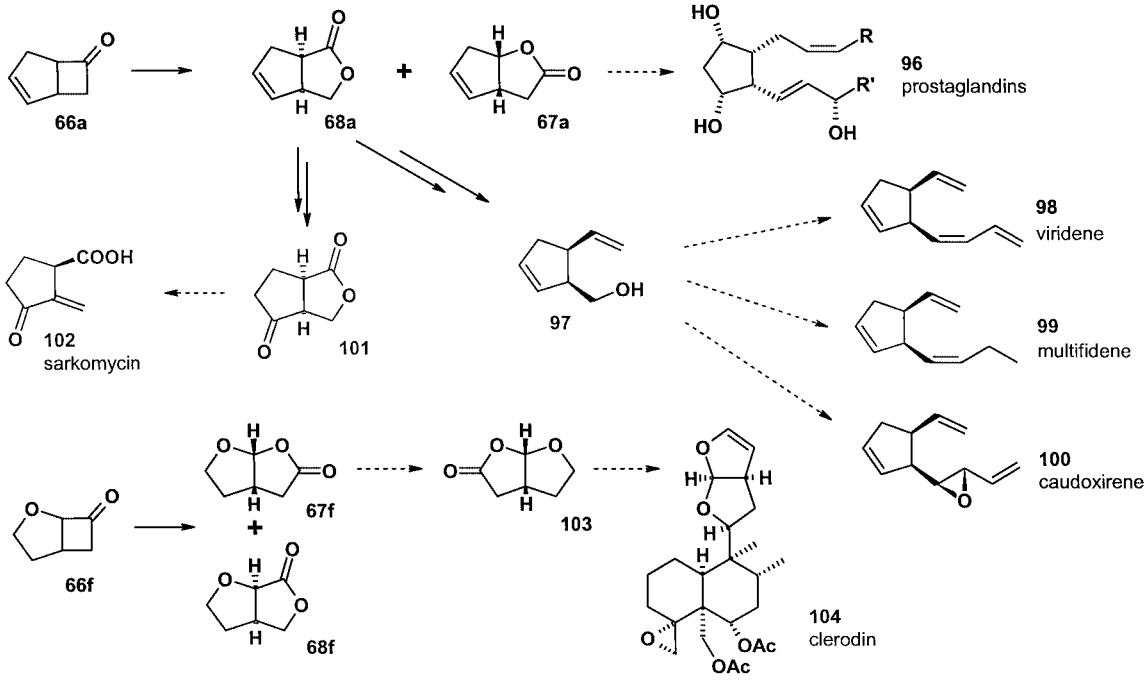


Scheme 23. Kinetic resolution of simple ketones to produce precursors utilized in synthetic routes

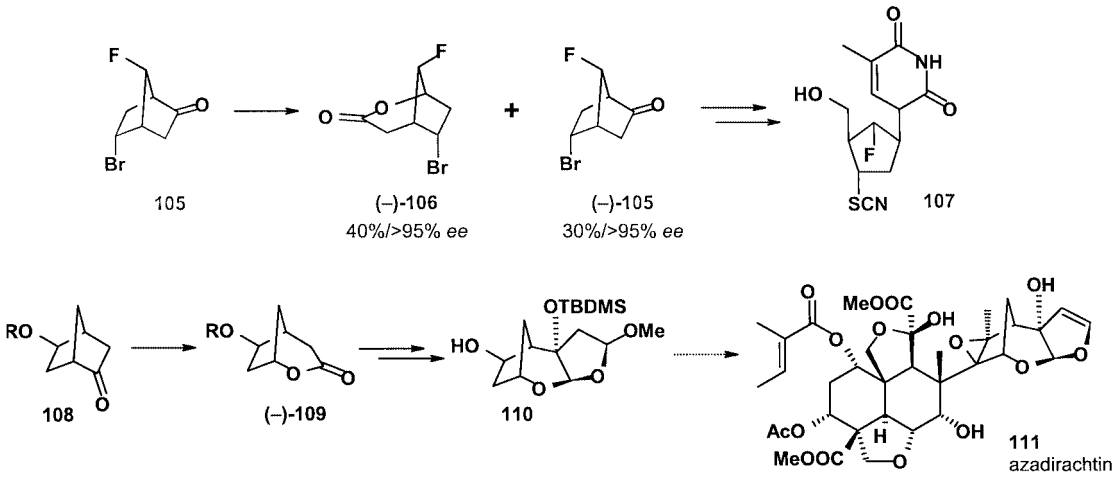
In a similar approach, the kinetic resolution of a side chain functionalized 2-substituted cyclohexanone **42m** was utilized to produce lactone **43m**. Both whole cells and isolated enzymes from various organisms were used for the biotransformation. While CPMO gave the (*R*)-lactone, DKCMO, MO2, and BVMOs from *Xanthobacter* oxidized the racemic substrate to the (*S*)-product.[172] This compound was subsequently used in a patented approach towards lipoic acid (**95**), a drug displaying activity for the treatment of hepatitis, pancreatitis, and induced carcinomas (Scheme 23).[173]

The "normal" Baeyer−Villiger product obtained by regiodivergent biotransformation of fused ketone **66a** by treatment with whole cells of *Acinetobacter* TD63 represents a first-row pivotal synthon for the synthesis of prostaglandins (**96**).[174] The "abnormal" lactone **68a**, accessible selectively in gram quantities by *Cunninghamella*-mediated oxidation, served as starting material for the straightforward synthesis of the brown algae pheromones viridiene (**98**), multifidene (**99**), and caudoxirene (**100**).[169] The same product was used for the preparation of cyclosarkomycin (**101**), a precursor for sarkomycin (**102**), a potential cytostatic (Scheme 24).[175]

A similar approach was used for the synthesis of the "northeast" part of the insect antifeedant clerodin (**104**); microbial oxidation of the heterocyclic fused system **66f** by *Acinetobacter* cells afforded lactones **68f** (40%, 100% *ee*) and **67f** (47%, 86% *ee*). However, the latter product possessed the wrong stereochemical configuration, and so an



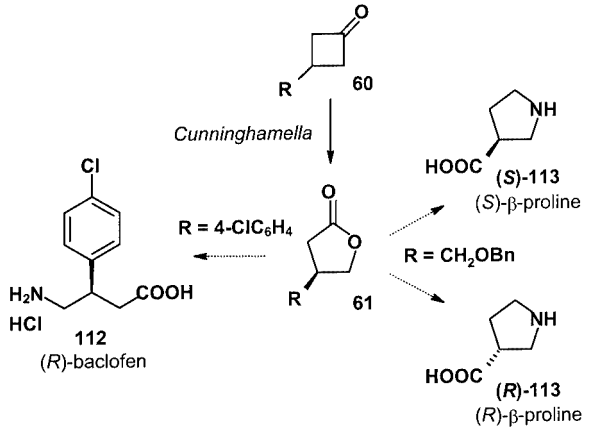
Scheme 24. "Normal" and "abnormal" fused lactones as precursors in natural product synthesis



Scheme 25. Kinetic resolution of bicyclic substrates for the production of synthetic precursors

elaborate chemical detour was finally required to obtain the correct precursor (**103**) for clerodin.[176]

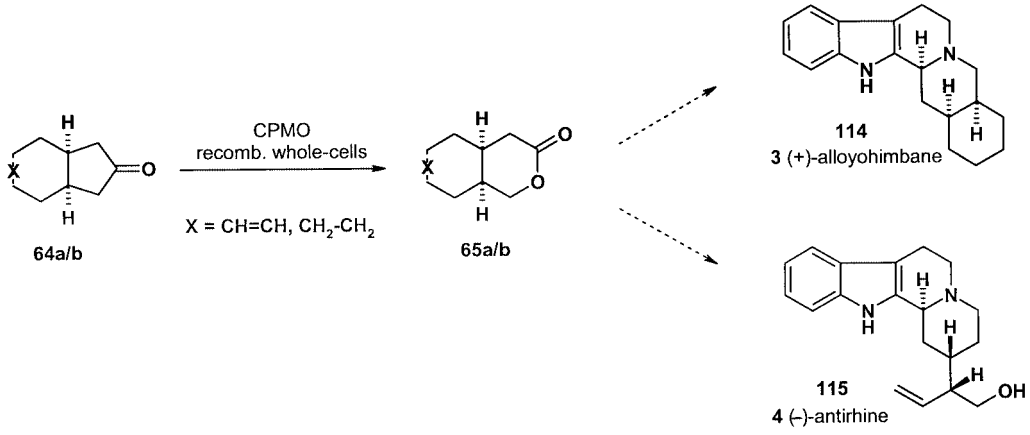
CHMO has been demonstrated to oxidize selected bicyclic ketones.[177,178] Kinetic resolution of **105** in the presence of CHMO-producing *Acinetobacter* cells was used to obtain the (−)-ketone **105** in optically pure form as a key intermediate for the production of antiviral fluorocarbocyclic nucleosides (**107**).[179] Norbornane ketones of type **108** were biotransformed into lactones **109** by *P. putida* NCIMB 10007 and by both isolated MO1 and MO2 in acceptable yields and with excellent optical purity. Such compounds can be converted into acetal **110**, an advanced precursor of the potent antifeedant and growth regulator agent azadirachtin **111** (Scheme 25).[180]



Scheme 26. Prochiral cyclobutanone substrates in bioactive compound synthesis

Asymmetrization of prochiral substrates is even more appealing for synthetic applications, since this biotransformation generates chirality with a theoretical yield of 100%. Furstoss and co-workers used such an approach to generate optically pure valerolactones **61** from 3-substituted cyclobutenones **60** by fermentation with *Cunninghamella*. These compounds serve as precursors for the production of enantiopure (*R*)-baclofen (**112**), a selective and therapeutically useful agonist of the $GABA_B$ receptor.[100] A similar substrate was utilized in the development of multigram enantiodivergent routes to (*R*)- and (*S*)-β-proline (**113**) (Scheme 26).[181]

Recently, we have for the first time used recombinant whole cells producing BVMO to prepare a key intermediate for the synthesis of alkaloids. We obtained the optically



Scheme 27. Precursors for indole alkaloid synthesis

pure fused lactones **65a/b** by fermentation with an overexpression system for CPMO. These compounds represent crucial precursors to access a family of indole alkaloids and we were able to present formal total syntheses of (+)-alloyohimbane (**114**) and (−)-antirhine (**115**) (Scheme 27).[102]

7. Outlook

With the rapid developments in molecular biology, an increasing number of genomes will be decoded in the years to come. In view of the many biological processes in which Baeyer−Villiger oxidations have so far been observed, and of the multitude of organisms capable of performing such oxygen insertions, a vast number of BVMOs must still remain to be discovered, characterized, and profiled. For the first time, novel techniques to identify such enzymes from large data sets of genomic sequences now allow rapid access to recombinant overexpression systems,[182] which can either be used as whole-cell biocatalysts or serve as sources for isolated protein.

Site-directed mutagenesis and gene shuffling enable controlled tuning of the biocatalytic activity of enzymes. The improvement of P450 proteins and other monooxygenases for the production of industrial chemicals has been demonstrated recently.[183] A multitude of novel biocatalytic entities, based on a large array of different genes for BVMOs, can be designed.

Rapid screening methods against representative substrate libraries employing microscale processing techniques[184] are a prerequisite to establish substrate profiles for such new proteins, and progress in this field will become a major challenge in the years to come. A vast new area of research is currently opening up, and recombinant whole-cell based approaches tackling the key issues of profiling and future synthetic applications − as currently being addressed by our group − offer an interesting option from the viewpoint of a synthetic chemist.

[1] A. Baeyer, V. Villiger, *Ber. Dtsch. Chem. Ges.* **1899**, *32*, 3625−3633.
[2] G. R. Krow, *Org. React.* **1993**, *43*, 251−798.
[3] M. Renz, B. Meunier, *Eur. J. Org. Chem.* **1999**, 737−750.
[4] R. Criegee, *Justus Liebigs Ann. Chem.* **1948**, *560*, 127−141.
[5] [5a] C. Bolm, G. Schlingloff, K. Weickhardt, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1848−1849. [5b] A. Gusso, C. Baccin, F. Pinna, G. Strukul, *Organometallics* **1994**, *13*, 3442−3451. [5c] C. Bolm, G. Schlingloff, *J. Chem. Soc., Chem. Commun.* **1995**, 1247−1248. [5d] C. Bolm, G. Schlingloff, F. Bienewald, *J. Mol. Catal. A: Chem.* **1997**, *117*, 347−350. [5e] C. Bolm, T. K. K. Luong, G. Schlingloff, *Synlett* **1997**, 1151−1152. [5f] G. Strukul, A. Varagnolo, F. Pinna, *J. Mol. Catal. A: Chem.* **1997**, *117*, 413−423. [5g] C. Bolm, O. Beckmann, T. Kühn, C. Palazzi, W. Adam, P. B. Rao, C. R. Saha-Möller, *Tetrahedron: Asymmetry* **2001**, *12*, 2441−2446. [5h] T. Uchida, T. Katsuki, *Tetrahedron Lett.* **2001**, *42*, 6911−6914. [5i] C. Bolm, O. Beckmann, A. Cosp, C. Palazzi, *Synlett* **2001**, 1461−1463. [5j] C. Bolm, O. Beckmann, C. Palazzi, *Can. J. Chem.* **2001**, *79*, 1593−1597.
[6] K. Faber, *Biotransformations in Organic Chemistry*, Springer, Berlin, **1980**.
[7] J. H. Dawson, M. Sono, *Chem. Rev.* **1987**, *87*, 1255−1276
[8] C. Walsh, *Acc. Chem. Res.* **1980**, *13*, 148−155.
[9] T. C. Bruice, *Acc. Chem. Res.* **1980**, *13*, 256−262
[10] S. Ghisla, V. Massey, *Eur. J. Biochem.* **1989**, *181*, 1−17.
[11] S. Colonna, N. Gaggero, C. Richelmi, P. Pasta, *NATO Sci. Ser. 1* **2000**, *33*, 133−160.
[12] [12a] D. R. Light, D. J. Waxman, C. Walsh, *Biochemistry* **1982**, *21*, 2490−2498. [12b] F. Secundo, G. Carrea, S. Dallavalle, G. Franzosi, *Tetrahedron: Asymmetry* **1993**, *4*, 1981−1982. [12c] J. Beecher, P. Richardson, A. Willetts, *Biotechnol. Lett.* **1994**, *16*, 909−912. [12d] P. Pasta, G. Carrea, H. L. Holland, S. Dallavalle, *Tetrahedron: Asymmetry* **1995**, *6*, 933−936. [12e] D. R. Kelly, J. C. Knowles, J. G. Mahdi, I. N. Taylor, M. A. Wright, *Tetrahedron: Asymmetry* **1996**, *7*, 365−368. [12f] S. Colonna, N. Gaggero, P. Pasta, G. Ottolina, *Chem. Commun.* **1996**, 2303−2307. [12g] S. Colonna, N. Gaggero, G. Carrea, P. Pasta, *Chem. Commun.* **1997**, 439−440. [12h] H. L. Holland, J.-X. Gu, A. Kerridge, A. Willetts, *Biocatal. Biotrans.* **1999**, *17*, 305−317.
[13] [13a] S. Colonna, N. Gaggero, A. Bertinotti, G. Carrea, P. Pasta, A. Bernardi, *J. Chem. Soc., Chem. Commun.* **1995**, 1123−1124. [13b] V. Alphand, N. Gaggero, S. Colonna, R. Furstoss, *Tetrahedron Lett.* **1996**, *37*, 6117−6120. [13c] V. Alphand, N. Gaggero, S. Colonna, P. Pasta, R. Furstoss, *Tetrahedron* **1997**, *53*, 9695−9706.
[14] S. Colonna, N. Gaggero, G. Carrea, P. Pasta, *Chem. Commun.* **1998**, 415−416.
[15] G. Ottolina, S. Bianchi, B. Belloni, G. Carrea, B. Danieli, *Tetrahedron Lett.* **1999**, *40*, 8483−8486.
[16] J. A. Latham, Jr., C. Walsh, *J. Chem. Soc., Chem. Commun.* **1986**, 527−528.
[17] J. A. Latham, Jr., B. P. Branchaud, Jr., Y.-C. J. Chen, C. Walsh, *J. Chem. Soc., Chem. Commun.* **1986**, 528−530.
[18] S. Colonna, N. Gaggero, G. Carrea, G. Ottolina, P. Pasta, F. Zambianchi, *Tetrahedron Lett.* **2002**, *43*, 1797−1799.
[19] C. T. Walsh, Y.-C. J. Chen, *Angew. Chem.* **1988**, *100*, 342−352.
[20] M. J. Taschner, L. Peddada, P. Cyr, Q.-Z. Cjem. D. J. Black, in *Microbial Reagents in Organic Synthesis* (Ed.: S. Serve), Kluwer Academic, Dordrecht, **1992**, p. 347−360.
[21] A. Willetts, *Trends Biotechnol.* **1997**, *15*, 55−62.
[22] S. M. Roberts, P. W. H. Wan, *J. Mol. Catal. B: Enzym.* **1998**, *4*, 111−136.
[23] [23a] D. R. Kelly, *Chim. Oggi* **2000**, *18*, 33−37. [23b] D. R. Kelly, *Chim. Oggi* **2000**, *18*, 52−56.
[24] S. Flitsch, G. Grogan, in *Enzyme Catalysis in Organic Synthesis* (Eds.: K. Drauz, H. Waldmann), Wiley-VCH Weinheim, **2002**, p. 1202−1245.
[25] G. E. Turfitt, *Biochem. J.* **1948**, *42*, 376−383.
[26] A. I. Laskin, P. Grabowich, C. L. Meyers, J. Fried, *J. Med. Chem.* **1964**, *7*, 406−409.
[27] J. Winter, B. Schneider, S. Meyenburg, D. Strack, G. Adam, *Phytochemistry* **1999**, *51*, 237−242.
[28] J. Fried, R. W. Thoma, A. Klingsberg, *J. Am. Chem. Soc.* **1953**, *75*, 5764−5765.
[29] D. H. Peterson, S. H. Eppstein, P. D. Meister, H. C. Murray, H. M. Leigh, A. Weintraub, L. M. Reinecke, *J. Am. Chem. Soc.* **1953**, *75*, 5768−5769.
[30] G. S. Fonken, H. C. Murray, L. M. Reineke, *J. Am. Chem. Soc.* **1960**, *82*, 5507−5508.
[31] M. A. Rahim, C. J. Sih, *J. Biol. Chem.* **1966**, *241*, 3615−3623.
[32] K. Carlström, *Acta Chem. Scand.* **1973**, *27*, 1622−1628.
[33] R. L. Prairie, P. Talalay, *Biochemistry* **1963**, *2*, 203−208.
[34] O. El-Tayeb, S. G. Knight, C. J. Sih, *Biochim. Biophys. Acta* **1964**, *93*, 411−417.
[35] E. Itagaki, *J. Biochem.* **1986**, *99*, 815−824.
[36] E. Itagaki, *J. Biochem.* **1986**, *99*, 825−832.
[37] S. Morii, S. Sawamoto, Y. Yamauchi, M. Miyamoto, M. Iwami, E. Itagaki, *J. Biochem.* **1999**, *126*, 624−631.
[38] J. M. Urbanczyk, J. Soloducho, A. Zabza, G. Sodano, A. Grassi, *J. Basic Microbiol.* **1993**, *33*, 141−146.
[39] M. J. van der Werf, A. M. Boot, *Microbiology* **2000**, *146*, 1129−1141

[40] C. A. Townsend, S. B. Christensen, S. G. Davis, *J. Am. Chem. Soc.* **1982**, *104*, 6154−6155.
[41] J. L. C. Wright, T. Hu, J. L. McLachlan, J. Needham, J. A. Walter, *J. Am. Chem. Soc.* **1996**, *118*, 8757−8758.
[42] S. Damtoft, H. Franzyk, S. R. Jensen, *Phytochemistry* **1995**, *40*, 773−84.
[43] G.-P. Chen, L. L. Poulsen, D. M. Ziegler, *Drug Metab. Dispos.* **1995**, *23*, 1390−1393.
[44] N. A. Donoghue, D. B. Norris, P. W. Trudgill, *Eur. J. Biochem.* **1976**, *63*, 175−192.
[45] C. C. Ryerson, D. P. Ballou, C. Walsh, *Biochemistry* **1982**, *21*, 2644−2655.
[46] D. Sheng, D. P. Ballou, V. Massey, *Biochemistry* **2001**, *40*, 11156−11167.
[47] S. Chandrasekhar, C. D. Roy, *Tetrahedron Lett.* **1987**, *28*, 6371−6372.
[48] For an excellent review on the concept of stereoelectronic effects see: P. Deslongchamps, *Organic Chemistry Series*, vol. 1 ("Stereoelectronic Effects in Organic Chemistry"), Pergamon, Oxford, **1983**.
[49] R. Noyori, T. Sato, H. Kobayashi, *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2661−2679.
[50] [50a] J. M. Schwab, *J. Am. Chem. Soc.* **1981**, *103*, 1876−1878. [50b] J. M. Schwab, W.-B. Li, L. P. Thomas, *J. Am. Chem. Soc.* **1983**, *105*, 4800−4808.
[51] K. Mislow, J. Brenner, *J. Am. Chem. Soc.* **1953**, *75*, 2318−2322.
[52] R. Wichmann, C. Wandrey, A. F. Bückmann, M.-R. Kula, *Biotechnol. Bioeng.* **1981**, *23*, 2789−2802.
[53] C.-H. Wong, G. M. Whitesides, *J. Am. Chem. Soc.* **1981**, *103*, 4890−4899.
[54] V. I. Tishkov, A. G. Galkin, G. N. Marchenko, Y. D. Tsyganokov, H. M. Egorov, *Biotechnol. Appl. Biochem.* **1993**, *18*, 201−207.
[55] K. Seelbach, B. Riebel, W. Hummel, M.-R. Kula, V. I. Tishkov, H. M. Egorov, C. Wandrey, U. Kragl, *Tetrahedron Lett.* **1996**, *37*, 1377−1380.
[56] S. Rissom, U. Schwarz-Linek, M. Vogel, V. I. Tishkov, U. Kragl, *Tetrahedron: Asymmetry* **1997**, *8*, 2523−2526.
[57] U. Schwarz-Linek, A. Krödel, F.-A. Ludwig, A. Schulze, S. Rissom, U. Kragl, V. I. Tishkov, M. Vogel, *Synthesis* **2001**, 947−951.
[58] A. J. Willetts, C. J. Knowles, M. S. Levitt, S. M. Roberts, H. Sandey, N. F. Shipston, *J. Chem. Soc., Perkin Trans. 1* **1991**, 1608−1610.
[59] G. Grogan, S. Roberts, A. J. Willetts, *Biotechnol. Lett.* **1992**, *14*, 1125−1130.
[60] F. Secundo, G. Carrea, S. Riva, E. Battistel, D. Bianchi, *Biotechnol. Lett.* **1993**, *15*, 865−870.
[61] M. C. Hogan, J. M. Woodley, *Chem. Eng. Sci.* **2000**, *55*, 2001−2008.
[62] V. Alphand, A. Archelas, R. Furstoss, *J. Org. Chem.* **1990**, *55*, 347−350.
[63] J. D. Stewart, *Curr. Opinion Biotechnol.* **2000**, *11*, 363−368.
[64] M. Kayser, G. Chen, J. Stewart, *Synlett* **1999**, 153−158.
[65] G. Chen, M. M. Kayser, M. D. Mihovilovic, M. E. Mrstik, C. A. Martinez, J. D. Stewart, *New J. Chem.* **1999**, *23*, 827.
[66] N. A. Donoghue, P. W. Trudgill, *Eur. J. Biochem.* **1975**, *60*, 1−7.
[67] H. Iwaki, Y. Hasegawa, M. Teraoka, T. Tokuyama, H. Bergeron, P. C. K. Lau, *Appl. Environ. Microbiol.* **1999**, *65*, 5158−5162.
[68] Q. Cheng, S. M. Thomas, K. Kostichka, J. R. Valentine, V. Nagarajan, *J. Bacteriol.* **2000**, *182*, 4744−4751.
[69] J. D. Stewart, *Curr. Org. Chem.* **1998**, *2*, 195−216.
[70] Y.-C. J. Chen, O. P. Peoples, C. T. Walsh, *J. Bacteriol.* **1988**, *170*, 781−789.
[71] J. F. Davey, P. W. Trudgill, *Eur. J. Biochem.* **1977**, *74*, 115−127.
[72] S. D. Doig, L. M. O'Sullivan, S. Patel, J. M. Ward, J. M. Woodley, *Enzyme Microb. Technol.* **2001**, *28*, 265−274.
[73] A. Z. Walton, J. D. Stewart, *Biotechnol. Prog.* **2002**, *18*, 262−268.
[74] H. D. Simpson, V. Alphand, R. Furstoss, *J. Mol. Catal. B: Enzym.* **2001**, *16*, 101−109.
[75] C. Slawson, J. Stewart, R. Potter, *J. Chem. Educ.* **2001**, *78*, 1533−1534.
[76] S. S. Barclay, J. M. Woodley, M. D. Lilly, P. L. Spargo, A. J. Pettman, *Biotechnol. Lett.* **2001**, *23*, 385−388.
[77] O. Abril, C. C. Ryerson, C. Walsh, G. M. Whitesides, *Bioorg. Chem.* **1989**, *17*, 41−52.
[78] M. J. Cheesman, B. Kneller, E. J. Kelly, S. J. Thompson, C. K. Yeung, D. L. Eaton, A. E. Rettie, *Protein Expression Purif.* **2001**, *21*, 81−86.
[79] M. B. Kneller, M. J. Cheesman, A. E. Rettie, *Biochem. Biophys. Res. Commun.* **2001**, *282*, 899−903.
[80] F. Zambianchi, P. Pasta, G. Carrea, S. Colonna, N. Gaggero, J. M. Woodley, *Biotechnol. Bioeng.* **2002**, *78*, 489−496.
[81] B. P. Branchaud, C. T. Walsh, *J. Am. Chem. Soc.* **1985**, *107*, 2153−2161.
[82] J. A. Latham, C. Walsh, *J. Am. Chem. Soc.* **1987**, *109*, 3421−3427.
[83] M. Wright, C. Knowles, F. Petit, R. Furstoss, *Biotechnol. Lett.* **1994**, *16*, 1287−1292.
[84] M. J. Taschner, D. J. Black, *J. Am. Chem. Soc.* **1988**, *110*, 6892−6893.
[85] M. J. Taschner, D. J. Black, Q.-Z. Chen, *Tetrahedron: Asymmetry* **1993**, *4*, 1387−1390.
[86] J. D. Stewart, K. W. Reed, M. M. Kayser, *J. Chem. Soc., Perkin Trans. 1* **1996**, 755−757.
[87] M. Vogel, U. Schwarz-Linek, *Bioorg. Chem.* **1999**, 102−110.
[88] J. D. Stewart, K. W. Reed, C. A. Martinez, J. Zhu, G. Chen, M. M. Kayser, *J. Am. Chem. Soc.* **1998**, *120*, 3541−3548.
[89] M. D. Mihovilovic, G. Chen, S. Wang, B. Kyte, F. Rochon, M. M. Kayser, J. D. Stewart, *J. Org. Chem.* **2001**, *66*, 733−738.
[90] M. D. Mihovilovic, P. Stanetty, H. Spreitzer, unpublished results.
[91] V. Alphand, R. Furstoss, S. Pedragosa-Moreau, S. M. Roberts, A. J. Willetts, *J. Chem. Soc., Perkin Trans. 1* **1996**, 1867−1872.
[92] J. D. Stewart, W. R. Kieth, J. Zhu, G. Chen, M. M. Kayser, *J. Org. Chem.* **1996**, *61*, 7652−7653.
[93] [93a] C.-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299. [93b] V. S. Martin, S. S. Woodard, T. Katsuki, Y. Yamada, M. Ikeda, K. B. Sharpless, *J. Am. Chem. Soc.* **1981**, *103*, 6237−6240. [93c] G. Gredig, K. Fajans, *Ber. Dtsch. Chem. Ges.* **1908**, *41*, 752−763.
[94] V. Alphand, R. Furstoss, *Tetrahedron: Asymmetry* **1992**, *3*, 379−382.
[95] M. D. Mihovilovic, B. Müller, M. M. Kayser, J. D. Stewart, J. Fröhlich, P. Stanetty, H. Spreitzer, *J. Mol. Catal. B: Enzym.* **2001**, *11*, 349−353.
[96] M. D. Mihovilovic, F. Rudroff, M. M. Kayser, J. D. Stewart, P. Stanetty, H. Spreitzer, unpublished results.
[97] M. M. Kayser, G. Chen, J. Stewart, *J. Org. Chem.* **1998**, *63*, 7103−7106.
[98] R. Gagnon, G. Grogan, E. Groussain, S. Pedragosa-Moreau, P. F. Richardson, S. M. Roberts, A. J. Willetts, V. Alphand, J. Lebreton, R. Furstoss, *J. Chem. Soc., Perkin Trans. 1* **1995**, 2527−2528.
[99] V. Alphand, C. Mazzini, J. Lebreton, R. Furstoss, *J. Mol. Catal. B: Enzym.* **1998**, *5*, 219−221.
[100] C. Mazzini, J. Lebreton, V. Alphand, R. Furstoss, *Tetrahedron Lett.* **1997**, *38*, 1195−1196.
[101] M. J. Taschner, L. Peddada, *J. Chem. Soc., Chem. Commun.* **1992**, 1384−1385.
[102] M. D. Mihovilovic, B. Müller, M. M. Kayser, P. Stanetty, *Synlett* **2002**, 700−702.
[103] M. D. Mihovilovic, B. Müller, M. M. Kayser, J. D. Stewart, P. Stanetty, *Synlett* **2002**, 703−706.
[104] V. Alphand, R. Furstoss, *J. Org. Chem.* **1992**, *57*, 1306−1309.

[105] F. Petit, R. Furstoss, *Tetrahedron: Asymmetry* **1993**, *4*, 1341−1352.
[106] A. J. Carnell, S. M. Roberts, V. Sik, A. J. Willetts, *J. Chem. Soc., Chem. Commun.* **1990**, 1438−1439.
[107] A. J. Carnell, S. M. Roberts, V. Sik, A. J. Willetts, *J. Chem. Soc., Perkin Trans. 1* **1991**, 2385−2389.
[108] M. J. Taschner, L. Peddada, P. Cyr, Q. Z. Chen, D. J. Black, *NATO ASI Ser., Ser. C* **1992**, *381*, 347−360.
[109] D. R. Kelly, C. J. Knowles, J. G. Mahdi, I. N. Taylor, M. A. Wright, *J. Chem. Soc., Chem. Commun.* **1995**, 729−730.
[110] D. R. Kelly, C. J. Knowles, J. G. Mahdi, M. A. Wright, I. N. Taylor, D. E. Hibbs, M. B. Hursthouse, A. K. Mish′al, S. M. Roberts, P. W. H. Wan, G. Grogan, A. J. Willets, *J. Chem. Soc., Perkin Trans. 1* **1995**, 2057−2066.
[111] D. R. Kelly, C. J. Knowles, J. G. Mahdi, M. A. Wright, I. N. Taylor, S. M. Roberts, P. W. H. Wan, G. Grogan, S. Pedragosa-Moreau, A. J. Willetts, *Chem. Commun.* **1996**, 2333−2334.
[112] D. R. Kelly, *Tetrahedron: Asymmetry* **1996**, *7*, 1149−1152.
[113] G. Ottolina, P. Pasta, G. Carrea, S. Colonna, S. Dallavalle, H. L. Holland, *Tetrahedron: Asymmetry* **1995**, *6*, 1375−1386.
[114] G. Ottolina, G. Carrea, S. Colonna, A. Rückmann, *Tetrahedron: Asymmetry* **1996**, *7*, 1123−1136.
[115] F. Zambianchi, P. Pasta, G. Ottolina, G. Carrea, S. Colonna, N. Gaggero, J. M. Ward, *Tetrahedron: Asymmetry* **2000**, *11*, 3653−3657.
[116][116a] D. B. Norris, P. W. Trugdill, *Biochem. J.* **1971**, *121*, 363−370. [116b] D. B. Norris, P. W. Trugdill, *Eur. J. Biochem.* **1976**, *63*, 193−198.
[117] Y. Hasegawa, K. Hamano, H. Obata, T. Tokuyama, *Agric. Biol. Chem.* **1982**, *46*, 1139−1143.
[118] P. W. Trugdill, H. J. Ougham, *J. Bacteriol.* **1982**, *150*, 1172−1182.
[119] H. Iwaki, M. Shimizu, T. Tokuyama, Y. Hasegawa, *Appl. Environ. Microbiol.* **1999**, *65*, 2232−2234.
[120][120a] M. K. Trower, R. M. Buckland, R. Higgins, M. Griffin, *Appl. Environ. Microbiol.* **1985**, *49*, 1282−1289. [120b] A. M. Magor, J. Warburton, M. K. Trower, M. Griffin, *Appl. Environ. Microbiol.* **1986**, *52*, 665−671.
[121] M. K. Trower, R. M. Buckland, M. Griffin, *Eur. J. Biochem.* **1989**, *181*, 199−206.
[122] M. A. Wright, I. N. Taylor, M. J. Lenn, D. R. Kelly, J. G. Mahdi, C. Knowles, *FEMS Microbiol. Lett.* **1994**, *116*, 67−72.
[123] M. J. van der Werf, H. J. Swarts, J. A. M. de Bont, *Appl. Environ. Microbiol.* **1999**, *65*, 2092−2102.
[124] M. J. van der Werf, *Biochem. J.* **2000**, *347*, 693−701.
[125] J. D. Schumacher, R. M. Fakoussa, *Appl. Microbiol. Biotechnol.* **1999**, *52*, 85−90.
[126] M. Griffin, P. W. Trugdill, *Biochem. J.* **1972**, *129*, 595−603.
[127][127a] M. Griffin, P. W. Trugdill, *Eur. J. Biochem.* **1976**, *63*, 199−209. [127b] P. W. Trugdill, *Methods Enzymol.* **1990**, *88*, 77−81.
[128] H. Sandy, A. J. Willetts, *Biotechnol. Lett.* **1989**, *2*, 615−620.
[129] S. Wang, G. Chen, M. M. Kayser, H. Iwaki, C. K. Lau, Y. Hasegawa, *Can. J. Chem.* **2002**, *80*, 613−621.
[130] M. T. Bes, R. Villa, S. M. Roberts, P. W. H. Wan, A. J. Willetts, *J. Mol. Catal. B: Enzym.* **1996**, *1*, 127−134.
[131] H. Iwaki, Y. Hasegawa, P. C. K. Lau, S. Wang, M. M. Kayser, submitted to *Appl. Environ. Microbiol*.
[132] M. D. Mihovilovic, B. Müller, M. M. Kayser, P. Stanetty, unpublished results.
[133] W. H. Bradshaw, H. E. Conrad, E. J. Corey, I. C. Gunsalus, D. Lednicer, *J. Am. Chem. Soc.* **1959**, *81*, 5507.
[134] H. E. Conrad, R. DuBus, M. J. Namtvedt, I. C. Gunsalus, *J. Biol. Chem.* **1965**, *240*, 495−503.
[135] D. G. Taylor, P. W. Trugdill, *J. Bacteriol.* **1986**, *165*, 489−497.
[136] H. E. Conrad, K. Lieb, I. C. Gunsalus, *J. Biol. Chem.* **1965**, *240*, 4029−4037.
[137] P. W. Trugdill, R. DuBus, I. C. Gunsalus, *J. Biol. Chem.* **1966**, *241*, 4288−4297.
[138] H. J. Ougham, D. G. Taylor, P. W. Trugdill, *J. Bacteriol.* **1983**, *153*, 140−152.
[139] J. G. Rheinwald, A. M. Chakrabarty, I. C. Gunsalus, *Proc. Nat. Acad. Sci. USA* **1973**, *70*, 885−889.
[140] K. H. Jones, R. T. Smith, P. W. Trugdill, *J. Gen. Microbiol.* **1993**, *139*, 797−805.
[141] J. Beecher, A. Willetts, *Tetrahedron: Asymmetry* **1998**, *9*, 1899−1916.
[142] P. J. Chapman, G. Meerman, I. C. Gunsalus, *Biochem. Biophys. Res. Commun.* **1965**, *20*, 104−108.
[143] G. Grogan, S. Roberts, P. Wan, A. J. Willetts, *Biotechnol. Lett.* **1993**, *15*, 913−918.
[144] J. Becher, G. Grogan, S. Roberts, A. Willetts, *Biotechnol. Lett.* **1996**, *18*, 571−576.
[145] G. Grogan, S. Roberts, A. J. Willetts, *J. Chem. Soc., Chem. Commun.* **1993**, 699−701.
[146] M. J. Lenn, C. J. Kowles, *Enzyme Microb. Technol.* **1994**, *16*, 964−969.
[147] G. Grogan, S. Roberts, P. Wan, A. J. Willetts, *Biotechnol. Lett.* **1994**, *16*, 1173−1178.
[148] P. Pasta, G. Carrea, N. Gagero, G. Grogan, A. Willetts, *Biotechnol. Lett.* **1996**, *18*, 1123−1128.
[149] C. Brosa, C. Rodiguez-Santamarta, J. Salva, E. Barbera, *Tetrahedron* **1998**, *54*, 5781−5788.
[150] R. E. Cripps, *Biochem. J.* **1975**, *152*, 233−241.
[151] R. E. Cripps, P. W. Trudgill, J. G. Whateley, *Eur. J. Biochem.* **1978**, *86*, 175−186.
[152] F. K. Higson, D. D. Focht, *Appl. Environ. Microbiol.* **1990**, *56*, 3678−85.
[153] A. Tanner, D. J. Hopper, *J. Bacteriol.* **2000**, *182*, 6565−6569.
[154] N. M. Kamerbeek, M. J. H. Moonen, J. G. M. van der Ven, W. J. H. van Berkel, M. W. Fraaije, D. B. Janssen, *Eur. J. Biochem.* **2001**, *268*, 2547−2557.
[155] M. Casellas, M. Grifoll, J. M. Bayona, A. M. Solanas, *Appl. Environ. Microbiol.* **1997**, *63*, 819−826.
[156] S. Hartmans, J. A. M. DeBont, *FEMS Microbiol. Lett.* **1986**, *36*, 155−158.
[157] F. W. Forney, A. J. Markovetz, R. E. Kallio, *J. Bacteriol.* **1967**, *93*, 649−655.
[158] F. W. Forney, A. J. Markovetz, *J. Bacteriol.* **1968**, *96*, 1055−1064.
[159] F. W. Forney, A. J. Markovetz, *Biochem. Biophys. Res. Commun.* **1969**, *37*, 31−38.
[160] L. N. Britton, J. M. Brand, A. J. Markovetz, *Biochim. Biophys. Acta* **1974**, *369*, 45−49.
[161] H. J. Rehm, I. Reiff, *Adv. Biochem. Eng.* **1981**, *19*, 175−215.
[162] L. N. Britton, A. J. Markovetz, *J. Biol. Chem.* **1977**, *252*, 8561−8566.
[163] J. E. Allen, A. J. Markovetz, *J. Bacteriol.* **1970**, *103*, 426−434.
[164][164a] G. Fronza, C. Fuganti, G. Pedrocchi-Fantoni, V. Perozzo, S. Servi, G. Zucchi, *J. Org. Chem.* **1996**, *61*, 9362−9367. [164b] F. Donzelli, C. Fuganti, M. Mendozza, G. Pedrocchi-Fantoni, S. Servi, G. Zucchi, *Tetrahedron: Asymmetry* **1996**, *7*. 3129−3134. [164c] C. Fuganti, M. Mendozza, D. Joulain, J. Minut, G. Pedrocchi-Fantoni, V. Piergianni, S. Servi, G. Zucchi, *J. Agric. Food Chem.* **1996**, *44*, 3616−3619. [164d] C. Fuganti, J. Minut, G. P. Fantoni, S. Servi, *J. Mol. Catal. B: Enzym.* **1998**, *4*, 47−52.
[165] J. Quazzani-Chahdi, D. Buisson, R. Azerad, *Tetrahedron Lett.* **1987**, *28*, 1109−1112.
[166] A. Carnell, A. J. Willetts, *Biotechnol. Lett.* **1990**, *12*, 885−890.
[167] A. Carnell, A. J. Willetts, *Biotechnol. Lett.* **1992**, *14*, 17−21.
[168] K. Königsberger, G. Braunegg, K. Faber, H. Griengl, *Biotechnol. Lett.* **1990**, *12*, 509−514.
[169][169a] J. Lebreton, V. Alphand, R. Furstoss, *Tetrahedron Lett.* **1996**, *37*, 1011−1014. [169b] J. Lebreton, V. Alphand, R. Furstoss, *Tetrahedron* **1997**, *53*, 145−160.

[170] V. Alphand, R. Furstoss, *J. Mol. Catal. B: Enzym.* **2000**, *9*, 209−217.

[171] Y. Hasegawa, Y. Nakai, T. Tokuyama, H. Iwaki, *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2696−2698.

[172][172a] B. Adger, T. Bes, G. Grogan, R. McCague, S. Pedragosa-Moreau, S. M. Roberts, R. Villa, P. W. H. Wan, A. J. Willetts, *J. Chem. Soc., Chem. Commun.* **1995**, 1563−1564. [172b] B. Adger, M. T. Bes, G. Grogan, R. McCague, S. Podragosa-Moreau, S. M. Roberts, R. Villa, P. W. H. Wan, A. J. Willetts, *Bioorg. Med. Chem.* **1997**, *5*, 253−261.

[173] B. M. Adger, R. McCague, S. M. Roberts, *Int. Pat. Appl.* WO 96/38437, **1996**, *Chem. Abstr.* **1997**, *126*, 89208.

[174] V. Alphand, A. Archelas, R. Furstoss, *Tetrahedron Lett.* **1989**, *30*, 3663−3664.

[175] L. Andrau, J. Lebreton, P. Viazzo, V. Alphand, R. Furstoss, *Tetrahedron Lett.* **1997**, *38*, 825−826.

[176] F. Petit, R. Furstoss, *Synthesis* **1995**, 1517−1520.

[177] M. Levitt, H. Sandy, A. J. Willetts, *Biotechnol. Lett.* **1990**, *12*, 197−200.

[178] M. S. Levitt, R. F. Newton, S. M. Roberts, A. J. Willetts, *J. Chem. Soc., Chem. Commun.* **1990**, 619−620.

[179] R. Gagnon, G. Grogan, M. S. Levitt, S. M. Roberts, P. W. H. Wan, A. J. Willetts, *J. Chem. Soc., Perkin Trans. 1* **1994**, 2537−2543.

[180] R. Gagnon, G. Grogan, S. M. Roberts, R. Villa, A. J. Willetts, *J. Chem. Soc., Perkin Trans. 1* **1995**, 1505−1511.

[181] C. Mazzini, J. Lebreton, V. Alphand, R. Furstoss, *J. Org. Chem.* **1997**, *62*, 5215−5218.

[182] P. C. Brzostowicz, K. L. Gibson, S. M. Thomas, M. S. Blasko, P. E. Rouviere, *J. Bacteriol.* **2000**, *182*, 4241−4248.

[183] J. A. Affholter, C. Davis, S. A. Selifonov, *Int. Pat. Appl.* WO 00/09682, **2000**, *Chem. Abstr.* **2000**, *132*, 162018.

[184] G. J. Lye, P. A. Dalby, J. M. Woodley, *Org. Process Res. Dev.* **2002**, *6*, 434−440.

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