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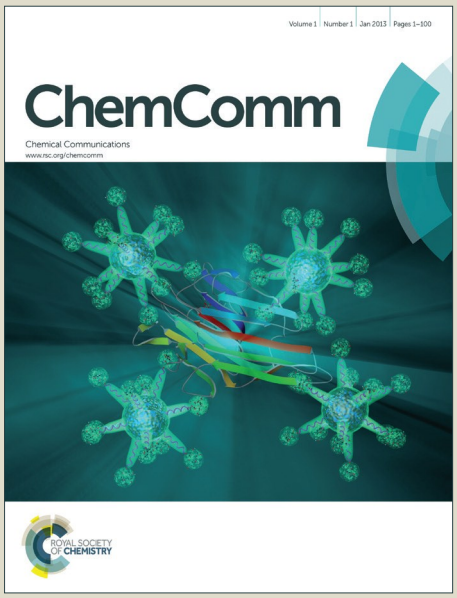

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Chemo-enzymatic Baeyer-Villiger oxidation of 4-methylcyclohexanone *via* kinetic resolution of racemic carboxylic acids: direct access to enantioenriched lactone

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Agnieszka Drożdż,[a] Anna Chrobok*[a]

A new method for the asymmetric chemo-enzymatic Baeyer-Villiger oxidation of prochiral 4-methylcyclohexanone to (*R*)-4-methylcaprolactone in the presence of (±)-4-methyloctanoic acid, Candida Antarctica lipase B and 30% aq. H_2O_2 has been developed. A mechanism for the asymmetric induction based on kinetic resolution of racemic carboxylic acids is proposed.

The Baeyer-Villiger (BV) oxidation of ketones to lactones with peroxide derivatives is a powerful methodology for oxygen insertion into the carbon-carbon bond breaking process.[1] Of particular importance are new protocols for the asymmetric version of this reaction which allows rapid access to chiral lactones. Natural products containing the lactone skeleton attract considerable attention from scientists. Lactones are valuable building blocks for the natural synthesis of biologically important substances such as alkaloids, steroids, pheromones, macrocyclic antibiotics, lignan lactones and antileukaemics.[2] In recent years, strategies to perform Baeyer-Villiger reactions in an enantioselective manner have been continuously improved. Organometallic approaches and biocatalytic methods have been described.[3]

The first data concerning asymmetric BV reactions catalysed by metal complexes date back to 1994.[4] Since then, synthetic methods for chiral lactones using both chiral catalysts and chiral hydroperoxides have been described. Chiral complexes of metals such as Al, Cu, Zr, Hf, Pt, Sn, Zn with different chiral ligands, *e.g.*, based on BINOL (1,1′-bi-2-naphthol) or VANOL (3,3′-diphenyl-2,2′-bi-1-naphthol) are known to catalyse the synthesis of lactones with high enantiomeric excess *ee* up to 87%.[3, 5-7]

Biocatalysis offers a "green chemistry" alternative for BV transformation.[8,9] The enzymes that are used for the transformations described are known as Baeyer-Villiger monooxygenases.[10-12] These enzymes utilise molecular oxygen to generate a peroxo-flavin species, which attacks the carbonyl group in a nucleophilic reaction similar to
the classical chemical Baeyer-Villiger reaction mechanism.[13] In this method lactones are obtained with enantioselectivities up to 100%. However, Baeyer-Villiger monooxygenases are relatively expensive and poorly stable enzymes for practical synthetic use.

Another interesting method for the synthesis of lactones is the chemo-enzymatic approach involving relatively stable and inexpensive lipase. Several early examples for lipase-mediated Baeyer-Villiger oxidation were reported. [14-22] In the chemo-enzymatic method the enzyme is used in only one step of the reaction, and therefore, the reaction is called chemo-enzymatic. This reaction involves the oxidation of long- or medium-chain carboxylic acids with hydrogen peroxide in the presence of lipase to generate *in situ* peracid, which is used to oxidise ketones to lactones in the second step. The chemo-enzymatic approach is an attractive alternative, avoiding handling of often unstable, hazardous and fairly expensive organic percarboxylic acids, which are typical oxidants used in the Baeyer-Villiger reaction.

Lipases are used in the pharmaceutical industry for the preparation of single-isomer chiral drugs, either by kinetic resolution of racemic molecules, or by the desymmetrisation of prochiral compounds.[23]

However, to date, there is only one example of chiral lactones synthesis in a chemo-enzymatic BV reaction utilising ethyl acetate as a peracetic acid precursor and chiral ketone levoglucosenone as a starting material that is converted to (S)-γ-hydroxymethyl-α,β-butenolide.[24]

Another study describes autocatalytic Baeyer–Villiger oxidation of cyclic ketones to lactones mediated by lipases using urea–hydrogen peroxide as the primary oxidant without the addition of carboxylic acid.[15] A different mechanism for this reaction was proposed. In this method the lactone that is created can undergo lipase-mediated perhydrolysis to peroxy acids that can oxidise ketones, thereby achieving an autocatalytic process. Lipase-catalysed perhydrolysis is enantiospecific and gives 6-substituted caprolactones or the corresponding hydroxy acids in enantiomerically enriched form. The best results were obtained for the oxidation of 2-methylcyclohexanone where the *S* isomer of the lactone was obtained with 44% yield and 60% enantioselectivity.[15]

To date, no examples of an asymmetric version of the Baeyer-Villiger reaction can be found in the literature with the application of chiral acid as the precursor of a chiral peracid or racemic carboxylic acids which after kinetic resolution serve as an effective chiral oxidant.

For other known chemo-enzymatic approaches, only one report of

[a] *Silesian University of Technology, Department of Chemical Organic Technology and Petrochemistry, Krzywoustego 4, 44-100 Gliwice, Poland; Fax: 48 3223 71032; Tel: 48 3223 72917; E-mail: anna.chrobok@polsl.pl*
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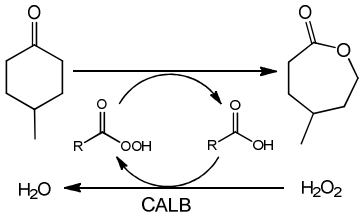
a chiral epoxide synthesis uses chiral acid ((2*R*,3*S*,4*R*,5*S*)-(–)-2,3:4,6-di-O-isopropylidiene-2-keto-L-gulonic acid monohydrate) in oxidation step has been found.[25]

Building on this, we propose herein for the first time the asymmetric chemo-enzymatic Baeyer-Villiger reaction for the synthesis of chiral lactones with the application of simple and inexpensive racemic carboxylic acids.

In order to develop a clean asymmetric Baeyer-Villiger process the racemic mixture of enantiomers of carboxylic acids as chiral peracid precursors was applied. As shown in Scheme 1, the chemo-enzymatic method for chiral lactone synthesis involves lipase as the biocatalyst for chiral peracid formation and 30% aq. H_2O_2 as the primary oxidant. In contrast to published works concerning the chemo-enzymatic BV reaction, the lipase was not attached to a solid support (commercial name Novozyme-435) but was used in the free form as commercially available water solution of Candida Antarctica lipase B (CALB).



Scheme 1 Chemo-enzymatic Baeyer–Villiger oxidation of 4-methylcyclohexanone

First, the influence of the structure of racemic acids on the asymmetric induction in this process was studied (Table 1). The experiments were carried out at 25°C as well as at 18°C which are the optimum rage of temperatures for CALB. Toluene was used as the best organic solvent for this process.[22] The oxidation of 4-methylcyclohexanone was chosen as a model reaction using 0.10 g native lipase (CALB) for 0.5 mmol of ketone. The molar ratio of the ketone to the carboxylic acid to H_2O_2 was fixed as 1:2:2. The progress of the reaction was checked by GC.[26]

Several acids were chosen for the model oxidation reaction of 4-methylcyclohexanone. In the literature only non-chiral medium chain and unbranched long chain acids were studied in chemo-enzymatic processes. In this work, the chiral carbon was located as first, second or third in the alkyl chain of the acid. The longer alkyl chain and the later location of the chiral carbon from the carboxylic group in the acid caused the better performance of the peracid precursor. Acids with phenyl or branched substituent as well as esters were much less reactive (Table S1, S2, ESI). Higher enantiomeric excess of (*R*)-4-methylcaprolactone was observed at a lower temperature 18°C. Then, the reaction was slower, allowing higher *ee*. The best results were obtained for the application of (±)-4-methyloctanoic acid at 18°C which allows full conversion of 4-methylcyclohexanone and the formation of 4-methylcaprolactone with 97% yield and an enantiomeric excess of 96% (*R* isomer) in 8 days (Table 1, entry 6). The faster the reaction is with completion after 3, 4 or 5 days, the lower the enantiomeric excess that is observed.

For further studies, (±)-4-methyloctanoic acid was chosen as the peracid precursor. The influence of the amount of (±)-4-methyloctanoic acid on the yield and *ee* of (*R*)-4-methylcaprolactone was determined (Fig. 1). In each case during the reaction time chiral induction was observed and the enantioselectivity had grown. The rate of reaction for the application of 2, 4 and 8 equivalents of acid to the ketone was similar, while 1 equivalent was not enough (Fig. S1, ESI). The yield of lactone for the application of 4 and 8 equivalents of acid after 8 days are slightly lower. That can be the result of saturation of active centres of lipase. The enantiomeric excess was growing during the reaction time to reach 90-96% in the presence of 2 and 4 equivalents of acid after 8 days (Fig. 1). In this case the 1 eq was also sufficient. A large excess of acid (8 eq) caused lowering of the *ee*. As a result, 2 equivalents of acid was sufficient to obtained high yields and enantioselectivity.

Table 1 The influence of the structure of peracid precursor on the yield and *ee* of (*R*)-4-methylcaprolactone[a]

No.	Carboxylic acid	25°C Yield [%]	25°C *ee (R)* [%]	18°C Yield [%]	18°C *ee (R)* [%]
1		99 (4 days)	62	99 (8 days)	80
2		99 (5 days)	61	95 (8 days)	83
3		94 (7 days)	40	95 (8 days)	82
4		89 (3 days)	51	87 (8 days)	89
5		89 (4 days)	55	77 (8 days)	81
6		95 (5 days)	67	97 (8 days)	96
7	rac.	92 (8 days)	43	82 (8 days)	57
	(*R*)-	-	-	86 (8 days)	66
	(*S*)-	-	-	4 (8 days)	-
8	rac.	52 (8 days)	44	30 (8 days)	49
	(*R*)-	-	-	39 (8 days)	72
	(*S*)-			- (8 days)	-
9		-	-	85 (2 days)	14
				99 (8 days)	50

[a] Reaction conditions: 4-methylcyclohexanone (0.5mmol), 30% aq. H_2O_2 (1mmol), acid (1mmol), 0.10 g of CALB, 18 °C, toluene (1ml), yields and *ee* determined by GC.

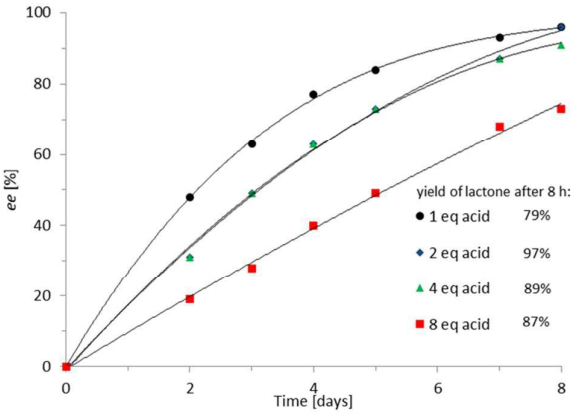


Fig. 1 The influence of the amount of (±)-4-methyloctanoic acid on the *ee* of (*R*)-4-methylcaprolactone obtained during the chemo-enzymatic oxidation of 4-methylcyclohexanone (0.5mmol) with 30% aq. H_2O_2 (1mmol) in the presence of 0.10g of CALB at 18 °C in toluene (1ml).

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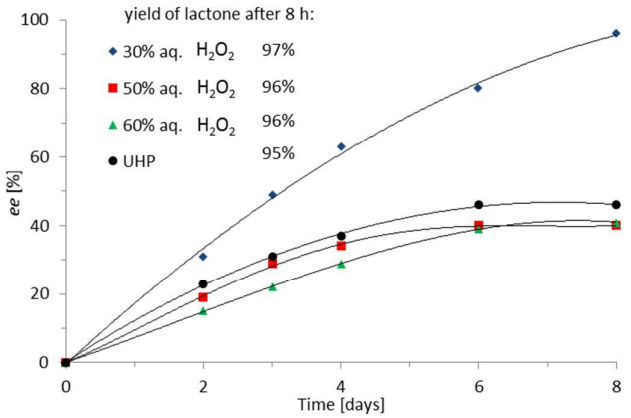
In order to study the influence of the primary oxidant 30%, 50% and 60% aq. H_2O_2 as well as anhydrous urea–hydrogen peroxide (UHP) were used in the model oxidation of 4-methylcyclohexanone at 18°C. The molar ratio of ketone to (±)-4-methyloctanoic acid to oxidant was 1:2:2. The type of oxidant only slightly influenced the reaction rate (Fig. S2, ESI) but in each case the high conversion of



ketone was achieved. The type of oxidant had a tremendous impact on the enantiomeric excess (Fig. 2). Asymmetric synthesis can be achieved only by using 30% aq. H_2O_2.

Fig. 2 The influence of the structure of primary oxidant on the *ee* of (*R*)-4-methylcaprolactone obtained during the chemo-enzymatic oxidation of 4-methylcyclohexanone (0.5mmol) in the presence of oxidant (1mmol), (±)-4-methyloctanoic acid (1mmol) and 0.10 g of CALB at 18 °C in toluene (1ml).

The optimum molar ratio of ketone to oxidant was proven to be 1:2. The molar excess of oxidant was varied from 1 to 6 (Fig. S3, ESI). The reaction rate increases rapidly with the higher molar ratio of oxidant (3-6 equivalents) but in all cases except equimolar amounts the full conversion of the ketone was achieved.

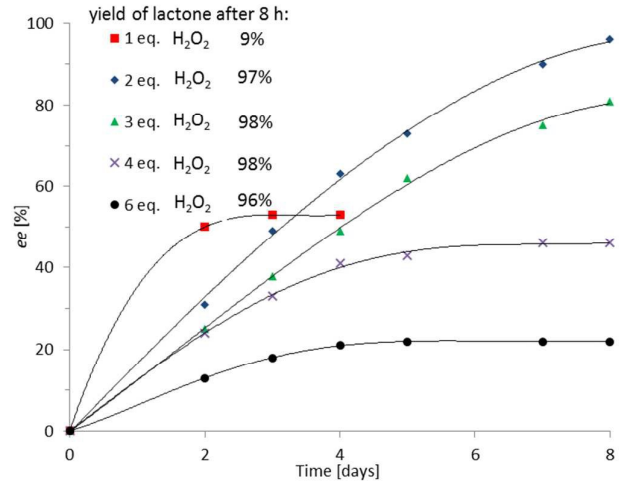


Fig. 3 The influence of the amount of 30% aq. H_2O_2 on the *ee* of (*R*)-4-methylcaprolactone obtained during the chemo-enzymatic oxidation of 4-methylcyclohexanone (0.5mmol) in the presence of (±)-4-methyl-octanoic acid (1mmol) and 0.10g of CALB at 18 °C in toluene (1ml).

Interestingly, the *ee* reached 96% after 8 days only with the application of a 2-fold molar excess of oxidant to ketone (Fig. 3). When using a triple excess of oxidant *ee,* was still growing after 8 days; fourfold and sixfold excess of oxidant deactivated the reaction system and stopped at the level *ee* of 40% and 20%, respectively.

Referring to the initial assumption that a further increase of the amount of enzyme over 0.1 g did not influence the yield of the lactone formed, a study of the influence of the amount of CALB on the reaction course was performed (Fig. 4 and Fig. 4S, ESI). Below 0.1 g of CALB per 0.5 mmol of ketone, the full conversion of ketone was reached but the *ee* was poor (37%). With higher amounts of CALB, above 0.1 g, the reactions were slower and for 0.2 g of CALB only 44% of lactone was obtained after 8 days. In all cases high *ee* were obtained. Lower yields of lactone obtained for the application of higher amounts of CALB can be caused by detrimental effect of high amounts of water which is introduced to the system with the native CALB.

The 0.10 g of CALB per 0.5 mmol of ketone was appropriate to carry out the reaction in the region where the reaction kinetic is controlled by the formation of lactone, not by the formation of peracid in the enzyme-catalysed reaction.

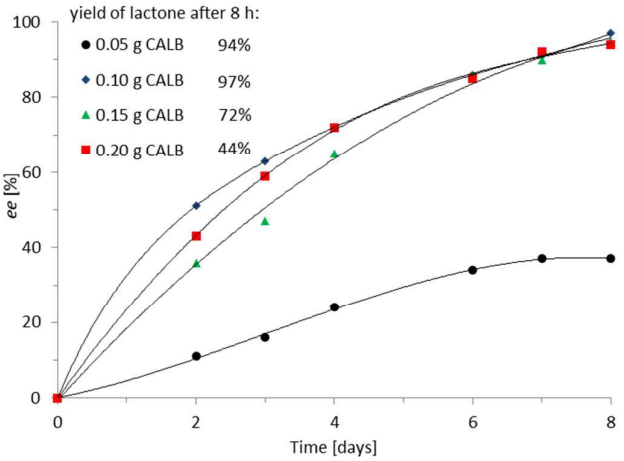


Fig. 4 The influence of the amount of CALB on the *ee* of (*R*)-4-methylcaprolactone obtained during the chemo-enzymatic oxidation of 4-methylcyclohexanone (0.5mmol) with 30% aq. H_2O_2 (1mmol) in the presence of (±)-4-methyloctanoic acid (1mmol) at 18 °C in toluene (1ml).

In this work, the native lipase was used because the rate of the model reaction under the conditions used in this work were much higher with the application of Novozyme-435, *e. g.,* with 0.03 g of Novozyme-435 per 0.5 mmol of ketone just after 4 days, the full conversion of ketone was obtained with only 15% of *ee*.

We also noticed that the solvent has an immense impact on the enantiomeric excess (Table 2). The best solvent for this reaction was found to be toluene, and the concentration of the reagents also has an influence on enantiomeric purity. The application of diethyl ether and hexane resulted in lower yields and *ee* in the same reaction time.

Table 2 The influence of the solvent on the reaction[a]

No.	Solvent	V [ml]	Time [days]	Yield [%]	*ee* (*R*) [%]
1	toluene	0.5	8	95	87
	toluene	1.0	8	97	96
	diethyl ether	1.0	6	79	37
2	diethyl ether	1.0	7	79	48
	diethyl ether	1.0	8	79	56
	hexane	1.0	6	86	40
3	hexane	1.0	7	88	45
	hexane	1.0	8	89	55

[a] Reaction conditions: 4-methylcyclohexanone (0.5mmol), 30% aq. H_2O_2 (1 mmol), (±)-4-methyloctanoic acid (1mmol), 0.10g of CALB, 18 °C,

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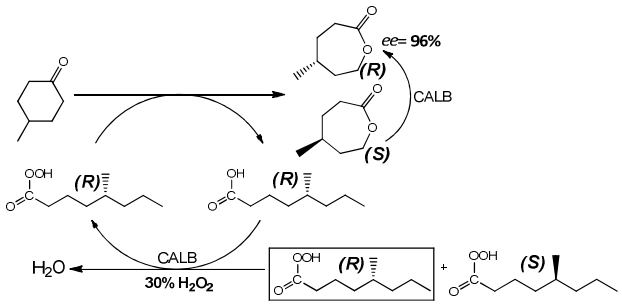
To learn more about the mechanism, the reactions with the application of optically pure *R* and *S* as well as a racemic mixtures of the acids: 2-methyl-3-hydroxybutyric and 2-phenylbutyric were performed (Table 1, entry 7, 8). These acids are not the most reactive in the chemo-enzymatic reaction but both pure isomers were commercially available, and we could learn about the influence of the *R* and *S* isomers on the reaction course. Additionally, the test reaction with octanoic acid without any substituents was performed (Table 1, entry 9).

As shown in Table 1, the pure *S* isomers of the acids do not work in this reaction. Thus, the lipase does not catalyse the conversion of acid to peracid. However, the pure *R* isomers of the acids work almost as well as the racemic mixture. Based on these experiments, the proposed mechanism of the asymmetric Baeyer-Villiger reaction *via* kinetic resolution of racemic carboxylic acids is depicted in Scheme 2. Lipase converts only the *R* isomer from the racemic mixture of acid to peracid (*R* isomer). Next, the optically pure peracid oxidises prochiral 4-methylcyclohexanone to (*R*)-4-methylcaprolactone with high *ee*. After one cycle of the reaction, the waste *R* isomer of acid is again converted with the aid of lipase to *R* isomer of peracid enable to form the lactone with high yield and enantiomeric excess. To enriched the created mixture of isomers in the isomer *R* the additional kinetic resolution takes place catalysed by CALB. Finally, high *ee* of lactone is obtained (96%).



Scheme 2 The proposition of the mechanism of asymmetric chemo-enzymatic Baeyer–Villiger oxidation of 4-methylcyclohexanone

To confirmed the possibility of kinetic resolution of isomers of lactone the additional experiment was performed. (±)-4-methylcaprolactone was mixed with CALB in toluene for 8 days. After this time *ee* of *R* isomer reached 45%.

Two other cyclic ketones were also oxidised using this method and optimised conditions (ESI, Table S3). The oxidation of 4-ethylcyclohexanone during 6 days gave 4-ethylcaprolactone with 99% yield and 69% *ee* of *R* isomer. In the same reaction time the 4-phenylbutyrolactone was obtained with 99% yield and 76% *ee* of *R* isomer.

In summary, lipase-mediated Baeyer–Villiger oxidation procedure offers mild reaction conditions, low toxicity and a green oxidant that make this method cost-effective, and a greener alternative to the metallorganic conventional asymmetric Baeyer-Villiger reaction. The ability for the chiral induction in lactones has not yet been explored with the application of racemic mixtures of carboxylic acids. This enzymatic oxygen insertion represents a promising approach for the synthesis of chiral lactones. The additional studies under the development of lipase-mediated asymmetric Baeyer-Villiger oxidation using different ketones are being undertaken in our laboratory.

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26 General method for Baeyer-Villiger oxidation: the ketone (0.5 mmol), carboxylic acid (1 mmol) and 1 ml of toluene were introduced into a 25 ml round-bottom flask and the contents of the flask was shaken. Next, 0.1 g of native CALB was introduced, and 30% H_2O_2 (1 mmol) was added dropwise. The flask was sealed with a septum and mixed in a thermostatted shaker (±0.5ºC) with orbital stirring at 250 rpm at 18 and 25°C for 3-8 days, depending on the reaction rate. Periodically, 15 µl of the sample diluted with 1 ml of dichloromethane was collected during the reaction to monitor the progress of the reaction (yield and *ee*) utilising GC (SUPELCOWAX™ 10 column (30 m×0.2 mm×0.2 µm) with *n*-decane as an external standard or Astec CHIRALDEX™ G-TA

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capillary column (30 m×0.25 mm×0.12 μm)). When the reaction was completed, the post-reaction mixture was washed with 5 ml of a 10% $NaHCO_3$ solution in water, dried over anhydrous $MgSO_4$ and concentrated under vacuum. The yield of 4-methylcaprolactone after purification by column chromatography with a hexane:ethyl acetate ratio of 4:1 as an eluent was 93%.