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Lipase catalyzed oxidations in a sugar-derived Natural Deep Eutectic Solvent

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Abstract
Chemoenzymatic oxidations involving the CAL-B/H_2O_2 system was developed in a sugar derived Natural Deep Eutectic Solvent (NaDES) composed by a mixture of glucose, fructose and sucrose. Good to excellent conversions of substrates like cyclooctene, limonene, oleic acid and stilbene to their corresponding epoxides, cyclohexanone to its corresponding lactone and 2-phenylacetophenone to its corresponding ester, demonstrate the viability of the sugar NaDES as a reaction medium for epoxidation and Baeyer-Villiger oxidation.

Keywords
Chemoenzymatic oxidations, green solvents, Natural Deep Eutectic Solvents, Epoxidation, Baeyer-Villiger, Lipase

1. Introduction

Oxidation reactions have always been a major area of research due to their tremendous industrial applications. However, several oxidation processes present sustainability issues from the point of view of oxidants, catalysts and solvents used. (Cavani & Teles 2009) For example, a peracid is often used as the oxidizing agent, (Swern 1949) but transportation and storage of organic peracids leads to significant safety issues and costs; when achievable, molecular oxygen or air are for sure the ideal oxidants, with hydrogen peroxide as the second-best choice, in terms of atom economy and applicability to various oxidation systems. (O. Burek et al. 2019) In this context, enzymes, that can work in sustainable solvents with mild oxidants, can contribute to increase the greenness of the oxidation reactions. (Niku-Paavola & Viikari 2000; Constable et al. 2007; Gorke et al. 2008; Kotlewska et al. 2011; Silva et al. 2011; Hollmann et al. 2011; Drożdż et al. 2015; Qin et al. 2015; Yin et al. 2015; Yang & Duan 2016; García et al. 2018)

In fact, a very interesting system for obtaining peracids *in situ* is the chemoenzymatic system lipase/H_2O_2 that continuously forms the peracids through a lipase-catalyzed perhydrolysis of carboxylic acids or their esters. (Björkling et al. 1990; Yadav & Devi 2002; Busto et al. 2010) A broad range of hydrolases has been investigated for the peracid formation and among them the lipase B from *Candida Antarctica* (CAL-B), immobilized onto an acrylic resin (Novozyme 435) is the most reactive. (Ortiz et al. 2019) This system has been successfully applied to both epoxidations of alkenes (Prileshajev-epoxidation) and Baeyer-Villiger (B-V) oxidations (Scheme 1). (Lemoult et al. 1995; Aouf et al. 2014)

Epoxides are fundamental intermediates in organic synthesis but, despite their relevance, their industrial synthesis is scarcely sustainable (both environmentally and economically). Epoxidation of some natural products is industrially carried out by the Prileshajev-epoxidation (epoxidation of an alkene with a peracid) using either preformed or in-situ-generated short chain peroxy acids. (Rüsch gen. Klaas & Warwel 1999; Hilker et al. 2001) Nevertheless, the need for a strong acid to catalyze peroxy acid formation in this process can result in unsatisfactory selectivity and undesiderable side reactions *via* oxirane ring opening, leading to diols, hydroxyesters, and dimers. Prileshajev-epoxidation can be chemoenzymatically carried out on various substrates with CAL-B, a carboxylic acid as precursor of the peracid, and H_2O_2 as oxidant (Scheme 1); this method allows an improvement in terms of sustainability, mild reaction conditions, limited side products and use of less toxic reagents. (Niku-Paavola & Viikari 2000; Moreira & Nascimento 2007; Silva et al. 2011; Hollmann et al. 2011)

The B-V oxidation is a very well-known and useful reaction for the synthesis of esters and lactones starting from ketones, important building blocks in pharmaceutical and polymer synthesis. (Renz & Meunier 1999; Brink et al. 2004; Woodruff & Hutmacher 2010) Peracids such as meta-chloroperbenzoic acid or peracetic acid are used as stoichiometric reagents, but also various catalytic methods that use metals have been studied. (Strukul 1998; Ma et al. 2014) Protocols based on B-V monooxygenases have also been developed, but given their need for oxygen, cofactor

NADPH and their intrinsic low stability, they are considered unpractical. (Alphand et al. 2003; Leisch et al. 2011; Balke et al. 2012) Nevertheless, the simple chemoenzymatic method based on CAL-B, used for epoxidation of alkenes described above, has been also applied to this kind of oxidations (Scheme 1). (Lemoult et al. 1995; Ríos et al. 2007; Rios et al. 2008)
Green solvents exploited in chemoenzymatic oxidation reactions can be categorized into two main groups: i) water and ii) non-aqueous solvents like ionic liquids, (Moniruzzaman et al. 2010; Elgharbawy et al. 2020) supercritical fluids and fluorinated solvents. (Hobbs & Thomas 2007) Despite their interesting properties and application possibility, ionic liquids suffer from several drawbacks like cost, toxicity, low biodegradability, complexity in preparation and handling. (Samorì et al. 2015; Lei et al. 2017; B. Wang et al. 2017) Deep Eutectic Solvents (DESs), described for the first time by *Abbott et al.,* (Abbott et al. 2001) are low melting mixtures based on a combination of readily-available and inexpensive components, like quaternary ammonium salts as hydrogen bond acceptors (HBA), and acids, amides, amines, carbohydrates and alcohols as hydrogen bond donors (HDB). They are liquid at or below 100 °C, thanks to H-bond interactions between the single components that create specific supramolecular structures. The number and the spatial positions of hydrogen atoms in the donor and acceptor groups, available for hydrogen bonding, influence the formation and stability of the DES itself. (Nkuku & LeSuer 2007; Zhang et al. 2012; Paiva et al. 2014; Smith et al. 2014; Tommasi et al. 2017; Samorì et al. 2019) Dai *et al.* reported numerous preparations of Natural Deep Eutectic Solvents (NaDESs) by using plant metabolites. Interestingly, when water is added to the mixtures, in different proportions according to the NaDES, it can be incorporated into this structure and becomes strongly bound, reducing the viscosity of the NaDES while retaining its original characteristics. (Dai et al. 2013)
The chemoenzymatic oxidation systems described above (Scheme 1) have been studied in several solvents, including DESs (T. Gorke et al. 2008; Kotlewska et al. 2011; Durand et al. 2012; Drożdż et al. 2015; Yang & Duan 2016; Zhou et al. 2017; Ranganathan et al. 2017; Gotor-Fernández & Paul 2019; Ma et al. 2019), in which it has been demonstrated that the enzymatic activity, stability, and selectivity can be enhanced. (Zhou et al. 2017; Ülger & Takaç 2017; Oh et al. 2019; Guajardo et al. 2019; Gotor-Fernández & Paul 2019; Nian et al. 2020). Among the various NaDES proposed by Dai *et al.* we focused on the only one sugar-derived and chlorine-free combination, composed by glucose, fructose sucrose and water (1:1:1:11), that we called with the acronym GFS. To the best of our knowledge the lipase/H_2O_2 system was never reported in a solvent like GFS and, following our interest in biocatalysis in sustainable reaction media, (Galletti et al. 2007) herein we report on its application in the epoxidation of alkenes and B-V oxidation of ketones.

2. Materials and methods

2.1 Material: all chemicals and solvents were purchased from Sigma-Aldrich or Alfa Aesar and used without any further purification.
CAL-B (Lipase B from *Candida antarctica*) immobilized on Immobead 150, recombinant from yeast, 4000 U/g was used.
2.2 DESs preparation: the components were mixed with the appropriate stoichiometric ratios, heated at about 80-90 °C (120 °C for GFS) and magnetically stirred until homogeneous liquid was obtained; for GFS, distilled water (up to 30 wt %) was then added to get a homogeneous colorless liquid phase. All the DESs were cooled to rt (20 °C) before the use and stored in the fridge (4 °C).
2.3 Representative procedure for enzymatic epoxidations of alkenes: in a 4-mL vial, the immobilized CAL-B (amounts reported in Tables 1-4 and Scheme 4) and 400 mg of DES (200 mg for **1d**, 800 mg for **1e**) were weighted, followed by the addition of 1.6 mmol of alkene (0.8 mmol for **1f**), carboxylic acid (amounts reported in Tables 1-4 and Scheme 4) and 1 equivalent (eq) of H_2O_2 (30% aqueous solution). For entries 17 and 18 in Table 1, H_2O_2 has been added in 4 aliquots in 4 h, for substrate **1f** 1.5 eq has been used.
The vial was heated at 45 ºC (or rt, 20 °C, for **1d**) for various reaction times, then crudes were extracted with cyclohexane or ethyl acetate and analyzed by GC-MS. Extraction residues were

checked after derivatization by silylation for the presence of other by-products (see section 2.5). Conversions were calculated as ratios between products areas and total areas. ^{1}H and ^{13}C NMR spectra of some products have been acquired after purification of the crude by flash-column chromatography (see section 2.6), some isolated yields are also reported in the Tables. All products are known, they were recognized by comparison with standards or through mass spectra matching to what reported in NIST database. Formation of byproducts was checked by GC-MS and NMR.

2.4 Representative procedure for B-V oxidation of ketones: in a 4-mL vial, the immobilized CAL-B (amounts in Tables 3-4) and 400 mg of GFS for **3a, 3b, 3c**, (700 mg of GFS for **3d**) were weighted, followed by the addition of 0.8 mmol of ketone (0.4 mmol for **3d**), carboxylic acid (amounts in Tables 3-4) and 1 eq of H_2O_2 (30% aqueous solution), different amounts of H_2O_2 are reported in Table 3. The vial was heated at 45 ºC or at kept at rt, 20 °C, for various reaction times, then crudes were extracted with ethyl acetate and analyzed by GC-MS as described above. ^{1}H and ^{13}C NMR spectra of some purified products have been acquired after purification (see section 2.6) of the crude by flash-column chromatography, some isolated yields are also reported in Tables 3-4.

2.5 Silylation procedure: 50 μL of silylating agent *N*,*O*-bis(trimethylsilyl)trifluoroacetamide and 1% chlorotrimethylsilane, (BSTFA + 1% TMCS), 100 μL of CH_3CN and 20 μL of pyridine were added to 1–10 mg of sample into a GC-MS vial. The vial was heated at 60–80 °C for 30-40 min. The sample was then diluted with CH_3CN before the injection.

2.6 Purification procedure of selected products: reaction mixtures were extracted with ethyl acetate or cyclohexane then washed with a $NaHCO_3$ solution to remove the octanoic acid (OA). After evaporating the solvent, the crude was purified by flash chromatography. The fractions containing the product were mixed, the solvent evaporated, and the purified products were analyzed by GC-MS and NMR (See spectra in supplementary information, SI).

2.7 Instrumentation: GC-MS analysis of epoxides and ester **4d** were performed using an Agilent HP 6850 gas chromatograph connected to an Agilent HP 5975 quadrupole mass spectrometer. Analytes were separated on a HP-5MS fused-silica capillary column (stationary phase 5%-Phenyl)-methylpolysiloxane, 30 m, 0.25 mm i.d., 0.25 μm film thickness), with helium as the carrier gas (at constant pressure, 36 cm s^{-1} linear velocity at 200 °C). Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan s^{-1} within the 12−600 m/z range. The injection port temperature was 250 °C. The temperature of the column was kept at 50 °C for 5 min, then increased from 50 to 250 °C at 10 °C min^{-1} and the final temperature of 250 °C was kept for 12 min.

GC-MS analysis of Baeyer-Villiger products (except **4d**) were performed using an Agilent 7820A gas chromatograph connected to an Agilent 5977E quadrupole mass spectrometer. Analytes were separated on a DB-FFAP polar column (30 m length, 0.25 mm i.d., 0.25 μm film thickness), with helium flow of 1 mL min^{-1}. Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan s^{-1} within the 29−450 m/z range. The injection port temperature was 250 °C.

The temperature of the column was kept at 50 °C for 5 min, then increased from 50 to 250 °C at 10 °C min^{-1} and the final temperature of 250 °C was kept for 15 min.

^{1}H NMR spectra were recorded on Varian 400 (400 MHz) spectrometers. ^{13}C NMR spectra were recorded on a Varian 400 (100 MHz) spectrometers. Chemical shifts were reported in ppm from trimethylsilane (TMS) with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm).

3. Results and discussion

3.1 Alkenes epoxidation

We studied the chemoenzymatic epoxidation of various alkenes (focusing for each of them on the enzyme amount, peracid precursors and H_2O_2 amount and additions, and reaction time): cyclic alkenes (Table 1), poorly-reactive stilbene (Table 2) and oleic acid (Scheme 2) .

*3.1.1 Cyclic alkenes **1 a-d***

Studying CAL-B mediated chemoenzymatic epoxidation in various NaDESs, Zhou *et al.* showed that amine-based DESs (i.e. choline chloride-urea, 1:2 molar ratio, called Reline) significantly reduced the stability of CAL-B in a wide temperature range whereas the polyol-based ones increased it. (Zhou et al. 2017) For this reason, we focused our initial experiments on polyol-based NaDESs. Cyclohexene **1a** (Table 1, entry 1) was epoxidized with immobilized CAL-B (100 mg per 1.6 mmol of alkene), octanoic acid OA (one eq respect to the alkene), and H_2O_2 (one eq respect to the alkene) in choline chloride-sorbitol (ChCl-Sorb), 1:1 molar ratio (400 mg) (similarly to Zhou *et al*). We observed a complete conversion of the starting material but a very low selectivity towards the epoxide; in fact the most of epoxide was converted into the chlorinated by-product and the diol after 20h. This unexpected result prompted us to turn our attention towards chloride-free, sugar-based NaDESs as the GFS. We tested both solvents (ChCl-Sorb and GFS) in the same conditions with more easily detectable cyclodocedene **1b** and we observed that GFS gave better conversions (Table 1, entries 2 and 3); the same held true for other cyclic substrates (cyclooctene **1c** and limonene **1d**) (see in SI Table S1, entries 1 and 5, and Table S2, entry 1). So, we decided to test various substrates to check the viability of the system.

When Z/E mixtures were used in the starting alkene (as in **1b**), no diastereoselectivity was observed and the final product diastereomeric ratio reflected the diastereomeric distribution in the reagent.

OA resulted the most reactive acid precursor under our conditions (Table 1, entries 9-12), confirming the literature results, and its amount can be significantly lowered from 1 eq to 0.1 eq with all the substrates (Table 1, entries 6, 8, 13, 14, 18). Considering aliphatic acids with different chain lengths, butanoic acid BA (Table 1, entry 10) gave very good results on **1c** while acetic acid AA (Table 1, entry 11) was poorly reactive; the biobased levulinic acid (LA) gave **2c** in good conversion (Table 1, entry 12), prompting us to include 40% of LA as a component of the GFS instead of water, with the aim of using it both as peracid precursor and solvent component; however in this case the epoxidation of cyclododecene **1b** was not satisfactory (Table 1, entry 5). We also tested GFS-LA in combination with OA as peracid precursor on **1b**; results were good but lower than using GFS (Table 1, entries 3 and 4). The same happened with **1c** (see SI, Table S1, entry 4). Dimethyl carbonate DMC was also tested as peracid precursor but without good results (see SI, Table S1, entries 1 and 3). The amount of the enzyme could be lowered till 30-25 U/mmol without significant loss of reactivity (Table 1, **1b** entries 7 and 8, **1c** entry 14, **1d** entries 18 and 19).

Limonene **1d** is a very important biobased substrate, whose epoxy derivative is having some relevance in the field of polymer synthesis (Auriemma et al. 2015). Its internal double bond is much more reactive than the terminal one, being electron-richer; in all the tested condition the product **2d** was obtained. Since we initially observed the formation of the diol as by-product (Table 1, entry 15), milder conditions were tested: i) halving the amount of NaDES; ii) keeping the temperature below 25 °C; iii) lowering addition rate of H_2O_2. All these conditions allowed to avoid the diol formation (Table 1, entries 17 and 18). The use of a catalytic amount of acid precursor (Table 1, entry 16) and a lower amount of the enzyme defined the best conditions to obtain **2d** in very good conversion (Table 1, entry 18).

As expected from limonene results, terminal bonds of styrene and itaconic anhydride were not reactive in the mild condition we tested for the other substrates (see SI, Table S2, entries 5 and 6).

*3.1.2 trans-Stilbene **1e***

trans-Stilbene **1e** is a challenging substrate because its double bond is electron-poor and it is poorly soluble in polar solvents like GFS. OA and other linear aliphatic carboxylic acids with shorter (hexanoic HA, butanoic BA and acetic acid AA) and longer (dodecanoic acid, DA) chain lengths were tested as peracid precursors (Table 2). In all cases OA resulted the most effective acid precursor also in this case but 1 eq was needed to obtain an effective conversion (Table 2, entry 2). A decrease of the enzyme amount was possible, but a conversion of 75% was reached only after 48 h (Table 2, entry 4). Longer reaction times did not increase the conversion (SI, Table 2, entries 3 and 4). Differently, the electron-poor, α-β double bonds of crotonic acid and methyl crotonate were very difficult to be epoxidized (see SI, Table S2, entries 7 and 8) and we obtained just traces of the products. We also tested substrates carrying hydroxyl groups such as 1-octen-3-ol or *trans*-2-hexen-1-ol but, as expected, the main product was the ester formed by OA and the alcohol under CAL-B catalysis (data not shown).

*3.1.3 Oleic acid **1f***

Oleic acid **1f** is a very interesting substrate since its epoxide (9,10-epoxystearic acid) is a highly-valuable oleochemical due to its wide range of industrial applications, including cosmetics, personal care, and pharmaceutical products. The epoxidation worked very well and without the addition of OA (Scheme 2), thanks to an autocatalytic mechanism that formed the peroxy acid from the oleic acid itself. (Rüsch gen. Klaas & Warwel 1999) A temperature of 45 °C was required not only to catalyze the reaction but also to avoid the product solidification. The condition used are the same suggested and used by the recent literature (temperature at maximun 50 °C, an excess of H_2O_2, short reaction time), except for the use of the solvent, which is generally toluene. (Milchert et al. 2015) The epoxidation can also be carried out in a solvent-free system, but the process is more efficient for methyl oleate since the corresponding epoxide is liquid respect to solid 9,10-epoxystearic acid. (Orellana-Coca et al. 2005)

<u>3.2 Baeyer-Villiger oxidations</u>

The first use of CAL-B as catalyst for B-V oxidations was performed in toluene with myristic acid as peracid precursor. (Lemoult et al. 1995) Recent examples report ethyl acetate both as solvent and peracid precursor (therefore in large excess with respect to the starting material) (Ríos et al. 2007; Rios et al. 2008; Chávez et al. 2013; Drożdż et al. 2013) and combination of ionic liquids and OA as solvent and peracid precursor, respectively (OA in excess with respect to the starting ketone). (Kotlewska et al. 2011; Drożdż et al. 2015) Urea-hydrogen peroxide is considered a milder oxidant than H_2O_2 alone and it was used to reduce the formation of water in the reaction, (Ríos et al. 2007; Rios et al. 2008) nevertheless other studies showed no significant improvement in product conversion and enzyme recycling. (Chávez et al. 2013) Considering that water is already present in our GFS, the availability and the lower cost of hydrogen peroxide, this last one was thus chosen as oxidant in our study. As for reaction times, when the reaction was carried out at room temperature it generally required very long reaction times (in the order of days) to reach effective conversions. (Ríos et al. 2007; Rios et al. 2008; Chávez et al. 2013; Drożdż et al. 2013; Drożdż et al. 2015)
We tested B-V oxidation on various substrates (see section 2.4), using the same chemoenzymatic method in GFS previously described for epoxidation reactions: CAL-B, H_2O_2 (30% aqueous solution) and OA as peracid precursor (scheme in Table 3). Also in this case, a detailed study was conducted on the reaction conditions, with the aim of reducing the use of the reagents in excess and to use the mildest possible conditions.

*3.2.1 Cyclic ketones **3a-c***

Since highly reactive in B-V oxidations, cyclohexanone **3a** was the first substrate tested. By carrying out the reaction at 20 °C, lactone **4a**, (ε-caprolactone, Table 3, entry 1) was obtained but the reaction proceeded very slowly and an increase in time lead to the formation of the by-product, 6-hydroxyhexanoic acid **4a'**, caused by the ring-opening of **4a**. Increasing the amount of catalyst or temperature did not increase the selectivity towards **4a** formation (Table 3, entries 2 and 3). Differently from the epoxidation reaction of cyclic alkenes (Table 1), the use of the peracid precursor in catalytic amount did not give good results (Table 3, entry 4). Indeed, ω-hydroxy acid formation is the main drawback in CAL-B mediated B-V oxidation (amounts reported in Table 3). (X.-P. Wang et al. 2017) Increasing the amount of H_2O_2 to 2 eq (Table 3, entries 5 and 6) gave higher conversions, without the formation of any by-product, while a shorter reaction time was achieved by conducting the reaction at 45 °C (Table 3, entry 6). The use of both OA and H_2O_2 in excess (Table 3, entries 7 and 8) gave the best conversion: 74% at 20 °C and 58% at 45 °C. As previous observed when the reaction was conducted at 45 °C, the reaction must be stopped after a few hours to avoid by-product formation (Table 3, entry 8). Further increasing of both oxidant and acid amounts was not effective (Table 3, entry 9).

The expected higher reactivity of cyclopentanone **3b** prompted us to lower the enzyme amount but also tuning temperature, oxidant and OA amounts (Table 3, entries 11-13) the high reactivity of the substrate caused a rapid formation of the by-product **3b'**. As expected from the literature, (Chávez et al. 2013; Drożdż et al. 2013; Drożdż et al. 2015) substrates with larger rings, as cyclooctanone **3c,** are unreactive in all the tested conditions (Table 3, entry 14).

*3.2.2 2-phenylacetophenone **3d***

When using 2-phenylacetophenone **3d** the regioselectivity issue must be considered, due to the formation of two possible regioisomers **4d** and **4d'** (structure in Table 4 foot) caused by the migration of the phenyl group instead of the benzyl one (favored). As expected, we predominantly obtained regioisomer **4d** in 50% conversion at long reaction times (Table 4, entry 1). Higher temperature did not increase the conversion but significantly increased the reaction rate (Table 4, entries 1 and 3). Conversions decreased by lowering the amount of OA and enzyme (Table 4, entries 2 and 4). Using 2 eq of OA and H_2O_2 was not effective (Table 4, entry 5), while a great excess of H_2O_2 gave 60% of **4d** (Table 4, entry 6). Linear ketones and levulinic acid were tested but the reactin did not work under the developed conditions (data not shown).

Conclusions

We demonstrated that chemoenzymatic oxidations using lipase CAL-B to form the active oxidant from carboxylic acid/H_2O_2 pair can be performed in a sugar-based NaDES composed by an equimolar mixture of glucose, fructose, sucrose and water (GFS). Specific conditions to perform the reaction on selected substrates in good conversion and selectivity were found. The best conditions for epoxidations proved to be related to the substrate reactivity; reaction conditions were tuned and catalysts amounts decreased to obtain epoxides from poorly reactive and steric-hindered double bonds (as *trans*-stilbene) and to control the formation of byproducts in more reactive alkenes (like internal double bond of R-limonene). Baeyer-Villiger oxidations always required at least stoichiometric amount of the peracid precursor to proceed and an excess of both oxidant and acid to obtain good conversions.

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Disclosure of interest

The authors report no conflict of interest.

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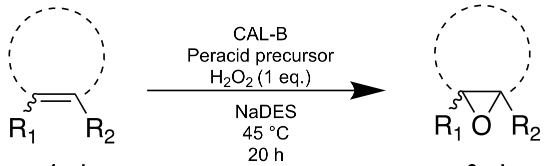
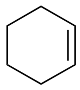
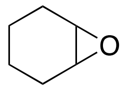
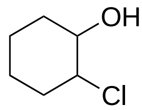
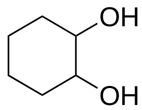
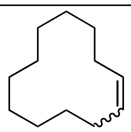
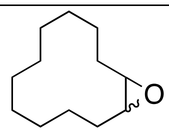
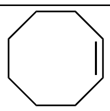
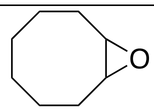
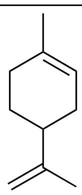
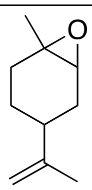
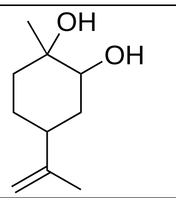
Tables

Table 1. Epoxidation of cyclic alkenes with chemoenzymatic method in NaDES.



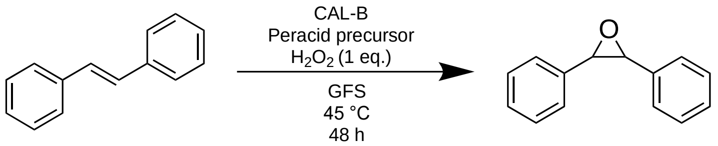
1a-d 2a-d

Entry	Alkene	NaDES	CAL-B (U/mmol)	Peracid precursor (eq)	Product conversion (%)[a]	By-products conversion (%)[a]	
	 **1a**				 **2a**	 **2a'**	 **2a''**
1	**1a**	ChCl- Sorb [1:1]	250	OA, 1	6	34	59
	 **1b**				 **2b**[b]	-	
2	**1b**	ChCl- Sorb [1:1]	250	OA, 1	68		
3	**1b**	GFS	250	OA, 1	71		
4	**1b**	GFS [1:1:1] -LA	250	OA, 1	64		
5	**1b**	GFS [1:1:1] -LA	250	LA	15		
6	**1b**	GFS	250	OA, 0.1	75		
7	**1b**	GFS	25	OA, 1	80		
8	**1b**	GFS	25	OA, 0.1	79 (77)		
	 **1c**				 **2c**	-	
9	**1c**	GFS	250	OA, 1	>99		
10	**1c**	GFS	250	BA, 1	99		
11	**1c**	GFS	250	AA, 1	48		
12	**1c**	GFS	250	LA, 1	87		
13	**1c**	GFS	250	OA, 0.1	93		
14	**1c**	GFS	25	OA, 0.1	95 (91)		
							

	1d				**2d**	**2d’**
15[c]	**1d**	GFS	60	OA, 1	76	14
16[c]	**1d**	GFS	60	OA, 0.1	73	16
17[c,d]	**1d**	GFS	60	OA, 1	89	**-**
18[c,d]	**1d**	GFS	30	OA, 0.1	96	**-**
19[c]	**1d**	GFS	30	OA, 0.1	53	31

[a] conversion by GC-MS, isolated yield in brackets; [b] diastereomeric ratio Z/E in **1b** and **2b** is always 2:1; [c] Room temperature (20 °C); [d] H_2O_2 total amount divided into 4 portions added in 4 hours.
Acronyms: ChCl= choline chloride, Sorb = sorbitol, GFS = glucose, fructose, sucrose, H_2O (1:1:1:11), OA= octanoic acid, LA= levulinic acid, AA = acetic acid, BA=butyric acid.

Table 2. Epoxidation of *trans*- stilbene **1e** with chemoenzymatic method in GFS NaDES.

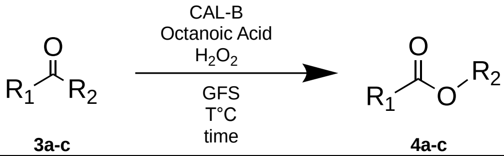
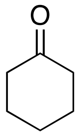
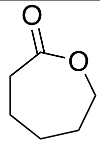
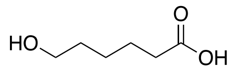
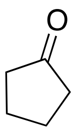
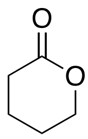
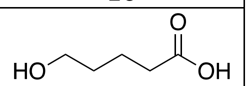
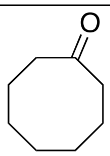
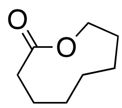


1e **2e**

Entry	CAL-B (U/mmol)	Peracid precursor (eq)	2e conversion (%)[a]
1[b]	250	OA, 1	60
2[b]	250	OA, 0.1	traces
3	250	OA, 1	74 (70)
4	25	OA, 1	73
5	25	DA, 1	11
6	25	HA, 1	54
7	25	BA, 1	**-**
8	25	AA, 1	**-**

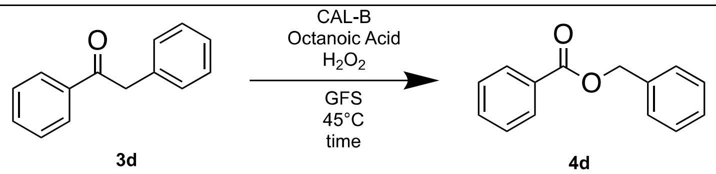
[a] conversion by GC-MS, isolated yield in brackets; [b] time (20h)
Acronyms: GFS = glucose, fructose, sucrose, water (1:1:1:11), OA= octanoic acid, DA = dodecanoic acid, HA= Hexanoic Acid, BA=butyric acid, AA = acetic acid.

Table 3. Baeyer-Villiger oxidation of lactones with chemoenzymatic method in GFS.

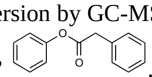
 **3a-c** → **4a-c**								
Entry	**Ketone**	**CAL-B (U/mmol)**	**OA (eq)**	**H_2O_2 (eq)**	**T (°C)**	**time (h)**	**Product conversion (%)[a]**	**By-product conversion (%)l[a]**
	 **3a**						 **4a**	 **4a'**
1	**3a**	125	1	1	20	20 70	32 23	- 53
2	**3a**	250	1	1	20	20	21	16
3	**3a**	200	1	1	45	5 15	35 36	- 28
4	**3a**	125	0,1	1	20	20 40	15 15	- -
5	**3a**	125	1	2	20	20 40	37 55	- -
6	**3a**	125	1	2	45	5 20	30 20	- 8
7	**3a**	125	2	2	20	20 40 64	49 61 74	- - -
8	**3a**	125	2	2	45	5 20	52 29	- 21
9	**3a**	125	1	3	20	20 4d	36 54	- 16
10	**3a**	125	3	3	20	20 4d	58 54	- 16
	 **3b**						 **4b**	 **4b'**
11	**3b**	65	1	1	20	20 40	15 7	- 50
12	**3b**	65	1	1	45	20	10	30
13	**3b**	65	0.1	1	20	40	4	15
	 **3c**						 **4c**	**Not found**
14	**3c**	65	various	various	20	various	traces	**-**

[a] conversion by GC-MS, isolated yield in brackets;
Acronyms: GFS = glucose, fructose, sucrose, H_2O (1:1:1:11), OA= octanoic acid.

Table 4. Baeyer-Villiger oxidation of 2-phenylacetophenone **3d** in GFS

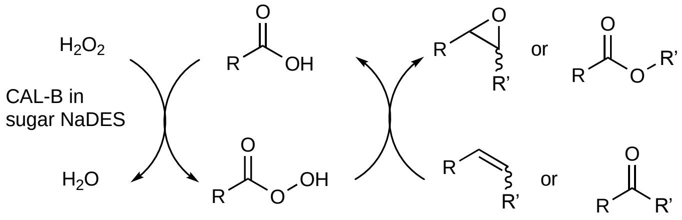


Entry	Ketone	CAL-B (U/mmol)	OA (eq)	H_2O_2 (eq)	time (h)	Conversion 4d (%)[a,c]
1[b]	**3d**	250	1	1	40 50 7 days	7 17 50
2[b]	**3d**	250	0.1	1	50	12
3	**3d**	250	1	1	40 7 days	40 (34) 50
4	**3d**	100	1	1	20 5 days	18 27
5	**3d**	250	2	2	20 50 3 days	38 42 44
6	**3d**	250	1	3	23 4 days	55 60

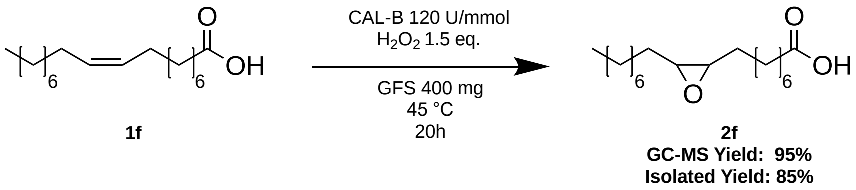
[a] conversion by GC-MS, isolated yield in brackets; [b] temperature (20 °C); [c] in all the entries there are traces of the regiosomer of 4d, **4d'** .
Acronyms: GFS = glucose, fructose, sucrose, H_2O (1:1:1:11), OA= octanoic acid.

Schemes

Scheme 1



Scheme 2



Schemes Captions

Scheme 1. Chemoenzymatic pathway for epoxidations and Baeyer-Villiger oxidations.

Scheme 2. Epoxidation of oleic acid with chemoenzymatic method in GFS.