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Lipase catalysed oxidations in a sugar-derived natural deep eutectic solvent

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

3  
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58 **Abstract**

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

65  
66 **Keywords**

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

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71 **1. Introduction**

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

83 In fact, a very interesting system for obtaining peracids *in situ* is the chemoenzymatic system  
84 lipase/H<sub>2</sub>O<sub>2</sub> that continuously forms the peracids through a lipase-catalyzed perhydrolysis of  
85 carboxylic acids or their esters. (Björkling et al. 1990; Yadav & Devi 2002; Busto et al. 2010) A  
86 broad range of hydrolases has been investigated for the peracid formation and among them the  
87 lipase B from *Candida Antarctica* (CAL-B), immobilized onto an acrylic resin (Novozyme 435) is  
88 the most reactive. (Ortiz et al. 2019) This system has been successfully applied to both epoxidations  
89 of alkenes (Prileshajev-epoxidation) and Baeyer-Villiger (B-V) oxidations (Scheme 1). (Lemoult et  
90 al. 1995; Aouf et al. 2014)

91 Epoxides are fundamental intermediates in organic synthesis but, despite their relevance, their  
92 industrial synthesis is scarcely sustainable (both environmentally and economically). Epoxidation of  
93 some natural products is industrially carried out by the Prileshajev-epoxidation (epoxidation of an  
94 alkene with a peracid) using either preformed or in-situ-generated short chain peroxy acids. (Rüsch  
95 gen. Klaas & Warwel 1999; Hilker et al. 2001) Nevertheless, the need for a strong acid to catalyze  
96 peroxy acid formation in this process can result in unsatisfactory selectivity and undesirable side  
97 reactions *via* oxirane ring opening, leading to diols, hydroxyesters, and dimers. Prileshajev-  
98 epoxidation can be chemoenzymatically carried out on various substrates with CAL-B, a carboxylic  
99 acid as precursor of the peracid, and H<sub>2</sub>O<sub>2</sub> as oxidant (Scheme 1); this method allows an  
100 improvement in terms of sustainability, mild reaction conditions, limited side products and use of  
101 less toxic reagents. (Niku-Paavola & Viikari 2000; Moreira & Nascimento 2007; Silva et al. 2011;  
102 Hollmann et al. 2011)

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

109 NADPH and their intrinsic low stability, they are considered unpractical. (Alphand et al. 2003;  
110 Leisch et al. 2011; Balke et al. 2012) Nevertheless, the simple chemoenzymatic method based on  
111 CAL-B, used for epoxidation of alkenes described above, has been also applied to this kind of  
112 oxidations (Scheme 1). (Lemoult et al. 1995; Ríos et al. 2007; Rios et al. 2008)  
113 Green solvents exploited in chemoenzymatic oxidation reactions can be categorized into two main  
114 groups: i) water and ii) non-aqueous solvents like ionic liquids, (Moniruzzaman et al. 2010;  
115 Elgharbawy et al. 2020) supercritical fluids and fluorinated solvents. (Hobbs & Thomas 2007)  
116 Despite their interesting properties and application possibility, ionic liquids suffer from several  
117 drawbacks like cost, toxicity, low biodegradability, complexity in preparation and handling.  
118 (Samorì et al. 2015; Lei et al. 2017; B. Wang et al. 2017) Deep Eutectic Solvents (DESs), described  
119 for the first time by *Abbott et al.*, (Abbott et al. 2001) are low melting mixtures based on a  
120 combination of readily-available and inexpensive components, like quaternary ammonium salts as  
121 hydrogen bond acceptors (HBA), and acids, amides, amines, carbohydrates and alcohols as  
122 hydrogen bond donors (HDB). They are liquid at or below 100 °C, thanks to H-bond interactions  
123 between the single components that create specific supramolecular structures. The number and the  
124 spatial positions of hydrogen atoms in the donor and acceptor groups, available for hydrogen  
125 bonding, influence the formation and stability of the DES itself. (Nkuku & LeSuer 2007; Zhang et  
126 al. 2012; Paiva et al. 2014; Smith et al. 2014; Tommasi et al. 2017; Samorì et al. 2019) Dai *et al.*  
127 reported numerous preparations of Natural Deep Eutectic Solvents (NaDESs) by using plant  
128 metabolites. Interestingly, when water is added to the mixtures, in different proportions according to  
129 the NaDES, it can be incorporated into this structure and becomes strongly bound, reducing the  
130 viscosity of the NaDES while retaining its original characteristics. (Dai et al. 2013)  
131 The chemoenzymatic oxidation systems described above (Scheme 1) have been studied in several  
132 solvents, including DESs (T. Gorke et al. 2008; Kotlewska et al. 2011; Durand et al. 2012; Drożdż  
133 et al. 2015; Yang & Duan 2016; Zhou et al. 2017; Ranganathan et al. 2017; Gotor-Fernández &  
134 Paul 2019; Ma et al. 2019), in which it has been demonstrated that the enzymatic activity, stability,  
135 and selectivity can be enhanced. (Zhou et al. 2017; Ülger & Takaç 2017; Oh et al. 2019; Guajardo  
136 et al. 2019; Gotor-Fernández & Paul 2019; Nian et al. 2020). Among the various NaDES proposed  
137 by Dai *et al.* we focused on the only one sugar-derived and chlorine-free combination, composed by  
138 glucose, fructose sucrose and water (1:1:1:11), that we called with the acronym GFS. To the best of  
139 our knowledge the lipase/H<sub>2</sub>O<sub>2</sub> system was never reported in a solvent like GFS and, following our  
140 interest in biocatalysis in sustainable reaction media, (Galletti et al. 2007) herein we report on its  
141 application in the epoxidation of alkenes and B-V oxidation of ketones.

## 142 143 **2. Materials and methods**

144 2.1 Material: all chemicals and solvents were purchased from Sigma-Aldrich or Alfa Aesar and  
145 used without any further purification.

146 CAL-B (Lipase B from *Candida antarctica*) immobilized on Immobead 150, recombinant from  
147 yeast, 4000 U/g was used.

148 2.2 DESs preparation: the components were mixed with the appropriate stoichiometric ratios,  
149 heated at about 80-90 °C (120 °C for GFS ) and magnetically stirred until homogeneous liquid was  
150 obtained; for GFS, distilled water (up to 30 wt %) was then added to get a homogeneous colorless  
151 liquid phase. All the DESs were cooled to rt (20 °C) before the use and stored in the fridge (4 °C).

152 2.3 Representative procedure for enzymatic epoxidations of alkenes: in a 4-mL vial, the  
153 immobilized CAL-B (amounts reported in Tables 1-4 and Scheme 4) and 400 mg of DES (200 mg  
154 for **1d**, 800 mg for **1e**) were weighted, followed by the addition of 1.6 mmol of alkene (0.8 mmol  
155 for **1f**), carboxylic acid (amounts reported in Tables 1-4 and Scheme 4) and 1 equivalent (eq) of  
156 H<sub>2</sub>O<sub>2</sub> (30% aqueous solution). For entries 17 and 18 in Table 1, H<sub>2</sub>O<sub>2</sub> has been added in 4 aliquots  
157 in 4 h, for substrate **1f** 1.5 eq has been used.

158 The vial was heated at 45 °C (or rt, 20 °C, for **1d**) for various reaction times, then crudes were  
159 extracted with cyclohexane or ethyl acetate and analyzed by GC-MS. Extraction residues were

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

166 2.4 Representative procedure for B-V oxidation of ketones: in a 4-mL vial, the immobilized CAL-B  
167 (amounts in Tables 3-4) and 400 mg of GFS for **3a**, **3b**, **3c**, (700 mg of GFS for **3d**) were weighted,  
168 followed by the addition of 0.8 mmol of ketone (0.4 mmol for **3d**), carboxylic acid (amounts in  
169 Tables 3-4) and 1 eq of  $\text{H}_2\text{O}_2$  (30% aqueous solution), different amounts of  $\text{H}_2\text{O}_2$  are reported in  
170 Table 3. The vial was heated at 45 °C or at kept at rt, 20 °C, for various reaction times, then crudes  
171 were extracted with ethyl acetate and analyzed by GC-MS as described above.  $^1\text{H}$  and  $^{13}\text{C}$  NMR  
172 spectra of some purified products have been acquired after purification (see section 2.6) of the crude  
173 by flash-column chromatography, some isolated yields are also reported in Tables 3-4.

174 2.5 Silylation procedure: 50  $\mu\text{L}$  of silylating agent *N,O*-bis(trimethylsilyl)trifluoroacetamide and  
175 1% chlorotrimethylsilane, (BSTFA + 1% TMCS), 100  $\mu\text{L}$  of  $\text{CH}_3\text{CN}$  and 20  $\mu\text{L}$  of pyridine were  
176 added to 1–10 mg of sample into a GC-MS vial. The vial was heated at 60–80 °C for 30-40 min.  
177 The sample was then diluted with  $\text{CH}_3\text{CN}$  before the injection.

178 2.6 Purification procedure of selected products: reaction mixtures were extracted with ethyl acetate  
179 or cyclohexane then washed with a  $\text{NaHCO}_3$  solution to remove the octanoic acid (OA). After  
180 evaporating the solvent, the crude was purified by flash chromatography. The fractions containing  
181 the product were mixed, the solvent evaporated, and the purified products were analyzed by GC-MS  
182 and NMR (See spectra in supplementary information, SI).

183 2.7 Instrumentation: GC-MS analysis of epoxides and ester **4d** were performed using an Agilent HP  
184 6850 gas chromatograph connected to an Agilent HP 5975 quadrupole mass spectrometer. Analytes  
185 were separated on a HP-5MS fused-silica capillary column (stationary phase 5%-Phenyl)-  
186 methylpolysiloxane, 30 m, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness), with helium as the carrier gas (at  
187 constant pressure, 36  $\text{cm s}^{-1}$  linear velocity at 200 °C). Mass spectra were recorded under electron  
188 ionization (70 eV) at a frequency of 1 scan  $\text{s}^{-1}$  within the 12–600  $m/z$  range. The injection port  
189 temperature was 250 °C. The temperature of the column was kept at 50 °C for 5 min, then increased  
190 from 50 to 250 °C at 10 °C  $\text{min}^{-1}$  and the final temperature of 250 °C was kept for 12 min.

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

196 The temperature of the column was kept at 50 °C for 5 min, then increased from 50 to 250 °C at 10  
197 °C  $\text{min}^{-1}$  and the final temperature of 250 °C was kept for 15 min.

198  $^1\text{H}$  NMR spectra were recorded on Varian 400 (400 MHz) spectrometers.  $^{13}\text{C}$  NMR spectra were  
199 recorded on a Varian 400 (100 MHz) spectrometers. Chemical shifts were reported in ppm from  
200 trimethylsilane (TMS) with the solvent resonance as the internal standard (deuteriochloroform: 7.26  
201 ppm).

202

### 203 **3. Results and discussion**

#### 204 3.1 Alkenes epoxidation

205 We studied the chemoenzymatic epoxidation of various alkenes (focusing for each of them on the  
206 enzyme amount, peracid precursors and  $\text{H}_2\text{O}_2$  amount and additions, and reaction time): cyclic  
207 alkenes (Table 1), poorly-reactive stilbene (Table 2) and oleic acid (Scheme 2) .

208

##### 209 3.1.1 Cyclic alkenes 1 a-d

210 Studying CAL-B mediated chemoenzymatic epoxidation in various NaDESs, Zhou *et al.* showed  
211 that amine-based DESs (i.e. choline chloride-urea, 1:2 molar ratio, called Reline) significantly  
212 reduced the stability of CAL-B in a wide temperature range whereas the polyol-based ones  
213 increased it. (Zhou et al. 2017) For this reason, we focused our initial experiments on polyol-based  
214 NaDESs. Cyclohexene **1a** (Table 1, entry 1) was epoxidized with immobilized CAL-B (100 mg per  
215 1.6 mmol of alkene), octanoic acid OA (one eq respect to the alkene), and H<sub>2</sub>O<sub>2</sub> (one eq respect to  
216 the alkene) in choline chloride-sorbitol (ChCl-Sorb), 1:1 molar ratio (400 mg) (similarly to Zhou *et al.*)  
217 *al.* We observed a complete conversion of the starting material but a very low selectivity towards  
218 the epoxide; in fact the most of epoxide was converted into the chlorinated by-product and the diol  
219 after 20h. This unexpected result prompted us to turn our attention towards chloride-free, sugar-  
220 based NaDESs as the GFS. We tested both solvents (ChCl-Sorb and GFS) in the same conditions  
221 with more easily detectable cyclododecene **1b** and we observed that GFS gave better conversions  
222 (Table 1, entries 2 and 3); the same held true for other cyclic substrates (cyclooctene **1c** and  
223 limonene **1d**) (see in SI Table S1, entries 1 and 5, and Table S2, entry 1). So, we decided to test  
224 various substrates to check the viability of the system.

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

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

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

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

251

### 252 3.1.2 *trans*-Stilbene **1e**

253 *trans*-Stilbene **1e** is a challenging substrate because its double bond is electron-poor and it is poorly  
254 soluble in polar solvents like GFS. OA and other linear aliphatic carboxylic acids with shorter  
255 (hexanoic HA, butanoic BA and acetic acid AA) and longer (dodecanoic acid, DA) chain lengths  
256 were tested as peracid precursors (Table 2). In all cases OA resulted the most effective acid  
257 precursor also in this case but 1 eq was needed to obtain an effective conversion (Table 2, entry 2).  
258 A decrease of the enzyme amount was possible, but a conversion of 75% was reached only after 48  
259 h (Table 2, entry 4). Longer reaction times did not increase the conversion (SI, Table 2, entries 3  
260 and 4). Differently, the electron-poor,  $\alpha$ - $\beta$  double bonds of crotonic acid and methyl crotonate were

261 very difficult to be epoxidized (see SI, Table S2, entries 7 and 8) and we obtained just traces of the  
262 products. We also tested substrates carrying hydroxyl groups such as 1-octen-3-ol or *trans*-2-hexen-  
263 1-ol but, as expected, the main product was the ester formed by OA and the alcohol under CAL-B  
264 catalysis (data not shown).

265

### 266 3.1.3 Oleic acid **1f**

267 Oleic acid **1f** is a very interesting substrate since its epoxide (9,10-epoxystearic acid) is a highly-  
268 valuable oleochemical due to its wide range of industrial applications, including cosmetics, personal  
269 care, and pharmaceutical products. The epoxidation worked very well and without the addition of  
270 OA (Scheme 2), thanks to an autocatalytic mechanism that formed the peroxy acid from the oleic  
271 acid itself. (Rüsch gen. Klaas & Warwel 1999) A temperature of 45 °C was required not only to  
272 catalyze the reaction but also to avoid the product solidification. The condition used are the same  
273 suggested and used by the recent literature (temperature at maximum 50 °C, an excess of H<sub>2</sub>O<sub>2</sub>,  
274 short reaction time), except for the use of the solvent, which is generally toluene. (Milchert et al.  
275 2015) The epoxidation can also be carried out in a solvent-free system, but the process is more  
276 efficient for methyl oleate since the corresponding epoxide is liquid respect to solid 9,10-  
277 epoxystearic acid. (Orellana-Coca et al. 2005)

278

### 279 3.2 Baeyer-Villiger oxidations

280 The first use of CAL-B as catalyst for B-V oxidations was performed in toluene with myristic acid  
281 as peracid precursor. (Lemoult et al. 1995) Recent examples report ethyl acetate both as solvent and  
282 peracid precursor (therefore in large excess with respect to the starting material) (Ríos et al. 2007;  
283 Rios et al. 2008; Chávez et al. 2013; Drożdż et al. 2013) and combination of ionic liquids and OA  
284 as solvent and peracid precursor, respectively (OA in excess with respect to the starting ketone).  
285 (Kotowska et al. 2011; Drożdż et al. 2015) Urea-hydrogen peroxide is considered a milder oxidant  
286 than H<sub>2</sub>O<sub>2</sub> alone and it was used to reduce the formation of water in the reaction, (Ríos et al. 2007;  
287 Rios et al. 2008) nevertheless other studies showed no significant improvement in product  
288 conversion and enzyme recycling. (Chávez et al. 2013) Considering that water is already present in  
289 our GFS, the availability and the lower cost of hydrogen peroxide, this last one was thus chosen as  
290 oxidant in our study. As for reaction times, when the reaction was carried out at room temperature it  
291 generally required very long reaction times (in the order of days) to reach effective conversions.  
292 (Rios et al. 2007; Rios et al. 2008; Chávez et al. 2013; Drożdż et al. 2013; Drożdż et al. 2015)

293 We tested B-V oxidation on various substrates (see section 2.4), using the same chemoenzymatic  
294 method in GFS previously described for epoxidation reactions: CAL-B, H<sub>2</sub>O<sub>2</sub> (30% aqueous  
295 solution) and OA as peracid precursor (scheme in Table 3). Also in this case, a detailed study was  
296 conducted on the reaction conditions, with the aim of reducing the use of the reagents in excess and  
297 to use the mildest possible conditions.

298

#### 299 3.2.1 Cyclic ketones **3a-c**

300 Since highly reactive in B-V oxidations, cyclohexanone **3a** was the first substrate tested. By  
301 carrying out the reaction at 20 °C, lactone **4a**, ( $\epsilon$ -caprolactone, Table 3, entry 1) was obtained but  
302 the reaction proceeded very slowly and an increase in time lead to the formation of the by-product,  
303 6-hydroxyhexanoic acid **4a'**, caused by the ring-opening of **4a**. Increasing the amount of catalyst or  
304 temperature did not increase the selectivity towards **4a** formation (Table 3, entries 2 and 3).  
305 Differently from the epoxidation reaction of cyclic alkenes (Table 1), the use of the peracid  
306 precursor in catalytic amount did not give good results (Table 3, entry 4). Indeed,  $\omega$ -hydroxy acid  
307 formation is the main drawback in CAL-B mediated B-V oxidation (amounts reported in Table 3).  
308 (X.-P. Wang et al. 2017) Increasing the amount of H<sub>2</sub>O<sub>2</sub> to 2 eq (Table 3, entries 5 and 6) gave  
309 higher conversions, without the formation of any by-product, while a shorter reaction time was  
310 achieved by conducting the reaction at 45 °C (Table 3, entry 6). The use of both OA and H<sub>2</sub>O<sub>2</sub> in  
311 excess (Table 3, entries 7 and 8) gave the best conversion: 74% at 20 °C and 58% at 45 °C. As



312 previous observed when the reaction was conducted at 45 °C, the reaction must be stopped after a  
313 few hours to avoid by-product formation (Table 3, entry 8). Further increasing of both oxidant and  
314 acid amounts was not effective (Table 3, entry 9).

315 The expected higher reactivity of cyclopentanone **3b** prompted us to lower the enzyme amount but  
316 also tuning temperature, oxidant and OA amounts (Table 3, entries 11-13) the high reactivity of the  
317 substrate caused a rapid formation of the by-product **3b'**. As expected from the literature, (Chávez  
318 et al. 2013; Drożdż et al. 2013; Drożdż et al. 2015) substrates with larger rings, as cyclooctanone  
319 **3c**, are unreactive in all the tested conditions (Table 3, entry 14).

320

### 321 3.2.2 2-phenylacetophenone **3d**

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

331

## 332 Conclusions

333 We demonstrated that chemoenzymatic oxidations using lipase CAL-B to form the active oxidant  
334 from carboxylic acid/H<sub>2</sub>O<sub>2</sub> pair can be performed in a sugar-based NaDES composed by an  
335 equimolar mixture of glucose, fructose, sucrose and water (GFS). Specific conditions to perform the  
336 reaction on selected substrates in good conversion and selectivity were found. The best conditions  
337 for epoxidations proved to be related to the substrate reactivity; reaction conditions were tuned and  
338 catalysts amounts decreased to obtain epoxides from poorly reactive and steric-hindered double  
339 bonds (as *trans*-stilbene) and to control the formation of byproducts in more reactive alkenes (like  
340 internal double bond of R-limonene). Baeyer-Villiger oxidations always required at least  
341 stoichiometric amount of the peracid precursor to proceed and an excess of both oxidant and acid to  
342 obtain good conversions.

343

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348

349

## 350 Disclosure of interest

351 The authors report no conflict of interest.

352

353

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## Tables

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

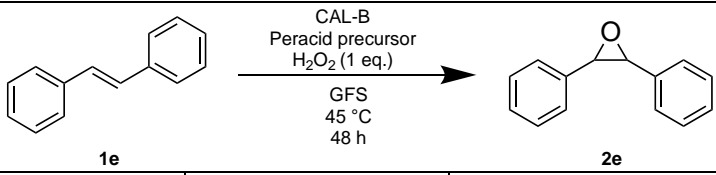
Entry	Alkene	NaDES	CAL-B (U/mmol)	Peracid precursor (eq)	Product conversion (%) <sup>a</sup>	By-products conversion (%) <sup>a</sup>
1	<b>1a</b>	ChCl- Sorb [1:1]	250	OA, 1	6	34      59
						-
2	<b>1b</b>	ChCl- Sorb [1:1]	250	OA, 1	68	
3	<b>1b</b>	GFS	250	OA, 1	71	
4	<b>1b</b>	GFS [1:1:1] -LA	250	OA,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

	<b>1d</b>				<b>2d</b>	<b>2d'</b>
15 <sup>c</sup>	<b>1d</b>	GFS	60	OA, 1	76	14
16 <sup>c</sup>	<b>1d</b>	GFS	60	OA, 0.1	73	16
17 <sup>c,d</sup>	<b>1d</b>	GFS	60	OA, 1	89	-
18 <sup>c,d</sup>	<b>1d</b>	GFS	30	OA, 0.1	96	-
19 <sup>c</sup>	<b>1d</b>	GFS	30	OA, 0.1	53	31

<sup>a</sup> conversion by GC-MS, isolated yield in brackets; <sup>b</sup> diastereomeric ratio Z/E in **1b** and **2b** is always 2:1; <sup>c</sup> Room temperature (20 °C); <sup>d</sup> H<sub>2</sub>O<sub>2</sub> total amount divided into 4 portions added in 4 hours.

Acronyms: ChCl= choline chloride, Sorb = sorbitol, GFS = glucose, fructose, sucrose, H<sub>2</sub>O (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.

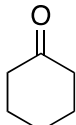
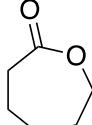
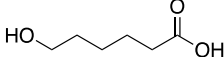
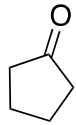
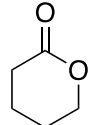
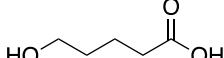
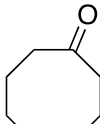
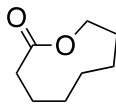
			
Entry	CAL-B (U/mmol)	Peracid precursor (eq)	<b>2e conversion (%)</b> <sup>a</sup>
1 <sup>b</sup>	250	OA, 1	60
2 <sup>b</sup>	250	OA, 0.1	traces
3	250	OA, 1	74 (70)
4	25	OA, 1	73
5	25	DA, 1	11
6	25	HA, 1	54
7	25	BA, 1	-
8	25	AA, 1	-

<sup>a</sup> conversion by GC-MS, isolated yield in brackets; <sup>b</sup> 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.

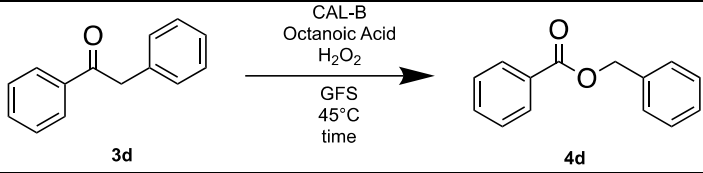


Table 3. Baeyer-Villiger oxidation of lactones with chemoenzymatic method in GFS.

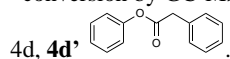
$  \begin{array}{ccc}  \text{R}_1-\text{C}(=\text{O})-\text{R}_2 & \xrightarrow[\text{GFS}]{\text{CAL-B, Octanoic Acid, H}_2\text{O}_2} & \text{R}_1-\text{C}(=\text{O})-\text{O}-\text{R}_2 \\  \text{3a-c} & & \text{4a-c} \\  \text{T}^\circ\text{C} & & \text{time}  \end{array}  $								
Entry	Ketone	CAL-B (U/mmol)	OA (eq)	H <sub>2</sub> O <sub>2</sub> (eq)	T (°C)	time (h)	Product conversion (%) <sup>a</sup>	By-product conversion (%) <sup>a</sup>
	 <b>3a</b>						 <b>4a</b>	 <b>4a'</b>
1	<b>3a</b>	125	1	1	20	20 70	32 23	- 53
2	<b>3a</b>	250	1	1	20	20	21	16
3	<b>3a</b>	200	1	1	45	5 15	35 36	- 28
4	<b>3a</b>	125	0,1	1	20	20 40	15 15	- -
5	<b>3a</b>	125	1	2	20	20 40	37 55	- -
6	<b>3a</b>	125	1	2	45	5 20	30 20	- 8
7	<b>3a</b>	125	2	2	20	20 40 64	49 61 74	- - -
8	<b>3a</b>	125	2	2	45	5 20	52 29	- 21
9	<b>3a</b>	125	1	3	20	20 4d	36 54	- 16
10	<b>3a</b>	125	3	3	20	20 4d	58 54	- 16
	 <b>3b</b>						 <b>4b</b>	 <b>4b'</b>
11	<b>3b</b>	65	1	1	20	20 40	15 7	- 50
12	<b>3b</b>	65	1	1	45	20	10	30
13	<b>3b</b>	65	0.1	1	20	40	4	15
	 <b>3c</b>						 <b>4c</b>	<b>Not found</b>
14	<b>3c</b>	65	various	various	20	various	traces	-

<sup>a</sup> conversion by GC-MS, isolated yield in brackets;  
 Acronyms: GFS = glucose, fructose, sucrose, H<sub>2</sub>O (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 <sub>2</sub> O <sub>2</sub> (eq)	time (h)	Conversion <b>4d</b> (%) <sup>a,c</sup>
1 <sup>b</sup>	<b>3d</b>	250	1	1	40 50 7 days	7 17 50
2 <sup>b</sup>	<b>3d</b>	250	0.1	1	50	12
3	<b>3d</b>	250	1	1	40 7 days	40 (34) 50
4	<b>3d</b>	100	1	1	20 5 days	18 27
5	<b>3d</b>	250	2	2	20 50 3 days	38 42 44
6	<b>3d</b>	250	1	3	23 4 days	55 60

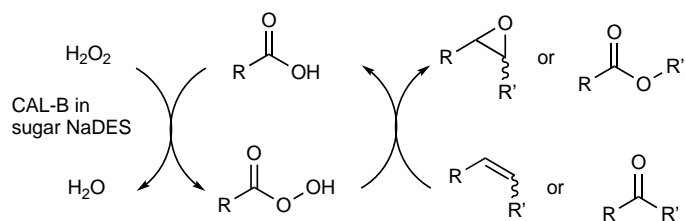
<sup>a</sup> conversion by GC-MS, isolated yield in brackets; <sup>b</sup> temperature (20 °C); <sup>c</sup> in all the entries there are traces of the regioisomer of



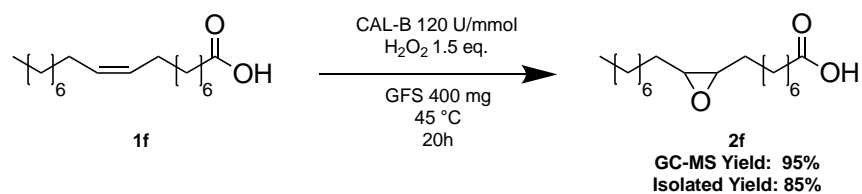
Acronyms: GFS = glucose, fructose, sucrose, H<sub>2</sub>O (1:1:1:11), OA= octanoic acid.

## 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.