Lipase-catalysed enantioselective kinetic resolution of *rac*-lipidic alkynylcarbinols and a C₅ synthon thereof *via* a hydrolysis approach

Diana Kelly Castro de Almeida^a, Marcos Reinaldo da Silva^a, Marcos Carlos de Mattos^a, Fátima Miranda Nunes^a, Stéphanie Ballereau,^b Yves Génisson,^b Valérie Maraval,^c Remi Chauvin^d and Maria Conceição Ferreira Oliveira^{a,*}

^a *Federal University of Ceará, Department of Organic and Inorganic Chemistry, Laboratory of Biotechnology and Organic Synthesis (LABS), Campus do Pici, Postal Box 6044, 60455-970, Fortaleza, Ceará, Brazil*

^b *Laboratory SPCMIB UMR 5068, CNRS, Paul Sabatier - Toulouse III University, 118 route de Narbonne, 31062 Toulouse CEDEX 9, France*

^c *LCC-CNRS, Université de Toulouse, CNRS, Toulouse, France*

^d *LCC-CNRS, Université de Toulouse, CNRS, Université Toulouse 3-Paul Sabatier, Toulouse, France*

ABSTRACT

Lipase-mediated kinetic resolution (LMKR) of three racemic alkynylcarbinol (LAC) acetates, *rac*-heptadeca-1,4-diyn-3-yl acetate (*rac*-**2**-Ac), *rac*-heptadeca-1,4,6-triyn-3-yl acetate (*rac*-**3**-Ac) and *rac*-heptadeca-1-yn-3-yl acetate (*rac*-**4**-Ac), besides the versatile C₅ synthon dialkynylcarbinol (DAC) acetate, *rac*-(triisopropylsilyl)penta-1,4-diyn-3-yl acetate (*rac*-**5**), were studied. CAL-B immobilized on acrylic resin was investigated as catalyst on LMKR of *rac*-**2-5**-Ac. Fourteen commercial lipases (immobilized and free forms), besides one esterase, were also investigated on the LMKR of *rac*-**5**-Ac. After investigation of reaction parameters, optimal conditions were established for each compound, yielding both (*R*)- and (*S*)-alcohols in 50% conversions, enantioselectivities (*E*) >200 and high *e.e.* values [(*R*)-**2** and (*S*)-**2**-Ac, *e.e.* > 99%); (*R*)-**3** and (*S*)-**3**-Ac, *e.e.*

98%); (*R*)-**4** and (*S*)-**4**-Ac, *e.e.* 96%); (*R*)-**5** and (*S*)-**5**-Ac, *e.e.* > 99%]. Except for *rac*-**5**-Ac that had *Thermomyces lanuginosus* immobilized on imobead-150 as best catalyst, CAL-B was the most efficient lipase on LMKR of *rac*-**2-4**-Ac. (*S*)-Alcohols were obtained by lipase-mediated hydrolysis of (*S*)-acetate compounds, using CAL-B for (*S*)-**4**-Ac (57.5% yield and *e.e.* 96%) and lipase from *Candida rugosa* for (*S*)-**5**-Ac (53% yield and *e.e.* > 99%).

* Corresponding author. Tel.: +55 85 999245221; fax: +55 85 33669782

E-mail address: mcfo@ufc.br (M.C.F. Oliveira)

Keywords: Biocatalysis; lipases; enzymatic kinetic resolution; lipidic alkynylcarbinols, chiral propargylic alcohols.

1. Introduction

Lipidic alkynylcarbinols (LACs) are found as acetylenic natural products well known for their outstanding cytotoxic activities [1]. Compounds from this class display a chiral secondary propargylic alcohol motif bearing a lipidic unit attached to the carbinol center (**1**, Fig.1). The terminal alkynylcarbinol moiety was identified as the chiral pharmacophore inducing the cytotoxicity of LACs and studies of the structure-activity relationship with a set of bio-inspired synthesized compounds have proved that the absolute configuration of the stereogenic center, besides the degree of unsaturation at the internal propargylic position and the length of the lipidic chain, plays a key role on their bioactivities [2–4].

The remarkable antitumor potential of naturally occurring LACs isolated from different sources has motivated the asymmetric synthesis of a variety of chiral analogues

for biological evaluation [4]. Compounds **2-4** (Fig. 1), displaying C₁₄ lipidic chains, α -unsaturated or not, are examples of synthetic LACs that were designed and prepared for cytotoxicity studies. Both enantio-enriched lipidic dialkynylcarbinols (DACs) (*R*)-**2** (76% *e.e.*) and (*S*)-**2** (91% *e.e.*) were obtained from two different approaches, employing (–)-*N*-methylephedrine ((–)-NME) as chiral inducer in modified Carreira reactions [2]. Later, a similar strategy was used on the syntheses of particular DACs, i.e. the butadiynylalkynylcarbinols (BACs) (*R*)-**3** (84% *e.e.*) and (*S*)-**3** (84% *e.e.*), embedding two conjugated triple bonds in the lipidic chain [5]. A different approach was considered for the enantioselective synthesis of the simple LAC (*S*)-**4** (> 99% *e.e.*). This saturated LAC was prepared through asymmetric reduction of the corresponding ynone (TIPS-protected alkyne) with Noyori Ru^{II} catalyst [2]. Therefore, the chemical asymmetric syntheses of these three LACs yielded enantiomers either in moderate yields and enantiomeric excesses (*e.e.*'s) for **2** and **3**, or in one single configuration but in a low chemical yield for **4**.

Fig. 1. General representation of LACs (**1**) and chemical structures of the bio-inspired synthetic compounds **2-4**.

Biocatalysis (enzyme catalysis) has proved to be a green alternative to conventional chemical catalysis to produce chiral pharmaceuticals and intermediates. The versatility of

enzymes to catalyze a variety of reactions to produce chiral compounds with high added value is exploited in Biocatalysis. In the context of green chemistry and sustainable development, the use of biocatalyst is extremely attractive since the enzymes are obtained from renewable resources and are non-hazardous, biocompatible and biodegradable. Besides being sustainable, enzymes catalyze a broad range of reactions with high chemo-, regio- and enantio-selectivities [6,7], and their optimal properties are achieved only in physiological conditions (reactions and substrates). Because the latter characteristic may be a problem in industrial scale, several tools for improving the enzyme limitation were developed, such as metagenomic, genetic (site-directed mutagenesis, directed evolution) and immobilization [8].

Among the enzymes used in biocatalysis, lipases (EC 3.1.1.3) are highlighted for their enantioselectivity in the kinetic resolution of either *rac*-alcohols or *rac*-esters to produce enantiopure alcohols and esters without the need of cofactors. Another advantage of these biocatalysts is their capability of being active in organic solvents, favoring the solubility of the organic substrate to be transformed [7,9]. Interfacial activation mechanism is considered an important characteristic of lipases. Conditions for optimal reaction with this class of enzyme involve an association between enzyme activity and substrate solubility. It is known that the lipase active site (catalytic triad Ser-His-Asp/Glu) is covered by a polypeptide chain lid that is opened in water-oil interface, a phenomenon known as interfacial activation. Thus, immobilized lipases with fixed open conformations present enhanced enzymatic activity. Moreover, immobilization may strongly modulate the lipase properties since the active center of this kind of enzyme is very flexible. It is worth mentioning that structural investigations of *Candida antarctica* lipase type B (CAL-B), one of the most used lipase, revealed that this enzyme displays an open (active) and closed (inactive) form and that its active site is covered by a very small lid [8,10].

Lipase-mediated kinetic resolution (LMKR) of some *rac*-LACs was also reported as alternative strategy for the production of enantiopure compounds [11–23] most of the examples involving acetylation approach in organic solvent [11–22]. Recently, the LMKR strategy *via* hydrolytic approach was used by our research group to resolve the *rac*-acetates of the LACs **2** and **3** (Fig. 1) with commercial CAL-B immobilized on acrylic resin [5]. Both resolved compounds, the reaction product (*R*)-**2** and the starting (*S*)-**2**-Ac, were obtained in > 99% *e.e.* After chemical hydrolysis of the latter acetate, both the alcohols (*R*)-**2** and (*S*)-**2** were evaluated as cytotoxic agents against HCT-116 cancer cell lines. The activity of enantiopure (*S*)-**2** (IC₅₀ 40 nM for *e.e.* > 99%, obtained by LMKR with CAL-B) [5] was improved when compared with the previously reported activity [1] of a moderately scalemic sample (IC₅₀ 91 nM for *e.e.* 76%, obtained by asymmetric synthesis using (-)-NME as chiral inducer). It is worth mentioning that (*R*)-**2** (*e.e.* > 99%) was not considered cytotoxic (IC₅₀ 3000 nM). These results illustrate the relevance of the biocatalytic approach to produce this promising antitumor compound (*S*)-**2** in the enantiomerically pure form. Since the reported LMKR of *rac*-**2**-Ac was performed using MeCN as co-solvent of buffer medium in the process, we therefore decided to investigate a greener protocol for the LMKR of *rac*-**2**-Ac in pure aqueous medium (without co-solvent).

The same approach (LMKR *via* hydrolysis) was used for the resolution of the *rac*-acetate of the BAC **3**, providing the enantio-enriched (*R*)-**3** and its (*S*)-**3**-Ac acetate derivative in 96 and 95% *e.e.*, respectively [5]. The biocatalytic approach was also essential in this case to generate the alcohol (*S*)-**3**. This is particularly worth of note since efficient chemical hydrolysis of the (*S*)-**3**-Ac was hampered by its high sensitivity to basic conditions [5]. The unique cytotoxicity of (*S*)-**3** (IC₅₀ 15 nM for *e.e.* 95%) against colon cancer cells (HCT-116) prompted us to investigate the resolution of *rac*-**3**-Ac using

different LMKR conditions in order to achieve efficient production of (*S*)-**3** in higher enantiomeric purity.

Another approach to obtain lipidic dialkynylcarbinols [24] and lipidic alkynyl-allenylcarbinols [4] is to use a chiral silyl-protected small (C_5) DAC synthon, such as **5**, as versatile precursor (Fig. 2). Attempts at resolving the TBDMS derivative *rac*-**5**-TBDMS through LMKR by an acylation approach were first performed using lipases from *Pseudomonas fluorescens* and *Candida rugosa* [24]. Nevertheless, the best result achieved was still modest, with both moderate values of conversion ($c = 35\%$) and enantiomeric excesses (*e.e.*_p 96%; *e.e.*_s 71%). The TIPS analogue *rac*-**5**-TIPS was also subjected to LMKR, *via* an acylation approach, using CAL-B immobilized on acrylic resin as enzyme [3]. In this case, the resolution achieved 31% of conversion and the desired (*S*)-**5**-TIPS was obtained in only 42% *e.e.* [3,4].

Fig. 2. Chiral silylated "small" (C_5) DACs **5**, that can be used as key precursors for the syntheses of various chiral lipidic alkynylcarbinols (LACs).

The aforementioned results show that the development of biocatalytic methodologies for producing enantiopure LACs in addition to the small chain silyl-protected dialkynylcarbinol building blocks remains a synthetically relevant challenge. Accordingly, the main objective of the work was to produce both (*R*)- and (*S*)-enantiomers in their enantiopure/enantiorich forms and. Evaluation of different lipases (free and immobilized forms) in the LMKR (via hydrolysis) of the LACs **2-4** as well as that of the high added-value TIPS-protected precursor **5** was performed. Commercially

available lipases from *C. antarctica* type B, *T. lanuginosus*, *R. oryzae*, *P. fluorescens*, *B. cepacia* and *R. miehei* were used in immobilized forms, besides unsupported lipases from *P. fluorescens*, *P. camemberti*, *R. niveus*, *M. javanicus*, *A. niger*, Porcine pancreas lipase and *C. rugosa*. Additionally, immobilized esterase from hog liver was investigated. It is highlighted the use of immobilized enzymes as a way of tuning enzyme property in the biocatalytic process.

2. Experimental

2.1. Enzymes

Commercially available lipases were used as follows: (i) Immobilized lipases: *Candida antarctica* lipase type B immobilized on acrylic resin (CAL-B, 7,300.0 U/g), *Thermomyces lanuginosus* lipase immobilized on imobead-150 (TLL, 250.0 U/g), *Rhizopus oryzae* lipase immobilized on imobead-150 (ROL, 340.0 U/g), *Pseudomonas fluorescens* lipase immobilized on imobead-150 (PFL, ≥ 600 U/g), *Pseudomonas fluorescens* lipase immobilized in Sol-Gel (AK, ≥ 30 U/g), *Pseudomonas fluorescens* lipase immobilized in Sol-Gel on pumice (AK, ≥ 8.0 U/g) and Amano lipase PS from *Burkholderia cepacia* immobilized on diatomaceous earth (PS-IM, ≥ 500 U/g) were acquired from Sigma-Aldrich®, *Rhizomucor miehei* lipase immobilized in anionic resin (RML, 150.0 U/g) was purchased from Novozymes®. (ii) Unsupported lipase preparations: *Pseudomonas fluorescens* lipase (AK, 22,100.0 U/g), *Penicillium camemberti* lipase (G, 50.0 U/g), lipase from *Rhizopus niveus* (RNL, ≥ 1.5 U/mg), Amano lipase from *Mucor javanicus* (MJL, $\geq 10,000$ U/g) and lipase from *Aspergillus niger* (ANL, 200 U/g) were acquired from Sigma-Aldrich®. Porcine pancreas lipase (PPL, 46.0 U/g solid), and *Candida rugosa* lipase (CRL, 1.4 U/g) were obtained from Sigma-

Aldrich[®]. (iii) Esterase, immobilized in Eupergit[®] C from hog liver (~200 U/g) was obtained from Sigma-Aldrich[®].

2.2. Chemical materials

Chemical reagents were purchased from different commercial sources and used without further purification. Acetone, *n*-butanol, heptane, hexane and dichloromethane (DCM) were acquired from Biograde[®]. Tetrahydrofuran (THF) and acetonitrile (MeCN), HPLC grade, were purchased from TEDIA[®]. Acetic anhydride (Ac₂O) was acquired from Vetec[®]. Anhydrous sodium sulfate (Na₂SO₄) and 4-(*N,N*-dimethylamino)pyridine (DMAP) were acquired from Sigma-Aldrich[®]. Analytical TLC analyses were performed on aluminum sheets pre-coated with silica gel 60 F254 (0.2 mm thick) from Merck[®]. Flash chromatography using eluting mixtures of DCM, hexane, petroleum ether (PE) or ethyl acetate (EtOAc), was performed using silica gel 60 (230-240 mesh) from Biograde[®]. Except for *rac*-4, all *rac*-alcohols (**2**, **3** and **5**) and *rac*-**2**-Ac and *rac*-**3**-Ac were prepared as previously reported [1,5,25].

2.3. Analyses

¹H and ¹³C NMR spectra were obtained using Spectrometer Bruker model Avance 300, operating at frequency of 300 MHz for ¹H and frequency of 75 MHz for ¹³C. The chemical shifts are given in delta (δ) values (positive at low field), and the absolute values of coupling constants (*J*) in Hertz (Hz). High-resolution mass spectra (HRMS) were performed on a Waters GCT Premier spectrometer. Optical rotations were measured on a Jasco P-2000 polarimeter. [α]_D values are given in deg.dm⁻¹.cm³.g⁻¹.

2.3.1. Gas-chromatography (GC-FID) analyses of *rac*-alcohols and *rac*-acetates

Gas chromatography (GC-DIC) analyses were carried out on a Shimadzu chromatograph model GC 2010, with a DIC detector, using a self-injector of the brand Shimadzu, model AOC-20i. The mobile phase used was nitrogen gas at 0.5 bar.

For the stationary phase, a CP-ChiraSil-DEX CB (25 m x 0.25 mm I.D.) chiral column was used for the separation of the enantiomers:

- ✓ *rac-2-Ac* – Injector temperature: 250 °C; Pressure 110 kPa; Column flow: 9.2 (nitrogen); Detector temperature: 220 °C; Temperature Ramp: 155 °C (30 min.); 0.1 °C/min. up to 190 °C and 5 °C/min. up to 220 °C; Retention times were: for (*R*)-**2-Ac**, 65.43 min; for (*S*)-**2-Ac**, 64.29 min.
- ✓ *rac-3-Ac* – Injector temperature: 220 °C; Pressure 110 kPa; Column flow: 9.8 (nitrogen); Detector temperature: 230 °C; Temperature Ramp: 160 °C (10 min.); 0.1 °C/min. up to 162 °C and 0.01 °C/min. up to 165°C. Retention times were: for (*R*)-**3-Ac**, 90.05 min.; for (*S*)-**3-Ac**, 88.09 min.
- ✓ *rac-4*; *rac-4-Ac* - Injector Temperature: 250 °C; Pressure 100 kPa; Column flow: 4.0 (nitrogen); Detector temperature: 220 °C; Temperature Ramp: 155 °C (30 min.); 0.1 °C/min. up to 190 °C and 5 °C/min. up to 220 °C. Retention times were: for (*S*)-**4**, 51.86 min; for (*R*)-**4**, 53.94 min; for (*S*)-**4-Ac**, 65.83 min; for (*R*)-**4-Ac**, 68.40 min.
- ✓ *rac-5*; *rac-5-Ac* - Injector Temperature: 200 °C; Pressure 100 kPa; Column flow: 1.19 (nitrogen); Detector temperature: 250 °C; Temperature Ramp: 120 °C; 0.3 °C/min. to 135 °C (3 min) and 0.5 °C/min. to 155 °C (5 min). Retention times were: for (*R*)-**5**, 40.13 min; for (*S*)-**5**, 39.43 min, for (*R*)-**5-Ac**, 30.44 min; for (*S*)-**5-Ac**, 28.72 min.

Additionally, the Rt[®]bDEXcst (30 m x 0.25 mm I.D.) chiral column was used to separate the following enantiomers:

- ✓ *rac-2* – Injector temperature: 210 °C; Pressure 75 kPa; Column flow: 0.95 (nitrogen); Detector temperature: 220 °C; Temperature Ramp: 175 °C (3 min.); 1 °C / min. 190 °C; 5 °C/min. 220 °C. Retention times were: for (*R*)- **2**, 16.94 min; for (*S*)- **2**, 17.98 min.
- ✓ *rac-3* – Injector temperature: 250 °C; Pressure 110 kPa; Column flow: 4.6 (nitrogen); Detector temperature: 300 °C; Temperature Ramp: 198 °C (10 min), 2 °C/min. to 210 °C (10 min). Retention times were: for (*R*)-**3**, 26.99 min; for (*S*)-**3**, 25.84 min.

2.4. Calculation of enantiomeric excess, conversion and enantioselectivity

Enantiomeric excess (*e.e.*), conversion (*c*) and enantioselectivity (*E* = enantiomeric ratio [26] of the LMKR were calculated as previously reported [27], using the following equations.

Enantiomeric excess (*e.e. %*), where A represents the chromatographic peak area of the major enantiomer and B denotes the chromatographic peak area of the minor enantiomer.

$$e.e._s = \frac{A-B}{A+B} \times 100 \quad (1)$$

$$e.e._p = \frac{A-B}{A+B} \times 100 \quad (2)$$

Conversion (*c*), where *e.e._s* and *e.e._p* are the enantiomeric excesses of the substrate and product, respectively.

$$c = \frac{e.e._s}{e.e._s + e.e._p} \quad (3)$$

Enantiomeric ratio (*E*)

$$E = \frac{\ln[1 - c(1 + e.e._p)]}{\ln[1 - c(1 - e.e._p)]} \quad (4)$$

2.5. Synthesis of *rac-4*

Ethynyl magnesium bromide (10 mL, 0.5 M in THF, 5.0 mmol, 1.2 equiv) was added to a solution of pentadecanal (941 mg, 4.16 mmol) in THF (43 mL) at 0°C and stirred for 30 min at this temperature, then for 1 h at room temperature. The reaction mixture was treated with a saturated NH₄Cl solution (20 mL) and was extracted with Et₂O (2x20 mL). The combined organic extracts were dried over MgSO₄. Purification by flash column chromatography (Petroleum ether:EtOAc, 95:5) gave 958 mg (91% yield) of propargylic alcohol **4** as a white solid. All analyses were in agreement with the data reported in the literature [2].

2.6. Synthesis of racemic acetates (*rac-4-Ac* and *rac-5-Ac*)

DMAP (24 mg, 0.20 mmol) and acetic anhydride (190 µL, 2.0 mmol) were added to a solution of the *rac*-alcohol (**4** or **5**; 50 mg) in DCM (2.0 mL). The reaction mixture was stirred at room temperature during 10 min, then the solvent was evaporated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel, using hexane:DCM (1:1) as eluents, to afford the desired *rac*-acetate (*rac-4-Ac*: 74%; *rac-5-Ac*: 90%).

2.7. LMKR of *rac-2-Ac* without co-solvent

A suspension of *rac*-heptadeca-1,4-diyn-3-yl acetate (*rac-2-Ac*: 4.4 mg, 0.015 mmol) and 4.4 mg of lipase from CAL-B in 152 µL of phosphate buffer 100 mM (pH 7.0) was shaken at 30 °C and 250 rpm for 48 h. After this time, the products were extracted with EtOAc (3 x 200 µL) and the organic phases were combined and dried with anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The crude product was analyzed by GC-FID and the chromatogram revealed (*R*)-**2** (*e.e.* > 99%) and (*S*)-**2-Ac** (*e.e.* > 99%).

2.8. Optimized LMKR of *rac*-3-Ac

A suspension of *rac*-heptadeca-1,4,6-triyn-3-yl acetate (*rac*-3-Ac: 4 mg, 0.014 mmol) and 8 mg of lipase from CAL-B in a mixture of 112 μ L of phosphate buffer 100 mM (pH 7.0) and 28 μ L of MeCN (80/20 v/v) was shaken at 20 °C and 250 rpm for 7 h. After this time, the products were extracted with EtOAc (3 x 200 μ L) and the organic phases were combined and dried with anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The crude product was analyzed by GC-FID and the chromatogram revealed (*R*)-3 (*e.e.* 98%) and (*S*)-3-Ac (*e.e.* 98%).

2.9. LMKR of *rac*-4-Ac

A suspension of *rac*-heptadeca-1-yn-3-yl acetate (*rac*-4-Ac: 20 mg, 0.068 mmol) and 40 mg of lipase from CAL-B in a mixture of 544 μ L of phosphate buffer 100 mM (pH 7.0) and 136 μ L of MeCN (80/20 v/v) was shaken at 25 °C and 250 rpm for 5 h. After this time, the products were extracted with EtOAc (3 x 5.0 mL) and the organic phases were combined and dried with anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using DCM as eluent, yielding 8.2 mg of (3*R*)-heptadeca-1-yn-3-ol ((*R*)-4: 48% yield and *e.e.* 96% and 7.5 mg of (3*S*)-heptadeca-1-yn-3-yl acetate ((*S*)-4-Ac: 37.5% yield and *e.e.* 96%).

2.10. Lipase-mediated hydrolysis of (*S*)-4-Ac

A suspension of (*S*)-4-Ac (7.5 mg, 0.0255 mmol) and 30 mg of lipase from CAL-B in a mixture of 204 μ L of phosphate buffer 100 mM (pH 7.0) and 51 μ L of MeCN (80/20 v/v) was shaken at 40 °C, 250 rpm for 96 h. After this time, the reaction mixture was centrifuged, the supernatant was removed, and the product was extracted with EtOAc (3 x 1.0 mL). The organic phases were combined and dried with anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The crude product was purified by

flash chromatography on silica gel using hexane:DCM (1:1) as eluent and yielded 3.8 mg of (*S*)-**4** (57.5% yield, *e.e.* 96%) and 2.3 mg of the remaining (*S*)-acetate.

2.11. *LMKR of rac-5-Ac*

A suspension of *rac*-(triisopropylsilyl)penta-1,4-diyn-3-yl acetate (*rac-5*: 30 mg, 0.108 mmol) and 40 mg of lipase from TLL in a mixture of 864 μ L of phosphate buffer 100 mM (pH 7.0) and 216 μ L of THF (80/20 v/v) was shaken at 40 °C and 250 rpm for 24 h. After this time, the products were extracted with EtOAc (3 x 5.0 mL) and the organic phases were combined and dried with anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using hexane:DCM (1:1) as eluent, and yielded 14 mg of (*R*)-**5** [41% yield, *e.e.* > 99] and 15 mg of (*S*)-**5-Ac** [48% yield, *e.e.* >99%, $[\alpha]_{\text{D}}^{25} = +36.9$ (*c* 1.35, CHCl₃); Lit. [3]: $[\alpha]_{\text{D}}^{20} = +34.8$ (*c* 13.5, CHCl₃)].

2.12. *Lipase-mediated hydrolysis of (S)-5-Ac*

A suspension of (*S*)-**5-Ac** (29 mg, 0.104 mmol) and 87 mg of lipase from CRL in a mixture of 832 μ L of phosphate buffer 100 mM (pH 7.0) and 208 μ L of MeCN (80/20 v/v) was shaken at 30 °C, 250 rpm for 96 h. After this time, the reaction mixture was centrifuged, the supernatant was removed, and the product was extracted with EtOAc (3 x 1.0 mL). The organic phases were combined and dried with anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using hexane:DCM (1:1) as eluent, and yielded 13 mg of (*S*)-**5** [53% yield, *e.e.* > 99%] and 11.1 mg of the remaining (*S*)-acetate.

2.13. *Chemical acetylation of (S)-5*

Enantiopure (*S*)-**5** (*e.e.* > 99%) was subjected to chemical acetylation reaction following the procedure described at Item 2.6, resulting in the corresponding (*S*)-**5-Ac** with *e.e.* > 99%.

2.14. General procedure for the LMKR of *rac*-**5**-Ac

A suspension of *rac*-(triisopropylsilyl)penta-1,4-diyn-3yl acetate (*rac*-**5**-Ac: 5 mg, 0.018 mmol), 10 mg of lipase in a mixture of 144 μ L of phosphate buffer 100 mM (pH 7.0) and 36 μ L of MeCN (80/20 v/v) was shaken at 30 °C, 250 rpm for 48 h. After this time, the products were extracted with EtOAc (3 x 200 μ L) and the organic phases were combined and dried with anhydrous Na₂SO₄, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using hexane:DCM (1:1) as eluent. This procedure yielded (*S*)-**5**-Ac and (*R*)-**5**, their enantiomeric excess being determined by GC-FID.

rac-heptadeca-1-yn-3yl acetate (*rac*-**4**-Ac): Oil. *R_f*(50% hexane/DCM): 0.60. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 5.32-5.29 (td, *J* 7.0, 0.8 Hz, 2H), 2.41 (d, *J* 2.4 Hz, 1H), 2.05 (s, 3H), 1.74 – 1.72 (m, 2H), 1.26 (m, 24 H), 0.85 (t, *J* 6.8 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): (CO) 170.20, (C $\equiv$ C) 81.57, (C $\equiv$ C) 73.59, (CH-OH) 64.12, (12 CH₂) 34.85, 32.14, 29.91, 29.89, 29.87, 29.83, 29.74, 29.65, 29.57, 29.30, 25.10, 22.87, (COOCH₃) 21.21, (CH₃) 14.33. HRMS *m/z* [M+H]⁺: calcd for C₁₉H₃₄O₂: 294.2559; found: 294.2565.

3. Results and Discussion

3.1. Chemical acetylation of *rac*-alcohols

The first step involved the chemical acetylation of the *rac*-alcohols (*rac*-**3**-**5**) to produce the corresponding *rac*-acetates (*rac*-**3**-**5**-Ac) in quite high yields (79, 74 and 90%, respectively). The appropriate conditions for resolving both *rac*-alcohols and *rac*-acetates on GC-FID were established, and their chromatograms are presented in the

supplementary material. Chemical acetylation of *rac*-**2** and *rac*-**3** were previously reported [5].

3.2. LMKR of *rac*-**2**-Ac without co-solvent: a greener approach

As already mentioned, most of the previously reported lipase-mediated resolutions of LACs were performed *via* acetylation in organic solvent [11–14,16–23]. Our research group has recently reported the LMKR of *rac*-**2**-Ac *via* hydrolysis using MeCN (20% in aqueous buffer medium) as co-solvent [5]. After screening 12 commercially available lipases, this LAC was efficiently resolved (*c* = 50% and *E* > 200) with CAL-B immobilized on acrylic resin and yielded both (*R*)-**2** (39% yield) and (*S*)-**2**-Ac (39% yield) with *e.e.* > 99% (determined by chiral GC analysis). After chemical hydrolysis of (*S*)-**2**-Ac, both the LACs (*R*)- and (*S*)-**2** revealed a relative cytotoxicity against HCT116 cells with an eudismic ratio of 0.013 in favor of (*S*)-**2** (IC₅₀ eutomer = 40 nM *vs.* IC₅₀ distomer = 3000 nM) [5].

Inspired by the Green Chemistry concept, we sought for an even more sustainable process to produce the promising antitumor LAC (*S*)-**2**. In accordance with the Green Chemistry requirements, when the reaction was run in strictly aqueous medium (no co-solvent) (Fig. 3), the same results were achieved (*c* = 50%, *E* > 200, *e.e.*_s and *e.e.*_p > 99%).

Fig. 3. Lipase-mediated kinetic resolution of *rac*-**2**-Ac without a co-solvent.

Therefore, the results herein presented for resolving *rac*-**2**-Ac are the first indication that the co-solvent-free LMKR hydrolytic process can be a viable approach to develop a greener production of enantiopure LACs.

3.3. Optimization of the LMKR of *rac*-**3**-Ac

Influenced by the stunning results obtained in the hydrolysis of the diyne DAC acetate *rac*-**2**-Ac with CAL-B without co-solvent (see Section 3.2), we decided to extend this enzymatic approach to the triyne BAC version *rac*-**3**-Ac (Fig. 1).

As mentioned in the introduction, the LMKR of *rac*-**3**-Ac, mediated by CAL-B immobilized on acrylic resin, was previously performed *via* hydrolysis reaction in the presence of MeCN as co-solvent, at 25 °C for 3 h, to furnish the enantio-enriched (*R*)-**3** (*e.e.*_p 96%) and the remaining acetate (*S*)-**3**-Ac (*e.e.*_s 95%), Table 1 (Entry 1) [5].

Initially, LMKR of *rac*-**3**-Ac was run without co-solvent, keeping all other previously reported reaction conditions (CAL-B, 25 °C and 3 h), Table 1 (Entry 2). In this case, a slight improvement of *e.e.*_p from 96 to 98% was observed, while conversion (50%), *e.e.*_s (95%) and enantioselectivity (*E* > 200) remained unaffected.

In order to improve the *e.e.* values of both product and remaining substrate, we decided to lower the reaction temperature from 25 to 20 °C (Table 1, Entries 3-5), the transformation being monitored until 50% of conversion. At this lower temperature, the best result (*c* = 50%, *E* > 200, *e.e.*_s and *e.e.*_p 98%) was obtained after 7 h of reaction (Table 1, Entry 3). Thus, the optimal conditions for the LMKR *via* hydrolysis of *rac*-**3**-Ac (Fig. 4) was achieved at 20 °C, over 7 h, at a stirring speed of 250 revolutions per minutes (r.p.m.), with an enzyme:substrate ratio of 2:1, in phosphate buffer 100 mM (pH 7.0) (Table 1, Entry 3). It is worth noting that despite the optimized condition presents a lipase:substrate ratio (w:w) of 2:1, which can be considered a high enzyme load, the lipase used is in its immobilized form that allows further reuses.

Table 1. Results from the kinetic resolutions of *rac*-**3**-Ac mediated by CAL-B [lipase:substrate (2:1), stirring speed 250 r.p.m, phosphate buffer 100 mM (pH 7.0)] with or without co-solvent [MeCN (buffer/co-solvent 80:20, v:v)] at varied reaction temperature and time.

Entry	Temperature (°C)	Reaction time (h)	<i>e.e.</i> _p (%)	<i>e.e.</i> _s (%)	<i>c</i> (%)	E
1 ^a	25	3	96	95	49	>200
2		3	98	95	49	>200
3		7	98	98	50	>200
4	20	5	98	91	45	>200
5		3	97	77	44	154

^a With co-solvent

Fig. 4. Optimized LMKR of *rac*-**3**-Ac via hydrolysis to produce (*R*)-**3** and (*S*)-**3**-Ac.

3.4. LMKR of *rac*-**4**-Ac

As opposed to the two other LACs (**2** and **3**), which display one or two triple bonds on each side of the stereogenic center (C-3), compound **4** has a fully saturated lipidic chain (C₁₄) directly linked to the chiral carbinol center. Again, CAL-B was the selected lipase to be used on the kinetic resolution of *rac*-**4**-Ac and the first test was run at 25 °C, using MeCN as co-solvent. Under these conditions, the ideal conversion (*c* = 50%) was achieved in 5 h, resulting in *E* = 194 and both enantiomeric excesses (*e.e.*_p and *e.e.*_s) of 96% (Table 2, Entry 1).

The effect of the co-solvent on both the conversion and selectivity of CAL-B was then investigated in the kinetic resolution of *rac*-4-Ac. The reaction was repeated under the same conditions (25 °C, 5 h), but without co-solvent and, surprisingly, no conversion was observed (Table 2, Entry 2). This result is in marked contrast with the afore-discussed observations made for the DAC substrates **2** and **3**. Therefore, we decided to investigate the influence of other organic co-solvents in the reaction (entries 3-6 in Table 2, and discussion in section 3.5.1).

Table 2. Results from the kinetic resolutions of *rac*-4-Ac mediated by CAL-B [25 °C, 5 h, lipase:substrate (2:1), stirring speed 250 r.p.m], with and without co-solvents (buffer/co-solvent 80:20, v:v).

Entry	Co-solvent	ϵ	$\log P$	<i>e.e.</i> _p (%)	<i>e.e.</i> _s (%)	<i>c</i> (%)	E
1	MeCN	37.5	-0.33	96	96	50	194
2	None	-	-	-	-	0	-
3	Acetone	20.7	-0.23	97	67	41	129
4	THF	7.58	0.50	95	88	48	114
5	<i>n</i> -Butanol	17.5	0.80	81	51	39	16
6	Heptane	1.9	4.00	90	97	52	78

Significant variations of conversion and selectivity with the co-solvent used were observed. Nevertheless, no correlation is evidenced with the corresponding lipophilicity index $\log P$, in spite of its relevance from both the standpoints of the reagent/catalyst (the efficiency of an enzyme is directly dependent on its compatibility with organic solvents *vs* aqueous biological media) and the substrates (LACs are slightly amphiphilic but mainly lipidic). For example, when the reaction was performed with acetone ($\log P = -0.23$) as co-solvent, having a polarity similar to that of MeCN ($\log P = -0.33$), conversion was lower (*c* = 41%), although with similar selectivity (*e.e.*_p 97%; E = 129), Table 2

(Entry 3). Moreover, THF ($\log P = 0.50$) and heptane ($\log P = 4.00$), with quite different $\log P$ values, gave similar conversions (48 and 52%, respectively: Table 2, entries 4 and 6). Nevertheless, a highly lipophilic co-solvent (heptane) reduced significantly the enantioselectivity ($E = 78$, Table 2, entry 6), while a protic aliphatic co-solvent (*n*-butanol) resulted in the lowest value ($E = 16$, Table 2, entry 5). The latest result could be attributed to an interference of hydrogen bonds in the active site of the enzyme.

To the best of our knowledge, there is not a general rule in enzymatic hydrolysis that explains the direct effects of the addition of co-solvent to the aqueous systems on the enzyme conformation. Despite of this, higher enzyme flexibility is normally observed in hydrolysis performed in aqueous media (without co-solvent), where water molecules induce conformational changes in the active site [28–30]. It is suggested that addition of a co-solvent capable of stripping off water molecules from the enzyme active site changes its microenvironment, inducing changes in the enzyme conformation [27]. Besides promoting changes in enzyme conformation, addition of a co-solvent to the reaction aqueous system also influences the mass-transfer of substrate between the aqueous and organic phases due to the presence of an interface. Therefore, in some cases, addition of an organic co-solvents to lipase-catalyzed reactions can improve conversion values [31].

As previously discussed, LMKR of *rac*-**2**-Ac and *rac*-**3**-Ac, both displaying unsaturated long chains, also produced good results in totally aqueous medium (Figs. 3 and 4, respectively). Differently, *rac*-**4**-Ac has its longer chain completely saturated and its LMKR worked only by addition of co-solvent (Table 2). In this case, it may be suggested that the addition of co-solvent is responsible for promoting an adequate enzyme conformation and improving mass-transfer of *rac*-**4**-Ac between the aqueous and organic phases.

Therefore, the optimal conditions for the LMKR (*via* hydrolysis) of *rac*-**4**-Ac (Fig. 5) was achieved using an enzyme:substrate ratio of 2:1, phosphate buffer:MeCN at pH 7.0 (buffer/co-solvent 80:20, v:v), at 25 °C, a stirring speed of 250 r.p.m. over 5 h. Hydrolysis of (*S*)-**4**-Ac with *C. antarctica* B immobilized on acrylic resin produced the enantiopure (*S*)-**4** in 57% yield (Fig. 5).

Fig. 5. Optimized LMKR of *rac*-**4**-Ac *via* hydrolysis to produce (*R*)-**4** and (*S*)-**4**.

3.5. LMKR of *rac*-**5**-Ac

Besides the immobilized pig liver esterase, fifteen commercially available lipases were evaluated in the kinetic resolution of *rac*-(triisopropylsilyl)penta-1,4-diyne-3-ol acetate (*rac*-**5**-Ac) *via* hydrolysis (Fig. 6). The reactions were conducted at 30 °C during 48 h, using a 1:2 substrate:lipase ratio (w:w), in phosphate buffer 100 mM (pH 7.0) with MeCN as a co-solvent (buffer/co-solvent 80:20, v:v), and monitored by chiral GC-FID. The results are summarized in Table 3.

Fig. 6. Enzymatic kinetic resolution of *rac*-**5**-Ac *via* hydrolysis reaction.

Table 3. Kinetic resolution of *rac*-**5**-Ac via hydrolysis using lipases or pig liver esterase: 30 °C, 48 h, 250 r.p.m. stirring speed, 1:2 substrate:lipase ratio (w:w), phosphate buffer 100 mM (pH 7.0) and MeCN as a co-solvent (buffer/co-solvent 80:20, v:v).

Entry	Lipase	<i>e.e._p</i> (%) ^a	<i>e.e._s</i> (%)	<i>c</i> (%) ^b	<i>E</i> ^c
1	<i>P. camemberti</i>	-	-	0	-
2	Porcine pancreas	-	-	0	-
3	<i>R. niveus</i>	-	-	0	-
4	Esterase immob. on Eupergit [®] C from hog liver	-	-	0	-
5	<i>P. fluorescens</i> immob. in sol-gel AK pumice	93	1	1	26
6	<i>C. rugosa</i>	49	61	45	7
7	<i>A. niger</i>	0	0	90	1
8	<i>R. oryzae</i> immobilized on immovead-150	>99	9	9	>200
9	Amano lipase from <i>P. fluorescens</i>	>99	86	46	>200
10 ^a	Amano lipase from <i>P. fluorescens</i>	98	87	47	>200
11	Amano lipase PS-IM	>99	88	47	>200
12 ^a	Amano lipase PS-IM	>99	13	35	>200
13	<i>T. lanuginosus</i> immob. on immovead-150	98	94	49	>200
14 ^a	<i>T. lanuginosus</i> immob. on immovead-150	>99	53	33	>200
15	<i>C. antarctica</i> B immob. on acrylic resin	90	>99	53	>200
16 ^a	<i>C. antarctica</i> B immob. on acrylic resin	96	98	50	>200
17	Amano lipase from <i>M. javanicus</i>	>99	22	18	>200
18	<i>R. miehei</i> immob. on anionic resin	>99	12	11	>200
19	<i>P. fluorescens</i> immob. on immovead 150	>99	45	31	>200
20	<i>P. fluorescens</i> immob. in sol-gel AK	>99	21	17	>200

^a Conditions: 40 °C, 24 h.

In the kinetic resolution of *rac*-**5**-Ac, lipases from *P. camemberti*, porcine pancreas and *R. niveus* were found as inactive as the immobilized pig liver esterase (Table 3, entries 1-4). Additionally, lipases from *P. fluorescens* immobilized in sol-gel AK pumice and *R. oryzae* immobilized on immovead-150 displayed only low activities (Table 3, entries 5 and 8). Among the tested enzymes, lipase from *A. niger* was the most active, reaching 90% conversion (Table 3, entry 7). All other enzymes showed high enantioselectivity (*E* > 200), highlighting Amano lipase from *P. fluorescens*, Amano lipase PS-IM, *T.*

lanuginosus immobilized on imobead-150 and *C. antarctica* B immobilized on acrylic resin with conversion values close to 50% (Table 3, entries 9, 11, 13 and 15). From these, only two Amano lipases (entries 9 and 11) yielded the product with *e.e.* values of > 99%.

In order to reduce the reaction time to 24 h, the LMKR was repeated at higher temperature (40 °C) using Amano lipase from *P. fluorescens*, Amano lipase PS-IM, *T. lanuginosus* immobilized on imobead-150 and *C. antarctica* B immobilized on acrylic resin (Table 3, entries 10, 12, 14 and 16). In this case, CAL-B showed the best conversion value (*c* = 50%), keeping high enantiomeric excesses for both the substrate (*e.e.*_s 98%) and product (*e.e.*_p 96%).

3.5.1. Co-solvent effect

Initially, the LMKR of *rac*-**5**-Ac was repeated at 40 °C and 24 h, replacing MeCN (log *P* = -0.33) by the less polar co-solvent THF (log *P* = 0.49), using the lipases Amano PS from *B. cepacia* immobilized on diatomaceous earth, Amano lipase from *P. fluorescens*, lipase from *T. lanuginosus* immobilized on imobead-150 and lipase from *C. antarctica* B immobilized on acrylic resin (Table 4). Among the tested enzymes, only *T. lanuginosus* immobilized on imobead-150 showed significant improvement on the results when compared with the experiment performed in MeCN as co-solvent, yielding both (*R*)-**5** and (*S*)-**5**-Ac with *e.e.* values > 99%, 50% conversion and *E* > 200 (Table 4, Entry 1).

Table 4. Kinetic resolution of *rac*-**5**-Ac via hydrolysis using lipases: 40 °C, 24 h, 250 r.p.m., 1:2 substrate:lipase ratio (w:w), in phosphate buffer 100 mM (pH 7.0) and THF as co-solvent (buffer/co-solvent 80:20, v:v)].

Entry	Lipase	<i>e.e.</i> _p (%)	<i>e.e.</i> _s (%)	<i>c</i> (%)	<i>E</i>
1	<i>T. lanuginosus</i> immob. on imobead-150	>99	>99	50	>200
2	Amano lipase from <i>P. fluorescens</i>	>99	94	48	>200
3	<i>C. antarctica</i> B immob. on acrylic resin	98	55	36	>200

4	Amano lipase PS-IM	>99	42	30	>200
---	--------------------	-----	----	----	------

Comparison of the results with the lipase from *T. lanuginosus* immobilized on immovead-150 in MeCN and THF clearly revealed the great influence of the organic co-solvent in the LMKR of *rac*-**5**-Ac and prompted us to investigate the same reaction with other co-solvents. Therefore, the reaction was repeated using *n*-butanol, acetone and heptane, besides in the absence of co-solvent (Table 5).

Table 5. Kinetic resolution of *rac*-**5**-Ac via hydrolysis using lipase from *T. lanuginosus* immobilized on immovead-150: 40 °C, 24 h, 250 r.p.m., 1:2 substrate:lipase ratio (w:w), phosphate buffer 100 mM (pH 7.0), with and without co-solvents (buffer/co-solvent 80:20, v:v).

Entry	Co-solvent	log <i>P</i>	<i>e.e.</i> _{<i>p</i>} (%) ^a	<i>e.e.</i> _{<i>s</i>} (%)	<i>c</i> (%)	E
1	THF	0.50	>99	>99	50	>200
2	MeCN	-0.33	>99	53	33	>200
3	Acetone	-0.24	>99	98	49	>200
4	Heptane	4.00	>99	94	49	>200
5	<i>n</i> -Butanol	0.83	>99	72	42	>200
6	None	-	94	97	51	131

Although all co-solvents provided the achievement of higher *e.e.*_{*s*} values and conversions (Table 5, entries 3-5) than MeCN (Table 5, entry 2), it was only in the presence of THF that both the product (*R*)-**5** and remaining substrate (*S*)-**5**-Ac were produced in their enantiomerically pure forms (Table 5, Entry 1). Remarkably, a considerable decrease in the enantioselectivity (E = 131) and *e.e.* values were observed when the LMKR of *rac*-**5**-Ac was carried out in the absence of co-solvent (Table 5, entry 6).

Likewise previously discussed for *rac*-**4**-Ac (Item 3.4), concerning the influence of the co-solvent in the LMKR of this compound, no correlation was evidenced with the corresponding lipophilicity index $\log P$ for the same study with *rac*-**5**-Ac. Even so, it is evident that co-solvent plays an important role in the LMKR of this latter synthon.

3.5.2. Study on enzyme:substrate ratio

LMKR of *rac*-**5**-Ac using the lipase from *T. lanuginosus* immobilized on immovead-150 in THF (co-solvent) was also investigated varying the enzyme:substrate ratio (from 2:1 to 1:1 and 0.5:1; Table 6). In this case, despite keeping the enantioselectivity ($E > 200$) and enantiomeric excess of products ($e.e._p > 99\%$), the reduction of the amount of enzyme resulted in lower $e.e._s$ and conversion values.

Table 6. Kinetic resolution of *rac*-**5**-Ac *via* hydrolysis using lipase from *T. lanuginosus* immobilized on immovead-150: 40 °C, 24 h, 250 r.p.m., THF (co-solvent), phosphate buffer 100 mM at pH $\approx$ 7.0 (buffer/co-solvent 80:20, v:v), with variable enzyme:substrate ratio.

Entry	enzyme:substrate (w:w)	$e.e._p$ (%)	$e.e._s$ (%)	c (%)	E
1	2:1	>99	>99	50	>200
2	1:1	>99	92	48	>200
3	0.5:1	>99	22	18	>200

Thus, the optimal conditions for the LMKR *via* hydrolysis of *rac*-**5**-Ac (Fig. 7) was achieved using *T. lanuginosus* immobilized on immovead-150 at 40 °C, for 24 h, under stirring at 250 r.p.m., with a 2:1 enzyme:substrate ratio, in a THF:phosphate buffer (pH 7.0) mixture (buffer/co-solvent 80:20, v:v). Enzymatic hydrolysis of (*S*)-**5**-Ac with lipase from *C. rugosa* produced the enantiopure (*S*)-**5** in 53% yield (Fig. 7).

Fig. 7. Optimized LMKR of *rac*-**5**-Ac *via* hydrolysis to produce (*R*)-**5** and (*S*)-**5**.

4. Conclusion

In summary, the lipase-mediated kinetic resolutions (LMKR), through hydrolytic approach, of racemic alkynylcarbinol acetates (*rac*-**2-5-Ac**) yielded both (*R*)-alcohols and the resolved (*S*)-acetates in high enantiomeric excesses (*e.e.* 96 - > 99%), ideal conversions (*c* = 50%) and enantioselectivities (*E* > 200). It is pointed out that *C. antarctica* B lipase immobilized on acrylic resin proved to be a robust biocatalyst in the LMKR of *rac*-**2-4-Ac** in buffer medium (pH 7.0). In the case of *rac*-**4-Ac**, the use of MeCN as co-solvent was a key requirement for the process efficiency. It is noteworthy that for *rac*-**5-Ac**, the lipase from *T. lanuginosus* immobilized on imobead-150 was efficient in providing optimal kinetic resolution (*e.e.*_s and *e.e.*_p > 99%; *c* = 50% and *E* > 200) only when 20% THF was added to the buffering medium. Accordingly, the use of co-solvents in some cases can be a powerful strategy for tuning the enantioselectivity of the lipase. Finally, it is highlighted the ability of CAL-B and lipase from *C. rugosa* to hydrolyze (*S*)-**4-Ac** and (*S*)-**5-Ac**, respectively, in the corresponding (*S*)-alcohols, with maintenance of the optical purity. Further studies are needed aiming to decrease the enzyme load used, including tests to verify product inhibition, enzyme activity or its inactivation during the biocatalytic process.

Acknowledgements

The Brazilian authors thank to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for sponsorships of D. K. C. Almeida (doctoral fellow, Process: 147073/2016-2), M. C. de Mattos (PQ-2, Process: 306043/2018-1) and M. C. F. de Oliveira (PQ-2, Process: 307667/2017-0). R.C. is grateful to the CAPES-COFECUB program, his French leader Prof. Vania Bernardes-Génisson and Brazilian leader Prof. Luiz Gonzaga de França Lopes, for exchange facilities with M. C. F. O. through a visit in Fortaleza in 2018.

References

- [1] D. Listunov, V. Maraval, R. Chauvin, Y. Génisson, Nat. Prod. Rep. 32 (2015) 49-75.
- [2] D. El Arfaoui, D. Listunov, I. Fabing, M. Oukessou, C. Frongia, V. Lobjois, A. Samson, F. Ausseil, A. Ben-Tama, E.M. El Hadrami, R. Chauvin, Y. Génisson, ChemMedChem 8 (2013) 1779-86.
- [3] D. Listunov, E. Joly, C. Duhayon, N. Saffon-Merceron, I. Fabing, Y. Génisson, V. Maraval, R. Chauvin, ChemMedChem 13 (2018) 1711-1722.
- [4] D. Listunov, E. Joly, P. Rulhière, H. Gaspard, V. Bernardes-Génisson, Y. Génisson, V. Maraval, R. Chauvin, Synthesis 50 (2018) 3114-3130.
- [5] M. Bourkhis, H. Gaspard, P. Rulhière, D.K.C. de Almeida, D. Listunov, E. Joly, R. Abderrahim, M.C. de Mattos, M.C.F. de Oliveira, V. Maraval, R. Chauvin, Y. Génisson, ChemMedChem 13 (2018) 1124-1130.
- [6] R.N. Patel, Bioorganic Med. Chem. 26 (2018) 1252-1274.
- [7] B. Thangaraj, P.R. Solomon, ChemBioEng Rev. 6 (2019) 157-166.
- [8] C. Ortiz, M.L. Ferreira, O. Barbosa, J.C.S. Dos Santos, R.C. Rodrigues, Á.

- Berenguer-Murcia, L.E. Briand, R. Fernandez-Lafuente, *Catal. Sci. Technol.* 9 (2019) 2380-2420.
- [9] A.C.L.D.M. Carvalho, T.D.S. Fonseca, M.C. De Mattos, M.D.C.F. De Oliveira, T.M.L.G. De Lemos, F. Molinari, D. Romano, I. Serra, *Int. J. Mol. Sci.* 16 (2015) 29682-29716.
- [10] M.P. Pinheiro, N.S. Rios, T. de S. Fonseca, F. de A. Bezerra, E. Rodríguez-Castellón, R. Fernandez-Lafuente, M. Carlos de Mattos, J.C.S. dos Santos, L.R.B. Gonçalves, *Biotechnol. Prog.* 34 (2018) 878-889.
- [11] S.H. Huang, W. Li, L. Chen, J. Xu, R. Hong, *Bioresources and Bioprocessing* 2 (2015) 10.
- [12] J.G. Ferreira, C.R. Princival, D.M. Oliveira, R.X. Nascimento, J.L. Princival, *Org. Biomol. Chem.* 13 (2015) 6458-6462.
- [13] J.G. Hernández, M. Frings, C. Bolm, *ChemCatChem* 8 (2016) 1769-1772.
- [14] T. Saravanan, S. Jana, A. Chadha, *Org. Biomol. Chem.* 12 (2014) 4682-4690.
- [15] M.C. Pischl, C.F. Weise, S. Haseloff, M.A. Müller, A. Pfaltz, C. Schneider, *Chem. - A Eur. J.* 20 (2014) 17360-17374.
- [16] C. Kim, J. Lee, J. Cho, Y. Oh, Y.K. Choi, E. Choi, J. Park, M.J. Kim, *J. Org. Chem.* 78 (2013) 2571-2578.
- [17] P. Chen, X. Zhu, *Journal Molecular Catalysis B: Enzymatic* 97 (2013) 184-188.
- [18] I.M.R.G. Princival, J.G. Ferreira, T.G. Silva, J.S. Aguiar, J.L. Princival, *Bioorg. Med. Chem. Lett.* 26 (2016) 2839-2842.
- [19] C. Raminelli, J. V. Comasseto, L.H. Andrade, A.L.M. Porto, *Tetrahedron: Asymmetry* 15 (2004) 3117-3122.
- [20] J.L. Abad, C. Soldevila, F. Camps, P. Clapés, *J. Org. Chem.* 68 (2003) 5351-5356.
- [21] D. Xu, Z. Li, S. Ma, *Tetrahedron Letters* 44 (2003) 6343-6346.

- [22] S. Ma, B. Wu, X. Jiang, *J. Org. Chem.* 70 (2005) 2588-2593.
- [23] N.P. McLaughlin, E. Butler, P. Evans, N.P. Brunton, A. Koidis, D.K. Rai, *Tetrahedron* 66 (2010) 9681-9687.
- [24] A. Sharma, S. Chattopadhyay, *Tetrahedron Asymmetry* 9 (1998) 2635-2639.
- [25] D. Listunov, C. Billot, E. Joly, I. Fabing, Y. Volovenko, Y. Génisson, V. Maraval, R. Chauvin, *Tetrahedron* 71 (2015) 7920-7930.
- [26] A.J.J. Straathof, J.A. Jongejan, *Enzyme Microb. Technol.* 21 (1997) 559-571.
- [27] G.V. Lima, M.R. da Silva, T. de Sousa Fonseca, L.B. de Lima, M. da C.F. de Oliveira, T.L.G. de Lemos, D. Zampieri, J.C.S. dos Santos, N.S. Rios, L.R.B. Gonçalves, F. Molinari, M.C. de Mattos, *Appl. Catal. A Gen.* 546 (2017) 7-14.
- [28] C.L. Tsou, in: *Ann. N. Y. Acad. Sci.*, New York Academy of Sciences, 1998, pp. 1-8.
- [29] R. V. Rariy, A.M. Klibanov, *Biocatal. Biotransformation* 18 (2000) 401-407.
- [30] J. Broos, *Biocatal. Biotransformation* 20 (2002) 291-295.
- [31] K. Faber, *Biotransformations in Organic Chemistry*, Springer Berlin Heidelberg, 2011.