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## Microwave-Assisted Kinetic Resolution of Homochiral (Z)-Cyclooct-5-ene-1,2-diol and (Z)-2-Acetoxyoct-4-enyl Acetate Using Lipases

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6 *Article*

7 **Microwave-Assisted Kinetic Resolution of Homochiral (Z)-**  
8 **Cyclooct-5-ene-1,2-diol and (Z)-2-Acetoxy-cyclooct-4-enyle**  
9 **Acetate using Lipases**

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18

19 **Abstract:** Over the last decade, the use of biocatalysts has become an attractive alternative  
20 to conventional chemical methods, especially for organic synthesis, due to their unusual  
21 properties. Among these enzymes, lipases are the most widely used, because they are  
22 cheap, easily available, cofactor-free, and have broad substrate specificity. Combined to  
23 microwave heating in non-aqueous medium, recent results suggest that irradiation may  
24 influence the enzyme activity. This Letter reports the benefits of lipases and the microwave  
25 irradiation on the kinetic resolution of racemic homochiral (Z)-cyclooct-5-ene-1,2-diol and  
26 (Z)-2-acetoxy-cyclooct-4-enyle acetate. In order to best achieve the kinetic resolution,  
27 different parameters were studied including the type of lipase, the temperature, the impact  
28 of microwave power compared to conventional heating. Optimization of the reaction  
29 parameters lead to the obtainment of highly enriched or enantiopure diols and diesters in a  
30 clean, efficient and safe way.

31 **Keywords:** biocatalysis; microwave irradiation; lipase; homochiral diols; kinetic  
32 resolution

33

34 **1. Introduction**

35 Enantiomerically pure vicinal diols are versatile chemical scaffolds for the production of flavors  
36 and fragrances. As part of our work on the enantioselective synthesis of methyl jasmonate derivatives  
37 from optically active bicyclo[3.3.0]octane derivatives by transannular cyclization, we first needed to  
38 prepare enantiopure homochiral (1*R*,2*R*) and (1*S*,2*S*) 5-cyclooctene-1,2-diols. In recent years, the

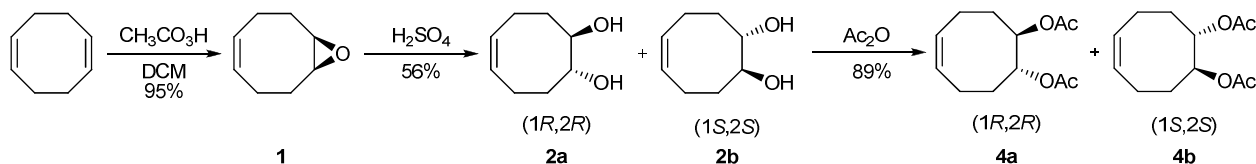
1 development of biocatalysts for organic synthesis has become an attractive alternative to conventional  
 2 chemical methods. Among those biocatalysts, lipases have become very popular in both academic and  
 3 industrial sectors because they are inexpensive, easily available, cofactor free and have a broad  
 4 substrate specificity [1]. We decided to focus our interest on microwave-assisted lipase- mediated  
 5 kinetic resolution involving CaLB (lipase B from *Candida antarctica*) or PS (*Pseudomonas cepacia*)-  
 6 catalyzed acetylation of diol. The use of microwave irradiation in biocatalysis can enhance the enzyme  
 7 activity, for example in resolution reaction, in specific oxido-reduction reaction or hydrolysis.  
 8 Combined to non-aqueous medium, recent results suggest that microwave irradiation can have also  
 9 influence the enzyme stability and activity, in addition to altering/enhancing reaction rates and/or  
 10 enantioselectivities, called non thermal microwave effects [2-8]. However, the exact role of microwave  
 11 irradiation on enzymes still remains unraveled [9]. To have a better comprehension of the influence of  
 12 the microwave irradiation on a biocatalyst, we decided to compare the lipase-catalyzed resolution of a  
 13 difunctionalized compound, under conventional and microwave irradiation heating We herein report  
 14 our studies on lipase resolution of homochiral (1*R*,2*R*) and (1*S*,2*S*) 5-cyclooctene-1,2-diols (**2**) and  
 15 their diesters (**4**), by varying the irradiation power and reaction temperature

16

## 17 2. Results and Discussion

18 In the context of the growing general interest for reducing energy costs, heating chemical reactions  
 19 under microwave irradiation is a useful approach for achieving higher reaction kinetics and  
 20 synthesizing cleaner products [10-13]. In combination with microwave technology and lipases, we  
 21 wished to examine the synthesis of chiral cyclooctenic diols and diesters starting from cycloocta-1,5-  
 22 diene using microwave technology. Rac-diol (**2**) and rac-diacetate (**4**) were initially prepared from  
 23 cycloocta-1,5-diene in a three-steps sequence including epoxidation, ring opening with aqueous  
 24 sulfuric acid followed by acetylation with acetic anhydride [14-15].

25

26  
27

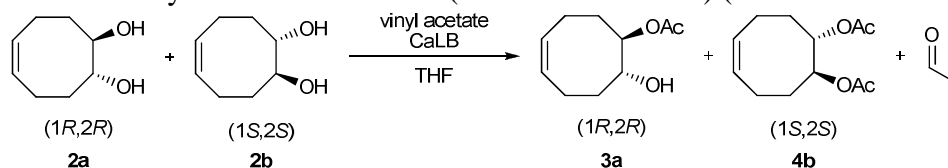
28 **Scheme 1.** Epoxidation and hydrolysis of cyclo-octa-1,5-diene leading to racemic diols (**2**) and  
 29 acetylation in diacetate (**4**)

30

31 The preparation of optically active 5-cyclooctene-1,2-diol **2** was first conceived by using microwave-  
 32 assisted lipase-catalyzed desymmetrization of *meso*-symmetric diol **2** using vinyl acetate as the  
 33 acylating agent in THF as solvent rather than isooctane or 2-methylbutan-2-ol, that gave lower results.  
 34 In order to perform the reaction under microwave irradiation, we decided to choose thermostable  
 35 immobilized lipases capable of withstanding microwave irradiation: Novozyme 435® (CaLB  
 36 immobilized on acrylic resin) and PS-D (*Pseudomonas cepacia* immobilized on diatomite). The goal  
 37 was to obtain an enantioselective enhancement with immobilized lipases, as previous studies  
 38 performed in our laboratory showed that resolution of rac-diol **2** with free *Pseudomonas cepacia* lipase  
 39 at 55°C in THF during 7 days afforded with a good conversion rac-monoacetate **3** (47%, 0% ee) and  
 40 rac-diol **2** (51%, 0% ee) but with no selectivity at all. Indeed, by immobilization of enzymes onto solid  
 41 supports, enhanced enzyme activity, selectivity, stability, and reusability in organic media may be  
 42 achieved compared to the native enzyme [16].

43

1 Performed at 35°C under conventional heating immobilized CaLB-enzymatic acylation of rac-diol (**2**)  
 2 afforded after 3 weeks 28% of (1*R*,2*R*)-monoacetate **3a** (42% ee) and 6 % of (1*S*,2*S*)-diacetate **4b** with  
 3 an excellent 99% ee. A higher temperature (50°C) led after 7 days to modest yields of monoacetate **3a**  
 4 and a real enhancement of yield for diacetate **4b** (20% with ee >99%) (Scheme 2 and Table 1).

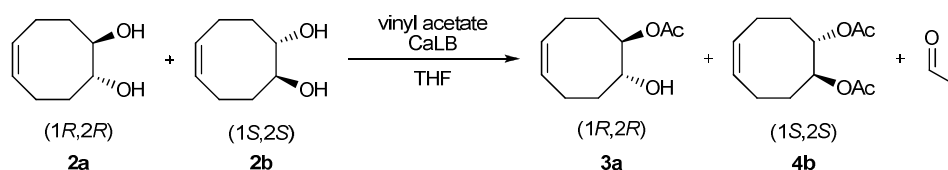


5  
 6 **Scheme 2.** : Enantioselective acetylation of diol (**2**) using immobilized CaLB lipase and vinyl acetate  
 7 by classical heating at various temperatures  
 8

| Heating mode | Temperature (°C) | Time     | Monoacetate <b>3a</b> |        | Diacetate <b>4b</b> |        |
|--------------|------------------|----------|-----------------------|--------|---------------------|--------|
|              |                  |          | yield (%)             | ee (%) | yield (%)           | ee (%) |
| Classical    | 35               | 3 weeks  | 28                    | 42     | 6                   | >99    |
| Classical    | 50               | 7 days   | 30                    | 50     | 20                  | >99    |
| Classical    | 50               | 14 hours | traces                |        | -                   |        |

9 **Table 1.** : Enantioselective acetylation of diol (**2**) using immobilized CaLB lipase and vinyl acetate by  
 10 classical heating at various temperatures

11  
 12 Under microwave irradiation at 35°C (5W), racemic diol **2** proceeded to (1*R*,2*R*)-monoacetate **3a**  
 13 (32%, 45% ee), trace amounts of (1*S*,2*S*)-diacetate **4b** (5%, >99% ee) and 65% of diol **2** (23% ee). In  
 14 order to study the influence of the irradiation power on the biocatalytic media, we decided to apply a  
 15 constant power (up to 300W) while maintaining the temperature at 35 °C by using a microwave oven  
 16 combined with a Cool mate®. We noticed an enhancement of the yield and the enantiomeric ratio of  
 17 (1*R*,2*R*)-monoacetate **3a** (42%, 67% ee) and diol **2** (51%, 50% ee) and only 2% of diacetate **4b** (99%  
 18 ee). At 50°C (10W, 14 hours) the same reaction yielded only esters: 58% of (1*R*,2*R*)-monoacetate **3a**  
 19 with 55% ee and 37% of diacetate **4b** (99% ee). A higher temperature (80°C, 40W, 14h) afforded a  
 20 decrease of diacetate yield with a lower enantiomeric excess (30% of **4b** with 94% ee) (Scheme 3 and  
 21 Table 2).



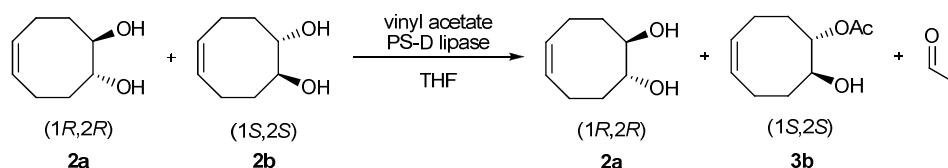
23  
 24 **Scheme 3.** : Enantioselective acetylation of diol **2** using immobilized CaLB with vinyl acetate lipase  
 25 under microwave irradiation

| Power (W) | Incubation time (h) | Temperature (°C) | Monoacetate <b>3a</b> |        | Diacetate <b>4b</b> |        |
|-----------|---------------------|------------------|-----------------------|--------|---------------------|--------|
|           |                     |                  | yield (%)             | ee (%) | yield (%)           | ee (%) |
| 5*        | 14                  | 35               | 32                    | 45     | 5                   | 99     |
| 10*       | 14                  | 50               | 58                    | 55     | 37                  | 99     |
| 20*       | 14                  | 80               | 55                    | 57     | 30                  | 94     |
| 40*       | 14                  | 100              | -                     | -      | -                   | -      |
| 300*      | 14                  | 35               | 42                    | 67     | 2                   | >99    |

**Table 2** : Enantioselective acetylation of diol **2** using immobilized CaLB lipase under microwave irradiation in a large range of temperatures. \*Constant power when the temperature is reached (max :300 W and 2 minutes) \*\* Using Cool mate

These results suggest that, at higher temperature (100°C), there is a loss of enzyme activity due to its denaturation. At 80°C, it was shown that the immobilized-CalB (Novozym 435) is very stable, keeping 90% of its activity after an incubation in diphenylether for 24h[17]. The irradiation power appears to display a key role in enzyme properties, and best enhancement of monoacetate yield and ee was observed at 35°C with 300W. Compared to conventional heating, the microwave irradiation as already shown to lead to much better conversion and enantiomeric excesses, in the case of lipase-catalyzed kinetic resolution of racemic secondary alcohols through acetylation.[18]

At 50°C under conventional heating, enzyme-catalyzed acylation of rac-diol **2** with immobilized PS-D provided both (1*S*,2*S*)-monoacetate **3b** and (1*R*,2*R*)-diol **2a** in poor enantiomeric purity (respectively 45% ee for **3b** and 3% ee for **2a**) and poor conversion (6%). At higher temperatures (80, 100°C), PS-D proved to be ineffective and lost all enzymatic activity. Under microwave irradiation at 50°C (15W), lipase resolution of racemic diol **2** afforded (1*S*,2*S*)-monoacetate **3b** (41%, 50% ee) and (1*R*,2*R*)-diol **2a** (57% , 35% ee). The microwave irradiation method gave a higher conversion compared to conventional heating. At 80°C (35W), the reaction yielded 12% of **3b** (35% ee) and 66% of diol with no selectivity (5% ee) (Scheme 4 and Table 3).



**Scheme 4.** : Acetylation of diol **2** using immobilized PS lipase (PS-D)

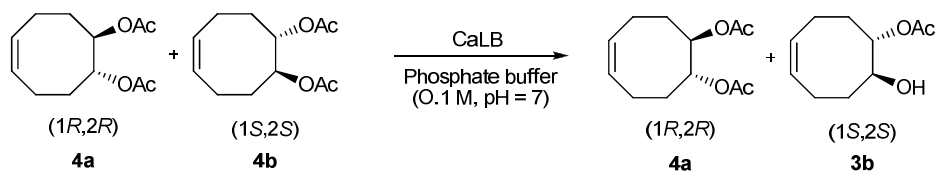
| Heating mode | Temperature (°C)           | Diol <b>2a</b><br>ee (%) | Monoacetate <b>3b</b> |        |
|--------------|----------------------------|--------------------------|-----------------------|--------|
|              |                            |                          | yield (%)             | ee (%) |
| Classical    | 50                         | 3                        | 6                     | 45     |
|              | 80                         | -                        | -                     | -      |
|              | 100                        | -                        | -                     | -      |
| Microwave    | 50 (15W*)                  | 35                       | 41                    | 50     |
|              | 80 (35W*)                  | 5                        | 12                    | 35     |
|              | 100 (closed vessel) (40W*) | -                        | -                     | -      |

**Table 3:** Acetylation of diol **2** using immobilized PS lipase (PS-D) (incubation time :14h). Effect of the heating mode and temperature on the conversion and kinetic resolution of rac-**2** into diol **2a** and monoacetate **3b**. \*Constant power when the temperature is reached (max :300 W and 2 minutes).

Noteworthy, PS-D exhibited a reverse enantiopreference for the monoacetate (1*S*,2*S*) compared to CaLB.

Following these preliminary results, we then investigated the lipase-catalyzed kinetic hydrolytic resolution of rac-diacetate **4** according to Suemune procedure [19]. Instead of using PFL (*Pseudomonas fluorescens* lipase), we used immobilized CaLB and PS-D in order to perform later the

1 reaction under microwave. Performed at various temperature (35, 50 and 80°C) under conventional  
 2 heating CaLB-catalyzed hydrolysis of rac-diacetate (**4**) in phosphate buffer (0.1M, pH 7.0) led only to  
 3 traces of monoacetate at 50°C. Compared to conventional heating, the microwave irradiation at 50°C.  
 4 during 14 hours led to a higher conversion (20%) with excellent enantiomeric excess for monoacetate  
 5 (ee: 97% for **3b** (1*S*,2*S*)) besides diacetate **4a** (ee: 34%) (Scheme 5 and Table 4).



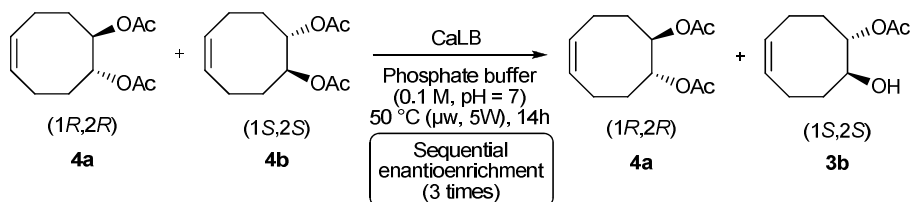
9  
10 **Scheme 5.** : Hydrolysis of diacetate **4** using immobilized CaLB lipase in phosphate buffer (0.1 M, pH  
 11 7). For detailed conversion and ee, see table 4.

12

| Heating mode | Temp. (°C) | Conversion | Diacetate <b>4a</b><br>ee (%) | Monoacetate <b>3b</b><br>ee (%) |
|--------------|------------|------------|-------------------------------|---------------------------------|
| Classical    | 35         | -          | -                             | -                               |
|              | 50         | Traces     | -                             | -                               |
|              | 80         | -          | -                             | -                               |
| Microwave    | 35 (1-5W*) | -          | -                             | -                               |
|              | 50 (5W*)   | 20%        | 34<br>[α] <sub>D</sub> = +29° | 97<br>[α] <sub>D</sub> = +6°    |
|              | 80 (10W*)  | -          | -                             | -                               |

13 **Table 4** : Hydrolysis of rac-diacetate **4** using immobilized CaLB lipase (incubation time : 14h). Effect  
 14 of the heating mode and temperature on the conversion and kinetic resolution of rac-**4** into diacetate **4a**  
 15 and monoacetate **3b**. \*Constant power when the temperature is reached (max :300 W and 2 minutes).

16  
17 Given the promising results obtained during the first enantioenrichment of rac-**4** with CaLB, the  
 18 recovered (1*R*,2*R*)-diacetate **4a** with 34% ee was submitted twice to the same enzymatic hydrolysis  
 19 under microwave irradiation (2 x 14h, 50°C, 5W). Enantiopure (1*R*,2*R*)-diacetate **4a** was obtained in  
 20 49% yield from rac-**4** (Scheme 6 and Table 5).



22  
23  
24 **Scheme 6.** : Sequential enantioenrichment of diacetate **4a**.

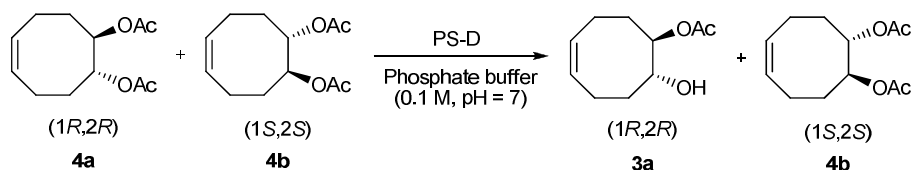
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|                            | Monoacetate <b>3b</b> |        |                  | Diacetate <b>4a</b> |        |                  |
|----------------------------|-----------------------|--------|------------------|---------------------|--------|------------------|
|                            | yield (%)             | ee (%) | [α] <sub>D</sub> | yield (%)           | ee (%) | [α] <sub>D</sub> |
| 1 <sup>st</sup> enrichment | 20                    | 97     | +5,8°            | 80                  | 34     | +25°             |

|                            |    |    |       |    |     |      |
|----------------------------|----|----|-------|----|-----|------|
| 2nd enrichment             | 41 | 98 | +6°   | 57 | 72  | +57° |
| 3 <sup>rd</sup> enrichment | 51 | 97 | +5,7° | 49 | >99 | +81° |

**Table 5** : Sequential enantioenrichment of diacetate **4a**.

CaLB and the PS-D were finally compared for their ability to carry out an effective kinetic resolution of rac-**4** diester. The hydrolysis using PS-D was conducted in the same manner. In classical conditions whatever the temperature (35, 50, 80°C) and the reaction time (14h, 7 days), in no case conversion to monoacetate **3** was observed. Interestingly, under microwave irradiation at 50°C (14h), rac-**4** was desymmetrized into (1*R*,2*R*) monoacetate **3a** with 81% ee and (1*S*,2*S*)-diacetate **4b** (28% ee), highlighting a non-thermal effect. (Scheme 7 and Table 6)

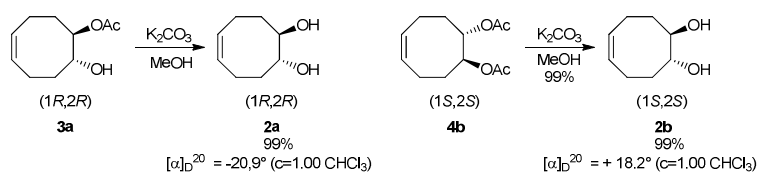
**Scheme 7** : Hydrolysis of diacetate **4** using PS-D lipase in phosphate buffer (0.1M, pH 7)

| Mode of heating | Temp. (°C) | Conversion | Monoacetate <b>3a</b><br>ee (%) | Diacetate <b>4b</b><br>ee (%) |
|-----------------|------------|------------|---------------------------------|-------------------------------|
| Classical       | 35         | -          | -                               | -                             |
|                 | 50         | -          | -                               | -                             |
|                 | 80         | -          | -                               | -                             |
| Microwave       | 35 (1-5W*) | -          | -                               | -                             |
|                 | 50 (5W*)   | 10%        | 81<br>[α] <sub>D</sub> = -4°    | 28<br>[α] <sub>D</sub> = -24° |
|                 | 80 (10W*)  | -          | -                               | -                             |

**Table 6** : Hydrolysis of diacetate **4** using PS-D lipase (incubation time : 14h). Effect of the heating mode and temperature on the conversion and kinetic resolution of rac-**4** into monoacetate **3a** and diester **4b**. \*Constant power when the temperature is reached (max :300 W and 2 minutes).

The microwave-assisted hydrolysis seems to enhance the enzyme activity compared to classical heating. Comparing the results with Suemune et al. [19], the CaLB seems to be a convenient enzyme in microwave irradiation resulting in the obtention of enantiopure monoacetate **3a** in a quick and clean way. However, the same reaction with PS-D needed to be optimized to obtain a better ee.

Finally, the isolated enantiopure monoacetates **3a**, **3b** and diacetates **4a** and **4b** were then quantitatively converted into (1*R*,2*R*)-diol **2a** and (1*S*,2*S*)-diol **2b** with >99% ee by methanolysis[7] (Scheme 8).



1 **Scheme 8.** : Isolation of enantiopure diols **2a** and **2b** by methanolysis of monoacetate **3a** and diacetate  
2 **4b**.

### 3 **3. Experimental Section**

4 Lipases from *Pseudomonas cepacia* (immobilized on diatom MKBB3465, 500 PLU<sup>-1</sup>) and *Candida*  
5 *antarctica* (immobilized on acrylic resin 077K1155, 10 000 PLU<sup>-1</sup> Novozym 435<sup>®</sup> or free form) were  
6 purchased from Sigma Aldrich. All chemicals were purchased from Sigma Aldrich and were used  
7 without further purification except in the case of vinyl acetate which was used after fresh distillation.

8 IR spectra were recorded on a Perkin–Elmer Spectrum 100 IRFT-ATR instrument. <sup>1</sup>H and <sup>13</sup>C NMR  
9 were recorded on a JEOL JNM LA400 (400 MHz) spectrometer. Chemical shifts (δ) are reported in  
10 parts per million (ppm) downfield from tetramethylsilane (TMS) which was used as internal standard.  
11 Coupling constants J are given in Hz. The high resolution mass spectra (HRMS) were recorded on a  
12 Varian MAT311 spectrometer in the Centre Régional de Mesures Physiques de l’Ouest (CRMPO),  
13 Université de Rennes. Analytical thin layer chromatography (TLC) was performed on Merck Kieselgel  
14 60 F254 aluminum packed plates.

15 Enantiomeric ratios were determined by gas chromatography (Agilent 7890A) equipped with an  
16 autosampler (7688B) and flame ionization detector (FID). For the experiment, a CP-Chirasil-Dex  
17 (0.25mm x 25m x 0.25µm, Chromopack) column was used. The injector and the detector were kept at  
18 180°C. Nitrogen was used as gas carrier at a flow of 1.5 mL/min. Hydrogen, air and nitrogen were  
19 supplied to the FID at 35 mL.min<sup>-1</sup>, 350 mL.min<sup>-1</sup> and 25 mL.min<sup>-1</sup> respectively. The products are  
20 analyzed at 110°C. The enantiomeric ratio and yields were calculated by taking the average of two  
21 duplicates, with an error <2%.

22 High performance liquid chromatography (HPLC) was carried out in a Waters 600s combined with  
23 an autosampler (Waters 717 plus). The Chiralpak–AD column (Amylose tris-(3,5-  
24 dimethylphenylcarbamate) coated on 10 µm silica-gel, Daicel Chemical, 250 x 4.6 mm) at a flowrate  
25 (n-Heptane/EtOH, 9/1) of 1 mL.min<sup>-1</sup> is used. Products were analyzed using a differential  
26 refractometer (Waters 410)

27 Optical rotations were measured on a Perkin Elmer 341 polarimeter.

28 Microwave reactions were conducted using a CEM Discover<sup>®</sup>, mode operating systems working at  
29 2.45 GHz, with a programmable power ranging from 1 to 300W. The microwave can be equipped with  
30 a Cool mate<sup>®</sup> system allowing reactions with high power input (up to 300 W) while maintaining the  
31 reaction media at 35 °C. This system is cooled by a cryogenic fluid (Galden HT-55<sup>®</sup>).

32 In closed vessel mode, microwave irradiation experiments were carried out using a single-mode  
33 microwave instrument (Initiator, Biotage) working at 2.45 GHz, with a power programmable from 1 to  
34 450 W (0-20 bars).

35

#### 36 *(Z)*-(1*S*,8*R*)-9-oxa-bicyclo[6.1.0]non-4-ene (**1**)

37 Under inert atmosphere, to a solution of cycloocta-1,5-diene (20 g, 0.161 mol) and sodium carbonate  
38 (117.6 g, 1.11 mol) in dichloromethane (560 mL) stirred at 0°C was added dropwise a solution of  
39 peracetic acid (42.7 mL, 0.222 mol) in dichloromethane (500 mL) during 2 hours. The mixture was  
40 stirred for 8 hours at 0°C and 12 hours at room temperature. The mixture was quenched with 500mL of



1 water and extracted with (3x250 mL) of dichloromethane. The organic layers were dried with  
2 magnesium sulfate anhydrous and concentrated under reduced pressure. The crude residue was  
3 purified by column chromatography (silica gel, petroleum ether/ethyl acetate 90/10) to provide the (Z)-  
4 (1*S*,8*R*)-9-oxa-bicyclo[6.1.0]non-4-ene **1** (5.697g, 67% yield) as a colorless oil;  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3003,  
5 2906, 2887, 1655, 1445, 1428, 1228, 1669, 1099, 1039, 934, 762, 743;  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ) 5.56–  
6 5.60 (2H, m,  $\text{CH}=\text{CH}$ ), 3.00–3.02 (2H, m,  $\text{CHCH}$ ), 2.41–2.47 (2H, m,  $\text{CH}_2$ ), 2.08–2.15 (2H, m,  $\text{CH}_2$ ),  
7 1.98–2.17 (4H, m,  $2\times\text{CH}_2$ );  $\delta_{\text{C}}$  (100 MHz,  $\text{CDCl}_3$ ) 128.6, 56.2, 27.8, 23.4.

8

9 *(Z)*-cyclooct-5-en-1,2-diol (**2**)

10 Under inert atmosphere, to the (Z)-(1*S*,8*R*)-9-oxa-bicyclo[6.1.0]non-4-ene (**1**) (5.697 g, 45.9 mmol)  
11 vigorously stirred was added a solution of sulfuric acid 2M (25.3 mL, 50.47 mmol). After 4 hours  
12 under stirring, the mixture was extracted with 3x75 mL of ethyl acetate. The organic layers were  
13 washed with a saturated solution of sodium hydrogenocarbonate (40 mL), brine (40 mL), dried with  
14 magnesium sulfate and concentrated under vacuum. The crude residue was purified by column  
15 chromatography (silica gel, petroleum ether/ethyl acetate 60/40) to give the rac-(Z)-cyclooct-5-en-1,2-  
16 diol **2** (3.691 g, 57% yield) as a colorless oil;  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3362, 3014, 2964, 2861, 1651, 1427, 1429,  
17 1400, 1271, 1202, 1010, 994, 976, 947, 868, 732, 719;  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ): 5.55–5.59 (2H, m,  
18  $\text{CH}=\text{CH}$ ), 3.57–3.61 (4H, m,  $\text{CHOHCHOH}$ ), 2.29–2.35 (2H, m,  $\text{CH}_2$ ), 2.00–2.12 (4H, m,  $2\times\text{CH}_2$ ),  
19 1.52–1.58 (2H, m,  $\text{CH}_2$ );  $\delta_{\text{C}}$  (100 MHz,  $\text{CDCl}_3$ ): 128.9, 73.8, 33.1, 22.6; HRMS: calculated for  
20  $\text{C}_8\text{H}_{14}\text{O}_2$  [ $\text{M}$ ] $^+$ : 142.09938, Found : 142.1001 (5 ppm).

21

22 *(1*R*,2*R*)-(Z)*-1-hydroxy-cyclooct-4-enyle acetate (**3a**)

23 Compound **3a** was obtained as a colorless oil [ $\alpha$ ] $_{\text{D}}^{20}$   $-3.0^\circ$  (*c* 1.00  $\text{CHCl}_3$ ).  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3449, 3012,  
24 2936, 1717, 1654, 1430, 1235, 1030, 971, 933, 720;  $\delta_{\text{H}}$  NMR (400 MHz,  $\text{CDCl}_3$ ): 5.59–5.69 (2H, m,  
25  $\text{CH}=\text{CH}$ ), 4.95 (1H dt,  $J=8.8$  Hz,  $4.0$  Hz,  $\text{CHOAc}$ ), 3.90 (1H, dt,  $J=2.1$  Hz,  $\text{CHOH}$ ), 2.54 (1H, s,  $\text{OH}$ ),  
26 2.38–2.42 (m, 2H,  $\text{CH}_2$ ), 2.04–2.25 (m, 7H,  $2\times\text{CH}_2$  and  $\text{CH}_3$ ), 1.68–1.75 (m, 2H,  $\text{CH}_2$ );  $\delta_{\text{C}}$  (100 MHz,  
27  $\text{CDCl}_3$ ): 170.9, 129.6, 128.6, 77.3, 72.1, 32.8, 30.0, 22.8, 22.8, 21.2. GC: Cromopack column  $t_{\text{R}} = 59.7$   
28 min HRMS: calcd for  $\text{C}_8\text{H}_{14}\text{O}_2$  [ $\text{M}-\text{CH}_2\text{CO}$ ] $^+$ : 142.09938, Found : 142.0993 (0 ppm).

29

30 *(1*S*,2*S*)-(Z)*-1-hydroxy-cyclooct-4-enyle acetate (**3b**)

31 Compound **3b** was obtained as a colorless oil. [ $\alpha$ ] $_{\text{D}}^{20}$   $+3.2^\circ$  (*c* 1.00  $\text{CHCl}_3$ ).  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3449, 3012,  
32 2936, 1717, 1654, 1430, 1235, 1030, 971, 933, 720;  $\delta_{\text{H}}$  NMR (400 MHz,  $\text{CDCl}_3$ ): 5.59–5.69 (2H, m,  
33  $\text{CH}=\text{CH}$ ), 4.95 (1H dt,  $J=8.8$  Hz,  $4.0$  Hz,  $\text{CHOAc}$ ), 3.90 (1H, dt,  $J=2.1$  Hz,  $\text{CHOH}$ ), 2.54 (1H, s,  $\text{OH}$ ),  
34 2.38–2.42 (m, 2H,  $\text{CH}_2$ ), 2.04–2.25 (m, 7H,  $2\times\text{CH}_2$  and  $\text{CH}_3$ ), 1.68–1.75 (m, 2H,  $\text{CH}_2$ );  $\delta_{\text{C}}$  (100 MHz,  
35  $\text{CDCl}_3$ ): 170.9, 129.6, 128.6, 77.3, 72.1, 32.8, 30.0, 22.8, 22.8, 21.2. GC: Cromopack column  $t_{\text{R}} = 56.6$   
36 min HRMS: calcd for  $\text{C}_8\text{H}_{14}\text{O}_2$  [ $\text{M}-\text{CH}_2\text{CO}$ ] $^+$ : 142.09938, Found : 142.0993 (0 ppm).

37

38 *(1*R*,2*R*)-(Z)*-2-acetoxy-cyclooct-4-enyle acetate (**4a**)

39 Compound **4a** was obtained as a colorless oil. [ $\alpha$ ] $_{\text{D}}^{20}$   $+81.0^\circ$  (*c* 1.00  $\text{CHCl}_3$ ).  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3016,  
40 2938, 2866, 1732, 1654, 1431, 1370, 1226, 1244, 1032, 978, 946, 735, 722;  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ):  
41 5.69–5.62 (2H, m,  $\text{CH}=\text{CH}$ ), 5.05–5.08 (2H, m,  $2\times\text{CHOH}$ ), 2.35–2.41 (2H, m,  $\text{CH}_2$ ), 2.12–2.18 (2H,  
42 m,  $\text{CH}_2$ ), 2.01–2.04 (2H, m,  $\text{CH}_2$ ), 1.96 (s, 6H,  $2\times\text{CH}_3$ ), 1.73–1.76 (2H, m,  $\text{CH}_2$ ).  $\delta_{\text{C}}$  (100 MHz,

1 CDCl<sub>3</sub>): 170.1, 128.6, 73.6, 29.9, 22.7, 20.9; [ $\alpha$ ]<sub>D</sub>: +83.9° (c = 1.00 CHCl<sub>3</sub>) GC Cromopack column t<sub>R</sub>  
2 = 61.2 min

3  
4 *(1S,2S)-(Z)-2-acetoxy-cyclooct-4-enyle acetate (4b)*

5 Compound **4b** was obtained as a colorless oil; [ $\alpha$ ]<sub>D</sub>: -79.0° (c 1.00 CHCl<sub>3</sub>). v<sub>max</sub> (cm<sup>-1</sup>): 3016, 2938,  
6 2866, 1732, 1654, 1431, 1370, 1226, 1244, 1032, 978, 946, 735, 722;  $\delta$ <sub>H</sub> (400 MHz, CDCl<sub>3</sub>): 5.69–  
7 5.62 (2H, m, CH=CH), 5.05–5.08 (2H, m, 2xCHOH), 2.35–2.41 (2H, m, CH<sub>2</sub>), 2.12–2.18 (2H, m,  
8 CH<sub>2</sub>), 2.01–2.04 (2H, m, CH<sub>2</sub>), 1.96 (s, 6H, 2x CH<sub>3</sub>), 1.73–1.76 (2H, m, CH<sub>2</sub>).  $\delta$ <sub>C</sub> (100 MHz, CDCl<sub>3</sub>):  
9 170.1, 128.6, 73.6, 29.9, 22.7, 20.9; GC: Cromopack column t<sub>R</sub> = 59.4 min; HRMS calcd for C<sub>10</sub>H<sub>16</sub>O<sub>3</sub>  
10 [M-CH<sub>2</sub>CO]<sup>+</sup>: 184.10994, Found: 184.1091 (4 ppm).

11

12 *General procedure for enantioselective acetylation of racemic (Z)-cyclooct-5-en-1,2-diol (2) using*  
13 *lipase*

14

15 Under inert atmosphere, to a solution of racemic diol **2** (0.2 g, 1.41 mmol) solubilized in 2.5 mL of  
16 THF were added the vinyl acetate (1.3 mL, 14 mmol) and the appropriate immobilized lipase (50 mg).  
17 The mixture was stirred at the required temperature under classical heating or microwave irradiation  
18 (see Table 1, Table 2, Table 3). The mixture was filtrated, extracted with ethyl acetate (3x7 mL). The  
19 organic layers were washed with 3 mL of HCl 5%, 3 mL of sodium hydrogenocarbonate, brine, dried  
20 with magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by  
21 column chromatography (silica gel, petroleum ether/ethyl acetate 60/40) to give the (1R,2R)-(Z)-1-  
22 hydroxy-cyclooct-4-enyle acetate **3a**, **3b**, **4b**.

23

24 *General procedure for the hydrolysis of (Z)-2-acetoxy-cyclooct-4-enyle acetate (4) by Candida*  
25 *antarctica lipase B*

26

27 Under inert atmosphere, to a solution of racemic (Z)-2-acetoxy-cyclooct-4-enyle acetate **4** (0.2 g,  
28 0.88 mmol) in 2.5 mL of phosphate buffer 0.1 M, pH=7.0, was added the lipase (50 mg). The mixture  
29 was stirred at 50°C under microwave irradiation (see Table 4, Table 5, Table 6). The mixture was  
30 filtrated, extracted with ethyl acetate (3x7 mL). The organic layers were washed with 3 mL of HCl 5%,  
31 3 mL of sodium hydrogenocarbonate, brine, dried with magnesium sulfate and concentrated under  
32 reduced pressure. The crude residue was purified by column chromatography (silica gel, petroleum  
33 ether/ethyl acetate 60/40) to give **3a**, **3b** and **4a**, **4b**.

34

35 *Diacetate enrichment*

36 The enriched (Z)-2-acetoxy-cyclooct-4-enyle **4a** is solubilized in 2.5 mL of phosphate buffer 0.1M,  
37 pH=7.0. *Candida antarctica* lipase B (50 mg) was added and the mixture was stirred at 50°C during 14  
38 hours by microwave irradiation (open vessel). The mixture was filtrated, extracted with ethyl acetate  
39 (3x7mL), The combined organic layers were washed with 3 mL of HCl 5%, 3 mL of sodium  
40 hydrogenocarbonate, brine, dried with magnesium sulfate and concentrated under reduced pressure.  
41 The crude residue was purified by chromatography column (silica gel, petroleum ether/ethyl acetate

60/40) to give the (Z)-(1S,2S)-2-hydroxy-cyclooct-4-enyle **3b** acetate in 49% overall yield (0.098g, ee>99%) and **4a** in 51 % overall yield (0.083g, ee>99%).

#### *Influence of the power of the microwave for the acetylation of (Z)-cyclooct-5-en-1,2-diol (2)*

The cryogenic fluid (Garlon 80<sup>®</sup>) of Cool mate<sup>®</sup> was cooled by dry ice, and maintained at 7°C. To a solution of racemic diol **2** (0.2 g, 1.406 mmol) solubilized in 2.5 mL of THF was added vinyl acetate (1.3 mL, 14 mmol) and *Candida antarctica* lipase (50 mg). The mixture was irradiated with an internal temperature set to 35°C, leading to an irradiation power of 300 W. After 7 hours of irradiation, the mixture was filtrated, extracted with ethyl acetate (3x7 mL). The organic layers were washed with 3 mL of HCl 5%, 3 mL of sodium hydrogenocarbonate, brine, dried with magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 60/40) to give the (Z)-(1R,2R)-1-hydroxy-cyclooct-4-enyle acetate **3a** in 42% yield (0.108 g, ee=67%), (Z) (1S,2S)-2-acetoxy-cyclooct-4-enyle acetate **4b** in 2% yield (ee=99%) and (Z)-cyclooct-5-en-1,2-diol in 51% yield (0.102g, ee=50%).

#### *Synthesis of diols by saponification of esters*

Under inert atmosphere, to a solution of enantiopure (Z)-(1R,2R)-2-acetoxy-cyclooct-4-enyle acetate **4b** (0.2 g, 17.3 mmol) or enantiopure monoacetate **3a** (0.2g, 12.0 mmol) in methanol (10 mL) was added potassium carbonate anhydrous (4 mg, 0.86. mmol). The mixture was stirred 8 hours at 0°C, and 10 mL of hydrochloric acid 1M are added. The aqueous layer was extracted with ethyl acetate (3x8 mL), washed with a saturated solution of sodium hydrogenocarbonate (5 mL) and brine (5 mL). The organic layers were dried with magnesium sulfate, concentrated under reduced pressure.

#### *(1R,2R)-(Z)-Cyclooct-5-ene-1,2-diol (2a)*

The crude residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 60/40) to give the (Z)-(1R,2R)-cyclooct-5-ene-1,2-diol **2a** ( 0.152g, 99% yield) as a white solid;  $[\alpha]_D^{20}$  -20,9° (c 1,00 CHCl<sub>3</sub>);  $\nu_{\max}$  (cm<sup>-1</sup>): 3362, 3014, 2964, 2861, 1651, 1427, 1429, 1400, 1271, 1202, 1010, 994, 976, 947, 868, 732, 719;  $\delta_H$  (400 MHz, CDCl<sub>3</sub>): 5.55–5.59 (2H, m, CH=CH), 3.57–3.61 (m, 4H, 2xCHOH), 2.29–2.35 (2H, m, CH<sub>2</sub>), 2.00–2.12 (2H, m, CH<sub>2</sub>), 1.52–1.58 (2H, m, CH<sub>2</sub>);  $\delta_c$  (100 MHz, CDCl<sub>3</sub>): 128.9, 73.8, 33.1, 22.6; HRMS: calculated for C<sub>8</sub>H<sub>14</sub>O<sub>2</sub> [M]<sup>+</sup>: 142.09938, Found : 142.1001 (5 ppm). HPLC t<sub>R</sub> = 11.9 min

#### *(1S,2S)-(Z)- Cyclooct-5-ene-1,2-diol (2b)*

The crude residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate 60/40) to give the (Z)-(1S,2S)-cyclooct-5-en-1,2-diol **2b** (0.153 g, 99% yield) as a white solid.  $[\alpha]_D$  : +18.2° (c = 1,00 CHCl<sub>3</sub>) $\nu_{\max}$  (cm<sup>-1</sup>): 3362, 3014, 2964, 2861, 1651, 1427, 1429, 1400, 1271, 1202, 1010, 994, 976, 947, 868, 732, 719;  $\delta_H$  (400 MHz, CDCl<sub>3</sub>): 5.55–5.59 (2H, m, CH=CH), 3.57–3.61 (m, 4H, 2xCHOH), 2.29–2.35 (2H, m, CH<sub>2</sub>), 2.00–2.12 (2H, m, CH<sub>2</sub>), 1.52–1.58 (2H, m, CH<sub>2</sub>);  $\delta_c$  (100 MHz, CDCl<sub>3</sub>): 128.9, 73.8, 33.1, 22.6; HRMS: calculated for C<sub>8</sub>H<sub>14</sub>O<sub>2</sub> [M]<sup>+</sup>: 142.09938, Found : 142.1001 (5 ppm). HPLC t<sub>R</sub> = 10.8 min.

1

## 2 4. Conclusions

3 The kinetic resolution of homochiral (*Z*)-cyclooct-5-ene-1,2-diols and (*Z*)-2-acetoxy-cyclooct-4-  
4 enyle acetates has been fulfilled in a clean and rapid way using the microwave-assisted biocatalysis.  
5 The enantioselectivity was controlled using two different lipases (CaLB or PS-D) in order to obtain  
6 one or the other useful enantiomer. The role of the microwave power has also been highlighted.  
7 Finally, by microwave irradiation, this eco-efficient optimization for the resolution of racemic diols,  
8 leads to a reduction of the reaction time and a decrease of power consumption, without any toxicity.

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## 13 Author Contributions

14 V.T., L.D. and M.G. designed the research. H.R. and E.D. performed the experimental work and  
15 participated equally to this work and should be considered as primary co-authors. V.T. wrote the  
16 manuscript with the cooperation of E.D. and H.R. All authors discussed, edited and approved the  
17 submitted version.

## 18 Conflicts of Interest

19 State any potential conflicts of interest here or “The authors declare no conflict of interest”.

## 20 References and Notes

- 21 1. Bornscheuer, U.T.; Kazlauskas, R.J. *Hydrolases in Organic synthesis* 2nd Ed. WILEY-VCH,  
22 Verlag GmbH & Co. KGaA, Weinheim, **2006**, 61-184.
- 23 2. Gotor-Fernandez, V.; Brieva, R.; Gotor, V. Lipases: Useful biocatalysts for the preparation of  
24 pharmaceuticals. *J. Mol. Cat. B: Enzymatic*, **2006**, *40*, 111-120.
- 25 3. Pchelka, B.; Loupy, A.; Petit, A. Preparation of various enantiomerically pure (benzotriazol-1-  
26 yl)-and (benzotriazol-2-yl)-alkan-2-ols. *Tetrahedron : Asymmetry*. **2006**, *17*, 2516-2530.
- 27 4. Bachu, P.; Sperry, J.; Brimble, M. Synthesis of a C8 oxygenated pyranonaphthoquinone: a  
28 useful precursor to dimeric pyranonaphthoquinones. *Tetrahedron*. **2008**, *64*, 3343-3350.
- 29 5. Leadbeater, N.; Stencel, LM.; Wood, E.C. Probing the effects of microwave irradiation on  
30 enzyme-catalysed organic transformations: the case of lipase-catalysed transesterification  
31 reactions. *Org. Biomol. Chem*. **2007**, *5*, 1052-1055.
- 32 6. Parvulescu, A.N.; Jacobs, P.A.; De Vos, D.E. Support influences in the Pd-catalyzed  
33 racemization and dynamic kinetic resolution of chiral benzylic amines. *Appl. Catal. A: Gen.*,  
34 **2009**, *368*, 9-16.

- 1 7. DeO Souza, O.M.A.; Antunes, O.A.C.; Kroutil, W.; Kappe, C.O. Kinetic Resolution of *rac*-1-  
2 Phenylethanol with Immobilized Lipases: A Critical Comparison of Microwave and  
3 Conventional Heating Protocols. *J. Org. Chem.*, **2009**, *74*, 6157-6162.
- 4 8. Yadav, G.D.; Pawar, S.V. Insight into microwave irradiation and enzyme catalysis in  
5 enantioselective resolution of DL-(±)-3-phenyllactic acid. *Appl Microbiol. Biotechnol.*, **2012**,  
6 *96* (1), 69-79.
- 7 9. Rouillard, H.; Chérouvrier J.R.; Thiéry, V. Ultrasound and Microwave: Recent Advances in  
8 Organic Chemistry. Research Signpost, Chapter 6: Biocatalysis under microwave irradiation,  
9 **2011**, 175-214. ISBN : 978-81-7895-532-2. 4.
- 10 10. Bóday, V.; Orovecz, O.; Szakács, G.; Novák, L.; Poppe, L. Kinetic resolution of trans -2-  
11 acetoxycycloalkan-1-ols by lipase-catalysed enantiomerically selective acylation. *Tetrahedron:*  
12 *Asymmetry*. **2003**, *14*, 2605-2612.
- 13 11. Onomura, O.; Arimoto, H.; Matsumura, Y.; Demizu, Y. Asymmetric oxidation of 1,2-diols  
14 using N -bromosuccinimide in the presence of chiral copper catalyst. *Tetrahedron Lett.* **2007**,  
15 *48*, 8668-8672.
- 16 12. Demizu, Y.; Matsumoto, K.; Onomura, O.; Matsumura, Y. Copper complex catalyzed  
17 asymmetric monosulfonylation of meso-vic-diols. *Tetrahedron Lett.* **2007**, *48*, 7605-7609.
- 18 13. Wakita, N.; Hara, S. Selective mono-acylation of 1,2- and 1,3-diols using ( $\alpha,\alpha$  -  
19 difluoroalkyl)amines. *Tetrahedron*. **2010**, *66*, 7939-7945.
- 20 14. Suemune, H.; Hizuka, M.; Kamashita, T.; Sakai K. Preparation of optically active  $\gamma$ -  
21 hydroxyethyl  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone using an enzymatic procedure. *Chem. Pharm. Bull.*  
22 **1989**, *37*, 1379-1381.
- 23 15. Suemune, H.; Hasegawa A.; Sakai, K. Highly Enantio- and Diastereo-selective Synthesis of  
24 C2-Symmetric 3,5-Cyclohexadiene-1,2-diol and D2-Symmetric Cyclohexane-1,2,4,5-tetrol.  
25 *Tetrahedron: Asymmetry*. **1995**, *6*, 55-58.
- 26 16. C. Mateo, J.M.; Palomo, G.; Fernandez-Lorente, J.M.; Guisan, R.; Fernandez-Lafuente.  
27 Improvement of enzyme activity, stability and selectivity via immobilization techniques.  
28 *Enzyme Microb. Technol.* **2007**, *40*, 1451-1463.
- 29 17. Poojari, Y., Clarson, S. Thermal stability of *Candida antarctica* lipase B immobilized on  
30 macroporous acrylic resin particles in organic media. *Biocatal. Agr. Biotechnol.* **2013**, *2*, 7-11.
- 31 18. Bachu, P., Gibson, J.S., Sperry, J., Brimble, M.A. The influence of microwave irradiation on  
32 lipase-catalyzed kinetic resolution of racemic secondary alcohols. *Tetrahedron: Asymmetry*.  
33 **2007**, *18*, 1618-1624.
- 34 19. Horikawa, T.; Norimine, Y.; Tanaka, M.; Sakai, K.; Suemune, H. Synthesis of Optically Active  
35 Bicyclo[3.3.0]octane Skeleton Using Transannular Reaction. *Chem. Pharm. Bull.* **1998**, *46*, 17-  
36 21.

37

38 *Sample Availability:* Samples of compounds are available from the authors.

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41 (<http://creativecommons.org/licenses/by/3.0/>).