Microwave-assisted kinetic resolution of homochiral diols using lipase

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Abstract: Since last decade, biocatalysts have become an attractive alternative to conventional chemical methods, especially for organic synthesis, due to their great properties. Among these enzymes, lipases are the most widely used, because they are cheap, easily available, cofactor free and have broad substrate specificity. Combined to microwave irradiation in non-aqueous medium, the published results suggest that microwave irradiation can have an influence on enzyme stability and activity, in addition to altering/enhancing reaction rates and/or enantioselectivities, called nonthermal microwave effects. However, the role of the microwave irradiation on enzyme still reminds controversial. This presentation will deal with the benefits of the use of lipases and the microwave irradiation. To have a better understanding of the system, different parameters were studied and analyzed, such as the impact of the microwave power, the temperature. The optimization of the reaction parameters will lead to the obtainment of useful chiral homochiral diols in clean, efficient and safe way.

Keywords: biocatalysis; microwave irradiation; lipase; homochiral diols; resolution

1. Introduction

Enantiomerically pure vicinal diols are versatile chemical intermediates for the production of flavors and fragances. As part of our work concerning the synthesis of enantioselective synthesis of methyl jasmonate derivatives from optically active bicycle[3.3.0]octane derivatives by transannular reaction, we needed first to prepare enantiopure homochiral (*1R, 2R*) and (*1S, 2S*) 5-cyclooctene-1,2-diols. In recent years, the employment of biocatalysts for organic synthesis has become an attractive alternative to conventional chemical methods. Lipases are the most widely used because they are inexpensive, easily available, cofactor free and have broad substrate specificity.[1] We decided to turn our interest to microwave-assisted lipase mediated kinetic resolution involving CaLB (lipase B from *Candida antartica*) or PS (*Pseudomonas cepacia*)-catalyzed acetylation of diol. The use of microwave irradiation in biocatalysis can enhance the enzyme activity: for example in resolution reaction, in specific oxido-reduction reaction or in hydrolysis. Combined to microwave irradiation in non-aqueous medium, the published results suggest that microwave irradiation can have also an influence on enzyme stability and activity, in addition to altering/enhancing reaction rates and/or enantioselectivities, called nonthermal microwave effects.[2] However, the role of the microwave irradiation on enzyme still reminds controversial.[3] We herein report our studies on microwave-assisted

lipase resolution of diol. To have a better understanding of the system, different parameters were studied and analyzed, such as the impact of the microwave power or the temperature.

2. Methods/Experimental section

Lipases from *Pseudomonas cepacia* (immobilized on diatome MKBB3465, 500 PLU^{-1}) and *Candida antarctica* (immobilized on acrylic resin 077K1155, 10 000 PLU^{-1} *Novozym 435®* or free form) were purchased from Sigma Aldrich. The entire commercials available were purchased from Sigma Aldrich.

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

Enantiomeric excesses were calculated by gas chromatography (Agilent 7890A) equipped with an autosampler (7688B) and flame ionization detector (FID) for the products detection. For the experiment, a CP-Chirasil-Dex (0.25mm x 25m x 0.25µm, Cromopack) column was used. The injector and the detector were kept at 180 °C. Nitrogen was used as gas carrier at a flow of 1.5 mL/min. Hydrogen, air and nitrogen were supplied to the FID at 35 $mL.min^{-1}$, 350 $mL.min^{-1}$ and 25 $mL.min^{-1}$ respectively. The products are analyzed at 110 °C.

High performance liquid chromatography (HPLC) was carried out in a Watters 600s combined with an autosampler (Watters 717 plus). The Chiralpak–AD column (Amylose tris-(3,5-dimethylphenylcarbamate) coated on 10 µm silica-gel, Daicel Chemical, 250 x 4.6 mm) in a flowrate (n-Heptane/EtOH, 9/1) of 1 $mL.min^{-1}$ is used. Products are analyzed by a differential refractometer (Watters 410)

Optical rotation was measured on a Perkin Elmer 341 polarimeter.

Microwave reactions were conducted using a CEM Discover®, mode operating systems working at 2.45 GHz, with a power programmable from 1 to 300W. The microwave can be equipped with a Cool mate® system allowing reactions at high irradiation (up to 300 W). This system is cooled by a cryogenic fluid (Galden HT-55®).

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

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

Under inert atmosphere, to a solution of cyclo-octa-1,5-dien (20 g, 0.161 mol) and sodium carbonate (117.6 g, 1.11 mol) in dichloromethane (560 mL) stirred at 0°C is added dropwise a solution of peracetic acid (42.7 mL, 0.222 mol) in dichloromethane (500 mL) during 2 hours. The mixture was stirred during 8 hours at 0°C and 12 hours at room temperature. The mixture was washed with 500mL of water and extracted with (3x250 mL) of dichloromethane. The organic layers were dried with magnesium sulfate anhydrous and concentrated under reduced pressure.

The crude residue was purified by chromatography column (silica gel, petroleum ether/ethyl acetate 90/10) to provide the (Z)-(*1S,8R*)-9-oxa-bicyclo[6.1.0]non-4-en **1** (5.697g, 67% yield) as a colorless oil; ν_{max} (cm^{-1}): 3003, 2906, 2887, 1655, 1445, 1428, 1228, 1669, 1099, 1039, 934, 762, 743; δ_H (400 MHz, $CDCl_3$) 5,56–5.60 (2H, m, C<u>H</u>=C<u>H</u>), 3.00-3.02 (2H, m, C<u>H</u>C<u>H</u>), 2.41–2.47 (2H, m, C<u>H</u>$_2$), 2.08-2.15 (2H, m, C<u>H</u>$_2$), 1.98–2.17 (4H, m, 2xC<u>H</u>$_2$); δ_C (100 MHz, $CDCl_3$) 128.6, 56.2, 27.8, 23.4.

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

Under inert atmosphere, to the (Z)-*(1S,8R)*-9-oxa-bicyclo[6.1.0]non-4-en **(1)** (5.697 g, 45.9 mmol) vigorously stirred is added a solution of sulfuric acid 2M (25.3 mL, 50.47 mmol). After 4 hours under stirring, the mixture is extracted with 3x75 mL of ethyl acetate. The organic layers were washed with a saturated solution of sodium hydrogenocarbonate (40 mL), brine (40 mL), dried with magnesium sulfate and concentrated under vacuum. The crude residue was purified by chromatography column (silica gel, petroleum ether/ethyl acetate 60/40) to give the rac-(Z)-cyclooct-5-en-1,2-diol **2** (3.691 g, 57% yield) as a colorless oil; ν_{max} (cm^{-1}): 3362, 3014, 2964, 2861, 1651, 1427, 1429, 1400, 1271, 1202, 1010, 994, 976, 947, 868, 732, 719; δ_H (400 MHz, $CDCl_3$): 5.55–5.59 (2H, m, C<u>H</u>=C<u>H</u>), 3.57–3.61 (4H, m, C<u>H</u>O<u>H</u>C<u>H</u>O<u>H</u>), 2.29–2.35 (2H, m, C<u>H</u>$_2$), 2.00–2.12 (4H, m, 2xC<u>H</u>$_2$), 1.52–1.58 (2H, m, C<u>H</u>$_2$); δ_C (100 MHz, $CDCl_3$): 128.9, 73.8, 33.1, 22.6; HRMS: calculated for $C_8H_{14}O_2$ $[M]^+$: 142.09938, Found : 142.1001 (5 ppm).

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

Compound **3a** is obtained as a colorless oil $[\alpha]_D^{20}$ -3.0° (*c* 1.00 $CHCl_3$). ν_{max} (cm^{-1}): 3449, 3012, 2936, 1717, 1654, 1430, 1235, 1030, 971, 933, 720; δ_H NMR (400 MHz, $CDCl_3$): 5.59–5.69 (2H, m, C<u>H</u>=C<u>H</u>), 4.95 (1H dt, *J=8.8 Hz, 4.0Hz*, C<u>H</u>OAc), 3.90 (1H, dt, *J=2.1 Hz*, C<u>H</u>OH), 2.54 (1H, s, O<u>H</u>), 2.38–2.42 (m, 2H, C<u>H</u>$_2$), 2.04–2.25 (m, 7H, 2xC<u>H</u>$_2$ and C<u>H</u>$_3$), 1.68–1.75 (m, 2H, C<u>H</u>$_2$); δ_C (100 MHz, $CDCl_3$): 170.9, 129.6, 128.6, 77.3, 72.1, 32.8, 30.0, 22.8, 22.8, 21.2. GC: Cromopack column t_R = 59.7 min HRMS: calcd for $C_8H_{14}O_2$ $[M\text{-}CH_2CO]^+$: 142.09938, Found : 142.0993 (0 ppm).

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

Compound **3b** is obtained as a colorless oil. $[\alpha]_D^{20}$ +3.2° (*c* 1.00 $CHCl_3$). ν_{max} (cm^{-1}): 3449, 3012, 2936, 1717, 1654, 1430, 1235, 1030, 971, 933, 720; δ_H NMR (400 MHz, $CDCl_3$): 5.59–5.69 (2H, m, C<u>H</u>=C<u>H</u>), 4.95 (1H dt, *J=8.8 Hz, 4.0Hz*, C<u>H</u>OAc), 3.90 (1H, dt, *J=2.1 Hz*, C<u>H</u>OH), 2.54 (1H, s, O<u>H</u>), 2.38–2.42 (m, 2H, C<u>H</u>$_2$), 2.04–2.25 (m, 7H, 2x<u>CH</u>$_2$ and <u>CH</u>$_3$), 1.68–1.75 (m, 2H, C<u>H</u>$_2$); δ_C (100 MHz, $CDCl_3$): 170.9, 129.6, 128.6, 77.3, 72.1, 32.8, 30.0, 22.8, 22.8, 21.2. GC: Cromopack column t_R = 56.6 min HRMS: calcd for $C_8H_{14}O_2$ $[M\text{-}CH_2CO]^+$: 142.09938, Found : 142.0993 (0 ppm).

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

Compound **4a** is obtained as a colorless oil. $[\alpha]_D^{20}$ +81.0° (*c* 1.00 $CHCl_3$). ν_{max} (cm^{-1}): 3016, 2938, 2866, 1732, 1654, 1431, 1370, 1226, 1244, 1032, 978, 946, 735, 722; δ_H (400 MHz, $CDCl_3$): 5.69–5.62 (2H, m, C<u>H</u>=C<u>H</u>), 5.05–5.08 (2H, m, 2xC<u>H</u>OH), 2.35–2.41 (2H, m, C<u>H</u>$_2$), 2.12–2.18 (2H, m, C<u>H</u>$_2$), 2.01–2.04 (2H, m, C<u>H</u>$_2$), 1.96 (s, 6H, 2x C<u>H</u>$_3$), 1.73–1.76 (2H, m, C<u>H</u>$_2$). δ_C (100 MHz, $CDCl_3$): 170.1, 128.6, 73.6, 29.9, 22.7, 20.9; $[\alpha]_D$: +83.9° (c = 1.00 $CHCl_3$) GC Cromopack column t_R = 61.2 min

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

Compound **4b** is obtained as a colorless oil; $[\alpha]_D$: -79.0° (*c* 1.00 $CHCl_3$). ν_{max} (cm^{-1}): 3016, 2938, 2866, 1732, 1654, 1431, 1370, 1226, 1244, 1032, 978, 946, 735, 722; δ_H (400 MHz, $CDCl_3$): 5.69–5.62 (2H, m, C<u>H</u>=C<u>H</u>), 5.05–5.08 (2H, m, 2xC<u>H</u>OH), 2.35–2.41 (2H, m, C<u>H</u>$_2$), 2.12–2.18 (2H, m, C<u>H</u>$_2$), 2.01–2.04 (2H, m, C<u>H</u>$_2$), 1.96 (s, 6H, 2x C<u>H</u>$_3$), 1.73–1.76 (2H, m, C<u>H</u>$_2$). δ_C (100 MHz, $CDCl_3$): 170.1, 128.6, 73.6, 29.9, 22.7, 20.9; GC: Cromopack column t_R = 59.4 min; HRMS calcd for $C_{10}H_{16}O_3$ $[M\text{-}CH_2CO]^+$: 184.10994 , Found : 184.1091 (4 ppm).

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

Under inert atmosphere, to a solution of racemic diol **2** (0.2 g, 1.41 mmol) solubilized in 2.5 mL of THF were added the vinyl acetate (1.3 mL, 14 mmol) and the appropriated immobilized lipase (50 mg). The mixture was stirred at various temperature under classical heating or microwave irradiation (see Table 1, Table 2, Table3). 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 chromatography column (silica gel, petroleum ether/ethyl acetate 60/40) to give the (*1R,2R*)-(Z)-1-hydroxy-cyclooct-4-enyle acetate **3a**, **3b**, **4b.**

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

Under inert atmosphere, to a solution of racemic (Z)-2-acetoxy-cyclooct-4-enyle acetate **4** (0.2 g, 0.88 mmol) in 2.5 mL of phosphate buffer 0.1 M, pH=7.0, was added the lipase (50 mg). The mixture was stirred at 50°C under microwave irradiation (see Table 4, Table 5, Table 6). 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 chromatography column (silica gel, petroleum ether/ethyl acetate 60/40) to give **3a**, **3b** and **4a, 4b**.

Diacetate enrichment

The (Z)-2-acetoxy-cyclooct-4-enyle **4a** obtained in section 1.10.2 is solubilized in 2.5 mL of phosphate buffer 0.1M, pH=7.0. The *Candida antarctica* lipase B (50 mg) is added and the mixture is stirred at 50°C during 14 hours by microwave irradiation (open vessel). The mixture was filtrated, extracted with ethyl acetate (3x7mL), 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 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®) of Cool mate® is 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 is added the vinyl acetate (1.3 mL, 14 mmol) and the *Candida antarctica* lipase (50 mg). The mixture is irradiated with an intern temperature set at 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 chromatography column (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 **4a** (0.2 g, 17.3 mmol) or enantiopure monoacetate **3b** (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 is 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-en-1,2-diol ***(2a)***

The crude residue was purified by chromatography column (silica gel, petroleum ether/ethyl acetate 60/40) to give the (Z)–(*1R,2R*)-cyclooct-5-en-1,2-diol **2a** (0.152g, 99% yield) as a white solid; $[\alpha]_D^{20}$ -20,9° (*c* 1,00 $CHCl_3$); ν_{max} (cm^{-1}): 3362, 3014, 2964, 2861, 1651, 1427, 1429, 1400, 1271, 1202, 1010, 994, 976, 947, 868, 732, 719; δ_H (400 MHz, $CDCl_3$): 5.55–5.59 (2H, m, C<u>H</u>=C<u>H</u>), 3.57–3.61 (m, 4H, 2xC<u>H</u>O<u>H</u>), 2.29–2.35 (2H, m, C<u>H</u>$_2$), 2.00–2.12 (2H, m, C<u>H</u>$_2$), 1.52–1.58 (2H, m, C<u>H</u>$_2$); δ_c (100 MHz, $CDCl_3$): 128.9, 73.8, 33.1, 22.6; HRMS: calculated for $C_8H_{14}O_2$ $[M]^+$: 142.09938, Found : 142.1001 (5 ppm).HPLC t_R = 11.9 min

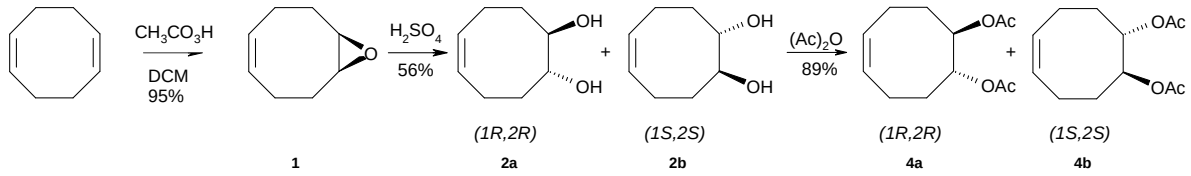
(1S,2S)-(Z)- Cyclooct-5-ène-1,2-diol ***(2b)***

The crude residue was purified by chromatography column (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 $CHCl_3$)ν_{max} (cm^{-1}): 3362, 3014, 2964, 2861, 1651, 1427, 1429, 1400, 1271, 1202, 1010, 994, 976, 947, 868, 732, 719; δ_H (400 MHz, $CDCl_3$): 5.55–5.59 (2H, m, C<u>H</u>=C<u>H</u>), 3.57–3.61 (m, 4H, 2xC<u>H</u>O<u>H</u>), 2.29–2.35 (2H, m, C<u>H</u>$_2$), 2.00–2.12 (2H, m, C<u>H</u>$_2$), 1.52–1.58 (2H, m, C<u>H</u>$_2$); δ_c (100 MHz, $CDCl_3$): 128.9, 73.8, 33.1, 22.6; HRMS: calculated for $C_8H_{14}O_2$ $[M]^+$: 142.09938, Found : 142.1001 (5 ppm). HPLC t_R = 10.8 min.

3. Results and Discussion

In the context of the growing general interest for reducing energy costs, heating chemical reactions under microwave irradiation is a useful approach for achieving higher reaction kinetics and the formation of cleaner products.[4]

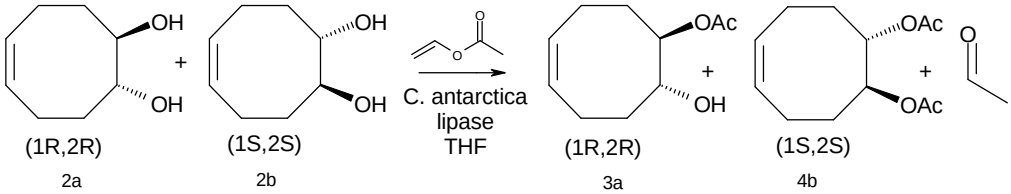
Rac-diol (**2**) and rac-diacetate (**4**) were prepared from cycloocta-1,5-diene by a three-steps sequence including epoxidation, ring opening with aqueous sulfuric acid followed by acetylation with acetic anhydride. [5]



*Epoxidation and hydrolysis of cyclo-octa-1,5-dien leading to diols **2** and diacetate **4***

The preparation of optically active 5-cyclooctene-1,2-diol **2** was first envisaged by using microwave-assisted lipase-catalyzed desymmetrization of *meso*-symmetric diol **2** using vinyl acetate as the acylating agent in THF as solvent among isooctane, M2B2. In order to perform the reaction under microwave irradiation, we decided to choose thermostable immobilized lipases that could to be used under microwave irradiation: Novozyme 435® (CaLB immobilized on acrylic resin) and PS-D (*Pseudomonas cepacia* immobilized on diatomite). In the literature by enzyme immobilization, enhanced enzyme activity, selectivity, stability, and reusability in organic media may be achieved compared to the native enzyme. It must be noted that Deau previously showed that the resolution of rac-diol **2** using free *Pseudomonas cepacia* at 55°C in THF during 7 days afforded with a good conversion rac-monoacetate **3** (47%, 0% ee) and rac-diol **2** (51%, 0% ee) but with no selectivity at all.

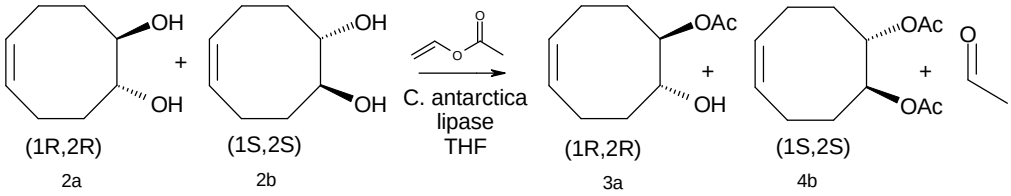
Performed at 35°C under conventional heating CaLB-enzymatic acylation of rac-diol (**2**) afforded after 3 weeks 28% of (*1R,2R*)-monoacetate **3a** (42% ee) and 6 % of (1*S*,2*S*)-diacetate **4b** with an excellent 99% ee. A higher temperature (50°C) led after 7 days to modest yields of monoacetate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



Mode of heating	Temperature (°C)	Time	Monoacetate **3a** yield(%)	ee (%)	Diacetate **4b** yield(%)	**ee (%)**
Classical	35	3 weeks	28	42	6	>99
Classical	50	7 days	30	50	20	>99
Classical	50	14 hours	traces		-	

***Table 1** : Enantioselective acetylation of diol **(2)** using CaLB lipase by classical heating*

Under microwave irradiation at 35°C (5W), racemic diol **2** proceeded to (*1R,2R*)-monoacetate **3a** (32%, 45% ee), trace of (*1S,2S*)-diacetate **4b** (5%, >99% ee) and 65% of diol **2** (23% ee). In order to study the influence of the irradiation power on the biocatalytic media, we decided to apply maximum of power (300W) in maintaining the temperature at 35°C by using a microwave oven combined with a Cool mate®. We noticed an enhancement of the yield and the enantiomeric ratio of (*1R,2R*)-monoacetate **3a** (42%, 67% ee) and diol **2** (51%, 50% ee) and only 2% of diacetate **4b** (99% ee). At 50°C (10W, 14hours) the same reaction yielded only esters: 58% of (*1R,2R*)-monoacetate **3a** with 55% ee and 37% of diacetate **4b** (99% ee). A higher temperature (80°C, 40W, 14h) afforded a decrease of diacetate yield with a lower enantiomeric excess (30% of **4b** with 93% ee).

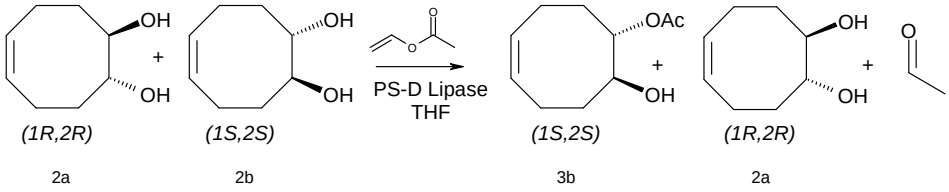


Power (W)	Temperature (°C)	Monoacetate yield (%)	3a ee (%)	Diacetate Yield (%)	4b ee (%)
5	35	32	45	5	99
10	50	58	55	37	99
20	80	55	57	30	94
40	100	-	-	-	-
300	35	42	67	2	>99

***Table 2** : Enantioselective acetylation of diol (**2**) using CaLB lipase by microwave irradiation*

These results suggest that at higher temperature (80-100 °C), there is a loss of enzyme activity and selectivity due to its denaturation. The power of irradiation displays key role in enzyme properties: enhancement of monoacetate yield and ee being observed at 35°C with 300W.

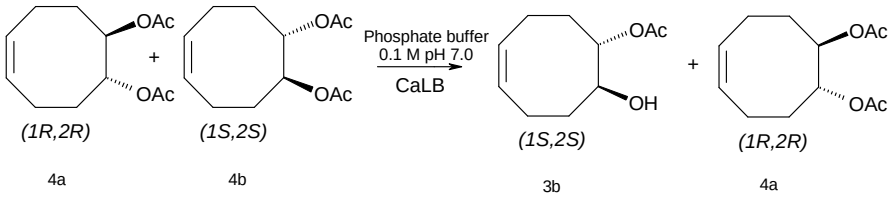
At 50°C under conventional heating, enzyme-catalysed acylation of rac-diol **2** with PSD provide both (*1S,2S*)-monoacetate **3b** and (*1R,2R*)-diol **2a** in poor enantiomeric purity (respectively 45% ee for **3b** and 3% ee for **2a**) and poor conversion (6%). At higher temperature (80, 100°C) there is a loss of activity. Under microwave irradiation at 50°C (15W), lipase resolution of racemic diol **2** afforded (1S,2S)-monoacetate **3b** (41%, 50% ee) and (*1R,2R*)-diol (57% , 35% ee). The microwave irradiation method gave a higher conversion value compared using conventional heating. At 80°C (35W), the reaction yielded 12% of 3**b** (35% ee) and 66% of diol with no selectivity (5% ee).



Mode of heating	Temperature (°C)	yield (%)	Monoacetate 3b ee (%)	Diol 2a ee (%)
Classical	50	6	45	3
	80		-	-
	100		-	-
Microwave	50	41	50	35
	80	12	35	5
	100 (closed vess.)	-	-	-

Table 3*: Acetylation of diol (**2**) using immobilized PS lipase (PS-D)*

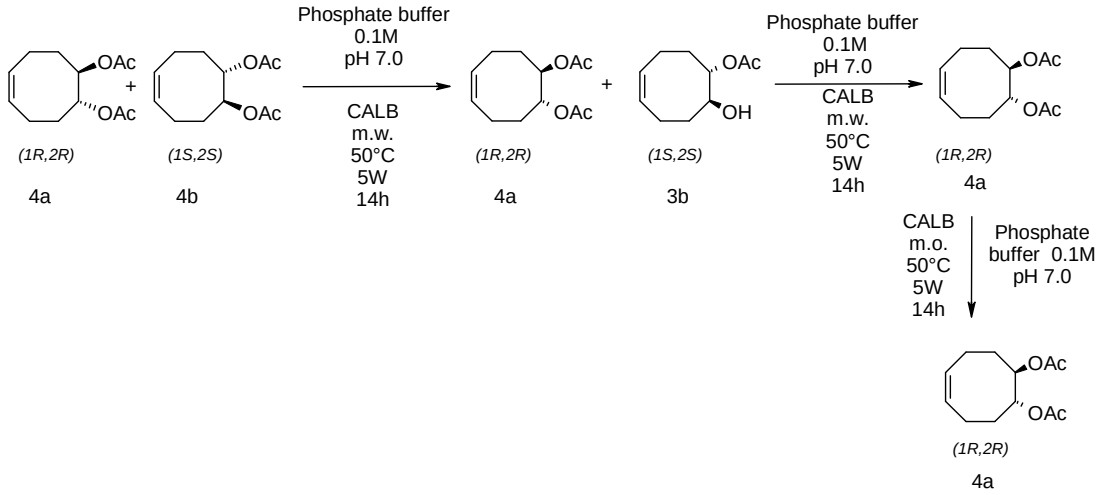
Compared to CalB, PSD exhibited a reverse enantiopreference for the monoacetate (*1S,2S*).
Then, we investigated the enzymatic enantioselective hydrolysis from rac-diacetate **4** according to Suemune procedure.[6] Instead of using PFL (*Pseudomonas fluorescens* lipase) as described by Suemune, we used immobilized CaLB and PSD in order to perform later the reaction under microwave. Performed at various temperature (35, 50 and 80°C) under conventional heating CalB-catalyzed hydrolysis of rac-diacetate (**4**) in phosphate buffer (0.1M, pH 7.0) led only to traces of monoacetate at 50°C. Compared to conventional heating, the microwave irradiation at 50°C during 14 hours led to a higher conversion (20%) with excellent enantiomeric excesses for monoacetate (ee: 97% for **3b** (1*S*,2*S*)) besides diacetate **4a** (ee: 34%).



Mode of heating	Temp. (°C)	Conversion	Monoacetate 3b ee (%)	Diacetate 4a ee (%)
Classical	35	-	-	-
	50	Traces	-	-
	80	-	-	-
Microwave	35	-	-	-
	50	20%	97 $[\alpha]_D$ =+6°	34 $[\alpha]_D$ =+29°
	80	-	-	-

Table 4 *: Hydrolysis of diacetate (**4**) using CaLB lipase*

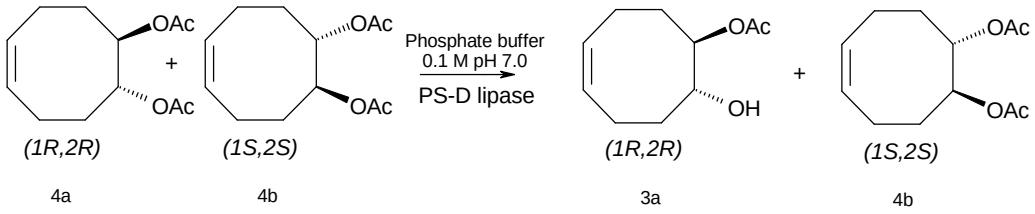
The recovered (1*R, 2R*)-diacetate **4a** of 34% ee was submitted twice to the same enzymatic hydrolysis under microwave irradiation (2 x 14h, 50°C, 5W). Pure (1*R*,2*R*)-diacetate **4a** was obtained in 49% yield from rac-**4**.



	Monoacetate **3b**			Diacetate **4a**		
	yield (%)	ee (%)	$[\alpha]_D$	yield (%)	ee (%)	$[\alpha]_D$
Before enrichment	20	97	+5,8°	80	34	+25°
1st enrichment	41	98	+6°	57	72	+57°
2nd enrichment	51	97	+5,7°	49	>99	+81°

***Table 5** : Enrichment of diacetate (**4a**)*

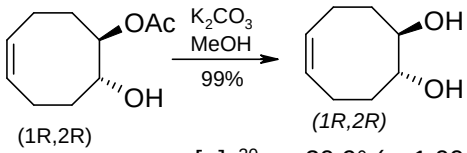
The hydrolysis using PS-D was conducted in the same manner. In classical conditions whatever the temperature (35, 50, 80°C) and the reaction time (14h, 7 days), in no case conversion to monoacetate **3** was observed. Interestingly, under microwave irradiation at 50°C (14h), rac-diacetate **4** was resolved into (-)-(*1R,2R*) monoacetate **3a** with 81% ee and (*S,S*)-diacetate **4b** (28% ee). This result highlights a non thermal effect.



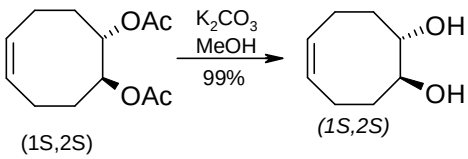
Mode of heating	Temp. (°C)	Conversion	Monoacetate **3a** ee (%)	Diacetate **4b** ee (%)
Classical	35	-	-	-
	50	-	-	-
	80	-	-	-
Microwave	35	-	-	-
	50	10%	81 $[\alpha]_D$ =-4°	28 $[\alpha]_D$ =-24°
	80	-	-	-

***Table 6** : Hydrolysis of diacetate (**4**) using PS-D lipase*

The obtained enantiopur monoacetates **3a**, **3b** and diacetates **4a** and **4b** after enantio-enrichments were then quantitatively converted into (*1R,2R*)-diol **2a** and (1*S*,2*S*)-diol **2b** of >99% ee by methanolysis.[6]



$[\alpha]_D^{20}$ = -20,9° (c=1.00 $CHCl_3$)



$[\alpha]_D^{20}$ = + 18.2° (c=1.00 $CHCl_3$)

Conclusions

The resolution of homochiral diol has been fulfilled in clean and rapid way using the microwave-assisted biocatalysis. The enantiopreference was guide using two lipases to obtain one or the other useful enantiomer. The role of the microwave power has also been highlighted. Finally, by microwave irradiation, this eco-efficient optimization for the resolution of racemic diols, leads to a reduction of the reaction time, a decrease of power consumption, without any toxicity

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References and Notes

1. Bornscheuer, U.T., Kazlauskas, R.J. Hydrolases in Organic synthesis 2nd Ed. WILEY-VCH, Verlag GmbH & Co. KGaA, Weinheim, **2006**. 99.

2. (a) G.F. Vincente, R. Brieva, V. Gotor, *Journal of Molecular Catalysis B: Enzymatic*, **2006**, *40*, 111. (b) Pchelka, B.; Loupy, A.; Petit, A. *Tetrahedron : Asymmetry*. **2006**, 17, 2516. (c) Bachu, P.; Sperry, J.; Brimble, M. *Tetrahedron*. **2008**, 64, 3343. (d) Leadbeater, N.; Stencel, L.; Wood, E. *Organic and Biomolecular Chemistry.* **2007**, 5, 1052. (e) A. Parvulescu, P. Jacobs, D. De Vos, *Applied Catalysis A: General*, **2009**, *368*, 9. (f) Octavio, R., Souza, M.A., Antunes, O.A., Kroutil, W., Kappe, C.O. *J. Org. Chem*., **2009**, 74, 6157. (g) G.D. Yadav, S. V. Pawar, *Appl Microbiol Biotechnol*.,**2012**, 96, 69.

3. H. Rouillard, J.R. Chérouvrier, V. Thiéry, Ultrasound and Microwave: Recent Advances in Organic Chemistry. Research Signpost, Chapter 6: Biocatalysis under microwave irradiation, **2011**, 175. ISBN : 978-81-7895-532-2.

4. (a) Bódai, V.; Orovecz, O.; Szakács, G.; Novák, L.; Poppe, L. *Tetrahedron: Asymmetry*. **2003**, 14, 2605. (b) Onomura, O.; Arimoto, H.; Matsumura, Y.; Demizu, Y. *Tetrahedron Letters*. **2007**, 48, 8668. (c) Demizu, Y.; Matsumoto, K.; Onomura, O.; Matsumura, Y. *Tetrahedron Letters*. **2007**, 48, 7605. (d) Wakita, N.; Hara, S. *Tetrahedron*. **2010**, 66, 7939.

5. (a) Suemune, H.; Hizuka, M.; Kamashita, T.; Sakai K. *Chemical and Pharmaceutical Bulletin*. **1989**, 37, 1379. (b) Suemune, H. ; Hasegawa A. ; Sakai, K. *Tetrahedron: Asymmetry*. **1995**, 6, 55.

6. Horikawa, T.; Norimine, Y.; Tanaka, M.; Sakai, K.; Suemune, H. *Chemical and Pharmaceutical Bulletin.* **1998**, 46, 17.