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Stereoselective Chemoenzymatic Synthesis of Enantiopure 2-(1*H*-imidazol-yl)cycloalkanols under Continuous Flow Conditions

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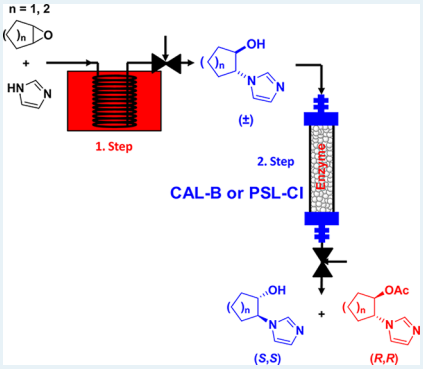
Supporting Information

ABSTRACT: The development of continuous flow processes for the synthesis of chiral enantiopure 1-(2-hydroxycycloalkyl)imidazoles is reported. For the ring-opening reaction microwave batch processes and continuous flow reactions have led to similar results in terms of conversion, although the productivity is clearly improved under flow. The use of continuous flow systems for the lipase-catalyzed kinetic resolution of the racemic 2-(1*H*-imidazol-yl)cycloalkanols with either immobilized CAL-B or PSL-C has been demonstrated to be significantly more efficient than the corresponding batch processes. The continuous flow biotransformations have allowed us to easily increase the production of these chiral imidazoles, adequate building blocks in the synthesis of chiral ionic liquids.



KEYWORDS: *continuous flow, microwave, biocatalysis, enantiopure imidazole*

INTRODUCTION

In the past few years, the number of continuous processes for the synthesis organic molecules has significantly increased.[1] Continuous flow processes present inherent advantages when compared with conventional batch processes. These include the improvement in mass and heat transfer, a significant intensification of the process, making available systems working 24 h a day, 7 days a week, or their easier optimization through the adjustments of simple parameters such as flow, pressure or temperature. Most experimental variables can be easily automated or controlled leading to purer and more reproducible products, and a far greater productivity, from a fixed amount of catalyst, than the one achieved in the related batch process. Additionally, the scale-up of flow processes is in general more easily attainable than for batch processes, via different approaches such as the scale-out or the number-up.[2−6] In general, reactor design is an important feature in continuous flow processes. The appropriate reactor design helps not only to facilitate the mass-transfer, to control the reaction conditions (pressure and temperature), and to facilitate the product isolation and recovery but also allows the process to be technically and economically feasible thinking in a scaling-up.

Here we report the development of continuous flow processes for the synthesis of chiral 1-(2-hydroxycycloalkyl)-imidazoles, which can be used for the preparation of chiral enantiopure ionic liquids.[7−9] The synthesis of racemic imidazole alcohols (*trans*-**3a-b**) has been performed by the ring-opening reaction of the corresponding epoxides using imidazole as nucleophile under microwave (MW) and conventional heating in either batch or continuous flow conditions followed by their enzymatic kinetic resolution (KR), which is the key step to obtain the enantiopure products. The optimization of these two reaction steps under continuous flow conditions allow to significantly increase the productivity for both the ring-opening reactions and the stereoselective lipase-catalyzed acetylation of the corresponding alcohols.

RESULTS AND DISCUSSION

Batch Synthesis of 1-(2-Hydroxycycloalkyl)-imidazoles. Conventional vs MW Heating. The epoxide ring-opening reaction is generally carried out under harsh conditions requiring either strong acid or base,[10] high temperature[11] or high pressure.[12] Alternatively, the MW-assisted ring-opening reaction of epoxides with nitrogen heterocycles[13,14] and the Lewis acid-catalyzed [$Yb(OTf)_3$] reaction in the presence of an excess of epoxide (200 mol %) have also been reported.[15] The synthetic pathway chosen by us for the synthesis of chiral *trans*-2-(1*H*-imidazol-1-yl)-cycloalkanols implies the ring-opening reaction of epoxides by

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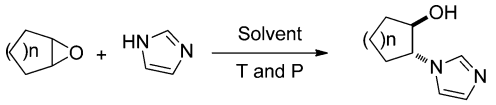
the nucleophilic attack of imidazole (Scheme 1) in the absence of any catalyst.

Scheme 1. Synthesis of Racemic 1-(2-Hydroxyalkyl)imidazoles by the Nucleophilic Ring-Opening of Epoxides 1a-b



1a, n = 1 **2** (±)-*trans*-**3a**, n = 1
1b, n = 2 (±)-*trans*-**3b**, n= 2

Table 1 summarizes the results obtained in the synthesis of (±)-*trans*-2-(1*H*-imidazol-1-yl)cyclohexanol (**3b**) obtained by the ring-opening reaction of cyclohexene oxide (**1b**) with imidazole (**2**) (Scheme 1). Both conventional and MW heating were used to facilitate the reaction. Low yields of *trans*-**3b** were obtained under conventional conditions, even at prolonged reaction times, when the reaction was performed in tetrahydrofuran (THF) as the solvent (entry 1, Table 1). Under such conditions, high conversions were only attained when the reaction was performed under solventless conditions (entry 2, Table 1).[16]

Alternatively, MW heating was also assayed to carry out the epoxide ring-opening reaction. A low concentration of reactant was initially used as the solubility in THF of the corresponding racemic alcohol *trans*-**3b** is about 0.08 M in THF. The reaction was carried out at a fixed temperature of 150 °C and a nominal power of 150 W for 1 h (entry 3, Table 1). Unfortunately, under those conditions, only traces of the final product were detected by ^{1}H NMR on the reaction crude. However, by increasing the concentration of the reactants to about 2 M, a full conversion of the epoxide to the alcohol was observed (entry 4, Table 1). Besides, the reaction time can be further reduced to only 5 min achieving a full conversion of the reagents (entries 5 and 6, Table 1).

Similar trends were found for the reaction between the cyclopentene oxide (**1a**) and imidazole (**2**). Hence, under conventional conditions a low yield of the corresponding alcohol (*trans*-**3a**) was observed except when solventless conditions and 25 h of reaction time were used (entries 7 and 8, Table 1). However, a high conversion was achieved, under MW heating, after only 1 h of reaction (entry 9, Table 1). We can conclude that the synthesis under MW heating is much more efficient in terms of imidazole conversion than when conventional heating is used. Although good conversions at short reaction times can be achieved under these conditions, applicability of this system is limited to small volumes of solution (about 5 mL) using standard equipment. Thus, to obtain a large amount of the products, repetitive reaction cycles would be needed. A way to overcome this limitation and to further improve the productivity of the system is to move from MW batch runs to a continuous flow process.

Figure 1 shows, schematically, the configuration of the continuous flow reactor used to evaluate the ring-opening epoxide reaction under flow conditions. The setup uses a PTA-Teflon coiled tubing (3 m length and 1000 μm i.d., 5.84 mL total volume) as a tubular reactor that is inserted in the CEM oven.[17,18] The reactor is connected to a High performance liquid chromatography (HPLC) pump to continuously deliver the reactants, and to a pressure restrictive valve (300 psi) at the reactor outlet to ensure a constant pressure in the system allowing the work with superheated solvents, in this case THF.[19] Thus, a THF solution was pumped at 0.3 mL/min working in constant power mode. Different power values were initially evaluated, 75, 50, 30, and 20 W, allowing in all the cases, with the use of pure THF, to achieve a steady temperature reading (130 °C). However, when a solution of the reactants in THF was pumped through the system under the same conditions, in all the cases the temperature of the system rose above the set temperature, leading to an automatic disconnection of the MW source. However, when the power was reduced to 10 W, the temperature of the system, after the stabilization time, reached a steady state with a temperature reading of about 140 °C (see Supporting Information, Figure SI-1).

Table 2 summarizes the results obtained for the different flows assayed at a fixed power of 10 W once the system reached the steady state. Under these conditions the infrared temperature reading was 135−140 °C and the pressure of the system was kept at 17−20 bar. As it was expected, excellent conversions (97−99%, entries 1−3, Table 2) were obtained at low flows (high residence times), the conversions being reduced as the flow was further increased (entries 4−6, Table 2). In all the cases, when the samples collected at the outlet of the reactor were cooled down, a crystallization of the product was observed, according to its low solubility in THF (see Supporting Information, Figure SI-3).

Table 1. Ring-Opening Reaction between Cycloalkene Oxides 1a-b and Imidazole (2)

entry	ratio	solvent	heating method	time (h)	T (°C)	concentration (M)[a]	yield (%)[b]	productivity (kg·L^{-1} h^{-1})[c]
1	**1b**	THF	conventional	24	67	1.07	38 (26)	2.82
2	**1b**		conventional	22	60	9.68	99 (60)	72.40
3	**1b**	THF	MW[d]	1	150	0.08		
4	**1b**	THF	MW[d]	1	150	1.96	99	≥322.53
5	**1b**	THF	MW[d]	0.25	150	1.96	99	≥1290.13
6	**1b**	THF	MW[d]	0.08	150	1.96	99	≥3885.94
7	**1a**	THF	conventional	24	67	0.98	19	1.18
8	**1a**		conventional	25	60	11.40	97	67.32
9	**1a**	THF	MW[d]	1	150	2.01	87	≥266.13

[a]Concentration referred to imidazole. [b]Conversion values calculated by ^{1}H NMR and isolated yields in brackets. [c]Productivity = concentration (g/L) × (yield/100)/time (h). [d]All the experiment working at fixed MW nominal power = 150 W.

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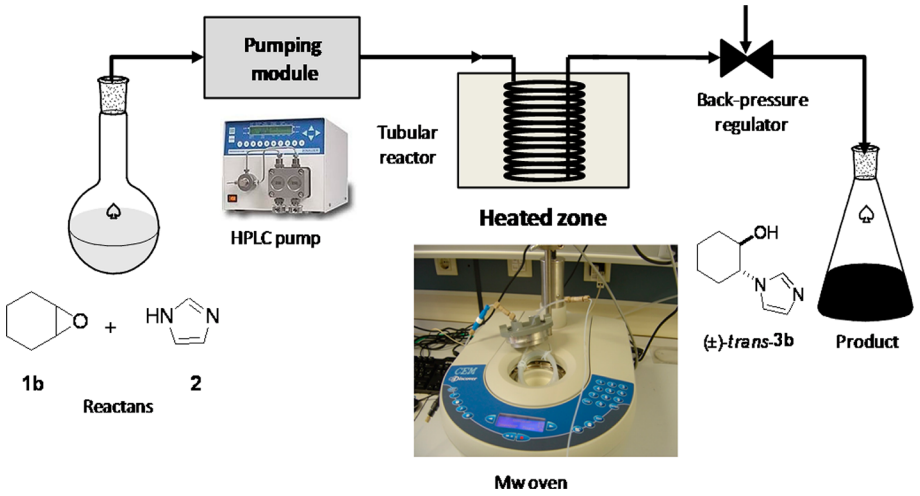


Figure 1. Schematic setup for the epoxide ring-opening reaction under flow conditions using the MW system as the heating source.

Table 2. Continuous Flow Ring-Opening Reaction of Cyclohexene Oxide (2.2 M in THF) with Imidazole (2 M in THF) Using MW Heating[d]

entry	flow (mL/min)	$t_{residence}$ (min)[a]	conversion (%)[b]	productivity (g/h)[c]
1	0.25	23	97	4.9
2	0.3	19	98	5.9
3	0.5	12	99	9.9
4	1	6	92	18.4
5	1.5	4	79	23.6
6	2	3	58	23.2

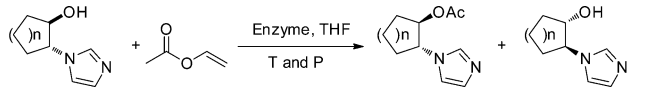
[a]Residence time in the reactor = reactor volume (mL)/flow (mL/min). Reactor volume (mL) = 5.84 mL (300 × 0.15748 cm). [b]Conversion values calculated by ^{1}H NMR of the reaction crude. [c]Productivity = flow (mL/min) × concentration (mmol/mL) × (% conversion/100) × M. weight (product, mg/mmol) × 60 (min/h). [d]10 W, 135−140 °C, 17−20 bar.

We also evaluated the same experimental setup under conventional heating, immersing the reactor in an oil-bath at the same reaction temperature. The results obtained for this system at 135 °C and 19 bar pumping 0.3 mL/min of a solution of imidazole (**2**, 2 M) and cyclohexene oxide (**1b**, 2.2 M) in THF, were very similar compared to the ones achieved for the MW system, with conversions over 95%.

Regarding productivity, the MW batch addition of the imidazole to the epoxide takes place in a quite efficient manner when working at a fixed temperature of 150 °C and a nominal power of 150 W (25−30 W experimental), being a quantitative conversion achieved in only 5 min of reaction. However, this time does not account for the heating and cooling time required to get the set temperature and cool the reaction down. Thus, the complete cycle (heating-reaction-cooling) requires about 10 min. Hence, in 1 h, a maximum of 6 consecutive batch experiments can be carried out, which means a productivity of 9.9 g/h. This productivity is the same achieved at 0.5 mL/min under flow conditions as the residence time is quite similar than the time we are accounting for a single batch cycle (∼ 12 min). However, if the volumetric productivity is considered, the STY obtained in the flow system (10.3 mol × L^{-1} × h) was 5 times higher than the one obtained under batch conditions (2 mol × L^{-1} × h). Thus, a certain degree of process intensification can be claimed for the flow process as a higher amount of product per unit of time is produced with the same reactor volume. Furthermore, at a flow rate of 1 mL/min a productivity of about 18 g/h (with an associated 92% yield) can be achieved. It must be noted that the same productivity is obtained with the flow system being heated either under MW or conventional conditions (Flow: 0.3 mL/min, reactor volume: 5.84 mL, Yield/Productivity: MW, 98%/5.9 g/h; conventional heating: 93%/5.6 g/h). Our results agree with other studies from the recent literature, which demonstrate that small-scale homogeneous MW batch chemistry can be successfully translated to a scalable high-temperature/pressure continuous-flow format utilizing conventionally heated micro- or mesofluidic platforms.[19]

KR of (±)-*trans*-2-(1*H*-imidazol-1-yl)cycloalkanols. The synthesis of enantiopure imidazole derivatives by enzymatic KR of the corresponding racemic alcohols (Scheme 2) is possible

Scheme 2. Enzymatic Kinetic Resolution of Racemic *trans*-2-(1*H*-imidazol-1-yl)cycloalkanols *trans*-3a-b



using *Candida antarctica* lipase type B or *Pseudomonas cepacia* lipase (PSL-C I, also known as *Burkholderia cepacia* lipase) as the biocatalyst in the presence of an organic solvent (TBME or THF).[7−9] The continuous flow processes can help to increase the efficiency of biotransformations as the use of continuous biocatalytic processes present some distinct advantages such as the easy separation of products and the continuous reutilization of the enzyme in consecutive cycles, avoiding downtime, and the consequent loss of productivity.[20−23] The productivity is also favored by the instant enzyme/substrate ratios achieved in a flow system, which may be significant higher in a fixed-bed packed continuous reactor than in a conventional batch one.[24−27] Furthermore, as some enzymatic reactions are inhibited by the products, the constant removal of the potentially inhibitory substance may clearly improve the efficiency of the process.

Figure 2 shows a schematic representation of the continuous flow reactor used to perform the KR of the racemic alcohols

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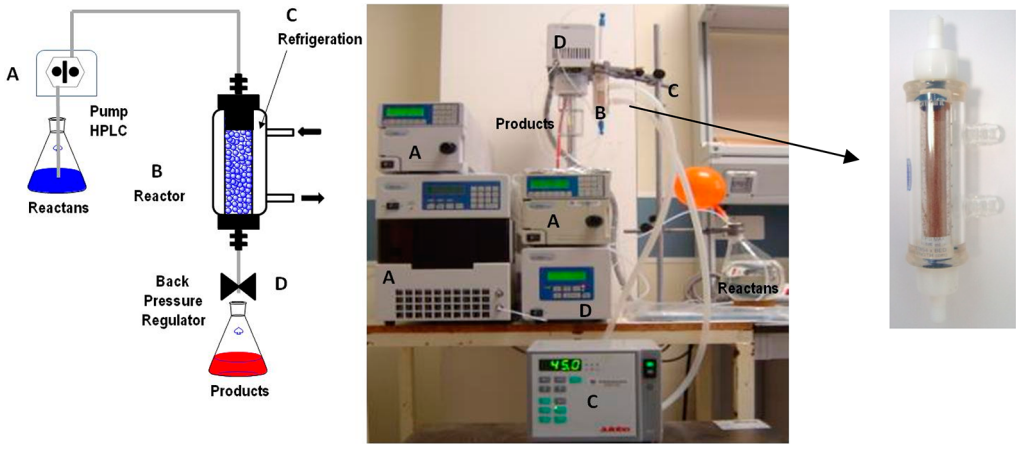

Figure 2. Continuous flow reactor setup used for the flow KR studies. A: pumping system; B: Packed bed reactor Omnifit (100 × 10 mm); C: Refrigeration system; D: Back pressure regulator.

trans-**3a**–**b**. The system is formed by a HPLC pump that allows feeding the reactor, which is a fix-packed bed reactor loaded with a commercial supported enzyme.[28] At the reactor exit, a back pressure regulator is installed to allow working at a variable moderate pressure (600 psi). Thus, a solution of the racemic alcohol *trans*-**3b** (0.02 M) and the acylating agent (5 equiv, 0.10 M) in THF was pumped through the catalytic bed, which is packed with PSL-C I, at 45 °C and 40 bar, using different flow rates. Samples were collected after the back pressure regulator at constant time intervals and analyzed by chiral HPLC.

The results obtained for the continuous KR *trans*-**3b** catalyzed by PSL-C I are summarized in Table 3. Thus, for a flow rate of 0.1 mL/min, a conversion of the alcohol *trans*-**3b** of 50% was found (entry 1, Table 3) with excellent enantiopurities for both product [(*R*,*R*)-*trans*-**5b**] and substrate [(*S*,*S*)-*trans*-**3b**]. However, when the flow rate was increased 10 times (from 0.1 mL/min to 1 mL/min, entry 2, Table 3), only the racemic alcohol *trans*-**3b** was detected. A flow rate of 0.5 mL/min led to a conversion of 43% recovering the product (*R*,*R*)-*trans*-**5b** in enantiomerically pure form (entry 3, Table 3). The efficiency of the system could be further improved using a larger excess of acylating agent (10 equiv of vinyl acetate), a more concentrated reactants solution (0.08 M of *trans*-**3b** in THF). The resolution was performed at 45 °C and 40 bar using flow rates ranging from (0.1 to 5 mL/min). The results demonstrate that it is possible to adjust the flow rate to achieve the KR of the alcohol *trans*-**3b** with a 50% of conversion and excellent enantioselectivities (>99%) for both alcohol (*S*,*S*)-*trans*-**3b** and acylated product (*R*,*R*)-*trans*-**5b**.

In a similar way, it was possible to perform the KR of the five-membered ring racemic alcohol *trans*-**3a**. The reaction was assayed at 45 °C, 40 bar, and flow rates ranging from 0.025 mL/min to 1.25 mL/min. The lower flow rate (0.025 mL/min) was needed to reach 50% of conversion with excellent enantiopurity for both substrate (*S*,*S*)-*trans*-**3a** and product (*R*,*R*)-*trans*-**5a** (see Table 4). These results suggest that the KR for the alcohol *trans*-**3a** is significantly slower than the KR for the *trans*-**3b** using PSL-C I.

Table 3. Continuous Flow Kinetic Resolution of the Racemic Alcohol *trans*-3b with Vinyl Acetate (4) Catalyzed by PSL-C I in THF at 45 °C and 40 ± 1 bar

entry	ratio (**3b**/**4**)	conc. (M)[a]	flow (mL/min)	ee_s (%)[b]	ee_p (%)[b]	*c* (%)[c]	*E*[d]
1	1:5	0.02	0.1[e]	>99	>99	50	>200
2	1:5	0.02	1[e]	0	0	0	0
3	1:5	0.02	0.5[e]	76	>99	43	>200
4	1:10	0.08	0.1[f]	>99	95	51	>200
5	1:10	0.08	0.25[f]	>99	97	51	>200
6	1:10	0.08	0.5[f]	>99	99	50	>200
7	1:10	0.08	0.75[f]	>99	99	50	>200
8	1:10	0.08	1[f]	>99	>99	50	>200
9	1:10	0.08	1.25[f]	92	99	48	>200
10	1:10	0.08	1.5[f]	87	99	43	>200
11	1:10	0.08	2[f]	51	99	34	>200
12	1:10	0.08	3[f]	32	99	24	>200
13	1:10	0.08	4[f]	21	99	18	>200
14	1:10	0.08	5[f]	20	99	17	>200

[a]Concentration of racemic alcohol. [b]Enantiomeric excesses (ee's) calculated by HPLC. [c]Conversion values: $c = ee_s/(ee_s+ee_p)$ [d]$E = \ln [(1 - c) \times (1 - ee_p)]/[(1 - c) \times (1 + ee_p)]$.[29] [e]Fixed-bed reactor packed with 3.4 g of PSL-C I. [f]Fixed-bed reactor packed with 4.4 g of PSL-C I.

Table 4. Continuous Flow KR of (±)-*trans*-3a with Vinyl Acetate (4) Catalyzed by PSL-C I[e]

entry	flow (mL/min)[a]	ee_s (%)[b]	ee_p (%)[b]	*c* (%)[c]	*E*[d]
1	1.25	12	>99	11	>200
2	1	14	>99	12	>200
3	0.75	17	>99	15	>200
4	0.5	25	>99	20	>200
5	0.025	>99	>99	50	>200

[a]Column packed with 4.4 g of PSL-C I. [b]Enantiomeric excesses (ee's) determined by HPLC. [c]Conversion values: $c = ee_s/(ee_s + ee_p)$. [d]$E = \ln [(1 - c) \times (1 - ee_p)]/[(1 - c) \times (1 + ee_p)]$.[29] [e]Ratio **3a**/**4** = 1:10, THF (0.08 M of alcohol), 45 °C and 40 ± 1 bar.

Additionally, the batch KRs of the alcohols *trans*-**3a**–**b** were also studied using a ratio substrate/PSL-C I (1:1) using a fixed concentration of 0.08 M of the corresponding racemic alcohols in THF at 45 °C. The kinetics of the reactions were followed taking samples at regular time intervals that were then analyzed by HPLC. The results obtained for those studies are depicted in Figure 3.

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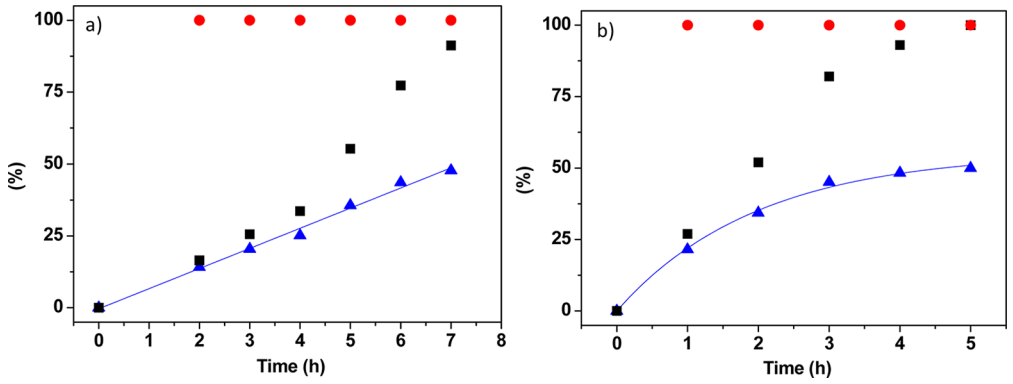

Figure 3. Kinetic profiles for the KR of racemic alcohols *trans*-**3a-b** with vinyl acetate (**4**) in batch at 45 °C. Circle: ee % product; Square: ee % substrate; Triangle: % alcohol conversion. (a) 20.46 mL, *trans*-**3b** (THF) = 13.2 mg/mL, 274 mg PSL-C I. (b) 20 mL, *trans*-**3a** (THF) = 12.5 mg/mL, 256 mg PSL-C I.

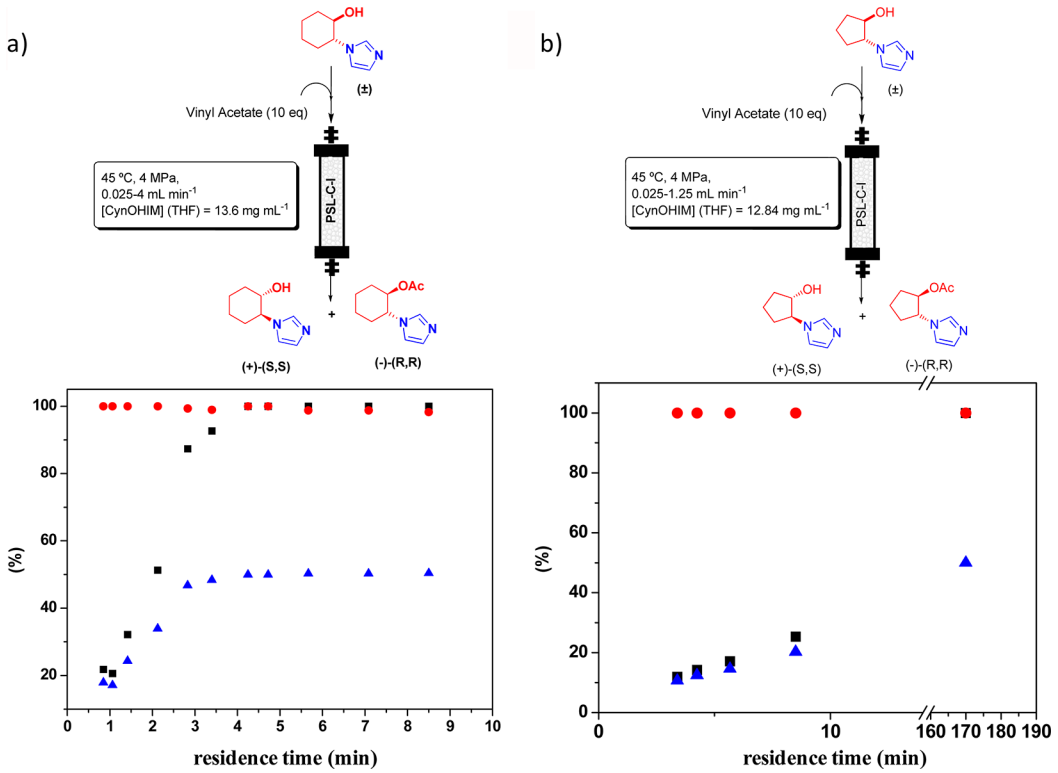

Figure 4. Kinetic profiles found for the continuous flow KR of the racemic alcohols *trans*-**3a-b** catalyzed by PSL-C I. Circle: ee % product; Square: ee % substrate; Triangle: % alcohol conversion. (a) Data for **3b**. (b) Data for **3a**.

Unexpectedly the batch results showed that, under batch condition, the activity found for the alcohol *trans*-**3a** was much higher than the one observed for *trans*-**3b**. This trend is opposite to the one found for the flow system, where the reactivity for alcohol *trans*-**3b** was higher than that observed for *trans*-**3a**. To rationalize these results and properly compare the KR carried out either at batch or under continuous flow conditions additional residence time distribution (RTD) studies for the flow system were performed. These studies allow us to estimate the mean residence time and, therefore, the free volume of the fix-bed reactor. Thus, for the fix bed-reactor packed with 4.4 g of PSL-C I in a column of 100 × 10 mm the free volume was 4.257 mL (see Supporting Information). This volume allows us to calculate the residence time for each flow rate used. Thus, the conversion can be plotted vs the residence time (reaction time) to calculate the kinetic parameters for the continuous flow systems (see Figure 4).

Table 5 shows the values of specific activity, turnover frequency (TOF) calculated for the 50% of conversion, and space time yield (STY) values obtained for both batch and continuous KR of the alcohols *trans*-**3a-b**. The results showed that the specific activity for the alcohol *trans*-**3a** is higher for the batch process than for the KR performed under flow conditions, while the opposite situation is found for the alcohol *trans*-**3b**. The reactor configuration can minimize this effect due to enhanced mixing and a higher and optimized contact substrate/enzyme, since the continuous-flow configuration ensures the accumulation of products toward the end of the column and the constant contact of fresh substrate with the enzyme. Regarding the process intensification when going from batch to continuous flow process, it should be mentioned that

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Table 5. Batch vs Continuous Kinetic Resolution of Racemic Alcohols *trans*-3a−b Catalyzed by PSL-C I

entry	substrate	specific activity[a]	t_{50} (min)[b]	STY ($g \cdot L^{-1}\ h^{-1}$)[c]
1	**3a** (batch)	24.17	450	1.5
2	**3a** (flow)	2.24	170	2.9
3	**3b** (batch)	6.75	540	0.92
4	**3b** (flow)	12.91	4	117.1

[a]Specific Activity in μmol g^{-1} min^{-1}. [b]In batch: time to achieve 50% conversion. *In flow: residence time necessary to achieve 50% conversion.* [c]STY (Space time yield) (batch) = (mmol of substrate (mmol) × (% conversion/100) × M. weight (product))/(volume (mL) × time (h)); STY (Space time yield) (flow) = (concentration substrate (mmol/mL) × flow (mL/min) × (% conversion/100) × M. weight (product) × (60 min/h))/volume (mL); volume reactor (batch) = 20 mL, volume reactor (flow) = 4.26 mL.

for the KR of the alcohol *trans*-**3b** only 4 min are required to achieve a 50% of conversion with a reactor of a small volume (4.26 mL) under flow conditions. On the contrary, 9 h are needed to get the same degree of conversion for the process in batch. The flow system is able to produce, during this time (9 h), about of 4.5 g of the acylated product (*R,R*)-*trans*-**5b** compared with the 0.116 g yielded by the batch process. For the substrate *trans*-**3a** as a result of the low specific activity found for the flow process, the increase on productivity going from batch to flow process is not so significant. However, a small increase on the STY is found moving from batch reaction ($1.5\ g \cdot L^{-1} \cdot h^{-1}$, entry 1, Table 5) to a continuous flow process ($2.9\ g \cdot L^{-1} \cdot h^{-1}$, entry 2, Table 5).

Alternatively, the KRs of the alcohols *trans*-**3a** and *trans*-**3b** were performed using CAL-B instead of PSL-C I as the immobilized biocatalyst. This biocatalyst has already proved to be active for the resolution of these substrates under batch conditions.[7] The KR was optimized, once again, using different flow rates (see Supporting Information) allowing us to obtain conditions in which the conversion of the alcohols was about 50%, yielding both product and substrate with excellent enantiopurities (>99%). Table 6 shows the optimized flow

Table 6. Optimized Continuous Flow Conditions for the Stereoselective Acetylation of Racemic Alcohols *trans*-3a-b Catalyzed by Different Supported Biocatalysts

entry	substrate	enzyme	flow (mL/min)	c (%)[a]	STY ($g \cdot L^{-1}\ h^{-1}$)[b]
1	**3a**	CAL-B	0.5	50	58.7
2	**3a**	PSL-C I	0.025	50	3.1
3	**3b**	CAL-B	0.05	50	6.1
4	**3b**	PSL-C I	1	50	117.7

[a]Conversion values $c = ee_s/(ee_s + ee_p)$. [b]STY (Space time yield) = (concentration substrate (mmol/mL) × flow (mL/min) × (conversion/100) × M. weight (product) × (60 min/h))/volume (mL); volume reactor = 4.26 mL.

conditions leading to efficient continuous KR of the corresponding racemic alcohol using either PSL-C I or CAL-B as the biocatalyst. In this case, CAL-B shows a significant higher STY values for the substrate *trans*-**3a** than those achieved with the PSL-C I under equivalent experimental conditions ($58.7\ g \cdot L^{-1} \cdot h^{-1}$ for CAL-B vs $3.1\ g \cdot L^{-1} \cdot h^{-1}$ for PSL-C I). For the substrate *trans*-**3b** the opposite trend is observed, the KR process being more efficient when PSL-C I is used as the biocatalyst ($117.7\ g \cdot L^{-1} \cdot h^{-1}$ for PSL-C I vs $6.1\ g \cdot L^{-1} \cdot h^{-1}$ for CAL-B). Hence, using the right set of conditions and biocatalyst a very efficient continuous flow KR of *trans*-**3a** or *trans*-**3b** can be carried out in terms of productivity and stereoselectivity. Indeed, for a reactor of about 4.4 mL, it is possible to produce in 10 h about of 2.5 g of the enantiopure ester (*R,R*) *trans*-**5a** (>99% ee) and 2 g of enantiopure alcohol (*S,S*)-*trans*-**3a** (>99% ee) using CAL-B as the biocatalyst or alternatively about 5 g of enantiopure acylated product (*R,R*) *trans*-**5b** (>99% ee) and 4 g of the enantiopure alcohol of (*S,S*)-*trans*-**3b** (>99% ee) using PSL-C I as the biocatalyst.

The RTD studies suggest that the reactor packed with PSL-C I is more efficient from an engineering point of view than the analogous one based on CAL-B, taking into account the shapes of the residence time distribution curves ($E(\theta)$) as function of a dimensional time and of the σ^2 values obtained at the different flow rates assayed in the pulse tracer experiments (see Supporting Information).[30] However, the two biocatalyst preparations are very different in several aspects, like the type of protein, nature of the support, enzyme loading, specific surface area, and so forth, which should also have a strong influence on the catalytic efficiency. Indeed, for the substrate *trans*-**3a** the more efficient reactor is obtained when CAL-B was used as the biocatalyst even when the behavior of the reactor does not fit so well to an ideal plug-flow reactor than the analogous system packed with PSL-C I.

Finally the stability of the biocatalytic systems was assayed. Figure 5a shows the different HPLC spectra obtained during 9 h of continuous KR of the racemic alcohol *trans*-**3b** catalyzed by PSL-C I at a flow rate of 0.9 mL/min, 45 °C, and 40 bar. The acylated product and the substrate were obtained with excellent enantiopurities. This catalytic cycle can be run at least during two additional cycles of 9 h reaction without observing any lost in the biocatalytic performance. Additionally, the KR of the racemic alcohol *trans*-**3a** catalyzed by CAL-B was carried out at least 11 consecutive days working 24 h a day without showing any significant decay in the activity (50%) or enantioselectivity (>99%) of the process, highlighting the excellent stability of the system for the continuous synthesis of the enantiopure (*R,R*)-*trans*-**5b** and (*S,S*)-*trans*-**3b** (Figure 5b).

CONCLUSIONS

The use of continuous flow systems has allowed us to develop a simple methodology to efficiently synthesize enantiopure 1-(2-hydroxycycloalkyl)imidazoles with good productivities, which facilitates the preparation of these building blocks for the overall synthesis of chiral imidazolium ionic liquids. The syntheses of both racemic five- and six-membered ring alcohols were first optimized using MW heating under batch conditions. The use of MW heating allows reducing the reaction times by several hours, in comparison to the use of conventional heating under reflux conditions, so as to reach complete conversions in a few minutes. To scale-up those protocols, a system for continuous ring-opening reaction under MW heating was developed. Under these conditions, it was possible to easily scale-up the production of the racemic alcohols. It should be mentioned, however, that the same results can be obtained using the continuous flow setup under conventional heating. These results suggest that small-scale homogeneous MW batch chemistry can be successfully translated to a scalable high-temperature/pressure continuous-flow format utilizing conventionally heated mesofluidic platforms according to the "microwave-to-flow" concept.[19] Finally, the KR to obtain the enantiopure 1-(2-hydroxycycloalkyl)imidazoles can be performed very efficiently under continuous flow conditions. In

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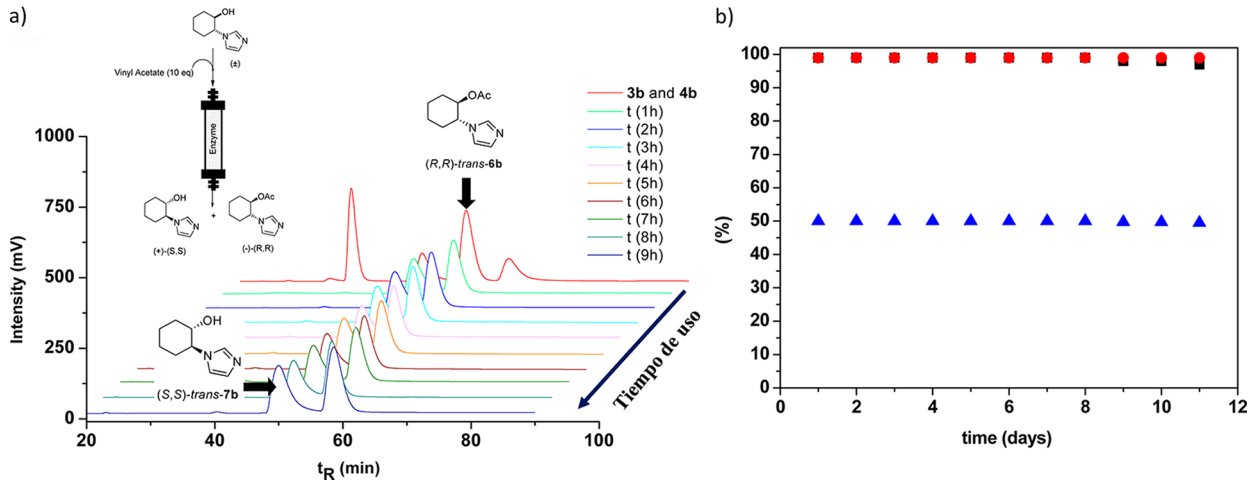

Figure 5. Stability study for the KR of the racemic alcohol *trans*-**3b** with PSL-C I (panel a) or CAL-B (panel 5b) at 45 °C and 40 ± 1 bar. Circle: ee % product; Square: ee % substrate; Triangle: % alcohol conversion.

all the cases assayed, it was possible to obtain a set of optimized conditions (type of the biocatalyst, flow rate, temperature, pressure, etc.) for which the results obtained in terms of productivity were significantly better than the ones found for the analogous batch process. Furthermore, the high stability of the biocatalytic systems allowed us to easily produce appreciable amounts of the enantiopure imidazoles to be used for future applications in the preparation of novel chiral Ionic Liquids (CILs), asymmetric phase transfer catalysts, or as precursors for gas or liquid chromatography stationary phases.

EXPERIMENTAL SECTION

C. antarctica lipase type B (CAL-B, Novozyme 435, 7300 PLU/g) was a gift from Novozymes and PSL-C I (1638 U/g) was purchased from Sigma-Aldrich. All other reagents were obtained from different commercial sources and used without further purification. Dry solvents were distilled over an adequate desiccant under nitrogen. Flash chromatographies were performed using silica gel 60 (230−240 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR experiments were obtained using a Varian INOVA 500 (^{1}H, 500 MHz and ^{13}C, 125 MHz) spectrometer. The chemical shifts are given in delta (δ) values and the coupling constants (J) in hertz (Hz). The mass spectrometry experiments were performed in a hybrid QTOF (quadrupole-TOF-hexapolo) with an orthogonal Z-spray interface-electrospray (Micromass, Manchester, U.K.). HPLC analyses were carried out in a Merck HITACHI LaChrom chromatograph UV detector at 210 nm using a using a Daicel CHIRALCEL OD-H column (25 cm ×4.6 mm I.D.). Further detailed data are provided in the Supporting Information.

General Procedure for the Preparation of Racemic Alcohols *trans*-3a-b under Batch Conditions. A solution of the imidazole **2** (2.11 g, 30.88 mmol) and the cycloalkene oxide (**1a** or **1b**, 33.94 mmol) in THF (18 mL) under a nitrogen atmosphere was refluxed for the required time. Then, the solvent was evaporated under reduced pressure, and the resulting crude purified by flash chromatography on silica gel (2−10% MeOH/CH_2Cl_2) yielding the racemic alcohols (±)-*trans*-**3a-b** as white solids (26−19%).

Solvent-Free Reaction. A mixture of the cycloalkene oxide (**1a** or **1b**, 0.23 mol) and the imidazole (**2**, 15 g, 0.22 mol) under a nitrogen atmosphere, was heated at 60 °C for 22−25 h. After that time, a white-yellow solid was formed, and the resulting crude was purified by flash chromatography on silica gel (2−10% MeOH/CH_2Cl_2), isolating the racemic alcohols yielding (±)-*trans*-**3a-b** as white solids (60%).

MW Reaction. A solution of the imidazole (**2**, 12.50 mmol) and the cycloalkene oxide (**1a** or **1b**, 13.75 mmol) in THF (5 mL) was heated in a CEM MW oven at 150 °C, high stirring speed, and variable power (Power (set) = 150 W). After that, the solvent was evaporated under reduced pressure, and the resulting crude purified by flash chromatography on silica gel (2−10% MeOH/CH_2Cl_2) yielding (±)-*trans*-**3a** or (±)-*trans*-**3b** as white solids.

General Procedure for the Synthesis of *trans*-3b under Continuous Flow Conditions. A mixture of the imidazole (**2**, 47.6 mL, 2.5 M) and the cyclohexene oxide (**1b**, 2.8 M) in THF was pumped through the tubular reactor heated by a MW oven (constant power P = 10 W) or by conventional heating at different flow rates, 17 bar and 140 °C. The samples were collected at the reactor's outlet and analyzed by NMR. All the fractions obtained under the same experimental conditions were mixed, the solvent was concentrated under reduced pressure, and the crude purified by flash chromatography on silica gel (2−10% MeOH/CH_2Cl_2), isolating the racemic alcohol (±)-*trans*-**3b** as a white solid.

General Procedure for the Enzymatic Kinetic Resolution of the Racemic Alcohols *trans*-3a-b under Batch Conditions. A suspension of racemic alcohol (±)-*trans*-**3a-b** (6.02 mmol), vinyl acetate (**4**, 1.67 mL, 18.07 mmol), and the corresponding enzyme (PSL-C I or CAL-B, 1:1 ratio by weight respect to the alcohol) in dry THF (60.2 mL) was shaken under nitrogen atmosphere for the time required at 25−45 °C and 250 rpm. Aliquots were regularly analyzed by HPLC until around 50% conversion was reached; then the reaction was stopped, the enzyme filtered off, and washed with CH_2Cl_2 (3 × 50 mL). The solvent was evaporated under reduced pressure, and the reaction crude purified by flash chromatography on silica gel (2−10% MeOH/CH_2Cl_2) affording the corresponding optically enriched acetates (*R*,*R*)-*trans*-**5a-b** and alcohols (*S*,*S*)-*trans*-**3a-b**.

General Procedure for KR of the Alcohols *trans*-3a-b under Continuous Flow Conditions. A solution of the racemic alcohol (±)-*trans*-**3a-b** (36.06 mmol) and vinyl acetate **4** (ratio **3a-b**: **4** 1:10 in weight) in dry THF (408 mL) was

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pumped, under nitrogen atmosphere, through a column packed with the corresponding enzyme (PSL-C I or CAL-B) at 45 °C, 40 bar and different flows. Aliquots were regularly taken at the exit of the reactor and analyzed by HPLC. All the aliquots obtained under the same conditions having the same degree of conversion and enantiopurity were mixed, the solvent was evaporated under reduced pressure, and the resulting crude purified by flash chromatography on silica gel (2−10% MeOH/CH_2Cl_2) affording the corresponding optically enriched acetates (*R*,*R*)-*trans*-**5a**-**b** and alcohols (*S*,*S*)-*trans*-**3a**-**b**.

■ ASSOCIATED CONTENT

Supporting Information

Further experimental details and Figures SI-1 to SI-7 and Tables SI-1 and SI-2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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