EurJOC
European Journal of Organic Chemistry

A Journal of
ChemPubSoc Europe

Accepted Article

Title: High-Throughput Preparation of Optically Active Cyanohydrins Mediated by Lipases

Authors: Leandro Piovan; Juliana Christina Thomas; Bruno Bernardi Aggio; Alfredo Ricardo Marques De Oliveira

To be cited as: Eur. J. Org. Chem. 10.1002/ejoc.201601028

Link to VoR: http://dx.doi.org/10.1002/ejoc.201601028

Supported by
ACES Asian Chemical Editorial Society

WILEY-VCH

FULL PAPER WILEY-VCH

High-throughput preparation of optically active cyanohydrins mediated by lipases

Juliana Christina Thomas,[a] Bruno Bernardi Aggio,[a] Alfredo Ricardo Marques de Oliveira,[a] Leandro Piovan*[a]

Abstract: Cyanohydrins are versatile compounds with high applicability in organic synthesis, being employed as starting materials for other chemical targets with high industrial added value. Lipase-mediated kinetic resolution reactions are a promising route for the synthesis of optically active cyanohydrins. These reactions can be performed via cyanohydrin acylation or deacylation of cyanohydrin esters, with different biocatalysts and reaction conditions. Unfortunately, depending on substrate structure, high reaction times are required to achieve suitable enantiomeric excesses. In this context, we present a high throughput protocol to produce optically active cyanohydrins in continuous-flow mode. Compounds were obtained with moderate to good enantioselectivity (E values from 8 up to >200) and with productivity values 2.4 to 8.7-fold higher in continuous-flow than in batch mode. Moreover, reaction time was reduced from hours, in batch mode, to minutes in continuous-flow mode.

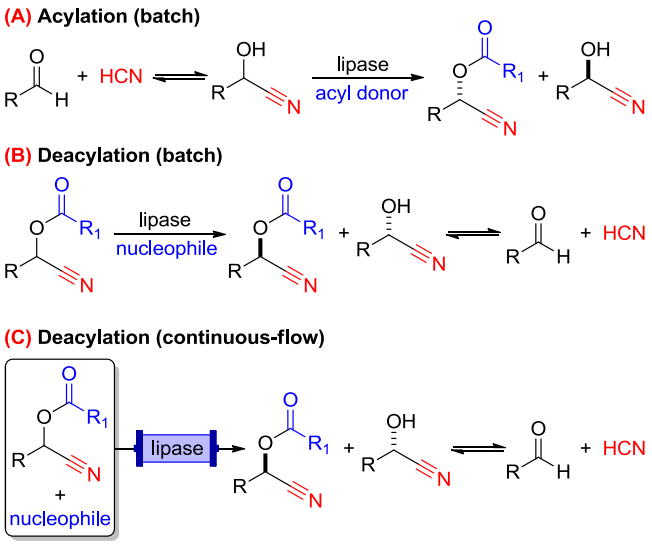
Introduction

Cyanohydrins are versatile compounds with several applications as chiral building blocks;[1] in this context, they can be employed as precursors of β-aminoalcohols,[2] α-hydroxyacids,[3] α-hydroxyketones[4] and other compounds with high added value in the pharmaceutical industry.[5] Cyanohydrins have already been employed as precursors of different drugs, such as fluoxetine, duloxetine and derivatives,[6] capuramycin,[7] bufuralol,[8] denopamine and salbutamol.[9]

Optically active cyanohydrins can be prepared either via asymmetric catalysis – mediated by metal-organic frameworks[8] and metal complexes[10] – or biocatalytic methods based on the use of oxynitrilases[11] and peptide based catalysis.[12] Another relevant way to achieve optically active cyanohydrins employs lipase-mediated kinetic resolution (EKR) reactions, which can be performed via acylation of cyanohydrins (Scheme 1 – A) or by hydrolysis/alcoholysis of cyanohydrin esters (Scheme1 - B), in both cases with classical[13-25] or dynamic resolution.[26]

[a] J. C.Thomas, B. B. Aggio, A. R. M. de Oliveira, L. Piovan
Department of Chemistry
Universidade Federal do Paraná
Paraná, Brazil
E-mail: lpiovan@quimica.ufpr.br

Supporting information for this article is given via a link at the end of the document.



Scheme 1. Cyanohydrins EKR via acylation (A), deacylation (B) and deacylation in continuous-flow mode (C).

Several examples of EKR reactions involving cyanohydrins have already been described; their involve acylation by *Burkholderia cepacia*[13] and *Candida antarctica* lipase B;[14] hydrolysis by *Bacillus coagulans*,[15] *Bacillus licheniformis*,[16] *Candida rugosa*,[17] *Pseudomonas aeruginosa*,[18] metagenome-derived esterases[19] and several other proteases;[20] alcoholysis by *Candida antarctica* lipase A,[21] *Candida antarctica* lipase B,[22] *Candida rugosa*[23] and *Pseudomonas fluorescens*;[24] and aminolysis by *Candida antarctica* lipase A.[25] Results indicate that, depending on substrate structure, despite of good enantioselectivity, long reaction times are required to achieve high enantiomeric excesses.

Promising dynamic kinetic resolution (DKR) reactions of cyanohydrins have also been reported. They involve a racemization agent and have the theoretical advantage of total conversion. For them, different racemization agents and biocatalysts have been described in the literature.[26] In this case reaction times are even larger than those observed for EKR reactions and high enantiomeric excess are strongly dependent of racemization agent and substrate structure.

Currently, a trend in biocatalytic transformations is their performance in continuous-flow mode and several enzymes have already been employed as biocatalysts in this mode, such as aldolases,[27] amidases,[28] transaminases,[29] oxidases,[30] peroxidases[31] and especially lipases, in esterification,[32] interesterification,[33] transesterification[34] and EKR reactions [35]

Although resolution reactions have been increasingly carried out in continuous-flow (CF) mode,[36] cyanohydrins have so far been

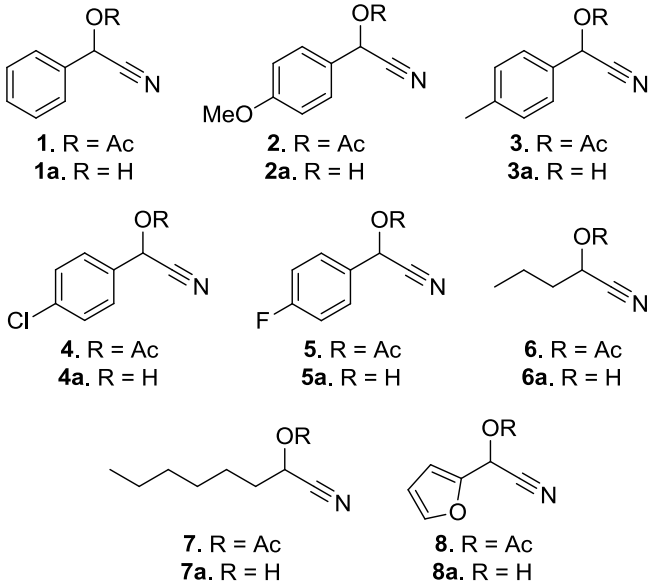
underexplored as CF substrates.[37] Considering that continuous-flow can offer several advantages over batchwise reactions, including reproducibility, efficient control of reaction parameters, fast/homogeneous heating, lower costs in reaction optimization and the observation of green chemistry principles,[38] the development of fast CF protocols for cyanohydrin resolution is highly desirable.

This work investigates the performance of the two modes in the biocatalytic resolution of benzylic, aliphatic and heterocyclic cyanohydrins; for this, we employed deacylation reactions with *Candida antarctica* lipase B (Scheme 1 – A and B). Calculated enantioselectivity and productivity parameters revealed that the continuous-flow mode provides higher productivity despite some loss of enantioselectivity. All reactions carried out in CF presented lower reactions time than batch mode. Particularly good results were obtained with benzylic cyanohydrins employing both batch and CF modes, with a surprising increase on enantioselectivity in CF mode for halogenated compounds.

Results and Discussion

Selection of Substrates

A series of compounds was synthesized in order to investigate the effect of aromatic (**1-5**), aliphatic (**6** and **7**) and heterocyclic (**8**) substituents attached to stereogenic center (Figure 1) on the lipase-mediated EKR. For aromatic compounds, the effect of electron donor and electron withdrawing groups was also investigated.



1. R = Ac
1a. R = H

2. R = Ac
2a. R = H

3. R = Ac
3a. R = H

4. R = Ac
4a. R = H

5. R = Ac
5a. R = H

6. R = Ac
6a. R = H

7. R = Ac
7a. R = H

8. R = Ac
8a. R = H

Figure 1. Cyanohydrin esters (**1-8**) employed as substrates and their cyanohydrin parent compounds (**1a-8a**).

Chemical Synthesis

Cyanohydrin esters (**1**-**8**) were synthetized in two consecutive reactions. Firstly, cyanohydrins were prepared from their corresponding aldehydes and then submitted to acylation with acetic anhydride as acyl donor and 4-(*N,N*-dimethylamino)pyridine (DMAP) as catalyst (see experimental section and supporting information for details).

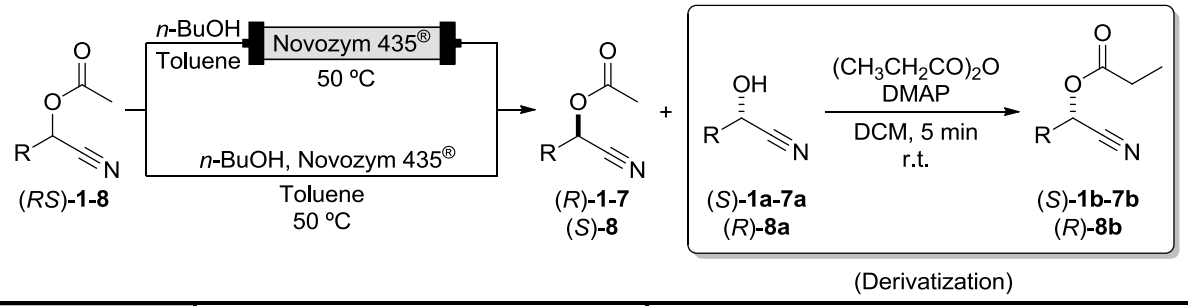
Enzymatic Kinetic Resolution (EKR) Reactions

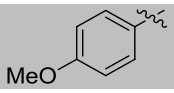
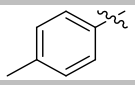
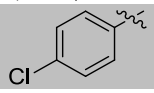
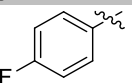
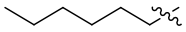
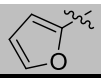
Cyano(phenyl)methyl acetate (**1**) was chosen as a model compound for preliminary assays. It was employed as acyl donor and *n*-butanol as nucleophile in the acylation reaction mediated by *Candida antarctica* lipase B. Cyanohydrin product was derivatized to its corresponding propionate in order to determine enantioselectivity and conversion parameters.

After optimization of reaction conditions, EKR of **1** reached 50% of conversion with high enantioselectivity (E > 200) in 8 h (Table 1 – entry 1). When the same reaction was performed in continuous-flow, results were even better, since the productivity parameter was higher than in batch mode (12.5 in continuous-flow and 5.2 in batch; Table 1 – entry 1) and reaction time was 48-fold lower in continuous-flow than in batch mode.

Based on the high enantioselectivy and productivity, as well as the short reaction time observed in EKR reactions employing cyano(phenyl)methyl acetate (**1**) as acyl donor, the strategy was expanded to include the cyanohydrin esters **2**-**9** (Table 1).

Table 1. EKR reactions with cyanohydrin esters (**1-9**) in batch and continuous-flow modes



#	R	Batch						Flow							
		t / h	c [a] / %	e.e. [b] / %		E [d]	r [e] / µmol min^{-1} g^{-1}	f / mL min^{-1}	CY	t [f] / h	c [a] / %	e.e. [b] / %		E [d]	r [g] / µmol min^{-1} g^{-1}
				$e.e._s$	$e.e._p$ [c]							$e.e._s$	$e.e._p$ [c]		
1		8	50	>99	98	>200	5.2	0.1	2	0.14	50	97	98	>200	12.5
2		12	50	98	97	>200	3.5	0.1	2	0.14	50	97	93	116	12.8
3		12	50	98	97	>200	3.5	0.1	2	0.14	50	96	94	127	12.5
4		24	62	86	53	9	1.8	0.1	2	0.14	52	90	82	31	13.0
5		24	53	97	96	55	1.5	0.1	2	0.14	52	96	90	74	13.0
6		4	54	99	84	60	11.3	0.1 (0.1)	1 (2)	0.07 (0.13)	66 (51)	90 (88)	47 (83)	8 (31)	33.0 (25.5)
7		24	54	98	88	71	1.9	0.1 (0.1)	2 (2)	0.14 (0.13)	54 (28)	92 (37)	78 (96)	26 (70)	13.5 (14.0)
8		12	60	90	61	12	4.2	0.1	1	0.07	53	88	78	23	26.5

Reaction conditions: batch mode: cyanohydrin ester (0.1 mmol), *n*-butanol (0.4 mmol), toluene (2 mL) and Novozym 435® (20 mg). Flow mode: cyanohydrin ester (0.1 mol L^{-1}), *n*-butanol (0.4 equivalents), toluene (5 mL) and Novozym 435® (200 mg). Temperature for both modes: 50 °C. [a] Conversion: ee_s / (ee_s + ee_p). [b] Enantiomeric excess: (R – S) / (R + S) x 100 (determined by chiral GC analysis). [c] Determined by derivatization to corresponding propionate. [d] Enantiomeric ratio: $E = \ln \{[ee_p (1 - ee_s)] / (ee_p + ee_s)\} / \ln \{[ee_p (1 + ee_s)] / (ee_p + ee_s)\}$. [e] Productivity (batch): n_P / t m_e. [f] Residence time: reactor volume / flow rate. [g] Productivity (flow): [P] *f* / m_e. CY: cycles of elution

EKR of cyano(phenyl)methyl acetate **1** (Table 1 – entry 1), as already described, presented high E values in both modes and productivity higher in continuous-flow than in batch. For cyano(4-methoxyphenyl)methyl acetate (**2**) and cyano(*p*-tolyl)methyl acetate (**3**), the presence of the methoxy or methyl groups led to higher reaction times (Table 1 – entries 2 and 3). This was already expected, because electron donor substituents such as –OMe and -Me, usually slow down this type of reaction. There are also steric effects when compounds **2** and **3** are compared to model compound **1**, which can also justify the lower reaction rate for *p*-substituted compounds. Selectivity in continuous-flow and batch mode was comparable for **2** and **3**, with higher productivity observed in continuous-flow. Halogenated compounds (4-chlorophenyl)(cyano)methyl acetate (**4**) and cyano(4-fluorophenyl)methyl acetate (**5**), in turn, presented the most significant differences between their corresponding results with higher selectivity in continuous-flow (Table 1 – entries 4 and 5). This fact could be justified by a possible lipase inhibition by reaction products (chloro- and fluoro-cyanohydrin and aldehyde) as already reported for other lipases[39] and other classes of enzymes.[40] Although this effect is strongly felt in batch mode, it can be minimized in continuous-flow, since the products are quickly removed from the contact with the biocatalyst. It is important to highlight that compounds **4** and **5** presented the longest reaction times in batch mode, corroborating the hypothesis of enzyme inhibition.

For aliphatic compounds 1-cyanobutyl acetate (**6**) and 1-cyanoheptyl acetate (**7**), continuous-flow EKR presented less selectivity than batch reactions (Table 1 – entries 6 and 7). Considering that the cyanohydrin EKR of aliphatic compounds is usually more challenging than EKR of benzylic compounds, *E* values are, usually, lower for these compounds,[41] and that the continuous-flow approach employs a larger amount of enzyme than batch mode, is possible that both enantiomers to be transformed. Accordingly, when compounds **6** and **7** were

submitted to EKR using a smaller amount (100 mg) of biocatalyst, *E* values were higher than those obtained with 200 mg (Table 1 – entries 6 and 7, values in parenthesis), although they were still lower than those obtained in batch mode. On the other hand, despite this lower enantioselectivity in continuous-flow, high productivity values were observed for both compounds **6** and **7**. Results gathered for the heterocyclic cyanohydrin ester **8**, in turn, were all very similar to those given by the chloro-substituted substrate **4**, also suggesting some degree of impairing interaction between the biocatalyst and the substrate (Table 1 – entry 8).

In general, the greatest difference between EKR in batch and CF modes was evidenced by the productivity parameter (*r*), which considers the quantity of biocatalyst and measure how much product can be obtained in 1 min using 1 g of enzyme.[42] For cyanohydrin esters **1**-**8**, productivity values were always higher in continuous-flow than in the batch mode (Figure 2). The most significant difference between productivities in batch and continuous-flow was observed for halogenated cyanohydrins **4** and **5**, which presented productivities 7.2 and 8.7-fold higher in continuous-flow than in batch, respectively.

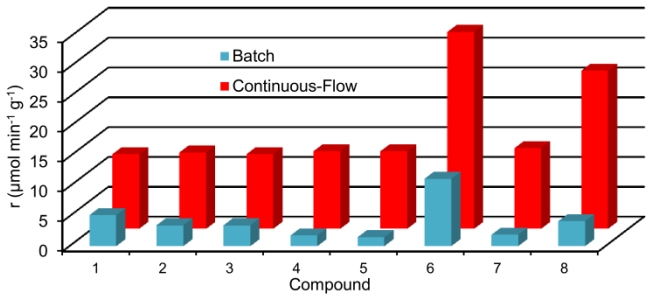


Figure 2. Comparative productivity values for EKR of cyanohydrin esters **1**-**8** in batch and continuous-flow modes.

Finally, a scaled-up reaction was performed in order to evaluate the reproducibility of results obtained in analytical scale and to demonstrate the robustness of our homemade continuous-flow system. For this purpose 1.051 g of cyanohydrin ester **1**, was resolved employing same reaction conditions described in Table 1 (entry 1). After the resolution reaction and compounds chromatographic separation, (*R*)-**1** and (*S*)-**1a** were obtained in 34% and 37% isolated yield, respectively. It is important to highlight that no significant changes in estereoselectivity parameters were observed since (*R*)-**1** and (*S*)-**1a** were isolated with 97% and 95% enantiomeric excess, respectively, in accordance with analytical scale.

In summary, a high-throughput protocol to achieve optically active cyanohydrins, together with a comparison between EKR reactions in continuous-flow and batch modes, have been presented in this work. Although EKR of all substrates presented here have already been described in literature, it is the first time they were performed in continuous-flow mode.

For all substrates employed, continuous-flow reactions presented higher productivities than their batch counterparts. Changes in enantioselectivity on going from the batch to the CF approach were observed - increase for compounds **4**, **5** and **8** and decrease for **6** and **7** - depending on steric and electronic demands of the substituent groups attached to the chiral center.

Conclusions

A successful protocol to achieve optically active cyanohydrins in continuous-flow mode was developed. Reaction times considerably decreased compared to batch mode and even with values found in literature. With this novel protocol, different optically active cyanohydrin esters can be achieved in few minutes of reaction. For compounds that may present inhibitory activity, the use of continuous-flow reactions is even more interesting, since compounds are quickly removed from contact with biocatalyst, as observed for halogenated cyanohydrin esters.

Experimental Section

*General Procedure for the Syntheses of Racemic Cyanohydrin Esters **1**-**5** and **8***

A solution of $Na_2S_2O_5$ (6 mmol, 1.141 g) in 5 mL of water was kept in an ice bath and received the dropwise addition of the suitable aldehyde (10 mmol). After magnetic stirring for 10 min, a solution of KCN (10 mmol, 0.652 g) in cold water (5 mL) was added dropwise, the ice bath was removed and the mixture was stirred at room temperature for 24 h. It was then extracted with dicloromethane (3 x 10 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure.[43] The resulting cyanohydrin was immediately submitted to a sequent acylation reaction, in which it was solubilized in dichloromethane (10 mL) and received the addition of both acetic anhydride (15 mmol, 1.42 mL) and DMAP (one crystal). This reaction medium was left stirring overnight at room temperature, after which it was washed with a solution of $NaHCO_3$ up to pH 8. The organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure. Residual aldehyde was removed by crystallization as its bisulphite salt.

Cyano(phenyl)methyl acetate (**1**). Yield: 62%. GC-MS (70 eV), m/z (relative intensity): 175 ($M^{\bullet+}$, 16%), 133 (76%), 115 (74%), 105 (35%), 89 (30%), 77 (21%), 63 (16%), 51 (19%), 43 (100%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ (ppm): 2.17 (s, 3H); 6.41 (s, 1H); 7.42-7.54 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 20.4; 62.9; 116.1; 127.9; 129.2; 130.4; 131.8; 168.9. IR (cm^{-1}): 3067, 3037, 2944, 1754, 1496, 1458, 1369, 1216, 1024, 756, 697.

Cyano(4-methoxyphenyl)methyl acetate (**2**). Yield: 35%. GC-MS (70 eV), m/z (relative intensity): 205 ($M^{\bullet+}$, 30%), 163 (25%), 146 (100%), 135 (20%), 116 (23%), 103 (17%), 91 (23%), 76 (21%), 63 (9%), 50 (10%), 43 (32%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ (ppm): 2.15 (s, 3H); 3.83 (s, 3H); 6.36 (s, 1H); 6.95 (d, *J* = 8.7 Hz, 2H); 7.45 (d, *J* = 8.7 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 20.5; 55.4; 62.6; 114.6; 116.3; 123.9; 129.6; 161.1; 169.0. IR (cm^{-1}): 3006, 2960, 2938, 2840, 1752, 1611, 1514, 1467, 1371, 1258, 1211, 1175, 1026, 960, 829.

Cyano(p-tolyl)methyl acetate (**3**). Yield: 63%. GC-MS (70 eV), m/z (relative intensity): 189 ($M^{\bullet+}$, 37%); 147 (85%); 129 (100%); 119 (36%); 103 (44%); 91 (27%); 77 (24%); 65 (17%); 51 (11%); 43 (48%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ (ppm): 2.16 (s, 3H); 2.39 (s, 3H), 6.37 (s, 1H) 7.25 (d, *J* = 8.2, 2H); 7.41 (d, *J* = 8.2 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$),

δ (ppm): 20.5; 21.3; 62.7; 116.2; 127.9; 128.9; 129.9; 140.7; 169.0. IR (cm^{-1}): 3032, 2926, 2862, 1754, 1613, 1515, 1372, 1214, 1020, 963, 812..

(4-chlorophenyl)(cyano)methyl acetate (**4**). Yield: 30%. GC-MS (70 eV), m/z (relative intensity): 209 (M•+, 17%); 167 (50%); 149 (63%); 139 (24%); 132 (10%); 123 (17%); 114 (45%); 88 (9%); 75 (16%); 63 (8%); 50 (8%); 43 (100%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ (ppm): ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 2.18 (s, 3H); 6.39 (s, 1H); 7.40-7.54 (m, 4H) IR (cm^{-1}): 3092, 3069, 2942, 1754, 1596, 1492, 1416, 1370, 1212, 1090, 1014, 964, 819.

Cyano(4-fluorophenyl)methyl acetate (**5**). Yield: 30%. GC-MS (70 eV), m/z (relative intensity): 193 ($M^{\bullet+}$, 12%); 151 (54%); 133 (100%); 123 (30%); 107 (35%); 95 (15%); 75 (12%); 57 (12%); 43 (94%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ (ppm): 2.17 (s, 3H); 6.39 (s, 1H); 7.09-7.20 (m, 2H); 7.48-7.57 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 20.4; 62.2; 116.0; 116.4 (d, *J* = 22.3 Hz); 127.8 (d, *J* = 3.0 Hz); 130.1 (d, *J* = 8.8 Hz); 163.8 (d, *J* = 250.1 Hz); 168.8. IR (cm^{-1}): 3079, 2947, 1755, 1606, 1511, 1425, 1373, 1218, 1161, 1023, 963, 832.

Cyano(furan-2-yl)methyl acetate (**8**). Yield: 39%. GC-MS (70 eV), m/z (relative intensity): 166 (8%); 139 (28%); 124 (4%); 108 (4%); 97 (100%); 81 (10%); 69 (15%); 52 (18%); 43 (69%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ (ppm): 2.18 (s, 3H); 6.45 (dd, *J* = 3.4; 1.9 Hz, 1H); 6.48 (s, 1H); 6.69 (dm, *J* = 3.4 Hz, 1H); 7.52 (dd, *J* = 1.9; 0.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 20.3; 55.7; 111.1; 112.5; 114.1; 145.5; 168.7. IR (cm^{-1}): 3154, 3129, 2955, 2922, 2851, 1749, 1496, 1368, 1209, 1016, 753.

*General Procedure for the Syntheses of Racemic Cyanohydrin Esters **6** and **7***

A solution of KCN (15 mmol, 0.977 g) in 20 mL of methanol was cooled down to 0 ^{o}C and received the dropwise addition of the corresponding aldehyde (10 mmol) in methanol (2 mL). After stirring for 15 min, glacial acetic acid (20 mmol, 1.15 mL) was added dropwise, the ice bath was removed and the mixture was warmed up to room temperature while stirring for 45 min. Dicloromethane (10 mL) was then added and the reaction medium was washed with a solution of $NaHCO_3$ up to pH 8. The solvent was dried over $MgSO_4$, filtered and evaporated under reduced pressure.[44] This reaction product was submitted to a subsequent acylation reaction, according to the previous experimental procedure. The cyanohydrins obtained from these syntheses were employed in EKR reactions without further purification.

1-cyanobutyl acetate (**6**). Yield: 49%. GC-MS (70 eV), m/z (relative intensity): 112 (1%); 99 (9%); 87 (2%); 81 (6%); 71 (4%); 61 (4%); 57 (11%); 43 (100%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ (ppm): 1.00 (t, *J* = 7.4 Hz, 3H); 1.45-1.63 (m, 2H); 1.84-1.95 (m, 2H); 2.14 (s, 3H); 5.33 (t, *J* = 6.7 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 13.3; 17.9; 20.3; 34.2; 60.9; 116.9; 169.2. IR (cm^{-1}): 2965, 2878, 1757, 1467, 1372, 1220, 1111, 1035.

1-cyanoheptyl acetate (**7**). Yield: 85%. GC-MS (70 eV), m/z (relative intensity): 184 ($M^{\bullet+}$, 1%), 154 (1%), 140 (2%), 122 (2%), 112 (7%), 95 (14%), 81 (23%), 70 (8%), 55 (27%), 43 (100%). ^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ (ppm): 0.89 (t, *J* = 6.6 Hz, 3H); 1.26-1.53 (m, 8H); 1.85-1.95 (m, 2H); 2.14 (s, 1H); 5.31 (t, *J* = 6.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 13.9; 20.4; 22.4; 24.4; 28.4; 31.4; 32.2; 61.1; 116.9; 169.2. IR (cm^{-1}): 2956, 2932, 2861, 1755, 1464, 1373, 1222, 1037.

General Procedure for the Syntheses of Propionates

Acylations with propionic anhydride (5 µL) and DMAP (one crystal) were carried out directly in aliquots taken from reaction mixture and followed a procedure similar to those described in the previous sections for reactions with acetic anhydride. After acylation, the reaction mixtures were stirred at room temperature for 5 min and neutralized with aqueous $NaHCO_3$. The organic layers were then dried over anhydrous $MgSO_4$ before analysis.

General Procedure for EKR Reactions of Cyanohydrin Esters in Batch Mode

In a 4 mL sealed vial, cyanohydrin esters **1**-**8** (0.1 mmol) were solubilized in toluene (2 mL). To this solution, *n*-butanol (0.4 mmol, 37 µL) and the supported enzyme CAL-B (Novozym 435®, 20 mg) were added. From the reaction mixture kept stirring at constant temperature (50 ºC), aliquots were periodically taken for analysis by chiral GC.

Continuous-Flow (CF) System

Our continuous-flow system consisted of a syringe pump connected to the reactor through a Teflon cannula. The reactor itself was an empty HPLC stainless steel column (74.0 x 4.6 mm) that was previously washed to remove the stationary phase. It was then filled with the biocatalyst, the supported lipase Novozym 435® (200 mg or 100 mg, internal volume 0.43 mL and 0.40 mL, respectively), and deactivated glass wool in both ends, in order to prevent enzyme agglomeration on the top of reactor. A homemade heating block (8.5 x 5.0 x 2.0 cm) controlled by a commercial thermostat was used to set the reaction temperatures.

General Procedure for EKR Reactions in Continuous-Flow Mode

The cyanohydrin esters **1**-**8** (0.5 mmol) and *n*-butanol (2 mmol, 0.18 mL) were dissolved in toluene (5 mL) and then eluted through a packed-bed column with the biocatalyst (200 mg) in a flow rate ranging from 0.1 to 1 mL min^{-1}. Aliquots (0.5 mL) collected for each flow rate were analysed by chiral GC. The reactor internal volume was equal to 0.43 mL.

*General Procedure for Scaled-up EKR of Cyanohydrin Ester **1***

Cyanohydrin ester **1** (6 mmol, 1.051 g) and *n*-butanol (24 mmol, 2.20 mL) were dissolved in toluene (60 mL) and then eluted through the reactor in a 0.1 mL min^{-1} flow rate. After 2 cycles, toluene was removed under reduced pressure and the crude material was chromatographed (10:1 hexanes/ethyl acetate). After solvent removing, (*R*)-**1** and (*S*)-**1a** were recovered in 34% and 37% isolated yield, respectively.

Absolute configuration assignment

Absolute configurations of compounds were attributed via optical rotation and comparison with the literature data. For this purpose, compounds **1**-**8** were submitted to EKR in preparative scale, employing continuous-flow mode, and compounds were separated via flash column (10:1 hexanes/ethyl acetate). Enantiomeric excesses were determined via chiral GC analyses.

(*R*)-*cyano(phenyl)methyl acetate* [(*R*)-**1**]. $[\alpha]_D^{20}$ 3.71 (c = 0.5, $CHCl_3$); e.e. 93%. Lit.: $[\alpha]_D^{25.8}$ 4.1 (c = 1.16, $CHCl_3$); e.e. 85%.[45]

(*S*)-*2-hydroxy-2-phenylacetonitrile* [(*S*)-**1a**]. $[\alpha]_D^{20}$ -27.29 (c = 0.5, $CHCl_3$); e.e. 96%. Lit.: $[\alpha]_D^{20}$ -8.6 (c = 0.50, $CHCl_3$); e.e. 81%.[46]

(*R*)-*cyano(4-methoxyphenyl)methyl acetate* [(*R*)-**2**]. $[\alpha]_D^{20}$ -15.11 (c = 0.5, $CHCl_3$); e.e. 97%. Lit.: $[\alpha]_D^{24.1}$ -4.7 (c = 1.14, $CHCl_3$); e.e. 27%.[45]

(*S*)-*2-hydroxy-2-(4-methoxyphenyl)acetonitrile* [(*S*)-**2a**]. $[\alpha]_D^{20}$ -31.07 (c = 0.5, $CHCl_3$); e.e. 99%. Lit.: $[\alpha]_D^{20}$ -43.6 (c = 1.25, $CHCl_3$); e.e. 93%.[47]

(*R*)-*cyano(p-tolyl)methyl acetate* [(*R*)-**3**]. $[\alpha]_D^{20}$ -6.09 (c = 0.5, $CHCl_3$); e.e. 97%. Lit.: $[\alpha]_D^{23.8}$ -2.5 (c = 1.27, $CHCl_3$); e.e. 30%.[45]

(*S*)-*2-hydroxy-2-(p-tolyl)acetonitrile* [(*S*)-**3a**]. $[\alpha]_D^{20}$ -40.17 (c = 0.5, $CHCl_3$); e.e. 96%. Lit.: $[\alpha]_D^{20}$ -31.5 (c = 0.51, $CHCl_3$); e.e. 68%.[48]

(*R*)-*(4-chlorophenyl)(cyano)methyl acetate* [(*R*)-**4**]. $[\alpha]_D^{20}$ -8.04 (c = 0.5, $CHCl_3$); e.e. 95%. Lit.: $[\alpha]_D^{25.2}$ -2.5 (c = 1.11, $CHCl_3$); e.e. 21%.[45]

(*S*)-*2-(4-chlorophenyl)-2-hydroxyacetonitrile* [(*S*)-**4a**]. $[\alpha]_D^{20}$ -33.10 (c = 0.5, $CHCl_3$); e.e. 96%. Lit.: $[\alpha]_D^{20}$ -28.5 (c = 1.10, $CHCl_3$); e.e. 70%.[49]

(*R*)-*cyano(4-fluorophenyl)methyl acetate* [(*R*)-**5**]. $[\alpha]_D^{20}$ 2.49 (c = 0.5, $CHCl_3$); e.e. 73%. Lit.: for (*S*) enantiomer $[\alpha]_D^{23}$ -6.7 (c = 0.012, $CHCl_3$); e.e. 92%.[14]

(*S*)-*2-(4-fluorophenyl)-2-hydroxyacetonitrile* [(*S*)-**5a**]. $[\alpha]_D^{20}$ -11.65 (c = 0.5, $CHCl_3$); e.e. 97%. Lit.: $[\alpha]_D^{20}$ -16.2 (c = 0.79, $CHCl_3$); e.e. 74%.[46]

(*R*)-*1-cyanobutyl acetate* [(*R*)-**6**]. $[\alpha]_D^{20}$ 81.48 (c = 0.5, $CHCl_3$); e.e. 85%. Lit.: for (*S*) enantiomer $[\alpha]_D^{25}$ -56.8 (c = 0.08, $CHCl_3$); e.e. 87%.[50]

(*S*)-*2-hydroxypentanenitrile* [(*S*)-**6a**]. $[\alpha]_D^{20}$ -19.24 (c = 0.5, $CHCl_3$); e.e. 60%. Lit.: $[\alpha]_D^{20}$ -21.8 (c = 0.97, $CHCl_3$); e.e. 98%.[46]

(*R*)-*1-cyanoheptyl acetate* [(*R*)-**7**]. $[\alpha]_D^{20}$ 40.94 (c = 0.5, $CHCl_3$); e.e. 65%.

(*S*)-*2-hydroxyoctanenitrile* [(*S*)-**7a**]. $[\alpha]_D^{20}$ -10.27(c = 0.5, $CHCl_3$); e.e. 90%. Lit.: $[\alpha]_D^{21}$ -13.3 (c = 1.00, $CHCl_3$); e.e. 98%.[51]

(*S*)-*cyano(furan-2-yl)methyl acetate* [(*S*)-**8**]. $[\alpha]_D^{20}$ -22.28 (c = 0.5, $CHCl_3$); e.e. 90%. Lit.: $[\alpha]_D^{20}$ -26.3 (c = 1.40, $CHCl_3$); e.e. 99%.[52]

(*R*)-*2-(furan-2-yl)-2-hydroxyacetonitrile* [(*R*)-**8a**]. $[\alpha]_D^{20}$ -1.06 (c = 0.5, $CHCl_3$); e.e. 90%. Lit.: $[\alpha]_D^{20}$ -36.1 (c = 1.07, $CHCl_3$); e.e. 83%.[49]

Acknowledgements

L. Piovan and A. R. M. Oliveira thank the Brazilian National Council for Scientific and Technological Development (CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support (Proc. 456834/2014). J. C. Thomas thanks CAPES for a Ph.D. fellowship.

Keywords: biocatalysis, continuous-flow, cyanohydrins, kinetic resolution, lipases.

[1] a) Gregory, R. J. H. *Chem. Rev.* **1999**, *99*, 3649-3682. b) North, M. *Tetrahedron: Asymmetry* **2003**, *14*, 147-176.

[2] Ziegler, T.; Hörsch, B.; Effenberger, F. *Synthesis* **1990**, *1990*, 575-578.

[3] Effenberger, F.; Eichhorn, J.; Ross, J. *Tetrahedron: Asymmetry* **1995**, *6*, 271-282.

[4] Krepski, L. R.; Jensen, K. M.; Heilmann, S. M.; Rasmussen, J. K. *Synthesis* **1986**, *1986*, 301-303.

[5] Gotor, V. *Org. Process Res. Dev.* **2002**, *6*, 420-426.

[6] Rej, R. K.; Das, T.; Hazra, S.; Nanda, S. *Tetrahedron: Asymmetry* **2013**, *24*, 913-918.

[7] Kurosu, M.; Li, K.; Crick, D. C. *Org. Lett.* **2009**, *11*, 2393-2396.

[8] Mo, K.; Yang, Y.; Cui, Y. *J. Am. Chem. Soc.* **2014**, *136*, 1746-1749.

[9] Brown, R. F. C.; Donohue, A. C.; Jackson, W. R.; McCarthy, T. D. *Tetrahedron* **1994**, *50*, 13739-13752.

[10] a) Achard, T. R. J.; Clutterbuck, L. A.; North, M. *Synlett* **2005**, *12*, 1828-1847. b) Belda, O.; Duquesne, S.; Fischer, A.; Moberg, C. *J. Organomet. Chem.* **2004**, *689*, 3750-3755. c) Khan, N. H.; Kureshy, R. I.; Abdi, S. H. R.; Agrawal, S.; Jasra, R. V. *Coord. Chem. Rev.* **2008**, *252*, 593-623. d) Niemeyer, J. Cloppenburg, J.; Fröhlich, R.; Kehr, G.; Erker, G. *J. Organomet. Chem.* **2010**, *695*, 1801-1812.

[11] a) Han, S. Q.; Ouyang, P. K.; Wei, P.; Hu, Y. *Biotechnol. Lett.* **2006**, *28*, 1909-1912. b) Fang, F.; Ji, A.; Meng, Z. *React. Kinet. Catal. Lett.* **2008**, *93*, 233-239. c) Huang, S. R.; Liu, S. L.; Zong, M. H.; Xu, R. *Biotechnol. Lett.* **2005**, *27*, 79-82. d) Hietanen, A.; Kanerva, L. T. *Eur. J. Org. Chem.* **2012**, *2012*, 2729-2737. e) Hietanen, A.; Ekholm, F. S.; Leino, R.; Kanerva, L. T. *Eur. J. Org. Chem.* **2010**, *2010*, 6974-6980. f) Gonzalo, G.; Brieva, R.; Gotor, V. *J. Mol. Catal. B: Enzym.* **2002**, *19-20*, 223-230. g) Holt, J.; Hanefeld, U. *Curr. Org. Synth.* **2009**, *6*, 15-37.

[12] a) Carrea, G.; Colonna, S.; Kelly, D. R.; Lazcano, A.; Ottolina, G.; Roberts, S. M. *Trends Biotechnol.* **2005**, *23*, 507-513. b) Ohno, H.; Nitta, H.; Tanaka, K.; Mori, A.; Inoue, S. *J. Org. Chem.* **1992**, *57*, 6778-6783. c) Mori, A.; Nitta, H.; Kudo, M.; Inoue, S. *Tetrahedron Lett.* **1991**, *32*, 4333-4336.

[13] a) Xu, Q.; Xie, Y.; Geng, X.; Chen, P. *Tetrahedron* **2010**, *66*, 624-630. b) Kamal, A.; Khanna, G. B. R. *Tetrahedron: Asymmetry* **2001**, *12*, 405-410. c) Shen, Z. L.; Zhou, W. J.; Liu, Y. T.; Ji, S. J.; Loh, T. P. *Green Chem.* **2008**, *10*, 283-286. d) Howell, J. A. S.; Humphries, K.; McArdle, P.; Cunningham, D.; Nicolosi, G.; Patti, A.; Walsh, M. A. *Tetrahedron: Asymmetry* **1997**, *8*, 1027-1030. e) Xu, Q.; Geng, X.; Chen, P. *Tetrahedron Lett.* **2008**, *49*, 6440-6441. f) Wang, Y. F.; Chen, S. T.; Liu, K. K. C.; Wong, C. H. *Tetrahedron Lett.* **1989**, *30*, 1917-1920. g) Gonzalo, G.; Lavandera, I.; Brieva, R.; Gotor, V. *Tetrahedron* **2004**, *60*, 10525-10532.

[14] Ribeiro, S. S.; Ferreira, I. M.; Lima, J. P. F.; Sousa, B. A.; Carmona, R. C.; Santos, A. A.; Porto, A. L. M. *J. Braz. Chem. Soc.* **2015**, *26*, 1344-1350.

[15] Ota, H.; Miyamae, Y.; Tsuchihashi, G. *Agric. Biol. Chem.* **1989**, *53*, 281-283.

[16] Holt, J.; Arends, I. W. C. E.; Minnaard, A. J.; Hanefeld, U. *Adv. Synth. Catal.* **2007**, *349*, 1341-1344.

[17] Maguire, N. M.; Ford, A.; Clarke, S. L.; Eccles, K. S.; Lawrence, S. E.; Brossat, M.; Moody, T. S.; Maguire, A. R. *Tetrahedron: Asymmetry* **2011**, *22*, 2144-2150.

[18] Sakai, T.; Miki, Y.; Nakatani, M.; Ema, T.; Uneyama, K.; Utaka, M. *Tetrahedron Lett.* **1998**, *39*, 5233-5236.

[19] Nguyen, G. S.; Kourist, R.; Paravidino, M.; Hummel, A.; Rehdorf, J.; Orru, R. V. A.; Hanefeld, U.; Bornscheuer, U. T. *Eur. J. Org. Chem.* **2010**, *2010*, 2753-2758.

[20] Konigsberger, K.; Prasad, K.; Repic, O. *Tetrahedron: Asymmetry* **1999**, *10*, 679-687.

[21] Bencze, L. C.; Paizs, C.; Toșa, M. I.; Vass, E.; Irimie, F. *Tetrahedron: Asymmetry* **2010**, *21*, 443-450.

[22] a) Belokon, Y. N.; Blacker, A. J.; Clutterbuck, L. A.; Hogg, D.; North, M.; Reeve, C. *Eur. J. Org. Chem.* **2006**, *2006*, 4609-4617. b) Veum, L.; Kuster, M.; Telalovic, S.; Hanefeld, U.; Maschmeyer, T. *Eur. J. Org. Chem.* **2002**, *2002*, 1516-1522. c) Hanefeld, U.; Li, Y.; Sheldon, R. A.; Maschmeyer, T. *Synlett* **2000**, *12*, 1775-1776.

[23] a) Lundell, K.; Raijola, T.; Kanerva, L. T. *Enzyme Microb. Technol.* **1998**, *22*, 86-93. b) Kanerva, L. T.; Kiljunen, E.; Hunhtanen, T. T. *Tetrahedron: Asymmetry* **1993**, *4*, 2355-2361.

[24] Zhou, R.; Xu, J. H. *Biochem. Eng. J.* **2005**, *23*, 11-15.

[25] Rodríguez, J. A. R.; Gotor, V.; Brieva, R. *Tetrahedron Asymmetry* **2011**, *22*, 1218-1224.

[26] a) Paizs, C.; Tähtinen, P.; Lundell, K.; Poppe, L.; Irimie, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2003**, *14*, 1895-1904. b) Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, J. *J. Am. Chem. Soc.* **1991**, *113*, 9360-9361. c) Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, J. *J. Org. Chem.* **1992**, *57*, 5643-5649. d) Sakai, T.; Wang, K.; Ema, T. *Tetrahedron* **2008**, *64*, 2178-2183. e) Sakulsombat, M.; Vongvilai, P.; Ramström, O. *Org. Biomol. Chem.* **2012**, *9*, 1112-1117. f) Paizs, C.; Toşa, M.; Majdik, C.; Tähtinen, P.; Irimie, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2003**, *14*, 619-627. g) Paizs, C.; Tähtinen, P.; Toşa, M.; Majdik, C.; Irimie, F.; Kanerva, L. T. *Tetrahedron* **2004**, *60*, 10533-10540. h) Veum, L.; Kanerva, L. T.; Haling, P. J.; Maschmeyer, T.; Hanefeld, U. *Adv. Synth. Catal.* **2005**, *347*, 1015-1021. i) Veum, L.; Hanefeld, U. *Tetrahedron: Asymmetry* **2004**, *15*, 3707-3709. j) Li, Y. X.; Straathof, A. J. J.; Hanefeld, U. *Tetrahedron: Asymmetry* **2002**, *13*, 739-743. k) Veum, L.; Hanefeld, U. *Synlett*, **2005**, *15*, 2382-2384. l) Pàmies, O.; Bäckvall, J. E. *Adv. Synth. Catal.* **2002**, *344*, 947-952. m) Laurell, A.; Moberg, C. *Eur. J. Org. Chem.* **2011**, *2011*, 3980-3984. n) Wen, Y. Q.; Hertzberg, R.; Gonzalez, I.; Moberg, C. *Chem. Eur. J.* **2014**, *20*, 3806-3812.

[27] Lamble, H. J.; Royer, S. F.; Hough, D. W.; Danson, M. J.; Taylor, G. L.; Bull, S. D. *Adv. Synth. Catal.* **2007**, *349*, 817-821.

[28] Preez, R.; Clarke, K. G.; Callanan, L. H.; Burton, S. G. *J. Mol. Catal. B: Enzym.* **2015**, *119*, 48-53.

[29] Andrade, L. H.; Kroutil, W.; Jamison, T. F. *Org. Lett.* **2014**, *16*, 6092-6095.

[30] a) Zambelli, P.; Tamborini, L.; Cazzamalli, S.; Pinto, A.; Arioli, S.; Balzaretti, S.; Plou, F. J.; Arrojo, L. F.; Molinari, F.; Conti, P.; Romano, D. *Food Chem.* **2016**, *190*, 607-613. b) Tomaszewski, B.; Schmid, A.; Buehler, K. *Org. Process Res. Dev.* **2014**, *18*, 1516-1526.

[31] Ruiz, I. R.; Codina, E. M.; Ackermann, T. N.; Llobera, A. *Microfluid Nanofluidics* **2015**, *18*, 1277-1286.

[32] a) Madarász, J.; Németh, D.; Bakos, J.; Gubicza, L.; Bakonyi, P. *J. Clean Prod.* **2015**, *93*, 140-144. b) Sutili, F. K.; Ruela, H. S.; Nogueira, D. O.; Leal, I. C. R.; Miranda, L. S. M.; Souza, R. O. M. A. *Sustain. Chem. Process.* **2015**, *3*, 6. c) Sutili, F.; Nogueira, D. O.; Leite, S. G. F.; Miranda, L. S. M.; Souza, R. O. M. A. *RSC Adv.* **2015**, *5*, 37287-37291. d) Junior, I. I.; Flores, M. C.; Sutili, F. K.; Leite, S. G. F.; Miranda, L. S. M.; Leal, I. C. R.; Souza, R. O. M. A. *Org. Process Res. Dev.* **2012**, *16*, 1098-1101. e) Wang, S. S.; Li, Z. J.; Sheng, S.; Wu, F. A.; Wang, J. J. *Chem. Technol. Biotechnol.* **2015**, *6*, 449-425. f) Woodcock, L. L.; Wiles, C.; Greenway, G. M.; Watts, P.; Wells, A.; Eyley, S. *Biocatal. Biotransform.* **2008**, *26*, 501-507.

[33] a) Varón, E. Y.; Joli, J. E.; Balcells, M.; Torres, M.; Garayoa, R. C. *RSC Adv.* **2012**, *2*, 9230-9236. b) Hernandez, A. L.; Otero, C.; Martín, E. H.; Garcia, H. S.; Hill, C. G. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 1147-1159.

[34] a) Tran, D. T.; Lin, Y. J.; Chen, C. L.; Chang, J. S. *Appl. Energ.* **2014**, *126*, 151-160. b) Wang, J.; Gu, S. S.; Cui, H. S.; Wu, X. Y.; Wu, F. A. *Bioresour. Technol.* **2014**, *158*, 39-47. c) Carnero, A.; Sanghvi, Y. S.; Gotor, V.; Fernández, S.; Ferrero, M. *Org. Process Res. Dev.* **2015**, *19*, 701-709.

[35] a) Manoel, E. A.; Pais, K. C.; Flores, M. C.; Miranda, L. S. M.; Coelho, M. A. Z.; Simas, A. B. C.; Freire, D. M. G.; Souza, R. O. M. A. *J. Mol. Catal. B: Enzym.* **2013**, *87*, 139-143. b) Liu, Z.; Burgess, K. *Tetrahedron: Lett.* **2011**, *52*, 6325-6327. c) Reetz, M. T.; Wiesenhöfer, W.; Franciò, G.; Leitner, W. *Chem. Commun.* **2002**, 992-993. d) Hellner, G.; Boros, Z.; Tomin, A.; Poppe, L. *Adv. Synth. Catal.* **2011**, *353*, 2481-2491. e) Boros, Z.; Falus, P.; Márkus, M.; Weiser, D.; Oláh, M.; Hornyánszky, G.; Nagy, J.; Poppe, L. *J. Mol. Catal. B: Enzym.* **2013**, *85-86*, 119-125. f) Boros, Z.; Weiser, D.; Márkus, M.; Abaháziová, E.; Magyar, A.; Tomin, A.; Koczka, B.; Kovács, P.; Poppe, L. *Process Biochem.* **2013**, *48*, 1039-1047.

[36] Junior, I. I.; Miranda, L. S. M.; Souza, R. O. M. A. *J. Mol. Catal. B: Enzym.* **2013**, *85-86*, 1-9.

[37] Hamberg, A.; Lundgren, S.; Wingstrand, E.; Moberg, C.; Hult, K. *Chem. Eur. J.* **2007**, *13*, 4334-4341.

[38] a) Schonstein, L.; Forro, E.; Fulop, F. *Tetrahedron: Asymmetry* **2013**, *24*, 202-206. b) Wiles, C.; Watts, P. *Green Chem.* **2012**, *14*, 38-52. c) Newman, S. G.; Jensen, K. F. *Green Chem.* **2013**, *15*, 1456-1472.

[39] a) Zhang, J.; Wu, J.; Yang, L. *J. Mol. Catal. B: Enzym.* **2004**, *31*, 67-72. b) Xiong, J.; Wu, J.; Xu, G.; Yang, L. *Chem. Eng.* **2008**, *138*, 258-263.

[40] Zhang, Z. J.; Pan, J.; Liu, J. F.; Xu, J. H; He, Y. C.; Liu, Y. Y. *J. Biotechnol.* **2011**, *152*, 24-29.

[41] a) Ferreira, H. V.; Rocha, L. C.; Severino, R. P.; Porto, A. L. M. *Molecules* **2012**, *17*, 8955-8967. b) Patel, R. N.; Banerjee, A.; Nanduri, V.; Goswami, A.; Comezoglu, F. T. *J. Am. Oil Chem. Soc.* **2000**, *77*, 1015-1019. c) Rodrigues, R. C.; Lafuente, R. F. *J. Mol. Catal. B: Enzym.* **2010**, *64*, 1-22. d) Ren, L.; Xu, T.; He, R.; Jiang, Z.; Zhou, H.; Wei, P. *Tetrahedron: Asymmetry* **2013**, *24*, 249-253.

[42] Csajági, C.; Szatzker, G.; Toke, E. R.; Ürge, L.; Darvas, F.; Poppe, L. *Tetrahedron: Asymmetry* **2008**, *19,* 237-246.

[43] Corson, B. B.; Dodge, R. A.; Harris, S. A.; Yeaw, J. S. *Org. Synth.* **1926**, *6*, 58.

[44] Gößnitzer, E.; Punkenhofer, A. *Monastsh. Chem.* **2003**, *134*, 1271-1282.

[45] Lin, Z. C.; Chen, C. *J. Chin. Chem. Soc.* **2010**, *57*, 726-737.

[46] Blocka, E.; Bosiak, M. J.; Welniak, M.; Ludwiczak, A.; Wojtczak, A. *Tetrahedron: Asymmetry* **2014**, *25*, 554-562.

[47] Sakai, Y.; Mitote, J.; Matsumoto, K.; Katsuki, T. *Chem. Commun.* **2010**, *46*, 5787-5789.

[48] Sakthivel, S.; Punniyamurthy, T. *Tetrahedron: Asymmetry* **2010**, *21*, 2834-2840.

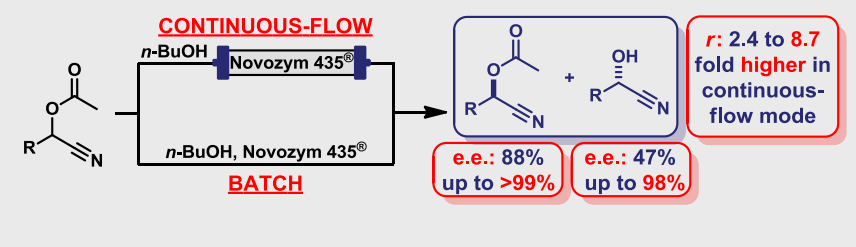
[49] Zeng, Z.; Zhao, G.; Zhou, Z.; Tang, C. *Eur. J. Org. Chem.***2008**, *2008*, 1615-1618.

[50] Zheng, Z.; Zi. Y.; Li, Z.; Zou, X. *Tetrahedron: Asymmetry* **2013**, *24*, 434-439.

[51] Hamashima, Y.; Sawada, D.; Nogami, H.; Kanai, M.; Shibasaki, M. *Tetrahedron* **2001**, *57*, 805-814.

[52] Lin, G.; Han, S.; Li, Z. *Tetrahedron* **1999**, *55*, 3531-3540.

FULL PAPER



A novel protocol to achieve optically active cyanohydrins in continuous-flow mode was developed. Cyanohydrin esters were employed as substrates in lipase-mediated enzymatic kinetic resolution (EKR) reactions. EKRs presented lower reaction times in continuous-flow than batch mode, as well as higher productivity in continuous-flow.

Cyanohydrins continuous-flow resolution*

*Juliana Christina Thomas, Bruno Bernardi Aggio, Alfredo Ricardo Marques de Oliveira, Leandro Piovan**

Page No. – Page No.

High-throughput preparation of optically active cyanohydrins mediated by lipases