



# Lipase-catalyzed Kinetic Resolution Using X-Cube™ Continuous Flow Reactor

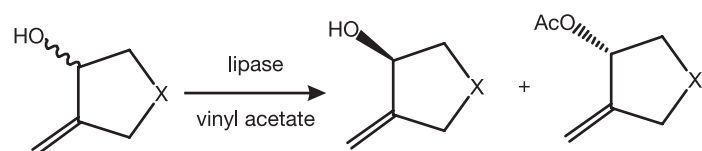


## INTRODUCTION

Enzyme catalyzed biotransformation reactions are now widely used in the chemical industry, most commonly in the synthesis of fine chemicals [1], but also in the production of drugs, agrochemicals and plastics. One of these enzymes is the lipase which can catalyze hydrolysis and also the esterification of ester chemical bonds in lipid substrates [2,3]. The main reason for using these enzymes is that they provide enantiomerically pure products during a reaction, often with higher purity than what can be achieved by non-enzyme enantiomer catalysts. Enzymes can be utilized in kinetic resolution, based on the different activity of enantiomers in certain reactions.

Prof. Poppe's group has previously performed experiments with different lipase enzymes where the immobilized and lyophilized powder forms of lipases were reacted in the kinetic resolution of benzene alcohol derivatives. Results clearly indicated that this reaction can be successfully performed under flow conditions with high conversion, enantiomeric excess (ee%), and enantiomer selectivity (E) [4]. In light of these results, the Poppe group decided to investigate further kinetic resolution reactions in ThalesNano's X-Cube™ continuous flow system. Racemic cycloalkane-1-ols, with ring sizes ranging from 5 to 7, were investigated. The influence of the methylene group and having an oxygen atom in the ring was also studied by repeating the reaction with 2-bromo-substituted cyclohexanol.

Finally, they used the results of these experiments to perform preparative scale synthesis in batch and also in flow [5].



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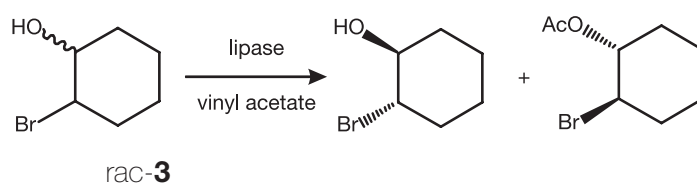
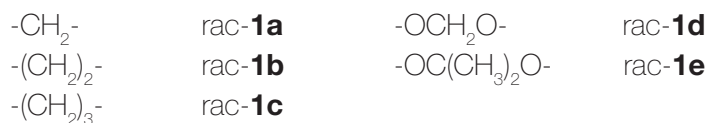


Figure 1. Lipase-catalyzed kinetic resolution

## BATCH EXPERIMENTS

Different enzymes were tested by adding 10 mg of the enzyme to a solution of a racemic alcohol (20 mg of each) in hexane-THF 2:1 (1 mL) and vinyl acetate (100 µL). The mixture was shaken in a sealed glass vial at 1000 rpm at room temperature.

## RESULTS OF BATCH EXPERIMENTS

The best selectivities were observed with lipases from the *Pseudomonas* strains (Lipase AK and Lipase PS), lipase B from *Candida antarctica*, and lipase BUTE 3b.  $\alpha$ -Chymotrypsin and papain were inactive in the acylation of the above molecules, although rac-**1e** was not accepted by the enzymes so it was not further investigated. The results detailed in the publication showed higher enantiomeric access in the kinetic resolution of five and six-membered ring substrates compared to the seven-membered ones. Having an oxygen heteroatom in the seven-membered rings increased the rate and the stereoselectivity of the reaction, apart from one exception. When compared with the bromide substituted compound, which does not contain a double bond function, the 2-methylene-substituted compound showed significantly faster reaction rates, although the stereoselectivities were almost the same for both of the six-membered ring alcohols.

## CONTINUOUS FLOW PROCEDURE

The solution of racemic alcohol (10 mg/mL; 0.090 M for rac-**1b**, 0.077 M for rac-**1d**, 0.057 M for rac-**3**) in a hexane-THF-vinyl acetate 2:1:1 mixture was pumped

through the lipase-filled CatCart® column. Runs were performed under various conditions [temperature (20–60 °C) and flow rate (0.1–0.3 mL/min)]. Samples were collected and analyzed during stable operation (GC samples taken every 10 min between 30 min and 90 min after changing the conditions).

## COMPARISON BETWEEN BATCH AND FLOW CONDITIONS

Based on the results of previous experiments, the comparison reactions were performed using substituted cyclohexane, seven-membered heterocycle ring and bromide substituted cyclohexane derivatives in the presence of CaLB and Sol-gel LAK enzymes. The batch reactions were carried out as described above and samples were taken at 2, 4, 8 and 24 hours. In Table 1, the results of each representative experiment shows that when using a flow reactor less time is needed to reach the same conversion and that the productivity of the flow reactor was always higher than the corresponding batch reaction. The productivity of the flow reactor could be increased by raising the flow rate in conjunction with elevated temperature.

Substrate	Mode	Enzyme	time / h	T / °C	v / mL / min	Conversion / %	ee / %	E	r / $\mu\text{mol} / \text{min} \cdot \text{g}$
rac- <b>1b</b>	Batch	CaLB	24	RT	-	53	98.5	65	2.0
rac- <b>1b</b>	Flow	CaLB	1.5	50	0.2	52	97.7	75	17.0
rac- <b>1d</b>	Batch	Sol-gel LAK	24	RT	-	48	>99	>200	2.6
rac- <b>1d</b>	Flow	Sol-gel LAK	1.5	40	0.1	35	99	>200	6.7
rac- <b>3</b>	Batch	CaLB	24	RT	-	54	98.8	65	1.7
rac- <b>3</b>	Flow	CaLB	1.5	60	0.3	52	96.9	79	17.6

Table 1. Results of comparison reactions between batch and flow methods

## PREPARATIVE SCALE SYNTHESIS IN BATCH

A 30 times larger sample (33 mL) was used compared to the previous experiments, but at a lower substrate concentration (10 mg/mL) to be able to compare the batch result with the flow. At the end of the reaction, the enzyme was filtered off and the filtrate was concentrated under vacuum. The residue was separated by column chromatography (silica gel, 5–40% gradient of EtOAc in hexane).

## PREPARATIVE SCALE SYNTHESIS IN FLOW

Similarly to the flow reactions above, a 10 mg/mL substrate concentration was used, but this time at 50 °C and at a flow rate of 0.2 mL/min. After collecting 36 mL of reaction mixture, the solvent was removed under vacuum and the residue was separated as described in the batch process.

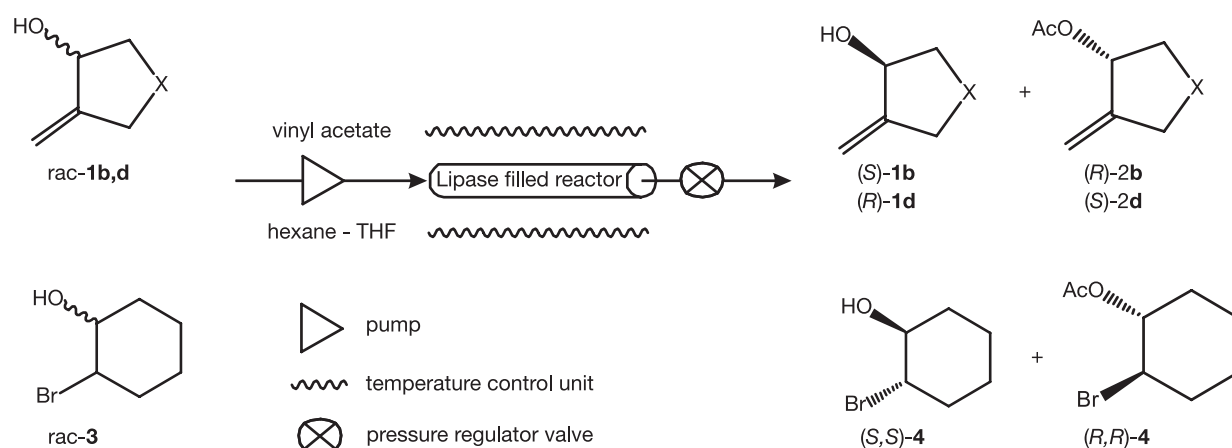


Figure 2. Preparative scale lipase-catalyzed kinetic resolution in flow

Substrate	Mode	Enzyme	time / h	Unreacted alcohol		Formed acetate		E	r / $\mu\text{mol} / \text{min} \cdot \text{g}$
				Yield / %	ee / %	Yield / %	ee / %		
rac-1b	Batch	CaLB	6	16	89.2	47	89.0	51	8.9
rac-1b	Flow	CaLB	3	17	92.9	48	90.0	93	16.9
rac-1d	Batch	Sol-gel LAK	24	42	40.8	19	98.8	>200	1.4
rac-1d	Flow	Sol-gel LAK	3	43	34.8	15	98.9	>200	5.7
rac-3	Batch	CaLB	8	34	92.1	48	91.0	65	4.2
rac-3	Flow	CaLB	3	35	98.0	48	85.0	55	11.9

Table 2. Results of preparative scale synthesis

## RESULTS OF PREPARATIVE SCALE SYNTHESIS

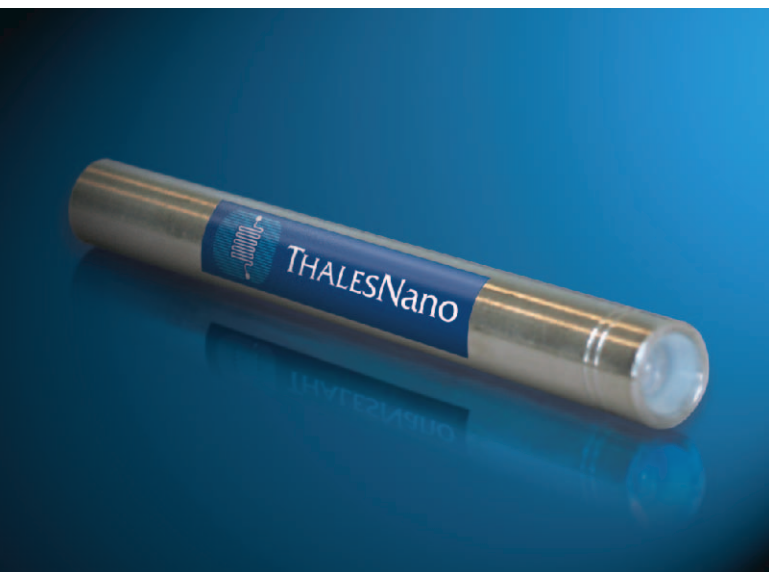
Table 2 clearly indicates that almost the same results could be achieved in batch and flow when comparing yield and ee of unreacted alcohol and formed acetate. The E values showed just a slight difference, but, in all cases, performing the study in flow resulted in 2-4 times higher productivity over batch.

## CONCLUSION

These experiments demonstrated the affect of the size and nature of the ring on enantiomeric selectivity in the kinetic resolution of secondary alcohols by lipase-catalyzed acetylation. The comparison reactions between batch and continuous flow showed less time was needed to perform the reactions in flow in order to achieve the same results in terms of enantiomeric excess and conversion. From the preparative scale experiments it can be seen that by using flow methodology higher productivity can be achieved.

## References

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- 5.) Tomin, A.; Hornyanszky, G.; Kupai, K.; Dorko, Zs.; Urge, L.; Darvas, F.; Poppe, L.; Lipase-catalyzed kinetic resolution of 2-methylene-substituted cycloalkanols in batch and continuous-flow modes; *Process Biochemistry*, **2010**, 45, 859-865



### ThalesNano's CatCarts®

ThalesNano's CatCart® columns contain sealed heterogeneous or immobilized homogeneous catalysts and reagents, which can be used in heterogeneously catalyzed reactions in the X-Cube™ flow reactor. Exposure to the catalyst is limited by removing the need for filtration, while the cartridges are easy to install and replace. In the CatCart® columns, the ratio of catalyst/reagent to substrate in the solution is significantly increased, which results in faster reaction rates and shortened processing time increasing productivity.



### CatCart Packer™

Users can now pack their own catalysts/reagents in a safe, easy and cost effective way with our new CatCart Packer™ instrument.

The CatCart Packer™ system enables users of the X-Cube™ flow reactor to pack their own catalysts into cartridges and screen them for rapid optimization.

Using the CatCart Packer™ instrument takes three simple steps: adding the catalyst/reagent, adding the filters and sealing the CatCart® column. After filling and sealing the CatCart® column is ready for use. After filling and sealing the CatCart® column.

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