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High-efficiency and minimum-waste continuous kinetic resolution of racemic alcohols by using lipase in supercritical carbon dioxide†

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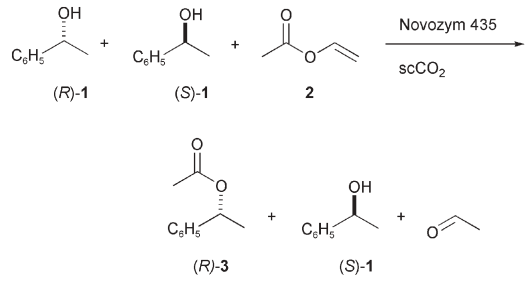
A novel continuous-flow $scCO_2$ process for kinetic resolution of racemic alcohols can be performed with an immobilized lipase to lead to a quantitative mixture of the corresponding optically active acetates with up to 99% ee and unreacted alcohols with up to 99% ee, in which the productivity of the optically active compounds was improved by over 400 times compared to the corresponding batch reaction using $scCO_2$.

Great efforts have been extended to homogeneous catalysis in $scCO_2$ since the early 1990's,[1] and the area of catalysis in the supercritical homogeneous phase has gained significant attention because of the intrinsic properties of $scCO_2$ including high miscibility of the gaseous reactants, favorable mass transfer, and tunable solvent power. $scCO_2$ is now realized to be a promising reaction medium for environmentally benign chemical processes. However, difficulties in separation of the homogeneous catalysts and products have remained unresolved except for recent advances in reaction and extraction techniques or multiphase catalysis systems including a SCF phase.[2]

Biocatalysis for organic synthesis has advantages in green chemistry because enzymes are reproducible and reusable catalysts with excellent chemo-, regio-, and enantioselectivities.[3] A combination of the concept of the heterogeneous biocatalysis with $scCO_2$ having unique properties has attracted considerable attention to achieve highly stereoselective molecular transformations. In fact, there have been many reports on asymmetric synthesis with enzymes in $scCO_2$ using batch type reactors since 1985.[4,5] However, continuous flow $scCO_2$ phase reactions over enzymatic catalysts as a practical procedure for synthesis of optically active compounds remain largely unexplored except for recent reports.[6] Here we report a novel continuous $scCO_2$ flow system for enzymatic asymmetric synthesis of optically active alcohols, in which racemic alcohols were efficiently converted to the corresponding optically pure acetates *via* kinetic resolution. The use of a continuous flow reaction system for the kinetic resolution resulted in a significant improvement in the productivity for long period of the reaction time, leading to virtually solventless reaction.[1]

First, kinetic resolution of racemic 1-phenylethanol (*R*/*S*)-**1** with vinyl acetate **2** catalyzed by an immobilized lipase from *Candida antarctica*, Novozym 435, in $scCO_2$ was examined using a batch system. The reaction of (*R*/*S*)-**1** (0.83 mmol) with vinyl acetate **2** (5.4 mmol) in the presence of the enzyme (5.0 mg) under 9 MPa of CO_2 at 40 °C proceeded to give a 1 : 1 mixture of optically active (*R*)-acetate (*R*)-**3** with 99.8% ee and unreacted (*S*)-alcohol (*S*)-**1** with 90.6% ee in 48% conversion (Scheme 1). The time courses of the reactions at 9 and 13 MPa revealed that (*R*)-enantiomer (*R*)-**1** reacted faster than (*S*)-enantiomer (*S*)-**1** and that the reaction stopped after about 7 h when the (*R*)-enantiomer was consumed. The ratio of the specificity constants of the enantiomers, *E*-value,[7] was more than 100 when the conversion was 48%.



Scheme 1

The utilization of a continuous flow reactor[8] (Fig. 1) for the kinetic resolution of (*R*/*S*)-**1** over the Novozym catalyst caused a significant improvement in the efficiency of the reaction. The reaction of (*R*/*S*)-**1** and vinyl acetate **2**, with the molar ratio of **1** : **2** = 1 : 0.5 at a flow rate of 0.70 mL min^{-1}, over the catalyst under 13 MPa of $scCO_2$ (1.5 mL min^{-1}) gave the desired acetate **3** with 99.7% ee in 47% yield.[9]‡ The *E* value exceeds 1800. The use of a slight excess of **2** resulted in an increase in the chemical yield of optically active (*R*)-**3** from 47% to 50%, in which the unreacted alcohol (*S*)-**1** with 98.8% ee was recovered quantitatively (Table 1). The NMR spectrum of the reaction mixture shows that no side reaction occurred. Noticeably, even when a large amount of **2** was used, the over-reaction of (*S*)-enantiomer hardly proceeded under the same conditions, providing a quantitative mixture of acetate **3** and unreacted **1** with over 99% ee. Changing the CO_2 pressure ranging from 8.9 to 20 MPa did not significantly change the outcome of the reaction.

Similarly, the aliphatic alcohols 2-undecanol **4a** and 1-tetralol **4b** were kinetically resolved with the Novozym catalyst under conditions similar to those described above (**4** : **2** = 1 : 0.6) to give a mixture of optically active *R*-ester of **4a** or **4b** and unreacted

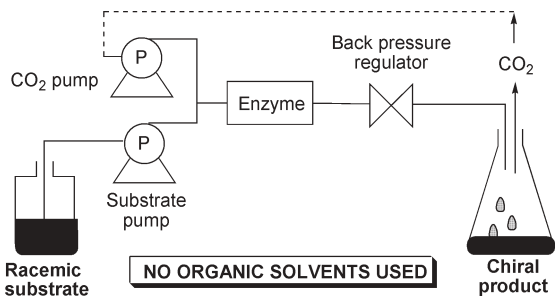


Fig. 1 Simplified illustration of experimental apparatus.[8]

† Electronic supplementary information (ESI) available: NMR spectrum of the product, (*S*)-1-phenylethanol and the corresponding (*R*)-acetate, without any purification procedure, figure and table showing kinetic resolution of (*R*/*S*)-**1** by Novozym and experimental details. See http://www.rsc.org/suppdata/cc/b4/b406882c/

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Table 1 The effect of reaction conditions on kinetic resolution of (*R*/*S*)-**1** by Novozym using $scCO_2$ flow reactor[a]

1 : **2** (mol/mol)	Pressure/MPa	(*S*)-**1** (% ee)	(*R*)-**3** (% ee)	Conv. (%)[e]	*E*
1:0.50[b]	13.0	89.6	99.7	47	1850
1:0.60[c]	13.0	98.8	99.2	50	1240
1 : 1.30[d]	12.9	>99.5	99.0	50	>1000
1:1.30[d]	8.9	>99.5	99.0	50	>1000
1:1.30[d]	20.0	>99.5	99.0	50	>1000

[a] Conditions: 42–43 °C, Novozym: 5.0 mL, $scCO_2$ flow: 1.5 mL min^{-1}, flow rate of **1**: 0.050 mL min^{-1}. [b] Flow rate of the mixture of **1** and **2**: 0.070 mL min^{-1} [c] Flow rate of the mixture of **1** and **2**: 0.074 mL min^{-1}. [d] Flow rate of the mixture of **1** and **2**: 0.100 mL min^{-1}. [e] Conversion to **3** based on the starting amount of **1**.

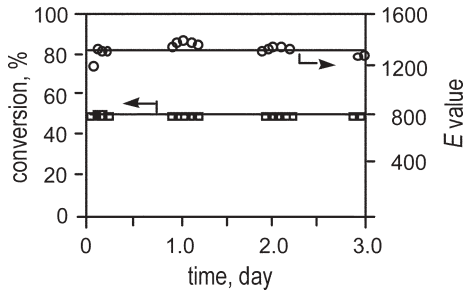

Fig. 2 The reaction profile of kinetic resolution of (*R*/*S*)-**1** with Novozym in continuous-$scCO_2$-flow reactor. (Conditions: Novozym: 5.0 mL (1.73 g), **1** : **2** = 1 : 0.6, flow rate of the mixture of **1** and **2**: 0.074 mL min^{-1}, $scCO_2$ flow: 1.5 mL min^{-1}, 12.9–13.0 MPa, 42 °C.)

(*S*)-**4a** or **4b**, respectively. Optically active acetate **5** was obtainable in 48–50% yield and with an excellent ee (*E* = 112–137 for **4a** and over 1500 for **4b**).

The present continuous asymmetric synthesis of optically active alcohols and their derivatives with the Novozym catalyst is characterized by a rapid and highly stereoselective transformation. Although the batch reaction using 10 mL of the reactor, produced 0.83 mmol of optically active compounds during 7 h reaction, the continuous-flow reactor (5 mL) gave the products at the rate of 25 mmol h^{-1} (3 mL h^{-1}). Furthermore, the reaction even with a small excess of vinyl acetate **2** proceeded smoothly to provide the desired product with an excellent ee, minimizing the use of unnecessary liquid materials and the production of waste during the reaction. This synthetic process is particularly useful for the large-scale production of optically active alcohols. As shown in Fig. 2, the present biocatalyst maintained its performance in terms of the reactivity and selectivity during 3 days' operation under a supercritical condition (12.9–13 MPa at 42 °C) and resulted in a quantitative transformation of (*R*/*S*)-**1** (221 g) to (*S*)-**1** with 99% ee and (*R*)-**3** with 99% ee using 1.73 g of the immobilized enzyme.[9]

Notes and references

‡ *Safety warning*: operators of high-pressure equipment should take proper precautions to minimize the risk of personal injury.
Experimental procedure: in a typical reaction, an immobilized lipase (Novozym, 5 mL, 1.89 g, approx. 10.000 U g^{-1} for propyl laurate synthesis) was introduced to the reactor and placed in the oven. The reaction temperature was increased to 42 õC and the CO_2 pressure was increased to 13.0 MPa, and the flow rate was set to 1.500 mL min^{-1}. Then a mixture of 1-phenylethanol (*R*/*S*)-**1** and vinyl acetate **2** (**1** : **2** = 1 : 0.5) was sent to the reactor at the flow rate of 0.070 mL min^{-1}. The sample was collected after 1.2 h without using any organic solvent at room temperature and was analyzed by GC (Chirasil-DEX CB), LC (Daisel Chiralcel OJ-H), and ^1H NMR (400 MHz) without any purification. Visual inspection of the reaction mixture in an autoclave equipped with sapphire windows showed that the reactants and products were all soluble in $scCO_2$ under the reaction conditions.

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9 Encouraged by this promising result, dynamic kinetic resolution (DKR) using both the lipase and the Ru catalyst in $scCO_2$ is in progress in our laboratory.