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Continuous-Flow Kinetic Resolution of (±)-*cis*-1-Amino-2-indanol by Lipase-Catalyzed N-Acetylation

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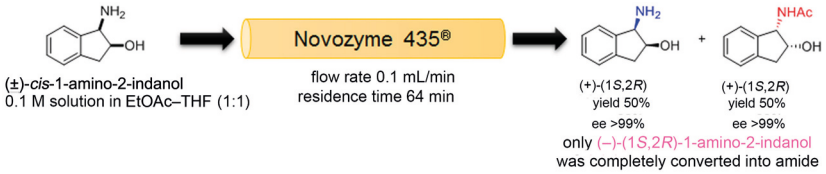

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Abstract Selective N-acetylation of (1*S*,2*R*)-1-amino-2-indanol by immobilized lipase B from *Candida antarctica* showed high enantiomeric excess when ethyl acetate was used as the acyl donor in a THF solution. Combining this process with continuous-flow system, we could obtain enantiomerically pure *N*-acetyl-aminoindanol at a flow rate of 0.1 mL/min (residence time of 64 min). It has been demonstrated to be more efficient compared to the flask mode.

Key words (±)-*cis*-1-amino-2-indanol, Novozyme 435®, continuous-flow system, kinetic resolution, selective N-acetylation

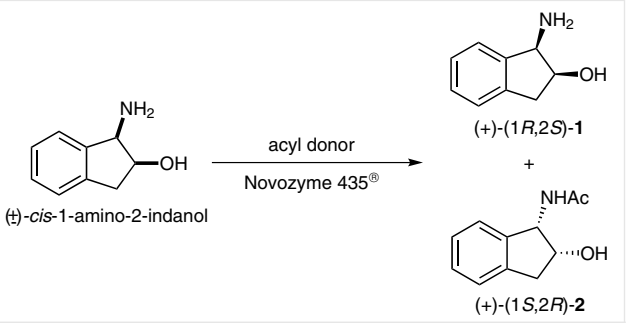
The bioactivity of compounds are strongly affected by their stereo structure; the kinetic resolution of active pharmaceutical ingredients or their small building-block precursors is very important in the pharmaceutical industry.[1] To achieve effective kinetic resolution, various approaches, such as using chiral auxiliaries, metal catalysts, and biocatalysts, have been attempted.[1,2] Among these, biocatalysts are attractive tools for improving the resolution of chiral compounds because they offer high regio- and stereocontrol under mild reaction conditions. Additionally, they usually perform well without toxic organic solvents or reagents, allowing a widely use in organic synthesis.[1,3]

Recently, a continuous-flow system represents an attractive approach for application in biotechnology and chemistry.[4] It employs excellent temperature control, a small footprint, easy workup process, reduced scale-up issues, and the ability to perform multiphase chemistry easily. For these reasons, many studies investigating the kinetic resolution of chemical compounds in continuous-flow systems have been reported.[5–7]

(1*S*,2*R*)-1-Amino-2-indanol is a key intermediate of the HIV protease inhibitor indinavir[8] and is also a component in chiral building blocks and chiral auxiliaries.[9] Herein, we reported the chiral resolution of (±)-*cis*-1-amino-2-indanol by enantioselective acetylation of a secondary amine. We applied a continuous-flow process to increase the effectiveness and productivity of kinetic resolution of (±)-*cis*-1-amino-2-indanol with lipase.

Lipase B from *Candida antarctica* is one of the most effective catalysts for the resolution of secondary amines and alcohols. In particular, Novozyme 435®, which is an immobilized lipase on an acrylic resin, has higher selectivity toward racemic amines than other variously immobilized forms.[10] In our small scale test reaction, it was confirmed that Novozyme 435® is also an appropriate catalyst for the acetylation of (±)-*cis*-1-amino-2-indanol (Scheme 1). There are many studies that lipase catalyzed acetylation of secondary alcohols under reaction conditions similar to that of amines.[6,11] So, we expected some O-acetylated side products might be produced. But O-acetylated products were not obtained in this study. It might be due to the reactivity difference between amine and alcohol or their position from the aromatic ring. Although it is needed more studies to identify the reasons for this, lipase-catalyzed acetylation of (±)-*cis*-1-amino-2-indanol has shown high selectivity between amine and alcohol.

Subsequently, in order to optimize the selective N-acetylation reaction conditions, acyl donors and solvents were screened via the flask mode. Four acyl donors were selected and tested, vinyl acetate and isopropenyl acetate showed no stereoselectivity. Other papers have also shown that vinyl acetate and isopropenyl acetate have low stereoselectivity for the acetylation of amine compounds.[7,12] Conversely, ethyl acetate and *n*-butyl acetate are suitable acyl donors for selective N-acetylation. The influence of differ-



Scheme 1 Kinetic resolution of (±)-*cis*-1-amino-2-indanol

ent equivalents of ethyl acetate and *n*-butyl acetate on the reaction conversion and the enantiomeric ratio are depicted in Table 1. Excess amounts of ethyl acetate have the highest conversion rate and enantioselectivity in the THF reaction solution (conversion rate 35%, *E* >200). *n*-Butyl acetate also has high enantioselectivity (*E* >200) but showed a lower conversion rate compared to ethyl acetate at the same reaction conditions.

Table 1 Screeening of the Acyl Donor for the Kinetic Resolution of (±)-*cis*-1-Amino-2-indanol[a]

Entry	Acyl donor (equiv)	Conv. (%)[b]	*E*[c]
1	EtOAc (1.0)	15	>200
2	EtOAc (50)	35	>200
3	*n*-BuOAc (1.0)	12	>200
4	*n*-BuOAc (50)	27	>200

[a] Enantiomeric excess (ee) was determined by HPLC analysis on a chiralcel OJ-H column.
[b] Conversion: $C = ee_r/(ee_p + ee_r)$, where ee_r and ee_p are enantiomeric excesses of amine **1** and amide **2**, respectively.
[c] Enantiomeric ratios (*E*) were calculated as $E = \ln[1 - C(1 - ee_p)]/\ln[1 - C(1 + ee_p)]$.[13] Reaction conditions: 0.1 M (±)-*cis*-1-amino-2-indanol solution in THF with 1.0 equiv of acyl donor or in EtOAc (or *n*-BuOAc) and THF (1:1, 50 equiv of acyl donor conditions), 30 °C, 125 rpm shaking incubation for 24 h.

Table 2 Solvent Effect on the Kinetic Resolution of (±)-*cis*-1-Amino-2-indanol[a]

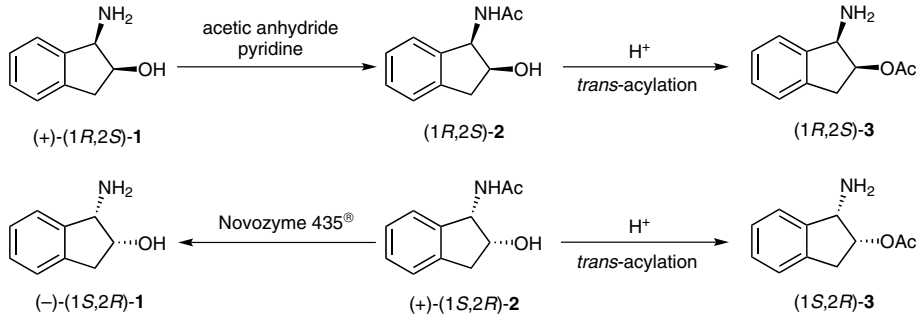
Entry	Solvent	Conv. (%)[b]	*E*[c]
1	THF	33	>200
2	CH_2Cl_2	32	>200
3	MTBE[d]	28	>200
4	toluene	39	>200

[a] Enantiomeric excess (ee) was determined by HPLC analysis on a chiralcel OJ-H column.
[b] Conversion: $C = ee_r/(ee_p + ee_r)$, where ee_r and ee_p are enantiomeric excesses of amine **1** and amide **2**, respectively.
[c] Enantiomeric ratios (*E*) were calculated as $E = \ln[1 - C(1 - ee_p)]/\ln[1 - C(1 + ee_p)]$.13 Reaction conditions: 0.1 M (±)-*cis*-1-amino-2-indanol solution in EtOAc and THF (1:1), 30 °C, 125 rpm shaking incubation for 6 h.
[d] MTBE: *tert*-butyl methyl ether.

Solvent screening was also conducted to verify the optimized conditions, and all four solvents were determined to be viable candidates because they all had excellent selectivity (Table 2). Although toluene showed the highest conversion rate (39%) at the same reaction time, the solubility of (±)-*cis*-1-amino-2-indanol in toluene was found to be lower than in THF. THF showed the second highest conversion rate (33%) but demonstrated great solubility of the reactant; therefore, THF was chosen to be the solvent in further experiments.

When the flask reaction[14] was processed for 6, 12, 24, and 48 hours in a flask with one equivalent of ethyl acetate, the conversion rates were 6%, 14%, 15%, and 22%, respectively. Alternatively, a conversion rate of 35% was shown at 12 hours when excess ethyl acetate was used. A reaction time of 24 hours gave the same conversion rate as 12 hours, and 48 h surprisingly showed a decreased conversion rate (Table 3, conditions A).

Thanks to the high enantioselectivitiy of Novozyme 435® to (1*S*,2*R*)-**1**, amide **2**[15] could be obtained as single enantiomer even the starting amine **1** was not completely converted into amide (Scheme 2). But the remained amine **1** was recovered only with ee <54% at 35% conversion. To get remained (1*R*,2*S*)-**1** as pure single enantiomer, full conversion of (1*S*,2*R*)-**1** was necessary. Moreover, it took more



Scheme 2 Transformation of chiral amine **1** and amide **2**

Table 3 Kinetic Resolution of (±)-*cis*-1-Amino-2-indanol under Flask Conditions (A) and Continuous-Flow Conditions (B)[a]

(A) Flask conditions						
Entry	Reaction time (h)	EtOAc (equiv)	ee_r (%)	ee_p (%)	Conv. (%)[b]	*E*[c]
1	6	1.0	6	>99	6	>200
2	12	1.0	16	>99	14	>200
3	24	1.0	18	>99	15	>200
4	48	1.0	28	>99	22	>200
5	6	50	43	>99	30	>200
6	12	50	54	>99	35	>200
7	24	50	54	>99	35	>200
8	48	50	29	>99	22	>200
(B) Continous-flow conditions						
Entry	Flow rate (residence time)	EtOAc (equiv)	ee_r (%)	ee_p (%)	Conv. (%)[b]	*E*[c]
1	2.0 mL/min (3.2 min)	50	15	>99	13	>200
2	1.0 mL/min (6.4 min)	50	26	>99	20	>200
3	0.5 mL/min (12.8 min)	50	52	>99	34	>200
4	0.25 mL/min (25.6 min)	50	70	>99	41	>200
5	0.1 mL/min (64 min)	50	>99	>99	50	>200

[a] Enantiomeric excess (*ee*) was determined by HPLC analysis on a chiralcel OJ-H column.
[b] Conversion: $C = ee_r / (ee_p + ee_r)$, where ee_r and ee_p are enantiomeric excesses of amine **1** and amide **2**, respectively.
[c] Enantiomeric ratios (*E*) were calculated as $E = \ln[1 - C(1 - ee_p)]/\ln[1 - C(1 + ee_p)]$.13

than 12 hours to achieve >35% conversion in the flask. A continuous-flow system was introduced to improve these unsatisfactory outcomes.

Novozyme 435® (2.0 g) was packed into a glass column followed by washing with THF. It was then fed with a 0.1 M solution of (±)-*cis*-1-amino-2-indanol in ethyl acetate and THF (1:1) at various flow rates.[16] HPLC analysis was performed directly to take reaction mixtures as analytical samples because an enzyme separating process was not required (Figure 1).

As the residence time increased, the conversion rate also gradually increased. A flow rate of 0.1 mL/min gave both high enantioselectivity (*E* >200) and complete conversion of (1*S*,2*R*)-1-amino-2-indanol [50% conversion of (±)-*cis*-1-amino-2-indanol] at a much shorter residence time (64 min) (Table 3, conditions B).

Because the ratio of the enzyme to the solution in the column of the continuous-flow system is much larger than in the flask, amine **1** had more chances to meet the lipase during the flow through the column packed with Novozyme 435® at same reaction time. As a result, higher conversion could be obtained by continuous-flow reaction in relatively short reaction time. Furthermore, amide **2** could be continually produced by feeding solution of amine **1** and acyl donor. Continuous production by immobilized enzymes using flow systems have been studied by some groups. In a study on the kinetic resolution of 1-phenylethanol, 72 days of continuous production by immobilized li-

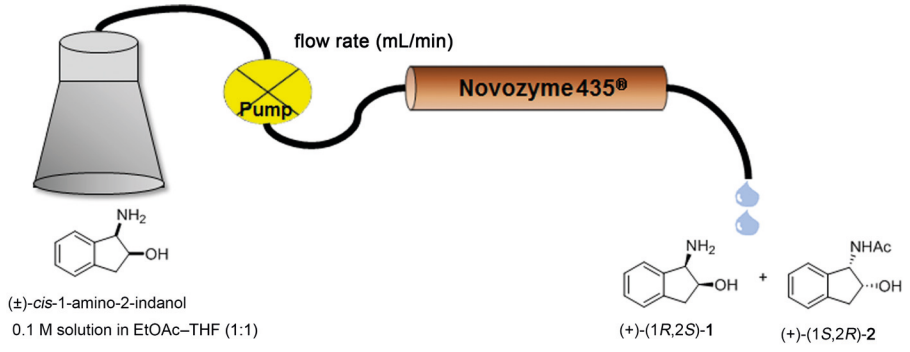


Figure 1 Schematic diagram of the continuous-flow system for kinetic resolution of (±)-*cis*-1-amino-2-indanol

pase has been reported.[11] We have confirmed that the activity and selectivity of Novozyme 435® catalyzed acetylation did not decrease during 21 days continuous production. Our reaction system is a very useful approach for kinetic resolution and suitable for large-scale reaction with a biocatalyst.

In summary, kinetic resolution of (±)-*cis*-1-amino-2-indanol was successfully carried out by lipase-catalyzed N-acetylation with high enantioselectivity. We could obtain enantiomerically pure N-acetyl-aminoindanol with a relatively short reaction time, higher productivity, and an easy workup process in a continuous-flow system. This method led to the complete conversion of (±)-*cis*-1-amino-2-indanol with >99% of enantiomeric excess in 64 minutes residence time at a flow rate of 0.1 mL/min. Furthermore, chiral amine **1** and amide **2** are expected to be converted easily into their N-acetylated form **2** or O-acetylated form **3**. And acetamide **2** could be transformed reversely into amine **1** by lipase under conditions without an acyl donor. All of these compounds could be utilized as functionalized chiral building blocks.

Acknowledgment

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0034-1380427.

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(14) **Flask Reaction – General Procedure**
To a separate small vials containing 0.2 M solutions of (±)-*cis*-1-amino-2-indanol (298 mg, 2.0 mmol) in THF–EtOAc (0.5 mL, 50 equiv), Novozyme 435® (20 mg) was added to each vial in one portion, and the turbid solutions were shaken (125 rpm) at 30 °C. After 6 h, 12 h, 24 h, and 48 h, the enzyme was filtered off from the samples. For HPLC analysis, samples were diluted with EtOH (0.5 mL).
(15) **(1*S*,2*R*)-1-Acetamido-2-indanol (2)**
White solid; $[\alpha]_D^{20}$ +11.7 (*c* 0.25, $CHCl_3$). IR (neat): 3444, 3299, 1539 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.22 (s, 4 H), 6.23 (d, *J* = 7.2 Hz, NH), 5.32 (dd, *J* = 8.2, 5.1 Hz, 1 H), 4.57 (td, *J* = 5.1, 2.3 Hz, 1 H), 3.13 (dd, *J* =16.5, 5.3 Hz, 1 H), 291 (dd, *J* = 16.5, 2.1 Hz, 1 H), 2.57 (br s, 1 H), 2.07 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 140.6, 139.9, 128.2, 127.2, 125.3, 124.5, 73.5, 57.6, 39.6, 23.3. ESI-HRMS: *m/z* calcd for $C_{11}H_{14}NO_2^+$: 192.1019; found: 192.1016 $[M + H]^+$.
(16) **Continuous-Flow Reaction, General Procedure**
Novozyme 435® (2.0 g) was packed into a glass column with an aluminum heating jacket for maintaining temperature at 30 °C. The column was fully washed with THF and then fed with a solution of (±)-*cis*-1-amino-2-indanol (0.1 M) in EtOAc and THF (1:1). At various flow rates (2.0, 1.0, 0.5, 0.25, and 0.1 mL/min), 1 mL of samples were collected. For HPLC analysis, samples were diluted with EtOH (0.5 mL).