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An efficient enzyme-catalyzed kinetic resolution: large-scale preparation of an enantiomerically pure indole-ethyl ester derivative, a key component for the synthesis of a prostaglandin D_2 receptor antagonist, an anti-allergic rhinitis drug candidate

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Abstract—Three racemic esters including indole-ethyl ester **1** as well as its related derivatives **3** and **4** in Scheme 1, all synthetic intermediates for the preparation of chiral compound **5**, were used as substrates to evaluate the catalytic potentials of a panel of commercial enzymes for asymmetric hydrolysis. After an extensive evaluation of the conversion rates (*C*), enantiomeric excesses (ee) and enantioselectivity determination (*E*), lipase from *Pseudomonas fluorescens* was identified. This lipase catalyzed the asymmetric hydrolysis of racemic indole-ethyl ester **1** affording the desired enantiomerically pure intermediate **1** with 97% ee and an *E* value of 425. Indole-ethyl ester **1**, with the following attributes: being early in the synthetic scheme, showing resistance to racemization in the later chemical reactions as well as the possibility of the recycling of the unwanted enantiomer, was therefore selected for optimization and establishment of an enzyme-catalyzed reaction for the industrial-scale synthesis of the compound **5**, a drug candidate for the treatment of allergic rhinitis.
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1. Introduction

Compound **5** (Scheme 1) is an antagonist of a prostaglandin DP receptor that is being developed for the treatment of allergic rhinitis.[1,2] In an early synthesis for the preparation of this compound, the cyclopentene-fused indoleacetate **1** was constructed via a two-step, non-isolation process using an imine condensation between 2-bromo-4-fluoroaniline and ethyl 2-(2-cyclopentanone)-acetate, followed by a palladium-catalyzed intramolecular Heck reaction. The resulting ester was then hydrolyzed and subjected to sequential bromination and benzylation to provide the penultimate intermediate **4**. The resolution to provide the stereogenic center of **4** was carried out at this stage using (*S*)-naphthylethylamine. The resolved intermediate (*R*)-**4** was then converted to the enantiomerically pure final compound **5** by sulfonylation.[3–5]

Although this route has been suitable for the preparation of **5** on a kilogram scale, it suffers from the large loss of material in the resolution step. To account for this loss at the end of the synthesis, more material must be carried through the early steps lowering the overall efficiency of the approach. A resolution at the beginning of the synthesis would therefore be much more practical. Of even greater benefit would be the ability to recycle the unwanted enantiomer through a racemization process. Fortunately, both issues were shown to be amenable in our study and are thus reported here.

Herein, we report a resolution/racemization approach to **5** using an enantioselective enzymatic hydrolysis of the

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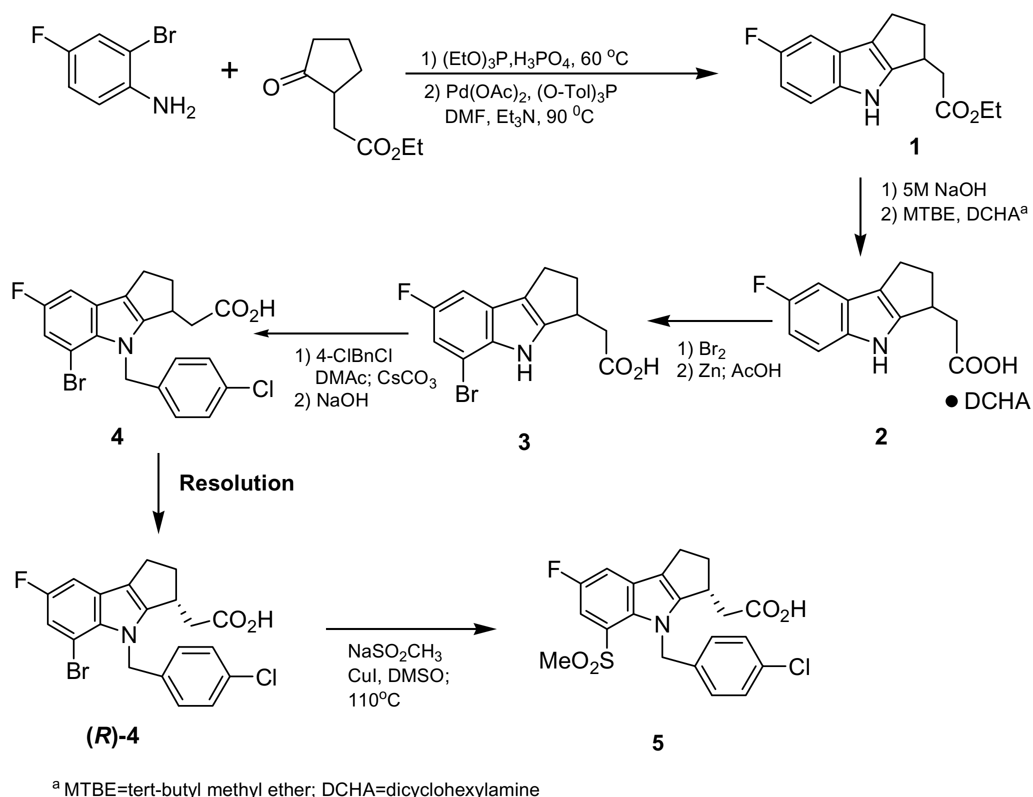
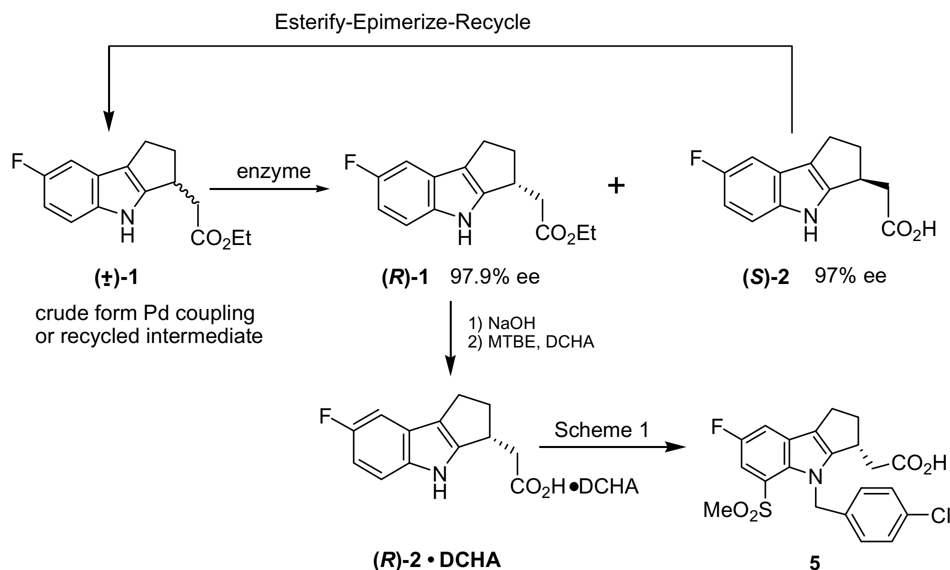


Scheme 1. First-generation synthesis of **5**.

unwanted enantiomer of the ester **1** and recovering the desired ester enantiomer in high enantiomeric excess (ee). As shown in the following discussion, by having a resolution early in the synthesis and with the ability to recycle the undesired enantiomer, the overall yield was improved resulting in the development of a process for an industrial-scale preparation of enantiomerically pure **5** (Scheme 2).

2. Results and discussion

In order to have the most efficient synthesis, it was important to resolve the stereogenic center early. From the previous work,[3] it was known that the enantiomerically pure cyclopentane acetate completely racemized during the Fischer-Indole reaction. Therefore, the next feasible intermediate, namely indole ester **1**, was selected



Scheme 2. Enzyme-catalyzed resolution/racemization approach to the asymmetric synthesis of **5**.

and used as a substrate to screen a library of enzymes for the identification of an appropriate biocatalyst. Additionally, precursor **3** and racemic **5** were similarly screened to ensure the availability of the appropriate enzyme catalysts in the event of the erosion of the chirality of intermediate **1** due to the subsequent bromination or sulfonylation reactions.

2.1. Screening for the identification of an appropriate enzymatic catalyst

A panel of commercial enzymes consisting of lipases, proteases and acylases were screened in order to determine their potential for the asymmetric hydrolysis of the racemic indole-ethyl ester **1**. After extensive evaluation of the conversion rates, enantiomeric excesses as well as the determination of the enantioselectivity ratio (*E*), the lipase from *Pseudomonas fluorescens* (*P. fluorescens*) was selected for the optimization and further development.

2.2. Optimization of *P. fluorescens* lipase-catalyzed reaction for the asymmetric hydrolysis of the indole-ethyl ester 1

Initially, by using an excess amount (50–200 mg) of biocatalyst (lipase from *P. fluoresens*), to force short reaction times, and substrate (10–15 mg) quantities, the reaction conditions for the enzyme-catalyzed asymmetric hydrolysis were optimized for the phosphate buffer concentration (0.1–0.5 M), pH (7–8) as well as incubation temperature (27–40 °C). Consequently, the reactions were routinely run in 0.1 or 0.5 M phosphate buffer, pH = 7.5, at ambient temperature to avoid any possible epimerization of the stereogenic center. Due to the high buffering capacity of the 0.5 M phosphate buffer, the pH monitoring of the reaction was no longer required. As shown in Table 1, a higher phosphate concentration than 0.5 M seemed to slow down the rate of the hydrolysis of the undesired enantiomer. However, due to the high selectivity of the enzyme, the reaction went to completion without significant decrease of the enantiomeric excess and yield after 45 h of aging. In phosphate experiments, the amount of the enzyme to substrate ratio was kept at 2 to 1.

Table 1. Effect of the phosphate buffer concentration on the enantiomeric excess of the desired indole-ethyl ester **1** enantiomer

Phosphate buffer (M)	Desired ester	
	(% ee—18 h)	(% ee—45 h)
0.10	77.7	97.3
0.50	84.7	97.3
0.75	61.0	93.2
1.00	57.0	97.0

2.3. Process development for *P. fluoresens* lipase-catalyzed asymmetric hydrolysis of the indole-ethyl ester 1

For the development of a large-scale enzyme-catalyzed reaction, a major issue was the solubilization and delivery of the substrate to the aqueous reaction medium. Quite often, water-insoluble organic compounds do not behave well in enzymatic reactions that take place in an aqueous medium. To circumvent this problem, various methods, including detergent solubilization of the substrate or use of appropriate organic co-solvent(s), have been exploited. Accordingly, Triton X-100 was tested for the solubilization and delivery of substrate **1**. Despite the fact that the desired transformation was achieved, the isolated, enantiomerically pure compound was contaminated with the detergent. Various solvent extractions, washes and crystallization failed to effectively remove the detergent from the ester. Even hydrolysis of the desired chiral ester and re-extraction of the chiral desired free acid could not eliminate Triton X-100 detergent. Therefore, the identification of an organic co-solvent(s), which could be used for the solubilization and the delivery of the substrate without deleterious effect to the activity of the enzymatic catalyst, was undertaken. Consequently, a triphasic solvent system consisting of an aqueous buffer/DMF/hexane (36:4:10) was developed. In this system, the substrate was dissolved in DMF and the resulting solution gradually added to the stirring solution of the enzyme. At the end, the substrate delivery vessel was washed with hexane, while the mixture was being stirred, either mechanically or by a magnetic stirring bar. Using this system, several experiments were successfully carried out at the 2–10 g scales, before larger scale experiments (36.6 g, 76.5 g, 83 g and kg) were undertaken (see below).

2.4. Evaluation of the enzymatic activity of the recovered lipase and the determination of its recycling potential

The recycling of the catalyst could have a beneficial impact on the cost of the process. To evaluate this potential, the enzyme catalyst that had been used in reactions under our standard conditions was recovered after ethanol precipitation. The precipitate was then lyophilized, after extensive washing with cold ethanol, and its catalytic activity was determined in gram-scale reactions using indole-ethyl ester substrate **1**. The recovered enzyme did not show any loss of catalytic potential after at least three sequential cycles of use.

2.5. Coupled esterification–racemization and recycling of DCHA salt of the enantiomerically pure undesired indole acid (*S*)-2

To have a truly effective and economically viable process for the asymmetric synthesis of chiral compound **5**, the undesired enantiomer, as (*S*)-**2**, would have to be recyclable. In this regard, a number of methods had previously been screened in our laboratories indicating that it was possible to epimerize this center in acid.[6] Additionally, the acid was to be converted to the ester for recycling. Based on this information, it was deemed logical to evaluate the possibility of the epimerization of this center while the acid was simultaneously converted to the ester. If successful, this would avoid a two-step operation of epimerization followed by esterification. Indeed this streamlined process worked well. Consequently, (*S*)-**2** acid was subjected to the normal esterification conditions with ethanol in the presence of a

catalytic amount of sulfuric acid at 95 °C, resulting in complete racemization and esterification in one pot.

2.6. Bromination of the DCHA salt of the enantiomerically pure indole acid (*R*)-2

Initially, it was not known whether the stereogenic center in intermediate **2** (Scheme 1) would survive the bromination conditions that were required for the preparation of chiral intermediate **3**. Thus, to ensure that the center did not epimerize, the enantiomerically pure DCHA salt of the indole acid (*R*)-**2** was subjected to bromination conditions. Upon completion, we were pleased to see the stability of the stereocenter as well as complete recovery of the enantiomerically pure brominated indole acid **3**.

3. Conclusions

The desired indole-ethyl ester enantiomer **1**, the required intermediate for the synthesis of chiral compound **5**, was prepared in near theoretical yield and greater than 97% ee by classical kinetic resolution using an enzyme-catalyzed asymmetric hydrolysis of racemic ester **1**. The undesired free acid antipode of **1**, (*S*)-**2**, was recycled via a racemization/esterification procedure to produce racemic ester **1**. Resubjection of the recovered racemic ester to an additional cycle of enzyme-catalyzed reaction provided an efficient source for the supply of the intermediate **2**. Using the optimized procedures of the reactions as outlined herein, a kilogram-scale process for the preparation of the desired ester enantiomer **1** was thus established. Additionally, the recyclability of the biocatalyst was demonstrated, making the process much more appealing economically as well as environmentally.

Finally, it is important to note that the lipase from *P. fluorescens* has previously been used in the kinetic resolution of primary[7] and secondary[8] alcohols, esters[9] as well as amines,[10] and it should be considered as a valuable catalyst for the preparation of a wide variety of chiral organic compounds.

4. Experimental

4.1. Analytical methods

1. Crude enzyme preparation (lyophilized powder with specific activity of 25,000U/g, according to the vendor for lot LAKZ1053102, was purchased from Amano Enzyme, USA).

2. HPLC monitoring: The time course of the reaction was monitored on a Zorbax C8 reverse-phase column (4.6 mm × 250 mm). The column operated at ambient temperature with a linear gradient solvent system with a 15 min run time, during which the concentration of solvent B was raised from 50% to 95% [A (2 mM ammonium formate, pH 3.5):B (acetonitrile/solvent A 9:1)] with a flow rate of 1 ml/min.

3. SFC Chiral column chromatography: Chirality evaluation of the product of the reaction examined on a Chiralcel OJ column running in 15% $MeOH/CO_2$ with a flow rate of 1.5 ml/min at 35 °C during a 20 min run time.

4. LC-mass: Mass spectrometric analysis was run on a C18 reverse-phase column-equipped mass spectrometer. This column (3.0 × 40 mm) was developed with an ammonium formate buffer, pH 3.5, as above, with a flow rate of 1 ml/min over 2.8 min during which the concentration of B was raised from 5% to 100%.

5. ^{1}H NMR were recorded at 400 MHz using a Bruker Avance 400. Circular dichroism (CD) samples were scanned between 200 and 400 nm in methanol at ambient temperature using JASCO spectropolarimeter, J810 (Japan).

6. The enzyme enantiomeric ratio (E) was determined according to the formula $E = [\ln[(1-c)(1-ee_s)]]/[\ln[(1-c)(1+ee_s)]]$, where $c = ee_s/ee_s + ee_p$.[11] Using this formula, the E value for the enzyme that was used in 36.6 g reaction run (see below) was calculated to be 455.

4.2. Enzymatic resolution of the racemic indole ester 1

The crude indole-ethyl ester **1** was obtained from the indolization reaction[3] as a DMAc solution. The mixture, after dilution with water (250 ml), was extracted with MTBE (500 ml × 3). The MTBE extract sequentially washed with 10% $NaHCO_3$ (250 ml × 2) and water (250 ml × 2), and dried over sodium sulfate. The dried solution containing 36.6 g racemic ester substrate **1** was concentrated under reduced pressure and the residue dissolved in DMF (40 ml). The DMF solution of the ester was used directly in the enzymatic hydrolysis. This reaction was run at room temperature using 150 g of *P. fluorescens* lipase suspended in 360 ml of 0.1 M phosphate buffer (pH 7.5). The crude DMF substrate solution was added to the lipase buffer suspension over a 30-min period using a dropping funnel. Finally, the dropping funnel was washed with 100 ml of hexane and the resulting solution was added to the reaction flask. The final mixture was stirred for an additional 30 min and the pH was adjusted to 7.5. After overnight incubation with stirring at room temperature, the pH of the reaction mixture was again adjusted to 7.5. The reaction was monitored by HPLC and the chirality of the reaction product was evaluated by chiral column equipped HPLC. The reaction was harvested when ee greater than 95% for the desired ester was achieved. The product was isolated by extraction with MTBE (500 ml × 3). The MTBE extract washed with water (500 ml × 3), and dried over sodium sulfate. A sample of the dried product was evaluated by NMR, chiral-HPLC, HPLC, LC-Mass, and CD. All of the results confirmed the structure of the desired ester, which had been obtained in an enantiomeric excess of 97.9%. The CD of this material showed a negative (−) optical rotation at 275 nm when examined in methanol.

The remaining MTBE extract was concentrated under reduced pressure. The residue was dissolved in a minimal amount of hexane with mild heating and stored at 4 °C. After an overnight incubation, rather soft crystals were recovered from the hexane solution. The recovered crystal washed with cold hexane and dried to give (*R*)-**1** (18.3 g LC assay, 50% yield with 97.9% ee).

4.3. Crystallization of desired ester (*R*)-1

A sample of the extract was worked up and the soft crystals, as described above, were recovered. The crystals thus obtained were cooled down and taken into a small amount of cold ethanol (acetone–dry ice). The resulting slurry was placed at 4 °C overnight and then washed with cold ethanol (acetone-dry ice) by filtration. The resulting beige colored firm crystal was dried under vacuum and its melting point was determined to be 83.7–84.5 °C.

4.4. Isolation of the DCHA salt of the undesired enantiomer of the indole free acid (*S*)-2

The pH of the aqueous phase containing 18.3 g of acid, after extraction of the desired ester, adjusted to 3.5 by the slow addition of neat orthophosphoric acid. The resulting solution was exhaustively extracted with MTBE. The MTBE extract was washed with acidified water (pH 3.5) and its volume adjusted to 250 ml. To the MTBE solution was gradually added DCHA (15.9 ml, 80 mmol) from a dropping funnel over 30 min while the mixture was mechanically stirred. Additional MTBE (250 ml) was added to the reaction mixture to convert the resulting thick paste, which was formed during the addition of the DCHA into a free-flowing uniform slurry. The resulting slurry was stirred for an additional 3 h and filtered. The recovered solid was washed with cold MTBE and dried under vacuum to provide (*S*)-**2**·DCHA salt (13.5 g LC assay, 74% yield with >97% ee).

4.5. Coupled esterification–racemization and recycling of the DCHA salt of the enantiomerically pure undesired indole acid (*S*)-2

In anticipation of the large-scale recycling of the undesired acid and its transformation into the enantiomerically pure desired ester **1**, 1.2 g of DCHA salt of indole acid was esterified and racemized using absolute ethanol (10 ml) and sulfuric acid (0.5 ml) at 95 °C for 7 h. The resulting racemic mixture of the indole-ethyl ester was isolated and then subjected to the enzyme-catalyzed asymmetric hydrolysis under our standard reaction condition. The enantiomeric excess of the isolated ester was established to be 93.5%. In this experiment, the lower than expected value for the ee might have been caused by an early harvest of the reaction.

4.6. Bromination of enantiomerically pure desired indole acid (*R*)-2

Bromination was carried out according to the published procedures. Briefly, 580 mg (~2.5 mmol) of the enantiomerically pure desired indole-acid (*R*)-**2** was dissolved in 10 ml of dichloromethane and the solution cooled to −20 °C. Pyridine (0.4 ml, ~5 mmol) was then added to the solution, followed by the dropwise addition of 0.32 ml of bromine (~6.2 mmol). After completion of the addition, the reaction mixture was aged for an additional hour at −15 °C, and then acidified with 4.3 ml (~7.5 mmol) of acetic acid, followed by the portionwise addition of 488 mg (4.5 mmol) zinc dust. The resulting reaction mixture was then warmed up to room temperature, aged overnight and then concentrated. The resulting concentrate was suspended in 20 ml of MTBE followed by 10 ml of 10% aqueous acetic acid and filtered through a bed of Solka-Floc. The filter-bed was rinsed with 20 ml of additional MTBE and the organic phase was recovered from the bi-phasic filtrate. Upon workup, 580 mg (75%) of the brominated free acid, showing greater than 93% ee, was recovered.

4.7. Demonstration of the process

In preparation for an industrial-scale enzyme-catalyzed asymmetric hydrolysis of the indole-ethyl ester **1**, 76.5 g, 83 g and multi-kg-scale reactions were also demonstrated using our optimized conditions. The final volumes of the reactions had been adjusted to a substrate concentration of 100 g/l in these reactions. From these reactions, the desired ester enantiomer was consistently recovered with greater than 93% ee and 48% yields. The recovery in all these reactions was noteworthy as the values were near the theoretical yields for both the desired ester enantiomer and its free acid antipode.

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References

1. Lewis, R. A.; Soter, N. A.; Diamond, P. T.; Austen, K. F.; Oates, J. A.; Roberts, L. J. *J. Immunol.* **1982**, *129*, 1627–1631.
2. Jones, T. WO 01/78697, 2001.
3. Campos, K. R.; Journet, M.; Lee, S.; Grabowski, E. J. J.; Tillyer, R. D. *J. Org. Chem.* **2005**, *70*, 268–274.
4. Guidon, Y.; Yoakim, C.; Gillard, Y.; Girard, Y. EP-0300676, 1989.
5. Hillier, M. C.; Marcoux, J-F.; Zhao, D.; Grabowski, E. J. J.; McKeown, A. E.; Tillyer, R. D. *J. Org. Chem.*, in press.
6. Grabowsky, E. J. J.; Shafiee, A.; Marcoux, J-F.; Jounet, M. Merck Research Laboratories, unpublished information.
7. Hof, R. P.; Kellog, R. M. *Tetrahedron: Asymmetry* **1994**, *5*, 565–568.
8. Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129–6139.
9. Burgess, K.; Jennings, L. D. *J. Org. Chem.* **1990**, *55*, 1138–1139.
10. Kato, K.; Gong, Y.; Saito, T.; Yokogawa, Y. *J. Mol. Catal. B: Enzymatic* **2004**, *30*, 61–68.
11. Chen, C. S.; Shih-Hsiung, W.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *102*, 2812–2817.