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Biotransformation-mediated synthesis of (1*S*)-1-(2,6-dichloro-3-fluorophenyl)ethanol in enantiomerically pure form

Carlos A. Martinez [a], Eric Keller [b], Renzo Meijer [b], Gerard Metselaar [b], Gerlof Kruithof [b], Curtis Moore [c], Pei-Pei Kung [d,*]

[a] *Pfizer Global Research and Development, Chemical Research Development, MS 4073 Eastern Point Road, Groton, CT 06340, USA*
[b] *Syncom B. V., Kadijk 3, 9747AT Groningen, The Netherlands*
[c] *University of California, San Diego, La Jolla, CA 92122, USA*
[d] *Medicinal Chemistry, Pfizer Global Research and Development, La Jolla, CA 92121, USA*

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ABSTRACT

An efficient four-step biotransformation-mediated synthesis of (1*S*)-1-(2,6-dichloro-3-fluorophenyl) ethanol in enantiomerically pure form is described. This compound is a key intermediate required for the preparation of PF-2341066, a potent inhibitor of c-Met/ALK that is currently in clinical development. The described synthesis was used to manufacture 6 kg of the title compound and can also be employed to produce the corresponding (1*R*)-enantiomer.

1. Introduction

The 2-aminopyridine-containing compound PF-2341066 **1** (Scheme 1) is a potent, orally bioavailable c-Met/ALK inhibitor that is currently in phase III clinical trials.[1a,b] To enable the large-scale preparation of this molecule, we required an efficient and scalable synthesis of (1*S*)-1-(2,6-dichloro-3-fluorophenyl)ethanol (*S*)-**2** in enantiomerically pure form for subsequent use in a Mitsunobu coupling reaction with 3-hydroxy-2-nitropyridine **3** (scheme 1). Herein, we report our unsuccessful efforts to produce (*S*)-**2** via chemical means along with the development of a biotransformation-mediated process that affords the target molecule in high yield and excellent enantiomeric excess.

2. Results and discussion

Our initial efforts to produce **2** in optically active form (*S*)-**2** focused on the asymmetric reduction of commercially available 2,6-dichloro-5-fluorophenacetone **4** employing the literature reagents and/or reactions known to transform achiral aryl ketones to the corresponding chiral alcohols (Scheme 2). These reagents/reactions included the CBS reduction, (employing (*R*)-tetrahydro-1-methyl-3,3-diphenyl-1*H*,3*H*-pyrrolo-[1,2-*c*][1,3,2]oxazaborole),[2] (−)-DIP-Cl,[3] and (*S*)-α,α-diphenylpyrrolidinemethane.[4] As shown in Table 1, however, the enantiomeric purity of alcohol (*S*)-**2** produced in these experiments was unacceptably low and was often accompanied by low conversion and/or isolated yield. Similar difficulties were previously reported by Brown et al. during the DIP-chloride-mediated transformation of 2′,6′-dichloroacetophenones to the corresponding alcohols and are believed to be caused by the lack of planarity between the ketone and phenyl moieties in such molecules.[5]

As a result of these difficulties, we abandoned our efforts to produce alcohol (*S*)-**2** chemically in enantiomerically enriched form and instead examined several enzyme-catalyzed processes to accomplish this objective.

Accordingly, we tested the ability of isolated and/or purified enzymes to enantioselectively convert acetophenone **4** to the desired alcohol (*S*)-**2** (Scheme 2). Twenty seven commercially available ketone reductases and four alcohol dehydrogenases were screened employing both NADH and NADPH. From all of the commercial enzymes tested, only horse liver alcohol dehydrogenase (HLADH) catalyzed the reduction of **4** to (*S*)-**2** with excellent enantioselectivity. In addition, 94 yeast strains containing various ketone reductases were also screened. Encouragingly, we found that a proprietary *Rhodutorula* sp. strain (Y2-UC2387) reduced acetophenone **4** to alcohol (*S*)-**2** in 100% ee. Unfortunately, the low substrate loading (1 mg/mL) tolerated by both the HLADH and the yeast mediated bioreductions rendered both reagents economically unsuitable for our scale-up purposes (the detailed screening of the reductases mentioned above has been described in detail elsewhere).[6]

Due to the challenges we encountered when preparing optically active (1*S*)-1-(2,6-dichloro-3-fluorophenyl)ethanol (*S*)-**2** from acetophenone **4**, we also explored the synthesis of (*S*)-**2** via the enzyme-mediated kinetic resolution of racemic acetic acid 1-(2,6-dichloro-3-fluoro-phenyl)ethyl ester **5**. Acetate **5** was conveniently prepared in good yield on a large scale from 2,6-dichloro-5-fluor-

* Corresponding author. Tel.: +1 858 526 4867.
E-mail address: peipei.kung@pfizer.com (P.-P. Kung).

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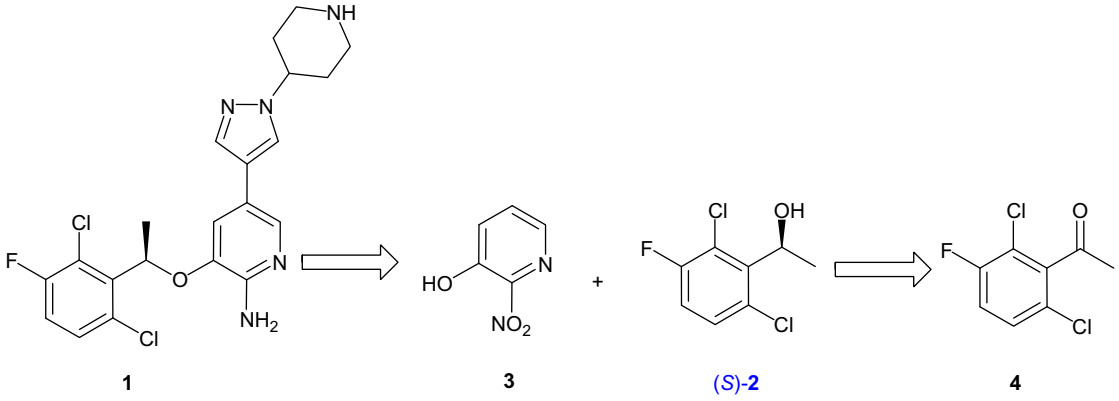
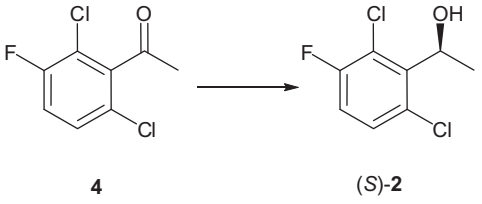


Scheme 1. Retrosynthesis of PF-2341066 **1** from 2,6-dichloro-5-fluorophenacetone **4**.



Scheme 2. Enantioselective reduction of 2,6-dichloro-5-fluorophenacetone **4** to chiral alcohol (*S*)-**2**.

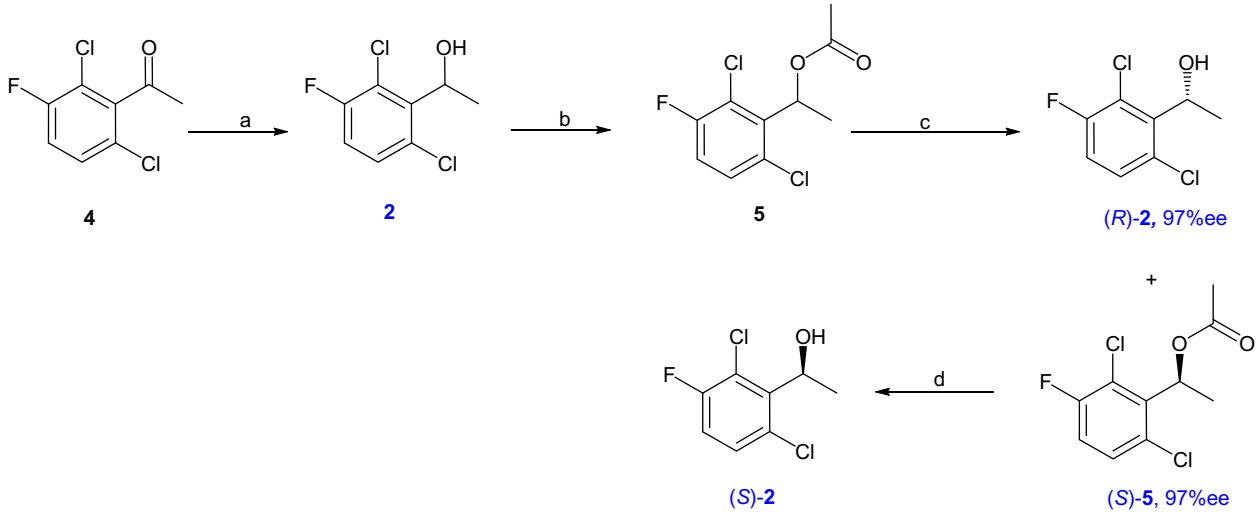
Table 1

Reagent(s)	% Conversion	% ee
BH_3·THF/(*R*)-methyl-CBS-oxazaborolidine (cat.)	90	22
((−)-B-chlorodiisopinocampheylborane ((−)-DIP-Cl)	10	ND
$NaBH_4$/TMSCl/(*S*)-α,α-diphenylpyrrolidinemethane	20	45

ophenacetone **4** by chemical reduction ($NaBH_4$) and acylation of the resulting alcohol **2** with acetic anhydride (Scheme 3). Several isolated and/or purified enzymes were then screened for their ability to selectively hydrolyze one enantiomer of acetate **5** (Table 2).[7] As shown in Table 2, eight of these enzymes effected the desired transformation with good enantioselectivity (*E* values >100). However, only pig liver esterase (PLE) exhibited rates of reaction that were sufficient enough for our scale-up purposes (data not shown). We therefore decided to focus our enzyme optimization efforts on the PLE-catalyzed hydrolysis of **5** since this enzyme showed the highest reactivity and was reported in the literature to selectively hydrolyze similar substrates with an (*R*)-configuration.[8]

During the early stages of optimization, our goal was to perform a reaction that could be carried out in less than 24 h and at substrate concentrations close to 1 M of **5** (207 g/L). That goal was achieved relatively easily, mainly because of the high catalytic activity of the PLE commercial preparation that was used (3.5 megaunits per liter). The reaction system in 0.1 M potassium phosphate buffer containing 175 kilounits of enzyme per liter (5% *v*/*v* PLE content in reactor), displayed good tolerance to high substrate concentration until 1 M concentration of **5** was reached; complete resolution (51–52%) was observed in less than 36 h (see Fig. 1). A 2 M substrate concentration yielded incomplete reactions after 36 h (42% conversion) and the reaction required 3 days to reach a complete resolution. A system utilizing 0.5 M (100 g/L) concentration of **5** allowed us to perform a reaction in 17–21 h. This reaction was then scaled multiple times on a 200–300 g scale to demonstrate the reproducibility of the enzymatic reaction.

Accordingly, the enantioselective hydrolysis of racemic **5** using pig liver esterase (PLE) afforded a close to 1:1 mixture of (1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethanol (*R*)-**2** (97.0% ee at 51% conversion) and (1*S*)-1-(2,6-dichloro-3-fluorophenyl)ethyl acetate



Scheme 3. Synthesis of (*S*)-**2** from **4** using PLE resolution. Reagents and conditions: (a) $NaBH_4$, methanol, 23 °C, 12 h, quant.; (b) $(CH_3O)_2CO$, pyridine, 23 °C, 12 h, 89%; (c) PLE-AS solution, K_2PO_4 buffer, NaOH (4 N), 23 °C, 12 h, 56% conversion; (d) LiOH, methanol, 0–5 °C, 45 min, 94%.

Table 2
E value comparison for different enzymes

Enzymes	Value E^{9}
Selective for hydrolysis of R acetate	
Candida antarctica lipase B (CAL-B), Pig Liver esterase, *Rhizopus delemar* lipase, Porcine Kidney acylase, cholesterol esterase, Bovine intestinal protease, sigma protease P6 type VIII, *Candida rugosa* lipase	(>100) High
Rhizopus orizae lipase, *Penicillum camembertii* lipase, *Pseudomonas* sp. Lipase, *Thermomyces* sp. Lipase, *Alcaligenes* sp. Lipase, *Chromobacterium viscosum* lipase, *Rhizomucor miehei* lipase, *Thermomyces lanuginosus* lipase, wheat germ lipase, Bromelain protease, Aspergillus niger protease, *Rhizopus niveus* protease, *Aspergillus saitoi* protease, acylase from aspergillus sp.	(<20) Low
Selective for hydrolysis of S acetate	
Candida antarctica lipase A (CAL-A), Subtilisin Carlsberg (protease from *Bacillus licheniformis*), *Rhizopus orizae* protease *Rhizopus delemar* lipase	(<10) Low

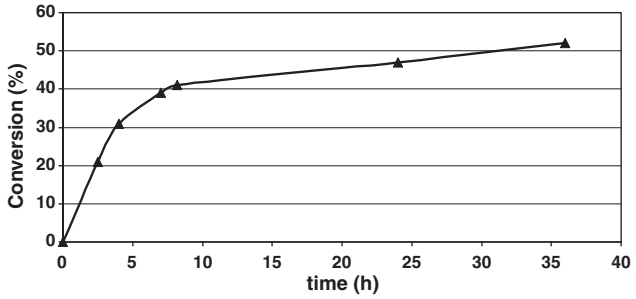


Figure 1. Reaction profile for the resolution of 250 g of **5** (1 M concentration) using PLE.

(*S*)-**5** (97.5% ee) in quantitative yield. These compounds were separated by simple silica gel chromatography, and acetate (*S*)-**5** was subjected to hydrolysis using lithium methoxide in methanol to give the desired (1*S*)-1-(2,6-dichloro-3-fluorophenyl)ethanol (*S*)-**2** in 87% yield and 99% ee as a white solid. The absolute configuration of alcohol (*S*)-**2** produced by this enzyme-mediated process was confirmed to be (*S*) by a single crystal X-ray diffraction study (Fig. 2).

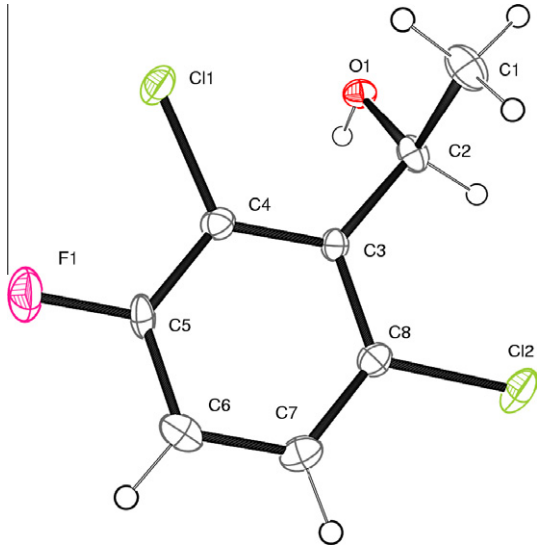


Figure 2. X-ray crystal structure of compound (*S*)-**2** (CCDC number 792017).

This process was successfully transferred to several large scale vendors, providing up to 6 kg of (*S*)-**2** as well as 6.5 kg of the corresponding (*R*)-isomer (*R*)-**2** from 15 kg of 2,6-dichloro-3-fluoroacetophenone.

In order to further maximize the production of enantiomerically pure (*S*)-**2** using this PLE resolution method, we also investigated the conversion of the (1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethanol (*R*)-**2** that was produced to the corresponding (1*S*)-isomer (*S*)-**2** by inversion of the stereogenic center (Scheme 4) as previously reported in the literature.[10] Thus, the mixture of (*R*)-**2** and (*S*)-**5** initially generated by PLE hydrolysis of **5** was treated with methanesulfonyl chloride (0.5 equiv based on the amount of acetate **5** employed) and the resulting mixture of methanesulfonate (*R*)-**6** and acetate (*S*)-**5** was exposed to potassium acetate at elevated temperature. This two-step process afforded acetate (*S*)-**5** in an acceptable yield (61% for both steps, procedure not optimised) and 97% ee. Acetate (*S*)-**5** was efficiently converted to alcohol (*S*)-**2**, using the hydrolysis procedure described above. Thus, we developed an efficient process to recycle (*R*)-**2** produced by the biotransformation of **5**, which could be used in future scale-up activities.

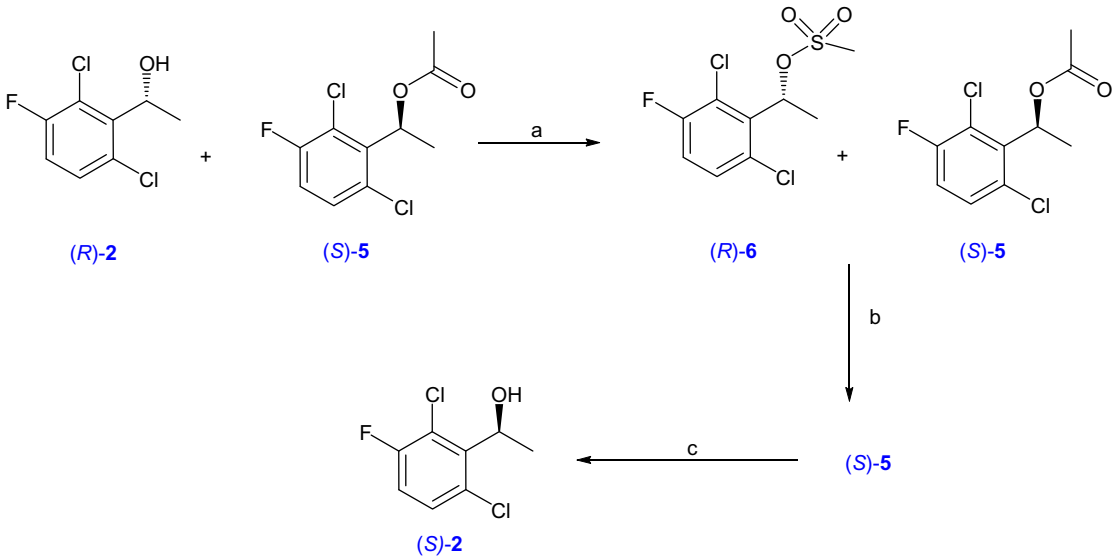
3. Conclusion

In conclusion, we have developed a novel procedure for the multi-kilogram preparation of enantiomerically pure (1*S*)-1-(2,6-dichloro-3-fluorophenyl)ethanol (*S*)-**2**, which is a key intermediate required for the synthesis of a promising clinical candidate (PF-2341066) for the c-Met/ALK targeted therapy. This biotransformation methodology has set the ground work for early phase clinical development of this compound.

4. Experimental

2,6-Dichloro-3-fluoroacetophenone was obtained from Aldrich (reagent quantities), SynQuest (bulk) and Apollo Scientific (bulk). Pig Liver Esterase (PLE) is an enzyme manufactured by Roche in Germany as a crude esterase preparation from pig liver and sold by biocatalytics (now Codexis Inc) under the name PLE-AS. Achiral HPLC performed using a Phenomenex luna C18 column, 3μ, C18, 4.6 × 30 mm; flow rate 2.0 mL/min; injection volume: 5 μL; mobile phases: A: Water-0.1% TFA B: Acetonitrile-0.1% TFA; using a gradient 5–95% B in 3 min, and monitoring at 254 nm. The enantiomeric purities of (*S*)-**2** and (*R*)-**2** were determined using Daicel Chiralpak columns. Chiral HPLC for racemic alcohol **2** was performed using Chiralcel ADR-H, 3μ, C18, 4.6 × 150 mm; flow rate 0.8 mL/min; injection volume: 10 μL; mobile phases: A: Water B: Acetonitrile; isocratic: 35% B for 31 min. Chiral HPLC for racemic acetate **5** was performed using Chiralcel OJ-RH, 3μ, C18, 4.6 × 150 mm; flow rate 0.6 mL/min; injection volume: 10 μL; mobile phases: A: Water B: Acetonitrile; isocratic: 50% B for 15 min.

All chemical reductions were performed in septum-sealed flasks under a slight positive pressure of dry nitrogen. All commercial reagents were used as received from their respective suppliers. All solvents were purchased from EMD in anhydrous form and used without additional purification. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 101 MHz. Chemical shifts are reported in ppm (δ) using internal solvent signals as references and coupling constants (when given) are reported in hertz (Hz). Optical rotations were determined using



Scheme 4. Recycling of compound (*R*)-**2**. Reagents and conditions: (a) CH_3SO_2Cl, pyridine, 23 °C, 3 h; (b) CH_3CO_2K, DMF, 100 °C, 12 h, 61%; (c) LiOH, methanol, 0–5 °C, 45 min, 94%.

a Perkin–Elmer polarimeter. The enantiomeric purities of compounds were determined by chiral SFC-MS (supercritical flash chromatography-mass spectrum). The type of chiral column and flow rate used in SFC were indicated in the individual experimental procedure.

4.1. Procedure for chemical reduction catalyst screening

2,6-Dichloro-5-fluoroacetophenone **4** was added to reducing agent(s) (2 equiv) in dichloromethane at −30 °C or room temperature and the reaction mixture was stirred at that temperature for 2 h. The reaction was then quenched with methanol. The solvents were evaporated to get an oil. This oil was analyzed by TLC to determine the % conversion and by SFC-MS to determine the % ee of the product. The enantiomeric purity of (*S*)-**2** was determined using a Chiralpak AD-H SFC column (4.6 × 250 mm); flow rate: 3 mL/min; mobile phase: 30% MeOH in CO_2 at 140 bar.

4.2. Procedure for enzyme screening

The resolution of racemic **5** was carried out as follows. A 96-well plate containing various esterases and hydrolases prepared in house[7] was thawed for 5 min. Next, 80 μL of potassium phosphate buffer (0.1 M, pH 7.2) was then dispensed into the wells using a multi-channel pipette. Then, 10 μL of the substrate stock solution (50 mg of 5 per mL acetonitrile) was added to each well via a multichannel pipette, and the 96 reactions were incubated at 30 °C and 750 rpm. The reactions were sampled after 16 h by the transfer of 50 μL of the reaction mixture into a new 96-well plate, which was then quenched by the addition of 150 μL of acetonitrile. The 96-well plate was then centrifuged, and the organic supernatant transferred from each well into another 96-well plate. Sampled reactions were then analyzed by HPLC. The same plate was used to analyze the samples for both fractional conversion and enantioselectivity using alternating columns on the HPLC simultaneously.

4.3. 1-(2,6-Dichloro-3-fluorophenyl)ethanol 2 and 1-(2,6-dichloro-3-fluorophenyl)ethyl acetate 5

To a solution of 2,6-dichloro-3-fluoroacetophenone **4** (1 kg, 4.8 mol) in methanol (8 L) was added in portions $NaBH_4$ (300 g, 7.9 mol) while maintaining the temperature below 15 °C. The reaction mixture was stirred at room temperature overnight. The reaction mixture was cooled in ice/water (0–5 °C) and acidified with hydrochloric acid (2 M) until a pH of 1–2 was reached. The methanol was evaporated in vacuo. The water layer was extracted with methylene chloride (3 × 2 L). The combined organic layers were dried over sodium sulfate, filtered, and evaporated to give compound **2** as a clear oil. Acetic anhydride (1 L, 10.6 mol) was added to a solution of **2** in pyridine (900 mL, 10.6 mol) in a single portion (caution: slightly exothermic). The reaction mixture was stirred at room temperature for 12 h. The organic layer (ethyl acetate) was washed with water (3 × 2 L) and once with brine. The solvent of the ethyl acetate layer was removed in vacuo and the residue was stripped with toluene. A yellow oil (**5**, 1100 g, 89%) was obtained with a purity of 98% according to HPLC-MS, ^{1}H NMR, and ^{13}C NMR spectra.

Compound **2**: ^{1}H NMR ($CDCl_3$) δ ppm 1.65 (d, J = 6.8, 3H), 5.58 (q, J = 6.8, 1H), 6.97–7.07 (m, 1H), 7.26 (dd, J = 8.8, 4.8, 1H); ^{13}C NMR ($CDCl_3$) δ ppm 21.3, 68.4, 115.6, 115.8, 121.4 (d, J = 19.0), 129.6 (d, J = 7.3), 140.6, 157.3 (d, J = 248.8).

Compound **5**: ^{1}H NMR ($CDCl_3$) δ ppm 1.88 (d, J = 6.8, 3H), 2.31 (s, 3H), 6.62 (q, J = 6.8, 1H), 7.25 (t, J = 8.46, 1H), 7.49 (dd, J = 8.8, 5.1, 1H); ^{13}C NMR ($CDCl_3$) δ ppm 17.8, 20.7, 69.5, 115.9, 116.1, 121.9 (d, J = 19.0), 129.5 (d, J = 8.1), 129.6, 137.7, 157.3 (d, J = 248.8), 170.1.

4.4. Enzymatic resolution on a 2.8 kg scale

4.4.1. (1*S*)-1-(2,6-Dichloro-3-fluorophenyl)ethyl acetate (*S*)-5 and (1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethanol (*R*)-2

To a 20 L reactor equipped with a pH-electrode connected to a Methrom titrino pH-stat, an overhead stirrer, and a base addition line were added the PLE-AS solution (0.75 L) and 13 L of potassium phosphate buffer solution. The pH of the solution was adjusted to 6.9 by the addition of NaOH (4 M). Compound **5** (2800 g, 11.2 mol) was added to the PLE-AS solution. This suspension was then stirred at room temperature for 30 h until the reaction was complete. *tert*-Butylmethylether (TBME, 2 L) was added to the reaction and the mixture was stirred for an additional 5–10 min. The resulting organic emulsion was passed through sand and then transferred to a separatory funnel/extractor. The aqueous layer was extracted four more times with TBME (2200 mL × 4). The combined organic

layers were dried with sodium sulfate and concentrated in vacuo to give a mixture of (*R*)-**2** and (*S*)-**5** as a yellow oil (2480 g).

The mixture was separated by column chromatography in 1100–1300 g batches on 25 kg of silica gel (MP silitech 32-63, 60A) using a gradient of heptanes and ethyl acetate. The column can be used 6 times without a loss of activity. The volume and gradient of the elution solvents are as follows: heptanes (40 L), 2% ethyl acetate in heptanes (20 L), 5% ethyl acetate in heptanes (40 L), 7% ethyl acetate in heptanes (20 L), 9% ethyl acetate in heptanes (20 L), 100% ethyl acetate in heptanes (10 L),and 0% ethyl acetate in heptanes (40 L to regenerate the column). Fractions (10 L/fraction) were collected [compound (*S*)-**5** was eluted in fractions 10–13 and compound (*R*)-**2** was eluted in fractions 15–17] and evaporated to give a colorless oil [compound (*S*)-**5**, 43%, >99% ee, R_f: 12.95 min.]. The enantiomeric purity of (*S*)-**5** was determined using a Chiracel OJ-RH (150 × 4.6 mm) CH_3CN/water (50:50; 0.6 mL/min). The other enantiomer of (*S*)-**5** (structure not shown) was eluted at 11.55 min.

Compound (*S*)-**5**: ^{1}H NMR ($CDCl_3$) δ ppm 1.66 (d, *J* = 7.1, 3H), 2.08 (s, 3H), 6.39 (q, *J* = 6.9, 1H), 7.02 (t, *J* = 8.5, 1H), 7.26 (dd, *J* = 9.0, 4.7, 1H); ^{13}C NMR ($CDCl_3$) δ ppm 17.8, 20.7, 69.5, 115.9, 116.1, 121.9 (d, *J* = 19.0), 129.5 (d, *J* = 7.3), 137.7, 157.4 (d, *J* = 248.8), 170.2.

4.4.2. (1*S*)-1-(2,6-Dichloro-3-fluorophenyl)ethanol (*S*)-2

Lithium hydroxide hydrate (307 g, 7.33 mol, 3 equiv) was added to a solution of (*S*)-**5** (213 g, 2.44 mol) in methanol (10 L) at 0–5 °C over 45 min. The mixture was stirred for 5 min and concentrated in vacuo (maintain water bath temperature at 45 °C). The resulting solid residue was partitioned between TBME (3 L) and sat. $NaHCO_3$ (aq) (2 L). The organic layer was separated and the milky aqueous layer was extracted again with MTBE (3 × 0.5 L). The combined organic layers were dried over Na_2SO_4 and concentrated to afford compound (*S*)-**2** as a white solid (98% ee, 479 g, 2.29 mol, 94% yield). $[\alpha]_D^{20} = +6.9 \pm 1.1$ (*c* 0.87, MeOH). ^{1}H NMR ($CDCl_3$) δ ppm 1.65 (d, *J* = 6.8 Hz, 3H), 2.94 (d, *J* = 9.9, 1H), 5.58 (dq, *J* = 10.0, 6.9, 1H), 6.92–7.08 (m, 1H), 7.26 (dd, *J* = 9.0, 4.9, 1H); ^{13}C NMR ($CDCl_3$) δ ppm 21.3, 68.4, 115.5, 115.8, 121.4 (d, *J* = 19.0), 129.6 (d, *J* = 8.1), 140.5, 157.3 (d, *J* = 248.8); Anal. Calcd for $C_8H_7Cl_2FO$: C, 45.96; H, 3.38. Found: C, 46.04; H, 3.32.

4.5. Synthesis of (*R*)-6 from (*R*)-2

Methanesulfonyl chloride (0.06 mL, 0.6 mmol) was added to a solution of a mixture of (*R*)-**2** and (*S*)-**5** (0.48 mmol) in 4 mL of pyridine under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3 h, and then evaporated to obtain an oil. Water (20 mL) was added to the mixture and then ethyl acetate (2 × 20 mL) was added to extract the aqueous solution. The organic layers were combined, dried, filtered, and evaporated to give a mixture of (*R*)-**6** and (*S*)-**5**. This mixture was used in the next step reaction without further purification. Compound (*R*)-**6**: ^{1}H NMR ($CDCl_3$) δ ppm 1.85 (d, *J* = 6.8, 3H), 2.92 (s, 3H), 6.46 (q, *J* = 7.0, 1H), 7.12 (dd, *J* = 8.8, 7.8, 1H), 7.34 (dd, *J* = 8.8, 4.8, 1H); ^{13}C NMR ($CDCl_3$) δ ppm 19.3, 38.4, 75.2, 117.1, 117.3, 122.3 (d, *J* = 19.8), 129.1 (d, *J* = 3.7), 135.6, 157.4 (d, *J* = 256.9).

4.6. Synthesis of (*S*)-5 from a mixture of (*R*)-6 and (*S*)-5

Potassium acetate (0.027 g, 0.26 mmol) was added to a solution of a mixture of (*R*)-**6** and (*S*)-**5** (0.48 mmol) in 4 mL of DMF under a nitrogen atmosphere. The reaction mixture was heated to 100 °C for 12 h. Water (20 mL) was added to the reaction mixture and EtOAc (2 × 20 mL) was added to extract the aqueous solution. The combined organic layers were dried, filtered, and evaporated to give an oil (*S*)-**5** (72 mg, 61% yield over two steps) in 97.6% ee.

4.7. Crystallization and X-ray structure determination of (*S*)-2

Vapor diffusion of hexanes into a diethyl ether solution of (*S*)-**2** yielded X-ray quality crystals in the form of colorless needles. The single crystal X-ray diffraction studies were carried out on a Bruker Kappa APEX-II CCD diffractometer equipped with Cu K_α radiation. A 0.18 × 0.08 × 0.05 mm colorless needle was mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using φ and ϖ scans. Crystal-to-detector distance was 45 mm and exposure time was 60 s/frame using a scan width of 1.0°. The data were integrated using the Bruker SAINT software program and scaled using the SADABS[11] software program. Solution by direct methods (SHELXS)[11] produced a complete phasing model consistent with the proposed structure. All nonhydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-97).[12] All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-97. Absolute configuration was established by anomalous dispersion effects in diffraction measurements on the crystal, giving a value of 0.04(2).

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