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Note

Preparative-scale Enzyme-catalyzed Synthesis of (*R*)-α-Fluorophenylacetic Acid

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A preparative-scale asymmetric synthesis of (*R*)-α-fluorophenylacetic acid, a useful chiral derivatizing reagent, is described. Starting from ethyl α-bromophenylacetate, α-fluorophenylmalonic acid dipotassium salt was prepared in three steps (54% yield), including nucleophilic substitution by the fluoride ion as the keystep. Both the purified form and crude preparation of arylmalonate decarboxylase in *E. coli* worked well on this substrate, and (*R*)-α-flurophenylacetic acid (>99% *e.e.*) was prepared in a quantitative yield.

Key words: (*R*)-α-fluorophenylacetic acid; arylmalonate decarboxylase; α-fluorophenylmalonic acid; asymmetric decarboxylation

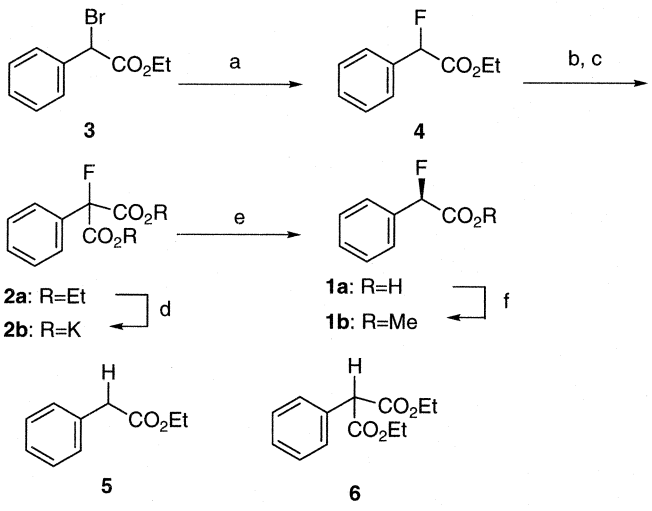
Enantiomerically enriched forms of α-fluorophenylacetic acid (**1**) have been developed as useful chiral derivatizing reagents for determinating enantiomeric excess (*e.e.*) as well as the absolute configuration of secondary alcohols and amines.[1-3] So far, the enantiomeric resolution of a racemate has been achieved by preferential crystallization of a diastereomeric amine salt with α-phenethylamine[3] and lipase-catalyzed enantioselective hydrolysis of the corresponding ester.[4] Our preliminary result that microorganism-catalyzed asymmetric decarboxylation yielded the (*R*)-enantiomer of **1**[5,6] prompted us to develop an enzyme-catalyzed asymmetric synthesis of (*R*)-**1**.

Toward this end, an inexpensive and efficient route to the substrate (**2**) was devised. Although elaborate procedures for direct fluorination of diethyl phenylmalonates and related compounds have been reported,[7,8] we chose the nucleophilic substitution of ethyl α-bromophenylacetate (**3**) by the fluoride ion as the keystep for introducting the fluorine atom, due to the inexpensiveness and ease of handling of fluorinating reagents.

Our first attempt, the conventional procedure, of treating **3** with KF in acetamide, however, resulted in contamination by non-fluorinated by-product (**5**) by as much as 20% in the desired product. As this by-product (**5**) apparently originated from **3** by homolytic cleavage of the C–Br bond, the reaction was carried out without oxygen and light. As a result, the contamination by **5** could be suppressed to only 3% of the desired product (**4**), and the yield of **4** was raised to 72%.

Diester **2a** was obtained by the ethoxycarbonylation of **4** in an 81% yield. Although all attempts to separate the non-fluorinated contaminant (**5** or **6**) from the desired product (**4** or **2a**) were unsuccessful, the dipotassium salt (**2b**) was fortunately obtained in a crystalline form and proved to be free from the non-fluorinated contaminant. The hydrolysis of **2a** was very fast in ethanol. As the very small amount of water contained in commercially available KOH effectively worked as a nucleophile, the dipotassium salt (**2b**) was precipitated in a pure state.

The asymmetric decarboxylation of **2b** by arylmalonate decarboxylase, which was overexpressed in *E. coli* JM 109 and subsequently purified, worked very well, and (*R*)-**1a** was obtained in a 93% yield. The *e.e.* value of **1a** was determined to be 99.1% by an HPLC analysis of the corresponding methyl ester (**1b**). Recrystallization of **1a** afforded the enantiomerically pure form with recovery of 69%. So far, due to a low enzyme activity of whole cells of the parent microorganism, *Alcaligenes bronchisepticus*, the reaction proceeded substantially slowly and the *e.e.* value of the product (**1a**) was not satisfactory (95%). This has probably been due to spontaneous non-enzymatic decarboxylation of the substrate occurring at the end of the reaction period



Scheme. a) KF/acetamide; b) LDA/THF; c) $ClCO_2Et$; d) KOH/EtOH-H_2O; e) arylmaronate decarboxylase; f) $TMSCHN_2$.

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(5 days). The moderate reactivity of this substrate deduced from the kinetic properties of K_m 22.1 mM and k_{cat} 13.9 s^{-1}, which was clarified in the present study, also supports this explanation. The asymmetric decarboxylation also worked successfully with a crude enzyme extract of *E. coli* overexpressing this enzyme in a quantitative yield.

In conclusion, an efficient preparative method for the asymmetric synthesis of (*R*)-α-fluorophenylacetic acid was established.

Experimental

Melting point (mp) data are uncorrected. Optical rotation values were recorded by a Jasco DIP 360 polarimeter. IR spectra were measured as films for oils and as KBr discs for solids with a Jasco FT/IR-410 instrument. ^{1}H-NMR spectra were recorded at 270 MHz with a JEOL JNM-EX 270 instrument unless otherwise stated. Column chromatography was carried out with Katayama 60 K070-WH silica gel of 70–230 mesh. Acetamide and potassium fluoride were purchased from Tokyo Chemical Industry (A0007) and Wako Chemical Ltd. (spray-dried, 166-13241), respectively.

Ethyl α-fluorophenylacetate (4). To a 300-ml three-necked flask was attached a Gymroth condenser, the other two necks being sealed by septam caps, and the reaction flask was flame-dried *in vacuo* and charged with Ar. Acetamide (14.8 g, 327 mmol) and potassium fluoride (14.3 g) were pre-dried under vacuum in a desiccator and added to the reaction flask. The mixture was heated to 90°C in an oil bath, and degassed four times by repetitively evacuating (60 mm Hg, 5 seconds) and re-charging with argon. The reaction flask was then shielded from light by a thin black flexible bag and the bath temperature was raised to 100°C. To the reaction mixture was added ethyl α-bromophenylacetate **3** (19.9 g, 82.0 mmol) and *via* a plastic syringe with a cannula that pierced the black bag and septam cap. The mixture was stirred for 6 hours at 100°C before being quenched by the addition of a phosphate buffer solution (100 ml, pH 6.0, 0.2 M) at room temperature and then extracted with ether. The organic layer was washed with brine, dried over anhydrous sodium sulfate and carefully concentrated under reduced pressure (100 mmHg). The residue was purified by chromatography on silica gel (400 g, hexane/ethyl acetate=20/1) and then by distillation to give **4** (10.7 g, 72%) as colorless oil, bp 82–85°C/5 mmHg; IR ν_{max} cm^{-1}: 3482, 2984, 1759, 1604, 1496, 1456, 1371, 1275, 1214, 1188, 1088, 1057; ^{1}H-NMR ($CDCl_3$) δ: 1.26 [3H, t, J=7.3 Hz, $C_6H_5CH(F)CO_2CH_2CH_3$], 4.22 [1H, dq, J=10.9, 7.3 Hz, $C_6H_5CH(F)CO_2CH_2CH_3$], 4.28 [1H, dq, J=10.9, 7.3 Hz, $C_6H_5CH(F)CO_2CH_2CH_3$], 5.77 [d, 1H, J=47.8 Hz, $C_6H_5CH(F)CO_2CH_2CH_3$], 7.29–7.48 [m, 5H, $C_6H_5CH(F)CO_2CH_2CH_3$].

This was contaminated with **5** (3%), as judged from a comparison of the signals δ 4.22 and 4.28 for **4**, and 3.61 for **5**, [2H, s, $C_6H_5CH_2CO_2CH_2CH_3$].

Diethyl α-fluorophenylmalonate (2a). To a solution of diisopropylamine (5 ml, 36.0 mmol) in tetrahydrofuran (100 ml) was added *n*-butyllithium (1.71 M in hexane, 19.3 ml) at 0°C under Ar, and the mixture was stirred for 5 min. After being cooled to −30°C, monoester **4** (5.07 g, 27.8 mmol) was added dropwise over 5 min, and the mixture was stirred for 5 min at that temperature. The mixture was then cooled to −78°C, and ethyl chloroformate (3.15 ml, 32.5 mmol) was added dropwise over 5 min. The mixture was stirred at that temperature for 15 min before being raised to 0°C. The reaction was quenched by additing a phosphate buffer solution (100 ml), and the mixture was extracted with ether. The organic layer was successively washed with a saturated sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (400 g, hexane/ethyl acetate=12/1) and then by distillation to give **2a** (5.65 g, 81%) as a colorless oil, bp 85°C/1 mmHg; IR ν_{max} cm^{-1}: 3502, 2984, 1755, 1597, 1451, 1368, 1271, 1097, 1071, 1045, 859, 735, 696, 631; ^{1}H-NMR ($CDCl_3$) δ: 1.31 [6H, t, J=7.2 Hz, $C_6H_5C(F)(CO_2CH_2CH_3)_2$], 4.33 [4H, q, J=7.2 Hz, $C_6H_5C(F)(CO_2CH_2CH_3)_2$], 7.38–7.61 [5H, m, $C_6H_5C(F)(CO_2CH_2CH_3)_2$].

This was contaminated with **6** (3%), as judged from the comparison of signals δ 4.33 for **2a**, and 4.61 for **6**, [1H, s, $C_6H_5CH(CO_2CH_2CH_3)_2$].

Dipotassium salt of α-fluorophenylmalonate (2b). Pottasium hydroxide (85% purity, 0.153 g, 9.15 mmol) was dissolved in ethanol (10 ml) at room temperature. The solution was cooled to 0°C, and diester **2a** (0.497 g, 1.96 mmol) was added. A white precipitate immediately appeared. The precipitate was collected by suction filtration, successively washed with cold ethanol and ether, and then dried *in vacuo* to give **2b** (0.493 g, 92%) as a colorless powder. IR ν_{max} cm^{-1}: 3292, 1635, 1405, 1327, 1044, 755, 699, 637; ^{1}H-NMR (D_2O) δ: 7.43–7.59 [5H, m, $C_6H_5C(F)(CO_2K)_2$].

Overexpression and purification of arylmalonate decarboxylase. To achieve greater production of the enzyme, plasmid pAMD101, which contains the gene coding for arylmalonate decarboxylase, was extracted from *E. coli* DH5α-MCR strain[9] by the conventional manner and subsequently transformed to competent *E. coli* JM109 cells. Cultivation of the transformed microorganism, induction of the gene, and purification of the enzyme was carried out according to the reported procedure.[10] A crude form of enzyme preparation (30 U/mg, total 24600 U) at the stage of ammonium sulfate precipitation and the purified enzyme (424 U/mg, total 15800 U) were obtained from a 1.5-liter culture of *E. coli* JM109/pAMD101.

(R)-α-Fluorophenylacetic acid (1a). Dipotassium salt **2b** (100 mg, 0.365 ml) was dissolved in a Tris-HCl buffer (3M, pH 8.5, 0.6 ml) and to this was added the purified enzyme preparation of arylmalonate decarboxylase (1.8 ml, 720 units). The mixture was incubated at 35°C for 40 min, before adding hydrochloric acid (2N, 2 ml). The

mixture was saturated with sodium chloride and extracted with ethyl acetate. The organic layer was concentrated *in vacuo* to give a solid (65.4 mg). The residue was purified by chromatography on silica gel (800 mg, ethyl acetate/acetic acid=99/1) to give **1a** (52.3 mg, 93%) as a slightly brownish solid. This was recrystallized from hexane-ether to give **1a** (36.2 mg, 64%) as colorless needles, mp 102–103°C [lit.[3] mp 103°C], $[\alpha]_D^{21}$-139° (*c* 1.24, $CHCl_3$) [lit.[3] $[\alpha]_D^{21}$-153° (*c* 1.25, $CHCl_3$)]; IR ν_{max} cm^{-1}: 2928, 1715, 1459, 1437, 1237, 1201, 1051, 739, 699, 646; ^{1}H-NMR (400 MHz, Jeol JNM-GX400, $CDCl_3$) δ: 5.83 (1H, d, J=47.4 Hz, $C_6H_5CH(F)CO_2H$)], 7.42–7.50 [5H, m, $C_6H_5CH(F)CO_2H$].

A portion of the recrystallized sample was converted to the corresponding methyl ester (**1b**) by being treated with trimethylsilyldiazomethane in methanol followed by a conventional workup. Crude **1b** was dissolved in a mixture of hexane-isopropyl alcohol (9/1), passed through a short column of silica gel, and analyzed by HPLC: column, Daicel Chiralcel OJ, 0.46 cm × 25 cm; eluent, hexane/isopropyl alcohol=9/1; flow rate, 0.5 ml/min; Rt 29.1 min [for the (*R*)-isomer as a single peak]. A crude sample of **1a** was revealed to be of 99.1% *e.e.*; Rt 29.1 min (99.55%), Rt 31.7 min [0.45%, for the (*S*)-isomer].

In a similar manner to that just described, the substrate (300 mg, 1.10 mmol) was incubated with the crude enzyme preparation (1.8 ml, 2760 U). Prior to the workup procedure, the enzyme protein was removed by ultrafiltration with an Amicon 8200 stirred ultrafiltration cell. The product (**1a**, 170 mg, quantitative) was obtained in an enantiomerically pure state, determined as already described.

Kinetic study. A kinetic study was performed according to the reported procedure.[10] The rate of decarboxylation was measured by HPLC at substrate concentrations of 15, 20, 25, 30 and 35 mM. HPLC: column, ODS-80 TM, 0.46 cm × 25 cm; eluent, water/acetonitrile=3/1 containing 0.05% trifluoroacetic acid; flow rate, 0.8 ml/min; Rt 6.7 min (**1a**), 10.5 min (**2a**). The results were presented on a Lineweaver-Burk plot to evaluate the K_m and k_{cat} values.

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