Enzymatic Synthesis of (*R*)-Flurbiprofen

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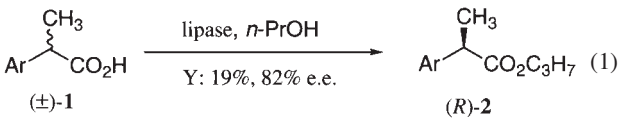
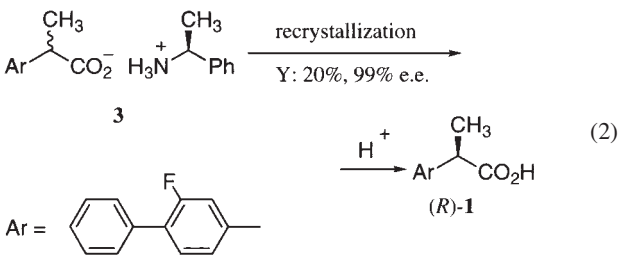
α-Methyl-α-(2-fluoro-4-biphenylyl)propionic acid (flurbiprofen) was prepared from the corresponding malonic acid derivative via asymmetric decarboxylation catalyzed by an enzyme, arylmalonate decarboxylase (EC 4.1.1.76), in high chemical and optical yields.

α-Arylpropionic acids constitute an important group of physiologically active compounds. The (*S*)-enantiomers are generally more important since they are active as non-steroidal anti-inflammatory drugs.[1–3] One exception is (*R*)-flurbiprofen **1** (Chart 1), to which much attention has recently been paid because of its anti-cancer activity.[4,5] Although it is now used as racemic mixture, development of an effective method of preparation of pure (*R*)-enantiomer is desirable.

(*R*)-Flurbiprofen has been already prepared via enzymatic kinetic resolution (Eq. 1)[6] and recrystallization of diastereomeric mixtures **3** (Eq. 2).[7] However, the yield and the enantiomeric excess of the product are not sufficiently high. In addition, this resolution method has its essential limitation, i.e., the maximum yield is only 50% and the separation of the desired enantiomer is often tedious.



(1)



(2)

On the other hand, racemic flurbiprofen is supplied via decarboxylation of the corresponding malonic acid produced according to the procedure shown in Scheme 1.[8,9] Thus if the decarboxylation step can be carried out in an enantioselective manner, only the desired enantiomer will be obtained by utilizing the established industrial process. However, no effective asymmetric decarboxylation reaction of malonic acid derivatives has been established via any ordinary chemical process.[10,11]

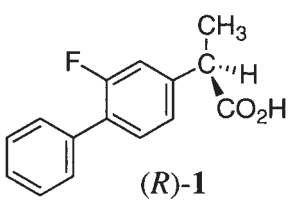
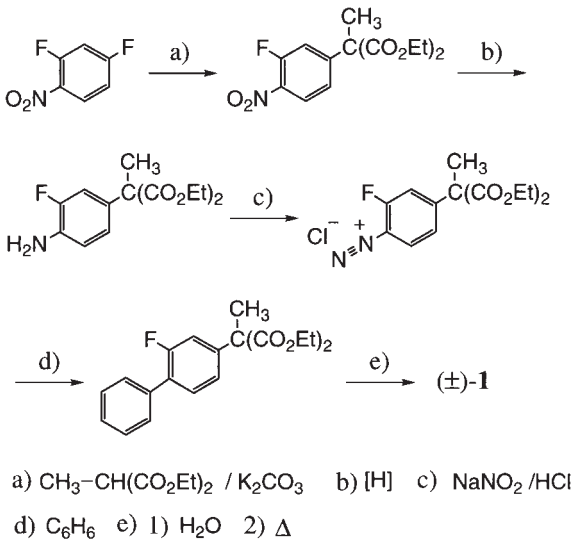


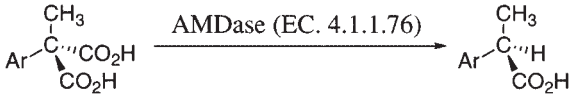
Chart 1.



Scheme 1. Industrial preparation of racemic flurbiprofen.

Results and Discussion

We have been doing research for several years on the reaction of a novel enzyme, arylmalonate decarboxylase (AMDase, EC 4.1.1.76), which gives (*R*)-α-arylpropionic acids starting from the corresponding malonates.[12,13] This enzyme catalyzes the reactions of a wide variety of arylmalonates, including those which have a substituent on *p*- or *m*-position, although it is inactive to *o*-substituted ones (Eq. 3).[14]



(3)

Thus we expected that this enzyme would be capable of cat-

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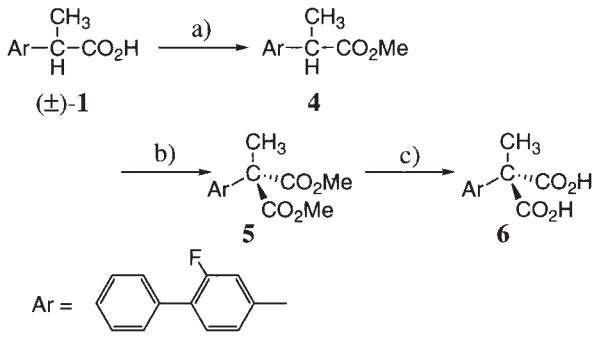

a) MeOH, H^+, Y: 97%; b) LDA, $ClCO_2Me$, Y: 88%; c) KOH/EtOH, 90%

Scheme 2. Preparation of the substrate.

Table 1. Reaction of **6** with AMDase[a)]

Entry	Time/h	Yield/%	e.e./%
1	0.6	22	89
2	24	24	82
3[b)]	24	35	90
4	1	90	92

a) [Substrate]: 100 mM for entries 1–3, 10 mM for entry 4. b) Additional AMDase was added after 2 h.

alyzing the asymmetric decarboxylation of **6** to give physiologically active (*R*)-enantiomer of flurbiprofen (**1**), although the steric bulkiness of the *p*-substituent of **6** is far larger than those of substrates (*p*- or *m*-CH_3, CH_3O, Cl, F) examined so far. The substrate of enzymatic reaction was prepared starting from commercially available (±)-flurbiprofen, as shown in Scheme 2, because this route is shorter than that of Scheme 1.

AMDase was produced by the transformed *E. coli* JM 109 (IPOD 12968) which had the plasmid containing the gene coding AMDase. The enzyme was purified via a sequence of precipitation with ammonium sulfate, and column chromatography (TOYOPEARL-DEAE then TOYOPEARL-Butyl).[15] The reaction of malonate **6** with AMDase was performed with 100 mM concentration of the substrate and 2500 unit/mL of the enzyme in 10 mM Tris–HCl buffer at pH 8.0. The isolated yield and the enantiomeric excess of the product determined by HPLC are summarized in Table 1. The absolute configuration of the product was revealed to be *R* by comparing the specific rotation ($[\alpha]_D^{27}$ −30.5° (*c* 0.1, $CHCl_3$)) with that of an authentic specimen.[16]

Although the e.e. of the product was fairly high (Table 1, entry 1), the yield of the product was disappointing. Prolonged reaction time had no effect to improve the yield of the optically active product (entry 2). This fact may be accounted for by supposing that the enzyme lost its activity in the early stage of the reaction, which has not been observed in the case of the other substrates. One possible reason is that the product itself might be acting as the inhibitor.

Thus, we examined the inhibitory effect of flurbiprofen, using phenylmalonic acid as the representative substrate. The reaction of phenylmalonic acid was carried out at several substrate concentrations (2, 2.5, 5, and 10 mM) in the presence of (±)-flurbiprofen (1, 10, and 20 mM) using 0.25 units of AM-

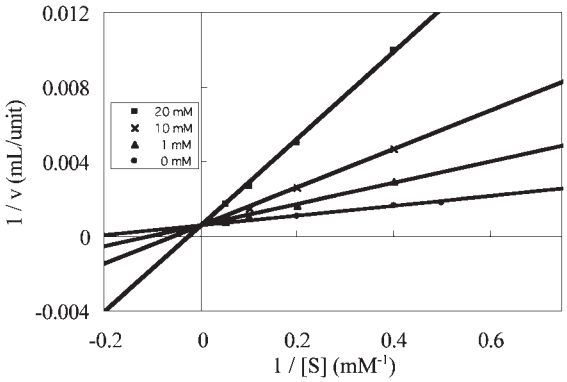

Fig. 1. Lineweaver–Burk plot of the reaction of phenylmalonic acid in the presence of (±)-**1**.

Dase in 500 μL of Tris–HCl buffer at pH 8.0. As shown in Fig. 1 the Lineweaver–Burk plot showed that (±)-flurbiprofen is indeed a competitive inhibitor to the enzyme. The K_i value was calculated to be 2.2 mM, which is smaller than the K_m value (4.3 mM) of the substrate **6**.

Some trials were made to lower the apparent concentration of the product. First we tried the reaction in a two phase-system with a hydrophobic organic solvent, such as toluene, hexane, and diisopropyl ether, expecting that some part of the product will move to the organic phase, because the product is less hydrophilic compared to the substrate. However, the enzyme did not work in the presence of organic solvents. Then a hydrophobic polymer (Amberlite XAD-2, -4, or -7) was added to the reaction system.[17] Based on the same reason described above, the polymer was expected to absorb selectively some part of the product. Thus we added 200 mg of the polymer to 57.7 mg of substrate in 2 mL of the buffer solution. Unfortunately, this method was not effective either. The only successful way at present is to perform the reaction in a relatively low substrate concentration. Thus, we obtained (*R*)-flurbiprofen of 92% e.e. in 90% yield by conducting the reaction in 4 mL of Tris–HCl buffer solution with the substrate concentration of 10 mM (11.5 mg). Under such conditions, the reaction was completed within 1 h.

Conclusion

We have succeeded in obtaining the (*R*)-enantiomer of flurbiprofen with anti-cancer activity from commercially available corresponding malonic acid derivatives. This process has great advantages compared to the hitherto known procedures because it is not only short but also directly gives optically active form.

Experimental

Preparation of Methyl 2-(2-Fluoro-4-biphenylyl)-2-methylpropionate (4). A solution of *rac*-flurbiprofen (2-(2-fluoro-4-biphenylyl)-2-methylpropionic acid) (3.00 g, 12.1 mmol) in MeOH (20 mL) containing a catalytic amount of conc. H_2SO_4 was refluxed for 3 h using a Dean–Stark trap. After evaporation of the solvent, the residue was extracted with diethyl ether. The ether layer was washed with brine and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent in vacuo gave a residue, which was purified by silica-gel column chromatography (hexane/ethyl acetate = 3/1) to afford 3.12 g of methyl 2-(2-fluoro-4-biphenylyl)-2-methylpropionate (**4**) as a colorless oil (99%

yield). ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.52 (3H, d, $J = 7.1$ Hz), 3.68 (3H, s), 3.75 (1H, q, $J = 7.1$ Hz), 7.07–7.52 (8H, m); IR ν_{max} cm^{-1}: 2982, 2951, 1737, 1642, 1582, 1563, 1515, 1484, 1418, 1376, 1333, 1198, 1132, 1073, 1010, 974, 920, 875, 834, 767, 725, 699, 577.

Preparation of Dimethyl 2-(2-Fluoro-4-biphenylyl)-2-methylmalonate (5). To a solution of diisopropylamine (3.39 mL, 24.2 mmol) in dry THF (20 mL) was added a solution of *n*-BuLi in hexane (1.56 M, 15.6 mL, 24.2 mmol) with stirring at −78 °C over a period of 50 min. **4** (3.12 g, 12.1 mmol) in THF (10 mL) was added at −78 °C and the mixture was stirred for 50 min. Then methyl chloroformate (2.11 mL, 24.2 mmol) was added and the stirring was continued for 2 h at 0 °C. The mixture was quenched with 2 M HCl and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by recrystallization from ethyl acetate/hexane to afford 3.44 g of **5** as colorless crystals (90% yield). Mp 116–117 °C; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.89 (3H, s), 3.78 (6H, s), 7.07–7.52 (8H, m); IR ν_{max} cm^{-1}: 3465, 2958, 1707, 1583, 1485, 1408, 1267, 1176, 1128, 940, 764, 724, 697, 578.

Preparation of 2-(2-Fluoro-4-biphenylyl)-2-methylmalonic Acid (6). Dimethyl 2-(2-fluoro-4-biphenylyl)-2-methylmalonate (**5**, 3.44 g, 10.9 mmol) was added to a mixture of 4 M aqueous KOH (20 mL) and EtOH (100 mL), and the resulting mixture was stirred for 1 h at 0 °C. After the mixture was acidified with conc. HCl, EtOH was evaporated and the residue was extracted with diethyl ether. The ether layer was washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by recrystallization from ethyl acetate/hexane to afford 2.68 g of **6** as colorless crystals (85% yield). Mp 155–156 °C; ^{1}H-NMR (270 MHz, CD_3OD) δ 1.76 (3H, s), 7.16–7.47 (8H, m); IR ν_{max} cm^{-1}: 3442, 1707, 1409, 1128, 937, 767, 751, 722, 697, 579, 457.

Enzymatic Decarboxylation. The substrate **6** (576 mg, 2 mmol) was dissolved in water (100 mL) and the pH of the mixture was adjusted to 8.0 with 2 M HCl and 2 M NaOH. To a 20-mL round-bottomed flask was added 1 mL of the 10 mM Tris–HCl buffer (pH = 8.0), 2 mL of the substrate solution (11.5 mg, 40 μmol) and 1 mL of AMDase solution. The reaction mixture was stirred at 30 °C for 40 min. The mixture was quenched with 1 mL of 2 M HCl and filtered through a pad of celite. The filtrate was extracted with diethyl ether. The ether layer was washed with brine and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by preparative thin layer chromatography (hexane/ethyl acetate/acetic acid = 33/66/1) to afford 8.79 mg of (*R*)-**1** as colorless crystals (90% yield). Mp 114 °C; ^{1}H-NMR (270 MHz, CD_3OD) δ 1.49 (3H, d, $J = 7.0$ Hz), 3.72 (1H, q, $J = 7.0$ Hz), 7.16–7.47 (8H, m); IR ν_{max} cm^{-1}: 2935, 1700, 1622, 1580, 1482, 1461, 1417, 1324, 1257, 1216, 1129, 1075, 1012, 959, 925, 874, 802, 766, 725, 698, 625, 574; $[\alpha]_D^{20}$ −30.5° (*c* 0.10, $CHCl_3$). The e.e. of (*R*)-**1** was determined by HPLC: Column, Chiralcel OJ (Daicel); Solvent, hexane/2-propanol = 9/1; Flow rate, 0.5 mL/min; Retention time, (*R*)-form 20.0 min, (*S*)-form 22.0 min.

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