The first synthesis of both enantiomers of [α-^{2}H]phenylacetic acid in high enantiomeric excess

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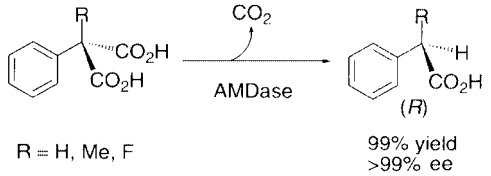
Arylmalonate decarboxylase (EC. 4. 1. 1. 76) catalysed decarboxylation followed by enantioface-differentiating protonation of [α-^{2}H]- and [α-^{1}H]phenylmalonic acid in ^1H_2O and ^2H_2O respectively, gave highly enantiomerically enriched (*R*)- and (*S*)-[α-^{2}H]phenylacetic acid in quantitative yields.

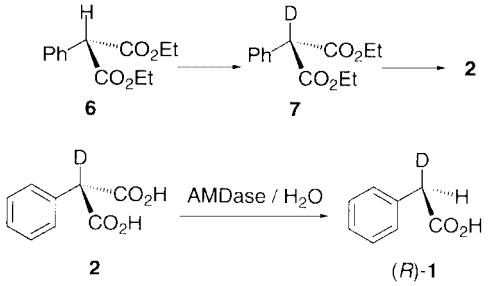
Regio- and stereospecifically [^{2}H]-labelled compounds are very useful in studying metabolic pathways and reaction mechanisms. Phenylacetic acid is the intermediary metabolite of aromatic amino acids, and also the starting material of amphetamine. For studies in this field, it will be of great use if both of the enantiomers of [α-^{2}H]phenylacetic acid **1** are available. So far, enantiomerically enriched forms of **1** have been synthesized *via* deuteriolysis of (*R*)-(−)-*N*,*N*-dimethylphenylglycine[1] and oxidative addition of optically active [α-^{2}H]benzyl halides to palladium complexes, followed by carbonyl insertion.[2] However, the products of these two methods suffer from the low enantiomeric excess (55[1], 76%[2]) and contamination of di-deuterated and non-deuterated by-products. It is practically impossible to raise the ee of the product and to separate these by-products by ordinary methods, as there are no chemical differences between the two enantiomers, and labeled and non-labeled compounds. Accordingly, high selectivity of the reaction is essential for obtaining the product in high enantiomeric excess.

Arylmalonate decarboxylase (AMDase, EC. 4. 1. 1. 76), which was found in our laboratory, catalyses decarboxylation of arylmalonate derivatives resulting in the formation of enantiomerically pure (*R*)-α-substituted phenylacetic acids (Scheme 1).[3] It is thought that enantioface-differentiating protonation to the enolate intermediate in the active site of the enzyme is the key step of this reaction. Thus, if [α-^{2}H]phenylmalonic acid was used as the substrate of this enzymatic reaction, it would give enantiomerically enriched (*R*)-[α-^{2}H]phenylacetic acid.



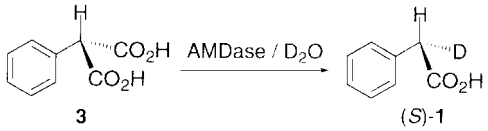
Scheme 1

[α-^{2}H]Phenylmalonic acid **2**† was prepared by deuteration of the corresponding [α-^{1}H]-diethyl ester followed by hydrolysis in ^2H_2O. The amount of non-deuterated diethyl ester was evaluated from ^{1}H-NMR to be about 3%, which was consistent with the peak intensity ratio of *m*/*z* 136 and 137 (parent ions corresponding to non- and mono-deuterated phenylacetic acids) of the enzyme-catalysed reaction product. The asymmetric decarboxylation of **2** by arylmalonate decarboxylase, which was obtained by overexpression in *E. coli* JM 109 and subsequent purification,[4] worked very well. The expected compound (*R*)-**1**‡ of over 95% ee was obtained in a quantitative yield (Scheme 2).



Scheme 2

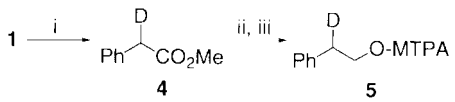
In this enzyme-catalysed reaction, it is considered that the origin of the proton source can be traced back to the solvent ^1H_2O. Consequently, when the reaction is performed in ^2H_2O, then ^{2}H will be incorporated enantioselectively into the product from the same direction as that of ^{1}H in the above case. As this enzyme is purified from the culture of *E. coli*, it is obtained as a solution in ^1H_2O. When the replacement of the solvent is incomplete, it will cause the formation of non-deuterated phenylacetic acid. Thus, we tried to replace ^1H_2O with ^2H_2O. Fortunately, as this enzyme was not inactivated by repeated lyophilization, it was possible to replace the ^1H_2O of the enzyme solution by ^2H_2O *via* successive freeze-drying and addition of ^2H_2O. When substrate **3** was added to this ^2H_2O-exchanged enzyme solution, the reaction proceeded smoothly in the same manner as for ^1H_2O, although the rate of reaction was not measured accurately. As expected, (*S*)-**1**§ in over 95% ee was obtained in a quantitative yield (Scheme 3).



Scheme 3

The absolute configuration of the product was determined by comparison of the sign of rotation with the one reported.[2] The ee of **1** was determined by ^{1}H NMR analysis of (*R*)-α-methoxy-α-trifluoromethylphenyl acetate (MTPA ester **5**¶), which was prepared by an esterification of the corresponding alcohol obtained by the reduction of methyl ester **4** (Scheme 4). The ^{1}H NMR spectra of the benzylic protons are shown in Fig. 1. The signals of the protons in question were simplified *via* decoupling by irradiation of the adjacent methylene protons.

In conclusion, an efficient method for the asymmetric synthesis of both enantiomers of [α-^{2}H]phenylacetic acid was established *via* decarboxylation of phenylmalonate catalysed by



Scheme 4

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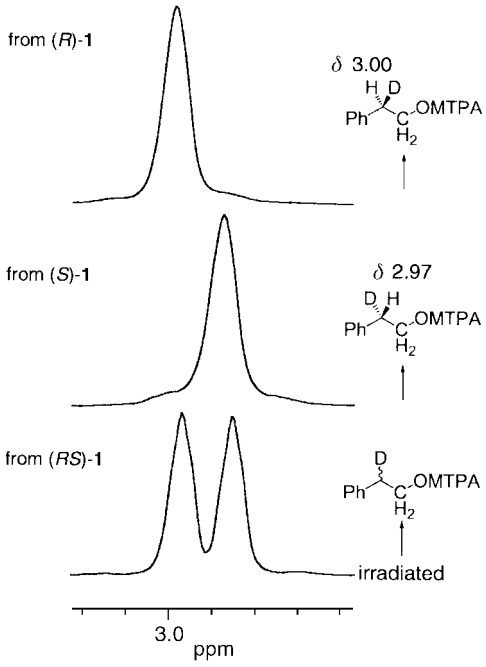

Fig. 1

arylmalonate decarboxylase. As some other analogous compounds having substituents on the aromatic ring also undergo this enzyme-catalysed reaction,[5] this procedure will be applicable to the preparation of the enantiomers of substituted [α-^{2}H]arylacetic acids.

Notes and references

† **2**: To a dispersion of NaH (12.7 mmol) in dry THF (10 ml) was added a solution of **6** (4.23 mmol) in dry THF (2 ml). The mixture was refluxed with stirring for 5 h and cooled to 0 °C. To this mixture was added D_2O (3.5 ml) and DCl (35% in D_2O, 1.4 ml). The product was extracted and purified by column chromatography on silica gel to give diethyl [α-^{2}H]phenylmalonate **7** (0.957 g, 96%). This was contaminated with **6** (3%), as judged from the signal at δ_H 4.61 [1H, s, $C_6H_5CH(CO_2CH_2CH_3)_2$]. The solution of **7** (0.747 g, 3.15 mmol) in CH_3OD (5 ml) was treated with NaOD (6.84 mmol) in D_2O (5 ml). The mixture was stirred at rt for 12 h, concentrated *in vacuo* and MeOH (15 ml) was added to the residue. The resulting precipitates were collected by suction filtration, washed with cold MeOH and ether, and then dried *in vacuo* to give the disodium salt of **2** (0.640 g, 90%) as a colorless powder. ν_{max}/cm^{-1} 3021, 1591, 1496, 1429, 1324, 1023, 910, 698; (400 MHz, D_2O) δ_H: 7.11–7.21 [m, 5H, $C_6H_5CD(CO_2Na)_2$].

‡ (*R*)-**1**: The disodium salt of **2** (0.450 g, 2.00 mmol) was added to a solution of purified arylmalonate decarboxylase (400 units) in Tris-HCl buffer (2 M, pH 8.5, 4 ml). This mixture was incubated at 35 °C for 30 min followed by acidification with 2 M HCl. The mixture was saturated with NaCl and extracted with ether. The organic layer was concentrated *in vacuo* to give (*R*)-**1** (0.279 g, quant.) as a white solid. This product was revealed to be pure by ^{1}H NMR. This was recrystallized from hexane to afford (*R*)-**1** (0.250 g, 92%) as colorless plates, mp 73.5–74.5 °C; $[\alpha]_D^{22}$ −1.1° (*c* 10.0, $CHCl_3$); ν_{max}/cm^{-1} 3033, 1700, 1412, 1263, 1224, 904, 700, 673; (400 MHz, $CDCl_3$) δ_H: 3.64 [t, 1H, *J* = 2.0 Hz, $C_6H_5CDHCO_2H$], 7.25–7.36 [m, 5H, $C_6H_5CDHCO_2H$]; MS (*m/z*, %) 92 (C_7H_6D, 100), 136 (1.2), 137 (M^+, 34.9), 138 (*M* + 1, 3.0). HRMS Found: *m/z* 137.0598. Calcd. for $C_8H_7DO_2$: 137.0586. Assuming the peak *m/z* 136 is entirely due to the presence of non-deuterated phenylacetic acid ($C_7H_8O_2$), the contamination is calculated to be about 3%. However, the peak corresponding to *M* − 1 (135) was also observed in the MS of non-deuterated phenylacetic acid. Thus the contamination of the non-deuterated compound in the enzymatic reaction product will be less than 3%.

MS of non-deuterated phenylacetic acid (*m/z*, relative intensity) 91 (C_7H_7, 100), 135 (1.2), 136 (M^+, 72), 137 (*M* + 1, 7.0), 138 (*M* + 2, 1.2).

§ (*S*)-**1**: The purified arylmalonate decarboxylase (600 units) was dissolved in a Tris-HCl buffer (2 M, pH 8.5, 5 ml) and lyophilized. The residue was dissolved in D_2O (5 ml) and incubated at 4 °C for 1 h and lyophilized again. The residue was dissolved in D_2O (5 ml) and the disodium salt of **3** (0.550 g, 2.45 mmol) was added. The mixture was incubated at 35 °C for 30 min and acidified with 2 M HCl. The mixture was saturated with NaCl and extracted with ether. The organic layer was concentrated *in vacuo* to give (*S*)-**1** (0.337 g, quant.) as a white solid. This was recrystallized from hexane to give (*S*)-**1** (0.300 g, 89%) as colorless plates, mp 74.5–75.5 °C [lit.[2] 75 °C]; $[\alpha]_D^{22}$ +1.1 (*c* 9.5, $CHCl_3$), +1.2 (*c* 25.7, $CHCl_3$) [lit.[2] for *S* enantiomer, $[\alpha]_D^{22}$ +1.5 ± 0.2 (*c* 25.7, $CHCl_3$)]; MS (*m/z*, %) 92 (C_7H_6D, 100), 136 (1.2), 137 (M^+, 34.9), 138 (*M* + 1, 3.0). HRMS Found: *m/z* 137.0566. Calcd. for $C_8H_7DO_2$: 137.0586. The IR and NMR spectra were identical with those of (*R*)-**1**. The racemic sample of **1** was prepared by the reported procedure.[6]

¶ **5** from (*R*)-**1**: (400 MHz, $CDCl_3$) δ_H: 3.00 [t, 1H, *J* = 6.3 Hz, $C_6H_5CDHCH_2OCOC(CF_3)(OCH_3)C_6H_5$], 3.47 [s, 3H, $C_6H_5CDHCH_2OCOC(CF_3)(OCH_3)C_6H_5$], 4.53 [d, 2H, *J* = 6.3 Hz, $C_6H_5CDHCH_2OCOC(CF_3)(OCH_3)C_6H_5$], 7.17–7.43 [m, 10H, $C_6H_5CDHCH_2OCOC(CF_3)(OCH_3)C_6H_5$]. **5** from (*S*)-**1**: (400 MHz, $CDCl_3$) δ_H: 2.97 [t, 1H, *J* = 7.3 Hz, $C_6H_5CDHCH_2OCOC(CF_3)(OCH_3)C_6H_5$], 3.46 [s, 3H, $C_6H_5CDHCH_2OCOC(CF_3)(OCH_3)C_6H_5$], 4.53 [d, 2H, *J* = 7.0 Hz, $C_6H_5CDHCH_2OCOC(CF_3)(OCH_3)C_6H_5$], 7.17–7.43 [m, 10H, $C_6H_5CDHCH_2OCOC(CF_3)(OCH_3)C_6H_5$].

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