A Biocatalytic Approach to the Enantioselective Synthesis of (*R*)- and (*S*)-Malic Acid

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(*S*)-Diethyl malate **1a** was prepared (70–80% yield; >98% optical purity) by an enantioselective reduction of sodium diethyl oxalacetate **2** by fermenting baker's yeast (*Saccharomyces cerevisiae*). Other microorganisms were tested for their capability of reducing **2**. Most of them afforded (*S*)-**1a**- with ee from 8 to 94% and only *Candida utilis, Aspergillus niger* and *Lactobacillus fermentum* ILC G18D preferentially reduced compound **2** to (*R*)-**1a**. (*R*)-Dimethyl malate **1b** was obtained from (*R,S*)-malate **1b** by hydrolysis with pig liver esterase (PLE), the highest ee (93%) being realized at 0 °C in 20% aqueous methanol. Enzymatic hydrolyses of protected malates **1d** and **1e** did not lead to improvement of the ee.

Malic acid has proven to be a valuable chiral synthon available as the (*S*)-(−)-enantiomer from the so called 'chiral pool'. Its use as building block for the synthesis of natural products and biologically active compounds is very well documented.[1] (*R*)-(+)-Malic acid is the unnatural stereoisomer and can be chemically prepared by several methods.[2]

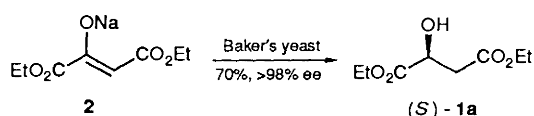
We describe here a new approach to the synthesis of both isomers through the application of biochemical methods, such as the use of microorganisms or purified enzymes as biocatalysts.

Results and Discussion

Diethyl (S)-*Malate* **1a**.—A simple method for the preparation of diethyl (*S*)-malate **1a** could take advantage of the well known capability of baker's yeast to realize enantioselective bioreductions.[3] From a structural point of view, (*S*)-malate **1a** could be prepared, in principle, by baker's yeast reduction of diethyl oxalacetate, which possesses a carbonyl function α and β to the ester groups.[4] Up to now, many successful examples of enantioselective reductions of 3-keto esters have been reported,[5] however, the same methods do not always apply with the same success to 2-keto esters.[6] Furthermore, several oxidoreductases

a; $R^1 = H, R^2 = R^3 = C_2H_5$
b; $R^1 = H, R^2 = R^3 = CH_3$
c; $R^1 = R^2 = H, R^3 = CH_3$
d; $R^1 = COCH_3, R^2 = R^3 = CH_3$
e; $R^1 = CH_2C_6H_5, R^2 = R^3 = CH_3$
f; $R^1 = CH_2C_6H_5, R^2 = H, R^3 = CH_3$
g; $R^1 = CH_2C_6H_5, R^2 = CH_3, R^3 = H$

can operate on the same substrate, sometimes with opposite stereochemistry.[7] This can lower the optical purity of the product obtained from the bioreductive process. In any event, we submitted to fermenting baker's yeast sodium diethyl oxalacetate **2**, which is the commercially available compound corresponding to diethyl oxalacetate. The optimum conditions were 24 h fermentation, working at 0.07 mol dm^{-3} concentration of compound **2** with a yeast–substrate ratio of 1.75 g mmol^{-1}. The (*S*)-malate **1a** was isolated in 70–80% yield (Scheme 1).† The optical purity of **1a** was ascertained by comparison of its chiroptical properties with reported constants for pure material[8] and was >98%. The enantiomeric excess (ee) of **1a** was also established by GLC analysis of its (*R*)-(+)-MTPA‡ ester[9] and no traces of the other enantiomer could be detected.



Scheme 1

It should be mentioned that the optical rotations of esters of malic acid had different values and opposite signs depending on the solvent. For instance, diethyl (*S*)-malate **1a** showed $[\alpha]_D$ +6° for $c = 2.3$ in chloroform and an optical rotation of −11.4° for $c = 2.5$ in ethanol. Dimethyl (*R*)-malate **1b** had $[\alpha]_D$ −3° for $c = 2.3$ in chloroform and +8.6° for $c = 2.5$ in ethanol. The optical rotations of (*R*)- and (*S*)-malates have been often recorded as pure liquid and for this reason one generally refers to them as to (*S*)-(−)- and (*R*)-(+)-**1a** or **1b**. The signs of the above optical rotations are the same when measured as pure liquid and in ethanol, and in both cases the absolute value is higher for ethanol than for chloroform solutions. For these reasons, ethanol was used for the measurement of optical rotations of malates **1a** and **1b** obtained from the biocatalytic routes.

(S)-Malic acid is an inexpensive and readily available form of malate, but diethyl (*S*)-malate is not readily available.[10] Our results show that diethyl (*S*)-malate **1a** can be prepared optically pure in good chemical yields, using as substrate the easily accessible enolate **2**.

Diethyl (R)-*Malate* **1a**.—*Microbial reduction of compound* **2**. Since the (*R*)-(+)-ester is the unnatural stereoisomer of malic acid esters, it was worthwhile trying the reduction of sodium

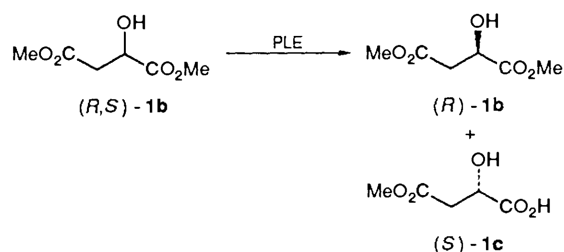
† Baker's yeast could efficiently reduce up to 2.6 mol dm^{-3} solutions of compound **2** at the same yeast–substrate ratio, but optical yields were lowered to 76%.

‡ MTPA = α-methoxy-α-trifluoromethyl-α-phenylacetic acid (β,β,β-trifluoro-α-methoxy-α-phenylpropionic acid).

oxalacetate **2** with different microorganisms. It was recently shown by us that several microorganisms belonging to Eumycetes[11] and lactic acid bacteria[12] can reduce a few non-cyclic carbonyl compounds, in some cases with the stereochemical outcome opposite to *Saccharomyces cerevisiae*. The screening was performed with the strains reported in Table 1. For most eumycetes, a strong hydrolytic activity toward diethyl malate was observed and therefore the experiments with these organisms were carried out for variable incubation times and the ee determined at the time of higher transformation yields. Results show that the substrate **2** was generally very quickly reduced by all the tested microorganisms, although none of them gave transformations higher than 65%. As regards the stereochemical course of the reduction, most of the considered microorganisms afforded the (*S*)-malate **1a** with ee from 8 to 94%. Only *Candida utilis*, *Aspergillus niger* and *Lactobacillus fermentum* G18D gave preferential reduction to the *R* form, but with ee not higher than 80%.

The results obtained in this preliminary screening were not sufficient to warrant the use of the above microorganisms for a high ee preparation of (*R*)-malate **1a** and a different approach to an enantioselective preparation of (*R*)-malate was attempted.

Enzymatic Resolution of Dimethyl (R,S)-*Malates* **1b**, **1d** *and* **1e**.—We investigated the porcine liver esterase (EC 3.1.1.1) (PLE)-mediated resolution of dimethyl (*R,S*)-malate **1b** (Scheme 2) and encountered unexpected difficulties in achieving



Scheme 2

a satisfactory recovery of the monoester formed. It has already been reported that the 100% hydrolysis of (*R,S*)- and (*S*)-diester **1b**, afforded the monoester **1c** in good yields.[13] We were able to recover **1c** in 70–75% yield only after chromatography on an anionic resin (Amberlite), the remaining material being malic acid. When the hydrolysis was stopped at 55% hydrolysis, the unchanged ester was (*R*)-(+)-**1b** with an ee of 50–60%.* In order to raise this ee, the incubation was repeated in 20% methanol[14] at room temperature and 0 °C and (*R*)-(+)-**1b** was obtained with 82 and 93% ee, respectively. All these results and the following enzymatic experiments are summarized in the Table 2.

Another possibility of raising the ee of an enzymatic reaction relied upon the structural modification of the substrate. A few successful applications of this concept are already available in the literature.[15] The acetate of dimethyl (*R,S*)-malate, compound **1d**, was prepared and subjected to PLE hydrolysis. In water and in 20% methanol at 25 and 0 °C, (*R*)-(+)-malate **1b** could be obtained with no more than 84% ee, but yields suffered because of competitive hydrolysis at the carboxymethyl moiety. Interestingly, α-chymotrypsin (CHY), which gave the most satisfactory results on a similar diester, namely 3-acetoxyglutarates,[15b] afforded a complex mixture of hydrolysis products.[16]

In principle, the chiral acetate **1d** could be obtained by the recently developed vinylacetate transesterification in the presence of a lipase from *Pseudomonas* species.[17] In order to achieve the highest ee of the acetate **1d**, we worked at a 40% conversion into the acetate in different solvents such as chloroform, benzene and tetrahydrofuran, using a *Pseudomonas fluorescens* lipase (PFL). The (*S*)-(−)-acetate **1d** was formed in good yields (38%) and the ee ranged between 30 and 60%, the most suitable solvent being chloroform. At 60% conversion to acetate **1d**, the unchanged ester was (*R*)-(+)-**1b** with unsatisfactory enantioselectivity (42% yield, 52% ee).

Also (*R,S*)-2-*O*-benzylmalate **1e** was prepared according to the method reported by Widmer.[18] With PLE, the yields of untransformed (*R*)-(+)-**1e** were around 40% and the ee was increased only from 50 to 59% in water and 20% methanol at 25 and 0 °C, respectively. From the last experiment, the (*S*)-(−)-monoester **1f** was obtained in 42% yield (58% ee) and the absence of the other monoester **1g** was established by ^{1}H NMR spectroscopy.

Thus, our data are different from the results obtained from the relatively regioselective hydrolysis of the diester **1b**.[13] It is likely that the bulky benzyl group in **1e** drives the hydrolysis toward complete regioselectivity.

Furthermore, in contrast with the monoester **1c**, the recovery of the monoester **1f** is excellent and this derivative may constitute the compound of choice, when the regioselective hydrolysis of malate is needed for synthetic applications.[19] In fact, when this regioselective hydrolysis was applied to the (*R*)- or (*S*)-malate **1e** in water at 25 °C, with complete hydrolysis of the substrate, the yields of the corresponding (*R*)- or (*S*)-monoester **1f** were 90–94%.

Finally, we expected a successful hydrolysis of **1e** by the CHY-catalysed reaction, in accord with the reaction on 2-benzylsuccinate[20] and 3-benzyloxyglutarate.[21] However, only a 8:2 ratio of stereoisomers (*S*)- and (*R*)-**1e** could be obtained. It should be noted that the stereochemical outcome of the CHY- and PLE-catalysed hydrolyses were opposites.

Conclusions

We have shown that different biocatalytic approaches can give access to both (*R*)- and (*S*)-malates. By use of microorganisms, reduction of oxalacetate **2** gives rise almost exclusively to the (*S*)-isomer and *Saccharomyces cerevisiae* gives the most satisfactory results from a preparative point of view. For the preparation of (*R*)-malate, a satisfactory chemical conversions and good optical purities can be obtained by PLE-catalysed hydrolysis of the (*R,S*)-malate **1b**, only when the reaction is carried out at 0 °C, in the presence of methanol (Scheme 2). Attempts to improve the enantioselectivity by protection of the hydroxy group was not successful. Also the irreversible transesterification of (*R,S*)-malate **1b** with vinyl acetate in organic solvents in the presence of PFL was not enantioselective enough for preparative purposes. Interestingly, a fully regioselective hydrolysis of benzyl derivative **1e** can afford in good yields the monoester **1f**.

Experimental

PLE was purchased from Boehringer Mannheim (West Germany), CHY from Fluka (Switzerland) and used without further purification. The activities were assayed as indicated in the catalogue. Infrared spectra were recorded on a 1420 Perkin-Elmer spectrometer for solutions in chloroform. Unless

* The hydrolysed product was the (*S*)-(+)-**1c**, $[\alpha]_D$ +1.1° (*c* 1.8 in chloroform) and when the hydrolysis was stopped at 40% conversion, the optical rotation was +1.3° (lit.,[13] +1.5° for 100% optically pure). Since the optically pure monoester **1c** could be obtained from quantitative PLE-mediated hydrolysis of dimethyl (*S*)-malate apparently in good yields (ref. 13), in the following experiments we did not isolate the (*S*)-monoester **1c**.

Table 1 Reduction of sodium oxalacetate **2** with different microorganisms

Microorganism	Incubation time/h	Yields[a] (%)	Configuration	ee[b] (%)
Candida boidinii CBS 2428[c]	5	50	*S*	28
Candida utilis CBS 621	5	44	*R*	43
Geotrichum candidum MIM	5	44	*S*	8
Hansenula glucozyma CBS 5766	5	58	*S*	40
Kluyveromyces fragilis CBS 397	48	42	*S*	57
Kluyveromyces marxianus MIM	24	42	*S*	90
Pachisolen thermophilus CBS 4044	5	65	*S*	34
Penicillium citrinum ATCC 9849	24	36	*S*	78
Pichia fermentans IMAP 2770	5	49	*S*	20
Aspergillus niger CBS 626.26	5	25	*R*	40
Rhizopus oryzae CBS 112.07	5	30	*S*	94
Lactobacillus fermentum ILC 1.f.	24	48	*S*	73
Lactobacillus fermentum ILC 3V11	24	15	*S*	64
Lactobacillus fermentum ILC G61D	24	63	*R,S*	0
Lactobacillus fermentum ILC G87	24	12	*S*	70
Lactobacillus fermentum ILC G18D	24	58	*R*	80

[a] By GLC (see Experimental section). [b] By GLC of MTPA esters. [c] CBS, Centralbureau voor Schimmelcultures, Baarn, Holland; MIM, Microbiologia Industriale, Milano, Italy; IMAP, Istituto Microbiologia Agraria, Perugia, Italy; ATCC, American Type Culture Collection, USA; ILC, Istituto Lattiero Caseario, Lodi, Italy.

Table 2 Enzyme-catalysed hydrolysis and transesterification of (*R*,*S*)-dimethyl malate **1b**; hydrolysis of derivatives **1d** and **1e**

Substrate	Enzyme (°C, solvent)	Product	Time/h	Yields[a] (%)	Configuration	ee[b] (%)
1b	PLE (25, H_2O)	**1b**	0.5	38	*R*	55
1b	PLE (25, 20% MeOH)	**1b**	1	40	*R*	82[c]
1b	PLE (0, 20% MeOH)	**1b**	3	42	*R*	93[c]
1d	PLE (25, H_2O)	**1b**	0.5	24	*R*	48
1d	PLE (0, H_2O)	**1b**	4.5	26	*R*	75
1d	PLE (0, 20% MeOH)	**1b**	7	26	*R*	84[d]
1d	CHY (25, H_2O)	**1d**	1	22	*S*	25[e]
1b	PFL (30, THF)	**1d**	24	40	*S*	30
1b	PFL (30, C_6H_6)	**1d**	24	40	*S*	40
1b	PFL (30, $CHCl_3$)	**1d**	48	42	*S*	60
1b	PFL (30, $CHCl_3$)	**1b**	52	42	*R*	52
1e	PLE (25, H_2O)	**1e**	0.5	40	*R*	50
1e	PLE (25, 20% MeOH)	**1e**	4	42	*R*	52
1e	PLE (0, 20% MeOH)	**1e**	10	44	*R*	59
1e	CHY (25, H_2O)	**1e**	3	32	*S*	62[e]

[a] Yields refer to pure, isolated product: 50% yield of product should correspond to 50% hydrolysis and complete recovery of product. [b] Except for the indicated case, the ee was established by comparison with reference optical rotations. [c] By GLC of (*R*)-(+)-MTPA ester. [d] Unchanged (*S*)-**1d** was isolated in 38% yield (88% ee). [e] Products mixture complicated or poorly recovered for preparative uses.

otherwise indicated, ^1H NMR refer to 60 MHz spectra, recorded on a Varian EM 360 L spectrometer for solution in $CDCl_3$, using $SiMe_4$ as internal standard. 200 MHz ^1H NMR spectra were recorded in $CDCl_3$ on a Varian XL 200 spectrometer. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. Distillations for analytical purposes were performed on a glass tube oven Buchi GKR-50. TLC analyses were carried out on silica gel Merck 60 F254 plates and column chromatography was performed on silica gel Merck 60 (230–400 mesh). HPLC was performed with a Jasco liquid chromatograph using a Merck RP C18 column. Gas chromatographic analyses of (*R*)-MTPA esters were carried out on a DANI gas chromatograph (Mod. 6500), using a capillary column (SE 52).

Diethyl (S)-*Malate* **1a** via *Baker's Yeast Reduction of Sodium Oxalacetate* **2**.—To a solution of saccharose (50 g) in water (900 cm^3) was added commercial baker's yeast (100 g) and the mixture set aside at 30 °C for 1 h, in order to start the fermentation. Sodium diethyl oxalacetate **2** (12 g, 57 mmol) was added and the mixture was set aside at 30 °C for 96 h, with vigorous stirring. The reaction was filtered through Celite and the aqueous phase continuously extracted with diethyl ether. The organic solution was dried over sodium sulphate and carefully evaporated at reduced pressure. The residue (8.8 g) was distilled (120 °C at 10 mmHg) to give pure (*S*)-malate **1a** (7.8 g, 72%) (Found: C, 50.4; H, 7.45. Calc. for $C_8H_{14}O_5$: C, 50.5; H, 7.36%); $[\alpha]_D$ −10.2° (neat) (lit.,[8] −10.18, neat); −11.4° (*c* 2.5 in ethanol) and +6° (*c* 2.3 in chloroform); δ_H 1.35 (6 H, t, *J*/Hz 7, Me), 2.90 (2 H, d, *J*/Hz 6, CH_2) and 4.10–4.80 (6 H, m); ν_{max}(neat)/cm^{-1} 3300 and 1740.

Microbial Reduction of Compound **2**.—The screening was performed with the eumycetes and lactic acid bacteria reported in Table 2. The eumycetes were cultured in 500 cm^3 Erlenmeyer flasks with 50 cm^3 of the previously described medium.[11] The cultures were incubated on a reciprocating shaker (100 strokes min^{-1}) for 24 h at 26–28 °C. Lactic acid bacteria were cultured on MRS broth and incubated for 24 h at the optimum temperature of each strain. For reduction experiments fresh cells from submerged cultures were collected by centrifugation and resuspended in a fifth of the original volume of sodium phosphate buffer (0.2 mol dm^{-3}) pH 5.8, in the presence of glucose (10%) for eumycetes, while lactic acid bacteria were resuspended in MRS broth with glucose added up to 5%. After 1 h incubation, the substrate was added (2 g dm^{-3}) and the

incubation continued for 5–48 h. The progress of the reduction was determined by gas-chromatographical analyses (Carbowax 1540), using 1-phenylethanol as internal standard. Diethyl malate was extracted with ethyl acetate and esterified with (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride in the presence of pyridine to determine its enantiomeric excess.

Dimethyl (R,S)-2-O-*Acetylmalate* **1d**.—This acetate was prepared from racemic dimethyl malate **1b**. To a solution of the hydroxy ester **1b** (0.324 g, 2 mmol) in anhydrous pyridine (1 cm^3), acetic anhydride (0.38 cm^3) was added at 0 °C. The solution was set aside overnight at room temperature and then poured into water. The aqueous solution was extracted with dichloromethane (3 × 10 cm^3), the solvent was dried over Na_2SO_4, and evaporated under reduced pressure. The acetoxy ester **1d** was distilled at 12 mmHg (0.367 g, 90%); b.p. 132–134 °C (Found: C, 47.2; H, 6.0. Calc. for $C_8H_{12}O_6$: C, 47.05; H, 5.88%); ν_{max}/cm^{-1} 1750; δ_H 2.10 (3 H, s, MeCOO), 2.90 (2 H, d, *J*/Hz Me 7, CH_2CO), 3.80 (3 H, s, COOMe), 3.90 (3 H, s, COOMe) and 5.60 (1 H, t, *J*/Hz 7, CH).

Dimethyl (S)-(−)-2-O-*Acetylmalate* **1d**.—This acetate was prepared from commercial methyl hydrogen (S)-(−)-malate **1b**, $[\alpha]_D$ −8.6° (*c* 2.5 in ethanol). The (S)-(−)-acetate **1d** was isolated and purified and showed $[\alpha]_D$ −21.6° (*c* 2.5 in ethanol), which was used as reference for optically pure material.

Dimethyl (R,S)- *and* (S)-2-O-*Benzylmalate* **1e**.—The racemic and optically pure substrates were prepared as described in ref. 18.

PLE-Catalysed Hydrolysis of Substrates: General Procedure.—(*a*) *In water*. A rapidly stirred solution of the substrate (3 mmol) in KH_2PO_4 (22.5 cm^3; 0.03 mol dm^{-3}) at 25 °C was neutralized with aqueous NaOH (0.5 mol dm^{-3}). PLE (150 U) was added and the pH of the solution maintained at 7 with continuous addition of aqueous NaOH (0.5 mol dm^{-3}). The reaction was stopped when 0.5 equiv. of base had been consumed. The reaction mixture was frozen at −78 °C, then thawed, and saturated with NaCl. The neutral mixture (pH 7) was extracted with ether (4 × 20 cm^3) and the ethereal extracts were washed with saturated aqueous sodium hydrogen carbonate (15 cm^3). The organic extracts were dried and evaporated, yielding the unchanged ester and the product of hydrolysis. If the product was an acid, the reaction mixture was then acidified to pH 1–2 with concentrated HCl, and extracted with diethyl ether (5 × 15 cm^3). Evaporation of dried (Na_2SO_4) extracts yielded the acidic product.

(*b*) *In 20% methanol*. A rapidly stirred solution of the substrate (3 mmol) in KH_2PO_4 (0.03 mol dm^{-3}) containing 20% methanol (22.5 cm^3) was neutralized (pH 7) with aqueous NaOH (0.5 mol dm^3). PLE was added (150 U) and the pH rapidly readjusted to 7 by addition of the appropriate amount of aqueous NaOH (0.5 mol dm^{-3}). Thereafter, the pH was maintained at 7 with continuous addition of NaOH (0.5 mol dm^{-3}). The enzymatic reaction was generally slower than in water and sometimes required additional amounts of enzyme (50 U). The reaction was stopped when 0.5 equiv. of base per mole of substrate was consumed. The reaction mixture was frozen at −78 °C, thawed and saturated with NaCl. The neutral fraction was extracted at pH 7 with diethyl ether (4 × 15 cm^3) and the organic extracts were washed with a saturated $NaHCO_3$ solution (15 cm^3), dried and evaporated to yield the unreacted ester and neutral product. If an acid was the product, the aqueous reaction mixture after extraction of the neutral products, was acidified to pH 1–2 with concentrated HCl and extracted with diethyl ether (5 × 15 cm^3), which was dried and evaporated.

PLE-Catalysed Hydrolysis of Dimethyl (R,S)-*Malate* **1b**.—(*a*) *In water*. The (*R,S*)-ester **1b** (0.486 g, 3 mmol) was incubated and the reaction stopped after 0.5 h. (*R*)-(+)-**1b** was obtained (0.184 g, 38%) after purification by silica gel chromatography (hexane–ethyl acetate, 8:2). $[\alpha]_D$ +4.7° (*c* 2.5 in ethanol, 55% ee).

(*b*) *In 20% methanol*. At 25 °C in 1 h, from (*R,S*)-ester **1b**. (3 mmol), (*R*)-(+)-**1b** was obtained (0.194 g, 40%) after purification by silica gel chromatography (hexane–ethyl acetate, 8:2). $[\alpha]_D$ +7° (82% ee). At 0 °C in 3 h, from the (*R,S*)-ester **1b** (3 mmol), (*R*)-(+)-**1b** was obtained (0.204 g, 42%); $[\alpha]_D$ +8° (93% ee).

PLE-Catalysed Hydrolysis of Dimethyl (R,S)-2-O-*Acetylmalate* **1d**.—(*a*) *In water*. At 25 °C in 0.5 h, from the (*R,S*)-acetate **1d** (0.612 g, 3 mmol), (*R*)-(+)-**1b** was obtained (0.117 g, 24%) after purification by silica gel chromatography (hexane–ethyl acetate, 8:2). $[\alpha]_D$ +4.1° (48% ee). At 0 °C, (*R*)-(+)-**1b** was obtained (0.126 g, 26%), $[\alpha]_D$ +6.45° (75% ee).

(*b*) *In 20% methanol*. At 0 °C in 7 h, from the (*R,S*)-acetate **1d** (0.612 g, 3 mmol), (*S*)-(−)-**1d** (0.232 g, 38%) and (*R*)-(+)-**1b** (0.126 g, 26%) were obtained after purification by silica gel chromatography (hexane–ethyl acetate, 9:1 and 8:2). For (*R*)-(+)-**1b**) $[\alpha]_D$ +7.2° (84% ee) and for (*S*)-(−)-**1d** $[\alpha]_D$ −19° (88% ee) were recorded (*c* 2.5 in ethanol).

PLE-Catalysed Hydrolysis of Dimethyl (R,S)-2-O-*Benzylmalate* **1e**.—(*a*) *In water*. From the (*R,S*)-ester **1e** (0.756 g, 3 mmol) in 0.5 h, unreacted (*R*)-(+)-**1e** was obtained (0.302 g, 40%) after purification by silica gel chromatography (hexane–ethyl acetate, 8:2); $[\alpha]_D$ +36.3° (*c* 1.6 in chloroform) (50% ee; lit.,[18] −72.6° at the same concentration).

(*b*) *In 20% methanol*. At 25 °C in 4 h, from the (*R,S*)-ester **1e** (3 mmol) unchanged (*R*)-(+)-**1e** was obtained (0.317 g, 42%) after purification by silica gel chromatography (hexane–ethyl acetate, 8:2). $[\alpha]_D$ +38° (*c* 1.6 in chloroform, 52% ee). At 0 °C in 10 h, from the (*R,S*)-ester **1e** (3 mmol), (*R*)-(+)-**1e** was obtained (0.332 g, 44%) $[\alpha]_D$ +43° (same *c* and solvent, 59% ee). By acidification of the incubation mixture and extraction, the monoester **1f** was isolated and directly esterified with an ethereal diazomethane solution. After silica gel chromatography, (*S*)-(−)-**1e** was isolated (0.3 g, 40%); $[\alpha]_D$ −42° (58% ee).

Regioselective PLE-Catalysed Hydrolysis of Dimethyl (S)-2-O-*Benzylmalate* **1e**.—The (*S*)-(−)-ester **1e** (0.756 g, 3 mmol) prepared according to ref. 18 and with $[\alpha]_D$ −73° was completely hydrolysed in the presence of PLE in 1 h, under the usual experimental conditions, monitoring the reaction by HPLC analysis (RP18 column, water–methanol, 1:1 as eluent). After extraction with dichloromethane, the incubation mixture was brought to pH 3 (conc. HCl) and extracted with dichloromethane. The pure monoester (*S*)-(−)-**1f** was obtained (0.64 g, 90%); $[\alpha]_D$ −60° (*c* 1.6 in chloroform).

CHY-Catalysed Hydrolysis: General Procedure.—The substrate (3 mmol) was suspended in a KH_2PO_4 (0.1 mol dm^{-3}) solution (1.5 cm^3) and CHY (0.27 g, 74 U mg^{-1}) in distilled water (12 cm^3) was added. The suspension was stirred for the time necessary for the hydrolysis keeping the pH constant at 7.8 by additon of NaOH (1 mol dm^{-3}) with a Radiometer automatic titrator. The extent of the reaction was estimated by the volume of base added and TLC. The reaction was then acidified (HCl) and the products were extracted with diethyl ether (3 × 5 cm^3). The purification was accomplished by column chromatography (silica gel, eluents indicated).

CHY-Catalysed hydrolysis of dimethyl (R,S)-2-O-*acetylmalate and* -2-O-*benzylmalate* **1d** *and* **1e**. From **1d** in 1 h, unchanged (*S*)-(−)-**1d** was isolated (0.135 g, 22%), $[\alpha]_D$ −3° (*c*

2.5 in ethanol, 25% ee). From **1e** in 3 h, unreacted (*S*)-(−)-**1e** was obtained (0.242 g, 32%), $[\alpha]_D$ −45° (*c* 1.6 in chloroform, 62% ee).

PFL-Catalysed Transesterification of Dimethyl (R,S)-*Malate* **1b**.—A solution of the racemic malate **1b** (0.324 g, 2 mmol), vinyl acetate (0.75 cm^3, 8.12 mmol) and PFL (22 mg, 924 U) in chloroform (4.5 cm^3) was stirred for 48 h at 30 °C. The reaction was monitored by ^1H NMR. When the desired conversion was reached, the enzyme was filtered off and the solvent evaporated. The acetate and the unchanged hydroxy ester were separated by silica gel chromatography: the fractions eluted with hexane–ethyl acetate (9:1) contained the (*S*)-(−)-acetate **1d** (0.171 g, 42%) $[\alpha]_D$ −13° (*c* 2.5 in EtOH, 60% ee). When the reaction was stopped at *ca.* 60% conversion to the acetate **1d**, the reaction was worked up and the mixture of products purified. Fractions eluted with hexane–ethyl acetate (8:2) contained the unchanged (*R*)-(+)-**1b** (0.136 g, 42%) $[\alpha]_D$ +4.5° (*c* 2.5 in EtOH, 52% ee).

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