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Asymmetric synthesis of (*R*)-(−)-chlozolinate through a chemoenzymatic procedure

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Abstract—A new asymmetric synthesis of (*R*)-chlozolinate **1**, an important antifungal agent, based on the enzymatic asymmetrization of diethyl 2-benzyloxy-2-methylmalonate, is reported.

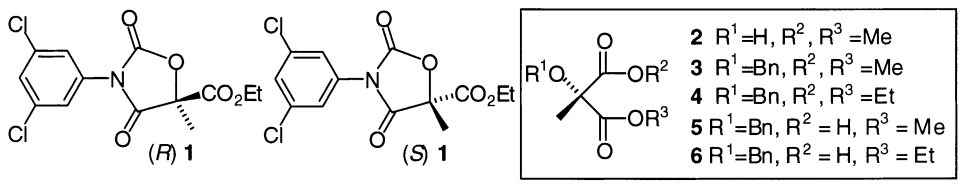
1. Introduction

Botrytis cinerea is one of the worst and most widespread fungal diseases in viticulture. The most useful pesticides currently used in order to prevent the growth of this microorganism belong to the dicarboximide family. These products were found to control *Botrytis* fairly well and additionally do not affect the alcoholic fermentation process.[1–5] Chlozolinate **1**[6] is particularly useful since it is rapidly degraded in soil and in wine. This ensures the absence of possible toxic residues in the final product, even when the statutory procedures are not followed (Scheme 1).[2]

Until now, chlozolinate has been produced and sold only in racemic form. In order to evaluate any differences in the biological behavior of its two enantiomers, we needed to develop a practical way to prepare multigram quantities of at least one of the two antipodal forms. Separation or resolution at the final product stage was foreseen to be difficult, due to the absence of suitable functionalities and the easy degradation of chlozolinate in solution.

Thus, we decided to develop an asymmetric synthesis based on the enzyme catalyzed asymmetrization of a substituted prochiral malonate of the type **2**–**4**.

There is only one literature report on asymmetrization of unprotected malonate **2**.[7] The results, however, were far from satisfactory in terms of enantiomeric excess (46%) of the resulting monoester. More recently a moderately good e.e. (up to 80.6% for the (*R*)-enantiomer) has been achieved in the asymmetrization of a series of 2-methyl-2-(2-nitrophenoxy)malonates.[8] However, the aryl groups bonded to oxygen in that study cannot be easily removed and thus are not suitable for chlozolinate synthesis.



Scheme 1.

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In the present study, we chose to employ malonates protected at the tertiary hydroxyl with an easily removable group. This strategy was adopted for two reasons: firstly, we expected that the protecting group may better differentiate the substituents at the prostereogenic center, thus allowing higher e.e.s to be achieved; and secondly, it was felt that the presence of the protecting group might facilitate the transformation of monoesters **5**–**6** into chlozolinate.

2. Results and discussion

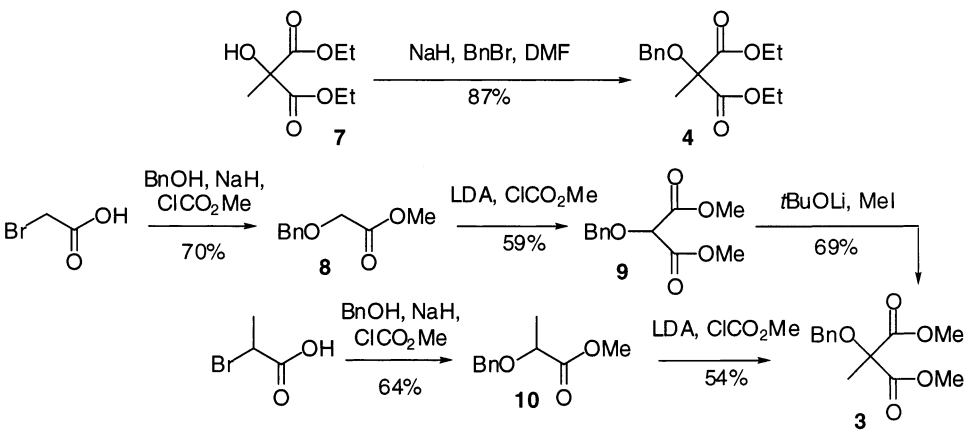
The required malonates **3** and **4** were prepared as depicted in Scheme 2. The benzyl protected diethyl ester **4**[9] was easily obtained from known 2-hydroxy-2-methylmalonate **7**,[10,11] which is an intermediate for the industrial synthesis of racemic chlozolinate.

For dimethyl malonate **3**, which had never been prepared before, we developed two new synthetic routes involving only two or three steps from low cost, commercially available, substrates (Scheme 2). The first step involved, for both routes, the one-pot conversion of a 2-bromoacid into the 2-benzyloxy esters **8** and **10** by an efficient procedure recently discovered in our laboratories. This involved treating an alkoxide with sodium bromoacetate, followed by reaction with an alkyl chloroformate in the presence of a catalytic amount of alkoxide. A mechanism for this conversion is proposed in Scheme 3. Chloroformate forms a mixed anhydride, which is then attacked by the catalytic alkoxide (in this case sodium methoxide). During this reaction, methoxide is reformed, and thus it is truly catalytic. Although compounds **8**[12,13] and **10**[14] are known in the literature, this one-pot procedure is convenient and comparable or more efficient than the previously known syntheses, especially in the case of **10**.

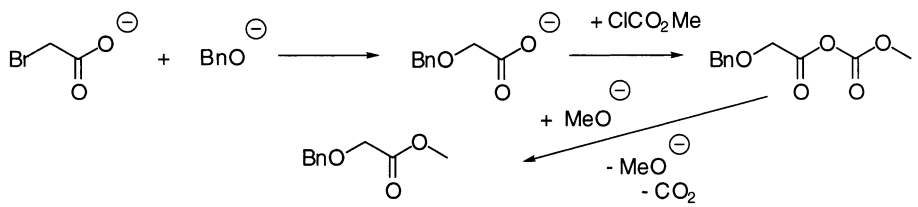
Table 1 reports the selected results of enzymatic asymmetrization of diesters **3** and **4**. The reactions were quenched (except for entry 5) when the starting malonate was no longer visible by TLC analysis. The reactions usually tended to stop after mono-hydrolysis. However, the lower yields obtained in some cases may be due to the formation of significant quantities of diacid (which was difficult to extract). As can be seen from entry 1, in the case of compound **3** PLE gave, under the usual conditions, poor results in terms of enantioselection, as reported by Tamm for the unprotected malonate **2**.[7] It should, however, be stressed that in Tamm's work the main enantiomer was probably (*S*)-configured although the absolute configuration was not demonstrated. By contrast, in this work, the (*R*)-enantiomer was always preferentially formed.

Since it is well known[15] that the presence of co-solvents can dramatically influence the enantioselectivity of PLE catalyzed reactions, we tried to optimize the e.e. by carrying out the reactions in mixed solvent systems (entries 2–4). No significant improvements were seen. We then explored other enzymes. However, of the many tested (lipases from *Candida anctarctica*, *Pseudomonas cepacia*, Pig liver acylase Amano 30.000, esterase from *Mucor miehei*), only lipase from *Candida cylindracea* did react, although quite slowly, with low e.e. A breakthrough was eventually made when we tested horse liver acetone powder (HLAP) (entry 6), which led to an encouraging e.e. of 89%.

Since for chlozolinate synthesis a monoethyl ester was expected to be more useful, we also attempted mono-



Scheme 2.

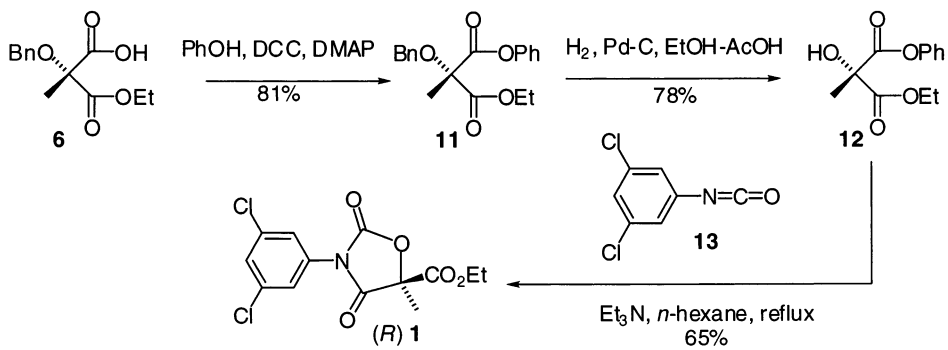


Scheme 3.

Table 1. Results of enzymatic asymmetrization of malonates **3**–**4** to give (*R*)-monoesters **5**–**6**[a]

Entry	Subst.	Enzyme[b]	Buffer[c]	Co-solvent (%)	Time (h)	% Conversion[d]	% Yield[e]	% E.e.[f]
1	**3**	PLE	A	None	6	>95	85	53
2	**3**	PLE	A	DMSO (10)	6	>95	82	59
3	**3**	PLE	A	*t*-BuOH (10)	4.5	>95	79	31
4	**3**	PLE	A	DMF (10)	4.5	>95	86	44
5	**3**	CCL	B	None	24	15	10	46
6	**3**	HLAP	A	None	3	>95	75	89
7	**4**	HLAP	B	None	15	>95	84	86
8	**4**	HLAP	B	*t*-BuOH (10)	3	>95	88	67
9	**4**	HLAP	B	DMF (10)	7	>95	92	83
10	**4**	HLAP	B	*i*-Pr_2O (10)	45	>95	62	96
11	**4**	HLAP	B	EtOH (10)	18	>95	54	96
12	**4**	HLAP[g]	B	*n*-Heptane (10)	25	>95	78	93
13	**4**	BLAP	B	None	19	>95	80	57

[a] All reactions were carried out at 20°C.
[b] PLE = pig liver esterase (600 U/mmol of substrate); CCL = *Candida cylindracea* lipase (300 mg/mmol of substrate); HLAP = horse liver acetone powder (500 mg/mmol of substrate); BLAP = bovine liver acetone powder (500 mg/mmol of substrate).
[c] A = phosphate buffer, pH 7; B = Tris buffer, pH 7.5.
[d] Means the percentage of initial diester which has reacted.
[e] Isolated yields after chromatography.
[f] Determined by ^{1}H NMR in the presence of (–)-ephedrine. The (*R*)-enantiomer was in all cases predominant.
[g] For preparative purposes 250 mg/mmol of substrate were used.



Scheme 4.

hydrolysis of **4** with HLAP. Interestingly (entry 7) the expected decrease in the reaction rate on changing from a methyl to an ethyl diester was not dramatic, and the e.e. was still good. We further optimized this reaction for an ethyl diester substrate. In this case, the addition of co-solvents had a remarkable effect on the rate of reaction, the product e.e. and the substrate selectivity (entries 8–12). For example, the presence of *tert*-BuOH increased the rate but decreased the enantioselectivity. The opposite effect was seen when *iso*-Pr_2O was added. Ethanol gave a very good e.e., but substrate selectivity (and thence the yield) dropped significantly because substantial amounts of diacid formed. The best co-solvent was found to be *n*-heptane, which afforded a high e.e. (93%) together with good yield and workable reaction times.

The absolute configuration of **6** was unambiguously established as (*R*) by $[\alpha]_D$ correlation with (*R*)- and (*S*)-**6**, that have been previously obtained by resolution and correlated with citramalic acid.[9,16] In the case of **5** the absolute configuration is also thought to be (*R*) on the basis of similar NMR behavior in the presence of (–)-ephedrine, on the same sign of specific rotation, and finally because it is highly likely that HLAP shows the same enantiopreference for both ethyl and methyl esters.

We then studied the conversion of mono-acid **6** into (*R*)-chlozolinate (Scheme 4). Our plan involved transformation of the free carboxylic acid into an active ester. The choice of active ester was not obvious because it was required to be active enough to be completely differentiated from the ethyl ester, but also be stable under the conditions required for benzyl ether deprotection by hydrogenolysis. After various experiments involving substituted phenyl esters, we found that the simple phenyl ester itself fulfilled all of our requirements, and hydrogenolysis of **11** took place without side-reactions to give **12**. Treatment of the latter with 3,5-dichlorophenyl isocyanate[1] in the presence of excess Et_3N afforded (*R*)-chlozolinate **1** in good yield. No analogous phenyl ester (from competitive cyclization onto the ethyl ester) was observed. ^{1}H NMR analysis of (*R*)-**1** in the presence of $Eu(hfc)_3$ showed (by comparison with a racemic sample) that no racemization had occurred during the sequence from **6** to **1**. Moreover, crystallization from absolute ethanol was found to increase the e.e. to $\geq 96\%$.

(*R*)-Chlozolinate **1** was then tested on two strains of *Botrytis cinerea* (IR004 and IR058). The former is sensitive to dicarboximide pesticides while the second one is resistant. As a control the same tests were carried out on racemic chlozolinate. The results, shown in Table 2, indicate no significant difference in activity between the two compounds. These data seem to suggest that the two enantiomers interact with the fungus equally well.

In conclusion an efficient asymmetric synthesis of (*R*)-(−)-chlozolinate was developed, based on an enzymatic asymmetrization step catalyzed by horse liver acetone powder.

3. Experimental

^1H and ^{13}C NMR spectra were taken in $CDCl_3$, at 200 and 50 MHz, respectively. Chemical shifts are reported in ppm (δ scale), using TMS as an internal standard. In ABX systems, the proton A is considered downfield and B upfield. GC–MS was carried out on an HP-5971A instrument, using an HP-1 column (12 m long, 0.2 mm wide), electron impact at 70 eV, a mass temperature of about 167°C and starting the mass range from $m/z=33$. Analyses were performed with a constant He flow of 0.9 mL/min, starting at 100°C for 2 min and then raising the temperature by 20°C/min; R_t are measured in minutes from injection. IR spectra were measured with a Perkin–Elmer 881 instrument. TLC analyses were carried out on silica gel plates, which were developed by UV or by dipping into a solution of $(NH_4)_4MoO_4$ tetrahydrate (21 g) and $Ce(SO_4)_2$ tetrahydrate (1 g) in H_2SO_4 (31 mL) and H_2O (469 mL) and warming. R_f values were measured after an elution of 7–9 cm. Chromatography was carried out on 220–400 mesh silica gel using the 'flash' methodology. Petroleum ether (40–60°C) is abbreviated as PE. All reactions employing dry solvents were carried out under a nitrogen atmosphere. *Candida cylindracea* lipase, Pig liver esterase, Horse liver acetone powder and Bovine liver acetone powder were purchased from Sigma and used as received.

3.1. Preparation of diethyl 2-(benzyloxy)-2-methylmalonate 4

To a suspension of NaH (60% in mineral oil) (2.11 g, 52.6 mmol) in dry DMF (80 mL) cooled in an ice bath, a solution of diethyl 2-hydroxy-2-methylmalonate **7** (10.01 g, 52.6 mmol) in dry DMF (10 mL) was added dropwise over 30 minutes.

After stirring the mixture for 15 minutes, benzyl bromide (6.40 mL, 52.6 mmol) was added and the mixture was stirred at room temperature for 5 hours, then quenched with H_2O/saturated aqueous NH_4Cl 1:1 (200 mL) and extracted with PE/Et_2O 1:1 (3×60 mL). The organic layer was then washed with water (100 mL). After drying (Na_2SO_4) and evaporation, the crude product was finally purified by silica chromatography with PE/Et_2O 75:25. The main product band to elute was evaporated to afford **4** as an oil (12.84 g, 87%).

$R_f=0.75$ (Et_2O/PE 1:1+2% CH_3COOH). Anal. found: C, 64.2; H, 7.2%. $C_{15}H_{20}O_5$ requires: C, 64.27; H, 7.19%. GC–MS: $R_t=6.76$ min; m/z: 207 (M^+-73, 0.6%), 174 (35.5), 128 (31.6), 100 (19.7); 92 (8.9), 91 (100), 65 (8.5), 43 (9.4). ^1H NMR: δ 7.46–7.22 [5H, m, Ar*H*]; 4.67 [2H, s, CH_2Ph]; 4.25 [4H, q, CH_2CH_3, $J=7.3$ Hz]; 1.72 [3H, s, CCH_3]; 1.29 [6H, t, CH_2CH_3, $J=7.3$ Hz].

3.2. Preparation of methyl 2-(benzyloxy)acetate 8

Sodium hydride (60% suspension in mineral oil, 8.47 g, 212 mmol) was placed in a two-necked flask equipped with a dropping funnel. It was washed twice with dry *n*-hexane (20 mL) under nitrogen, suspended in dry DMF (90 mL) and cooled to 0°C. A solution of benzyl alcohol (10 ml, 96.6 mmol) in dry DMF (20 mL) was then added over 20 min through a dropping funnel. After stirring for a further 15 min, a solution of bromoacetic acid (13.51 g, 97.2 mmol) in dry DMF (20 mL) was added over 20 min through the dropping funnel. The suspension was stirred for 30 min at 0°C, then for 3 h at rt, and a further 2 h at 60°C. After cooling again to 0°C, methyl chloroformate (7.5 mL, 97 mmol) was added over 15 min and the mixture stirred for 10 min. Sodium methoxide (500 mg, 9.3 mmol) was added. The suspension was allowed to warm to room temperature and stirred overnight. It was then cautiously poured into a mixture of saturated aqueous NH_4Cl (100 mL)+H_2O (100 mL). Three extractions with Et_2O, followed by washing of the ethereal extract with brine gave, after drying (Na_2SO_4) and evaporation to dryness, a crude oil which was distilled to afford pure **8** as a colorless liquid (12.19 g, 70%). Bp 85–90°C (at 0.6 mbar); $R_f=0.67$ (Et_2O/PE 4:6). Anal. found: C, 66.45; H, 6.8%. $C_{10}H_{12}O_3$ requires: C, 66.65; H, 6.71%. GC–MS: $R_t=4.52$ min; m/z: 180 (M^+, 0.55); 121 (3.2); 107 (77.7); 105 (3.0); 92 (9.2); 91 (100); 89 (4.8); 79

Table 2. Activity of (*R*) and racemic **1** toward *Botrytis cinerea* strains

IR004 (sensible strain)			IR058 (resistant strain)		
Dose (ppm)	Inhibition % (*R*)-**1**	Inibition % (*R,S*)-**1**	Dose (ppm)	Inhibition % (*R*)-**1**	Inhibition % (*R,S*)-**1**
1	84	86.5	50	96.5	95.5
0.5	50	65.5	25	90.5	93.5
0.25	12	13.5	12.5	71.5	77.5
0.125	5	5.5	6.25	9.5	19

(11.3); 77 (5.9); 74 (41.6); 65 (16.0); 51 (5.2); 43 (22.3); 39 (6.4). ^{1}H NMR: δ 7.40–7.25 [5H, m, Ar*H*]; 4.64 [2H, s, CH_2Ph]; 4.11 [2H, s, CH_2CO$_2$Me]; 3.76 [3H, s, OCH_3].

3.3. Preparation of dimethyl 2-(benzyloxy)malonate 9

A solution of lithium di-*iso*-propylamide in THF/*n*-hexane was prepared under Ar, at −15°C, from *n*-BuLi (1.6 M in *n*-hexane, 14.5 mL, 23.2 mmol), (*i*-Pr)$_2$NH (3.70 mL, 26 mmol) and dry THF (28 mL). To this solution, cooled to −78°C, a solution of ester **8** (3.22 g, 17.9 mmol) in THF (5 mL) was added dropwise over 5 min. In the meantime, methyl chloroformate (1.80 ml, 23.3 mmol) was dissolved under Ar in dry THF (20 mL) and cooled to −78°C in another flask, equipped with a dropping funnel. The dropping funnel was cooled by an acetone/dry ice mantle at −78°C. After complete addition of **8** to LDA (in 15 min), the resulting solution was transferred into the cooled dropping funnel and added over 5 min to the chloroformate solution. After 30 min, saturated NH_4Cl (100 mL) was added. After warming to rt, the mixture was extracted with Et_2O. After washing with brine, drying (Na_2SO_4) and evaporation to dryness, the crude product was chromatographed (PE/Et_2O 6:4→1:1) to give pure **9** as an oil (2.50 g, 59%). R_f=0.42 (Et_2O/ETP 4:6). Anal. found: C, 60.25; H, 6.1%. $C_{12}H_{14}O_5$ requires: C, 60.50; H, 5.92%. GC–MS: R_t=6.28 min; *m*/*z*: 179 (M^+−59, 0.21%); 132 (77.3); 107 (14.5); 101 (8.6); 100 (46.3); 91 (100); 79 (6.6); 77 (4.5); 74 (5.6); 69 (14.0); 65 (15.3); 59 (5.6); 39 (5.2). ^{1}H NMR: δ 7.40–7.25 [5H, m, Ar*H*]; 4.70 [2H, s, CH_2Ph]; 4.57 [1H, s, C*H*-CO$_2$Me]; 3.79 [6H, s, OCH_3].

3.4. Preparation of methyl 2-(benzyloxy)propanoate 10

Prepared using the same methodology employed for ester **8**, this time using 2-bromopropanoic acid instead of bromoacetic acid. The crude product was purified by chromatography (PE/Et_2O 6:4). Yield: 64% (oil). R_f=0.44 (Et_2O/ETP 4:6). Anal. found: C, 68.1; H, 7.4%. $C_{11}H_{14}O_3$ requires: C, 68.02; H, 7.27%. GC–MS: R_t=4.53 min; *m*/*z*: 194 (M^+, 0.07%); 135 (3.0); 107 (9.9); 91 (100); 88 (55.7); 65 (8.8); 57 (5.6). ^{1}H NMR: δ 7.40–7.25 [5H, m, Ar*H*]; 4.70 [2H, AB syst., CH_2Ph, J_{AB} 11.7 Hz]; 4.07 [1H, q, C*H*-CH$_3$, J=6.9 Hz]; 3.75 [3H, s, OCH_3]; 1.44 [3H, d, CH_3-CH, J=6.9 Hz].

3.5. Preparation of dimethyl 2-(benzyloxy)-2-methylmalonate 3

Method A: Dry *t*-BuOH (50 ml) was placed under N_2 in a two-necked flask equipped with a dropping funnel. *n*-BuLi (1.6 M in hexanes, 6.75 mL, 10.8 mmol) was added. After 5 min, a solution of ester **9** (2.20 g, 9.25 mmol) in *t*-BuOH (30 mL) was added. During the additions, the flask was externally water-cooled when needed. After 15 min, methyl iodide (675 μL, 1.08 mmol) was added. After stirring for 6 h at rt, the reaction was quenched with saturated aqueous NH_4Cl (60 mL). Most of the *t*-BuOH was evaporated under reduced pressure. Then the mixture was extracted three times with Et_2O/PE 1:1 and once with AcOEt. After drying (Na_2SO_4) and evaporation to dryness, the crude product was chromatographed (PE/Et_2O 6:4) to give pure **3** as an oil (1.605 g, 69%).

Method B: A solution of lithium di-*iso*-propylamide in THF/*n*-hexane was prepared under Ar at −15°C from *n*-BuLi (1.6N in *n*-hexane, 4.0 mL, 6.4 mmol), (*i*-Pr)$_2$NH (1.00 mL, 7.1 mmol) and dry THF (11 mL). To this solution, cooled to −78°C, was added a solution of ester **10** (1.04 g, 5.4 mmol) in THF (20 mL) dropwise over 5 min. After 15 min, methyl chloroformate (0.50 mL, 6.5 mmol) was added. After 30 min, saturated NH_4Cl (15 mL) was added. After warming to rt, the mixture was extracted with AcOEt. Purification as above gave pure **3** as an oil (737 mg, 54%).

R_f=0.37 (Et_2O/ETP 4:6). Anal. found: C, 61.65; H, 6.50%. $C_{13}H_{16}O_5$ requires: C, 61.90; H, 6.39%. GC–MS: R_t=6.27 min; *m*/*z*: 193 (M^+−59, 0.44%); 146 (46.1); 114 (44.0); 91 (100); 86 (13.3); 65 (12.0); 43 (10.1). ^{1}H NMR: δ 7.46–7.25 [5H, m, Ar*H*]; 4.65 [2H, s, CH_2Ph]; 3.78 [6H, s, OCH_3]; 1.73 [3H, s, CH_3]. ^{13}C NMR: δ 169.82 [*C*=O]; 137.51 [quat. aromatic]; 128.28, 127.94, 127.74 [aromatic *C*H]; 81.94 [*C*-OBn]; 68.52 [*C*H$_2$Ph]; 52.76 [O*C*H$_3$]; 21.24 [*C*H$_3$-C-O].

3.6. Preparation of (*R*)-2-benzyloxy-2-methylmalonic acid monomethyl ester 5

Diester **3** (305 mg, 1.21 mmol) was suspended in pH 7.5 Tris buffer (60 mL) (prepared dissolving 3.64 g of Tris–base in 50 mL of H_2O, adjusting to pH 7.5 with 1N HCl, and finally diluting to 100 mL). Horse liver acetone powder (300 mg) was then added and the suspension was stirred at rt for 6 h. It was then acidified with 0.5 M citric acid to pH 3, saturated with NaCl, and filtered through a Celite pad washing with Et_2O. The phases were separated and the aqueous extracted three times with Et_2O always keeping pH<3. In order to prevent the build-up of emulsions a few drops of EtOH were added. After drying (Na_2SO_4) and evaporation to dryness, chromatography (PE/Et_2O 1:1→PE/Et_2O/AcOH 29:69:2) gave pure monoacid **5** as an oil (217 mg, 75%). R_f=0.23 (Et_2O/PE/AcOH 39:59:2). $[\alpha]_D$: +5.27 (*c* 2.1, $CHCl_3$). Anal. found: C, 60.7; H, 6.05%. $C_{12}H_{14}O_5$ requires: C, 60.50; H, 5.92%. ^{1}H NMR: δ 7.42–7.30 [5H, m, Ar*H*]; 4.62 [2H, s, CH_2Ph]; 3.81 [3H, s, OCH_3]; 1.79 [3H, s, CH_3-C]. ^{13}C NMR: δ 172.58, 169.22 [*C*=O]; 136.89 [quat. aromatic], 128.43, 128.05, 127.96 [aromatic *C*H]; 81.76 [*C*-OBn]; 68.60 [*C*H$_2$Ph]; 53.10 [*C*H$_3$O]; 20.66 [*C*H$_3$-C-O].

E.e. was measured by ^{1}H NMR in the presence of 1.5 equiv. of (−)-ephedrine. Under these conditions the singlets given by OCH_3 were split with a $\Delta\delta$ of 0.037 ppm. Integration gave an e.e. of 89%.

3.7. Preparation of (*R*)-2-benzyloxy-2-methylmalonic acid monoethyl ester 6

A solution of diester **4** (1.04 g, 3.71 mmol) in *n*-heptane (10 mL) was treated with pH 7.5 Tris buffer (100 mL)

(prepared as described above), and with horse liver acetone powder (1.02 g). The two-phase suspension was stirred at rt for 25 h. It was then acidified with 0.5 M citric acid to pH 3, saturated with NaCl, and filtered through a Celite pad washing with Et_2O. The phases were separated and the aqueous extracted three times with Et_2O, keeping pH<3. In order to prevent the build-up of emulsions a few drops of EtOH were added. After drying (Na_2SO_4) and evaporation to dryness chromatography (PE/Et_2O 6:4→PE/Et_2O/AcOH 39:59:2) gave pure monoacid **6** as an oil (730 mg, 78%). R_f=0.30 (Et_2O/PE/AcOH 49:49:2). $[\alpha]_D$: +9.54 (*c* 2.2, EtOH); +7.13 (*c* 2.2, $CHCl_3$) (lit.: +9.65, *c*=2, EtOH).[16] Anal. found: C, 62.1; H, 6.4%. $C_{13}H_{16}O_5$ requires: C, 61.90; H, 6.39%. ^{1}H NMR: δ 7.40–7.25 [5H, m, Ar*H*]; 4.61, 4.59 [2H, AB syst., C*H*$_2$Ph, J_{AB} 12.7 Hz]; 4.29 [2H, q, C*H*$_2$-CH$_3$, *J*=7.2 Hz]; 1.79 [3H, s, C*H*$_3$-C]; 1.32 [3H, t, CH$_2$C*H*$_3$, *J*=7.3 Hz]. ^{13}C NMR: δ 173.59, 168.72 [*C*=O]; 136.97 [quat. aromatic]; 128.20, 127.76 [aromatic *C*H]; 81.68 [*C*-OBn]; 68.40 [*C*H$_2$Ph]; 62.20 [CH$_3$*C*H$_2$O]; 20.68 [*C*H$_3$-C-O]; 13.79 [*C*H$_3$CH$_2$O].

E.e. was measured by ^{1}H NMR in the presence of 1.5 equiv. of (−)-ephedrine. Under these conditions the signals given by C*H*$_2$CH$_3$ were splitted with a $\Delta\delta$ of 0.034 ppm. The signal of the (*R*)-enantiomer was downfield. Relative integration was done irradiating the CH$_2$C*H*$_3$ signal at 1.27 ppm. In this way the C*H*$_2$CH$_3$ signals become sharp singlets. Also, the signals due to C*H*$_3$-CH$_2$ are split ($\Delta\delta$=0.038 ppm; (*R*)-enantiomer downfield). They can be integrated as well upon irradiation at 4.23 ppm. However, traces of BHT coming from the solvents used for chromatography can alter the measurement. In this case e.e. was 93%.

3.8. Preparation of (*S*)-2-benzyloxy-2-methylmalonic acid ethyl ester phenyl ester 11

A solution of monoester **6** (982 mg, 3.89 mmol) in CH_2Cl_2 (8 mL), phenol (440 mg, 4.68 mmol) and 4-dimethylaminopyridine (48 mg, 0.39 mmol) was cooled in an ice bath and a solution of dicyclohexylcarbodiimide (964 mg, 4.67 mmol) in CH_2Cl_2 (23 mL) was added. The solution was stirred at 0°C for 30 min then at room temperature for 5 h, then filtered and concentrated under reduced pressure. The residue was purified by chromatography (PE/Et_2O 95:5→7:3) to give pure **11** (1.03 g, 81%) as an oil. R_f=0.42 (PE/Et_2O 85:15). $[\alpha]_D$: +9.46 (*c* 1.65, $CHCl_3$). Anal. found: C, 69.35; H, 6.1%. $C_{19}H_{20}O_5$ requires: C, 69.50; H, 6.14%. GC–MS: R_t=8.94 min; *m*/*z*: 255 (M^+−73, 0.04%); 222 (8.5); 184 (6.8); 128 (5.2); 94 (28.2); 92 (10.1); 91 (100); 65 (10.7); 43 (8.9). ^{1}H NMR: δ 7.50–7.20 [8H, m, Ar*H*]; 7.09 [2H, δ, *H ortho* to O, *J*=8.2 Hz]; 4.84, 4.75 [2H, AB syst., C*H*$_2$OPh, J_{AB}=11.1 Hz]; 4.32 [2H, q, C*H*$_2$-CH$_3$, *J*=7.1 Hz]; 1.87 [3H, s, C-C*H*$_3$]; 1.34 [3H, t, CH$_2$-C*H*$_3$, *J*=7.2 Hz]. ^{13}C NMR: δ 168.94, 168.24 [*C*=O]; 150.36, 137.59 [quat. aromatics]; 129.54, 128.37, 127.85, 127.82, 126.26, 121.16 [aromatic *C*H]; 82.09 [*C*-OBn]; 68.78 [*C*H$_2$Ph]; 62.15 [CH$_3$*C*H$_2$O]; 21.46 [*C*H$_3$-C-O]; 14.15 [*C*H$_3$CH$_2$O]. IR ($CHCl_3$): ν_{max} 3023, 1745, 1591, 1185, 1138, 1100, 1019 cm^{-1}.

3.9. Preparation of (*S*)-2-hydroxy-2-methylmalonic acid ethyl ester phenyl ester 12

A solution of phenyl ester **11** (1.005 g, 3.06 mmol) in 96% ethanol (50 mL) containing AcOH (0.5 mL) was hydrogenated using 10% Pd/C (200 mg) for 7.25 h. After removal of the catalyst and evaporation, the crude product was chromatographed (PE/AcOEt 85:15→6:4) to give pure **12** as an oil (569 mg, 78%). R_f=0.55 (PE/Et_2O 1:1). $[\alpha]_D$: −0.45 (*c* 2.1, $CHCl_3$). Anal. found: C, 60.45; H, 5.85%. $C_{12}H_{14}O_5$ requires: C, 60.50; H, 5.92%. GC–MS: R_t=8.94 min; *m*/*z*: 210 (M^+−28, 2.8%); 165 (1.21); 95 (14.7); 94 (100); 77 (5.4); 66 (4.4); 65 (5.1); 51 (3.3); 43 (51.8); 39 (5.4). ^{1}H NMR: δ 7.40 [2H, t, *H meta* to O, *J*=7.8 Hz]; 7.26 [1H, t, *H para* to O, *J*=7.3 Hz]; 7.09 [2H, d, *H ortho* to O, *J*=8.0 Hz]; 4.35 [2H, q, C*H*$_2$-CH$_3$, *J*=7.1 Hz]; 3.92 [1H, s, O*H*]; 1.78 [3H, s, C*H*$_3$-C]; 1.35 [3H, t, C*H*$_3$-CH$_2$]. ^{13}C NMR: δ 170.79, 169.39 [*C*=O]; 150.32, 129.57, 126.40, 120.93 [aromatics]; 76.27 [*C*-OH]; 62.85 [CH$_3$-*C*H$_2$]; 21.62 [*C*H$_3$-CH$_2$]; 14.10 [*C*H$_3$-C-OH]. IR ($CHCl_3$): ν_{max} 3527, 2986, 2940, 1743, 1590, 1478, 1445, 1379, 1261, 1159, 1106, 1015, 956 cm^{-1}.

3.10. Preparation of (*R*)-chlozolinate 1

A solution of alcohol **12** (1.088 g, 4.56 mmol) in dry *n*-hexane (60 mL) was treated, under N_2, with triethylamine (0.636 ml, 4.56 mmol) and 3,5-dichlorophenylisocyanate **13**[1] (1.20 g, 6.38 mmol). After stirring at rt for 30 min, the mixture was refluxed overnight. After cooling, the suspension was filtered and the filtrate evaporated to dryness. Trituration with absolute ethanol gave **1** as a solid (987 mg, 65%), pure by ^{1}H NMR and TLC, mp 127.2–130.0°C, $[\alpha]_D$: −14.9 (*c* 2.0, $CHCl_3$). Recrystallization from EtOH gave a purer sample (691 mg) with mp=135.5–136.1°C and $[\alpha]_D$=−16.79 (*c* 2.0, $CHCl_3$). The mother liquors had $[\alpha]_D$=−11.1. The enantiomeric excess of the sample obtained just after trituration was determined by ^{1}H NMR in the presence of Eu(hfc)$_3$ by observation of the split C*H*$_3$-C signals, and showed an e.e. of 90%. In the case of the recrystallized sample, the signal of the minor enantiomer was no longer visible. Thus, we estimated an e.e. of ≥96%. This compound was found to be identical to an authentic sample of the racemate by TLC, MS, ^{1}H NMR and IR. GC–MS: R_t=8.16 min; *m*/*z*: 333 (11.6%); 331 (M^+, 17.7); 261 (18.4); 259 (28.2); 216 (5.23); 190 (13.4); 189 (15.5); 188 (32.8); 187 (22.2); 186 (24.4); 124 (11.0); 43 (100). ^{1}H NMR: δ 7.45, 7.26 [2×1H, 2s, aromatics]; 4.34 [2H, q, C*H*$_2$-CH$_3$, *J*=7.2 Hz]; 1.91 [3H, s, C*H*$_3$-C]; 1.34 [3H, t, C*H*$_3$–CH$_2$, *J*=7.2 Hz].

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