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# Enzymatic Synthesis of Chiral Monosubstituted Malonates in Organic Solvents

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**Abstract:** Prochiral stereospecificity of enzymes in organic solvents was used to develop a strategy for the formation of heretofore unknown chiral monosubstituted malonate diesters with high enantiomeric excess. The enzymatic reaction involved transesterification of symmetrical monosubstituted dimethyl malonates with benzyl alcohol, exploiting the ability of lipases to discriminate between the enantiotopic ester groups of the symmetrical malonate molecule. This enzymatic approach is not feasible in aqueous solutions because the activated malonic hydrogen invariably undergoes fast exchange accompanied by racemisation. The synthetic utility of this method was further demonstrated by converting the configurationally unstable mixed methyl benzyl diesters into the corresponding half esters, which were in turn selectively reduced into configurationally stable and synthetically useful hydroxyesters.

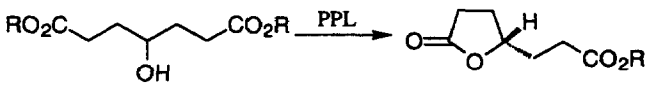
## Introduction

An increasingly important development in the field of organic chemistry is the application of enzymes to chemical reactions.[1] It is now well established that hydrolytic enzymes such as lipases, esterases, and proteases are highly stable in organic solvents and can be used to catalyse reactions of esterification and transesterification,[2] which have been used for preparative kinetic resolutions of chiral acids and alcohols,[3] as well as for the synthesis of homochiral lactones[4] and polyesters.[5] It has also been reported that some lipases and proteases are effective in catalysing the reaction between carboxylic esters and amines in anhydrous organic solvents,[6] which was useful for kinetic resolution of racemic amines[7] and for the synthesis of homochiral lactams.[8]

All these kinetic resolution experiments exploit the enzymes' enantioselectivety, i.e., their ability to discriminate between enantiomers of a racemic mixture. The theoretical yield of the wanted homochiral product from such reactions is 50%, although in practice it will be considerably lower, since the unfavoured enantiomer usually also reacts, all be it at a slower rate. This problem of loosing 50% of material may be avoided when it is possible to exploit the enzymes' prochiral stereospecificity, i.e. their ability to discriminate between enantiotopic groups or faces of a prochiral molecule. Several lipases and proteases were shown to exhibit prochiral selectivity under aqueous conditions,[9,10] for example when hydrolysing symmetrical C-3-substituted glutarate diesters.[9] It was rewarding to realise that enzymes can exhibit prochiral selectivity also in organic solvents, for example in the acylation reaction of symmetrical C-2 substituted 1,3-propanediols.[11]

Our recent studies extended the concept of prochiral selectivity in organic solvents to enzymatic lactonisation and oligomerisation of hydroxy diesters.[12] These reactions were shown to be enantioconvergent and enabled us to convert *all* of the precursor γ-hydroxypimelate diester **1** into a single enantiomer of the appropriate chiral lactone **2** (Scheme 1). It is important to note that in such cases work in organic solvents is an

absolute necessity, and that this approach cannot even in principle succeed if the reaction is attempted in reverse, in aqueous solutions, where enzymatic hydrolysis of lactones such as **2** would also hydrolyse the ester group and produce the symmetrical molecule of γ-hydroxypimelic acid.




**1** **2**

**Scheme 1**

Encouraged by these findings we identified another group of chiral compounds, the monosubstituted malonate mixed diesters **4** and half esters **6** (Figure 1), which would be useful as chiral synthons and as substrates for mechanistic and kinetic studies on racemisation, but their synthesis has not yet been reported. The failure to prepare these compounds in optically active form was due to the inapplicability of the conventional approach, i.e. enzymatic hydrolysis of the symmetrical monosubstituted diesters **3** to the corresponding half esters **6**. Although successfully used for the synthesis of homochiral disubstitued malonates,[13] this approach was bound to fail for the monosubstituted malonates, because under aqueous conditions, the activated malonic hydrogen undergoes fast exchange accompanied by racemisation.[13,14] A preliminary report[15] described our approach to these compounds, which exploited the lipases' prochiral stereospecificity in organic solvents. This strategy provided the first synthetic route to homochiral monosubstituted malonates and served as another example of a problem which was solved only by using enzymes in organic solvents.

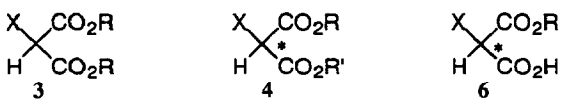


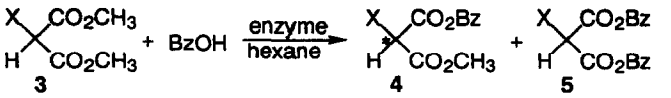

**Figure 1**

## Results and Discussion

In the present work we undertook to extend this study with a view to develop a general enzymatic method for the synthesis of this class of heretofore unknown chiral compounds. We also attempted to establish the synthetic utility of the method by selectively converting the configurationally unstable malonate half ester **6** into the corresponding configurationally stable and synthetically usefull hydroxyester **7**.

In order to check the feasibility of our approach we chose five monosubstituted symmetrical malonate diesters **3a-e** as substrates for the enzymatic reaction (Scheme 2). The methoxy and methyl substituted dimethyl esters **3a** and **3b** were commercially available, while the benzyl, phenylethyl and ethoxyethyl substituted dimethyl esters **3c-e** were prepared by alkylation of dimethyl malonate with the appropriate benzyl, phenylethyl or ethoxyethyl halides under basic conditions as described in the Experimental Section. In all cases the solvent of choice for the enzymatic reaction was hexane, which was chosen because of its high hydrophobicity. We believed this solvent to be suitable both from the point of view of enzyme activity[16] and in order to minimise the lability of the activated malonic hydrogen, which, under hydrophilic conditions, is known to undergo fast

exchange accompanied by racemisation.[14,15] Benzyl alcohol was chosen as the transesterification agent with a view to enable, in the following step, the conversion of the mixed alkyl benzyl ester **4** into the half ester of malonic acid **6** by the facile selective cleavage of the resultant benzyl ester using catalytic hydrogenation.




a, x = -$OCH_3$
b, x = -$CH_3$
c, x = -$CH_2C_6H_5$
d, x = -$CH_2CH_2C_6H_5$
e, x = -$CH_2CH_2OCH_2CH_3$

**Scheme 2**

Each of the prochiral symmetrical substrates **3a-e** with benzyl alcohol in hexane was submitted to the action of several commercially available enzyme preparations and progress of the transesterification reaction was followed by proton NMR. These preliminary screening experiments revealed, that lipases from *Candida cylindracea* (CCL), *Mucor miehei* (Lipozyme), *Candida antarctica* (Novozym) and two acetone powders - from horse liver (LAPH) and from porcine liver (LAPP) - catalysed the transesterification reaction. On the other hand, the lipases from porcine pancreas (PPL) and from *Pseudomonas fluorescens* (Amano PS and SAM 2) did not show any significant activity. For each substrate the enzyme (or enzymes) that showed the best results were chosen for further research.

As can be seen from Table 1, all the reactions proceeded with moderate to high stereoselectivity. For substrates **3a**, **3b** and **3e** the high stereoselectivity of the enzymatic reaction was reflected by the fact that only insignificant quantities of the byproduct - dibenzyl ester **5** - were formed, even after ca. 18 h. On the other hand, substrates **3c** and **3d** gave high ee values even though notable amounts of the corresponding byproducts **5c,5d** were formed. It can also be seen that the enantiomeric excess of the mixed diesters **4a-e** is markedly dependent on the degree of conversion, with higher conversions leading to reduced ee values. It is interesting to note that in all the substrates investigated, the enzymes - CCL, LAPP and LAPH gave the same enriched enantiomer while Novozym and Lipozyme gave the opposite.

The use of benzyl alcohol for the transesterification reactions was particularly rewarding, as it afforded the methyl benzyl esters **4**, which could be easily converted to the half esters **6** by catalytic hydrogenation (Scheme 3). Possessing two readily distinguishable functional groups, such half esters may provide versatile chiral building blocks. By using palladium oxide catalysed hydrogenation of **4a** and **4b** in a toluene - hexane mixture, the corresponding monosubstituted malonate half esters **6a** and **6b** respectively were prepared in optically active form.

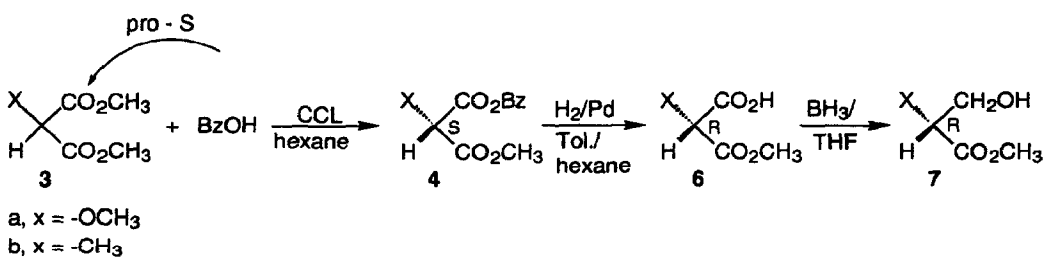
It must however be noted that the activated malonic hydrogen, which provided the sourse of configurational instability for the mixed diesters **4**, is still present in the half esters **6**. To stabilise the stereogenic centre it is necessary to reduce the acidity of the malonic hydrogen. This could be achieved, for example, by selectively converting either the ester or the acid end of **6** to an alcohol group. Our plan was to conduct a second chemical transformation, and selectively reduce the acid end of the enzymatically prepared non-racemic malonate half esters **6** into the corresponding hydroxyesters **7** (Scheme 3). This would convert the easily racemisable malonate structure

**Table 1.** Asymmetric Transesterification of Monosubstituted Dimethyl Malonates **3** with Enzymes in Hexane[a]

| entry | substrate | enzyme | initial rate[b] | reaction time (h) | % conv. to **4**[c] | % conv. to **5**[c] | % ee[d] | $[\alpha]^{28}_D$ (c in $CHCl_3$) |
|---|---|---|---|---|---|---|---|---|
| 1 | **3a** | CCL | 0.46 | 3.5 | 53 | <1 | 98 | -17.4 (1.43) |
| 2 | | | | 18.5 | 64 | <1 | 91 | |
| 3 | **3b** | CCL | 0.50 | 3.5 | 48 | <1 | e | -6.1 (1.08) |
| 4 | | | | 18.5 | 55 | 1.7 | e | |
| 5 | **3c** | Lipozyme | 0.17 | 1.0 | 14 | 1.2 | 71 | f +(α) |
| 6 | | | | 3.4 | 42 | 4.6 | 93 | |
| 7 | | | | 5.0 | 47 | 10.0 | 83 | |
| 8 | | LAPP | 0.38 | 2.3 | 30 | 2.5 | 60 | f -(α) |
| 9 | | | | 5.25 | 38 | 4.0 | 54 | |
| 10 | **3d** | LAPP | 0.55 | 0.58 | 21 | 1.7 | 88 | f -(α) |
| 11 | | | | 4.42 | 31 | 3.6 | 84 | |
| 12 | **3e** | Novozym | 0.17 | 2.33 | 12 | <1 | 98 | +4.2 (1.43) |
| 13 | | | | 17.42 | 29 | <1 | 90 | |
| 14 | | Lipozyme | 0.25 | 3.0 | 49 | 4.8 | 88 | |

[a] The experimental protocol was the same as described in the Experimental Section for **3a**. No reaction took place in the absence of the enzyme under the conditions used. [b] In $\mu mol\ h^{-1}$ (mg enzyme)$^{-1}$. [c] Reaction progress was monitored by integration of $^1H$ NMR signals, which enabled quantitative determination of the remaining dimethyl ester **3**, the mixed benzyl methyl ester **4** and the byproduct dibenzyl ester **5**. [d] Determined by HPLC on a chiral column (Chiralcel OJ, Daicel) with different mixtures of hexane and 2-propanol as described in the Experimental Section. [e] Conditions for separating enantiomers of **4b** have not yet been found. [f] The optical rotations of **4c** and **4d** were determined as mixtures with the corresponding achiral byproducts **5c** and **5d** respectively and therefore are not reported in this paper.

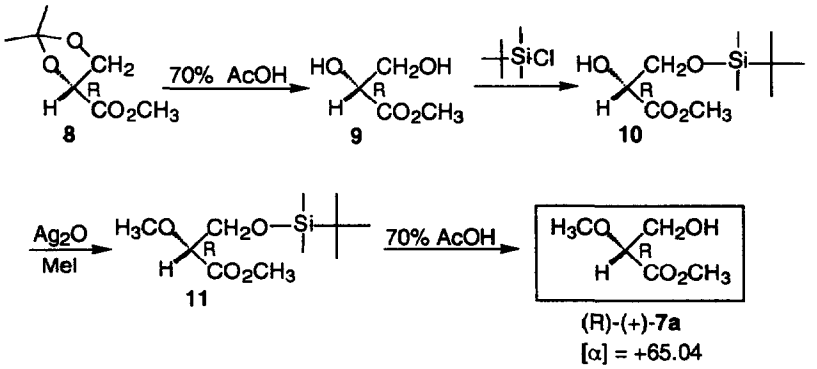
into a configurationally stable molecule. In addition to its potential synthetic utility, this transformation would also enable us to determine the heretofore unknown stereochemical course of the enzymatic reaction, which would be revealed from the absolute configuration of **6**, which in turn would be concluded from the absolute configuration of **7**. The latter could be determined by correlation of the optical rotation of **7** to that of a compound with a known absolute configuration.




**Scheme 3**

The transformation of choice for this purpose was the $BH_3$/THF reduction, that is known to selectively reduce acids in the presence of esters.[17] Submitting the half esters **6a** and **6b** to the $BH_3$/THF reduction (as

described in the Experimental Section) gave the optically active **7a** and **7b** respectively but with low to moderate ee values. The hydroxyester **7b** had an ee value of ca. 20% (as determined by NMR with a chiral shift reagent) and it showed a negative optical rotation. Its absolute configuration was assigned as (R)-(-)-**7b** by comparison of its optical rotation with published data for this compound.[18] The hydroxyester **7a** had an ee value of 70% and displayed positive optical rotation. To consider the consistency of the enzyme's prochiral stereospecificity it was intriguing to determine the absolute configuration of (+)-**7a** as well. Since this compound has not been reported previously, we embarked on its independent synthesis from a chiral precursor with a known absolute configuration, under conditions that excluded racemisation (Scheme 4).



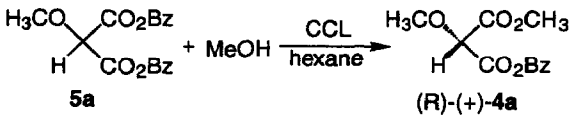

**Scheme 4**

The commercially available 2,3-O-isopropylidene-(R)-glyceric acid methyl ester **8** was chosen as the homochiral starting material for this synthesis. In the first step, its ketal protecting group was removed by treatment with 70% acetic acid to give the diol (R)-(+)-**9**.[19] The primary hydroxyl group was selectively protected by reaction of **9** with t-butyl-dimethyl-chlorosilane with triethylamine and a catalytic amount of dimethyl-aminopyridine in dichloromethane[20] to give (R)-(-)-**10**, which was then subjected to the etherification reaction with $Ag_2O$ and $CH_3I$ under reflux conditions[21] to give the silyl protected methoxy substituted intermediate (R)-(+)-**11**. Removing the silyl protecting group with 70% acetic acid afforded the desired (R)-(+)-**7a**. All the synthetic steps were carried out under conditions that did not involve the stereogenic centre and hence the absolute configuration of the synthetic **7a** was firmly established as (R). Comparing the specific rotation of this synthetic **7a** with that obtained in the enzymatic experiment confirmed that also in the enzymatic experiment the (R) enantiomer of **7a** was obtained.

Knowing the configuration of **7a** and **7b** to be (R), we could assign the absolute configuration of the former products to be - (R)-**6a** and (R)-**6b** and also (S)-**4a** and (S)-**4b** respectively. Noteworthy is the fact that in both substrates **3a** and **3b**, which were submitted to the same enzyme - CCL, the stereochemical course of the reaction was the same, beginning with the enzyme's attack on the pro-S ester group in the prochiral molecule. This stereochemical outcome is built into Scheme 3.

Assuming that the prochiral selectivity of lipases would not be changed as long as similar substrates are submitted to the enzyme, we envisaged that if the enzymatic transesterification is carried out in reverse, i.e. between monosubstituted dibenzyl malonates and methanol (instead of benzyl alcohol), under identical

conditions, the opposite enantiomer would be obtained. To verify this assumption we carried out the transesterification reaction of dibenzyl 2-methoxymalonate **5a** with methanol in hexane catalysed by CCL under the same conditions (Scheme 5). Determination of the enantiomeric excess of the product **4a** revealed that in this case the opposite (R)-(+)-**4a** enantiomer was formed, as expected, with high ee values (Table 2). Thus, the prochiral selectivity of the enzyme allows the preparation of either the (+) or the (-) enantiomer of the mixed diesters of monosubstituted malonates **4a-e** by following one of the two complementary routes.




**5a** (R)-(+)-**4a**

**Scheme 5**

**Table 2.** CCL-Catalysed Transesterification of Dibenzyl Methoxy Malonate **5a** with Methanol in Hexane[a]

| initial rate[b] | reaction time (h) | % conv. to **4a**[c] | % conv. to bypr. **3a** | % ee[d] |
|---|---|---|---|---|
| 0.68 | 0.75 | 48 | 7 | 90-95 |
| | 5.7 | 54 | 15 | 80-85 |
| | 24.0 | 50 | 24 | 70-75 |

[a] The experimental protocol was the same as described in the Experimental Section. No reaction took place in the absence of the enzyme under the conditions used. [b] In μmol $h^{-1}$ (mg enzyme)$^{-1}$. [c] Reaction progress was monitored by integration of $^1$H NMR signals, which enabled quantitative determination of the remaining dibenzyl ester **5a**, the mixed benzyl methyl ester **4a** and the byproduct dimethyl ester **3a**. [d] Determined by HPLC on a chiral column (Chiralcel OJ, Daicel) with a mixture of hexane and 2-propanol (95:5) as the mobile phase at the flow rate of 0.9 ml/min using detection at 258 nm.

As can be seen from Tables 1 and 2, in some cases (e.g. Table 1 entries 6, 7, 14 and Table 2) the formation of the mixed diester **4** was accompanied by significant quantities of the byproduct dibenzyl ester **5** or dimethyl ester **3a**. Formation of these byproducts indicates that the enzyme is not highly selective in distinguishing between the two enantiotopic ester groups, as a result of which one would expect to see a significant lowering in the ee values of the corresponding mixed diesters **4**. However, this was not always so (see for example substrate **3c** with Lipozyme, Table 1, entries 6, 7), and the ee value of **4c** remained high in spite of the considerable formation of the byproduct. This behaviour was reported previously,[22] and encouraged us to take a closer look at the investigated systems, for example at the benzyl substituted malonate.

The relatively unselective transesterification of **3c** initially leads to a significant fraction of (-)-**4c**, reducing the ee of the desired monoester (+)-**4c** for short reaction times (entry 5, Table 1). It must be assumed, however, that as the reaction proceeds the (-)-enantiomer of the mixed diester undergoes further benzylation more rapidly than its (+)-stereoisomer and is removed from solution, raising the enantiomeric excess of the desired (+)-benzyl methyl malonate at long reaction times (entry 6, Table 1).

This assumption was confirmed experimentally by conducting an enzymatic transesterification reaction on racemic **4c** with benzyl alcohol and Lipozyme at the same reaction conditions. It was found that indeed the enzyme preferentially transesterified the (-)-enantiomer to the corresponding dibenzyl ester **5c**. The decreasing of the ee value between entry 6 and entry 7 (Table 1) was probably due to temperature induced racemisation, as was observed for all the substrates investigated, at longer reaction times (see Tables 1 and 2).

## Conclusions

This study significantly extends the synthetic utility of lipases in organic solvents. It has been shown that prochiral stereospecificity can be achieved by enzymes in organic solvents and be used to solve a synthetically important problem. The method was used for the preparation of heretofore unknown chiral monosubstituted malonate diesters with high enantiomeric excess. The generality of this approach was established with five variously substituted malonic substrates and several different lipase preparations. Suitable enzymes and reaction conditions were found for the synthesis of either enantiomer of the aforementioned compounds. Although, due to the enhanced lability of the malonic hydrogen, the synthetic utility of these compounds as such is limited, it has been shown that it is possible to convert them, via a facile two step procedure, into the configurationally stable and synthetically useful 2-substituted 3-hydroxypropanoates. It is noteworthy that this enzymatic approach can work only in organic solvents and that the synthetic goal achieved in this work is even in principal not possible in aqueous solutions, where the activated malonic hydrogen undergoes fast exchange accompanied by racemisation.

## Experimental

### General.

$^1$H NMR spectra were recorded on a Bruker AM 200-MHz spectrometer in $CDCl_3$. All chemical shifts were reported in ppm. Optical rotations were determined on a JASCO digital polarimeter DIP-370. Solvent extracts of aqueous solutions were dried over $Na_2SO_4$. Solutions were concentrated under reduced pressure on a rotary evaporator. Column chromatography was performed on silica gel kieselgel 40, 0.063-0.200 mm (Merck). Distillations were performed on a glass tube oven Buchi GKR-50. The shaker used for enzymatic experiments was a G24 environmental incubator shaker from New Bronswick Scientific Co. GC-MS analyses were performed on a Magnum gas chromatograph (Finnigan Mat) with a fused silica capillari column DB-5. The lipases from *Candida cylindracea* (CCL, type VII), Porcine Pancreas (PPL, type II) and the acetone powders from porcine liver (LAPP) and from horse liver (LAPH) were purchased from Sigma Chemical Co. The lipases from *Mucor miehei* (Lipozyme) and *Candida antarctica* (Novozym) were kindly given to us by Novo Nordisk, A/S Denmark. The lipases from *Pseudomonas fluorescens* (Amano PS and Sam 2) were obtained from Amano Pharmaceutical Co. The hexane used in the enzymatic experiments was HPLC grade (Merck). Unless otherwise stated, materials were obtained from commercial suppliers and were used without further purification.

### Determination of enantiomeric excess (ee).

The enantiomeric excess of the chiral monosubstituted malonates **4a,c-e** was determined by Merck Hitachi HPLC on a chiral column (Chiralcel OJ, Daicel) with different mixtures of hexane and 2-propanol as the mobile phase. The detector used was l-4000 UV detector at 258 nm. The separation conditions and retention times ($R_f$) were: for **4a** - the mobile phase was hexane:2-propanol, 95:5, at the flow rate of 0.9 ml/min, the Rf values were 82 min for the (S)-(-) enantiomer and 91 min for the (R)-(+) enantiomer; for **4c** - hexane:2-propanol, 99:1, flow rate=0.9 ml/min, $R_f$=53 min for the (+) enantiomer, $R_f$=58 min for the (-) enantiomer; for

**4d** - hexane:2-propanol, 35:65, flow rate=0.74 ml/min, $R_f$=15.3 min for the (+) enantiomer, $R_f$=16.4 min for the (-) enantiomer; for **4e** - hexane:2-propanol, 90:10, flow rate=0.9 ml/min, $R_f$=13.8 min for the (-) enantiomer, $R_f$=18.8 min for the (+) enantiomer. It should be mentioned that the chiral column we used is very sensitive to temperature and therefore, the $R_f$ values changed significantly between analyses. To avoid confusion we often had to inject racemic mixtures during analyses.

The optical purity of **7a** was determined by NMR spectra in the presence of the chiral reagent (R)-(-)-2,2,2-trifluoro-1-(9-anthryl)-ethanol (0.8-0.9 equivalents). The solvent was $CCl_4$:$CDCl_3$, 3:1. The singlet common to the methyl protons of the methoxy group was separated and shifted up-field giving almost baseline separation. The optical purity of **7b** was determined by NMR spectra in the presence of the chiral reagent tris[3-(trifluoromethylhydroxy-methylene)-(+)-camphorato]europium (III) derivative (0.4-0.6 equivalents). The solvent was $CD_3CN$. The singlet corresponding to the esteric methyl protons was separated and shifted down-field.

**Synthesis of monosubstituted dimethyl malonates (3c,3d,3e) (representative procedure).**

Clean cut sodium (2.88 g, 0.125 mmol) was added in pieces to methanol (dried over magnesium) (250 ml). When the reaction had ceased, dimethyl malonate (14.3 ml, 0.125 mmol) was added dropwise and the mixture was stirred at room temperature for 0.5 h. Benzyl bromide (15 ml, 0.126 mmol) was added and the mixture was refluxed for 2 h. Then most of the solvent was removed under reduced pressure and aqueous HCl (0.5 M, 140 ml) was added. Extraction with $CHCl_3$ (3X50 ml), drying, followed by distillation (0.05 mm Hg, 120-130 °C) gave dimethyl 2-benzylmalonate **3c**, (13.6 g, 49% yield).

**Dimethyl 2-benzylmalonate 3c**: $^1$H NMR δ 7.17-7.25 (5 H, m), 3.69 (6 H, s), 3.68 (1 H, t, J=7.68 Hz), 3.22 (2 H, d, J=7.79 Hz).

**Dimethyl 2-(2-phenylethyl)-malonate 3d**: $^1$H NMR δ 7.14-7.30 (5 H, m), 3.72 (6 H, s), 3.37 (1 H, t, J=7.45 Hz), 2.64 (2 H, t, J=7.12 Hz), 2.19-2.27 (2 H, m); distillation conditions: 0.02 mm Hg, 105-110 °C; 15% yield.

**Dimethyl 2-(2-ethoxyethyl)-malonate 3e**: $^1$H NMR δ 3.71 (6 H, s), 3.56 (1 H, t, J=7.31 Hz), 3.44 (2 H, t, J=5.83 Hz), 3.39 (2 H, t, J=6.96 Hz), 2.10-2.20 (2 H, m), 1.13 (3 H, t, J=7.01 Hz); distillation conditions: 0.02 mm Hg, 60-65 °C; 20% yield.

**Synthesis of dibenzyl 2-methoxymalonate 5a.**

The substrate was prepared by titanate-mediated transesterification as follows: dimethyl 2-methoxymalonate **3a** (5 g, 30.8 mmol) was dissolved in 100 ml of benzyl alcohol, tetraisopropyl-ortho-titanate (2.63 g, 9.25 mmol) was added, and the mixture was heated to reflux temperature for 24 h. It was then cooled to room temperature, quenched with 1N HCl (150 ml), and extracted with ether (3x50 ml). The organic extract was washed with aqueous sodium hydrogen carbonate (2x50 ml), dried and evaporated. Distillation afforded a pure fraction of **5a** (2.42 g, 25% yield). $^1$H NMR δ 7.31 (10 H, broad s), 5.20 (4 H, s), 4.49 (1 H, s), 3.50 (3 H, s).

**Kinetic Measurements.**

Enzyme-catalysed transesterification reactions in organic solvents were carried out as follows: a suspension of the enzyme (50 mg) in 1 ml hexane containing the substrates (70 μmol of the ester **3** or **5a** and 320 μmol alcohol) was placed in a 4 ml screw-cap vial. The vial was shaken at 200 rpm and 40 °C. Periodically, different vials were withdrawn, the enzyme filtered off, the solvent evaporated and NMR spectra

recorded. Integration of the NMR signals afforded quantitative determination of the reaction progress. For example, in the reaction of **3a** the following groups of signals were examined:
(1) singlets at 3.51 ppm and at 3.50 ppm common to the methyl protons of the methoxy group of **3a** and **4a** respectively; (2) singlets at 3.82 ppm and at 3.77 ppm corresponding to the esteric methyl protons of **3a** and **4a** respectively; and (3) singlet at 5.26 ppm corresponding to the methylene protons of the benzyl ester of **4a**. Progress was monitored by comparing the relative intensities of the above mentioned signals.

**Enzymatic transesterification of monosubstituted malonates 3a-e with benzyl alcohol (representative procedure).**

Lipase from *Candida cylindracea* (4 g) was added to a solution of dimethyl 2-methoxymalonate **3a** (1 g, 6.2 mmol) and benzyl alcohol (2.9 ml, 28 mmol) in 30 ml of hexane. The suspension was shaken at 40 °C and 200 rpm. The reaction was terminated by filtering off the enzyme, followed by evaporation of the solvent and separation by bulb to bulb distillation between the remaining benzyl alcohol and dimethyl ester **3a**, and the optically active product **4a**. The distillations were carried out within 1-2 hours at relatively low oven temperatures (50-70 °C) and high vacuum (0.02-0.05 mm Hg) to avoid temperature induced racemisation. Under these conditions the benzyl alcohol and **3a** were distilled out while the desired product **4a** remained in the original bulb. In the cases of **3c** and **3d** we did not separate the desired products (**4c**, **4d** respectively) from the unwanted byproducts - dibenzyl esters **5c**, **5d**.

Using this procedure homochiral monosubstituted benzyl,methyl malonates **4a-e** were obtained from **3a-e** respectively, their structures confirmed by NMR, the optical rotations obtained (except for **4c** and **4d**) and the optical purities determined by HPLC as discussed earlier.
**(S)-(-)-Benzyl,methyl 2-methoxymalonate 4a**: $^1$H NMR δ 7.36 (5 H, s), 5.26 (2 H, s), 4.47 (1 H, s), 3.77 (3 H, s), 3.50 (3 H, s); $[\alpha]_D^{28}$=-17.4 (c=1.43, $CHCl_3$); 83% yield.
**(S)-(-)-Benzyl,methyl 2-methylmaloate 4b**: $^1$H NMR δ 7.33 (5 H, s), 5.16 (2 H, s), 3.69 (3 H, s), 3.49 (1 H, q, J=7.2 Hz), 1.43 (3 H, d, J=7.3 Hz); $[\alpha]_D^{28}$=-6.1 (c=2.25, $CHCl_3$); 95% yield.
**(+)-Benzyl,methyl 2-benzylmalonate 4c**: $^1$H NMR δ 7.12-7.32 (10 H, m), 5.11 (2 H, s), 3.71 (1 H, t, J=7.90 Hz), 3.66 (3 H, s), 3.22 (2 H, d, J=7.85 Hz).
**(-)-Benzyl,methyl 2-(2-phenylethyl)-malonate 4d**: $^1$H NMR δ 7.10-7.32 (10 H, m), 5.16 (2 H, s), 3.68 (3 H, s), 3.39 (1 H, t, J=7.55 Hz), 2.61 (2 H, t, J=7.07 Hz), 2.23 (2 H, t, J=7.82 Hz).
**(+)-Benzyl,methyl 2-(2-ethoxyethyl)-malonate 4e**: $^1$H NMR δ 7.32 (5 H, broad s), 5.16 (2 H, s), 3.69 (3 H, s), 3.61 (1 H, t, J=7.48 Hz), 3.36-3.45 (4 H, m), 2.13-2.22 (2 H, m), 1.12 (3 H, t, J=6.88 Hz); $[\alpha]_D^{30}$=+4.2 (c=1.43, $CHCl_3$); 80% yield.

**Catalytic hydrogenation of monosubstituted benzyl,methyl malonates 4a-b (representative procedure).**

Palladium on carbon (5% Pd/C) (64 mg) was added to a solution of benzyl,methyl 2-methoxymalonate **4a** (96 mg, 0.4 mmol) in 20 ml of hexane:toluene mixture (1:1). Hydrogenation was carried out at atmospheric pressure and room temperature until one equivalent of hydrogen was taken up (20-30 min.). The catalyst was removed by filtration and evaporation of the solvent afforded the methyl 2-methoxymalonate **6a** (58 mg, 96% yield).
**(R)-(+)-Methyl 2-methoxymalonate 6a**: $^1$H NMR δ 8.14 (1 H, broad s), 4.48 (1 H, s), 3.85 (3 H, s), 3.55 (3 H, s); $[\alpha]_D^{28}$=+15.1 (c=0.53, $CHCl_3$).

**(R)-(-)-Methyl 2-methylmalonate 6b**: $^1$H NMR δ 7.40 (1 H, broad s), 3.77 (3 H, s), 3.51 (1 H, q, J=7.1 Hz), 1.47 (3 H, d, J=7.2 Hz); $[\alpha]_D^{28}$=-2.5 (c=1.66, $CHCl_3$); 90% yield.

**Enzymatic transesterification of dibenzyl 2-methoxymalonate 5a with methanol.**

Lipase from *Candida cylindracea* (2.4 g) was added to a solution of dibenzyl 2-methoxymalonate **5a** (400 µl, 1.9 mmol) and methanol (0.32 g, 9.8 mmol) in 20 ml hexane. The suspension was shaken at 40 °C and 200 rpm. The reaction was terminated by filtering off the enzyme, followed by evaporation of the solvent. The components of this reaction were not separated, and the ee values of (R)-(+)-**4a** were determined from the mixture of the remaining starting materials, the product **4a** and the byproduct **3a**.

**$BH_3$/THF Reduction of monosubstituted malonate half esters 6a,6b (representative procedure).**

All glassware was dried thoroughly in a drying oven and cooled in a dessicator. All reduction experiments were carried out under a dry argon atmosphere. Syringes were used to transfer the reagent solution. Into a 25 ml two necked flask equipped with a rubber syringe cap, a magnetic stirring bar and a gas buret was placed 0.84 g of methyl 2-methoxymalonate **6a** (5.69 mmol), followed by 3-5 ml of toluene (when the reaction was carried out immediately after the $H_2$/Pd hydrogenation, the toluene was not evaporated and was used directly in the $BH_3$/THF reduction). The flask was immersed in an ice bath and cooled to 0-5 °C. Then 3.2 ml of 2.0 M borane solution in THF (1.1 eq.) were added dropwise over a period of 10 min. Hydrogen was evolved. The reaction mixture was stirred under argon for 20 h and the ice bath was allowed to equilibriate slowly to room temperature. The reaction was terminated by adding a few drops of water. The THF and excess borane were evaporated and 25 ml of water were added. The solution was titrated with a 6N aqueous solution of NaOH to pH 8-8.5 and extracted with $CH_2Cl_2$ (4x30ml). The combined $CH_2Cl_2$ extracts were dried and the solvent evaporated. The crude product was purified by column chromatography on silica gel with $CHCl_3$ as eluent (the toluene eluting first) to give a pure fraction of **7a** (0.15 g, 15-20% yield).

**(R)-(+)-Methyl 3-hydroxy-2-methoxypropanoate 7a**: $^1$H NMR δ 3.79-3.94 (3 H, m), 3.79 (3 H, s), 3.50 (3 H, s), 2.18 (1 H, t, J=5.63 Hz); the structure of **7a** was confirmed by GC-MS analyses; $[\alpha]_D^{28}$=+45 (c=0.48, $CHCl_3$); ee=70% (determined by NMR as discussed earlier).

**(R)-(-)-Methyl 3-hydroxy-2-methylpropanoate 7b**: $^1$H NMR δ 3.72 (3H, s), 3.69-3.75 (2H, m), 2.61-2.78 (1 H, m), 2.27 (1H, t, J=6.48 Hz), 1.18 (3 H, d, J=7.23 Hz); $[\alpha]_D^{28}$=-5.2 (c=2.4 MeOH); ee=20% (determined by NMR as mentioned earlier); 15-20% yield.

**Independent synthesis of (R)-(+)-7a:**

**(R)-(+)-Methyl 2,3-dihydroxypropanoate 9.**

2 g of 2,3-O-isopropylidene-(R)-glyceric acid methyl ester **8** (12.5 mmol) were added to 34 ml of 70% acetic acid and stirred at room temperature overnight. The reaction mixture was evaporated to dryness and coevaporated two times with toluene to give 1.35 g of crude **9** as a colourless oil in 90% yield. $^1$H NMR δ 4.26 (1 H, t, J=3.54 Hz), 3.84-3.88 (2 H, m), 3.82 (3 H, s); $[\alpha]_D^{31}$= +4.1 (c=1.7, $CHCl_3$).

**(R)-(-)-Methyl 3-(O-tert-butyldimethylsilyl)-2-hydroxypropanoate 10.**

The foregoing diol **9** (1 g, 8.3 mmol) was dissolved (without further purification) in 5 ml of $CH_2Cl_2$. Then, 1.28 ml of triethylamine (9.13 mmol) and 40.7 mg of 4,4-dimethyl-aminopyridine (0.33 mmol) were added with stirring. The reaction mixture was kept under nitrogen and 1.38 g of t-butyl-dimethyl-chlorosilane (9.13 mmol) were added and the reaction was allowed to proceed overnight under nitrogen at room temperature. Work-up involved washing the organic solution with water, saturated ammonium chloride, drying and evaporating the solvent. The crude product was purified by silica gel chromatography, eluting with dichloromethane-hexane, 2:1 to give a pure fraction of **10** as a colourless oil (0.47 g, 24% yield). $^1$H NMR δ 4.17-4.24 (1 H, m), 3.80-3.95 (2 H, m), 3.77 (3 H, s), 3.01 (1 H, d, J=8.05 Hz), 0.85 (9 H, s), 0.038 (3 H, s), 0.023 (3 H, s); $[\alpha]_D^{29}$=-9.3 (c=1.52, $CH_2Cl_2$).

**(R)-(+)-Methyl 3-(O-tert-butyldimethylsilyl)-2-methoxypropanoate 11.**

To a 25 ml flask were added 0.18 g of **10** (0.77 mmol), 2.2 ml $CH_3I$ (35 mmol) and 0.18 g of $Ag_2O$ (0.77 mmol) (freshly prepared from $AgNO_3$ and NaOH according to the published method[21b]). The reaction mixture was refluxed for 1 h and then the same amounts of $CH_3I$ and $Ag_2O$ were added again and the reflux was continued for one more hour. The reaction was terminated by filtering off the solids and evaporating the excess $CH_3I$, giving 0.17 g of **11** as a white solid in 89% yield. $^1$H NMR δ 3.86 (3 H, s), 3.74 (3 H, s), 3.42 (3 H, s), 0.86 (9 H, s), 0.035 (6 H, s); $[\alpha]_D^{28}$=+20.3 (c=1.9, $CH_2Cl_2$).

**(R)-(+)-Methyl 3-hydroxy-2-methoxypropanoate 7a.**

0.14 g of **11** (0.56 mmol) were dissolved in 70% acetic acid (8 ml) and stirred at room temperature overnight. Then, the reaction mixture was evaporated to dryness and coevaporated twice with toluene to give 64 mg of **7a** in 85% yield. $^1$H NMR δ 3.79-3.94 (3 H, m), 3.79 (3 H, s), 3.50 (3 H, s), 2.18 (1 H, t, J=5.63 Hz); $[\alpha]_D^{33}$=+65.0 (c=1.86, $CHCl_3$).

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## References

1. (a) Whitesides, G. M.; Wong, C.- H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617-638. (b) Jones, J. B.*Tetrahedron* **1986**, *42*, 3351-3403. (c) Yamada, H.; Shimizu, S. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 622-642. (d) Boland, W.; Frobl, C.; Lorenz, M. *Synthesis* **1991**, 1049-1072. (e) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071-1140. (f) Faber, K.; Franssen, M. TIBTECH **1993**, *11*, 461-470.
2. (a) Chen, C.- S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695-707. (b) Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194-211. (c) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114-120. (d) Margolin, A. L. CHEMTECH **1991**, 160-167. (e) Xie, Z. -F. *Tetrahedron Asymmetry* **1991**, *2*, 733-750. (f) Faber, K.; Riva, S. *Synthesis* **1992**, 895-910.
3. (a) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072-7076. (b) Stokes, T. M.; Oehlschlager, A. C. *Tetrahedron Lett.* **1987**, *28*, 2091-2094. (c) Chen, C. -S.; Wu, S. -H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812-2817. (d) Wang, Y. -F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C. -H. *J. Am. Chem. Soc.* **1988**, *110*, 7200-7205. (e) Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129-6139. (f) Ferraboschi, P.; Brembilla, D.; Grisenti, P.; Santaniello, E. *J. Org. Chem.* **1991**, *56*, 5478-5480. (g) Ader, U.; Schneider, M. P.*Tetrahedron Asymmetry* **1992**, *3*, 205-208. (h) Frykman, H.; Ohrner, N.; Norin, T.; Hult, K. *Tetrahedron Lett.* **1993**, *34*, 1367-1370. (i) Gutman, A. L.; Brenner, D.; Boltanski, A. *Tetrahedron Asymmetry* **1993**, *4*, 839-844.
4. (a) Gutman, A. L.; Zuobi, K.; Boltanski, A. *Tetrahedron Lett.* **1987**, *28*, 3861-3864. (b) Makita, A.; Nihira T.; Yamada, Y. *Tetrahedron Lett.* **1987**, *28*, 805-808. (c) Guo, Z.-W.; Sih, C. J. *J. Am. Chem.*

*Soc.* **1988,** *110,* 1999-2001. (d) Guo, Z.-W.; Ngooi, T. K.; Scilimati, A.; Fulling, G.; Sih, C. J. *Tetrahedron Lett.* **1988,** *29,* 5583-5586. (e) Ngooi, T. K.; Scilimati, A.; Guo, Z.-W.; Sih, C. J. *J. Org. Chem.* **1989**, *54,* 911-914.

5. (a) Gutman, A. L.; Oren, D.; Boltanski, A.; Bravdo, T. *Tetrahedron Lett.* **1987**, *28,* 5367-5368. (b) Margolin, A. L.; Crenne, J.-Y.; Klibanov, A. M. *Tetrahedron Lett.* **1987**, *28,* 1607-1610. (c) Wallace, J. S.; Morrow, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **1989**, *27,* 2553-2567.
6. Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. USA* **1985**, *82,* 3192-3196.
7. (a) Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111,* 3094-3095. (b) Gutman, A. L.; Meyer, E.; Kalerin, E.; Polyak, P.; Sterling J. *Biotechnol. Bioeng.* **1992**, *40,* 760-767. (c) Pozo, M.; Gotor, V. *Tetrahedron* **1993**, *49,* 4321-4326.
8. Gutman, A. L.; Meyer, E.; Yue, X.; Abell, C. *Tetrahedron Lett.* **1992**, *33,* 3943-3946.
9. Huang, F. C.; Lee, L. F. H.; Mittal, R. S. D.; Ravi Kumar, P. R.; Chan, J. A.; Sih, C. J.; Caspi, E.; Eck, C. R. *J. Am. Chem. Soc.* **1975**, *97,* 4144-4245.
10. (a) Ohno, M.; Kobayashi, S.; Limori, T.; Wang, Y. F.; Izawa, T. *J. Am. Chem. Soc.* **1981**, *103,* 2405-2406. (b) Lok, K. P.; Jakovak, I. J.; Jones, J. B. *J. Am. Chem. Soc.* **1985**, *107,* 2521-2526.
11. Tombo, G. M. R., Schar, H. P., Fernandez, X., Busquets, I., Ghisalba, O. *Tetrahedron Lett.* **1986**, *27,* 5707-5710.
12. (a) Gutman A. L.; Bravdo, T. *J. Org. Chem.* **1989**, *54,* 4263-4265. (b) Gutman A. L.; Bravdo, T. *J. Org. Chem.* **1989**, *54,* 5645-5646. (c) Gutman, A. L.; Zuobi, K.; Bravdo, T. *J. Org. Chem.* **1990**, *55,* 3546-3552.
13. (a) Schneider, M.; Engel, N.; Boensmann, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23,* 66. (b) Bjorkling, F.; Boutelje, J.; Gatenbeck, S.; Hult, K.; Norin, T.; Szmulik, P. *Tetrahedron* **1985**, *41,* 1347-1352. (c) Kitazume, T.; Sato, T.; Kobayashi, T.; Lin, J. T. *J. Org. Chem.* **1986**, *51,* 1003-1006. (d) Luyten, M.; Muller, S.; Herzog, B.; Keese, R. *Helv. Chim. Acta* **1987**, *70,* 1250-1254. (e) De Jeso, B.; Belair, N.; Deleuze, H.; Rascle, M. C.; Maillard, B. *Tetrahedron Lett.* **1990**, *31,* 653-654. (f) Toone, E. J.; Jones, J. B. *Tetrahedron Asymmetry* **1991**, *2,* 1041-1052.
14. Thomas, N. R.; Rose, J. E.; Gani, D. J. *J. Chem. Soc., Chem. Commun.* **1991**, 908-909.
15. Gutman, A. L.; Shapira, M.; Boltanski, A. *J. Org. Chem.* **1992**, *57,* 1063-1065.
16. Laane, C.; Boeren, S.; Vos, K.; Veeger, C. *Biotechnol. Bioeng.* **1987**, *30,* 81-87.
17. Yoon, H. M.; Pak, C. S.; Brown, H. C.; Krishnamurthy, S.; Stocky, T. P. *J. Org. Chem.* **1973**, *38,* 2786-2792.
18. Ohta, H.; Tetsukawa, H.; Noto, N. *J. Org. Chem.* **1982**, *47,* 2400-2404.
19. Lucas, H.; Basten, J. E. M.; van Dinther, T. G.; Meuleman, D. G.; van Aelst, S. F.; van Boeckel, C. A. A. *Tetrahedron* **1990**, *46,* 8207-8228.
20. Chaudhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979**, 99-102.
21. (a) Mislow, K. *J. Am. Chem. Soc.* **1951**, *73,* 4043-4044. (b) Schmitz, E.; Ohme, R. *Organic Synthesis,* Collective volume V, 897-898.
22. (a) Wang, Y. -F.; Chen, C. -S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1984**, *106,* 3695-3696. (b) Kazlaukas, R. J. *J. Am. Chem. Soc.* **1989**, *111,* 4953-4959. (c) Guo, Z. -W.; Wu, S. -H.; Chen, C. -S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112,* 4942-4945. (d) Chen, C. -S.; Liu, Y. -C.; *J. Org. Chem.* **1991**, *56,* 1966-1968. (e) Harris, K. J.; Gu, Q. -M.; Shih, Y. -E.; Girdaukas, G.; Sih, C. J. *Tetrahedron Lett.* **1991**, *32,* 3941-3944.

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