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Enzymes in Organic Synthesis 50.[1] Probing the Dimensions of the Large Hydrophobic Binding Region of the Active Site of Pig Liver Esterase Using Substituted Aryl Malonate Substrates.

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Abstract: The active site model reported recently for the synthetically useful enzyme pig liver esterase (PLE) permits the structural specificity and stereoselectivity of the enzyme to be interpreted and predicted for a wide range of substrates. The specifications of the dimensions of this model were based on the specificity data available at that time. In order to test the model further, and to delineate more accurately the dimensions of its large hydrophobic (H_L) binding pocket, PLE-catalyzed hydrolyses of dimethyl ortho- and para-methyl-, ethyl-, isopropyl-, and tert-butylphenyl 2-methylmalonate substrates have been carried out. Each of these malonate diesters proved to be a good substrate of the enzyme. In every case, the pro-*S* ester group was hydrolyzed to give *R*-acid-ester products of 78-$\geq$97% ee. The results show that the initial volume specified for the H_L pocket was too small to accommodate the larger aryl groups of this substrate series. A modified model with an appropriately enlarged H_L region is presented. The sizes of the other binding pockets remain unchanged.

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INTRODUCTION

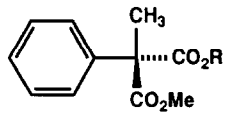
Enzymes are an attractive group of catalysts for asymmetric synthesis since they can catalyze a wide spectrum of organic reactions, and can operate on a very broad structural range of substrates with very high regiospecificity and stereoselectivity.[2,3] Furthermore, they are capable of achieving their large rate enhancements under very mild reaction conditions. Hydrolytic enzymes are one of the most useful groups of enzymes from the asymmetric synthetic point-of-view, with the carboxylesterase from pig liver (PLE, EC 3.1.1.1) having emerged as an especially useful catalyst for resolution and the creation of chiral synthons.[3] PLE is commercially available at relatively low cost ($0.005/U), requires no cofactors, and can be used in aqueous or organic solvent media.

Recently, we reported a generally applicable active site model of PLE that permits its structural specificity and stereoselectivity to be interpreted and predicted with confidence for a broad range of structurally dissimilar substrates.[4] While the validity of the model has received independent verification,[5] the dimensions assigned to the binding pockets represent minimum values since they were based on an analysis of the specificity data available at that time. Accordingly, we have initiated a series of investigations to refine the model's specification more accurately, particularly with respect to specifying/identifying the maximum volume and dimensions available for accommodating hydrophobic moieties of substrates in the key, large hydrophobic (H_L) site. In this paper, the substituted arylmalonates **1a-6a** are employed as probes of the H_L-pocket.

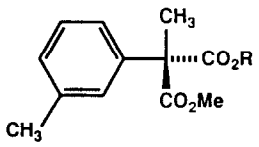
Aryl-substituted malonates are appropriate probes for several reasons. Malonates are good PLE-substrates[6] and their binding at the active site is readily interpretable by the cubic space model.[4] Furthermore, the relative inflexibility of the three-carbon malonate chain ensures a unique binding mode, that in the case of **1a-6a** directs the aryl groups into the H_L pocket. Introduction of alkyl groups of graded sizes at different positions on the rigid phenyl ring thus makes exploration of the H_L dimensions straightforward.

RESULTS

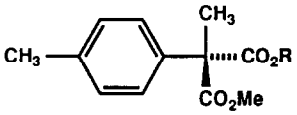
The substrates **1a-6a** were synthesized according to literature methods. All were satisfactory substrates for PLE and were subjected to preparative(>0.7 mmol)-scale PLE-catalyzed hydrolysis. The hydrolyses were carried out in distilled water at pH 7.0 and 21°C. The pH was maintained at 7.0 by addition of base with a pH stat unit, and the reaction progress was



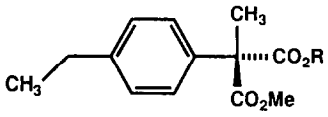
1a, R=CH_3
1b, R=H



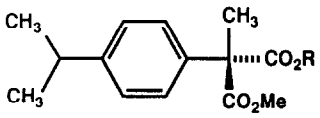
2a, R=CH_3
2b, R=H



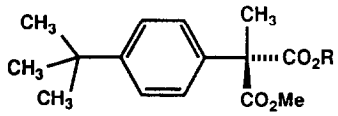
3a, R=CH_3
3b, R=H



4a, R=CH_3
4b, R=H



5a, R=CH_3
5b, R=H



6a, R=CH_3
6b, R=H

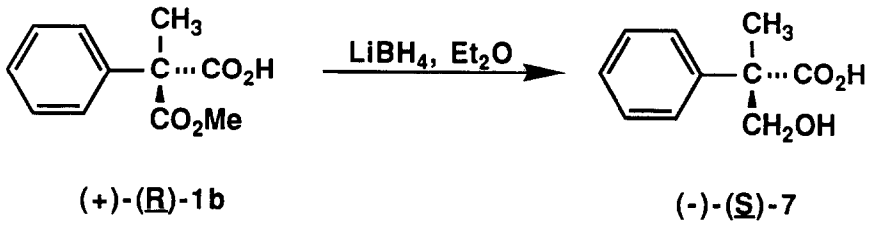
monitored by consumption of base. In all cases, hydrolysis ceased after uptake of one equivalent of base. The results are recorded in Table 1.

Table 1. PLE-Catalyzed Hydrolyses of 1a-6a.*

Substrate	Product	Yield(%)	EE(%)
1a	1b	90	81
2a	2b	100	92
3a	3b	92	82
4a	4b	100	78
5a	5b	96	>97
6a	6b	83	>97

*at pH 7.0, 25 °C, aqueous conditions.

Enantiomeric excess values of all products were determined by integration of diastereotopic proton NMR signals in the presence of (+)-*R*-α-methylbenzylamine.[7] The absolute configuration of (+)-**1b** was determined by its selective reduction to (-)-**7** of known absolute configuration,[8] as shown in Scheme 1.[9] The absolute configurations of the remaining products **2b-6b** were assigned using Brewster's rules,[10] based on the positive rotation of (+)-*R*-**1b** as the benchmark.



(+)-(R)-1b (-)-(S)-7

Scheme 1. Absolute configuration assignment of **1b**

DISCUSSION

PLE was an effective catalyst for the hydrolysis of each of the malonates **1a-6a**, although the rates of reaction slowed considerably for the most hydrophobic substrates, such as **6a**, whose low water solubility was undoubtedly a major contributor to the 11 day reaction period needed to achieve its complete hydrolysis to **6b**.

Analyses of the stereoselectivity of PLE-catalyzed hydrolysis of substrates **1a-6a** in terms of the active site model reveals that the original specifications[4] of the H_L site were too small. As Figure 1(a) demonstrates, the initial limits of H_L are reached with binding of the 4-methylphenyl group of **3a** in this pocket. The 3-methylphenyl function of **2a** also fits within the originally specified H_L volume. However, for the substrates **4a-6a**, the ethyl, *iso*-propyl, and *tert*-butyl substituents at the 4-positions of the phenyl groups breach the left-side boundary of H_L formulated in the initial model. This transgression is illustrated in Figure 1(b) for binding of **6a**. Since the current results establish that each of **4a-6a** is a substrate, each must be accommodated by the active site. This dictates a new minimum size and shape for H_L, with the left-hand boundary extended by 0.77Å as shown in Figure 1(b). The updated dimensions of the

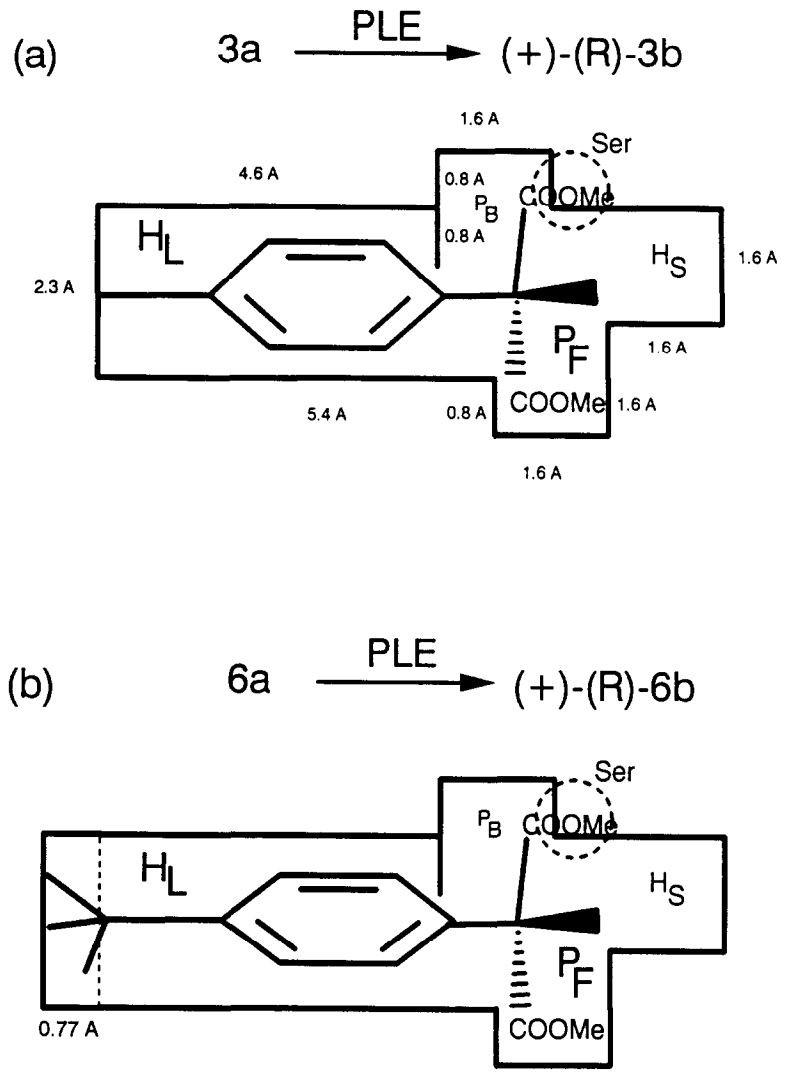


Figure 1. The top perspective view of the active site model initially specified[4] for PLE is used (cf. Figure 2). (a) The 4-phenyl substituent of **3a** extends to the end of the H_L pocket originally[4] specified. The phenyl functions of **1a** and **2a** are also accommodated satisfactorily in this volume. (b) For the malonate substrates **4a-6a**, the substituents on the phenyl ring are too big to fit in the initial H_L dimensions. The left boundary of H_L must therefore be extended by 0.77Å before the largest phenyl substituent, the 4-t-butyl group of 6a has enough room. The position of the old boundary is shown by the dotted line.

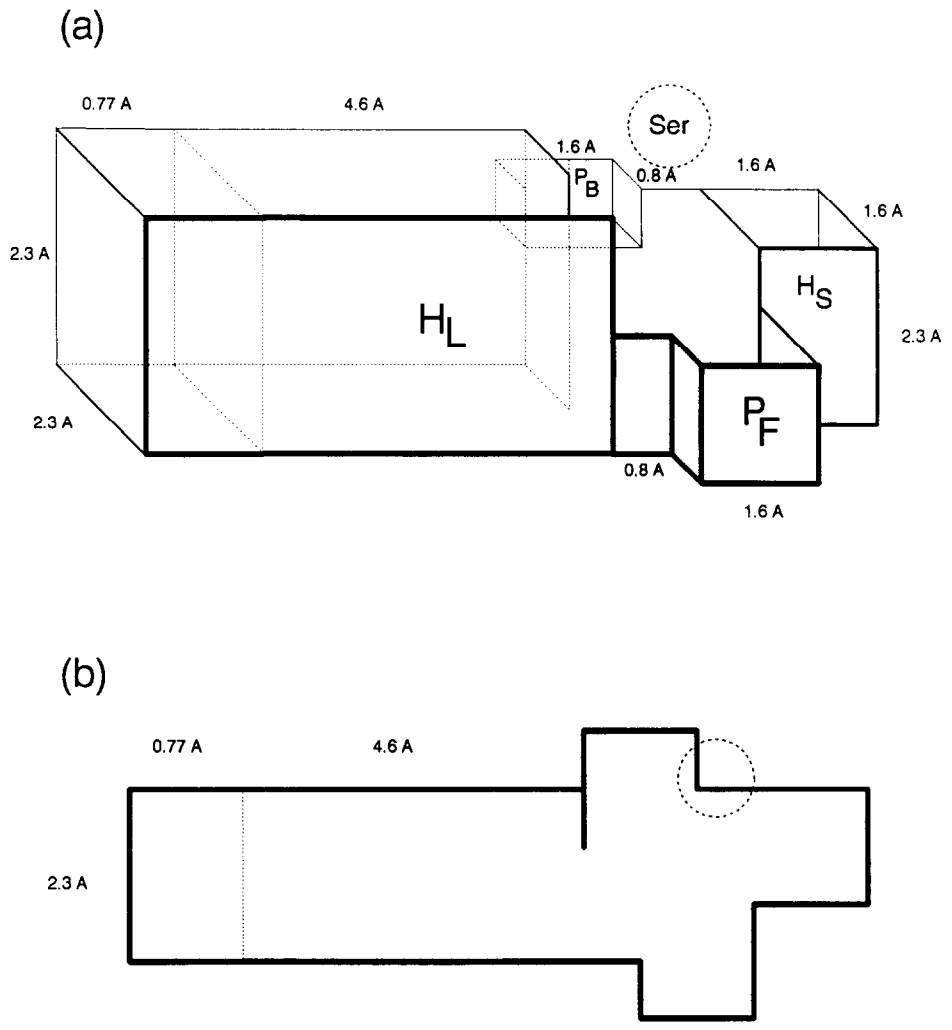


Figure 2. In (a) is shown the modified active site model for PLE, incorporating the new H_L dimensions. The original[4] H_L boundary is indicated by the dotted line. The mode of application of the model specified before[4] for predicting and analyzing substrate specificity is unchanged. The top perspective of the model, of which the new view is given in (b), is the most convenient for depicting substrate fit.

whole active site model are given in Figure 2. In this form, the model now correctly interprets all structural and stereospecificity data available for PLE substrates at this time. The expansion of the H_L pocket has no effect on the way substrate analyses are carried out,[4] but merely allows larger hydrophobic groups to be accommodated in H_L. Work is currently underway to delineate further the limits and properties of the binding pockets of the active site of PLE.

The present data provide some supplementary insights into PLE specificity that are of asymmetric synthetic significance. A major barrier to further analysis is the insolubility of large, hydrophobic substrates. For example, as illustrated by the case of dimethyl 2-methyl-2-(-4-t-butylphenyl)malonate (**6a**), even when a substrate is so hydrophobic that its rate of PLE-catalyzed hydrolysis is very slow due to its extremely low water-solubility, the stereoselectivity is not affected by the long reaction times needed, and high product ee's can still be obtained. In such cases, shorter reaction times can be induced by the addition of appropriate organic solvents. However, this strategy should be adopted cautiously since solvent-induced changes in stereoselectivity are well documented.[11] Furthermore, within the substrate series **1a-6a**, the ee levels are generally highest for the diesters with the largest hydrophobic ring substituent (Table 1). This is in accord with earlier observations in the malonate[6] and glutarate[12] series that the best ee's are seen for substrates with the largest H_L-binding groups.[4] In fact, when the H_L-binding needed to ensure the desired stereospecificity does not occur because the group in question is small, thus resulting in its obligatory binding in the H_S pocket,[4] it is possible to compel its H_L binding by increasing its hydrophobic bulk appropriately by incorporating a protecting group that can be removed subsequently. Spectacular redirections of stereoselectivities and high product ee's have been achieved in this way for malonate substrates.[6b] While this approach is clearly a generally applicable one, care must be taken not to exceed the H_L dimensions or substrate activity will be lost due to active site binding being precluded.

EXPERIMENTAL

All melting points were taken on an Electrothermal Capillary Melting Point Apparatus, and are uncorrected. IR spectra were recorded on a Nicolet 5DX FTIR spectrophotometer. Solids were all run as KBr disks; oils were run as neat films. All routine proton spectra were recorded on a Varian T60 instrument. The solvent in all cases was deuterochloroform, with tetramethylsilane added as an internal standard. High field proton spectra were recorded in

deuterochloroform on either a Varian XL200 or XL400 instrument. Optical rotations were measured in a Perkin-Elmer 243 B Polarimeter in a thermostatted cell. All samples were run in chloroform unless otherwise noted. Pig liver esterase was obtained from Sigma Chemical Co., in pH 8.0 phosphate buffer (type I). The enzyme throughout was from the same Sigma lot, number 45F-813. In all cases, substrates were ≥98% pure by capillary GLC. Elemental analyses were by Galbraith Laboratories, Knoxville, TN. Analytical GLC work was carried out on a Varian Series 3400 Capillary Gas Chromatograph. The pH stat unit used was either a Radiometer REA 270 with a TTT 80 titrator and an ABU 80 auto burette, or a Metrohm 655 Dosimat, with a Metrohm 632 pH meter and Metrohm 614 Impulsomat.

Synthesis of Substrates. Dimethyl 2-Phenyl-2-Methylmalonate (1a) was prepared in 21% overall yield from phenyl acetonitrile according to the method of Nelson and Cretcher,[13] bp 120 °C (0.1 mm Hg) (lit.[14] bp 144-7 °C (9 mm Hg)); IR *v* 1733 cm^{-1}; ^1H NMR δ 2.05 (s, 3H), 3.80 (s, 6H), 7.35 (br s, 5H) ppm.

The malonate substrates **2a-6a** were also prepared by the basic procedure of Nelson and Cretcher,[13] as follows: **Dimethyl 2-methyl 2-(3-methylphenyl)malonate (2a**, 49% overall yield from (3-methylphenyl)acetonitrile): bp 95 °C (0.1 mm Hg); mp 47-49 °C; IR (KBr) *v* 1725, 1748 cm^{-1}; ^1H NMR δ 1.90 (s, 3H), 2.25 (s, 3H), 3.65 (s, 6H), 6.90-7.25 (m, 4H) ppm. Anal. Calc'd for $C_{13}H_{16}O_4$: C 66.09%, H 6.83%. Found: C 66.43%, H 7.03%.

Dimethyl 2-methyl-2-(4-methylphenyl)malonate (3a, in 43% overall yield from (4-methylphenyl)acetonitrile): bp 120 °C (0.1 mm Hg); mp 51-2 °C; IR (KBr) *v* 1735, 1742 cm^{-1}; ^1H NMR δ 1.90 (s, 3H), 2.40 (s, 3H), 3.75 (s, 6H), 7.00-7.50 (m, 4H) ppm. Anal. Calc'd for $C_{13}H_{16}O_4$: C 66.09%, H 6.83%. Found: C 66.07%, H 6.97%.

Dimethyl 2-methyl-2-(4-ethylphenyl)malonate (4a, in 29% overall yield from (4-ethylphenyl)-acetonitrile, itself obtained in 66% overall yield from 4-ethylbenzyl alcohol by treatment with HBr in HOAc[15] followed by NaCN in ethanol[16]): bp 120 °C (0.05 mm Hg); IR *v* 1732 cm^{-1}; ^1H NMR δ 1.28 (t, $\underline{J}$ = 7 Hz, 3H), 1.95 (s, 3H), 2.65 (q, $\underline{J}$ = 7 Hz, 2H), 3.70 (s, 6H), 7.25 (m, 4H) ppm. Anal. Calc'd for $C_{14}H_{18}O_4$: C 67.18%, H 7.25%. Found: C 67.28%, H 7.41%.

Dimethyl 2-methyl-2-(4-isopropylphenyl)malonate (5a, in 49% overall yield from (4-isopropylphenyl)acetonitrile, itself obtained in 67% overall yield from 4-isopropylbenzyl alcohol[15,16]): bp 120 °C (0.05 mm Hg); IR *v* 1731 cm^{-1}; ^1H NMR δ 1.20 (d, $\underline{J}$ = 7 Hz, 6H), 1.85 (s, 3H), 3.60 (s, 6H), 3.95 (septet, $\underline{J}$ = 7 Hz, 1H), 7.25 (br s, 4H) ppm. Anal. Calc'd for $C_{15}H_{20}O_4$: C 68.16%, H 7.63%. Found: C 68.40%, H7.92%.

Dimethyl 2-methyl-2-(4-tert-butylphenyl)malonate (**6a**, in 38% overall yield from (4-t-butylphenyl)acetonitrile, itself obtained in 86% yield from 4-t-butylbenzyl bromide[16]): bp 110 °C (0.05 mm Hg); IR ν 1736 cm^{-1}; ^1H NMR δ 1.25 (s, 9H), 1.80 (s, 3H), 3.65 (s, 6H), 7.10 (br s, 4H) ppm. Anal. Calc'd for $C_{16}H_{22}O_4$: C 69.04%, H 7.97%. Found: C 69.24%, H 8.06%.

PLE-Catalyzed Hydrolyses of 1a-6a.

The same procedure was used to hydrolyze each of the malonate substrates **1a-6a**. The following is representative. **Dimethyl 2-methyl-2-phenylmalonate** (**1a**, 1.0 g, 4.5 mmol) was suspended in distilled water (30 mL) and the pH adjusted to 7.0 with 0.2 N NaOH. PLE (1144 units) was added and the reaction stirred at 21 °C. The pH was maintained at 7.0 by addition of 0.2 N NaOH via a pH-stat unit. When one equivalent of base had been added (2.5 days), the reaction stopped and the suspension had become a homogeneous solution. The mixture was washed at pH 7.0 with diethyl ether (2 X 40 mL), then acidified to pH 2.0 with 6 N HCl. The solution was extracted with diethyl ether (4 X 50 mL). The combined organic phase from the acid extraction was dried ($MgSO_4$) and rotary evaporated to give methyl hydrogen (+)-(R)-2-methyl-2-phenylmalonate[17] ((+)-**1b**, 920 mg, 90% yield, 81% ee): $[\alpha]^{25}_D$ +9.7 (c 3.1, $CHCl_3$); IR ν 3600-3000 (br), 1734, 1716 cm^{-1}; ^1H NMR δ 2.01 (s, 3H), 3.75 (s, 3H), 7.20 (m, 5H), 11.75 (at 60 MHz,[18] br s, 1H) ppm.

Similarly:

Dimethyl 2-methyl-2-(3-methylphenyl) malonate (**2a**, 174 mg, 0.73 mmol) with PLE (286 units) during 53 h gave methyl hydrogen (+)-(R)-2-methyl-2-(3-methylphenyl) malonate ((+)-**2b**, 170 mg, quant. yield, 92% ee): $[\alpha]^{25}_D$ +2.97 (c 17, $CHCl_3$); IR ν 3600-3000, 1735, 1714 cm^{-1}; ^1H NMR δ 1.82 (s, 3H), 2.27 (s, 3H), 3.71 (s, 3H), 7.15-7.30 (m, 4H), 9.17 (at 60 MHz, br s, 1H) ppm.

Dimethyl 2-methyl-2-(4-methylphenyl) malonate (**3a**, 210 mg, 0.89 mmol) with PLE (572 units) during 53 h gave methyl hydrogen (+)-(R)-2-methyl-2-(4-methylphenyl) malonate ((+)-**3b**, 192 mg, 98% yield, 82% ee): $[\alpha]^{25}_D$ +4.45 (c 17.5, $CHCl_3$); IR ν 3600-3000, 1735, 1712 cm^{-1}; ^1H NMR δ 1.90 (s, 3H), 2.35 (s, 3H), 3.65 (s, 3H), 7.01-7.50 (m, 4H), 10.05 (at 60 MHz, br s, 1H) ppm.

Dimethyl 2-methyl-2-(4-ethylphenyl) malonate (**4a**, 202 mg, 0.80 mmol) with PLE (1144 units) during 24 h gave methyl hydrogen (+)-(R)-2-methyl-2-(4-ethylphenyl) malonate ((+)-**4b**, 188 mg, quant. yield, 78% ee): $[\alpha]^{25}_D$ +3.5 (c 14.5, $CHCl_3$); IR ν 3600-3000 (br), 1735, 1719 cm^{-1}; ^1H NMR δ 1.15 (t, J = 7 Hz, 3H), 2.00 (s, 3H), 2.70 (q, J = 7 Hz, 2H), 3.78 (s, 3H), 7.25 (m, 4H), 12.37 (at 60 MHz, br s, 1H) ppm.

Dimethyl 2-methyl-2-(4-isopropylphenyl) malonate (**5a**, 194 mg, 0.73 mmol) with PLE (670 units) during 5.5 days gave methyl hydrogen (+)-(R)-2-methyl-2-(4-isopropylphenyl) malonate ((+)-**5b**, 178 mg, 96% yield, ≥97% ee): $[\alpha]^{25}_D$ +7.43 (c 17, $CHCl_3$); IR *v* 3600-3000, 1735, 1714 cm^{-1}; ^1H NMR δ 1.25 (d, J = 6.5 Hz, 6H), 1.95 (s, 3H), 2.95 (septet, J = 6.5 Hz, 1H), 3.80 (s, 3H), 7.25 (m, 4H), 10.30 (at 60 MHz, br s, 1H) ppm.

Dimethyl 2-methyl-2-(4-t-butylphenyl) malonate (**6a**, 286 mg, 1.0 mmol) with PLE (1430 units) during 11 days gave methyl hydrogen (+)-(R)-2-methyl-2-(4-t-butylphenyl) malonate ((+)-**6b**, 220 mg, 83% yield, ≥97% ee): $[\alpha]^{25}_D$ +2.4 (c 22, $CHCl_3$; IR *v* 3600-3000, 1734, 1717 cm^{-1}; ^1H NMR δ 1.30 (s, 9H), 1.95 (s, 3H), 3.80 (s, 3H), 7.22 (br s, 4H), 11.58 (at 60 MHz, br s, 1H) ppm.

Enantiomeric Excess Determinations. The enantiomeric excesses of product acids **1b-6b** were determined by ^1H NMR examination of the unhydrolyzed ester OCH_3 resonance, or the C-2 CH_3 peak, in the presence of (+)-R-α-methylbenzylamine.[7]

Absolute Configuration Determinations. The (2R)-absolute configuration of (+)-**1b** was determined by its conversion to (-)-2-methyl-2-phenyl-3-hydroxypropanoic acid of known (2S)-absolute configuration,[8] as follows: methyl hydrogen 2-methyl-2-phenyl malonate ((+)-**7**, 400 mg, 1.9 mmol) was reduced to (-)-(2S)-2-methyl-2-phenyl-3-hydroxy-propanoic acid with $LiBH_4$ in diethyl ether according to the method of Cornforth et al.[9] (234 mg, 71% yield): $[\alpha]^{25}_D$ -19.04 (c 5.0 in $CHCl_3$) (lit.[13] $[\alpha]^{25}_D$ -23.4 (c 2 in $CHCl_3$)) IR *v* 3600-3000 cm^{-1}; ^1H NMR δ 2.10 (s, 3H), 3.45 (s, 2H), 4.05 (br s, 1H), 7.20 (br s, 5H), 13.15 (br s, 1H) ppm.

The absolute configurations of the remaining malonate half-acid esters **2b-6b** followed from Brewster's rules, which predict an identical signs of rotation for the same absolute configurations within this series.[10]

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REFERENCES

1. Part 49. Toone, E.J.; Jones, J.B. Tetrahedron Asymm., **1991**, 2, 207. Abstracted from the Ph.D. thesis of EJT, University of Toronto, 1988.
2. (a) Davies, H.G.; Green, R.H.; Kelly, D.R.; Roberts, S.M. Biotransformations in Preparative Organic Chemistry, Academic Press, London, **1990**. (b) Klibanov, A.M. Acc. Chem. Res., **1990**, 23, 114. (c) Crout, D.H.G.; Christen, M. in Modern Synthetic Methods, Vol. 5, Scheffold, R., Ed.; Springer Verlag, Berlin, **1989**, pp 1-114. (d) Wong, C.-H. Science, **1989**, 244, 1145. (e) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. Tetrahedron, **1989**, 45, 5365. (f) Jones, J. B. Tetrahedron **1986**, 42, 3351.
3. (a) Ohno, M.; Otsuka, M. Org. Reactions, **1989**, 37, 1. (b) Zhu, L.-M.; Tedford, M.C. Tetrahedron, **1990**, 46, 6587.
4. Toone, E. J.; Werth, M.; Jones, J. B. J. Am. Chem. Soc. **1989**, 112, 4946.
5. Moorlag, H.; Kellogg, R.M.; Kloosterman, M.; Kaptein, B.; Kamphuis, J. J. Org. Chem. **1990**, 55, 5878.
6. (a) De Jeso, B.; Belair, N.; Deleuze, H.; Rascle, M.-C.; Maillard, B. Tetrahedron Lett., **1990**, 31, 653. (b) Luyten, M.; Mueller, S.; Herthog, B.; Keese, R. Helv. Chim. Acta, **1987**, 70, 1250. (c) Boutelje, J.; Hjalmarsson, M.; Szmulik, P.; Norin, T. in Biocatalysis in Organic Media, Laane, C.; Tramper, J.; Lilly, M.D. Eds., Elsevier, New York, **1987**, pp 361-368. (d) Kitazume, T.; Sato, T.; Kobayashi, T.; Lin, J.T. J. Org. Chem.,**1986**, 51, 1003. (e) Bjoerkling, F.; Boutelje, J.; Gatenbeck, S.; Hult, K.; Norin, T. Tetrahedron Lett., **1985**, 26, 4857. (f) Bjoerkling, F.; Boutelje, J.; Gatenbeck, S.; Hult, K.; Norin, T.; Szmulik, P. Tetrahedron, **1985**, 41, 1347. (g) Schneider, M.; Engel, N.; Boensmann, H. Angew. Chem. Int. Ed. Engl., **1984**, 23, 66. (h) Iriuchijima, S.; Hasegawa, K.; Tsuchihashi, G. Agric. Biol. Chem., **1982**, 46, 1907.
7. Schneider, M.; Engel, N.; Honicke, P.; Heinemann, G.; Gorisch, H. Angew. Chem. Int. Ed. Engl. **1984**, *23*, 67.
8. Knabe, J.; Junginger, H.; Geismar, W. Arch. Pharm. **1971**, 304, 1.
9. Cornforth, J. W.; Cornforth, R. H.; Popjak, G.; Yvngouyan, L. J. Biol. Chem. **1966**, 241, 3970.
10. Brewster, J. H. J. Am. Chem. Soc. **1959**, 81, 5475.

11. (a) Hersh, L.B.; J. Biol. Chem., **1971**, 246, 7804. (b) Lam, L.K.P.; Hui, R.A.H.F.; Jones, J.B. J. Org. Chem., **1986**, 51, 2047. (c) Klunder, A.J.H.; Huizinga, W.B.; Hulsof, A.J.M.; Zwanenburg, B. Tetrahedron Lett., **1986**, 27, 2543. (d) Kitaguchi, H.; Fitzpatrick, P.A.; Huber, J.E.; Klibanov, A.M. J. Am. Chem. Soc., **1989**, 111, 3094. (e) Virden, R.; Tan, A.K.; Fink, A.L. Biochemsitry, **1990**, 29, 145.
12. Adachi, K.; Kobayashi, S.; Ohno, M. Chimia, **1986**, 40, 311.
13. Nelson, W. L.; Cretcher, L. H. J. Am. Chem. Soc. **1928**, 50, 2758.
14. Cervinka, O.; Hub, L. Collect. Czech. Chem. Commun. **1967**, 32, 2295.
15. Crowley, J. I.; Rapoport, H. J. Org. Chem., **1980**, 45, 3215.
16. Adams, R.; Thal, A. H. Org. Synth. **1921**, 2, 9.
17. Because of the acid-ester malonate products undergo slow decarboxylation at room temperature, elemental analyses could not be obtained on **1b-6b**.
18. The -COOH protons of **1b-6b** were detectable only in the initial 60 MHz spectra that contained a trace of ether.