Tetrahedron: Asymmetry Vol. 4, No. 9, pp. 2025-2040, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00

Enzymes in Organic Synthesis 51.[1] Probing the Dimensions of the Large Hydrophobic Pocket of the Active Site of Pig Liver Esterase

Louis Provencher, Hla Wynn, J. Bryan Jones*
Department of Chemistry, University of Toronto, Toronto, Ontario, Canada, M5S 1A1

and

Andrzej R. Krawczyk
Department of Chemistry, Warsaw University, 02093 Warsaw, Poland

(*Received in UK* 16 *June* 1993; *accepted* 27 *July* 1993)

Abstract: The dimensions of the large hydrophobic pocket (H_L) of the active site model of pig liver esterase (PLE) were probed using a series of aliphatic and phenylic malonates. Results from the hydrolyses of these new unnatural substrates permitted the extension of the H_L pocket to give the new dimensions of 6.2 x 2.3 x 3.9 Å for a total volume of ~ 56 $Å^3$.

INTRODUCTION

Enzymes are highly efficient biocatalysts which have become of prime importance in the production of new chirons of significant synthetic interest.[2] Of the classes of enzymes that are synthetically useful, hydrolases are often preferred because of their ease of use and their acceptance of a wide range of substrate structures. Pig liver esterase (PLE, EC 3.1.1.1) is of particular interest in this regard because its catalysis of a highly diverse structural range of ester substrates is often accompanied by high stereospecificity.[3] Until recently, the use of PLE was hampered by its seemingly erratic specificity, which was particularly manifest in reversals of stereospecificity within homologous series of ester substrates. However, such apparent anomalies in the structural and stereospecificity of PLE are now successfully rationalizable in terms of the active site model proposed recently[4] for which specificity can be interpreted in terms of substrate interactions with two polar binding sites, designated as $P_{F(ront)}$ and $P_{B(ack)}$, and two hydrophobic pockets, $H_{L(arge)}$ and $H_{S(mall)}$. This model is of predictive value for new, unnatural, substrates for PLE[1,4,5] and has been independently applied successfully by several groups.[6,7,8,9]

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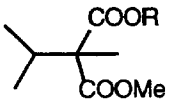

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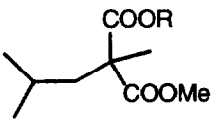

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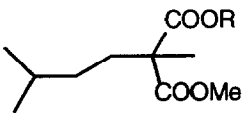

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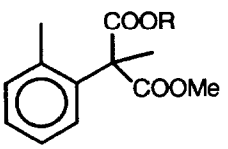

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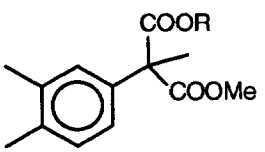

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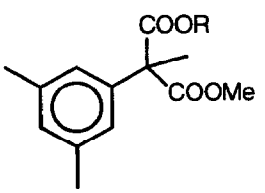

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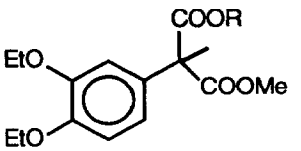

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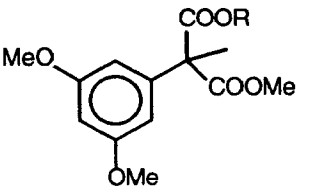

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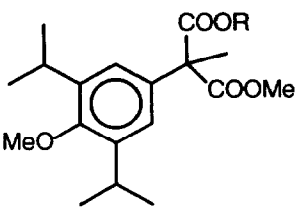

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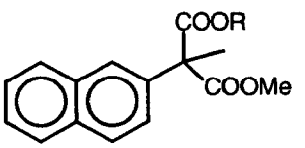

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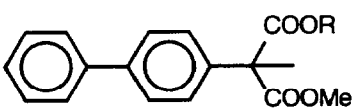

a, R = Me

b, R = H

Although the model as initially formulated functions well, its pocket-dimensions clearly represent the minimum values. In particular, the size of the large hydrophobic pocket H_L as first specified is now known to be too small, and probing the true limits of its volume with substrates of prescribed shapes and structures has already begun. The first refinements of H_L dimensions obtained in this way have recently been reported.[1,6] In this paper, systematic probing of the dimensions and properties of the H_L-pocket is continued, using dimethyl malonates **1a-11a** bearing aliphatic and aromatic substituents designed to probe the steric tolerances of the H_S and H_L pockets in three dimensions.

RESULTS AND DISCUSSION

The substrates **1a-11a** were prepared in good yields by unexceptional routes that are fully described in the Experimental section. Each compound was then evaluated as a PLE-substrate under preparative-scale conditions in distilled water at pH 7.0. In no case was hydrolysis observed in the absence of PLE. The progress of each reaction was monitored by the uptake of NaOH from a pH-stat unit. All hydrolyses were terminated after the consumption of one equivalent of base. The half acid-ester products **1b-10b** were isolated, and their enantiomeric excesses determined by NMR,[10] as described previously.[1,6] The racemates of **1b-10b** needed as reference standards for the %ee determinations were prepared by treating their diester precursors **1a-10a** with one equivalent[11] of KOH in MeOH for 3-7 days. The absolute configurations of **3b-10b** were assigned from the signs of the observed optical rotations, according to literature precedents for aliphatic[12] and phenyl[1,13] malonates and from Brewster's rules.[14] The results are summarized in Table 1.

The dimensions of the hydrophilic (P_F and P_B) and small hydrophobic (H_S) pockets specified for the original active site model[4] remain valid as originally delineated, and it is only the maximum volume of the large hydrophobic, H_L, pocket that remains to be fully established.[1,6] H_L clearly has the capacity to accept larger groups than confirmed up till now[1,4,6] and malonate structures **4a-11a** were selected for their suitability for progressively probing the maximum group-size tolerances in all three dimensions of H_L. The substrates **1a-3a** were also included because they provided additional evidence on the group size limits of H_S. That hydrolysis of **1a,2a** gave products **1b,2b** respectively that were racemic, while the **3b** obtained from **3a** was of >95%ee of the predicted (*R*)-configuration, confirmed the original conclusion[4] that H_S accepts hydrophobic groups up to a C_4-size, as in **1a,2a**, but that side chains that are $\geq C_5$, as in **3a**, exceed the H_S volume and are thus obliged to locate in H_L. The data on other malonates substituted with small unbranched alkyl groups[15] are also in accord with this interpretation.

The substituted-phenyl malonates **4a-9a**, and the naphthyl malonate **10a**, were excellent substrates. Each hydrolysis proceeded with high enantiotopic selectivity to give the half acid-ester products **4b-10b** of excellent enantiomeric purities, and of the *R*-configurations predicted by the model. Thus the aromatic groups of these substrates, even of the sterically most demanding 3,5-diisopropyl-6-methoxy-phenyl residue of **10a**, are evidently quite readily accommodated by H_L. As a consequence, it is necessary to further expand the specifications of the H_L pocket.

Table 1: PLE-Catalyzed Hydrolyses* of Malonate Diesters **1a-11a**

Substrates	Products	Yields %	A.C.	ee's %
1a	**1b**	99	±	6
2a	**2b**	98	±	7
3a	**3b**	97	R	>95
4a	**4b**	92	R	>95
5a	**5b**	80	R	>95
6a	**6b**	90	R	94
7a	**7b**	92	R	>95
8a	**8b**	92	R	81
9a	**9b**	70	R	>95
10a	**10b**	95	R	>95
11a	**N.S.****	---	---	---

*pH 7.0, H_2O, 23 °C. **Non Substrate

In order to provide sufficient volume to bind all side chains of the **4a-10a** series, both the height and length of the H_L pocket must be extended by 0.77 Å from the original[4] specifications, as shown in Figure 1. The new height dimension is largely imposed by the requirements of the **4a** side chain and the extra length requirement by the **9a** group. At this time, there is no reason to extend the H_L-length further since the biphenyl malonate **11a** was not a PLE-substrate. This is interpreted as being due to the fact that the 8.5 Å-long biphenyl group cannot bind in H_L because its length exceeds that of the left-hand side boundary of the H_L pocket.

The improved specifications shown in Figure 1 represent a significant improvement to the active site model for PLE and add further to its predictive value. The true maximum dimensions of H_L remain to be defined and further work towards this goal is continuing. However, at its present level of development, and when applied according to the specified protocols,[6] the model can be relied on to interpret and predict stereospecificity correctly, with very few exceptions.[16]

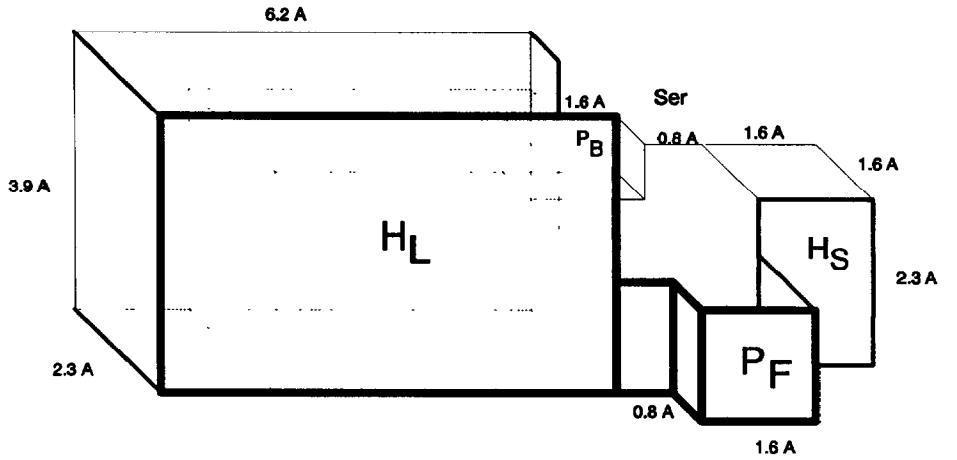

FIGURE 1: Extended PLE Active Site Model. The original H_L-pocket specifications are indicated by the dotted line.

EXPERIMENTAL

General: The starting materials 4-hydroxymethylbiphenyl, 2-hydroxymethylnaphthalene, 3,4-diethoxybenzoic acid, 3,3-dimethyl-1-butanol, dimethyl malonate, and pivalaldehyde were purchased from Aldrich Chemicals and were used as received. Pig liver esterase (EC 3.1.1.1, from porcine liver, suspension in 3.2 M aqueous $(NH_4)_2SO_4$ solution, pH 8 (11 mg protein/mL, 260 Units/mg protein; Lot 45F-8130) was purchased from Sigma Chemical Co.

Melting points were measured on a Fisher-Johns hot plate melting point apparatus, and are uncorrected. IR spectra were recorded on a Nicolet 5DX FT-IR system. Liquid and solid samples were run as neat liquid films or KBr wafers respectively. Proton NMR spectra were obtained using Varian T60, XL200, or XL400 instruments. Unless otherwise noted, NMR spectra were of deuteriochloroform solutions with tetramethylsilane as internal standard. Optical rotations were measured on a Perkin-Elmer 141 polarimeter in 10 cm cells. PLE-catalyzed hydrolyses were controlled by a Metrohm 655 Dosimat pH stat.

SYNTHESIS OF SUBSTRATES

Dimethyl 2-isopropyl-2-methylmalonate (1a).

Dimethyl malonate (16.6 mL, 0.148 moL), acetic anhydride (17.1 mL), and anhydrous $ZnCl_2$ (2.96 g, 0.022 moL) in acetone (16 mL) was heated under reflux for 20 h, then cooled to 20 °C and diluted with EtOAc. The resulting dark-coloured solution was washed with water and extracted with EtOAc (4x100 mL). The organic extracts were dried ($MgSO_4$), filtered, and

concentrated. The crude product was Kugelrohr-distilled, first at 40 °C/0.6 mmHg to remove unreacted dimethyl malonate, and then at 70 °C/0.6 mmHg to afford dimethyl 2-isopropylidene malonate (10.7 g, 43%). IR ν 2955, 1735, 1727, 1642, 1437, 1246, 1214, 1062 cm^{-1}. ^1H NMR δ 2.05 (6H,s), 3.74 (6H,s) ppm.

The above isopropylidene (2 g, 11.6 mmoL) and 10% Pd-C catalyst (0.2 g) were stirred in MeOH (25 mL) under a H_2 atmosphere (1 atm)[17] for 7 h. The mixture was filtered and the filtrate was concentrated. Purification by silica gel column chromatography (EtOAc/hexanes (1:4) elution) yielded dimethyl 2-isopropyl malonate (1.74 g, 86%). IR ν 2957, 1755, 1736, 1436, 1204, 1157 cm^{-1}. ^1H NMR δ 1.00 (6H,d,*J*=8Hz), 2.40-2.60 (1H,m), 3.16 (1H,d,*J*=8Hz), 3.65 (6H,s) ppm.

To a stirred solution of the above 2-isopropyl malonate (1.7 g, 9.77 mmoL) in dry THF (40 mL) at 0 °C under N_2 was added NaH (50% dispersion in oil; 0.56 g, 11.7 mmoL). After 10 min, MeI (1.8 mL, 28.9 mmoL) was added and stirring continued for 2 h at 20 °C The solution was poured into ice-cold 1M aqueous HCl (40 mL) and extracted with CH_2Cl_2 (3x60 mL). The extracts were washed again with aqueous saturated sodium thiosulfate solution, dried ($MgSO_4$), filtered, and concentrated. Purification by silica gel column chromatography (EtOAc/hexanes (1:4) elution) gave **dimethyl 2-isopropyl-2-methyl malonate (1a,** 410 mg, 54%) as a colorless oil. IR ν 2964, 1735, 1443, 1264, 1137 cm^{-1}. ^1H NMR δ 0.90 (6H,d,*J*=6Hz), 1.32 (3H,s), 2.30-2.80 (1H,m), 3.65 (6H,s) ppm.

Dimethyl 2-(2,2-dimethyl propyl)-2-methyl malonate (2a).

Pivaldehyde (5.0 g, 58 mmoL), dimethyl malonate (11.48 g, 87 mmoL), ammonium acetate (6.7 g, 87 mmoL), and glacial acetic acid (5 mL, 87 mmoL) in benzene (200 mL) was refluxed, with azeotropic removal of water, for 16 h, cooled to 20 °C, poured into ice-cold saturated aqueous sodium bicarbonate and then extracted with EtOAc (3x80 mL). The crude product was purified by flash column chromatography on silica gel (EtOAc/hexanes (1:4) elution) to give methyl-2-carbomethoxy-4,4-dimethyl-2-pentenoate (5.0 g, 43%). IR ν 1642, 1736 cm^{-1}. ^1H NMR δ 1.20 (9H,s), 3.82 (3H,s), 3.84 (3H,s), 6.85 (1H,s, vinylic proton) ppm.

To a solution of the above pentenoate (4.0 g, 20 mmoL) in absolute EtOH (60 mL) at 0 °C under N_2 was added $NaBH_4$ (190 mg, 5.0 mmol).[17] The solution was stirred for 1 h at 0 °C and was then poured into ice-cold saturated aqueous ammonium chloride. The aqueous phase was extracted with CH_2Cl_2 (20x4 mL). The extracts were dried ($MgSO_4$), filtered, and concentrated. Purification on silica gel column chromatography (EtOAc/hexanes (1:4) elution) followed by Kugelrohr distillation, yielded dimethyl 2-(2,2-dimethyl propyl) malonate (2.5 g, 62%). bp 50 °C/0.5 mmHg. IR ν 2955, 1737 cm^{-1}. ^1H NMR δ 1.00 (9H,s), 2.00 (2H,d,*J*=6.5Hz), 3.43 (1H,t,*J*=6.5Hz), 3.80 (6H,s) ppm.

Using the same procedure as for the final step in the preparation of **1a**, the above malonate (2.5 g, 12.4 mmoL) was methylated using NaH (0.71 g, 14.80 mmoL) and MeI (1.15 mL, 18.46 mmoL) in THF (40 mL); to yield **dimethyl 2-(2,2-dimethyl propyl)-2-methyl malonate (2a,** 2.0 g,

75%). IR ν 2957, 1735, 1436 cm^{-1}. ^1H NMR δ 0.9 (9H,s), 1.80 (3H,s), 2.01 (2H,s), 3.70 (6H,s) ppm. Anal. Calcd. C 61.07, H 9.33. Found C 61.31, H 9.12.

Dimethyl 2-(3,3-dimethyl butyl)-2-methyl malonate (3a).

To a stirred solution of 3,3-dimethyl-1-butanol (4.0 g, 39 mmoL) in dry pyridine (45 mL) at 0 °C under N_2 was added p-toluenesulfonyl chloride (freshly recrystallized from hexane; 8.2 g, 43 mmoL). The mixture was stirred for 5 h at 0 °C, then kept at 4 °C for 20 h, poured into ice-water (200 mL) and extracted with Et_2O (3x100 mL). The ethereal extracts were washed twice with ice-cold 1M HCl, dried ($MgSO_4$), and concentrated to give 1-(p-toluenesulfonyloxy)-3,3-dimethylbutane (3 g, 30%). IR ν 2957, 1596, 1363, 1177, 958, 666 cm^{-1}. ^1H NMR δ 0.92 (9H,s), 1.55 (2H,t,*J*=7Hz), 2.42 (3H,s), 4.05 (2H,t,*J*=7Hz), 7.22 (2H,d,*J*=8Hz), 7.72 (2H,d,*J*=8Hz) ppm.

To a mixture of dimethyl malonate (1.8 g, 13.6 mmoL), NaH (50% dispersion in oil; 0.972 g, 40.86 mmoL) and distilled HMPA (3.6 mL, 19.8 mmoL) in dry THF (35 mL) at 0 °C under N_2 was added dropwise a solution of the above tosylate (3 g, 11.7 mmoL) in THF (10 mL). The mixture was warmed to 20 °C, and then heated under reflux for 16 h. The reaction mixture was then cooled to 20 °C and poured into ice-cold 1M HCl . The solution was extracted with CH_2Cl_2 (3x40 mL) and the extracts dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexanes, then EtOAc/hexanes (1:4) elution) yielded dimethyl 2-(3,3-dimethyl butyl) malonate (884 mg,35%). IR ν 2964, 1742, 1436, 1151 cm^{-1}. ^1H NMR δ 0.98 (9H,s),1.20-2.20 (4H,m), 3.30 (1H,t,*J*=7Hz), 3.75 (6H,s) ppm.

Again with the **1a** methylation method, the above malonate (1.0 g, 4.63 mmoL), NaH (0.26 g, 5.42 mmoL), and MeI (0.9 mL, 14.45 mmoL) yielded **dimethyl 2-(3,3-dimethyl butyl)-2-methyl malonate (3a,** 0.662 g, 62%, spectroscopically identical with literature values[18]). IR ν 2957, 1735, 1463, 1244, 1171, 1118 cm^{-1}. ^1H NMR δ 0.90 (9H,s), 0.90-1.20 (2H,m), 1.40 (3H,s), 1.70-2.20 (2H,m), 3.75 (6H,s) ppm.

Dimethyl 2-methyl-2-(2-methylphenyl)malonate (4a).

Diethyl 2-methylphenylmalonate[12] (1.25 g, 5.0 mmol) was refluxed in MeOH (100 mL) containing NaOMe (6 eq.) for 1 h. The MeOH solvent was rotary evaporated and the residue was taken up in 1M HCl. The aqueous phase was extracted with CH_2Cl_2, the organic phase dried ($MgSO_4$) and then rotary evaporated, flash column-chromatographed on silica gel (EtOAc/hexanes (19:1) elution) to give dimethyl 2-(2-methylphenyl)malonate (0.67g, 60%). mp 73-6 °C. IR ν 1733, 1747 cm^{-1}. ^1H NMR δ 2.31 (3H,s), 3.73 (6H,s), 4.88 (1H,s), 7.10-7.40 (4H,m) ppm. Anal. Calcd. for $C_{12}H_{14}O_4$: C 64.86, H 6.31. Found C 64.87, H 5.86.

The above malonate (0.4 g, 1.8 mmol) and NaH (3 eq) were stirred in dry THF (50 mL) at 20 °C for 30 min then MeI (3 eq) was added. The solution was stirred for 2 h at 20 °C. The solvent was rotary evaporated and the residue was taken up in 1M HCl. The aqueous phase was extracted with CH_2Cl_2. The organic phase was washed with aqueous sodium thiosulfate, then

dried ($MgSO_4$) and rotary evaporated and flash chromatographed on silica gel (EtOAc/hexanes (19:1) elution) to give **dimethyl 2-methyl-2-(2-methylphenyl)malonate (4a** , 0.21 g, 50%). IR ν 1731-7 cm^{-1}. ^1H NMR δ 1.87 (3H,s), 2.27 (3H,s), 3.80 (6H,s), 7.05-7.20 (4H,m) ppm. Anal. Calcd for $C_{13}H_{16}O_4$: C 66.10, H 6.78. Found C 66.55, H 7.47.

Dimethyl 2-(3,4-dimethylphenyl)-2-methylmalonate (5a).

3,4-Dimethylbenzylcyanide[19] (8.3 g, 57.3 mmol); and $NaNH_2$ (2 eq) were refluxed for 3 h in dry Et_2O (120 mL) until the evolution of ammonia had ceased. The mixture was cooled to 20 °C and dimethyl carbonate (2 eq) added, and the mixture then refluxed for an additional 1h, and then poured into cold 1M HCl. The aqueous phase was extracted with Et_2O and the organic phase washed with cold 10% aqueous NaOH, dried ($MgSO_4$), and then rotary evaporated to give methyl 2-cyano-2-(3,4-dimethylphenyl) acetate (7.3 g, 63%). IR ν 2256, 1750 cm^{-1}. ^1H NMR δ 2.27 (6H,s); 3.77 (3H,s), 4.66 (1H,s), 7.20 (3H,m) ppm.

The above cyanoacetate (7.3g) and MeI (5 eq) in MeOH containing NaOMe (2 eq.) was refluxed for 1.5 h. The solvent was rotary evaporated, the residue acidified with 1M HCl, and extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$) then rotary evaporated and recrystallized from aqueous MeOH to give methyl 2-cyano-2-(3,4-dimethylphenyl) propionate (6.3 g, 83%). mp 60-3 °C. IR ν 2249, 1750 cm^{-1}. ^1H NMR δ 1.92 (3H,s), 2.26 (6H,s), 3.77 (3H,s), 7.13-7.30 (3H,m) ppm.

Into a solution of the above cyanopropionate (2.0 g, 9 mmol) in MeOH (50 mL) containing H_2O (2 mL) was bubbled gaseous HCl for 3 h at reflux followed by further HCL saturation for 1 h at 0 °C. The solution was then refluxed for 2.5 h. The mixture was rotary evaporated and the residue acidified with 1M HCl and extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$) then rotary evaporated and flash chromatographed on silica gel to give **dimethyl 2-(3,4-dimethylphenyl)-2-methylmalonate (5a,** 0.6 g; 26%). IR ν 1735 cm^{-1}. ^1H NMR δ 1.87 (3H, s), 2.23 (6H,s), 3.70 (6H,s), 7.00 (3H,m) ppm. Anal. Calcd. for $C_{14}H_{18}O_4$: C 67.20, H 7.20. Found C 67.35, H 7.02.

Dimethyl 2-(3,5-dimethylphenyl)-2-methylmalonate (6a).

Using the above **5a** procedure, 3,5-dimethylbenzyl cyanide[20] (6.8 g, 47 mmol) was converted into methyl 2-cyano-2-(3,5-dimethylphenyl) acetate (6.5 g, 68%) bp 150 °C/0.05 mmHg. IR ν 3356, 1750 cm^{-1}. ^1H NMR δ 2.33 (6H,s), 3.77 (3H,s), 4.63 (1H,s), 7.00 (3H,m) ppm. This cyanoacetate (5.0 g, 23 mmol) then gave methyl 2-(3,5-dimethylphenyl)-2-cyano propionate (4.2 g, 78%). IR ν 2242, 1750 cm^{-1}. ^1H NMR δ 1.93 (3H,s), 2.30 (6H,s), 3.80 (3H,s), 6.90-7.20 (3H,m) ppm. Conversion of this cyanopropionate (0.6 g, 27 mmol) yielded **dimethyl 2-(3,5-dimethylphenyl)-2-methylmalonate (6a,** 0.3 g, 43% - after a six-fold increase in reaction time) mp 56-9 °C. IR ν 1735 cm^{-1}. ^1H NMR δ 1.87 (3H,s), 2.33 (6H,s), 3.75 (6H,s), 6.90 (3H,m) ppm. Anal. Calcd for $C_{14}H_{18}O_4$: C 67.20, H 7.20. Found C 67.59, H 7.47.

Dimethyl 2-(3,4-diethoxyphenyl)-2-methylmalonate (7a).

To a stirred solution of 3,4-diethoxybenzoic acid (7.1 g, 33.8 mmoL) in dry Et_2O (150 mL) at 0 °C under N_2 was added $LiAlH_4$ (1.25 g, 32.89 mmoL). The temperature was allowed to warm to 20 °C over a 1.5 h period followed by reflux for 3 h. The mixture was cooled to 20 °C and cold saturated aqueous sodium potassium tartarate was added slowly, followed by extraction with Et_2O (4x80 mL). The Et_2O extracts were further washed with brine, dried ($MgSO_4$), filtered, and then concentrated to give 3,4-diethoxybenzyl alcohol (6.36 g, 96%) as a clear oil. IR ν 3395, 2977-2877, 1589, 1044-1137 cm^{-1}. ^1H NMR δ 1.40 (6H,br.t,*J*=7Hz), 1.76 (1H,s, exchangeable with D_2O), 4.10 (4H,br.q,*J*=7Hz), 4.55 (2H,s), 6.79-6.85 (3H,m, aromatic protons) ppm.

To a stirred mixture of $SOCl_2$ (freshly distilled, 2.77 mL, 37.9 mmoL) and $CaCl_2$ (0.129 g, 1.16 mmoL) at 35 °C under N_2 was slowly added a solution of the above benzyl alcohol (6.36 g, 32.45 mmoL) in benzene (100 mL). After stirring for 2 h, Et_2O (10 mL) and calcium carbonate (0.18 g) were added and the solution was stirred overnight at 20 °C. Ice and water were added and the organic layer washed with water, then with dilute aqueous sodium bicarbonate. The solvents were removed under reduced pressure to give 3,4-diethoxybenzyl chloride, which wasdissolved immediately in benzene (100 mL) and water (14 mL) and NaCN (3 g, 61.22 mmoL) added.[21] The mixture was refluxed for 6 h with vigorous stirring, then cooled to 20 °C, the organic layer separated, washed with water, dried ($MgSO_4$), and concentrated. The crude product was chromatographed on silica gel (10%- then 20%-EtOAc in hexane as eluent) to give 3,4-diethoxybenzyl cyanide (4.18 g, 63% over two steps). IR ν 2984, 2253, 1589 cm^{-1}. ^1H NMR δ 1.41 (6H,br.t,*J*=7Hz), 3.62 (2H,s), 4.00 (4H,br.q,*J*=7Hz), 6.66 (3H,s, aromatic protons) ppm.

To a solution of $NaNH_2$ (1.18 g, 30.26 mmoL) in dry THF (50 mL) at 20 °C under N_2 was added a solution of the above cyanide (4.18 g, 20.3 mmoL) in dry THF (20 mL). The mixture was heated to reflux for 1 h then cooled to 20 °C. Dimethylcarbonate (2.6 mL, 30.88 mmoL) was then added and the mixture was refluxed for another 1 h, then cooled to 20 °C. Ice-cold 1M HCl (50 mL) was added followed by extraction with Et_2O (3x80 mL) which in turn was washed with brine. The ether extracts were dried ($MgSO_4$), concentrated, and purified by column chromatography on silica gel (20% EtOAc in hexane elution) to give methyl 2-cyano-2-(3,4-diethoxyphenyl) acetate (2.5 g, 48%). IR ν 2249, 1750, 1595 cm^{-1}. ^1H NMR δ 1.42 (6H,br.t,*J*=7Hz), 3.78 (3H,s), 4.00 (4H,br.q,*J*=7Hz), 4.59 (1H,s), 6.80 (3H,br s, aromatic protons) ppm.

To a stirred solution of the above cyano acetate (1.0 g, 3.8 mmoL) in dry THF (30 mL) at 0 C under N_2 was added sodium hydride (50% dispersion in oil; 0.27 g, 5.7 mmoL). After 10 min, MeI (0.47 mL, 7.6 mmoL) was added and stirring continued for 3 h at 20 °C The solution was poured into ice-cold 1M HCl (30 mL) and extracted with CH_2Cl_2 (3x60 mL). The extracts were washed again with aqueous saturated sodium thiosulfate solution, dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (100% hexane, then20% EtOAc in hexane elution) gave methyl-2-cyano-2-(3,4-diethoxyphenyl) propionate (0.71 g, 67%). IR ν 2246,

1748, 1257 cm^{-1}. ^1H NMR δ 1.44 (6H,br.t,*J*=7Hz), 1.95 (3H,s), 3.80 (3H,s), 4.10 (4H,br.q,*J*=7Hz), 6.80-7.00 (3H,m, aromatic protons) ppm.

The above cyano propionate (700 mg, 2.52 mmoL) was Fischer-esterified in HCl-MeOH to yield **dimethyl 2-(3,4-diethoxyphenyl)-2-methyl malonate (7a,** 199 mg, 25%) as a yellowish oil after column chromatography on silica gel (EtOAc/hexanes (1:9) elution). IR ν 2990, 1735, 1728, 1516, 1257, 1111 cm^{-1}. ^1H NMR δ 1.40 (6H,br.t,*J*=7Hz), 1.82 (3H,s), 3.79 (6H,s), 4.20 (4H,br.q,*J*=7Hz), 6.80-7.00 (3H,m, aromatic protons) ppm. Anal. Calcd. C 61.90, H 7.15. Found C 61.65, H 7.19.

Dimethyl 2-(3,5-dimethoxyphenyl)-2-methylmalonate (8a).

Using the malonate **5a** method,[22] 3,5-dimethoxybenzyl cyanide[17] (2.4 g, 13.5 mmol) was treated with $NaNH_2$ (2 eq) and dimethyl carbonate (2 eq) to give methyl 2-cyano-2-(3,5-dimethoxyphenyl) acetate (1.27 g, 6.2 mmol, 46%). IR ν 2256, 1750 cm^{-1}. ^1H NMR δ 3.84 (9H,s), 4.70 (1H,s), 6.50-6.70 (3H,m) ppm

Applying the malonate **6a** procedure to the above cyano acetate (1.27g, 6.2 mmol), NaOMe (2 eq), and MeI (5 eq) in MeOH afforded methyl 2-cyano-2-(3,5-dimethoxyphenyl) propionate (1.23 g, 91%). IR ν 2242, 1743 cm^{-1}. ^1H NMR (200 MHz) δ 1.90 (3H,s), 3.83 (9H,s), 6.46-6.62 (3H,m) ppm. By further application of the **6a** method, treatment of this cyano propionate (1.23 g, 5.7 mmol) in MeOH (40 mL), H_2O (1.5 mL) with gaseous HCl gave **dimethyl 2-(3,5-dimethoxyphenyl)-2-methylmalonate (8a,** 298.0 mg, 21%). IR ν 1728 cm^{-1}. ^1H NMR δ 1.86 (3H,s), 3.77-3.80 (12H, two s), 6.40-6.55 (3H,m) ppm. Anal. Calcd for $C_{14}H_{18}O_6$: C 59.57, H 6.38. Found C 59.63, H 6.57.

Dimethyl 2-(3,5-diisopropyl-4-methoxyphenyl)-2-methylmalonate (9a).

To a stirred mixture of NaH (1.2 g, 50 mmol) in Et_2O (120 mL) was added dropwise a solution of 4-bromo-2,6-diisopropylphenol,[23] (10.45 g, 40.7 mmol) in Et_2O (30 mL) followed by NaI (30 g, 0.21 mol) and the mixture was refluxed for 5 h then stirred overnight at 20 °C. Water (30 mL) was added slowly under N_2 and the aqueous phase extracted with Et_2O (2x), the organic phase dried ($MgSO_4$) then rotary evaporated and purified by flash chromatography on alumina (hexanes elution) to give 4-bromo-2.6-diisopropyl-1-methoxy-benzene (4.52 g, 91%). ^1H NMR δ 1.21 (12H,d,*J*=6Hz), 3.27 (2H,m,*J*=6Hz), 3.70 (3H,s), 7.13 (2H,s,broad) ppm.

The above bromobenzene (3.64 g, 13.4 mmol) was coupled[24] with diethyl malonate (2.6 g, 16.1 mmol) in the presence of NaH (0.65 g, 16.2 mmol) and CuBr (2.5 g, 17.4 mmol) in dioxane (20 mL) under reflux for 5 h, then flash chromatographed on silica (EtOAc/hexanes (19:1) elution) to give diethyl 2-(3,5-diisopropyl-4-methoxyphenyl)malonate (0.88 g, 20%). IR ν 1735-55 cm^{-1}. ^1H NMR δ 1.25 (12H,d,*J*=6Hz), 1.27 (6H,t,*J*=7Hz), 3.31 (2H,m,*J*=6Hz), 3.73 (3H,s), 4.20 (4H,q,*J*=7Hz), 4.55 (1H,s), 7.06 (2H,s,broad) ppm.

This malonate (0.88 g, 2.5 mmol) in MeOH (50 mL) containing NaOMe (6 eq.) was refluxed for 1 h, then rotary evaporated and acidified with 1M HCl and extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$) then rotary evaporated and purified by flash column chromatography to yield dimethyl 2-(3,5-diisopropyl-4-methoxyphenyl) malonate (0.38 g, 47%, 1.2 mmol). mp 65-6 °C; ^1H NMR δ 1.25 (12H,d,*J*=6Hz), 3.33 (2H,m,*J*=6Hz), 3.75 and 3.78 (9H,two s), 4.60 (1H,s), 7.13 (2H,s) ppm.

Applying the **4a** method, treatment of this malonate (0.88mg, 2.5 mmol), with NaH (3 eq) in THF (50 mL) and then with MeI (3 eq) gave **dimethyl 2-(3,5-diisopropyl-4-methoxyphenyl)-2-methylmalonate (9a**, 139 mg, 35%). IR ν 1736 cm^{-1}; ^1H NMR δ 1.21 (12H,d,*J*=7Hz), 1.83 (3H, s), 3.29 (2H, m, *J*=7Hz), 3.70 and 3.74 (9H,two s), 7.00 (2H,s) ppm. Anal. Calcd. for $C_{19}H_{28}O_5$: C 67.86, H 8.33. Found C 67.44, H 8.27.

Dimethyl 2-Methyl-2-(2-Naphthyl) Malonate (10a).

Into a solution of 2-hydroxymethylnaphthalene (15 g,94.82 mmoL) in glacial acetic acid (30 mL) was bubbled HBr for 1 h. Work-up by CH_2Cl_2 extraction, rotary evaporation and recrystallization from absolute EtOH yielded 2-bromomethylnaphthalene (19.97 g, 95%) mp 55-56 °C (lit.[25] mp 56 °C). IR ν 3057, 1595, 1208, 1124, 821, 751, 660, 589, 477 cm^{-1}. ^1H NMR δ 4.62 (2H,s), 7.40-8.00 (7H,m, aromatic protons) ppm.

To a stirred solution of NaCN (5 g, 102 mmol) in distilled water (10 mL) at 20 °C was added the above bromide (19 g, 85.9 mmoL) in absolute EtOH (80 mL) and the mixture refluxed for 3 h. After rotary evaporation, saturated aqueous sodium bicarbonate (80 mL) was added and the mixture extracted with CH_2Cl_2 (4x100 mL). Evaporation of the dried ($MgSO_4$) extract and recrystallization from absolute EtOH yielded 2-cyanomethylnaphthalene (13.83 g, 96%) mp 79-80 °C. IR ν 2249, 1595, 1510, 1405, 828, 758, 477 cm^{-1}. ^1H NMR δ 3.82 (2H,s), 7.20-8.00 (7H,m, aromatic protons) ppm.

To a solution of $NaNH_2$ (2.05 g, 52.5 mmoL) in dry Et_2O (40 mL) was added the above cyanonaphthalene (5.85 g, 35 mmoL) in Et_2O (10 mL) while stirring under N_2. After 10 min the mixture was heated to reflux for 1 h, then cooled to 20 °C. MeI (4.4 mL, 70 mmoL) was added and the solution refluxed for an additional 1 h. The mixture was again cooled to 20 °C and acidified with 1M HCl . The layers were separated and the aqueous phase extracted further with Et_2O (3x50 mL). The combined ether extracts were dried ($MgSO_4$), concentrated, and subjected to flash column chromatography on silica gel (10%- then 20%-EtOAc in hexane elution) to give 2-(2-naphthyl)-propionitrile (3.0 g, 31%). mp 63-65 °C. IR ν 2242, 821, 751, 477 cm^{-1}. ^1H NMR δ 1.70 (3H,d,*J*=8Hz), 4.00 (1H,q,*J*=7Hz), 7.30-8.0 (7H,m, aromatic protons) ppm.

The above propionitrile (2.62 g, 14.4 mmoL) in MeOH (50 mL) was saturated with gaseous HCl and the mixture then refluxed. Distilled water (5 mL) was then added and, after further refluxing for 1 h, the solvents were rotary evaporated, 1M HCl (20 mL) added, and the mixture extracted with CH_2Cl_2 (3x20 mL). The organic extracts were dried ($MgSO_4$), concentrated, and column chromatographed on silica (10% EtOAc in hexanes elution) to yield methyl 2-(2-naphthyl)-

propionate (3.41 g, 82%). bp 95 °C/0.25 mmHg; mp 34-35 °C. IR ν 2984, 1735, 1195 cm^{-1}. ^1H NMR δ 1.58 (3H,d,*J*=7Hz), 3.63 (3H,s), 3.82 (1H,q,*J*=7Hz), 7.20-7.60 (7H,m, aromatic protons) ppm.

To a solution of LDA (from diisopropylamine (2.27 mL)) and n-BuLi (6.8 mL of 2.07 M solution in hexane, 14.1 mmol) in dry THF (20 mL) at -78 °C under N_2 was added the above propionate (2.5 g, 11.6 mmoL) in THF (10 mL). The mixture was gradually warmed, first to 0 °C and then to 20 °C, and stirred for 30 min. It was then cooled again to -78 °C and dimethyl carbonate (1.95 mL, 23 mmoL) was added via syringe and the solution warmed to 20 °C, at which temperature it was stirred for 3 h. Aqueous saturated NH_4Cl was added, giving two layers. The organic phase was washed with 1M HCl (30 mL) and extracted with Et_2O (3x50 mL) and the ether extracts washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel (10% EtOAc in hexane elution) followed by Kugelrohr distillation to give **dimethyl 2-methyl-2-(2-naphthyl) malonate (10a,** 2.7 g, 86%). bp 135-140 °C/0.25 mmHg. IR ν 2950, 1728, 1257 cm^{-1}. ^1H NMR δ 1.89 (3H,s), 3.70 (6H,s), 7.40-8.00 (7H,m, aromatic protons) ppm. MS $[M]^+$ 272.11 (77%). Anal. Calcd. C 70.56, H 5.93. Found C 70.92, H 6.18.

Dimethyl 2-(4-Biphenyl)-2-Methyl malonate (11a).

Using the initial procedure for the malonate **10a,** 4-hydroxymethylbiphenyl (10 g, 54 mmoL) was converted to 4-bromomethylbiphenyl (11.5 g, 86%). IR ν 1482, 850, 758, 723, 688, 603 cm^{-1}. ^1H NMR δ 4.50 (2H, s), 7.20-7.60 (9H,m, aromatic protons) ppm. To a stirred solution of NaCN (2.4 g, 49 mmoL) in distilled water (10 mL) at 23 °C was added dropwise a solution of this bromomethylbiphenyl(10.0 g, 40.4 mmoL) in absolute EtOH (60 mL). The mixture was heated under reflux for 3 h and EtOH was then removed under reduced pressure. Aqueous saturated sodium bicarbonate (80 mL) was added and the mixture extracted with CH_2Cl_2 (4x100 mL). The extracts were dried ($MgSO_4$), concentrated, and then recrystallized from hexane to give 4-cyanomethylbiphenyl (5.5 g, 71%). mp 93-95 °C. IR ν 2249, 1489, 1412, 751, 688 cm^{-1}. ^1H NMR δ 3.80 (2H, s), 7.20-7.80 (9H, m, aromatic protons) ppm.

To a solution of $NaNH_2$ (1.52 g, 38.9 mmoL) in dry THF (40 mL) under N_2 was added the above nitrile (5 g, 25.9 mmoL) in THF (10 mL). After 10 min the mixture was heated to reflux for 1 h, then cooled to 20 °C, and dimethyl carbonate (3.37 mL, 38.8 mmoL) added. After refluxing for an additional 1.5 h, the mixture was cooled to 20 °C, and 1M HCl added until the water phase was acidic to litmus. The aqueous layer was further extracted with EtOAc (3x40 mL). The organic extracts were dried ($MgSO_4$), concentrated, and purified by flash column chromatography on silica gel using 20% EtOAc in hexane as the eluent to give methyl 2-cyano-2-(4-biphenyl) acetate (2.5 g, 38%). mp 92-95 °C. IR ν 2910, 2249, 1750, 1482, 1433, 1278, 997, 765, 702 cm^{-1}. ^1H NMR δ 3.80 (3H, s), 4.75 (1H, s), 7.20-7.60 (9H, m, aromatic protons) ppm. Then, to a solution of NaOMe (prepared from Na (0.24 g, , 10.47 eq.) in MeOH (30 mL)) was added the above cyano acetate (1.5 g, 6.98 mmoL) in MeOH (5 mL). The mixture was heated under reflux for 1 h, then cooled to 20 °C and MeI (2.2 mL, 34.9 mmoL) added. The mixture was heated again under reflux for an

additional 1 h and then poured into 1M HCl (25 mL) and extracted with EtOAc (3x30 mL). The extracts were dried ($MgSO_4$), concentrated, and purified by column chromatography on silica gel (20% EtOAc in hexane elution) to give methyl 2-(4-biphenyl)-2-cyano-2-methyl acetate (650 mg, 35%). mp 112-114 °C. IR ν 2959, 2249, 1750, 1243, 1103, 730 cm^{-1}. ^1H NMR δ 2.0 (3H, s), 3.80 (3H, s), 7.20-7.60 (9H, m, aromatic protons) ppm. This cyano acetate (600 mg, 2.26 mmoL) was then dissolved in MeOH (20 mL) and Fischer-esterified with gaseous HCl. Column chromatography of the crude product on silica gel (10% EtOAc in hexane elution) and recrystallization from hexane:EtOAc (19:1 v/v) afforded **dimethyl 2-(4-biphenyl)-2-methyl malonate (11a,** 475 mg, 70 %) mp 83-84 °C. IR ν 2959, 1743-1735, 1468, 1433, 1264, 1117, 702 cm^{-1}. ^1H NMR δ 1.90 (3H,s), 3.79 (6H,s), 7.20-7.60 (9H,m, aromatic protons) ppm. Anal. Calcd. C 72.45, H 6.09: Found C 72.70, H 6.53).

PLE-CATALYZED HYDROLYSES OF MALONATE DIESTERS

The PLE-catalyzed hydrolyses of malonates **1a-10a** were all carried out by now-routine procedures,[1] and with the %ee's determined by NMR[10] and absolute configurations by Brewster's rules,[14] all as described previously.[1,5] The procedure for hydrolysis of **10a** is representative, as follows:

Malonate **10a** (205 mg, 0.75 mmoL) in distilled water (10 mL) was placed in a 50 mL two-necked flask fitted with a stirrer and a pH-stat-controlled burette containing 0.2M aqueous NaOH. The pH was set at 7.0 and PLE (200 μL, 572 Units) was added to the stirred solution at 23 °C. The course of the reaction was monitored by the pH-stat controlled volume of base added and was stopped after one equivalent of base (3.52 mL) had been added (36 h). The mixture was acidified to pH 2, extracted with Et_2O (5x10 mL), and the ether extracts dried ($MgSO_4$), and concentrated, The half-acid ester product **(+)-*R*-10b** (185 mg, 95%, >95% ee), which could be recrystallized from 10% CH_2Cl_2 in hexane, had mp 97-99 °C. $[\alpha]^{25}_D$ +8.3 (*c* 0.83, $CHCl_3$). IR ν 3437-2635, 1743, 1693, 1285, 1110 cm^{-1}. ^1H NMR δ 2.01 (3H,s), 3.80 (3H,s), 7.45-7.50 (3H,m, aromatic protons), 7.79-7.83 (4H,m, aromatic protons) ppm.

PLE-catalyzed hydrolysis of the other malonates gave the following results (see also Table 1).

Malonate **1a** (263 mg, 1.39 mmoL); PLE (200 μL, 572 U); 0.2M aqueous NaOH (6.8 mL); 24 h, yielded half-acid ester **1b** (240 mg, 99%, 6% ee) as a clear oil. $[\alpha]^{25}_D$ ~0 (*c* 1.3, $CHCl_3$) (lit.[20] $[\alpha]^{25}_D$ -0.55 (*c* 3.29,$CHCl_3$)). IR ν 3302-2638, 1715, 1463, 1257, 1144 cm^{-1}. ^1H NMR δ 0.92 (3H,d,*J*=6.8Hz), 0.94 (3H,d,*J*=7.0Hz), 1.35 (3H,s), 2.39-2.50 (1H,septet,*J*=6.6Hz), 3.75 (3H,s) ppm.

Malonate **2a** (204 mg, 0.94 mmoL); PLE (200 μL, 572 U); 0.2M aqueous NaOH (4.35 mL); 36 h, yielded half-acid ester **2b** (187 mg, 98%, 7% ee) mp 90-93 °C. $[\alpha]^{25}_D$ 0 (*c* 0.83, $CHCl_3$).

IR ν 3289-2645, 1742-1715, 1463, 1277, 1197, 1111 cm^{-1}. ^1H NMR δ 0.88 (9H,s), 1.54 (3H,s), 1.92 (1H,d,*J*=14.32Hz), 2.19 (1H,d,*J*=14.41Hz), 3.79 (3H,s) ppm.

Malonate **3a** (203 mg, 0.88 mmoL); PLE (200 µL, 572 U); 0.2M aqueous NaOH (4.33 mL); 72 h, yielded half- acid ester **(+)-*R*-3b** (180 mg, 97%, >95% ee). $[\alpha]^{25}_D$ +1.92 (*c* 0.83, $CHCl_3$) (lit.[26] $[\alpha]^{25}_D$ +0.85° (*c* 3.025, $CHCl_3$)). IR ν 3289-2632, 1715, 1469, 1244, 1118 cm^{-1}. ^1H NMR δ 0.86 (9H,s), 1.07-1.12 (2H,m), 1.42 (3H,s), 1.78-1.93 (2H,m), 3.74 (3H,s) ppm.

Malonate **4a** (195 mg, 0.82 mmol); PLE (570 U); 0.2M aqueous NaOH (3.94 mL); 4 h, yielded half-acid ester **(+)-*R*-4b**, 169 mg, 92%, >95% ee). $[\alpha]^{25}_D$ +29.0 (*c* 8.4, $CDCl_3$); IR ν 3450, 3200, 2635, 1750-1700 cm^{-1}; ^1H NMR δ 1.97 (3H,s), 2.22 (3H,s), 3.77 (3H,s), 7.20-7.40 (4H, m), 9.60 (1H, s,broad) ppm.

Malonate **5a** (190 mg, 0.76 mmol); PLE (570 U); 0.2 M aqueous NaOH (3.41 mL); 24 h, yielded half-acid ester **(+)-*R*-5b** (145 mg, 80%, 100% ee). $[\alpha]^{25}_D$ +12.2 (*c* 8.6, $CDCl_3$). IR ν 3200, 2628, 1735, 1714 cm^{-1}; ^1H NMR δ 1.86 (3H,s), 2.21 (6H,s), 3.73 (3H,s), 7.30 (3H,m), 10.03 (1H,s,broad) ppm.

Malonate **6a** (196 mg, 0.78 mmol); PLE (570 U); 0.2M aqueous NaOH (3.84 mL); 48 h, yielded half-acid ester**(+)-*R*-6b** (167 mg, 90%, 94% ee). $[\alpha]^{25}_D$ +14.9 (*c* 4.2, $CDCl_3$); IR ν 3200, 2635, 1735, 1714 cm^{-1}; ^1H NMR δ 1.88 (3H,s), 2.30 (6H,s), 3.78 (3H,s), 6.93 (3H,m), 8.95 (1H,s,broad) ppm.

Malonate **7a** (110 mg, 0.35 mmoL); PLE (150 µL, 429 U); 0.2M aqueous NaOH (1.51 mL); 16 h, yielded half-acid ester **(+)-*R*-7b** (96 mg, 92%, 81% ee) as a thick oil. $[\alpha]^{25}_D$ +13.69 (*c* 1.87, $CHCl_3$). IR ν 3455-2605, 1728, 1516, 1257 cm^{-1}. ^1H NMR δ 1.40 (6H,br.t,*J*=7Hz), 1.83 (3H,s), 3.76 (3H,s), 4.05 (4H,br.q,*J*=7Hz), 6.79-6.93 (3H,m, aromatic protons) ppm.

Malonate **8a** (263 mg, 1.05 mmol); PLE (1540 U), 0.2M aqueous NaOH (4.51 mL); 20 h, yielded half-acid ester **(+)-*R*-8b** (226.3 mg, 92%, >95% ee). $[\alpha]^{25}_D$ +9.6 (*c* 11.3, $CDCl_3$); IR ν 3200, 2635, 1735, 1714 cm^{-1}; ^1H NMR δ 1.92 (3H,s), 3.78 (9H,s), 6.40-6.60 (3H,m), 10.47 (1H,s,broad) ppm.

Malonate **9a** (117 mg, 0.35 mmol); PLE (570 U); 0.2M aqueous NaOH (1.50 mL); 20 h, yielded half-acid ester **(+)-*R*-9b** (78 mg, 70%, >95% ee). mp 116-8 °C; $[\alpha]^{25}_D$ +14.2 (*c* 5.2, $CDCl_3$); IR ν 3428, 3177, 1754-38, 1718 cm^{-1}; ^1H NMR δ 1.23 (12H,d,*J* = 6Hz), 3,85 (3H,s), 7.10 (2H,s), 10.73 (1H,s,broad) ppm.

ACKNOWLEDGEMENT

We thank the Natural Sciences and Engineering Council of Canada (NSERC) for financial support. The awards of NSERC Postgraduate and a Fonds pour la Formation de Chercheurs de Quebec scholarships (to LRP) are also gratefuly acknowledged.

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