A Concluding Specification of the Dimensions of the Active Site Model of Pig Liver Esterase[1]

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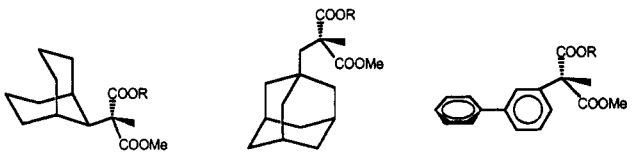
Using bicyclononyl-, (adamantylmethyl)-, and biphenylmalonate substrate probes, the dimensions of the large hydrophobic pocket of the active site model of pig liver esterase have been established as 6.2 × 3.1 × 4.7 Å^3. It is believed that the current refinement completes the basic specification of the active site model and that, for the majority of practical applications of the enzyme, no significant further modifications are likely to be required.

Introduction

Enzymes are highly efficient biocatalysts that are now widely used for creating new chiral synthons.[2] Of the enzymes that are synthetically useful, hydrolases are currently the most widely applied 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 of its broad substrate specificity and the high stereoselectivity of many of its catalyses.[2-4] Until recently, the use of PLE was hampered by its seemingly fickle specificity, most evident in reversals of stereoselectivity within homologous series of ester substrates. However, the apparent specificity anomalies of PLE have been successfully rationalized by the active-site model proposed[5] 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 substrate structures and has been independently applied successfully by several groups.[6] In the few cases where the model is reported to be invalid,[7] it has either not been applied as specified,[5] as in allegedly predicting the wrong stereochemistry,[7a] or has been applied outside its stated[5] limitations, as in attempts to interpret stereoselectivity preferences in low % ee product forming reactions.[7b]

Although the model as initially formulated functioned well, its dimensions delineated the minimum pocket volumes. While the volumes of the P_F, P_B, and H_S dimensions were confirmed as initially specified, the size of the large hydrophobic pocket, H_L, as first identified was found to be too small, and subsequent probing of the true limits of its volume with substrates of prescribed shapes and structures led to expansion of the H_L boundaries.[8] In this paper, we report the completion of our probing of the dimensions and properties of the H_L pocket, using the dimethyl bicyclononyl-, (adamantylmethyl)-, and biphenylyl malonate substrate probes **1a–3a** to delineate what we believe to be the *de facto* boundaries of H_L.



1a, R = Me b, R = H | 2a, R = Me b, R = H | 3a, R = Me b, R = H

Results and Discussion

The malonate probes **1a–3a** were prepared by unexceptional routes in good yields. Each was then subjected to preparative-scale PLE-catalyzed hydrolysis at pH 7 and 25 °C in the usual manner.[8] The reactions were terminated after 12 days, when conversion of the biphenyl compound **3a** to the half-ester **3b** was complete. Hydrolyses of the bicyclononyl **1a** and adamantyl **2a** malonates to the corresponding acid malonates **1b** and **2b** proceeded only to 25% and 9% completion, respectively, in this time period. As in the previous studies,[8] the enantiomeric excesses of the acid–ester products **1b–3b** were determined by NMR and their absolute configurations from literature precedents and Brewster's rules.[9] From these analyses, the PLE-catalyzed hydrolysis products were found to be (*R*)-**1b** (70% ee), (*R*)-**2b** (>98% ee), and (*R*)-**3b** (>98% ee).

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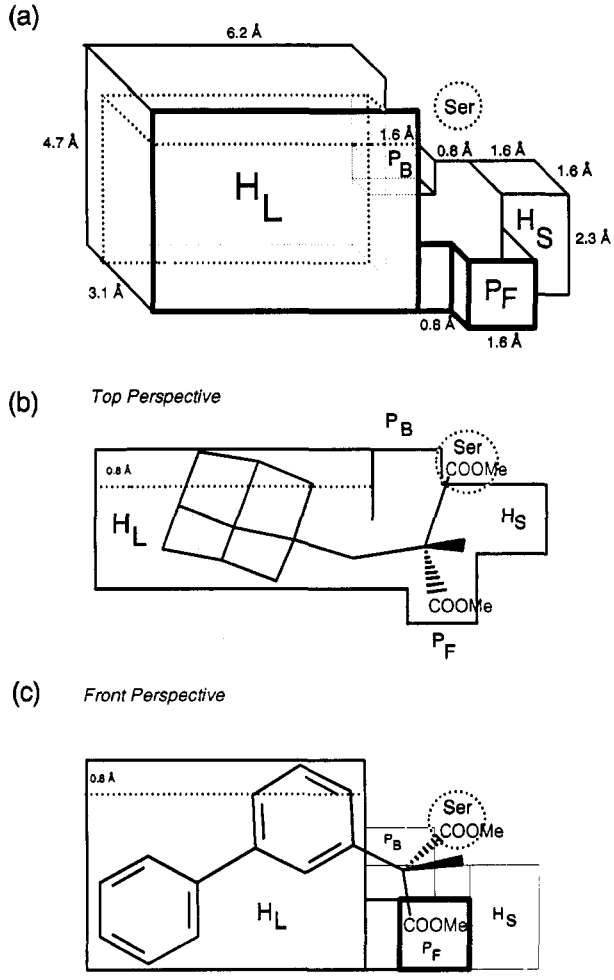

Figure 1. (a) Final active site model for PLE. The boundary limits of the H_L pocket established previously[8b] are shown in the dotted (···) lines. The new 3.1- × 4.7- × 6.2-Å^3 dimensions of H_L shown represent height and rear extensions of 0.8 Å, respectively, from these previous limits, giving a final H_L volume of 90 Å^3. (b) The binding of **2a** into model a is shown from the top perspective. In order for the adamantyl residue to fit into H_L as depicted, the earlier[8b] H_L boundary (···) had to be expanded by 0.8 Å as shown. (c) Binding of **3a** into model a is shown from the front perspective. For the biphenyl moiety to be accommodated by H_L, the pocket had to be stretched by 0.8 Å from its prior[8b] limit (···), as shown.

The fact that each of **1a–3a** was a substrate and was stereoselectively hydrolyzed by PLE to give a half-ester product of high ee and of the expected absolute configuration requires that the active site be able to bind each of the bulky hydrophobic groups present. However, the most recent H_L pocket specification[8b] is still not large enough to accommodate the bicyclononyl, adamantyl, or 3-biphenylyl groups of **1a–3a** and thus needs to be expanded further.

The active site model that now satisfies all known substrate specificity data is shown in Figure 1a. The H_L-dimensions depicted are those that now permit substrates **1a–3a** to fit satisfactorily. The substrate orientations depicted in Figure 1b and 1c illustrate the ES complexes envisaged when **2a** and **3a**, respectively, bind into the active site of PLE with their adamantyl and 3-biphenylyl groups in H_L and with their *pro-S* COOMe groups located in the serine locus, as required for hydrolysis to the observed (*R*)-**2b** and (*R*)-**3b** products. Since the Figure 1a model simply represents an enlargement of the earlier specifications, it is applied as specified originally[5] and all previous substrate specificity interpretations remain valid.

For consistency, all of our active site model studies[5,6,8] have been performed in aqueous solutions. At times, this has presented problems of solubility for very hydrophobic substrate probes. In such cases, the enhanced solubilities, and hence higher rates of hydrolysis, conferred by operating in aqueous, or water-deficient, organic solvents, are clearly desirable from a practical standpoint. While PLE conformations will undoubtedly be modified by the addition of organic solvents, the fundamental geometry of the active site should be preserved up to the denaturation point and it is envisaged that the basic validity of the model as a guide to interpreting PLE specificity will be retained for transformations involving organic solvents.

The new 6.2- × 4.7- × 3.1-Å^3 dimensions of H_L represent a significant expansion of the 6.2- × 3.9- × 2.3-Å^3 volume specified in the previous refinement.[8b] The extremely sluggish hydrolyses rates of **1a** and **2a** lead us to conclude that these substrates represent the limits of PLE's ability to locate large hydrophobic moieties in H_L and that bicyclononyl and adamantyl groups, together with 3- and 4- biphenyl,[8b] represent the maximum sizes that can be fitted into the H_L volume. The H_L dimensions shown in Figure 1 are thus believed to reflect closely its actual boundary limits. While new substrate structure types for PLE will undoubtedly continue to be identified, it is felt that any modifications to the Figure 1 active site model that may be required in the future will be minor.

Experimental Section

Analytical procedures and equipment used were as described previously.[7b] Chemicals were purchased from Aldrich and porcine liver esterase (PLE, EC 3.1.1.1, Lot 45F-8130 and Lot 107F-8235) from Sigma. NMR spectra were run in $CDCl_3$ unless specified otherwise.

Synthesis of Substrates. Dimethyl 2-(9-Bicyclo[3.3.1]nonyl)-2-methylmalonate (1a). Using a modification of the procedure of Cope and co-workers,[10] bicyclo[3.3.1]nonan-9-one (1 g, 7.24 mmol), cyanoacetic acid (615 mg, 7.24 mmol), and ammonium acetate (56 mg, 0.72 mmol) were refluxed in benzene (25 mL) for 1 day in a Dean–Stark apparatus. Et_2O (25 mL) was then added, and the organic phase was washed with H_2O (3 × 8 mL) and then with brine (8 mL), dried ($MgSO_4$), and then rotary evaporated to yield a white solid that was recrystallized from CCl_4 to give 2-(bicyclo[3.3.1]nonan-9-ylidene)-2-cyanoacetic acid (1.22 g, 82%): mp 175 °C; IR ν 3400–2400 (br), 2228, and 1700 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.46–1.58 (2 H, m), 1.80–2.13 (10 H, m), 3.23 (1 H, d, J = 1.8 Hz), ~9.0 (1 H, br s) ppm; MS calcd for $C_{12}H_{15}NO_2$ 205, found [M]$^+$ 205.

The above cyano acid (1.06 g, 5.15 mmol) and 5% Pd/C (53 mg) was stirred in MeOH (15 mL) at 22 °C under H_2 (1 atm) for 20 h. Filtration followed by rotary evaporation yielded (±)-2-(9-bicyclo[3.3.1]nonyl)-2-cyanoacetic acid (988 mg, 93%): mp 145 °C; IR ν 3500–2800 (br), 2256, 1757, 1222, 1152 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.50–1.68 (5 H, m), 1.68–2.00 (8 H, m), 2.15 (2 H, d, J = 11.0 Hz), 3.82 (1 H, d, J = 12.0 Hz), 10.54 (1 H, br s) ppm; MS calcd for $C_{12}H_{17}NO_2$ 207, found [M + 1]$^+$ 208.

Into a stirred solution of this (±)-cyanoacetic acid (2.14 g, 10.33 mmol) in MeOH (35 mL) containing H_2O (1 mL) at 0 °C was bubbled HCl for 30 min. After the solution was refluxed for 2 h the MeOH was rotary evaporated and the residue taken up in Et_2O (30 mL). The organic phase was successively washed with H_2O (2 × 5 mL), saturated aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), and brine (5 mL) and then dried ($MgSO_4$) and rotary evaporated to yield dimethyl 2-(9-bicyclo[3.3.1]nonyl)malonate (1.60 g, 61%) as a colorless oil bp 95–100 °C (0.025 mmHg); IR

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ν 1757 and 1735 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.40–1.94 (14 H, m), 2.28 (1 H, d, J = 12.1 Hz), 3.70 (6H, s), 3.83 (1 H, d, J = 12.1 Hz) ppm; ^{13}C NMR (50 MHz) δ 21.0, 21.9, 24.8, 29.7, 33.1, 42.0, 52.2, 53.6, 169.5 ppm; ^{13}C APT NMR (50 MHz) (up) δ 21.0, 21.9, 24.8, 33.1, 169.5 ppm; MS calcd for $C_{14}H_{22}O_4$ 254, found: $[M]^+$ 254.

To a stirred suspension of NaH (310 mg, 7.76 mmol) in dry THF (2 mL) at 22 °C under Ar was added a solution of the above malonate (988 mg, 3.88 mmol) in dry THF (4 mL). After 3 h, MeI (0.48 mL, 1.103 mg, 7.77 mmol) was added, and the solution was stirred for 19 h. Aqueous 1 M HCl was then added, followed by Et_2O (20 mL). The organic phase was successively washed with saturated aqueous $Na_2S_2O_3$ (8 mL), H_2O (8 mL), and brine (8 mL) and then dried ($MgSO_4$) and rotary evaporated to yield a yellowish oil (1.09 g) that on flash chromatography (SiO_2, EtOAc/hexanes (1:19) elution) yielded dimethyl 2-(9-bicyclo[3.3.1]nonyl)-2-methylmalonate (**1a**, 781 mg, 75%) as a colorless oil which later crystallized under vacuum (0.05 mmHg): mp 72 °C; IR ν 1757 (sh) and 1728 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.40–1.59 (4 H, m), 1.55 (3 H, s), 1.70–1.92 (10 H, m), 2.41 (1 H, s), 3.67 (6 H, s) ppm; ^{13}C NMR (50 MHz) δ 20.1(7), 20.2(3), 22.2, 25.4, 30.0, 35.9, 44.5, 52.2, 57.8, 173,5 ppm; ^{13}C APT NMR (50 MHz) (up) δ 20.2 (3), 22.2, 25.4, 35.9, 57.8, 173.5 ppm. Anal. Calcd for $C_{15}H_{24}O_4$: C, 67.14, H, 9.01. Found: C, 67.17, H, 9.19.

Dimethyl 2-(1-Adamantylmethyl)-2-methylmalonate (2a). Using the general procedure of Albarella,[11] 3-(1-adamantyl)-propanonitrile[12] (1.957 g, 10.3 mmol) in dry THF (10 mL) was added under N_2 to lithium isopropylamide (23.8 mmol) in dry THF (10 mL) at −78 °C. The solution was stirred at −78 °C for 30 min and then warmed to 22 °C and stirred for a further 30 min. The mixture was then cooled again to −78 °C, dimethyl carbonate (0.91 mL, 978 mg, 10.9 mmol) added, the reaction mixture stirred for 3.5 h at −78 °C and then for 1 h at 22 °C, and then saturated aqueous NH_4Cl (10 mL) added followed by Et_2O (100 mL). The organic phase was successively washed with 1 M aqueous HCl (3 × 20 mL), H_2O (3 × 20 mL), and brine (20 mL) and then dried ($MgSO_4$) and rotary evaporated to give, after Kugelrohr distillation, methyl 3-(1-adamantyl)-2-cyanopropanoate (2.17 g, 85%), bp 120 °C (0.25 mmHg), as a colorless oil that slowly crystallized during 12 h. Recrystallization from hexanes at 0 °C yielded crystalline leaflets: mp 38 °C; IR ν 2256 (w), 1771, and 1750 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.53 (6 H, s), 1.65 (6 H, d, J = 8.9 Hz), 1.75–1.79 (2 H, m), 1.98 (3 H, br s), 3.47 (1 H, dd, J = 7.7 Hz, J' = 5.5 Hz), 3.79 (3 H, s) ppm.

Into a stirred solution of this cyano ester (1.15 g, 4.63 mmol) in MeOH (25 mL) and H_2O (1 mL) at 0 °C was bubbled HCl for 30 min. The solution was then refluxed for 2 h and cooled, Et_2O (20 mL) added, and the organic phase washed with H_2O (3 × 5 mL) and then with brine (5 mL), dried ($MgSO_4$), and rotary evaporated. The oily residue was Kugelrohr distilled to yield dimethyl 2-(1-adamantylmethyl)malonate (1.133 g, 87%): bp 95–100 °C (0.05 mmHg); IR ν 1757 and 1735 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.41 (6 H, d, J = 2 Hz), 1.61 (6 H, dd, J = 19.8 Hz, J' = 12.1 Hz), 1.77 (2 H, d, J = 6.3 Hz), 1.91 (3H br s), 3.44 (1 H, t, J = 6.4 Hz), 3.70 (6 H, s) ppm; ^{13}C NMR (50 MHz) δ 28.3, 32.1, 36.7, 41.7, 42.4, 46.4, 52.44, 171.1 ppm; ^{13}C APT NMR (50 MHz) (up) δ 28.3, 46.4, 52.4 ppm; MS calcd for $C_{16}H_{24}O_4$ M 280, found [M] 280.

To a stirred suspension of NaH (180 mg, 4.49 mmol) in dry THF (30 mL) at 22 °C under N_2 was added a solution of the above dimethyl adamantyl malonate (1.05 g, 3.75 mmol) in dry THF (2 mL). After 30 min, MeI (0.47 mL, 1.063 g, 7.49 mmol) was added, the solution stirred for 4 h and then aqueous 1 M HCl (20 mL) was added, and the aqueous phase was extracted with Et_2O (8 mL). The organic phase was successively washed with saturated aqueous $Na_2S_2O_3$ (8 mL), H_2O (8 mL), and brine (8 mL) and then dried ($MgSO_4$), rotary evaporated, and flash chromatographed (SiO_2, hexanes to EtOAc/hexanes (1:9) gradient elution) to afford dimethyl 2-(1-adamantylmethyl)-2-methylmalonate (**2a**, 0.985 g, 89%, after recrystallization from MeOH): mp 82 °C; IR ν 1728 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.48 (6 H, s), 1.49 (3 H, s), 1.58 (6 H, d, J = 2.5 Hz), 1.81 (2 H, s), 1.85 (3 H, br s), 3.65 (6 H, s) ppm; ^{13}C NMR (50 MHz) δ 21.9, 28.5, 33.2, 36.6, 42.9, 48.4, 52.2, 52.9, 173.8 ppm; ^{13}C APT NMR (50 MHz) (up) δ 33.2, 36.6, 42.9, 48.4, 52.9, 173.8 ppm. Anal. Calcd for $C_{17}H_{26}O_4$: C, 69.36, H, 8.90. Found: C, 69.68, H, 8.98.

Dimethyl 2-(3-Biphenylyl)-2-methylmalonate (3a). To a stirred suspension of NaH (0.738 g, 18.45 mmol) in dry THF (25 mL) at 22 °C under Ar was added methyl (3-biphenylyl)acetate[13] (prepared from 3-phenylbenzoic acid[14] *via* Arndt–Eistert homologation, 1.815 g, 8.02 mmol) in dry THF (5 mL). After the mixture was stirred for 15 min, dimethyl carbonate (2.70 mL, 2.89 g, 32.08 mmol) was added, and the solution was stirred for a further 2 days at 22 °C. Aqueous 1 M HCl (1 mL) was added, the solution rotary evaporated, the residue taken up in Et_2O (100 mL), and the organic phase washed successively with aqueous 1 M HCl (3 × 10 mL), H_2O (1 × 10 mL), and brine (1 × 10 mL) and then dried ($MgSO_4$) and rotary evaporated. The residual oil was flash chromatographed (SiO_2, EtOAc/hexanes (1:9) elution) to yield dimethyl (3-biphenylyl)malonate (1.70 g, 75%): IR ν 1757 and 1743 cm^{-1}; ^{1}H NMR (200 MHz) δ 3.76 (6 H, s), 4.70 (1 H, s), 7.29–7.49 (5 H, m), 7.53–7.62 (4 H, m) ppm; MS calcd for $C_{17}H_{16}O_4$ 284, found $[M]^+$ 284.

To a stirred suspension of NaH (0.280 g, 6.99 mmol) in dry THF (30 mL) at 22 °C under Ar was added the above malonate (1.656 g, 5.82 mmol) in dry THF (5 mL). After 15 min, MeI (0.73 mL, 1.653 g, 11.65 mmol) was added and the solution stirred for a further 6 h. Aqueous 1 M HCl (2 mL) was added, the mixture rotary evaporated, Et_2O (100 mL) added, the organic phase washed successively with aqueous 1 M HCl (2 × 10 mL), saturated aqueous $Na_2S_2O_3$ (2 × 10 mL), H_2O (2 × 10 mL), and brine (10 mL), and then dried ($MgSO_4$) and rotary evaporated, the oily product flash chromatographed (SiO_2, EtOAc/hexanes (1:9) elution) to give, after Kugelrohr distillation, dimethyl 2-(3-biphenylyl)-2-methylmalonate (**3a**, 1.5 g, 87%): bp 115–20 °C (0.2 mmHg); IR ν 1728 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.91 (3 H, s), 3.76(5) (6 H, s), 7.29–7.59 (9 H, m) ppm. Anal. Calcd for $C_{18}H_{18}O_4$: C, 72.47, H, 6.08. Found: C, 72.59, H, 6.07.

PLE-Catalyzed Hydrolyses. The general procedure used is illustrated below using **3a** as the substrate. In all cases, control experiments established that no hydrolyses occurred at pH 7 in the absence of PLE. As described previously,[8] the enantiomeric excesses of the acid–ester products **1b–3b** were determined by NMR on the (*R*)-(+)-1-phenylethylamine salts,[15] using racemic **1b–3b**, prepared by treating the malonate substrates with 1 equiv of KOH in methanol,[16] as reference standards. The absolute configurations of **1b–3b** were assigned from literature precedents[4c,8,17] and Brewster's rules.[9]

PLE-Catalyzed Hydrolysis of Dimethyl 2-(3-Biphenylyl)-2-methylmalonate (3a). Dimethyl 2-(3-biphenylyl)-2-methylmalonate (**3a**, 510 mg, 1.71 mmol) was suspended in distilled H_2O (15 mL) at 22 °C, the pH adjusted to 7.0 with 0.2 M aqueous NaOH, and PLE (EC 3.1.1.1, 500 μL, 3180 units) added to the gently stirred mixture. The pH was maintained at 7.0 by pH-stat-controlled addition of 0.2 M aqueous NaOH. The progress of the reaction was monitored by the rate of addition of the base, which was plotted on a recorder. The reaction was stopped when 1 equiv of base had been added (12 days). The solution was filtered through Hyflo Supercel, washed with Et_2O (3 × 15 mL), acidified with 1 M aqueous HCl (2 mL), and then extracted with Et_2O (5 × 15 mL). The organic phase was washed with brine (15 mL) and then dried ($MgSO_4$) and rotary evaporated to yield (2*R*)-(+)-hydrogen methyl 2-(3-biphenylyl)-2-methylmalonate (**3b**, 398 mg, 82%, >98% ee) $[\alpha]^{25}_D$ +7.2° (*c* 1.49, $CHCl_3$); IR ν 3600–2700 (br), 1728, and 1714 cm^{-1}; ^{1}H NMR (200 MHz) δ 1.95 (3 H, s), 3.80 (3 H, s), 7.29–7.60 (9 H, m), 10.45 (1 H, br) ppm; ^{13}C NMR (50 MHz) δ 21.8, 53.2, 58.5, 126.3, 126.4, 127.1, 127.4, 127.6, 128.9, 138.0, 141.1, 141.7, 172.5, 177.0 ppm; ^{13}C APT NMR (50 MHz) (up) δ 58.5, 138.0, 141.1, 141.7, 172.5, 177.0 ppm. Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.58; H, 5.82.

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The other substrates were hydrolyzed in the same manner, as follows: **Dimethyl 2-(9-bicyclo[3.3.1]nonyl)-2-methylmalonate** (**1a**, 300 mg, 1.12 mmol), H_2O (15 mL), PLE (300 μL, 858 U), pH 7.0, 22 °C. After 2 days of hydrolysis more PLE (300 μL, 858 U) was added. The reaction mixture was worked up after 12 days (25% completion) to give recovered malonate **1a** (208 mg, 69%) and (2*R*)-(+)-hydrogen methyl 2-(9-bicyclo[3.3.1]-nonyl)-2-methylmalonate (**1b**, 55 mg, 19%, 70% ee): mp 133–4 °C; $[\alpha]^{25}_D$ +8.1° (*c* 0.94, $CHCl_3$); IR *ν* 3600–2600 (br), 1750, and 1707 cm^{-1}; ^1H NMR (400 MHz) δ 1.44–1.58 (4 H, m), 1.59 (3 H, s), 1.70–1.99 (10 H, m), 2.28 (1 H, br s), 3.74 (3 H, s) ppm; ^{13}C NMR (50 MHz) δ 20.1(6), 20.2(1), 22.1, 25.3, 25.4, 29.8, 30.2, 35.9(5), 36.0(1), 45.8, 52.6, 57.6, 174.1, 178.1 ppm; ^{13}C APT NMR (50 MHz) (up) δ 20.1 (6), 22.1, 25.3, 25.4, 35.9 (5), 36.0 (1), 57.6, 174.1, 178.1 ppm. Anal. Calcd for $C_{14}H_{22}O_4$: C, 66.12; H, 8.72. Found: C, 65.83; H, 8.60.

Dimethyl 2-(1-Adamantylmethyl)-2-methylmalonate (**2a**, 500 mg, 1.70 mmol), H_2O (15 mL), PLE (500 μL, 1430 U), pH 7.0, 22 °C. The reaction mixture was worked up after 7 days (9% completion) to give recovered malonate **2a** (449 mg, 90%) and (*R*)-(+)-hydrogen methyl 2-(1-adamantylmethyl)-2-methylmalonate (**2b**, 33 mg, 7%, 98% ee) as a white solid: mp 92–3 °C; $[\alpha]^{25}_D$ +17.0 °C (*c* 1.03, $CHCl_3$); IR *ν* 3600–2400 (br), 1743, and1707 cm^{-1}; ^1H NMR (200 MHz) δ 1.45 (6 H, d, *J* = 2.6 Hz), 1.53 (3 H, s), 1.59 (6 H, t, *J* = 2.7 Hz), 1.76 (1 H, d, *J* = 14.4 Hz), 1.88 (3 H, br s), 2.03 (1 H, d, *J* = 14.4 Hz), 3.78 (3 H, s), 10.6–12.5 (1 H, br s) ppm; ^{13}C NMR (50 MHz) δ 25.5, 28.4, 33.3, 36.5, 42.4, 49.8, 51.7, 52.8, 175.8, 178.0 ppm; ^{13}C APT NMR (50 MHz) (up) δ 33.3, 36.5, 42.4, 49.8, 51.7, 175.8, 178.0 ppm. Anal. Calcd for $C_{16}H_{24}O_4$: C, 68.55; H, 8.63. Found: C, 68.27; H, 8.73.

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