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Enzymes in Organic Synthesis 49.[1] Resolutions of Racemic Monocyclic Esters with Pig Liver Esterase.

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ABSTRACT. Pig liver esterase (PLE)- catalyzed hydrolyses of the racemic methyl esters of cyclobutane-, cyclohexane-, and cyclohex-4-ene-carboxylic acids bearing cis-2-methyl or cis-2-bromomethyl substituents are highly stereoselective, giving the corresponding acid products of $\geq$97% ee. The stereoselectivity of the enzyme exhibits the expected reversal for such compounds, with the absolute configurations of the cyclobutane and cyclohexane acids being of the opposite absolute configuration types, and cyclopentane substrates such as cis-1-carbomethoxy-2-methylcyclopentane representing the change-over structures and giving products of only 22%ee. This stereoselectivity reversal, and the absolute configurations preferred, are as predicted by the recently proposed active site model for the enzyme.

INTRODUCTION

The use of enzymes as chiral catalysts for the preparation of chiral synthons is now well established.[2] Although virtually every class of enzyme has been applied synthetically, it is the hydrolytic enzymes that are currently the most actively investigated group. Among the reasons for the popularity of hydrolytic enzymes are that they are readily available, are relatively inexpensive, require no expensive cofactors, and often exhibit high stereoselectivities in their catalyses of structurally varied substrates. One of the most useful hydrolytic enzymes for organic synthesis is the carboxylesterase from pig liver (PLE, EC 3.1.1.1).[3] PLE is relatively inexpensive ($0.005/unit), has no cofactor requirement, and shows broad substrate specificity, and high stereoselectivity with a wide range of substrates. Although the PLE sold commercially is a mixture of isozymes, it has been demonstrated that the stereospecificities of each major isozyme component are virtually identical, and that for synthetic purposes PLE may be used as though it were a single enzyme.[4]

Soon after its use in synthesis became widespread, information began to accumulate suggesting PLE was fickle in its stereoselectivity preferences. In particular, a number of examples where PLE exhibited reversals of stereoselectivity within homologous series of substrates were reported, triggered by apparently minor variations in substrate structure.[5a-c] This seemingly unpredictable behaviour detracted from the general utility of PLE in synthesis. Several models were devised in attempts to rationalize the stereochemical behaviour of PLE.[6a-e] However, none of these early models was generally applicable, and most worked only for the small range of substrates for which they were designed.

Recently we have developed a generally applicable active site model for PLE that allows the prediction of both the stereochemical sense of PLE-catalyzed hydrolyses, as well as the level of enantiomeric excess which can be expected.[7a] This simple-to-use yet comprehensive three-dimensional cubic space model (Figure 1) gives accurate predictions of PLE specificity, both structural and stereochemical, for a wide range of structurally dissimilar literature substrates, so far without exception.[7b] In order to further extend the validity of this model, as well as to better delineate the nature of the binding sites, we now report its application in predicting and interpreting the stereochemical outcome of the PLE-catalyzed hydrolyses of the racemic monocyclic esters (±)-**1** - **6**. PLE exhibits a number of substrate size-induced reversals in stereoselectivity. Such a reversal during the PLE- catalyzed hydrolyses of a series of monocyclic meso 1,2-diester substrates was the first clearly recognized example of this phenomenon.[5a] The 1,2-disubstituted monocyclic substrates, (±)-**1** - **6** were chosen as substrates that would shed further light on this aspect. In addition to possessing the potential for inducing another size-induced reversal of stereoselectivity, it was felt that the bromomethyl or methyl groups at C-2 would clarify the nature of the P_F binding site, and the types of groups this pocket could accommodate.

RESULTS AND DISCUSSION

The syntheses of the substrates **1** - **6** were carried out in straightforward fashion according to literature methods. Compounds **1** - **6** were all substrates for PLE, and each was hydrolyzed on a preparative scale (>3 mmol). Hydrolyses were carried out at pH 7.0, 21°C, in distilled water. The pH was maintained at 7.0 by continuous addition of base with a pH-stat unit, with the progress of reaction monitored by the uptake of base. Hydrolysis of all substrates except **2** stopped after one-half equivalent of base had been consumed. Hydrolysis of **2** was stopped at 50% conversion by extraction of the unhydrolyzed ester from the reaction mixture with diethyl ether. For the remaining substrates, following consumption of one-half equivalent of base, the unhydrolyzed esters were recovered by extraction of the reaction mixture at neutral pH. The carboxylic acid products were

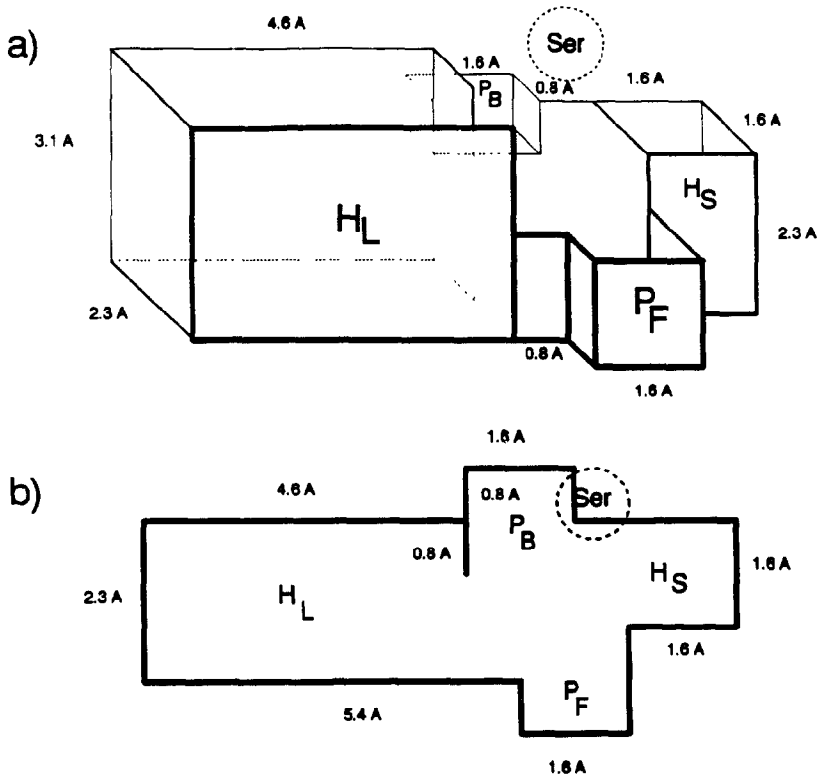
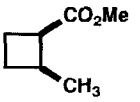
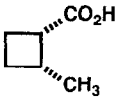
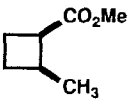
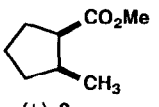
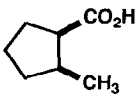
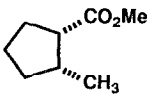
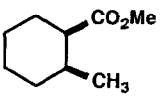
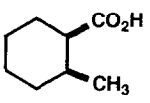
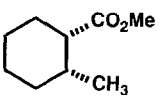
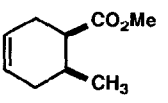
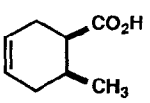
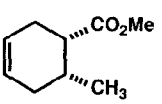
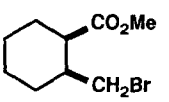
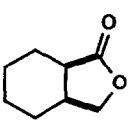
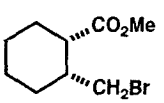
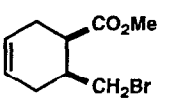
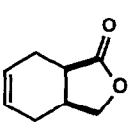
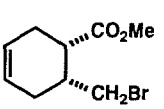
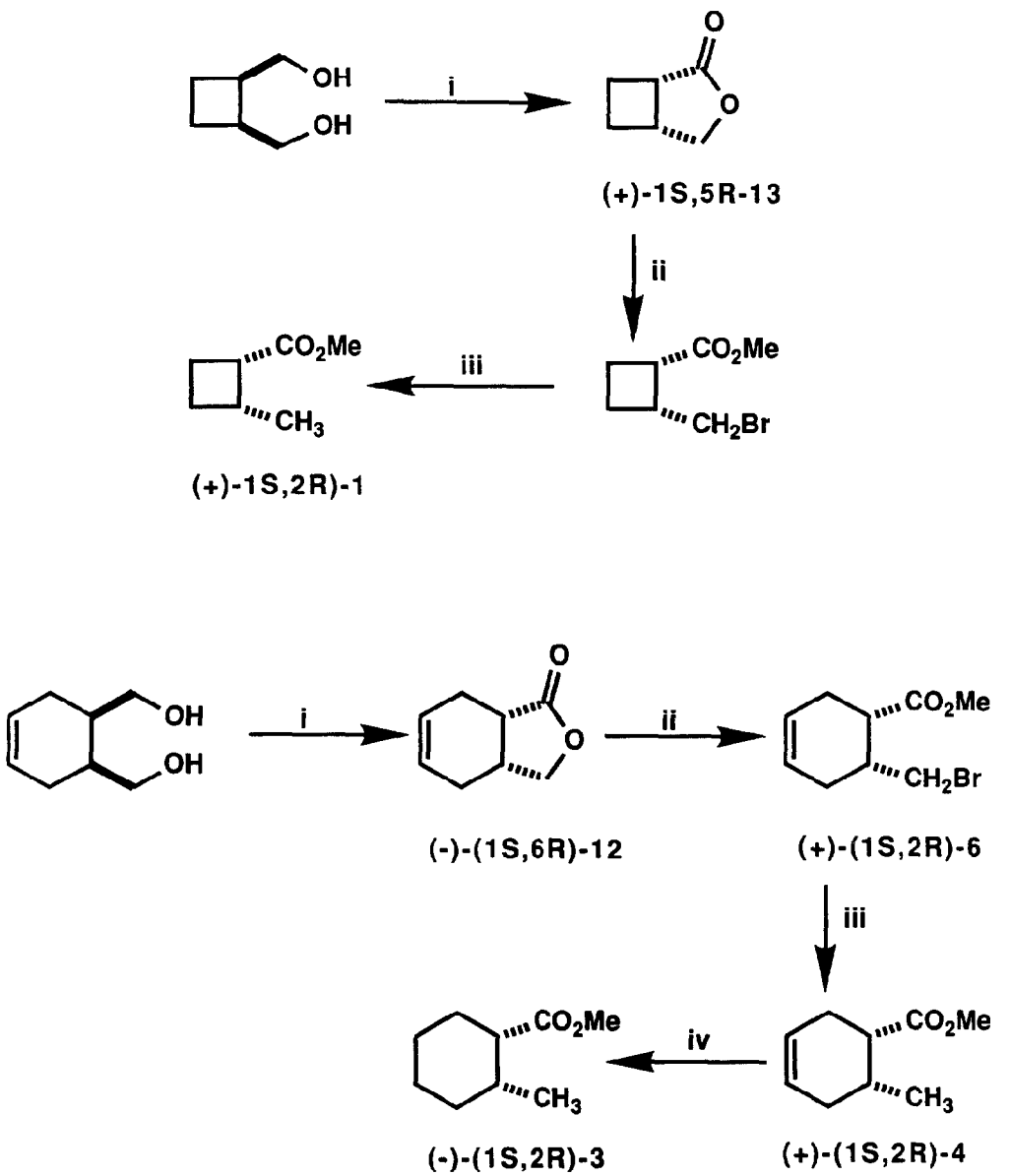


Figure 1. Perspective (a) and top (b) views of the active site model of PLE. The model, which is fully described in ref.7a, is composed of four binding regions - one large (H_L) and one small (H_S) hydrophobic pocket and two more polar cavities at the front (P_F) and back (P_B) of the active site that can accommodate electron rich functions and that can act as a hydrogen bond acceptors. The ester group to be hydrolyzed must locate in the serine nucleophile region (dotted circle). The boundaries of the pockets represent the physical constraints imposed by the side chains of the amino acids forming the pockets, and groups may not penetrate through them. Hydrophobic moieties of substrates bind preferentially in the smaller H_S site, unless they are too large to do so, when they bind by default in H_L. The model is utilized by placing the ester group to be hydrolyzed in the serine hydroxyl sphere, then fitting the remainder of the substrate into the appropriate binding regions. All of the stereochemically distinct binding possibilities are examined. The best fit represents the binding mode which will predominate and thereby forecasts the stereochemistry of the hydrolyzed product. In the binding analyses depicted in the subsequent Figures, the top perspective of the model is used throughout.

Table I. Results of PLE-Catalyzed Hydrolyses of (±)-1-6

 (±)-1	 (+)-(1S,2R)-7 90%, ≥97% ee	+  (-)-(1R,2S)-2 64%, ≥97% ee
 (±)-2	 (+)-(1R,2S)-8 57%, 22% ee	+  (+)-(1S,2R)-2 42%, 17% ee
 (±)-3	 (+)-(1R,2S)-9 93%, ≥97% ee	+  (-)-(1S,2RS)-3 91%, ≥97% ee
 (±)-4	 (-)-(1R,2S)-10 86%, ≥97% ee	+  (+)-(1S,2R)-4 94%, ≥97% ee
 (±)-5	 (-)-(1R,6S)-11 98%, ≥97% ee	+  (+)-(1S,2R)-5 100%, ≥97% ee
 (±)-6	 (+)-(1R,6S)-12 96%, ≥97% ee	+  (+)-(1S,2R)-6 97%, ≥97% ee



Scheme 1. Absolute configuration correlations. Reagents: (i) HLADH (ii) HBr/MeOH (iii) Bu_3SnH/AIBN (iv) H_2/Pd.

then recovered by acidification of the reaction mixtures, followed by re-extraction. In the case of substrates **5** and **6**, both unhydrolyzed ester and hydrolyzed product were recovered by extraction at pH 7.0, as the acid products cyclized spontaneously to the lactones (-)-**11** and (+)-**12** under the reaction conditions. The lactones were separated from the residual esters chromatographically. The results of the PLE-catalyzed hydrolyses are shown in Table 1.

Enantiomeric excesses of the product acids **7** - **10** were determined by NMR using the (+)-R-1-methylbenzylamine salt method of Schneider et al.[8] Enantiomeric excesses of the residual esters **1** - **6** were determined by NMR examination of diastereomeric signals in the presence of the chiral shift reagent $Eu(hfc)_3$. Enantiomeric excesses of the chiral lactones (-)-**11** and (+)-**12** were determined using the method of Jakovac and Jones.[9]

The absolute configuration of the products (+)-**2** and (+)-**8** from PLE-catalyzed hydrolysis of 1-carbomethoxy-2-cyclopentane ((±)-**2**) were determined by comparison of the rotation of the ester product with the rotation of optically active 1-carbomethoxy-2-methylcyclopentane of known absolute configuration.[10] Naturally, in a resolution of this type the hydrolyzed acid must have the opposite absolute configuration to the residual ester. Absolute configurations of the remaining products were determined by converting optically active lactone (-)-**12** of known (1S,6R) absolute configuration to (+)-**4** and (-)-**3**, and lactone (+)-**13** (also of known absolute configuration) to (+)-(1S,2R)-**1**, as outlined in Scheme 1. The reference lactones (-)-**12** and (+)-**13** were prepared using horse liver alcohol dehydrogenase (HLADH) according to the method of Jakovac and Jones.[9]

PLE is thus seen to exert excellent stereospecificity in the hydrolysis of the four-membered ring substrate (±)-**1** and the six-membered ring substrates (±)-**3** - **6**, with the ring-sized controlled reversal of stereoselectivity paralleling that previously noted for the analogous series of meso 1,2-diesters.[5a] The use of chiral, monocyclic, structures in synthesis is well documented,[11] and these products further extend the current range of enzymically available chirons of this category.

ACTIVE SITE MODEL ANALYSIS

Interpretation of the stereochemical course of PLE-catalyzed hydrolyses of (±)-**1** and (±)-**4** using the active site model of PLE are shown in Figures 2 and 3 respectively. The analyses are representative for all the Table I substrates and the model accounts completely for the experimentally observed results in each instance. As in the cases of both monocyclic and acyclic meso diester substrates, the size-induced reversal of the stereochemical sense of hydrolysis is accounted for on the basis of substrate orientations controlled by the binding properties of two different sized hydrophobic pockets, H_L and H_S. The initial model suggested that this

stereoselectivity reversal should be a general phenomenon. The current experimental results are in total accord with this prediction.

In the initial specification of the model,[7a] the P_F pocket functioned primarily to bind a second, non-hydrolyzed ester group of a meso-diester substrate. The Figure 3 analysis now shows that other groups, including hydrophobic moieties, can extend into the P_F binding site without adversely affecting the overall binding. Thus the ES-complex leading to hydrolysis of the 1R-ester group of (1R,2S)-4 places the 2S-methyl group entirely in the P_F pocket. This binding mode dominates completely because all catalytically productive orientations of the enantiomeric (1S,2R)-ester are unfavourable, such as that illustrated in Figure 3 (b) in which the (2R)-methyl group has to extend through the wall of the H_L pocket.

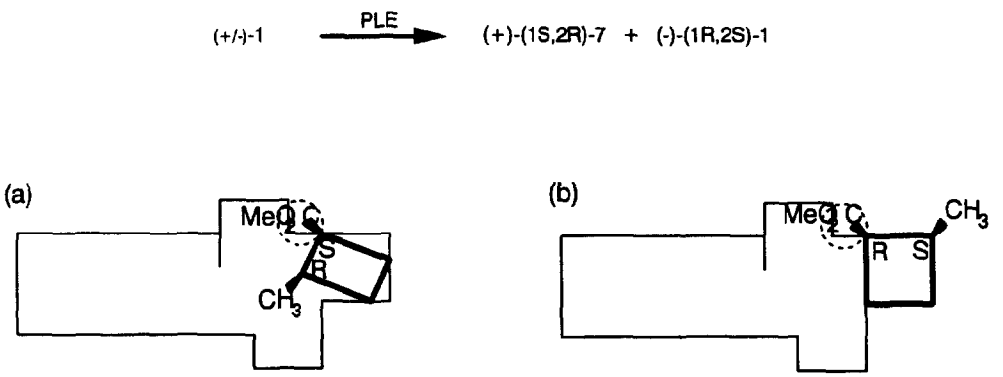


Figure 2. Hydrolysis of (±)-cis-1-Carbomethoxy-2-methylcyclobutane ((±)-1). In (a), the orientation shown leads to a preferred ES-complex since the cyclobutane ring of the (1S,2R)-enantiomer is nicely accommodated in H_S while the ester group sits in the serine region (dotted circle) and the methyl group in the empty space above and between the P-pockets. This ES-complex is fully allowed and thus hydrolysis to the observed product (+)-(1S,2R)-7 occurs readily. For the enantiomer, (1R,2S)-1, binding of the cyclobutane ring in the H_S pocket as shown in (b) cannot occur as it would require penetration of the 2S-methyl group through the pocket's boundary. Alternative orientations with cyclobutane in H_L are energetically much less preferred than for H_S binding[7a], and do not lead to a favoured ES-complex. Thus hydrolysis of this enantiomer does not occur.

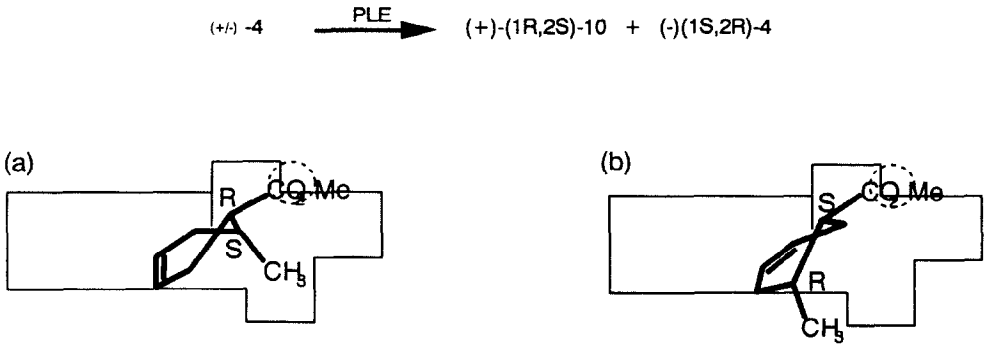


Figure 3. Hydrolysis of (±)-cis-1-Carbomethoxy-2-methyl-3-cyclohexene ((±)-4). With six-membered ring substrates, the ring binding must clearly take place in the larger H_L pocket. Also, it has been shown that equatorial, or pseudo-equatorial, ester orientation is preferred by PLE.[20] (a) This orientation of the (1R,2S)-**4** locates the 1R-ester group in the catalytic serine region (dotted circle) and places all other functions in allowed space, with the C-2 methyl group in P_F. This is therefore a preferred ES-complex, and leads to (1R,2S)-**10**. (b) The (1S,2R)-**4** enantiomer cannot bind with its ester group in the serine locus without violating the active site boundaries, as shown here with the methyl group breaching H_L. The analysis is analogous for the bromomethyl ester (±)-**6** and the cyclohexane derivatives (±)-**3,5**. In the case of the cyclopentane ester (±)-**2**, a racemic product is obtained because the cyclopentane ring marginally exceeds the optimum fit dimensions of H_S and thus binds with a slight partiality for H_L, thus leading to minor preference for hydrolysis of the (1R,2S)-**2** enantiomer (cf. ref. 7a), in accord with the 22% ee level of (1R,2S)-**8** observed experimentally.

EXPERIMENTAL

Melting points are uncorrected and were taken on an Electrothermal Capillary Melting Point Apparatus. IR spectra were recorded as KBr disks (for solids) or as films on a Nicolet 5DX FTIR spectrophotometer. 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 carried out 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

(±)-cis-1-Carbomethoxy-2-methylcyclobutane ((±)-1).

Cyclobutane-1,2-dicarboxylic acid anhydride (5.5 g, 11% yield) was prepared according to the method of Buchman et al.:[12] mp 75-77°C (lit.[12] mp 76.6-77°C); IR 1850, 1776 cm^{-1}; ^1H NMR (400 MHz) δ 2.35-2.42 (m, 2H), 2.71-2.79 (m, 2H), 3.49-3.53 (m, 2H) ppm. The anhydride was converted to the lactone (±)-**13** (1.4 g, 79% yield) according to the method of Bailey and Johnson:[13] bp 80°C (5.0 mm Hg) (lit.[13] bp 109-113°C (15 mm Hg)); IR 1764 cm^{-1}; ^1H NMR δ 2.00-2.80 (m, 4H), 2.95-3.40 (m, 2H), 4.02-4.50 (m, 2H) ppm. The lactone (±)-**13** (2.0 g, 17.9 mmol) was dissolved in methanol (15 mL). The stirred mixture was cooled to 0°C, and anhydrous HBr bubbled through the solution for 45 min. The solution was then warmed to 21°C and stirred for 18 h, then added to water (50 mL) and extracted with diethyl ether (5 X 50 mL). The combined organic extracts were dried ($MgSO_4$) and rotary evaporated. The residue was Kugelrohr-distilled to give (±)-cis-1-carbomethoxy-2-bromomethyl-cyclobutane (2.73 g, 74% yield): bp 70°C (10 mm Hg); IR 1742 cm^{-1}; ^1H NMR δ 2.10-2.75 (m, 4H), 2.90-3.45 (m, 2H), 3.55 (br d, J=7Hz, 2H), 3.75 (s, 3H) ppm. (±)-cis-1-Carbomethoxy-2-bromomethyl-cyclobutane (2.5 g, 12.1 mmol) in hexane (50 mL, containing catalytic AIBN) was reduced with tributyltin hydride (3.83 g, 13.3 mmol) in hexane (50 mL) to give cis-(±)-1-carbomethoxy-2-methylcyclobutane ((±)-**1**, 1.0 g, 68% yield): bp 80°C (100 mm Hg) (lit.[14] bp 62°C (30 mm Hg)); IR 1735 cm^{-1}; ^1H NMR δ 1.05 (d, J=6.5Hz, 3H), 1.50-3.30 (m, 6H), 3.75 (s, 3H) ppm.

(±)-cis-1-Carbomethoxy-2-methylcyclopentane ((±)-2).

6,6-Dichloro-bicyclo[3.2.0]-7-heptanone (14.3 g, 50% yield) was prepared according to the method of Hine and Hahn:[15] bp 80°C (0.8 mm Hg) (lit.[16] bp 73°C (1.5 mm Hg)); IR 1801 cm^{-1}; ^1H NMR δ 1.20-2.85 (m, 6H), 3.45-3.65 (m, 1H), 4.00-4.46 (m, 1H) ppm. 6,6-Dichloro-bicyclo[3.2.0]-7-heptanone (8 g, 45 mmol) was dissolved in methanol (50 mL) and KOH (48 mmol, 46mL 1.03 M solution in methanol) was added with stirring at 0°C . The reaction mixture was stirred at 0°C for

30 min. 12 M Hydrochloric acid (2 mL) was added, and the mixture rotary evaporated. The residue was taken up in diethyl ether (70 mL) and washed with saturated aqueous sodium bicarbonate (2 X 20 mL), and then with brine (1 X 20 mL), dried ($MgSO_4$) and rotary evaporated. The residual oil was Kugelrohr-distilled and chromatographed on silica gel (hexane-ethyl acetate (19:1) elution) to give (±)-cis-1-carbomethoxy-2-(dichloromethyl)cyclopentane (8.12 g, 86% yield): bp 60°C (6 mm Hg); IR 1731 cm^{-1}, ^1H NMR δ 1.80-2.10 (m, 6H), 2.85-3.20 (m, 2H), 3.75 (s, 3H), 6.15 (d, J=9Hz, 1H) ppm. (±)-1-Carbomethoxy-2-(dichloromethyl)cyclopentane (4.0 g, 19 mmol) in hexane (40 mL, containing catalytic AIBN) was reduced with tributyltin hydride (11.6 g, 40 mmol) in hexane (40 mL) to give (±)-cis-1-carbomethoxy-2-methylcyclopentane ((±)-**8**, 2.6 g, 96% yield): bp 80°C (30 mm Hg) (lit.[10] bp 80°C (27 mm Hg)); IR 1735 cm^{-1}; ^1H NMR δ 1.01 (br d, J=6.5Hz, 3H), 1.2-2.0 (m, 6H), 2.50-3.00 (m, 2H), 3.60 (s, 3H) ppm.

(±)-cis-1-Carbomethoxy-2-bromomethyl-4-cyclohexene ((±)-6).

The method of Bailey and Johnson[13] was used to prepare the lactone **12** (1.8 g, 67% yield) from commercially available 1,2,3,6-tetrahydrophthalic anhydride: bp 80°C (0.1mmHg) (lit.[16] bp 85°C (0.1 mmHg)); IR 1775 cm^{-1}; ^1H NMR δ 1.30-2.80 (m, 6H) 3.55-4.20 (m, 2H), 5.48 (br s, 2H) ppm. The lactone (±)-**12** (2 g, 14.5 mmol) was dissolved in methanol (50 mL). The stirred mixture was cooled to 0°C, and anhydrous HBr bubbled through the solution for 45 min. The solution was then warmed to 21°C and stirred for 18 h. Solid $NaHCO_3$ was added until cessation of effervescence. The mixture was filtered, and rotary evaporated. The residual solid was taken up in diethyl ether (80 mL), dried ($MgSO_4$) and rotary evaporated. The residual oil was purified by flash chromatography on silica gel ((19:1) hexane-ethyl acetate elution) to give (±)-cis-1-carbomethoxy-2-bromomethyl-4-cyclohexene ((±)-**6**, 2.72 g, 81% yield): bp 80°C (3 mm Hg); IR 1734 cm^{-1}; ^1H NMR δ 2.20-2.50 (m, 4H), 2.90-3.15 (m, 2H), 3.55-3.85 (m, 2H), 3.70 (s, 3H), 5.60 (br s, 2H) ppm. Anal. Calc'd for $C_9H_{13}O_2Br$: C 46.37%, H 5.62%, Br 34.28%; Found: C 46.14%, H 5.54%, Br 34.48%.

(±)-cis-1-Carbomethoxy-2-methyl-4-cyclohexene ((±)-4).

(±)-cis-1-Carbomethoxy-2-bromomethyl-4-cyclohexene ((±)-**6**, 5.0 g, 22 mmol) in hexane (50 mL, containing catalytic AIBN) was reduced with tri-n-butyl tin hydride (6.9 g, 24 mmol) in hexane (30 mL) to give (±)-cis-1-carbomethoxy-2-methyl-4-cyclohexene ((±)-**4**, 3.0 g, 92% yield): bp 85°C (2.5 mmHg); IR 1735 cm^{-1}; ^1H NMR δ 1.05 (br d, J=7Hz, 3H), 1.95 (m, 2H), 2.10-2.60 (m, 4H), 3.79 (s, 3H), 5.55 (br s, 2H) ppm. Anal. Calc'd for $C_9H_{14}O_2$: C 70.10%, H 9.15%; Found C 70.24%, H 9.01%.

(±)-cis-1-Carbomethoxy-2-bromomethylcyclohexane ((±)-5).

(±)-cis-1-Carbomethoxy-2-bromomethyl-4-cyclohexene ((±)-**6**) was reduced by catalytic hydrogenation (10% Pd/C, 50 psi H_2) in methanol to give (±)-cis-1-carbomethoxy-2-bromomethylcyclohexane ((±)-**5**, 1.8 g, 91% yield): bp 80°C (3 mm Hg); IR 1735 cm^{-1}; ^1H NMR δ 1.00-2.25 (m, 8H), 3.05-3.20 (m, 2H), 3.55-3.68 (br d, J=7Hz, 2H), 3.95 (s, 3H) ppm. Anal. Calc'd for $C_9H_{15}O_2Br$: C 45.98%, H 6.43%, Br 33.98%. Found; C 46.11%, H 6.30%, Br 33.79%.

(±)-cis-1-Carbomethoxy-2-methylcyclohexane ((±)-3).

(±)-cis-1-Carbomethoxy-2-bromomethyl-cyclohexane ((±)-**5**, 5.0 g, 21.4 mmol) in hexane (20 mL, containing catalytic AIBN) was reduced with tri-n-butyl tin hydride (6.8 g, 23.5 mmol) in hexane (40 mL) to give (±)-cis-1-carbomethoxy-2-methylcyclohexane ((±)-**3**, 2.99 g, 91%): bp 80°C (2.5 mm Hg); IR 1735 cm^{-1}; ^1H NMR δ 1.01 (br d, J=7Hz, 3H), 1.40-1.80 (m, 8H), 2.05-2.68 (m, 2H), 3.75 (s, 3H) ppm. Anal. Calc'd for $C_9H_{16}O_2$: C 69.19%, H 10.32%; Found: C 69.04%, H 10.48%.

PLE-Catalyzed Hydrolyses.

The following general procedure was used for all PLE-catalyzed hydrolyses: PLE (n units) was added to a stirred solution of substrate in distilled water, pH 7.0 at 21°C. The pH was maintained at 7.0 by addition of 0.2 M NaOH with a pH stat. The hydrolysis was permitted to continue until one-half equivalent of base had been consumed. The reaction mixture was immediately extracted with CH_2Cl_2 or diethyl ether at pH 7.0 (3 X 50 mL). The combined organic phases were dried ($MgSO_4$) and rotary evaporated to yield the residual ester products. The aqueous phase was acidified to pH 2.0 with 6 M HCl, and re-extracted with CH_2Cl_2 (3 X 50 mL). Evaporation of the dried ($MgSO_4$) organic phase yielded the acid products.

(±)-cis-1-Carbomethoxy-2-methylcyclobutane ((±)-**1**, 403 mg, 3.1 mmol) with PLE (268 units) during 18.5 h gave unhydrolyzed ester ((-)-(1R,2S)-**1**, 128 mg, 64% yield, ≥97% ee): $[\alpha]^{25}_D$ -19.4 (c 5.0, $CHCl_3$); spectra and bp identical to racemic material, and (+)-(1S,2R)-1-carboxy-2-methylcyclobutane ((+)-**7**, 160 mg, 90% yield, ≥97% ee): bp 75° (0.1 mm Hg); $[\alpha]^{25}_D$ +6.8 (c 16.2, $CHCl_3$); IR 3600-3000 (br), 1710 cm^{-1}; ^1H NMR δ 1.25 (d, J=7Hz, 3H), 1.55-2.10 (m, 5H), 3.05 (m, 1H), 11.25 (br s, 1H) ppm.

(±)-cis-1-Carbomethoxy-2-methylcyclopentane ((±)-**2**, 1.0 g, 7 mmol) with PLE (536 units) during 17 h gave unhydrolyzed ester ((+)-(1S,2R)-**2**, 210 mg, 42% yield, 17% ee): $[\alpha]^{25}_D$ +5.6 (c 14.8, $CHCl_3$) (lit.[9] $[\alpha]^{25}_D$ +21.8 (c 1.0, $CHCl_3$)); spectra and bp identical to racemic material, and (+)-

(1R,2S)-1-carboxy-2-methylcyclopentane ((+)-**8**, 254 mg, 57% yield, 22% ee): bp 90°C (0.1 mm Hg); $[\alpha]^{25}_{D}$ +2.4 (c 24.9, $CHCl_3$); IR 3600-3000 (br), 1702 cm^{-1}; ^1H NMR δ 1.10 (d, J=7Hz, 3H), 1.25-2.15 (m, 7H), 2.46-3.08 (m, 1H), 10.80 (br s, 1H) ppm.

(±)-cis-1-Carbomethoxy-2-methylcyclohexane ((±)-**3**, 848 mg, 5.6 mmol) with PLE (536 units) during 14 h gave unhydrolyzed ester ((-)-(1S,2R)-**3**, 386 mg, 91% yield, ≥97% ee: $[\alpha]^{25}_{D}$ -0.41 (c 32.4, $CHCl_3$); bp and spectra identical to racemic material), and (+)-(1R,2S)-1-carboxy-2-methylcyclohexane ((+)-**8**, 370 mg, 93% yield, ≥97% ee: bp 90°C (0.1 mm Hg) (lit.[17] bp 134-5°C (20 mm Hg)); $[\alpha]^{25}_{D}$ +7.40 (c 37.2, $CHCl_3$); lit.[18] $[\alpha]^{25}_{D}$ +10.6 (c 5.95, ethanol)); IR 3600-3000 (br), 1702 cm^{-1}; ^1H NMR δ 1.01 (br d, J=7Hz, 3H), 1.45-2.00 (m, 8H), 2.05-3.00 (m, 2H), 11.85 (br s, 1H) ppm).

(±)-cis-1-Carbomethoxy-2-methyl-4-cyclohexene ((±)-**4**, 817 mg, 5.3 mmol) with PLE (536 units) during 13 h gave unhydrolyzed ester ((+)-(1S,2R)-**4**, 386 mg, 94% yield, ≥97% ee): $[\alpha]^{25}_{D}$ +18.1 (c 34.0, $CHCl_3$); spectra and bp identical to racemic material, and (-)-(1R,2S)-1-carboxy-2-methyl-4-cyclohexene ((-)-**10**, 295 mg, 86% yield, ≥97% ee): bp 90-95°C (l mm Hg) (lit.[14] bp 70-75°C (0.05 mm Hg)); $[\alpha]^{25}_{D}$ -26.9 (c 26.0, $CHCl_3$) (lit.[19] $[\alpha]^{25}_{D}$ -31.77 (c 1.98, $CHCl_3$)); IR 3600-3000 (br), 1703, 1655 cm^{-1}; ^1H NMR δ 1.05 (br d, J=6.5Hz, 3H), 1.85-2.60 (m, 6H), 5.50 (br s, 2H), 11.08 (br s, 1H) ppm.

(±)-cis-1-Carbomethoxy-2-bromomethyl-cyclohexane ((±)-**5**, 800 mg, 3.4 mmol) and PLE (536 units) during 13.5 h gave unhydrolyzed ester and the hydrolyzed acid, present as its lactone derivative (+)-**11** in the pH 7.0 extract. The two products were separated by flash chromatography on silica gel ((19:1) hexane-ethyl acetate elution) to give (+)-(1S,2R)-1-carbomethoxy-2-bromomethylcyclohexane ((+)-**5**, 404 mg, 100% yield, ≥97% ee): $[\alpha]^{25}_{D}$ +11.3 (c 41.0, $CHCl_3$); spectra identical to racemic material above, and (-)-(1R,6S)-8-oxabicyclo[4.3.0]nonan-9-one ((-)-**11**, 234 mg, 98% yield, ≥97% ee): $[\alpha]^{25}_{D}$ -38.0 (lit.[10] for (1S,6R)-isomer $[\alpha]^{25}_{D}$ +38.8 (c 2.37, $CHCl_3$); IR 1770 cm^{-1}; ^1H NMR δ 1.20-2.60 (m, 10H), 4.05-4.65 (m, 2H) ppm.

(±)-cis-1-Carbomethoxy-2-bromomethyl-4-cyclohexene ((±)-**6**, 828 mg, 3.6 mmol) with PLE (536 units) during 13.5 h gave unhydrolyzed ester (+)-(1S,2R)-1-carbomethoxy-2-bromomethyl-4-cyclohexene ((+)-**6**, 402mg, 97% yield, ≥97% ee): $[\alpha]^{25}_{D}$ +29.0 (c 31.0, $CHCl_3$); spectra identical to racemic material above, and (+)-(1R,6S)-8-oxabicyclo[4.3.0]non-3-en-9-one ((+)-**12**, 236 mg, 96%

yield, ≥97% ee): $[\alpha]^{25}_D$ +50.9 (c 18.1 in $CHCl_3$) (lit.[10] for (1S,6R)-isomer $[\alpha]^{25}_D$ -55.5 (c 0.38, $CHCl_3$); IR 1765, 1655 cm^{-1}; ^1H NMR δ 1.80-2.80 (m, 6H), 3.85-4.60 (m, 2H), 5.85 (br s, 2H) ppm.

Enantiomeric Excess Determinations.

Enantiomeric excesses of ester products **1** to **6** were determined by examination of diastereotopic signals in the presence of the chiral shift reagent $Eu(hfc)_3$. The enantiomeric excesses of acid products **7** to **10** were determined by examination of ^1H NMR spectra in the presence of (+)-R-methylbenzylamine.[8] Enantiomeric excesses of the lactone products **11** and **12** were determined by ^1H NMR examination of the diol products after reaction with methyl lithium, in the presence of $Eu(tfc)_3$.[9]

Absolute Configuration Determinations.

The absolute configurations of ester **1** and acid **7** were determined by conversion of (+)-(1S,5R)-3-oxabicyclo[3.2.0]heptan-2-one of known absolute configuration, as follows: (+)-(1S,5R)-3-oxabicyclo[3.2.0]heptan-2-one ((+)-**13**, 111 mg, 57% yield) was prepared according to the method of Jakovac et al.[10] bp 80°C (5 mm Hg); $[\alpha]^{25}_D$ +116.7 (c 2.0, $CHCl_3$) (lit.[10] bp 100°C (10 mm Hg)$[\alpha]^{25}_D$ +118.7 (c 10, $CHCl_3$)); IR 1774 cm^{-1}; ^1H NMR δ 1.80-2.75 (m, 6H), 2.80-3.50 (m, 2H), 4.06-4.40 (m, 2H) ppm.

(+)-(1S,5R)-3-Oxabicyclo[3.2.0]heptan-2-one ((+)-**13**) was opened to the ester in exactly the same fashion as for (±)-**12** above to give (+)-(1S,2R)-1-carbomethoxy-2-bromomethylcyclobutane (207 mg, 94% yield): $[\alpha]^{25}_D$ +24.6 (c 2.9, $CHCl_3$); bp and spectra identical to racemic material above.

(+)-(1S,2R)-1-Carbomethoxy-2-bromomethylcyclobutane was debrominated in exactly the same fashion as (±)-**4** to give (+)-(1S,2R)-1-carbomethoxy-2-methylcyclobutane ((+)-**1**, 128 mg, 97%): $[\alpha]^{25}_D$ +22.4°C (c 12, HCl_3); bp and spectra identical to racemic material above.

The absolute configurations of **2** and **8** were determined by comparison of rotation of (+)-**2** with optically active 1-carbomethoxy-2-methyl-cyclopentane (**2**) of known absolute configuration.[10]

Absolute configurations of **3** and **4** were determined by transformation of (-)-(1S,6R)-8-oxabicyclo[4.3.0]non-3-en-9-one ((-)-**12**) of known absolute configuration.

The method of Jakovac et al.[10] was used to produce optically active lactone (-)-**12** (1.24 g, 65% yield): bp 80-90°C (0.5 mmHg); $[\alpha]^{25}_D$ -62 (c 35.0, $CHCl_3$) (lit.[10] bp 85°C (0.1 mmHg)$[\alpha]^{25}_D$ -67.1 (c 1, $CHCl_3$)); IR 1769, 1655 cm^{-1}; ^1H NMR δ 1.80-2.80 (m, 6H), 3.81-4.40 (m, 2H), 5.85 (br s, 2H) ppm.

The lactone (-)-(1S,6R)-**12** was ring-opened with HBr in exactly the same fashion as for (±)-**12** above, to give (+)-(1S,2R)-1-carbomethoxy-2-bromomethyl-4-cyclohexene ((+)-(1S,2R)-**6** (1.1 g, 66% yield): $[\alpha]^{25}_{D}$ +37.2 (c 13.9, $CHCl_3$); bp and spectra identical to racemic material above.

(+)-(1S,2R)-1-Carbomethoxy-2-bromomethyl-4-cyclohexene ((+)-**6**) was debrominated in exactly the same fashion as above for (±)-**6** to give (+)-(1S,2R)-1-carbomethoxy-2-methyl-4-cyclohexene ((+)-**4**, 530 mg, 94% yield): $[\alpha]^{25}_{D}$ +25.6 (c 14.5, $CHCl_3$); bp and spectra identical to racemic material above.

(+)-(1S,2R)-1-Carbomethoxy-2-methyl-4-cyclohexene ((+)-**4**) was hydrogenated in exactly the same fashion as (±)-**4** above, to give (-)-(1S,2R)-1-carbomethoxy-2-methylcyclohexane ((-)-(1S,2R)-**3**, 168 mg, quant. yield): bp 80°C (2 mm Hg); $[\alpha]^{25}_{D}$ -1.2 (c 8.3, $CHCl_3$); bp and spectra identical to racemic material above.

The absolute configurations of lactone products **11** and **12**, as well as the residual esters **5** and **6** respectively were determined by comparison of the optical rotations of the lactones with those of (+)-(1S,6R)-8-oxabicyclo[4.3.0]-7-nonanone and (-)-(1S,6R)-8-oxabicyclo[4.3.0]non-3-en-7-one respectively, both of known absolute configuration.[10]

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