Enzymes in Organic Synthesis. 35.[1] Stereoselective Pig Liver Esterase Catalyzed Hydrolyses of 3-Substituted Glutarate Diesters. Optimization of Enantiomeric Excess via Reaction Conditions Control

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Pig liver esterase catalyzed hydrolyses of C-3-substituted dimethyl glutarates are enantiotopically selective, giving acid ester products of 17–79% ee under normal (aqueous, pH 7, 20 °C) hydrolysis conditions. The stereoselectivity can be increased by optimizing the reaction conditions. For example, in 20% aqueous methanol of pH 7 at −10 °C hydrolysis of the 3-methyl diester gives the 3-methyl acid ester of 97% ee. The hydrolysis is *pro-S* selective for the diesters with small C-3 substituents and reverses to *pro-R* preference when C-3 is large. An active site model consistent with these data is presented.

The asymmetric synthetic opportunities provided by exploiting the chiral catalytic properties of enzymes are well documented.[2] Esterases, which do not require expensive cofactors, are particularly attractive in this regard. Pig liver esterase (PLE, E.C. 3.1.1.1) is one of the hydrolytic enzymes with considerable asymmetric synthetic potential whose value for the production of useful chiral synthons has already been demonstrated.[2,3] The abilities of enzymes such as PLE to induce stereospecific transformations on prochiral substrates are particularly important, as illustrated by the numerous examples of enantiotopically selective PLE-catalyzed hydrolyses of symmetrical diesters that have been documented.[3] 3-Substituted glutaric acid diesters **1** are attractive precursors for such reactions since the chiral acid ester products **2**, or their lactone **3** derivatives, are versatile chiral synthons for targets such as (*R*)- and (*S*)-mevalonolactone (from **2h**),[4] β-lactams (from **2i,j**),[5] verrucarinic acid (from **2a**),[6] and pimaricin (from **2k**).[7]

From the literature data,[4-7] it is clear that PLE-catalyzed hydrolyses of 3-substituted glutarate diester substrates **1** can provide acid esters **2** of very high or complete enantiomeric purities, and our initial studies on the conversions of **1a–g** to **2a–g** also appeared to be in this category.[8]

Table I. PLE-Catalyzed Hydrolyses of 1a–g[a]

substr	acid ester (%)	lactone (% yield, % ee)	abs config
1a	**2a** (95)	**3a** (72, 79)	*R*
1b	**2b** (67)	**3b** (66, 50)	*R*
1c	**2c** (78)	**3c** (68, 25)	*R*
1d	**2d** (98)	**3d** (78, 38)	*S*
1e	**2e** (95)	**3e** (91, 17)	*R*
1f	**2f** (98)	**3f** (53, 42)	*S*
1g	**2g** (95)	**3g** (98, 54)	*S*

[a] At pH 7, 20 °C.

Table II. Optimization of Enantiomeric Excess via Reaction Conditions Control[a]

substr	prod.	pH[b]	org solv	temp (°C)	ee (%)[c]
1a	**2a**	7		20	79
1a	**2a**	6		20	71
1a	**2a**	7	5% MeCN	20	70
1a	**2a**	7	5% Me_2CO	20	72
1a	**2a**	7	5% Me_2SO	20	81
1a	**2a**	7	5% MeOH	20	88
1a	**2a**	6	5% MeOH	20	86
1a	**2a**	6	10% MeOH	20	92
1a	**2a**	7	5% MeOH	0	93
1a	**2a**	6	5% MeOH	0	92
1a	**2a**	7	10% MeOH	0	93
1a	**2a**	7	20% MeOH	−10	97
1a	**2a**	7	30% MeOH	−20	[e]
1g	**2g**	7[d]		20	54
1g	**2g**	7[d]	20% MeOH	−10	81

[a] All reactions performed in 0.03 M KH_2PO_4. [b] Apparent pH in presence of organic solvents. [c] Determined on corresponding lactones **3a,g**. [d] No buffer present. [e] No reaction.

However, with our current enzyme we do not obtain the same high levels of ee (enantiomeric excess) of **2a–g**. This paper reports the ee's that are consistently achievable in the **1** → **2a–g** reactions and on how manipulation of reaction conditions can be used to optimize enantiomeric purities if the stereoselectivity of PLE under the initial conditions is inadequate.

Results

The diester substrates **1a–g** were prepared in a straightforward manner from their precursor diacids, as were the racemic lactones (±)-**3a–g** required as reference standards for the ee determinations.

PLE-catalyzed hydrolyses of **1a–g** were performed at pH 7. The pH was maintained at this level by the addition

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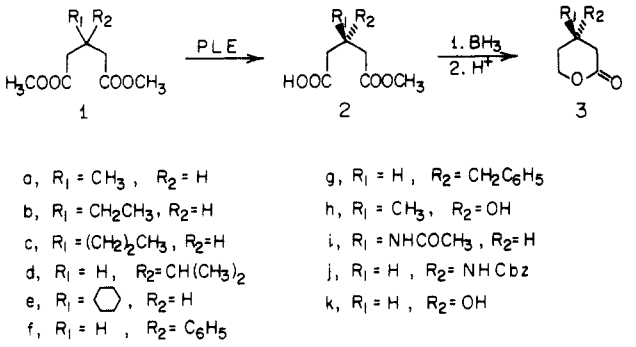

of 0.5 M aqueous sodium hydroxide, and each reaction was worked up after 1 equiv of base had been taken up. The acid ester products **2a–g** were converted to their corresponding lactones **3a–g**. The absolute configurations of **3a–g**, and hence of **2a–g**, were established by comparison with authentic samples.[9] The ee's of **3a–g** were determined by GLC analyses of their (2*R*,3*R*)-butanediol-derived ortho esters, by using the ortho esters of the racemic lactones as reference standards.[10] The results are summarized in Table I.

The effect of reaction conditions on ee was evaluated for the **1a,g** → **2a,g** conversions. The results are summarized in Table II. The best conditions for the **1a** → **2a** reaction were found to be 20% aqueous methanol, pH 7, −10 °C. These conditions also enhanced the product ee in the **1g** → **2g** hydrolysis to a significant extent.

Discussion

Because the acid esters **2a–g** are much poorer substrates than their diester precursors **1a–g**, the PLE-catalyzed reactions of Table I conveniently terminate themselves after 1 ester equiv has been hydrolyzed. The acid ester products **2a–g** were converted to their corresponding lactones **3a–g** to facilitate the ee[10] and absolute configuration[9] determinations. Attempts to determine the ee's of **2a–g** directly[3d] were unsuccessful.

The C-3 substituent of the diesters **1a–g** has a significant effect on both the rates and stereoselectivities of the PLE-catalyzed hydrolyses. The hydrolysis rates are fastest when the C-3 substituent is small and fall progressively as the C-3 group becomes larger. The fastest reaction (with **1a**) is over 25 times more rapid than that for the slowest substrate (**1f**). Increasing the size of the C-3 substituent also induces a reversal of the enzyme's stereoselectivity. Under the normal pH 7, 20 °C hydrolysis conditions (Table I), the 3-methyl substrate **1a** yields **2a** of 79% ee and of *R* absolute configuration. The absolute configuration sense remains *R* for the C-3-ethyl (**1b**) and -propyl (**1c**) cases, but the stereoselectivity decreases progressively. For the still more bulky substituents of **1d,f**, and **g**, the opposite *S* stereoselectivity predominates to an ever increasing extent. Reversals of PLE stereospecificity induced by substituent size have been observed previously with malonate,[3h] aminoglutarate,[5] and monocyclic[2g,i] diester substrates. All of these data indicate that, in addition to the ester hydrolysis region, the active site of PLE possesses at least two other binding sites.[11,12] One is relatively small

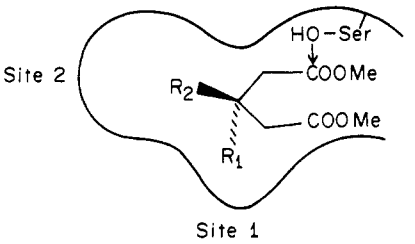

Figure 1. Active site orientations of glutarate diesters **1a–g**. The ester group to be hydrolyzed must locate adjacent to the nucleophilic serine function ↓. The C-3 substituents R_1 or R_2 can then locate in either the small site 1 or the large site 2. Site 1 binding dominates until its steric dimensions are exceeded.

and accommodates groups up to approximately *n*-propyl in size. The other site can accommodate much larger groups. Binding to the smaller site is dominant unless its steric dimensions are exceeded. Only then will a substituent prefer to locate in the larger binding site. The situation envisaged is represented schematically in Figure 1.[13]

The ee's of the chiral products **2a–g** of Table I are too low for them to be acceptable as synthons in asymmetric synthesis. However, the commercially available PLE is a mixture of isozymes that respond differently to variations in reaction conditions such as pH, temperature, and the presence of buffer and organic solvents.[14,15] Accordingly, we explored the possibility of exploiting these variations to expand the diastereomeric transition state energy differences between the competing enantiotopic ester group hydrolysis pathways.

The most detailed study was performed on the **1a** → **2a** reaction. As Table II shows, lowering the pH from 7 to 6 reduced the ee of **2a** from 79% to 71%. Although the nature of the buffer can influence ee,[16–18] in this case the phosphate buffer used served only to accelerate the rate of hydrolysis. Addition of acetonitrile and acetone was deleterious. In contrast, the presence of 5% dimethyl sulfoxide increased the ee to 81%. The addition of 5% methanol was even more beneficial. The presence of 5–10% methanol also overcame the negative effect of lowering the pH from 7 to 6. Consequently, the remaining optimization experiments were performed in methanolic solutions. The final parameter explored was temperature. Progressive lowering of the reaction temperature resulted in a steady increase of ee. The best results were observed at −10 °C in 20% aqueous methanol, under which condition **2a** of 97% ee was obtained. At temperatures lower than −10 °C, the hydrolysis rate became impracticably slow. This degree of enantiomeric purity is now acceptable for most asymmetric synthesis applications. A similar, albeit not complete, improvement of ee was observed with the 3-benzyl substrate **1g**, for which the enzymic hydrolysis is of opposite enantiotopic selectivity to that for **1a**. In this case rising of the ee of **2g** from 54% to 81% was induced by applying the −10 °C, 20% aqueous methanol

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(12) The two-binding site concept has been recognized independently by Bjorkling et al.[3h]

(13) The marginal *R* absolute configuration preference for the cyclohexyl product **2e** may be due to a favoring of a different ES complex in which the cyclohexyl group binds at site 2 and the *pro-R* ester group at site 1. Further evidence supporting and quantifying the Figure 1 active site concept, and its implications with respect to the other active site models that have been proposed,[14,15] will be reported shortly.[16]

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conditions. While further optimization was not explored at this time, the identification of still more beneficial reaction conditions leading to additional improvements in ee should not present a problem. The temperature coefficients for these PLE-catalyzed hydrolyses in the −10 to +20 °C range studied approximate the normal twofold per 10 °C values.

The reasons for the effects induced by the different solvents are not clear. For nucleophilic solvents such as methanol, both the influence on enzyme conformation and interference with acylation and deacylation steps must be considered.

The ability to optimize ee's when the stereoselectivity of an enzyme is inadequate under normal conditions represents an important and valuable technique that will extend the synthetic potential of enzymes considerably. Since the approach is based on identifying conditions that expand the energy difference between the transition states of competing diastereomeric pathways, it should be widely applicable. It has already been applied successfully to some extent with alcohol dehydrogenases[19] and with lipases,[20,21] for which the improvements achieved in epoxide ester resolutions represent the first major success of the reaction conditions manipulation technique.[20] It will be particularly important for enzymes such as PLE for which variations in ee with different enzyme batches are documented.[22] While the present optimization procedures were formulated empirically, statistical methods based on factorial[20,24] or simplex[25] approaches will be more reliable in the long term.[26]

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Boiling points refer to Kugelrohr distillations. IR spectra were recorded on films with a Nicolet FT-IR spectrometer. ^{1}H NMR spectra were obtained for $CDCl_3$ solutions ($(CH_3)_4Si$ internal standard) on a Varian T-60 instrument and ^{13}C NMR data for $CDCl_3$ solutions with a Varian CFT-20. All enzymic reactions were performed with a Radiometer or Metrohm pH-stat. GLC analyses were carried out either on a Varian 2700 chromatograph with a 10 ft × 2 mm 5% QF-1 column at 115–170 °C or on a Varian 3400 instrument equipped with a Supelcowax 10 capillary column at 170 °C, both with flame ionization detectors. Optical rotations were measured on $CHCl_3$ solutions with a Perkin-Elmer 141 polarimeter. Pig liver esterase (PLE, E.C. 3.1.1.1) was Sigma Chemical Company type II (Lot 123F-0240).

Preparations of Dimethyl 3-Substituted Glutarates 1a–g. Each substrate diester was prepared by esterification of the precursor diacid with diazomethane (**1a,g**) under Fischer conditions (**1e**), or by the general procedure of Riegel et al.[27] (**1b–d,f**).

3-Methylglutaric acid (Aldrich) gave 1a: 99% yield; bp 70–75 °C/0.05 mmHg (lit.[28] bp 110 °C/19 mmHg); IR ν 1737 cm^{-1}; ^{1}H NMR δ 0.99 (3 H, d, J = 6 Hz), 2.12–2.63 (5 H, m), and 3.70 (6 H, s).

3-Ethylglutaric acid[9c] yielded 1b: 95% yield; bp 55 °C/0.3 mmHg; IR ν 1740 cm^{-1}; ^{1}H NMR δ 0.9 (3 H, distorted t, J = 6 Hz), 1.4 (2 H, m), 2.37 (5 H, m), and 3.67 (6 H, s); ^{13}C NMR δ 10.1, 26.0, 33.0, 37.1, 50.5, and 172.0. Anal. Calcd for $C_9H_{16}O_4$: C, 57.43; H, 8.57. Found: C, 57.19; H, 8.62%.

3-*n*-Propylglutaric acid[9c] afforded 1c: 86% yield; bp 52–55 °C/0.1 mmHg; IR ν 1740 (s), 1780 (m) cm^{-1}; ^{1}H NMR[29] δ 0.9 (3 H, m), 1.1–1.5 (4 H, m), 2.37 (5 H, br s), and 3.70 (6 H, s); ^{13}C NMR[29] δ 13.7, 19.4, 31.6, 36.0, 38.0, 51.0, and 172.6. Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.39; H, 8.97. Found: C, 59.44; H, 8.85.

3-Isopropylglutaric acid[9a] yielded 1d: 93% yield; bp 55 °C/0.1 mmHg; IR ν 1735 cm^{-1}; ^{1}H NMR δ 0.9 (6 H, d, J = 6 Hz), 1.75 (1 H, m), 2.24 (5 H, m), and 3.68 (6 H, s); ^{13}C NMR δ 18.3, 29.9, 35.1, 37.2, 50.7, and 172.5. Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.39; H, 8.97. Found: C, 58.97; H, 8.92%.

3-Cyclohexylglutaric acid[9b] gave 1e: 94% yield; bp 65–70 °C/0.15 mmHg (lit.[30] bp 106–108 °C/17 mmHg); IR ν 1736 cm^{-1}; ^{1}H NMR δ 0.9–2.0 (11 H, m), 2.35 (5 H, br s), and 3.66 (6 H, s); ^{13}C NMR δ 26.1, 29.2, 35.5, 36.8, 40.6, 50.9, and 172.8. Anal. Calcd for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15. Found: C, 64.49; H, 8.92.

3-Phenylglutaric acid[9a,31] yielded 1f: 91% yield; mp 85–87 °C (lit.[29] mp 87–88 °C, lit.[32] mp 85–87 °C); IR (Nujol) ν 1732 cm^{-1}; ^{1}H NMR[29] δ 2.77 (4 H, m), 3.4–3.85 (1 H, m), 3.6 (6 H, s), and 7.23 (5 H, s); ^{13}C NMR[29] δ 38.1, 40.1, 51.2, 126.7, 127.0, 128.4, 142.4, and 171.7.

3-Benzylglutaric acid[33] afforded 1g: quant. bp 112–114 °C/0.4 mmHg; IR ν 1736 cm^{-1}; ^{1}H NMR δ 2.20–2.80 (7 H, m), 3.70 (6 H, s), and 7.0–7.5 (5 H, m). Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.00; H, 7.42%.

PLE-Catalyzed Hydrolyses of Diesters 1a–g. The following procedure is representative. A rapidly stirred solution of dimethyl 3-methylglutarate (**1a**) (1.0 g, 5.75 mmol) in 0.03 M KH_2PO_4 buffer (30 mL) at 20 °C was neutralized to pH 7 with 0.5 M aqueous NaOH by using a pH-stat. PLE (400 units) was then added, and the pH of the solution was maintained at 7. The hydrolysis proceeded until 1 equiv of base had been consumed (50 min), at which point it stopped. The reaction mixture was frozen at −78 °C and then just thawed and a saturating amount of NaCl was added. The resulting cold solution was washed with ether (2 × 30 mL) to remove unreacted diester then acidified to pH < 2.5 with concentrated HCl. Extraction with ether (3 × 70 mL) followed by evaporation of the dried ($MgSO_4$) ether extracts and Kugelrohr distillation yielded the acid ester **2a**:[34] (860 mg, 94% yield); bp 65–68 °C/0.05 mmHg; IR ν 1713, 1735, 2410–3712 cm^{-1}; ^{1}H NMR δ 1.02 (3 H, d, J = 6 Hz), 1.93–2.70 (5 H, m), 3.67 (3 H, s), and 9.45–10.33 (1 H, br s).

The above acid ester **2a** (850 mg, 5.3 mmol) was dissolved with stirring under N_2 in dry THF (15 mL) at −78 °C, and BH_3·Me_2S (5.3 mL of 2 M THF solution, 10.6 mmol, 2.0 equiv) was added dropwise with a syringe.[35] The mixture was then allowed to warm to 20 °C and stirred further for 3 h under N_2. Routine workup, by adding methanol (1.0 mL) to destroy excess BH_3 followed by rotary evaporation or by adding ether followed by saturated aqueous NaCl, yielded a mixture of methyl 5-hydroxy-3-methylpentanoate and lactone **3a**. This was dissolved directly in dry benzene (20 mL) containing toluene-*p*-sulfonic acid, and the mixture was refluxed for 4 h in a Dean-Stark apparatus. Evaporation of the benzene followed by Kugelrohr distillation gave (+)-(4*R*)-4-methyltetrahydropyran-2-one (**3a**): 440 mg, 73% yield, 79% ee; bp 78–80 °C/0.5 mmHg (lit.[9a] bp 93–94 °C/0.02 mmHg); $[\alpha]_D^{25}$ + 21.7° (*c* 4) (lit.[9a,b] $[\alpha]_D^{25}$ −23.6° (*c* 1) for (4*S*)-**3a** of 78% ee). Spectral characteristics of (4*S*)-**3a** and of **3b–g** below were as reported previously.[9]

The hydrolyses of the other diesters **1b–g** were carried out similarly, with the following results, which are summarized in Table I.

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Dimethyl 3-ethylglutarate (1b): 200 mg, 1.06 mmol; 0.1 M KH_2PO_4 buffer (20 mL); PLE (160 U); 20 °C; pH 7 for 6 h gave acid ester **2b**: 124 mg, 67% yield; bp 60–65 °C/0.01 mmHg; IR ν 1710, 1739, and 2300–3600 cm^{-1}; ^1H NMR δ 0.9 (3 H, app t, J = 6 Hz), 1.43 (2 H, br d of q, J = 6 Hz), 2.2–2.55 (5 H, m), 3.68 (3 H, s), and 10.4 (1 H, br s). Reduction of **2b** (120 mg, 0.69 mmol) with BH_3·Me_2S (0.38 mL of 2 M THF solution, 1.1 equiv) followed by lactonization gave (+)-(4*R*)-4-ethyltetrahydropyran-2-one (**3b**): 58 mg, 66% yield, 50% ee; bp 55–58 °C/0.05 mmHg (lit.[9c] bp (±) 84 °C/1.5 mmHg); $[\alpha]_D^{25}$ +12.7° (*c* 3.1) (lit.[9b] $[\alpha]_D^{25}$ +12.9° (*c* 1) for (**4*R*)-3b** of 53% ee).

Dimethyl 3-*n*-propylglutarate (1c): 200 mg, 0.99 mmol; suspension in 0.1 M KH_2PO_4 buffer (20 mL); PLE (160 units); 20 °C; pH 7 for 9 h yielded acid ester **2c**: 145 mg, 78% yield; bp 80–85 °C/0.03 mmHg; IR ν 1709, 1740, and 2400–3600 cm^{-1}; ^1H NMR δ 0.65–1.5 (7 H, m), 2.2–2.5 (5 H, m), 3.70 (3 H, s), and 10.9 (1 H, br s). Reduction of **2c** (140 mg, 0.74 mmol) with BH_3·Me_2S (0.41 mL of 2 M THF solution, 1.1 equiv) followed by lactonization afforded (+)-(4*R*)-4-*n*-propyltetrahydropyran-2-one (**3c**): 72 mg, 68% yield, 25% ee; bp 55–65 °C/0.025/mmHg (lit.[9c] bp (±) 90–91 °C/1.5 mmHg); $[\alpha]_D^{25}$ +5.6° (*c* 5.8) (lit.[9b] $[\alpha]_D^{25}$ +5.2° (*c* 1) for (**4*R*)-3c** of 24% ee).

Dimethyl 3-isopropylglutarate (1d): 500 mg, 2.47 mmol; suspension in 0.1 M KH_2PO_4 buffer (40 mL); PLE (320 U); 20 °C; pH 7 for 10 h gave acid ester **2d**: 460 mg, 99% yield; bp 90 °C/0.05 mmHg; IR ν 1710, 1739, and 2400–3600 cm^{-1}; ^1H NMR δ 0.93 (6 H, d, J = 7 Hz), 1.4–1.85 (1 H, m), 2.1–2.5 (5 H, m), 3.70 (3 H, s), and 11.1 (1 H, br s). Reduction of **2d** (360 mg, 1.9 mmol) with BH_3·Me_2S (1.05 mL of 2 M THF solution, 1.1 equiv) and then lactonization yielded (−)-(4*S*)-4-isopropyltetrahydropyran-2-one (**3d**): 211 mg, 78% yielded, 38% ee; bp 60–65 °C/0.01 mmHg (lit.[9a] bp (−)61–62 °C/0.05 mmHg, (±)98–105 °C/0.1 mmHg); $[\alpha]_D^{25}$ −11.1° (*c* 7.3) (lit.[9b] $[\alpha]_D^{25}$ −14.4° (*c* 1) for (**4*S*)-3d** of 46% ee).

Dimethyl 3-cyclohexylglutarate (1e): 500 mg, 2.06 mmol; suspension in 0.1 M KH_2PO_4 buffer (40 mL); PLE (320 U); 20 °C; pH 7 for 12.5 h yielded acid ester **2e**: 445 mg, 95% yield; bp 115–120 °C/0.04 mmHg; IR ν 1709, 1739, and 2300–3600 cm^{-1}; ^1H NMR δ 0.8–2.1 (11 H, m), 2.15–2.6 (5 H, m), 3.70 (3 H, s), and 11.5 (1 H, br s). Reduction of **2e** (345 mg, 1.51 mmol) with BH_3·Me_2S (0.83 mL of 2 M THF solution, 1.1 equiv) and then lactonization afforded (−)-(4*R*)-4-cyclohexyltetrahydropyran-2-one (**3e**): 250 mg, 91% yield, 17% ee; bp 85–90 °C/0.01 mmHg (lit.[9b] bp (±) 120–121 °C/0.2 mmHg), $[\alpha]_D^{25}$ −3.4° (*c* 8) (lit.[9b] $[\alpha]_D^{25}$ −1.5° (*c* 1) for (**4*R*)-3e** of 10% ee).

Dimethyl 3-phenylglutarate (1f): 500 mg, 2.12 mmol; vigorously stirred suspension in 0.1 M KH_2PO_4 buffer (40 mL); PLE (320 U); 20 °C; pH 7 for 25 h gave acid ester **2f** as a crystallizing syrup: 460 mg, 98% yield; IR (Nujol) ν 1697, 1731, and 2300–3400 cm^{-1}; ^1H NMR δ 2.55–2.85 (4 H, m), 3.35–3.85 (1 H, m), 3.55 (3 H, s), 7.23 (5 H, m), and 10.65 (1 H, br s). Reduction of **2f** (300 mg, 1.35 mmol) with BH_3·Me_2S (0.74 mL of 2 M THF solution, 1.1 equiv) followed by lactonization afforded, after chromatographic purification,[36] (+)-(4*S*)-4-phenyltetrahydropyran-2-one (**3f**): 127 mg, 53% yield, 42% ee; $[\alpha]_D^{25}$ +1.73° (*c* 6) (lit.[37] $[\alpha]_D^{25}$ +3.80° for (**4*S*)-3f** of 98% ee).

Dimethyl 3-benzylglutarate (1g): 1.0 g, 4 mmol; suspension in 0.03 M KH_2PO_4 (30 mL); PLE (200 U); 20 °C, pH 7 for 4 h yielded acid ester **2g**: 893 mg, 95% yield; bp 140–142 °C/0.25 mmHg; IR ν 1707, 1737, and 2500–3550 cm^{-1}; ^1H NMR δ 2.2–2.9 (7 H, m), 3.67 (3 H, s), 7.0–7.5 (5 H, m), and 9.8–10.8 (1 H, br s). Reduction of **2g** (850 mg, 3.6 mmol) with BH_3·Me_2S (3.6 mL of 2 M THF solution, 2.0 equiv) and then lactonization gave (−)-(4*S*)-4-benzyltetrahydropyran-2-one (**3g**): 672 mg, 98% yield, 54% ee; bp 136–138 °C/0.4 mmHg; mp 58–67 °C (lit.[9c] bp 161–163 °C/1 mmHg); $[\alpha]_D^{25}$ −20.1° (*c* 2) (lit.[9b] $[\alpha]_D^{25}$ −19.1 (*c* 1) for 20% ee).

Optimization of Enantiomeric Excess Study. The effects of buffer, pH, organic solvent, and temperature on the ee of the **1a** → **2a** → **3a** and **1g** → **2g** → **3g** reactions were studied by using the general procedure described above for **1a** hydrolyses. The PLE proportions used ranged from 70 U/mmol of substrate (S) for the fastest (50 min at 20 °C, pH 7, no organic solvent) reactions to 140 U/mmol of S for the slowest (8.5 h at −10 °C, pH (approximately) 7, 20% aqueous MeOH) one. For **1g**, 50 U of PLE/mmol of S required 3.5 h at 20 °C, pH 7, no organic solvent and 6 days with 100 U of PLE/mmol of S at −10 °C, pH (approximately) 7, 20% aqueous MeOH, with an additional 100 U of PLE/mmol of S added at day 3 and day 4. The results are summarized in Table II.

Enantiomeric Excess Determinations. The ee's of the optically active lactones **3a–f** derived from the PLE-hydrolysis reactions were determined by converting each into its corresponding ortho ester with (2*R*,3*R*)-butane-2,3-diol followed by GLC analysis.[10] The racemic lactones (±)-**3a–f**[9a–c] required as the reference standards were prepared by reductions of the corresponding anhydrides[9a–c,38] with $NaBH_4$.[39] The results are summarized in Tables I and II and are considered accurate to within ±2%.

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Registry No. **1a**, 19013-37-7; **1b**, 91478-78-3; **1c**, 58219-47-9; **1d**, 2338-44-5; **1e**, 91478-79-4; **1f**, 19006-47-4; **1g**, 91478-80-7; **2a**, 63473-60-9; **2b**, 91478-81-8; **2c**, 91478-82-9; **2d**, 101713-09-1; **2e**, 91478-84-1; **2f**, 101713-10-4; **2g**, 101713-11-5; **3a**, 61898-55-3; **3b**, 71301-88-7; **3c**, 71301-89-8; **3d**, 61949-74-4; **3e**, 71302-20-0; **3f**, 61198-49-0; **3g**, 71301-86-5.

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Chemiluminescence from Organic Reactions. Formation of Diphenoyl Peroxide as an Intermediate

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Visible, long-lasting chemiluminescence results when diphenic anhydride and *p*-nitroperoxybenzoic acid in THF are treated with powdered KOH in the presence of a catalytic chemiluminescence (CIEEL) activator. This chemiluminescent process is presumed to involve in situ generation of diphenoyl peroxide, which then undergoes chemiluminescent decomposition by the CIEEL mechanism. Carboxylate **4**, the precursor to diphenoyl peroxide in this reaction, was generated by an entirely different route from peroxide **5**, resulting again in chemiluminecence in the presence of a CIEEL activator.

In 1978 we fully delineated the chemically initiated electron-exchange (CIEEL) mechanism based upon our detailed investigation of the chemiluminescent decomposition of diphenoyl peroxide (DPP, Scheme I).[1] Since then

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