First Preparation of Enantiopure Indane Monomer, (*S*)-(−)- and (*R*)-(+)-2,3-dihydro-3-(4′-hydroxyphenyl)-1,1,3-trimethyl-1H-inden-5-ol, via a Unique Enantio- and Regioselective Enzymatic Kinetic Resolution

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Compound **1**, (2,3-dihydro-3- (4′-hydroxyphenyl)-1,1,3-trimethyl-1H-inden-5-ol), a highly valuable monomer was prepared for the first time into its two enantiomerically pure forms via enzyme catalyzed kinetic resolution of corresponding **1**-diesters. Hydrolysis of **1**-dipentanoate catalyzed by lipase from *Chromobacterium viscosum* (CVL) is highly regioselective (38:1) between two phenyl groups and highly enantioselective ($E = 48$) toward a remote quaternary chiral center (five bonds), yielding (*S*)-(−)-**1**-4′-monopentanoate and unreacted (*R*)-(+)-**1**-dipentanoate in high yield and excellent ee. Unlike other hydrolase-catalyzed reactions, the CVL-catalyzed reaction does not proceed to sequential hydrolysis of (*S*)-(−)-**1**-4′-monopentanoate to (*S*)-(−)-**1**, showing that the reaction is also highly chemical selective between **1**-diester and **1**-monoester. The structural preference of the reaction was clearly determined by ^{1}H and COSY NMR. The absolute configuration of the nonreacted (*R*)-(+)-**1**-dipentanoate was determined consistently by circular dichroism and X-ray crystallography after being chemically transformed to (*R*)-(+)-**1** and derivatives. Surprisingly, CVL favors the carbonyl group on the more substituted phenol, which has more steric hindrance, shorter bond length (1.39 Å compared to 1.41 Å on less substituted phenyl), and was believed to be the less favored group. In brief, in this reaction, the more substituted phenol group is preferred. (*S*)-enantiomer is preferred. **1**-Diesters are substrates while corresponding **1**-monoesters are not. The unique feature of CVL provides a simple access to enantiopure diol **1**, corresponding **1**-monoestesrs, **1**-homodiesters, as well as **1**-mixed diesters.

Introduction

Optical communication systems of the next generation of information technologies require a high quality of second-order nonlinear optical (NLO) materials for high-speed information transfer vehicles, namely, photons. These materials can be used for optoelectronic modulation or frequency doubling, information storage, optical signal processing and display, polarizing coating, optic fiber, and optical switches and devices. Research has been focused on four types of materials including inorganic crystals, semiconductors, glass optical fibers, and polymers.[1] Among these, polymers are exceptionally promising materials. Unlike other three types of materials, which are generally fragile, polymers are suitable for microfabrication and can be made inexpensively. To be practically useful, the polymers should possess large second-order nonlinear optical response, that is, nonvanishing first molecular hyperpolarizability and good thermal and mechanical stability for microfabrications. At the molecular level it is necessary that chromophores be incorporated into a polymer with rigid noncentrosymmetric macroscopic structures. Several approaches have been used in the development of such polymers, namely, embedding the chromophores in the backbone or alignment of NLO side chains as pendants.[2]

Chirality is another factor to consider in order to enhance NLO. It is expected that the use of enantiomerically pure monomer (one handed) in the polymer backbone might avoid NLO fading effects, because of the uniformity of the bond orientation compared to its racemic counterpart. Compound **1**, 2,3-dihydro-3- (4′-hydroxyphenyl)-1,1,3-trimethyl-1H-inden-5-ol, is a monomer bearing two asymmetrically located chromophores with hyperpolarizability. It has been widely used in polymeric materials. These materials are known for their inherent toughness, clarity, thermal and mechanical stability, and good optical properties and have been the subject of hundreds of patents.[3] The structure of compound **1** contains a quaternary chiral center. It is therefore a chiral molecule and readily available in racemic form. It is of great interest for both industry and academia to prepare this compound in its enantiopure forms. However, the preparation of enantiomers of this compound has been a challenge. Because of the lack of an efficient method for the formation of a quaternary chiral center, an asymmetric synthesis, if possible, needs a multistep sequence and an efficient method to introduce chirality and transfer it onto the quaternary carbon

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Scheme 1. Hydrolysis of Racemic 1-Dipentanoate May Yield Two Regioisomers of Monopentanoates (each of which consists of a pair of enantiomers) and Two Enantiomers of Diol 1

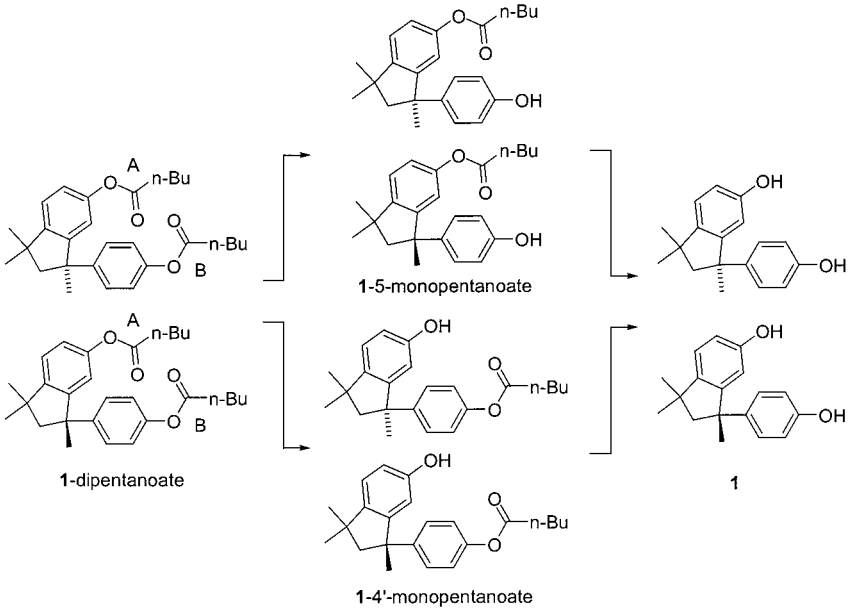


Table 1. Regioselectivity and Enantioselectivity of the Best Hydrolases toward 1-Dipentanoate and 1-Didecanoate[a]

lipase	**1**-diesters	rate (U/mg)	(de/me/diol)[b]	ee (ac) of **1**	ee (**1**-4′-me)	E[c]
bacterial lipase						
CVL (Sigma)	dipentanoate	5.1	55/45/0	na	92 (*S*)	38 ± 18[d]
CVL (Sigma)	didecanoate	6.8	87/12/0	na	60 (*S*)	4.3
CVL (Asahi)	dipentanoate	4.8	64/36/0	na	86 (*S*)	22 ± 4
CVL (ATCC 6918)	dipentanoate	nd	96/4/0	na	81 (*S*)	11
fungal lipases						
RDL (Amano D)	dipentanoate	0.1	33/14/51	64 (*S*)	nd	9[e]
RDL (Amano D)	didecanoate	0.12	45/12/43	67 (*S*)	nd	8[e]
ROL (Amano F)	dipentanoate	0.5	37/16/47	74 (*S*)	43	13[e]
ROL (Amano F)	didecanoate	0.5	84/7/9	71 (*S*)	nd	6[e]
CAL-A	dipentanoate	0.05	18/40/24	46 (*R*)	nd	3[e]
CAL-A	didecanoate	0.05	62/22/16	52 (*R*)	nd	8[e]

[a] de: diester; me: monoester; nd: not determined; na: not applicable; ac: absolute configuration; Amano D: lipase from *Rhizopus delemar* supplied by Amano; Amano F: lipase from *Rhizopus oryzae* supplied by Amano; CAL-A: lipase from *Candida antarctica*, fraction A supplied by Boehringer Mannheim Biochemicals as Chirazyme L-5. [b] Molar ratios of **1**-dipentanoate, **1**-monopentanoates, and diol **1** measured by HPLC. Peak areas were corrected for differences in extinction coefficient. [c] Enantiomeric ratio as defined by Chen *et al.* (ref 6). [d] Average and standard deviation of eight measurements. The data listed are for one of these measurements. [e] Approximate value of the overall enantioselectivity calculated from the amount and enantiomeric purity of diol using equations for a single-step resolution.

without racemization. Enzymatic kinetic resolution appears to be the simplest strategy. The technology uses enzymes to catalyze a reaction enantioselectively, leading two enantiomers in a racemic mixture of starting material into two different chemical forms, which are then easily separated. In this paper, we report the first preparation of enantiopure **1** and its derivatives via a unique lipase-catalyzed enantioselective and regioselective hydrolysis of corresponding **1**-diesters. This work has led to successful preparation of a series of chiral polymers with unparalleled optical properties.[4]

As shown in Scheme 1, there are two distinguishable ways that hydrolysis can proceed. Hydrolysis of the acyl group A or B may yield two different regioisomers of **1**-monoester, **1**-4′-monoester, and **1**-5-monoester, respectively. Each regioisomer consists of a pair of enantiomers. Sequential hydrolysis of the second acyl group on these monoesters yields **1**. Depending on the selectivity of each step and the reaction extension, the result might be a mixture of all possible compositions of the four different compounds and their enantiomers. To achieve a practically useful kinetic resolution of this complex system, at least one step of high enantioslectivity (*E*) is needed.[5] As molecular modeling is still in its infancy in the selection of biocatalysts, a screening was carried out in order to find the right enzyme.

Results

Selection of Hydrolases. Twenty-one mammalian, fungal, and bacterial hydrolases were screened for their ability to catalyze the hydrolysis of (±)-**1**-dipentanoate. Fourteen hydrolases showed catalytic activity. Of these, four gave moderate to high enantiomeric purity for the products. These four enzymes were tested again using (±)-**1**-didecanoate as substrate. The results are shown in Table 1.[6]

Unlike other hydrolases, which gave a mixture of diol **1**, **1**-monoesters and unreacted **1**-diester at the end of reaction, lipase from *Chromobacterium viscosum* (CVL)

(4) (a) U.S. 5, 856, 422 (**1999**). (b) U.S. 5, 777, 063 (**1998**). (c) U.S. 5, 883, 218 (**1999**).

(5) A rational analysis of possibilities of resolution is available as Supporting Information.

(Sigma) gave a single **1**-monopentanoate as product and unreacted **1**-dipentanoate. The product was determined to be (*S*)-(−)-**1**-4′-monopentanoate (see below for details on structure determination). The regioselectivity was higher than 30:1 and the enantioselectivity $E = 38 \pm 12$.[7] A remarkable drop of the *E* value was observed using (±)-**1**-didecanoate as substrate ($E = 4.3$), indicating that the chain length of acyl is an influential factor on *E*.

CVL from two other sources, Asahi and the culture supernatant from a lipase-producing strain of CVL (ATCC 6918), were also investigated, both of which were less enantioselective toward (±)-**1**-dipentanoate. The former demonstrated an *E* value of 22 and the latter a value of 11.

Three fungal lipases catalyzed the hydrolysis of (±)-**1**-dipentanoate or (±)-**1**-didecanoate to a mixture of **1**, corresponding **1**-monoesters, and unreacted **1**-diester. Both *Rhizopus delemar* lipases (RDL, Amano D) and *Rhizopus oryzae* lipase (ROL, Amano F) yielded (*S*)-(−)-**1** with similar enantiomeric purity. *Candida antarctica* lipase (CAL-A) yielded the (*R*)-(+)-**1** with lower enantiomeric purity. Hydrolysis of (±)-**1**-diesters to **1** requires removal of two ester groups and each step may be enantioselective. We did not determine the enantioselectivity of each step; instead, we estimated an overall enantioselectivity for both steps from the amount and ee of **1** using equations for a single-step hydrolysis. This estimate shows that the overall *E* for hydrolysis of (±)-**1**-dipentanoate or (±)-**1**-didecanoate to **1** by RDL, ROL, and CAL-A is substantially lower than the single step CVL (Sigma)-catalyzed hydrolysis of (±)-**1**-dipentanoate to (*S*)-(−)-**1**-4′-monopentanoate. Consistent with this estimate, the ee of **1** from these reactions was significantly lower than the ee of (*S*)-(−)-**1**-4′-monopentanoate from the CVL-catalyzed hydrolysis.

An attempted acylation of **1** with vinyl butanoate using CVL in *tert*-butyl methyl ether gave no detectable acylated product after 3 days incubation at room temperature.

Optimization of Enantioselectivity with Different Acyl Chain Length. The variation of the *E* value versus acyl chain lengths was examined. In the case of Amano D, the difference of chain length was less influential. A similar composition of products and almost the same ee of **1** were obtained using (±)-**1**-dipentanoate and (±)-**1**-didecanoate ($E = 9$ and 8, respectively). No remarkable improvement was observed. In the case of Amano F, a similar ee was obtained at a lower conversion using (±)-**1**-didecanoate. This indicates a decrease of enantioselectivity, which was reflected in the decrease on overall *E* (13 to 6. In the case of CAL-A, the enantioselectivity was slightly improved ($E = 3$ to 8). These data were shown in Table 1.

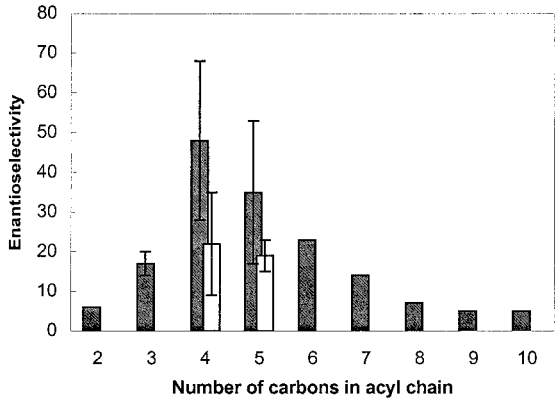


Figure 1. Variation in the enantioselectivity of CVL-catalyzed hydrolysis of **1**-diesters with different acyl chain length. CVL from Sigma (diagonal lines) showed the highest enantioselectivity, $E = 48 \pm 20$, for the dibutanoate. CVL from Asahi (white) showed lower enantioselectivity. The error bars represent standard deviations for three to eight separate measurements, some with different lots of lipase.

Table 2. Enantioselectivity of *Chromobacterium vicosum* Lipase toward 1-Diesters with Different Acyl Chain Length

ester	rate (U/mg)	ratio[a] (de/me)	ee of me (%)	E
diacetate	15	34.0/63.4[b]	42.3	5
dipropionate	11.5	62.6/37.4	78.5	14 ± 3
dibutanoate	8	63.9/36.1	93.2	48 ± 20[c]
(Asahi)[d]	(4.6)	(59.5/40.5)	(76.4)	13 (22 ± 13)[c]
dipentanoate	11	61.2/38.8	(86.5)	21 (19 ± 4)
(Asahi)[d]	(6.3)	(64.5/33.5)	92.4	45 ± 18[c]
dihexanoate	0.4	48.8/51.2	78.4	21
diheptanoate	10	96.6/3.4	86.4	12
dioctanoate	2.8	91.2/8.8	63.8	5
dinonanoate	6.4	53.2/46.8	51.1	5
didecanoate	6.8	79.6/20.4	60.1	5

[a] Measured by HPLC monitoring at 272 nm. Baseline resolution of two enantiomers of diol, four isomers of monoester, and overlapped two enantiomers of diesters were obtained on the same chromatogram using Chiralcel OD eluted with ethanol−hexane (8/92), see Supporting Information (1). Peak areas for the monoester were divided by 1.07 to account for the difference in extinction coefficient for diester and monester. [b] 2.6% diol noted in the hydrolysis of the diacetate. [c] Average and standard deviation for six different experiments. [d] Use of CVL supplied by Asahi.

However, the *E* value of CVL-catalyzed hydrolysis of (±)-**1**-diesters varies dramatically with the chain length of esters. This phenomenon was examined using the same source of CVL (Sigma). The highest enantioselectivity was obtained with (±)-**1**-dibutanoate. Both shorter and longer ester chains render the reaction less enantioselective. Some undesired sequential hydrolysis to the diol **1** was observed using (±)-**1**-diacetate. CVL from Asahi was less enantioselective than CVL from Sigma for both (±)-**1**-dibutanoate and (±)-**1**-dipentanoate. The rate of reaction remained constant within a factor of 2 for all chain lengths except (±)-**1**-dihexanoate (slower by a factor of 20) and (±)-**1**-dioctanoate (slower by a factor of 4). See Figure 1 and Table 2.

Structure of 1-Monopentanoate and Regioselectivity. The regioselectivity of the CVL-catalyzed hydrolysis was clearly determined using a simple method. ^{1}H NMR chemical shifts unambiguously show that the acyl

(6) Hydrolases with low selectivity toward (±)-**1**-dipentanoate: pen G acylase (12% ee (*R*) diol, no monoesters, very slow), porcine pancreatic cholesterol esterase (~5% ee diol, 2:2:6:1 ratio of monoesters), porcine liver esterase (22% ee (*S*) diol, 1:1:1:1 ratio of monoesters), lipase from *Candida antarctica* B (8% ee (*R*) diol, trace monesters, very slow), lipase from *Candida rugosa* (21% ee (*R*) diol, 0:1:1:0 ratio of monoesters), lipase from *Humicola lanuginosa* (29% ee (S) diol, 1:12:16:1 ratio of monoesters), lipase from *Pseudomonas cepacia* (0% ee diol, 1:3:3:9 ratio of monoesters), lipase from *Pseudomonas sp*. (Boehringer Mannheim) (35% ee (R) diol, 1:3:2:10 ratio of monoesters). Lipases from *Rhizopus niveus* and *Rhizomucor javanicus* (Amano lipase N and M) were very slow catalysts and yielded approximately equimolar mixtures of all four monoesters. The following lipases showed no detectable hydrolysis: Amano lipases K-10 (*Pseudomonas sp*.), AK (*Pseudomonas fluorescens*), G (*Penicillium camembertii*), R (*Penicillium roqueforti*), L (*Candida lipolytica*), Sigma wheat germ lipase.

(7) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

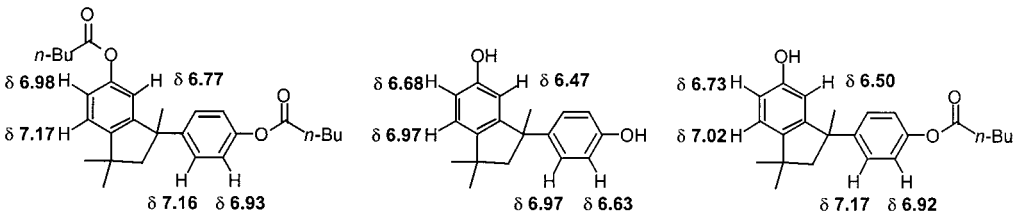


Figure 2. ^{1}H NMR chemical shifts of the aromatic protons in **1**, **1**-dipentanoate, and the **1**-monopentanoate isolated from a CVL-catalyzed hydrolysis of **1**-dipentanoate. All of the aromatic protons in **1** resonate 0.2−0.3 ppm upfield of those in **1**-dipentanoate. In the monopentanoate, the resonances of the more substituted ring lie 0.15−0.27 ppm upfield from those in the dipentanoate, while those in the less substituted ring lie within 0.01 ppm of the dipentanoate. Thus, the ester group lies on the less substituted ring. Resonances were assigned from ^{1}H-^{1}H COSY spectra.

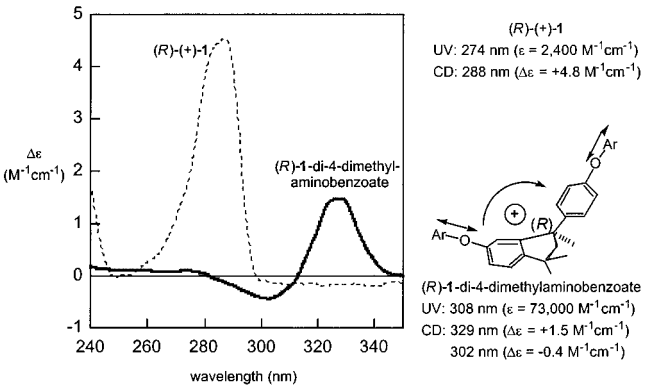
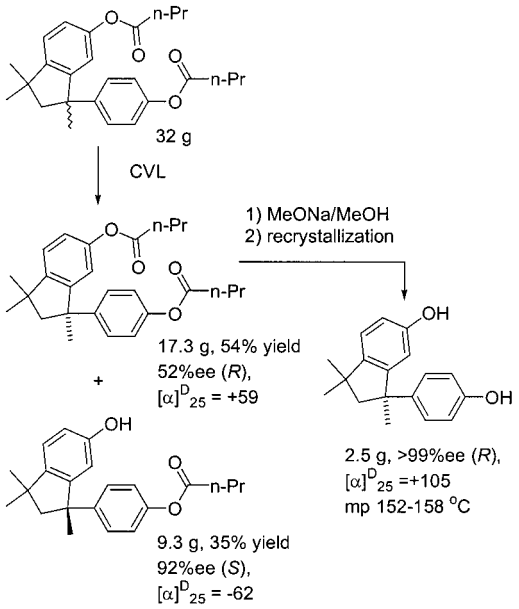


Figure 3. Assignment of absolute configuration using the exiton coupling method. Circular dichroism spectra of (*R*)-(+)-**1** (dotted line) and (*R*)-**1**-di-4-dimethylaminobenzoate (solid line). The bisgnate signal for (*R*)-**1**-di-4-dimethylaminobenzoate shows a positive peak at lower energy, which indicates positive helicity for the orientation of chromophores. Positive helicity corresponds to an (*R*)- absolute configuration.

group of the monoester isolated from CVL-catalyzed hydrolysis of (±)-**1**-pentanoate is on the less substituted phenol. This means, surprisingly, that CVL preferably hydrolyzes the acyl group on the more substituted phenol, which was believed to be the more hindered and less favored one. See Figure 2.

Absolute Configuration. The slow-reacting enantiomer (+)-**1**-dipentanoate was chemically transformed to (+)-**1.** The absolute configuration of the latter was unambiguously established to be (*R*)-(+)-**1** using two independent methods, circular dichroism (CD) and X-ray crystallography. The exiton chirality method assigns the absolute configuration of two chromophores in space when the electronic absorption couples, thereby causing a bisignate CD spectrum. One expects two split CD signals of opposite sign around UV maximum, one is slightly higher and the other slightly lower in energy. Compound (+)-**1** contains two chromophores − phenols − but does not show any split CD signals. Only the lower energy signal appears in the CD spectrum (dotted line) in Figure 3. The higher energy signal is presumably obscured by other electronic transitions. To overcome this obstacle, we added two new chromophores (4-dimethylaminobenzoyl), which has an UV absorption maximum at 308 nm, in the molecule by making (+)-**1**-di-(4-dimethylamino)benzoate. This moves the electronic transitions to lower energy. The (+)-**1**-di-(4-dimethylamino)benzoate shows, indeed, two weak but clear bisignate CD signals: positive at 329 nm and negative at 302 nm as expected for splitting due to exiton coupling. Since the lower energy signal is positive, the benzoate chromophores orient with a positive helicity, which corresponds to the (*R*)-configuration.[8] The helicity model of (+)-(*R*)-**1**-di-(4-dimethylamino)benzoate is shown also in Figure 3.

Scheme 2. Preparative Scale Resolution of Compound 1 via CVL-Catalyzed Hydrolysis of Corresponding (±)-1-Dibutanoate



This (*R*)-configuration was confirmed independently by X-ray crystallography method (see the Supporting Information for X-ray crystallography data and an ORTEP drawing of (*R*)-(+)-**1**).

32 g Scale Resolution of (±)-1-Dibutanoate. The selection of the biocatalysts was stopped on the CVL (Sigma) as it gave high enantioselectivity, high regioselectivity, and unique one-step resolution. The reaction was well characterized and optimized. A large-scale resolution was carried out using 32 g of (±)-**1**-dibutanoate. At 43% conversion, unreacted (*R*)-(+)-**1**-dibutanoate was isolated as a colorless oil (54%, 17.3 g, 52% ee) and (*S*)-(−)-**1**-4′-monobutanoate as a light yellow oil (35%, 9.3 g, 92% ee), corresponding $E = 50$. The (*R*)-(+)-**1**-dibutanoate was cleaved with sodium methoxide and the resulting (*R*)-(+)-**1** was recrystallized from methanol−dichloromethane, yielding pure enantiomer as white crystals (2.5 g, >99% ee), Scheme 2.

Conclusion

Compound **1**, a highly valuable monomer for NLO polymeric materials, was successfully prepared for the

(8) Harada, N., Nakanishi, K. *Circular Dichroic Spectroscopy*; University Science Books: Mill Valley, CA, 1983.

first time in its enantiomerically pure forms via an enzyme-mediated kinetic resolution method. The work has made it possible to prepare a series of polymers with improved NOL properties.[4] The method described in this work also provides simple access to monoesters, homo- and mixed diesters, and all possible derivatives of **1** in pure enantiomeric forms. The CVL-catalyzed hydrolysis of **1**-dibutanoate (**1**-pentanoate) is highly enantio- and regioselective toward the remote quaternary chiral center and it shows a certain unusual quality of the enzyme beyond our understanding. It favors the carbonyl group on the more substituted phenol, which is more hindered, the bond is shorter and is believed to be the less favored group. Nevertheless, the reaction does not proceed to sequential hydrolysis of monoesters to diol, excluding the monoesters as substrates. The origin of these enzyme features remains unknown. Revealing the fundamentals beneath these phenomena will certainly enrich current scientific knowledge.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded at 270 MHz in $CDCl_3$ and COSY NMR were recorded at 500 MHz in $CDCl_3$. Mass spectra were obtained in direct-inlet mode (FAB 6 kV Xe). Circular dichroism spectra were measured in $CHCl_3$, 0.1 cm path length, 5 accumulations at 100 nm/min scan speed, room temperature, 0.2 nm resolution. Bond lengths were obtained from simple modeling using CS Chem3D Pro (CambridgeSoft, Cambridge, MA). All chemicals were purchased from Aldrich-Sigma Canada unless otherwise indicated. THF was dried by distillation from sodium benzophenone ketyl under N_2. Enzymes were purchased from Sigma, Boehringer Mannheim, Amano, and Amresco. Lipase from *Chromobacterium viscosum* was also obtained as generous gift from Asahi (Japan) and prepared in our laboratory from a bacterial strain (ATCC 6918) purchased from American Type Culture Collection. Racemic **1** was a gift from Molecular OptoElectronics Corporation (Watervliet, NY).

Synthesis of 1-Diesters. (Example of 1-dibutanoate; the same procedure was used for all other diesters). Butyric acid chloride (8.9 mmol, 2.4 eq) in dry THF (25 mL) was added over 10 min to a solution of **1** (1.0 g, 3.7 mmol) and triethylamine (0.90 g, 8.9 mmol, 2.4 eq) in dry THF (25 mL). TLC showed complete consumption of **1** after stirring overnight at room temperature. HCl (50 mL, 1 M) was added and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined extracts were washed with $NaHCO_3$ (10%, 3 × 20 mL) and water (2 × 20 mL) and dried over magnesium sulfate (10 g). Chromatography on silica gel eluted with dichloromethane yielded colorless oils in 90−95% yield. The corresponding products are characterized as follows.

(±)-1-Diacetate. R_f = 0.74 (CH_2Cl_2), 0.40 (9/1 hexane/2-propanol); ^{1}H NMR δ 1.1 (s, 3H), 1.3 (s, 3H), 1.7 (s, 3H), 2.2−2.4 (m, 8H), 6.8 (s, 1H), 6.9−7.1 (m, 3H), 7.2 (m, 3H); ^{13}C NMR, δ 21.6, 30.9, 31.2, 31.3, 42.9, 50.6, 59.5, 117.3, 120.1, 120.4, 122.7, 126.9, 121.1, 146.9, 147.1, 147.6, 148.6, 148.9, 149.1, 168.35, 168.5; MS (FAB, M+H$^+$) 353.

(±)-1-Dipropanoate. ^{1}H NMR δ 1.0 (s, 3H), 1.1−1.4 (m, 12H), 1.7 (s, 3H), 2.2 (d, 1H, J = 13 Hz), 2.4 (d, 1H, J = 13 Hz), 2.5−2.6 (m, 4H), 6.8 (d, 1H, J = 2 Hz), 6.9−7.0 (m, 3H), 7.1−7.2 (m, 3H); MS (FAB, M+H$^+$) 381.

(±)-1-Dibutanoate. ^{1}H NMR δ 1.1 (m, 10 H), 1.3 (s, 3H), 1.7 (s, 3H), 1.8 (m, 4H), 2.2 (d, 1H), 2.4 (d, 1H), 2.5(dt, 4H), 6.8 (d, 1H), 7.0 (m, 3H), 7.2 (m, 3H); MS (FAB, M+H$^+$) 409.

(±)-1-Dipentanoate. ^{1}H NMR δ 0.8−1.1 (s+m, 10H), 1.3−1.5 (s+m, 6H), 1.6−1.9 (s+m, 6H), 2.1−2.3 (m, 1H), 2.4−2.6 (s, 6H), 6.8 (s, 1H), 6.9−7.2 (m, 3H), 7.1−7.2 (m, 3H); ^{13}C NMR, δ 14.3, 14.4, 20.4, 22.8, 27.6, 30.1, 31.0, 31.2, 34.6, 43.0, 50.7, 53.8, 59.7, 117.4, 120.08, 120.4, 122.7, 126.9, 147.0, 147.7, 148.5, 148.9, 149.1, 171.2, 171.3; MS (FAB, M+H$^+$) 437.

(±)-1-Dihexanoate. ^{1}H NMR δ 0.8−0.9 (m, 6H), 1.0 (d, 3H), 1.2−1.5 (m, 13H), 1.5−1.8 (m, 6H), 2.2 (m, 1H), 2.4 (m, 1H), 2.5 (m, 3H), 6.7 (d, 1H, J = 2 Hz), 6.9−7.0 (m, 3H), 7.1−7.2 (m, 3H); MS (FAB, M+ H$^+$) 465.

(±)-1-Diheptanoate. ^{1}H NMR δ 0.8−0.9 (m, 12H), 1.0 (s, 3H), 1.2−1.4 (m, 26H), 1.6 (s, 3H), 1.7 (m, 4H), 2.2 (s, 2H), 2.5 (m, 4H), 6.8 (d, 1H, J = 2 Hz), 6.9−7.0 (m, 3H). 7.1−7.2 (m, 3H); MS (FAB, M+ H+) 493.

(±)-1-Dioctanoate. ^{1}H NMR δ 0.9 (m, 6H), 1.1 (s, 3H), 1.2−1.5 (m, 20H), 1.7 (s, 3H), 1.7−1.9 (m, 3H), 2.2 (d, 2 H, J = 13 Hz), 2.4 (d, 1H, J = 13 Hz), 2.5−2.6 (m, 4H), 6.8 (d, 1H, J = 2 Hz), 6.9−7.0 (m, 3H), 7.1−7.2 (m, 3H); MS (FAB, M+ H$^+$) 521.

(±)-1-Dinonanoate. ^{1}H NMR δ 0.9 (m, 7H), 1.1 (s, 3H), 1.2−1.5 (m, 22H), 1.7−1.9 (m, 8H), 2.2 (d, 2 H, J = 13 Hz), 2.4 (d, 1H, J = 13 Hz), 2.5−2.6 (m, 4H), 6.8 (d, 1H, J = 2 Hz), 6.9−7.0 (m, 3H), 7.1−7.2 (m, 3H); MS (FAB, M+H$^+$) 549.

(±)-1-Didecanoate. ^{1}H NMR δ 0.9 (m, 6H), 1.1 (s, 3H), 1.2−1.5 (m, 30H), 1.6−1.8 (m, 8H), 2.2 (d, 2 H, J = 13 Hz), 2.4 (d, 1H, J = 13 Hz), 2.5−2.6 (m, 4H), 6.8 (d, 1H, J = 2 Hz), 6.9−7.0 (m, 2H), 7.0 (d, 1H, J = 2 Hz), 7.1−7.2 (m, 3H); MS (FAB, M+H$^+$) 577.

Mixture of (±)-1−4′-Monopentanoate and (±)-1−5-Monopentanoate. The same procedure as for **1**-diesters, except using one equivalent of pentanoyl chloride and of triethylamine yielded a mixture of diol **1**, **1**-monopentanoates and **1**-dipentanoate: R_f = 0.1, 0.28, 0.73 (CH_2Cl_2). The mixture of **1**-monopentanoates was isolated by flash chromatography on silica gel eluted with dichloromethane: oil, 1.30 g, 50%. The samples were used as the standard mixture for development of HPLC analytical method.

Screening of Hydrolases. Hydrolase (10 mg for most, 0.7 mg for CVL) was dissolved in 1 mL of phosphate buffer (10 mM, pH 7.5) and added to a mixture of the same buffer (3 mL) and **1**-dipentanoate solution (50 mg in 4 mL of *tert*-butylmethyl ether). A pH stat regulated the addition of NaOH (0.1 N) to maintain the pH at 7.5. After 1 h (2 h for reactions where no base was consumed in the first hour), the mixture was poured into 20 mL of diethyl ether. The phases were separated and the aqueous phase was extracted by ether (3 × 10 mL). The combined organic phases were washed with water (2 × 20 mL), dried over magnesium sulfate, filtered, and evaporated. The slurry was dissolved in 2 mL of ethanol for HPLC analysis. Enantioselectivity of CVL was calculated according to Sih's[7] equation from the degree of conversion measured and the enantiomeric purity of the product **1**-4′-monoester. The peak areas were corrected for the relative extinction coefficients at 272 nm: **1**-dipentanoate, 1.00; **1**-4′-monopentanoate, 1.06; **1**, 1.60; **1**-dibutanoate, 1.00; **1**-4′-monobutanoate, 1.07. **1**, 1.70. For other enzymes the hydrolysis proceeded to the formation of diol. We estimated the overall enantioselectivity using the same equation from the fraction of diol and its enantiomeric purity. Note that values for overall enantioselectivity are only estimates.

(*S*)-(−)-1-4′-Monopentanoate and (*R*)-(+)-1-Dipentanoate. Hydrolysis of (±)-**1**-dipentanoate (500 mg) by CVL (3.4 mg, 10000 units, Sigma) using the above-described screening procedure yielded crude product (435 mg). Chromotography of a portion (145 mg) on silica gel (10 g) yielded (*R*)-(+)-**1**-dipentanoate upon elution with CH_2Cl_2−hexane (80/20), followed by (*S*)-(−)-**1**-4′-monopentanoate upon elution with CH_2Cl_2−Et_2O (90/10). (*R*)-(+)-**1**-dipentanoate: oil, 70 mg, 48%; 83% ee, $[\alpha]^{D}_{25}$ +64.6 (c = 2.47, MeOH). NMR spectra were identical with the spectra of the racemate. (*S*)-(−)-**1**-4′-monopentanoate: oil, 45 mg, 40%; 92% ee; $[\alpha]^{D}_{25}$ 73.2 (c = 2.47, MeOH); ^{1}H NMR δ 1.0 (t, 3H, J = 7.4 Hz), 1.0 (s, 3H), 1.3 (s, 3H), 1.4−1.5 (m, 2H), 1.6 (s, 3H), 1.7−1.8 (m, 2H), 2.2 (d, 1H, J = 13 Hz), 2.4 (d, 1H, J = 13 Hz), 2.5 (t, 2H, J = 7 Hz), 6.5 (d, 1H, J = 2 Hz), 6.7 (dd, 1H, J_1 = 2, J_2 = 8 Hz), 6.9 (d, 2H, J = 9 Hz), 7.0 (d, 1H, J = 8 Hz), 7.2 (d, 2H, J = 9 Hz); ^{13}C NMR δ 13.7, 22.3, 27.1, 30.7, 31.1, 34.2, 42.4, 50.4, 59.8, 111.4, 114.7, 120.9, 123.5, 127.7, 144.5, 148.3, 148.6, 150.5, 154.9, 172.6.

(*S*)-(−)-1−4′-Monobutanoate and (*R*)-(+)-1. A 4 L Erlenmeyer flask containing (±)-**1** (21.8 g, 81 mmol) and triethylamine (19.7 g, 195 mmol, 2.4 equiv) dissolved in dry THF (500

mL) was stirred magnetically and cooled in an ice−water bath. Butanoyl chloride (21.0 g, 197 mmol, 2.43 equiv) was added over 20 min. The flask was removed from the ice−water bath and stirred for 1 h. The mixture was washed with $NaHCO_3$ (1 M, 2 × 150 mL) and water (1 × 160 mL) yielding a clear, slightly yellow solution. The solution was dried over $MgSO_4$ and evaporated to a yellow oil (32 g). The oily material was dissolved in *tert*-butyl methyl ether (240 mL) and transferred to a 1 L round-bottomed flask. Phosphate buffer (240 mL, 0.1 M, pH 7.5) was added and the pH was adjusted to 7.20 with sodium hydroxide (1 M). CVL (20.4 mg, 60 000 units, Sigma) dissolved in phosphate buffer (15 mL) was added. The mixture was stirred and the pH was maintained at 7.2 ± 0.3 by the manual addition of sodium hydroxide (1 M). Approximately 20 mL were required in the first 3 h, and an additional 18 mL over the next 2 h. HPLC analysis after 5 h showed 43% conversion and 92% ee of (*S*)-(−)-**1**-4′-monobutanoate. The reaction was stopped by separating the two phases and extracting the aqueous phase with ethyl acetate (4 × 100 mL) and dichloromethane (3 × 100). The aqueous phase containing CVL was stored at −20 °C. The combined organic phases were concentrated by rotary evaporation to a sticky gum which was dissolved in dichloromethane (200 mL), washed with sodium bicarbonate (1 M, 2 × 100 mL) and water (3 × 100 mL), dried over $MgSO_4$ (∼30 g), and concentrated by rotary evaporator to a thick oil (30 g). Chromatography in three portions of 10 g on silica gel column (500 g of 60−200 mesh packed in 7 cm diameter column) yielded *(R)-(+)*-**1**-dibutanoate eluting with dichloromethane: clear oil, 17.3 g, 54%, 51% ee; $[\alpha]^{D}_{25} = +59.3$ (c = 3.25, MeOH); R_f = 0.84; ^{1}H and ^{13}C NMR spectra are identical with the racemate. **1**-Monobutanoate was obtained by eluting with ethyl acetate (R_f = 0.24 in dichloromethane, R_f = 1 ethyl acetate): oil, 9.3 g, 35%, 92% ee; $[\alpha]^{D}_{25}$ −61.6 (c = 3.85, meow); ^{1}H NMR, δ 1.0 (m, 6H) 1.3 (s, 3H), 1.6 (s, 3H), 1.7−1.8 (DT, 2H), 2.2 (d, 1H, J = 13 Hz), 2.4 (d, 1H, J = 13 Hz), 2.5 (t, 2H, J = 7 Hz), 6.5 (d, 1H, J = 2.5 Hz), 6.7 (DD, 1H, J_1= 2.5, J_2= 8 Hz), 6.9 (d, 2H, J = 9 Hz), 7.0 (d, 1H, J = 8 Hz), 7.2 (d, 2H, J = 9 Hz); ^{13}C NMR, δ 13.7, 15.3, 18.5, 30.7, 31.1, 36.3, 42.4, 50.4, 53.5, 59.8, 66.0, 111.4, 114.7, 120.9, 123.5, 127.7, 144.5, 148.3, 148.5, 150.5, 154.8, 172.6. Sodium methoxide (5.1 g of 95% purity, 2.2 equiv) was added to (*R*)-(+)-**1**-dibutanoate (15.7 g, 38 mmol) dissolved in methanol (300 mL) and the solution was stirred for 2 h at room temperature. The reaction mixture was neutralized to pH 7 with HCl (1 M) and diluted water (300 mL) and extracted with ethyl acetate (3 × 250 mL). Evaporation of the combined extracts yielded (*R*)-(+)-**1**: white powder, 10.2 g, 52% ee. The solid was dissolved in a minimum amount of hot methanol and water was added dropwise until the solution became turbid. Upon cooling two crops of powder formed (2.1 g, 3% ee (*R*), 200 mg, 13% ee (*R*)), leaving the mother liquor with 62% ee (*R*). The mixture was then extracted by methylene chloride and the aqueous phase discarded. The solid was recrystallized from methanol−dichloromethane. The first crop yielded white crystals of (*R*)-(+)-**1**: 2.5 g, >99% ee; $[\alpha]^{D}_{25} = +105.2$ (c = 3.50, MeOH); mp 152−158 °C (racemic mp 203−206 °C); NMR spectrum was identical with racemic **1**. Circular dichroism spectrum shows a positive Cotton effect at 285 nm as shown (dotted line) in Figure 3.

(*R*)-(+)-1-Di-4-dimethylaminobenzoate. A solution of 4-dimethylaminobenzoyl chloride (88 mg, 0.47 mmol, 2.5 equiv) in dry THF (5 mL) was added over 10 min to a solution of (*R*)-(+)-**1** (50 mg, 0.19 mmol) and triethylamine (47 mg, 0.46 mmol, 2.5 equiv) in dry THF (10 mL). The reaction was stirred overnight at room temperature. The diol had reacted completely according to TLC. Sodium bicarbonate solution (20 mL, 10%) was added to the reaction mixture. The mixture was extracted with ethyl acetate (3 × 10 mL). The combined extracts were washed by water (3 × 20 mL) and dried over magnesium sulfate (5 g). Column chromatography on silica gel eluted with dichloromethane yielded a white solid: 51 mg, 48%; ^{1}H NMR, δ 1.0 (m, 3H) 1.3 (m, 3H), 1.6 (m, 3H), 2.2 (m, 1H), 2.4 (m, 1H), 3.1 (m, 18H), 6.7 (m, 6H), 6.9−7.2 (m, 3H), 7.9−8.1 (m, 6H). The CD spectrum shown in Figure 3 was run in chloroform, 0.1 cm path length, 5 accumulations at 100 nm/min scan speed, room temperature, 0.2 nm resolution.

Preparation of Lipase from *Chromobacterium viscosum* (ATCC 6918). *Chromobacterium viscosum* ATCC 6918 was grown on nutrient broth (Difco) at 37 °C for 24 h, then at 42 °C for 8 h. The culture was centrifuged at 5K rpm for 10 min. The pellet was discarded and the supernatant was used to catalyze hydrolysis of **1**-dibutanoate, see Table 1 in the text.

Quantitative HPLC Analysis of Enantiomeric Purity. Enantiomers of **1** were separated on a Chiralcel OD HPLC column (Chiral Technologies Inc., Exton, PA) eluted with hexanes−ethanol (90/10, 1 mL/min): δ = 1.89, k'_S = 1.94, k'_R = 3.66. Four isomers of **1**-monobutanoate and **1**-monopentanoate were separated on the Chiralcel OD column eluted with hexanes−ethanol (96/4, 1 mL/min): **1**-4′-monobutanoate, δ = 1.71, k'_R = 1.85, k'_S = 3.17; **1**-5-monobutanoate, δ = 3.01, k'_S = 0.92, k'_R = 2.77; **1**-4′-monopentanoate, δ = 1.63, k'_R = 1.71, k'_S = 2.78; **1**-5-monobutanoate, δ = 2.44, k'_S = 0.95, k'_R = 2.31.[9]

Absolute Configuration by X-ray Crystallography. The (+)-**1**-dipentanoate isolated from the CVL-catalyzed hydrolysis was transformed to (+)-**1** by treatment with sodium methoxide. Crystals of (+)-**1** were grown from dichloromethane. Data were collected over a whole sphere, to 2 of 140ø on a CAD4 diffractometer using copper radiation. Data were processed using the NRCVAX94 software package.[10] Structures were solved by direct methods (Shelxs-86) and refined against F2.

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Supporting Information Available: The following materials are available: (1) Figure S1. HPLC chromatograms showing separation of enantiomers of monoesters and diol. (2) A case by case rational discussion of possibilities to achieve a good resolution of the complex system. (3) Figure S2. X-ray crystallographic data and ORTEP drawing. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) At first we found it difficult to separate the four isomeric **1**-monopentanoates and used a ternary mixture of hexane-2-propanol-ethanol (97.5/1/0.25) to elute the Chiracel OD column. Once we started screening reaction mixtures, the separation improved dramatically and we used the conditions noted above. We do not know why separation improved.

(10) Gabe, E. J.; LePage, Y.; Charland, J. P.; Lee, F. L.; White, P. S. *J. Appl. Crystallogr.* **1989**, *22*, 384−389.