Organic Process Research & Development **2009**, *13*, 483–488

Enzymatic Desymmetrization of Dimethyl Cylcohex-4-ene-*cis*-1,2-dicarboxylate to (1*S*,2*R*)-2-(Methoxycarbonyl)cyclohex-4-ene-1-carboxylic Acid

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Abstract:

An efficient process for the synthesis of the monoester, (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid by *Candida antarctica* lipase (Novozym 435)-catalyzed desymmetrization of the corresponding diester, dimethyl-cyclohex-4-ene-*cis*-1,2-dicarboxylate was developed. The process was optimized and scaled-up to prepare a total of 3.15 kg of the 1*S*,2*R*-monoester from 3.42 kg of diester in two batches. The yield of the two batches ranged from 98.1−99.8% and ee of the 1*S*,2*R*-monoester was >99.9%.

Introduction

The chiral monoester, (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid **1** is a key chiral intermediate for the synthesis of a potential drug candidate for the modulation of chemokine receptor activity.[1] Both the 1*S*,2*R*-monoester **1** and its enantiomer 1*R*,2*S*-monoester **2** can be obtained by resolution[2] of the racemic acid with alkaloids, e.g. cinchonidine and ephedrine. However, as the maximum theoretical yield of resolution process can not be more than 50%, a process that affords desymmetrization of a meso compound would be greatly preferred. Such a meso desymmetrization process has been published affording either the 1*S*,2*R*-monoester **1** or its enantiomer 1*R*,2*S*-monoester **2** by desymmetrization of the meso-anhydride, *cis*-1,2,3,6-tetrahydrophthalic anhydride **3**, by alcoholysis catalyzed by cinchona alkaloids.[3] Cinchonine and quinine provided the 1*S*,2*R*-monoester **1**, while cinchonidine and quinidine provided 1*R*,2*S*-monoester **2**. To support the preparation of a drug candidate, we utilized this quinine-catalyzed alcoholysis of the anhydride **3** to prepare kilogram quantities of the 1*S*,2*R*-monoester **1** with 90.8% ee. While we continued to optimize the enantioselectivity of this process for the large-scale preparation of **1**, we simultaneously began to investigate alternative methods for the synthesis of **1**.

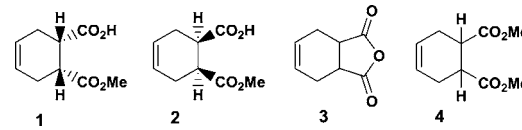

Figure 1. **Structures of compounds.**

There are several reports in the literature[2b,4] for the synthesis of the opposite enantiomer of the monoester, (1*R*,2*S*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid, **2**, by porcine liver enzyme-catalyzed hydrolytic desymmetrization of the dimethyl ester **4**.[5] We hypothesized that a broad screen of enzymes would identify an enzyme that would similarly afford the desired enantiomer **1** with high enantioselectivity. This report describes the development of an efficient process for synthesizing the monoester, (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid **1** by enzyme-catalyzed desymmetrization of the corresponding diester, dimethyl-cyclohex-4-ene-*cis*-1,2-dicarboxylate, **4** (Figure 1).

Results and Discussion

Hydrolytic enzymes (lipase, esterase and protease) from our collection were screened for the hydrolysis of the diester **4** to the desired 1*S*,2*R*-monoester **1**. Eleven enzymes (Table 1) afforded more than 5% conversion to monoester. Hydrolysis to the corresponding diacid was not observed with any enzyme.

The enzymes could be classified in three groups (Table 1):

- Entries 1 and 2: Porcine liver esterase[2b,4] and acylase I from porcine liver showed high (99%) and good (61%) conversions, respectively, to the undesired 1*R*,2*S*-monoester **2** in high (94−95%) ee.
- Entries 3 to 6: Novozym 435, lipoprotein lipase, and cholesterol esterase showed high conversion (93−100%) to the desired 1*S*,2*R*-monoester **1** with high (86−99%) ee. Lipase TL showed good (51%) conversion with moderate (39%) ee to the desired 1*S*,2*R*-monoester **1**.

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10.1021/op8002954 CCC: $40.75
Published on Web 02/16/2009

Table 1. **Result of enzyme screening for the hydrolysis of diester 4**

entry	enzyme	source	supplier	monoester **1** or **2** (%)	ee of **2** (%)	ee of **1** (%)
1	porcine liver esterase (PLE)	porcine liver	Sigma	99	93.8	
2	acylase I	porcine kidney	Sigma	61	94.7	
3	Novozyme 435	*Candida antarctica*	Novo	100		99.3
4	lipoprotein lipase, LPL-311	*Pseudomonas* sp	Toyobo	97		87.8
5	cholesterol esterase, COE-311	*Pseudomonas* sp	Toyobo	93		95.9
6	lipase TL	*Pseudomonas stutzeri*	Meito Sangyo	51		38.8
7	lipase PS/PP (immobilized PS-30)	*Pseudomonas cepacia*	Amano	11		85.5
8	pepsin A	porcine stomach mucosa	Sigma	15		50.3
9	lipase	*Chromobacterium viscosum*	Sigma	13		45.2
10	protease type I	bovine pancreas	Sigma	6		66.8
11	α-chymotrypsin	bovine pancreas	Sigma	9		66.1

Table 2. **Enzyme-catalyzed alcoholysis of the anhydride 3**

enzyme	source	supplier	monoester **1** or **2** (%) corr. for control 13%	ee of **1** (%)	ee of **2** (%)
porcine liver esterase (PLE)	porcine liver	Sigma	2	8.8	
acylase I	porcine kidney	Sigma	0	9.4	
lipase TL	*Pseudomonas stutzeri*	Meito Sangyo	72		86.1
lipoprotein lipase (LPL-311)	*Pseudomonas* sp	Toyobo	6		45.8
cholesterol esterase III (COE-311)	*Pseudomonas* sp	Toyobo	81		95.5
Novozym 435	*Candida antarctica*	Novo	58		33.2

- Entries 7 to 11: These enzymes showed low conversions (6−15%) in moderate to high (45−82%) ee to the desired 1*S*,2*R*-monoester (**1**).

A few enzymes identified as the best from the screening of diester hydrolysis above (entries 1−6, Table 1) were also evaluated for the enantioselective alcoholysis of the anhydride, *cis*-1,2,3,6-tetrahydrophthalic anhydride **3** to the desired 1*S*,2*R*-monoester **1**. The enzymatic alcoholysis was conducted with methanol using a relatively large ratio of enzyme to substrate anhydride **3** in MTBE. Even the control reaction without any enzyme showed the formation of some monoester (about 13%). The results are in Table 2. Cholesterol esterase, lipase TL, and Novozym 435 showed significant enzyme-catalyzed alcoholysis. As expected from the hydrolysis, these enzymes showed the preferential formation of the undesired 1*R*,2*S*-monoester **2**. Cholesterol esterase showed the highest ee, 95.5%, for the undesired 1*R*,2*S*-monoester **2**. We predicted that porcine liver esterase and acylase I, if active in an organic solvent, would yield preferentially the desired 1*S*,2*R*-monoester **1**. However, as reported elsewhere,[4e] porcine liver esterase showed very little enzyme-catalyzed ester formation activity in organic solvents vs a control experiment without enzyme. Acylase I also showed no additional monoester formation vs a control experiment without enzyme. Further efforts on enzyme-catalyzed alcoholysis of the anhydride **3** in MTBE showed only limited success. Since the desired 1*S*,2*R*-monoester **1** was not formed in high yield and ee and the enzymatic hydrolysis process showed promise, further evaluation of an enzymatic alcoholysis of anhydride approach was discontinued.

To develop a scalable process for the biocatalytic synthesis of the desired 1*S*,2*R*-monoester **1**, further studies were conducted following up on the initial screening results. After analyzing several reaction attributes, e.g. yield and ee of product, reaction rate, and cost of enzyme, the immobilized lipase from *Candida antarctica* (Novozym 435) was chosen for further development of the desymmetrization of diester **4** to the desired 1*S*,2*R*-monoester **1**.

Table 3. **Effect of pH and temperature on the hydrolysis by Novozym 435**[a]

pH	temp (°C)	time (h)	conversion (%)	ee (%)
7	21	2	ND	
		4	ND	
		24	55	99.2
8	21	2	ND	
		4	24	
		24	91	100
8	40	2	39	
		4	62	
		24	100	98.8
8.5	40	2	45	
		4	68	
		24	100	99.2
9	40	2	49	
		4	72	
		24	100	95.6

[a] ND = Not Determined.

The effect of pH on the hydrolysis of diester **4** catalyzed by Novozym 435 was investigated by carrying out the hydrolysis at a constant pH. It was found that at room temperature (21 °C) the conversion was faster at pH 8 than at pH 7. At pH 8, the reaction was much faster at 40 °C than at room temperature. In all cases the ee of 1*S*,2*R*-monoester **1** was >99%. After these initial experiments, a comparison of hydrolysis at a constant pH of 8, 8.5, and 9 was performed at 40 °C. In all cases, the reaction was complete within one day, and the reaction rate was found to increase modestly with increasing pH (Table 3). The ee of the product at pH 9 at earlier times during the reaction (2−6 h, 97.4−97.7%) and also that of the isolated 1*S*,2*R*-monoester **1** after 24 h (ee 95.6%) were significantly lower than the ee of **1** obtained after 24 at pH 8 or pH 8.5. Thus, pH 8.5 was chosen for further studies on the basis of the rate of hydrolysis and product ee. Yazbeck et al.[6] reported increasing rate

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Table 4. **Results of three (1−2 g) laboratory batches**

expt	diester (g)	completion (h)	conversion (%)	product (g)	yield (%)	ee of **1** (%)
1	1.45	14	100	1	94	98.9
2	2.29	22	99.6	1.75	82 (79[a])	99.2
3[b]	2.29	39	99.6	1.71	80	99.1

[a] Yield corrected for residual solvents from NMR. [b] Recovered Novozym 435 from expt 2 was used for expt 3.

at higher pHs, but a decrease in ee above pH 8.5 for the hydrolysis of an ester by *Candida antarctica* lipase B.

A simple isolation method involving separation of Novozym 435 (enzyme immobilized on a solid polymeric support) by filtration, acidification of the aqueous layer, followed by extraction of the product in organic solvent was developed. In order to optimize productivity, higher substrate concentrations and lower enzyme to substrate ratio were investigated. The first experiment evaluated hydrolysis of 1 mL (1.145 g) of diester **4** with Novozym 435 (200 mg, enzyme to substrate ratio 1:5.7) in 20 mL of 1 M phosphate buffer pH 8.5 (substrate concentration 55 g/L) at 40 °C. The slope of 1 M NaOH consumption suggested that the hydrolysis was essentially complete in about 14 h. After 21 h, the reaction mixture was filtered through a stainless steel screen to separate the Novozym 435 enzyme. The combined aqueous extract was acidified and extracted with MTBE. Removal of MTBE provided 1.0 g of 1*S*,2*R*-monoester **1** as an oil, 94% yield, 98.9% ee. Elution of the recovered Novozym 435 with acetonitrile showed only a negligible amount (∼0.2%) of the ester was extractable from the immobilized enzyme. A second hydrolysis experiment was conducted at a higher substrate concentration (2 mL, 2.29 g) of diester **4** and the same amount of enzyme (lower enzyme to substrate ratio 1:11.5) and buffer. The slope of 1 M NaOH consumption suggested that the hydrolysis was complete in about 22 h. After 27 h, the product was isolated by the same procedure to afford 1.75 g of **1** as an oil, 82% yield, 99.2% ee. During the enzymatic hydrolysis, some breakage of the immobilized enzyme beads was seen, and the fines also passed through the stainless steel screen during filtration resulting in less than 100% recovery of Novozym 435 after the reaction. The enzyme recovered from the second experiment was used for a third hydrolysis experiment. In addition to the loss of enzymes due to fines, loss of some activity of Novozym 435 due to mechanical agitation as reported recently[7] cannot be ruled out. The hydrolysis proceeded slowly with the recovered enzyme, and the reaction required more time (about 39 h) to complete. The 1*S*,2*R*-monoester **1** was still obtained in high yield and high ee. Optimization of the agitation and recovery of the enzyme would be required for optimal recycle of the immobilized enzyme. The results obtained in the above three 1−2 g laboratory batches are shown in Table 4. NMR (^{1}H and ^{13}C) spectra confirmed the structure of the compound. NMR also showed some residual solvent methyl *tert*-butyl ether (MTBE) as the only other component in the sample. The amount of residual MTBE was estimated to be about 5% by weight from the ^{1}H NMR.

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Table 5. **Results of the preparative-scale batches**

	batch 1	batch 2
diester, g	1732	1690
monoester "as is", g	1675	1720
MTBE, wt %	1.9	6.11
water, wt %	3.78	2.76
monoester corr for MTBE and H_2O, g	1580	1567
yield %, corr for MTBE and H2O	98.1	99.8
HPLC AP of monoester **1**	98.3	99.2
HPLC AP, *trans*-monoester impurity	0.7	0.16
ee of monoester **1**, %	>99.9	>99.9
specific rotation	−15.19	−15.16

Before conducting a kilogram-scale desymmetrization of diester **4**, an intermediate scale desymmetrization of 50 mL (57.2 g) of diester **4** was conducted to afford 53.19 g of the 1*S*,2*R*-monoester **1**. Proton NMR confirmed the structure of the product and found approximately 4% by weight of residual MTBE. The yield was 96% after correction for residual solvent. The chiral HPLC of the product showed only the peak for the desired 1*S*,2*R*-monoester **1**, >99.9% ee.

Preparative, kilogram-scale batches were performed to prepare 3.4 kg of 1*S*,2*R*-monoester **1** in two batches. The two batches were performed on a 1.7 kg scale with the conversion profile closely paralleling that observed in our smaller-scale batches with reaction completion in 24−27 h. A summary of the results of the batches is in Table 5. Chiral HPLC analysis found only the desired 1*S*,2*R*-monoester **1** in both batches, ee > 99.9%. The HPLC area % (AP) of the 1*S*,2*R*-monoester **1** was 98.3 and 99.2%. One impurity, tentatively assigned as the *trans*-monoester (LC−MS same molecular weight), was seen in 0.16 to 0.7 HPLC AP in the two batches. The residual solvent MTBE was 1.9−6.1%, and water level was 2.8−3.8%. The yield corrected for solvent and water ranged from 98 to 99%. Overall, we developed an efficient *Candida antarctica* lipase enzyme (Novozym 435)-catalyzed hydrolytic desymmetrization of the dimethyl ester, dimethyl cyclohex-4-ene-*cis*-1,2-dicarboxylate **4** to enable the synthesis of kilogram quantities of the required monoester, (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid **1** with >99% ee.

Experimental Section

The diester, dimethyl cyclohex-4-ene-*cis*-1,2-dicarboxylate **4** was purchased from Aldrich for initial laboratory experiments. Larger quantities of **4** were prepared according to a literature procedure.[5] The monoester, (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid, **2**, and the anhydride, *cis*-1,2,3,6-tetrahydrophthalic anhydride, **3**, were purchased from Aldrich. A sample of the monoester, (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid **1** was prepared by alkaloid-catalyzed alcoholysis of the anhydride **3**. Enzymes were obtained from commercial suppliers.

Analytical Methods. *HPLC Method 1 (Analyses of Diester and Monoester).* HPLC was done on a YMC Pack Pro C18 3 μ, 4.6 mm × 150 mm (Waters) column using gradient of solvent A (0.05% TFA in water/methanol, 80:20) and solvent B (0.05% TFA in acetonitrile/methanol, 80:20) with 0 to 100% B in 15 min at a flow rate of 1 mL/min at ambient temperature. The detection was done by UV at 210 nm. The retention times are as follows: dimethyl-cyclohex-4-ene-*cis*-1,2-dicarboxylate (**4**)

10 min, (1*S*,2*R*) or (1*R*,2*S*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid (**1** or **2**) 8.4 min.

*HPLC Method 2 (Chiral Analyses of Monoester **1** and **2**).* This method was used for the chiral analyses during initial enzyme screening work. A Chiralpak AS-RH, 4.6 mm × 150 mm (Chiral Technologies) with a gradient of solvent A (0.05% TFA in water/methanol, 80:20) and solvent B (0.05% TFA in acetonitrile/methanol, 80:20) was used. The gradient program was: 0 to 10% B in 30 min, to 50% B in 35 min, stay 50% B to 40 min. The flow rate was 0.5 mL/min, and the column was maintained at 25 °C. The detection was by UV at 210 nm. The retention times are as follows: (1*S*,2*R*)-2-(methoxycarbonyl) cyclohex-4-ene-1-carboxylic acid (**1**) 18.51 min; (1*R*,2*S*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid (**2**) 16.47 min. The separation between the two enantiomers was not ideal by this method. A better HPLC method (Method 3) was later developed and used after the initial screening studies.

*HPLC Method 3 (Chiral HPLC Analyses for 1S,2R-, **1**, and 1R,2S-monoester, **2**).* This method was used for chiral analysis after the initial screening work. Solutions of all compounds were made in heptane/isopropanol (1:1) at a concentration of 2.5 mg/mL. A solution of the racemic monoester was made by combining equal amounts of the solutions of **1** and **2**. HPLC was performed on a Chiralpak AD-H, 4.6 mm × 150 mm (Chiral Technologies) using heptane/isopropanol/trifluoroacetic acid (95:5:0.05) as eluent at a flow rate of 1 mL/min. The column was maintained at 25 °C. The detection was by at UV 210 nm. Racemic monoester (combination of **1** and **2**) showed two peaks at 6.92 and 7.79 min in the ratio of 50.4:49.6. The two peaks showed baseline separation. The first peak was due to the (1*R*,2*S*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid (**2**) and the second peak was due to the (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid (**1**). Dimethyl-cyclohex-4-ene-*cis*-1,2-dicarboxylate (**4**) eluted at 3.58 min in this system.

A sample of (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid (**1**) made by the cinchona alkaloid-catalyzed alcoholysis of the anhydride showed an ee of 90.8% by this method. This sample showed $[\alpha]^{D}_{20}$ −14.05° [*c* 1.06, EtOH]. A sample of (1*R*,2*S*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid (**2**) obtained from Sigma showed an ee of 98.1% by this method. This sample showed $[\alpha]^{D}_{20}$ +14.94° [*c* 1.037, EtOH].

*HPLC Method 4 (Analyses of the Diester **4** and Monoester **1** or **2**).* This method was used for the analysis of the 50 g and the two large-scale batches. A YMC S3 ODS-A 4.6 mm × 50 mm column (Waters) was used with a gradient of solvent A (0.2% aqueous phosphoric acid) and solvent B (90% acetonitrile 10% water); going from 0% to 100% B in 8 min, at a flow rate of 2.5 mL/min. The detection was by UV at 210 nm. The retention times are as follows: diester (**4**) 4.61 min, monoester (**1** or **2**) 2.69 min.

*HPLC Method 5 (Chiral HPLC Analyses of 1S,2R **1** and 1R,2S-monoester **2**).* This method was used for the 50 g and the two large-scale batches. This was a slight variation from Method 3. A Chiralpak AD-H, 4.6 mm × 150 mm (Chiral Technologies) and the isocratic eluent heptane/isopropanol/trifluoroacetic acid (95:5:0.05) was used at a flow rate of 1 mL/min at 15 °C, and the detection was by UV at 210 nm. The retention times are: 1*R*,2*S*-monoester (**2**) 8.5 min; 1*S*,2*R*-monoester (**1**) 9.5 min.

Enzyme Screening for the Hydrolysis of Dimethyl Ester 4. Enzyme screening was done on multiwell plates. Each well of a 24-well plate contained a different enzyme (10 mg each). The immobilized lipase enzymes from *Candida antarctica* (Novozym 435 from Novozyme Corporation), and *Pseudomonas cepacia* (lipase PS/PP) were added to the empty wells each in 50 mg quantities. A solution of 0.1 M phosphate buffer (pH 7, 1 mL) was added to each well. After mixing for 10 min, dimethyl-cyclohex-4-ene-*cis*-1,2-dicarboxylate (**4**, 10 μL) was added to each well. The hydrolysis was conducted by shaking the plate in a Thermomixer R shaker at 700 rpm at 28 °C. After 48 h, 1 mL of mobile phase B (0.05% TFA in acetonitrile/methanol, 80:20) was added to each well. The plate was closed by capmat and shaken at 400 rpm for 15 min. The content of each well was filtered (0.2 μ) and analyzed by HPLC (Methods 1 and 2). The area ratio of peaks of diester and monoester in reverse phase HPLC were used to determine the conversion. The summary of results from the experiments which afforded more than 5% conversions is in Table 1.

Alcoholysis of Anhydride 3. The enzymes (100 mg each, except 50 mg for cholesterol esterase) were charged separately to 50 mL Teflon flasks. A solution of 100 mg (0.66 mmol) of *cis*-1,2,3,6-tetrahydrophthalic anhydride (**3**) in MTBE (10 mL) was added to each flask. Methanol (53.2 μL, 1.32 mmol) was added to each flask. The flasks were closed, and the reaction was conducted by shaking the flasks in a shaker at 250 rpm at 28 °C. After 6 h, 1, 2, and 3 days, 0.5 mL samples were taken from each flask. The samples were evaporated and analyzed by HPLC (Methods 1 and 2). The results after one day of reaction are shown in Table 2.

Small-Scale Hydrolysis of Dimethyl Ester 4 with Novozym 435 with Substrate Input of 50 μL/mL, 100 μL/mL, and at 100 μL/mL Reusing the Enzyme. A solution of 1 M phosphate buffer (pH 8.5, 20 mL) was charged to a pH Stat vessel fitted with an overhead stirrer with automatic addition of 1 M NaOH to maintain the pH. Novozym 435 (200 mg) was added. Dimethyl cyclohex-4-ene-*cis*-1,2-dicarboxylate (**4**, 1 mL, 1.145 g, 5.776 mmol) was added to the vessel. The mixture turned slightly reddish-brown. The pH Stat was set at pH 8.5 and the temperature to 40 °C. NaOH was slowly consumed as the hydrolysis proceeded. The progress of the reaction was monitored by noting the NaOH consumption, and taking samples (1 mL) for HPLC analysis. The pH of each sample was adjusted to about 2 by addition of 5 N HCl and extracted with 2 mL of MTBE. A portion (0.5 mL) of the extract was evaporated for reverse phase HPLC analysis (Method 1); the remaining portion (1.5 mL) was evaporated for chiral HPLC analysis (Method 3). The slope of the NaOH addition curve suggested that the reaction was essentially complete at about 14 h when about 5.8 mL of NaOH was consumed. The reaction was stopped at 21 h when 5.9 mL of NaOH was consumed. The entire reaction mixture (solution and immobilized enzyme) was filtered through a stainless steel screen (40 mesh). The solid Novozym 435 (immobilized enzyme) was mixed with 5 mL of the same buffer for 10 min and passed through the screen.

This was repeated again. The buffer washings were mixed with the original filtrate, acidified to pH ≈ 2 by addition of 5 N HCl, and extracted with MTBE (2 × 30 mL). The MTBE extract was washed with water (2 × 30 mL). Removal of solvent from the MTBE extract by rotary evaporator followed by overnight drying under house vacuum provided 1.0 g of (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid (**1**) as a thick oil (5.429 mmol, yield 94%). No diester **4** was detected by HPLC in the product. The product showed an ee of 98.9% and $[\alpha]^{D}_{20}$ −15.68° [*c* 1.014, EtOH].

In the second experiment for substrate input of 100 μL/mL, hydrolysis of dimethyl ester (**4**, 2 mL) with Novozym 435 (200 mg) was carried out in the same way at pH 8.5 at 40 °C. The reaction was stopped at 27 h showing 99.6% conversion from NaOH consumption. Workup in the same way as described above afforded 1*S*,2*R*-monoester **1** as a thick oil (1.75 g, 9.50 mmol, yield 82%). The residual diester **4** was <0.5% by HPLC. The ee by HPLC was 99.2% and $[\alpha]^{D}_{20}$ −15.55° [*c* 1.048, EtOH]. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.22−2.48 (m, 2H), 2.49−2.78 (m, 2H), 2.85−3.16 (m, 2H), 3.69 (s, 3H), 5.68 (s, 2H) and for Residual MTBE estimated ∼5 wt % δ 1.3, 3.25; ^{13}C (75.47 MHz) δ 25.44, 25.62, 39.35, 39.50, 51.82, 124.96, 125.05, 173.60, 179.50, and for residual MTBE δ 26.79, 47, 73.

Breakage of the immobilized enzyme beads was observed during the reaction. The fine particulates of the immobilized enzyme passed through the screen after filtration and were present in the aqueous layer. The recovered wet Novozym 435 from the second experiment weighed only 208 mg and was used in a third experiment for the hydrolysis of dimethyl ester **4** at 100 μL/mL substrate input. The rate of the reaction was slower than that of the previous two experiments. The slope of the NaOH addition curve suggested that the reaction was complete at about 39 h. Workup of the reaction mixture provided 1.71 g of a thick oil, **1** (9.28 mmol, yield 80.3%). The residual diester **4** was about 0.4%. The ee was 99.1% and $[\alpha]^{D}_{20}$ −14.77° [*c* 1.022, EtOH].

Hydrolysis of 50 mL of Dimethyl Ester 4 with Novozym 435. Dimethyl ester **4** for this reaction was prepared according to the reported procedure[5] and distilled before use. To 1 M K_2HPO_4 buffer (500 mL, pH 8.5) at 40 °C in a 1 L jacketed reactor, was added Novozym 435 (5.00 g) under stirring. Dimethyl ester **4** (288.75 mmol; 57.24 g; 50 mL) was added. The mixture was slowly stirred overnight, keeping the pH constant at 8.5 with the aid of a pH stat (10 M NaOH). After 27 h HPLC showed 0.2 AP of starting diester **4**. The uptake of NaOH (28.45 mL) was negligible from 25 to 27 h with about 98.5% theoretical amount of NaOH consumed at the end showing that the reaction was over. The immobilized enzyme was separated by filtration. The aqueous layer was washed with MTBE (100 mL) to remove any unreacted dimethyl ester **4**. HPLC showed 0.1% product **1** plus a small amount of starting diester **4** in the MTBE wash. The aqueous layer was acidified to pH 2 with concd HCl (85 mL). The aqueous layer was extracted with MTBE (250 mL × 3). HPLC showed 80%, 17%, and 2% of the product **1** in the three successive MTBE extracts. The final aqueous layer contained 0.7% product **1**. The combined MTBE layers were washed with water (50 mL × 2). HPLC showed about 1.7% product **1** was lost in the aqueous washes. Removal of the solvent from the rich organic layer via rotary evaporation at 35 °C gave the 1*S*,2*R*-monoester **1** as colorless oil, 53.19 g (96% yield corrected for 8 M % MTBE). ^{1}H and ^{13}C NMR ($CDCl_3$) conform to the structure and showing the presence of 8 M %, 3.7 wt % MTBE. LC−MS 183 M − H, 185 M + H; HPLC monoester **1** AP 96.8, unknown impurity (same mass in LC−MS 185 M + H, likely the *trans*-isomer) 1.8 AP; chiral HPLC ee > 99.9%, no 1*R*,2*S*-enantiomer **2** detected; $[\alpha]^{20}_{D}$ −15.96° [*c* 1.015, EtOH].

Preparative Scale Batches: Hydrolysis of 1.5 L per Batch of Dimethyl Ester 4 with Novozym 435. Dimethyl ester **4** for this reaction was prepared according to a literature procedure[5] and distilled before use. A 30-L jacketed reactor fitted with a pH stat to maintain constant pH by automatic addition of 10 M NaOH was used for both batches. K_2HPO_4 buffer (1 M, 15 L, pH 8.5) was stirred at 40 °C in a 30 L reactor, and Novozym 435 (150 g) was added. The dimethyl ester (**4**, 8.74 mol; 1.73 kg) was added. The mixture was gently stirred for 27 h keeping the pH constant at 8.5 by addition of 10 M NaOH. After 27 h HPLC showed <1 AP of starting diester **4**. The rate of uptake of NaOH was very slow from 25 to 27 h. The reaction mixture was filtered through a Buchner funnel (no. 1 filter paper). The recovered enzyme was washed with water (500 mL × 2). The combined aqueous filtrate was washed with MTBE (3 L). The aqueous layer was acidified to pH 2 with concd HCl (2.33 L). The acidified aqueous layer was extracted with MTBE (5 L × 3). The combined MTBE layers were washed with water (500 mL × 2). The MTBE solution was evaporated to dryness on the rotary evaporator and dried (106 torr, bath 35 °C, 1 h) to provide the desired 1*S*,2*R*-monoester **1** as a yellow oil, 1.675 kg, "as is" yield 104%; 1.58 kg corrected for MTBE and water, corrected yield 98.1%. ^{1}H and ^{13}C NMR ($CDCl_3$) and elemental analysis were consistent with the structure and indicated the presence of 1.9 wt % MTBE and 3.78% H_2O; LC−MS 185 M + H; HPLC 1*S*,2*R*-monoester **1** AP 98.3; unknown impurity 0.7 AP (same mass in LC−MS 185 M + H, *trans*-isomer); chiral HPLC ee >99.9, no enantiomer **2** detected; $[\alpha]^{20}_{D}$ −15.19° [*c* 1.066, EtOH].

The second batch was run in the same reactor in the same fashion using dimethyl ester (**4**, 8.53 mol; 1.69 kg), 1 M K_2HPO_4 buffer (15 L, pH 8.5) and Novozym 435 (150 g). The mixture was gently stirred for 27 h, keeping the pH constant at 8.5 with a pH stat adding 10 M NaOH. After 27 h HPLC showed <1 AP of starting diester **4**. The rate of uptake of NaOH was very slow from 25 to 27 h. Workup of the reaction mixture in the same way provided the desired monoester **1** in MTBE. The MTBE solution was evaporated to dryness on a rotary evaporator and dried (106 torr, bath 35 °C, 1 h) to provide the desired 1*S*,2*R*-monoester **1** as a yellow oil, 1.720 kg, "as is"

yield 109.5%; 1.57 kg corrected for MTBE and water, corrected yield 99.8%. ^{1}H and ^{13}C NMR ($CDCl_3$) and elemental analysis were consistent with the structure and indicated the presence of 6.1 wt % MTBE and 2.76% H_2O; LC−MS 185 M + H; HPLC 1*S*,2*R*-monoester **1** AP 99.2, unknown impurity 0.16 AP (*trans*-isomer, same mass in LC−MS 185 M + H); Chiral HPLC ee > 99.9, no enantiomer **2** detected; $[\alpha]^{20}_{D}$ −15.06° [*c* 1.049, EtOH].

Acknowledgment

We are indebted to Robert Waltermire, Ramesh N. Patel, Richard H. Mueller, Percy Carter, Arvind Mathur, Janak Singh, Yadagiri Pendri, Ming-Hsing Huang, and Konstantinos Dambalas for their help in various stages of this work.

Received for review November 26, 2008.
OP8002954