C=O), 164.63 (amide C=O), 140.46, 130.53, 129.37, 128.97, 128.54, 128.07, 128.01, 127.94, 127.85, 125.61, 125.48 (Ph), 88.85, 84.63 (diolate OCH), 79.81 ($TiOCHMe_2$), 68.11, 67.50 (ester OCH), 25.37, 21.64, 21.58 (CH_3) ppm.

$Ti(ONPhBz)_2(O^iPr)_2$: ^1H NMR δ 7.42–7.21 (m, 10 H), 4.99 (h, 2 H), 1.32 (d, 12 H) ppm; ^{13}C NMR δ 163.40 (C=O), 140.94, 130.56, 129.19, 129.02, 128.89, 128.59, 128.17, 128.06, 127.88, 125.66 (Ph), 77.36 (OCH), 25.43 (CH_3) ppm.

$Ti_3DIPT_4(Et_3N)_2(O^iPr)_4$: ^1H NMR δ 5.24 (AB q, 8 H, J = 9.34 Hz, Δδ = 0.184 ppm, diolate OCH), 4.99 (2 h, 8 H, J = 6.32 Hz, ester OCH), 4.83 (h, 4 H, J = 5.91 Hz, $TiOCHCH_3$), 3.28 (b, 12 H, NCH_2), 1.69 (bs, 8 H, OH), 1.30–1.12, 1.07 (m+t, 138 H incl HO^iPr, CH_3) ppm; ^{13}C NMR δ 171.28, 170.79 (C=O), 85.64, 85.06 (diolate OCH), 77.28 ($TiOCHCH_3$), 67.82, 67.36 (ester OCH), 46.18 (NCH_2), 26.00, 25.64, 21.87, 21.70, 21.65 (CH_3) ppm.

$Ti_3DIPT_4(^iPr_2NH)_2(O^iPr)_4$: ^1H NMR δ 5.23 (AB q, 4 H, J = 9.07 Hz, Δδ = 0.196 ppm, diolate OCH), 4.99, 4.98 (2 h, 4 H, ester OCH), 4.83 (h, 2 H, $TiOCHCH_3$), 2.80 (h, NCH), 1.30–1.12 (m, $OCHCH_3$), 1.06 (d, $NCHCH_3$) ppm; ^{13}C NMR δ 171.28, 170.94 (C=O), 85.80, 85.04 (diolate OCH), 77.21 ($TiOCHCH_3$), 68.05, 67.66 (ester OCH), 45.28 (NCH), 26.04, 25.68, 23.23, 21.89, 21.74 (CH_3) ppm.

$Ti_2DMT_2(O^tBu)_4$: ^{13}C NMR δ 174.78, 171.81 (CO), 87.88, 83.92 (skeletal OCH), 86.02, 84.68 ($OCMe_3$), 52.70, 52.17 (OCH_3), 31.26, 31.12 (CCH_3) ppm.

$Ti_2DET_2(O^tBu)_4$: ^{13}C NMR δ 174.85, 171.45 (CO), 87.69, 84.00 (skeletal OCH), 86.08 ($OCMe_3$), 61.85, 61.12 (OCH_2), 31.27, 31.12 (CCH_3), 14.24, 14.03 (CH_2CH_3) ppm.

$Ti_2DIPT_2(O^tBu)_4$: ^{13}C NMR δ 173.28 (CO), 86.65, 84.45 (skeletal OCH), 84.95, 83.14 ($OCMe_3$), 69.24, 68.95 ($OCHMe_2$), 31.95, 31.01 (CCH_3), 22.06, 21.57 ($CHCH_3$) ppm.

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Notes

The Enzymatic Preparation of (2*R*,3*S*) Phenyl Glycidic Acid Esters

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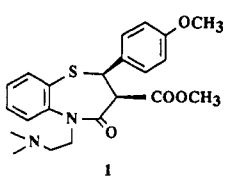
Due to new examples of compounds exhibiting different properties in their enantiomeric forms there is a current growing interest in the preparation of biologically active enantiomerically pure compounds, drugs, and pharmaceuticals. Only a few tenths of the pharmaceuticals on the market are present as single enantiomers, and they mainly arise from fermentation or from classical resolution at some stage of the synthetic sequence; however, their number is expected to grow in the future.[1] Recently, hydrolytic enzymes were successfully used to perform the kinetic resolution of racemates, thus allowing the preparation of enantiomerically pure drugs.[2]

We wish to report a resolution process applied to the synthesis of (2S,3S)-diltiazem 1, an enantiomerically pure drug with calcium antagonist activity.[3] The practical



1

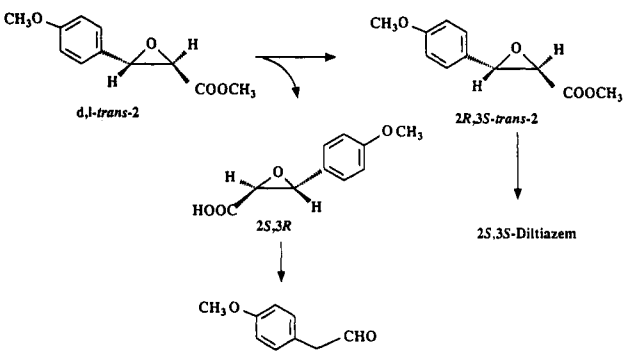


Figure 1.

synthesis of 1 involves the *trans-p*-methoxyphenylglycidic acid (PGA) ester 2 as the first intermediate.[4] Optically active 1 is currently prepared by the classical resolution of an intermediate at a late stage of the synthetic sequence;[5] in practice it would be preferable to carry out the resolution at an early stage of the synthesis so as to avoid processing twice the material necessary in the successive synthesis steps, as is required with a racemic intermediate. This prompted us to investigate the resolution of *rac*-2. Although optically active 2 is currently available through a classical resolution[6] or asymmetric synthesis,[7] we considered an alternative approach: an enzymatic way. As possible enzymatic reagents to perform such a resolution on PGA esters one would first consider the hydrolytic action of lipases because of the unspecificity of such enzymes and, for a more specific fit, α-chymotrypsin (α-CHT), a proteolytic and hydrolytic enzyme which has a preference for phenylpropionic acid derivatives. In fact,

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(2) See, for instance: Bertola, M. A.; Desmet, M. J.; Marx, A. F.; Phillips, G. T. Eur. Pat. 0299559-A to Gist-Brocades NV, 1987.
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Table I. Enzymatic Hydrolysis of PGA Methyl Ester with CCL

solvent	$t_{1/2}$ (h)	% ee[a]
CH_3CN/H_2O	2.5	30
CH_2Cl_2/H_2O	12	60
cyclohexane/water	1.5	96/98

[a] Recovered ester.

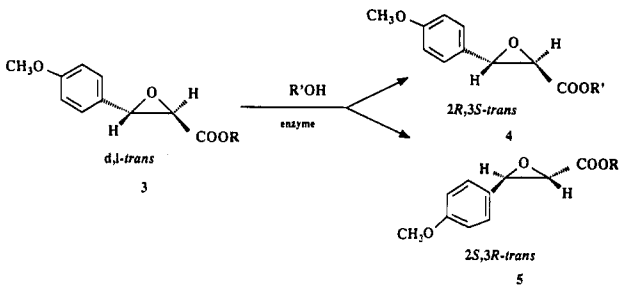

Figure 2.

we found that (2S,3R)-PGA ester 2 is enantioselectively hydrolyzed to the corresponding acid by lipase from *Candida cylindracea* (CCL), as well as by other lipases like pig pancreatic lipase (PPL), the hydrolysis resulting in the production of the (2R,3S) ester of varying enantiomeric purity. This transformation was effected in a biphasic system: the methyl ester in cyclohexane was stirred with an aqueous solution of the enzyme at a constant pH of 7.4. At room temperature the reaction had a $t_{1/2}$ = 90 min, and the methyl ester, with the desired (2R,3S) absolute configuration, was obtained with 96–99% ee and 35–40% yield.[8] It is worth noting that while lipase was not able to hydrolyze the esters in water, the transformation in a biphasic system was quite rapid. The acid obtained from the enzymatic hydrolysis decomposed to the corresponding aldehyde. Thus, the organic phase contained the survived ester contaminated with the products arising from the decomposition of the acid (aldehyde).[9] This process can be considered as a *subtractive process* that does not permit the recovery of the undesired stereoisomer, but which gives the methyl ester in the optically active form with the required absolute configuration. These results[10] were obtained independently from similar ones from other laboratories.[11]

Notwithstanding the good optical purity and recovery of 1 from the above process, serious drawbacks of the method lie in the fact that the acid decomposes and by-products are formed which require extensive purification,[12] moreover, the formation of a reactive aldehyde is likely to cause enzyme inactivation.[13] Since PGA esters are direct intermediates in the synthesis of Diltiazem, resolution via interesterification is more convenient[14] than the hydrolytic process having the following advantages: (i) separation of the esters allows recovery and then recycling of the undesired isomer (decomposed in the case of an hydrolytic process) and (ii) no formation of enzyme inactivating products.

Table II. Transesterification of PGA Esters

PGA ester	solvent		$t_{1/2}$ (h)	enzyme	% ee	abs config
methyl	ethanol	2	5.5	α-CHT	78	2R,3S
	buffer[b]	1			(85)[a]	
methyl	*n*-butanol	1	4.5	α-CHT	80	2R,3S
	buffer[b]	2				
methyl	propanol	2	6	α-CHT	70	2R,3S
	buffer[b]	1				
butyl	ethanol			α-CHT		
	methanol			CCL		
	buffer[b]					
methyl	*n*-butanol	1	24	CCL	60	2S,3R
	n-hexane	5		PPL		

[a] At 30% conversion. [b] Phosphate buffer, pH 7.4.

We submitted the PGA methyl ester 3 with various alcohols in a phosphate buffer–alcohol system to enzymatic transesterification using as enzymes lipases and proteases. With α-CHT alcohol transfer was effective and worked preferentially on the (2R,3S) enantiomer, giving optically active 4 of ee ranging from 60 to 80% at 50% conversion (Table II). Ester separation was possible through rapid chromatography or via selective extraction with mixtures of solvents. In the case of methyl and butyl ester mixtures, partition between a biphasic system, formed by hexane, ethyl acetate, methanol, acetonitrile, and water in ratios of 30/8/30/8/24, respectively, allowed the butyl ester to be obtained in good purity in the upper phase. Several of the higher alcohols up to *n*-heptanol were equally well transferred with similar efficiency, but benzyl alcohol was not. Other proteases were tested but no significant results were obtained. The use of lipases from *C. cylindracea* and from porcine pancreas in *n*-hexane and alcohols mixtures also led to esters enriched in one enantiomer, but these enzymes showed a preference opposite to the one displayed by α-CHT, giving esters of type 5 with (2S,3R) absolute configuration. The two methods are thus complementary, leading to either of the esters with the desired configuration. Attempts to transesterify PGA butyl ester with methanol or ethanol was not successful neither with α-CHT nor with lipases, showing that the compound is not, in any way, a substrate for those enzymes.

If α-CHT was immobilized, it was possible to reuse the enzyme several times with only a slight loss of activity. Good turnovers were obtained with α-CHT immobilized on Biofix-E in *n*-hexane solution. The same technique could also be applied to lipase from CCl or PPL immobilized on Celite. Although the reaction with the immobilized enzymes is possible in other organic solvents, no advantages were found in changing from hexane to cyclohexane or methylene chloride. Toluene proved unsuitable for these transformations.

The efficiency of the procedure presented compares favorably with the recent asymmetric synthesis of Diltiazem with a chiral auxiliary derived from an enzymatic resolution process.[15]

Experimental Section

The extent of esterification and hydrolysis was followed by HPLC using a Merck-Hitachi apparatus with a SiO_2 5-nm Lichrosorb-Merck column and mixtures of *n*-hexane and ethyl

(8) Small differences both in enantiomeric excess and chemical yield of the product were observed when different batches of enzyme were used. Chemical yield was calculated via HPLC with an internal standard.

(9) Singh, S. P.; Kagan, J. *J. Org. Chem.* **1970**, *53*, 2203.

(10) Ghirotto, L.; Servi, S.; Fuganti, C.; Gentile, A.; Giordano, C. Ital. Pat. Appl. 19584 A/90, March 6, 1990.

(11) Hulshof, L. A.; Roskam, J. H. Eur. Pat. Appl. PRAI NL 88-1311, 20 May 1988; PI EP 343714 A1, 29 Nov 1989. Dodds, D. R.; Lopez, J. L.; Zepp, C. H.; Brandt, S. PCP, WO 90/04643, 3 May 1990.

(12) Analytical samples were prepared by crystallization after short column chromatography. On a larger scale the aldehyde arising from the decomposition of PGA can be drawn into the aqueous phase as the bisulfite adduct. Different experiments were usually followed by HPLC without product isolation (see Experimental Section).

(13) Creighton, T. E. In *Proteins: Structures and Molecular Properties*; Freemann: New York, 1983.

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(15) Schwartz, A.; Madan, P. B.; Mohacsi, E.; O'Brien, J. P.; Todaro, L. J.; Coffen, L. D. *J. Org. Chem.* **1992**, *57*, 851.

acetate. Enantiomeric excess was determined by HPLC on a chiral column (CHIRACEL OD from DAICEL, using mixtures of *n*-hexane and 2-propanol). The absolute configuration of the PGA esters was established by comparing optical rotation values of analytical samples obtained by crystallization or flash chromatography of the crude material, with the ones of known compounds. The following enzymes were used: CCL lipase-grade VII and PPL grade II from SIGMA, α-CHT crystalline from SCLAVO, α-CHT on agarose from SIGMA. α-CHT on BIOFIX E_1 was prepared at room temperature by removing the water from a mixture obtained by mixing BIOFIX E_1 with a solution of α-CHT in water. In this way a powder containing 10% of the enzyme was obtained: this preparate was stable to storage and after five uses retained about 30% of the original activity. The following enzymes not mentioned in the text gave negative results or no significant improvement with respect to the one described above: Subtilisin, Trypsin, Papain, Acylase I, lipases from *Pseudomonas fluorescens*, *Rhizzopus arizus*, *Candida lipolitica*, and *Aspergillus niger*. PGA esters were prepared according to the literature.[7]

Hydrolysis of Racemic PGA Methyl Ester with CCL Lipase in CH_2Cl_2/H_2O. CCL (3g) was dissolved in water (75 mL), and the pH was set to 7.5 with a 0.1 N sodium hydroxide solution (1.5 mL). Methyl ester of racemic *trans*-(4-methoxyphenyl)-glycidic acid (1.5 g) in methylene chloride (50 mL) was then added and the mixture stirred at room temperature for 2 h. The organic layer was separated and the water phase extracted again with ethyl acetate. The combined organic phases were dried over sodium sulfate and evaporated to give a yellow oil as a residue (1.28 g) consisting of the methyl ester of (2*R*,3*S*)-*trans*-(4-methoxyphenyl)glycidic acid (50.2% assay, 41.5% yield, 60.4% ee) on the basis of HPLC analysis [Chiracel OD, 4.6 × 250, flow 0.7 mL/min, 35 °C, eluent: hexane/2-propanol = 85/15, t_R 12.2 min for the (2*S*,3*R*)-methyl ester of *trans*-(4-methoxyphenyl)glycidic acid and 9.8 min for the (2*R*,3*S*) enantiomer]. In parallel experiments the oily residue (1.5 g) (85% assay, 60% ee) was crystallized from ethanol to give, after two crystallizations, 0.4 g of the methyl ester of 99% ee. The enrichment in one enantiomer upon crystallization could not be reproduced and remained dependent on undetermined factors.

Hydrolysis of PGA Methyl Ester with CCL Lipase in Cyclohexane/H_2O. PGA methyl ester (10 g) was suspended in 500 mL of cyclohexane and then treated with a CCL lipase solution (10 g) in water (300 mL) at room temperature. After 1.5 h the mixture was treated with ethyl acetate (200 mL) and filtered through a Celite pad. The organic phase was dried and evaporated to give an oil (4.5 g) as a residue which consisted of the methyl ester of (2*R*,3*S*)-*trans*-(4-methoxyphenyl)glycidic acid (47% assay, 35% yield, 98% ee).

Hydrolysis of PGA Methyl Ester with CCL Lipase in Acetonitrile/H_2O. With a procedure analogous to that described, hydrolysis was performed in acetonitrile/water. The reaction arrested at 2 h gave the methyl ester as a 65:35 mixture of the two enantiomers.

Transesterification of PGA Methyl Ester with Ethanol in the Presence of α-CHT. PGA methyl ester (9.3 g) in ethanol (740 mL) was mixed with a solution of α-CHT (7.4 g) in phosphate buffer at pH 7.4 (370 mL), and the resulting biphasic system was stirred at room temperature. The reaction was followed by HPLC (SiO_2 column, *n*-hexane/ethyl acetate (9:1), t_R 8 min for the ethyl ester and 10 min for the methyl ester) which showed that after 5.5 h the ratio of the two esters was 1:1. The solution was rapidly evaporated in vacuum at room temperature to give a mixture of products from which by rapid chromatography crude ethyl ester was obtained (4.5 g). ^1H NMR spectra in the presence of chiral shift reagents showed the product to be a 9:1 mixture of two enantiomers. To this material the (2*R*,3*S*) absolute configuration was assigned on the basis of the negative rotation sign. If the reaction was interrupted at 30% conversion the optical purity of the ester was 85% ee.

Transesterification of PGA Methyl Ester with *n*-Propanol in the Presence of α-CHT. PGA methyl ester (1 g) in *n*-propanol (80 mL) was mixed with a solution of α-CHT (0.8 g) in phosphate buffer at pH 7.4 (40 mL), and the resulting biphasic system was stirred at room temperature. When the reaction approached 50% conversion the propyl ester was recovered by rapid chromatography and judged to be of 70% ee from chiral HPLC.

Transesterification of PGA Methyl Ester with *n*-Butanol in the Presence of α-CHT. PGA methyl ester (10 g) in *n*-butanol (200 mL) was mixed with a solution of α-CHT (9 g) in phosphate buffer at pH 7.4 (400 mL), and the resulting biphasic system was stirred at room temperature. When the reaction approached 50% conversion (4.5 h) the mixture was extracted with ethyl acetate and evaporated. Cooling of the mixture allowed the recovery of some unreacted methyl ester (3 g). The remainder was partitioned between the two phases obtained upon mixing hexane, ethyl acetate, methanol, acetonitrile, and water in ratios of 30/8/30/8/24, respectively. From the upper phase 92% chemically pure 4 was obtained which resulted of 80% ee from chiral HPLC (*n*-hexane/2-propanol 90/10, t_R 6.5 min for the (2*S*,3*R*) enantiomer and 7.02 min for the (2*R*,3*S*) enantiomer).

Transesterification of PGA Methyl Ester with *n*-Butanol in the Presence of CCL Lipase in *n*-Hexane. PGA methyl ester (4 g) in *n*-hexane containing 20% of *n*-butanol (100 mL) was treated with CCL lipase (10 g) and stirred at room temperature until the ratio of the esters was 1:1 (HPLC). Partition between solvents as above afforded the butyl ester 5 90% chemically pure of 60% ee to which the (2*S*,3*R*) absolute configuration was assigned from the positive rotation sign and the retention time in the HPLC. Other trials with different solvents were also performed: in **cyclohexane** the $t_{1/2}$ is slightly higher then in *n*-hexane. The product obtained is 58% ee. In ***n*-heptane** after 24 h only 15% of the butyl ester is formed with an ee of 70%. In **butyl acetate** the reaction is extremely slow.

Transesterification of PGA Methyl Ester with *n*-Butanol in the Presence of α-CHT on Biofix E_1 in *n*-Hexane. A preparate of α-CHT absorbed on Biofix E_1 as described (10 g) was suspended in *n*-hexane containing 10% of *n*-butanol (100 mL) and the mixture treated with the methyl ester (1 g). After 6 h the enzyme was filtered off and the mixture analyzed. The butyl ester was 70% ee. The preparate was reused several times, and it was evaluated that the loss of activity was approximately 20% for each run. After five times the retained activity was 30%.

Transesterification of PGA Methyl Ester with *n*-Butanol in the Presence of α-CHT Immobilized on Agarose. The preparate (90 mg, 50 U) was mixed with the methyl ester (40 mg), phosphate buffer pH 7.4 (1.6 mL), and 0.8 mL of *n*-butanol. The reaction was complete after 8 h. The product was of 68% ee as judged from HPLC. After seven runs the material had retained 20% of the original activity.

Kiheisterones, New Cytotoxic Steroids from a Maui Sponge

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Marine sponges have proven to be a veritable cornucopia of unusual steroids.[1] We now describe the isolation and structure elucidation of the kiheisterones A and B (1 and 2),[2] each containing an α,β-disubstituted furan in the side chain, cis-fused A/B ring, a monoenolized α-diketone in the A ring, and a C-21 carboxyl group.

A recent collection of marine invertebrates off the island of Maui, Hawaii, provided a sponge of the order Poecilosclerida[3] whose crude extracts were cytotoxic against several cell lines. Silica gel flash and C-18 gravity column

(1) Kerr, R. G.; Baker, B. J. *Nat. Prod. Rep.* **1991**, *8*, 465–497.
(2) The name of the compounds is derived from the town of Kihei in West Maui, the nearest place to the collection site.
(3) We are grateful to Dr. Shirley Pomponi, Harbor Branch Oceanographic Institution, for indentifying the sponge. A voucher specimen is available at HBOI.

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