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Lipase Catalyzed Kinetic Resolution of (±)-*cis*-4-Hydroxymethyl-2-phenyl-1,3-dioxane.

Bernardo Herradón [1]
Laboratorium für Organische Chemie, E.T.H., Universitätstrasse, 16, Zürich, CH-8092, Switzerland

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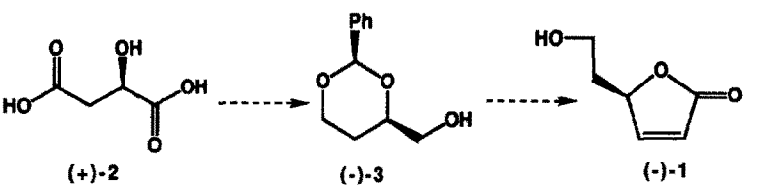
Abstract. The title compound has been kinetically resolved through a lipase catalyzed transesterification in organic solvents. The influence of the enzyme source, as well as the nature of the solvent on the enantioselectivity have been studied.

Recently [2] we reported the EPC-synthesis [3] of (R)-5-(2-hydroxyethyl)-2(5H)-furanone [(±)-**1**], a functionalized chiral building block for the synthesis of sugar analogues, pheromones [4] and other natural products [5]. The synthesis started from (R)(+)-malic acid [(+)-**2**]; a key intermediate was the chiral 1,3-dioxane (-)-**3** (*Scheme 1*).

Scheme 1.



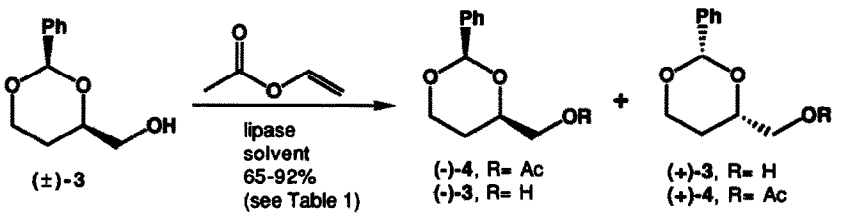
Although the overall transformation of (+)-**2** into the butenolide (-)-**1** is quite short (5 steps) and gives a high overall yield (48 %), the relatively high cost of unnatural (R)-malic acid is a drawback. This limits the applicability of the synthesis reported in *scheme 1*, and, in general, the utility of chiral building blocks prepared from (R)-malic acid [6]. Due to this drawback, and in connection with projects for the enantioselective synthesis of polyhydroxylated compounds using biocatalyts [7], we searched for alternative procedures to prepare the above mentioned chiral building blocks.

Lipases are noteworthy among enzymes [8]. This class of ester hydrolases are readily available, do not require expensive cofactors, and can accept a broad range of substrates, usually performing enantioselective transformations. Furthermore, lipases are remarkably stable in organic solvents; and, although the natural function of these enzymes is the hydrolytic reaction of water with esters of glycerol, it has been shown that the reaction can be reversed in poor water-content organic media, affording esters from an alcohol and an acylating agent [9]. Some additional advantages of lipase-catalyzed transformations in organic solvents when

compared with reactions in aqueous media are easier work-up, lack of water-promoted side reactions, higher solubility of organic substrates, and the possibility to alter the selectivity by changing the hydrophobicity and polarity of the media [10].

In this paper we report that (±)-cis-4-hydroxymethyl-2-phenyl-1,3-dioxane [(±)-3] [11] can be efficiently resolved through a lipase-catalyzed transesterification using vinyl acetate as irreversible acyl donor [12] (*Scheme 2*). To optimize the process, we have carried out the transformation using lipases from several sources as well as different solvents [13, 14]. The results are collected in table 1.

Scheme 2.



Several features are worth noting. The stereochemical outcome and the enantioselectivity depended on the source of the enzyme. Thus, while the pig pancreatic lipase (PPL) and the *Candida cylindracea* lipase (CCL) selectively acylated the (R,R)-enantiomer, affording (-)-**4** and (+)-**3** (entries 1-7), the lipase from *Pseudomonas fluorescens*, SAM-2 (PFL) [15] showed preference for the (S,S)-enantiomer as substrate (entries 8-20), furnishing (+)-**4** and (-)-**3**.

The efficiency of the resolution was calibrated by the value of the enantioselectivity E as defined by Sih and coworkers [16]. As shown in table 1, the selectivity of either PPL or CCL catalyzed acetylation was poor (entries 1-7). On the other hand, the reactions catalyzed by PFL displayed from medium to good levels of enantioselectivity, and the values of E depended on the nature of the solvent (entries 8-20). The highest selectivities (E >45) were achieved in the reactions carried out in THF (entry 14), THF/hexane (entries 16-18) or benzene (entry 20).

Another factor that influenced the enantioselectivity and the velocity of the process catalyzed by PFL is the water content of the solvent. Thus, while the reaction in anhydrous chloroform proceeded with low enantioselectivity, the transformation in wet chloroform was carried out with good enantioselectivity (entries 8 and 9). The hydration level of ethyl ether did not influence enantioselectivity, but only the rate of the reaction (entries 12 and 13). Furthermore, water was necessary for the reaction in methylene chloride (entry 12); the reaction in anhydrous methylene chloride is extremely slow (data not shown).

Summarizing, we have shown that proper choice of the solvent (entries 14, 17, and 20 of table 1) can provide (-)-**3** in high enantiometic purity (ee >98%) and acceptable chemical yield through a *Pseudomonas fluorescens* lipase catalyzed acetylation of (±)-**3** using vinyl acetate as acylating agent. This enzymatic method complements the reported chemical synthesis of (-)-**3** from (R)-malic acid [2]. Although the reactions have not been tried yet, the use of the conditions reported in entries 14, 16 and 20, and lower conversion degrees (*ca.* 45%) would provide (+)-**4** in high enantiomeric excess too.

Table 1. Results of the lipase-catalyzed acetylation of (±)-3.

Entry	Lipase [a] (Amount) [b]	Solvent [c] (Eq. vinyl acetate) [d]	Time (h)	% c [e]	Alcohol % ee (% y) [f]	Ester % ee (% y) [f]	E [g]
1	PPL (750)	$CHCl_3$ (2.5)	120	27	(+)-3 17 (56)	(-)-4 45 (18)	3.7
2	PPL (680)	wet $CHCl_3$ (2.5)	32.5	33	(+)-3 35 (47)	(-)-4 72 (24)	8.5
3	PPL (810)	Et_2O (2.5)	7	56	(+)-3 54 (46)	(-)-4 43 (45)	4.2
4	PPL (680)	wet Et_2O (1.0)	144	31 [h]	(±)-3 0 (59)	(±)-4 0 (22)	
5	PPL (680)	THF (2.5)	24	66	(+)-3 80 (21)	(-)-4 41 (57)	5.5
6	PPL (470)	EtOAc (2.5) [i]	9	47	(+)-3 39 (39)	(-)-4 44 (39)	3.7
7	CCL (310)	wet $CHCl_3$ (2.0)	44	19	(+)-3 5 (74)	(-)-4 23 (18)	1.6
8	PFL (410)	$CHCl_3$ (1.0)	72	64	(-)-3 39 (30)	(+)-4 71 (46)	4.5
9	PFL (120)	wet $CHCl_3$ (5.0)	80	47	(-)-3 74 (41)	(+)-4 84 (41)	25.7
10	PFL (120)	wet $CHCl_3$ (5.0) [k]	6	38	(-)-3 49 (53)	(+)-4 80 (31)	14.3
11	PFL (230)	wet CH_2Cl_2 (2.5)	28	50	(-)-3 70 (42)	(+)-4 69 (46)	11.2
12	PFL (220)	Et_2O (1.5)	9	48	(-)-3 68 (36)	(+)-4 74 (39)	13.3
13	PFL (220)	wet Et_2O (1.5)	67	45	(-)-3 59 (34)	(+)-4 72 (38)	11.1
14	PFL (230)	THF (2.5)	16.5	53	(-)-3 >98 (31)	(+)-4 86 (42)	>60
15	PFL (330)	THF (2.5) [k]	5	46	(-)-3 75 (46)	(+)-4 89 (44)	38.6
16	PFL (210)	THF-hexane (1:1, v/v) (1.5)	10.5	52	(-)-3 93 (33)	(+)-4 87 (42)	48.6
17	PFL (230)	THF-hexane (1:1, v/v) (1.5)	15	56	(-)-3 >99 (32)	(+)-4 77 (50)	45.8
18	PFL (210)	THF-hexane (1:2, v/v) (1.5)	22	51	(-)-3 91 (40)	(+)-4 87 (46)	45.8
19	PFL (230)	EtOAc (1.5) [i]	32.5	51	(-)-3 67 (43)	(+)-4 64 (46)	9.1
20	PFL (430)	benzene (1.5)	9	54	(-)-3 >98 (32)	(+)-4 83 (49)	>50

a) All the enzymes were commercially available (FLUKA). PPL refers to hog pancreas lipase (specific activity: 3.5 U/mg); CCL is *Candida cylindracea* lipase (specific acyivity: 31.6 U/mg); PFL denotes *Pseudomonas fluorescens* lipase (specific activity: 31.5 U/mg). An unit corresponds to the amount of enzyme which liberates 1μmol oleic acid per minute at pH 8.0 and 40°C (for CCL and PFL) or 37°C (for PPL) (as indicated in FLUKA catalogue). b) In units of enzyme per mmol of (±)-3. c) All the solvents were of *puriss.* quality (water-content< 1%). Wet solvents refer to water-saturated solvents. d) The amount of vinyl acetate with respect to (±)-3. At less otherwise stated, all the reactions were carried out at room temperature. e) Conversion degree, calculated from the relation $c = ee_S/(ee_S+ee_P)$ (ref. 16). f) All the yields refer to isolated products after flash-chromatography. The enantiomeric excesses (% ee) were determined by ^{1}H-NMR spectroscopy of any of the reaction products in the presence of Eu(hfc)$_3$. g) Calculated according to ref. 16. h) Determined by ^{1}H-NMR. i) The reaction in the absence of vinyl acetate was much slower. k) At 40°C.

Work is in progress in order to elucidate the role of the polarity, hydrophobicity, and water-content of the solvent on the enantioselectivity of lipase-catalyzed transesterifications [16].

References and footnotes.

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11 Synthesized in multigram scale (>0.25 mol, >35 g) by reaction of readily available (±)-butane-1,2,4-triol with benzaldehyde or benzaldehyde dimethyl acetal catalyzed by p-toluenesulfonic acid in refluxing toluene (>75% isolated yield; >90% regioselectivity; >98% diastereoselectivity).

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13 A typical procedure is as follows. A mixture of (±)-**3**, vinyl acetate (the amount indicated in table 1), and the amount of the corresponding lipase shown in table 1 was stirred at the temperature and for the time indicated in table 1. Then, the enzyme is filtered off and thoroughly washed with CH_2Cl_2. Evaporation of the solvents and flash-chromatography (hexane-EtOAc, 3:1 and 2:3) afforded the ester **4** and the alcohol **3**, which has identical spectroscopic data to the previously reported [2]. In all the cases the progress of the reaction was readily followed by ^{1}H-NMR (200 MHz), analyzing the integrals of the signal of H-2 in both compounds, the chemical shifts are 5.56 ppm for **3** and 5.53 ppm for **4**. The values of the optical rotation (at room temperature) are: for (-)-**3** (>98% ee): $[\alpha]_D$= -10.0 ($CHCl_3$, c= 1.18); for (+)-**4** (87% ee): $[\alpha]_D$= +23.5 ($CHCl_3$, c= 1.5). The value of the optical rotation of (-)-**3** is slighty higher than the one we reported formerly [2], which was supossed to be enantiomerically pure; we have found that the enantiomeric purity of starting (R)(+)-malic acid for the preparation of (-)-**3** is batch depending (between 96->99%). Also racemization during some of the steps for the synthesis of (-)-**3** as previously reported [2] can not be ruled out; this issue is being currently investigated.

14 Some selected spectroscopic data for **4** are as follows: ^{1}H-NMR ($CDCl_3$): δ= 7.61-7.46 (m, 2H), 7.44-7.32 (m, 3H), 5.53 (s, 1H), 4.40-4.27 (m, 1H), 4.25-4.09 (m, 3H), 4.00 (dt, 4.0, 12.7, 1H), 2.11 (s, 3H), 2.05-1.80 (m, 1H), 1.62-1.49 (m, 1H) ppm; ^{13}C-NMR ($CDCl_3$): δ= 171.4 (s), 138.7 (s), 129.2 (d), 128.6 (2C, d), 126.5 (2C, d), 101.5 (d), 75.1 (d), 66.8 (2C, t), 27.7 (t), 21.1 (q) ppm.

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