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Chemo-enzymatic Synthesis of Chiral Cyclic Compounds: Efficient Kinetic Resolution of 2-Bromo-2-Cyclohexenol.

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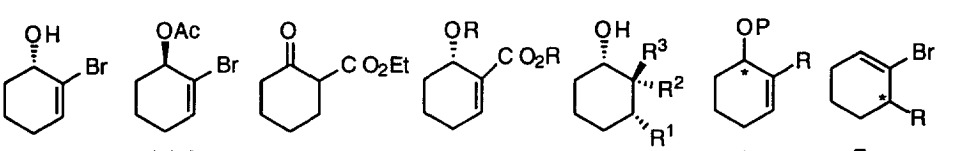
Abstract: The kinetic resolution of 2-bromo-2-cyclohexenol has been achieved through enzyme-mediated transesterification in organic solvents, using vinyl acetate as the irreversible acylating agent. The effect of the enzyme and the solvent on the velocity and the enantioselectivity of the transformation has been studied. Also the influences of the size of the carbocyclic ring and the substituent in the 2-position have been briefly examined.

In connection with current endeavours on the synthesis of analogs of morphine,[1] we required functionalized chiral derivatives of 2-cyclohexenol (e.g.; compounds **1** and **2**). Our previous experience in this area was the preparation of (*S*)-allylic alcohols **4**, which were synthesized using methodologies which involved the yeast-mediated enantioselective reduction of the β-keto ester **3**.[2] Although the α,β-unsaturated esters **4** are useful chiral building blocks for the synthesis of the densely functionalized chiral cyclohexanol derivatives **5**,[3] an inconvenience is that the yeast-promoted reduction affords only one enantiomer of the desired chiral building block.



(-)-1 (+)-2 **3** **4** **5** **6** **7**

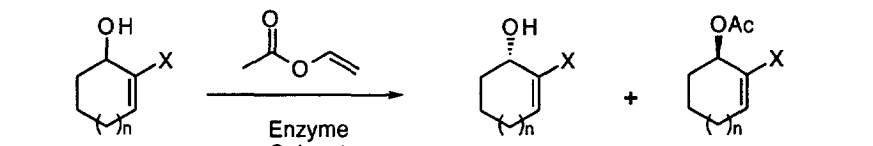
6 and **7** in any enantiomeric form

An alternative approach, that would provide derivatives of both enantiomers of the required chiral building block, is the kinetic resolution of suitable racemic compounds. An appropriate method would be the enzyme-mediated acylation of alcohols in organic solvents.[4] We have previously found that both the velocity and the selectivity of lipase- and acylase-catalyzed transesterifications[5] are dependent on the nature of the solvent, it being possible to obtain highly enantioselective transformations simply by changing the solvent.[6] One interesting 2-cyclohexenol derivative is the 2-bromo-derivative **1**, where the functionalities of vinyl bromide and allylic alcohols could provide access to a variety of derivatives, such as **6** and **7**.[7]

Because of this synthetic potential, it would be desirable to have practical routes to chiral derivatives of (-)-**1** and (+)-**1**. Previously, the *R*-enantiomer of the alcohol **1** was prepared by borane-reduction of the corresponding ketone using chiral oxazaborolidines as catalysts.[8] The biocatalytic synthesis of chiral derivatives of **1** include the low enantioselective hydrolysis of (±)-**2** using the mold *Rhizopus nigricans*,[9] and the acetylation of (±)-**1** catalyzed by a recombinant *Candida antarctica* lipase.[10]

In order to find an efficient route to these chiral building blocks, as well as to learn further about the influence of organic solvents on the selectivity of enzyme-mediated acylations,[5,6] we have carried out the acetylation of (±)-**1** in organic solvents, using vinyl acetate as the reagent and readily available enzymes as biocatalysts. The enzymes used are the lipases from *Pseudomonas fluorescens* (PFL) and from pig pancreas (PPL) as well as the acylase I from *Aspergillus* species (AA-I) (Scheme 1). The results of the kinetic resolution of (±)-**1** are indicated in entries 1-18 of Table 1.[11]

Scheme 1



(±)-**1**, n = 1, X = Br
(±)-**8**, n = 1, X = H
(±)-**10**, n = 2, X = Br

(-)-**1**, n = 1, X = Br
(-)-**8**, n = 1, X = H
(-)-**10**, n = 2, X = Br

(+)-**2**, n = 1, X = Br
(+)-**9**, n = 1, X = H
(+)-**11**, n = 2, X = Br

The efficiency of the kinetic resolution has been calibrated by the values of the enantioselectivity E, as defined by Sih and Wu.[12] It is observed that the use of AA-I, an enzyme recently shown to catalyze acylation reactions,[5] resulted in slow and low-selective reactions (entry 1).[16] The reactions catalyzed by PFL (entry 2-9, Table 1) proceed with moderate to good enantioselectivity (E = 18-38). It is observed that the selectivity depends on the solvent, in accordance with previous findings.[6] In the acylations of (±)-**1** catalyzed by PFL, it is possible to obtain the acetate (+)-**2** in high enantiomeric purity (entries 4 and 7), albeit in low chemical yield. On the other hand, the alcohol (-)-**1** is obtained enantiomerically pure when the reactions are carried out at relatively high conversions (entries 2 and 8, Table 1). The selectivity of the acetylation of (±)-**1** catalyzed by the inexpensive enzyme PPL (entries 10-18) depends on the water-content of the solvent. It is observed that the reactions in wet solvents are slow and not very selective (entries 11 and 16); on the other hand, the enantioselectivity of the acetylations in dry solvents ranges are good to excellent (E = 41-93). Although the selectivities in toluene (entry 10), *iso*propyl ether (entry 12), THF-hexane (entry 17), and vinyl acetate (entry 18) are good, and it is possible to obtain compounds with high enantiomeric purities, the reactions in these solvents are relatively slow. Faster reactions and excellent selectivities have been achieved when operating in dry ethyl ether (entries 13-15). The enantioselectivity is independent of the amount of PPL (compare entries 13 and 14), the only effect of increasing the amount of enzyme is a faster reaction.[17] Using these experimental conditions, it is possible to obtain both chiral building blocks (+)-**2** and (-)-**1** in high enantiomeric purities.

In order to understand the influence of the structure of the substrate (namely, the substituent in the 2-position and the size of the carbocyclic ring) on the enantioselectivity, we have carried out some acetylations of the allylic alcohols (±)-**8** (entries 19 and 20) and (±)-**10** (entries 21 and 22) catalyzed by PPL. A striking difference has been observed between the reactions of (±)-**1** and its analogs (±)-**8** and (±)-**10**, because these

Table 1. Results of the kinetic resolution of (±)-**1**, (±)-**12**, and (±)-**14** (Scheme 1).[a)]

Entry	Starting material	Enzyme (amount)[b)]	Solvent[c)]	Time (h)	%c[d)]	Acetate (ee)[e)]	Alcohol (ee)[e)]	E[d)]
1	(±)-**1**	AA-I (200)	toluene	25	15	(+)-**2** (68%)	(-)-**1** (12%)	6
2	(±)-**1**	PFL (600)	hexane	31.5	59	(+)-**2** (68%)	(-)-**1** (> **99%**)	32
3	(±)-**1**	PFL (600)	THF-hexane (1:1)	46.5	52	(+)-**2** (83%)	(-)-**1** (90%)	34
4	(±)-**1**	PFL (400)	toluene	24	18	(+)-**2** (94%)	(-)-**1** (21%)	38
5	(±)-**1**	PFL (400)	wet toluene	23.5	28	(+)-**2** (90%)	(-)-**1** (34%)	27
6	(±)-**1**	PFL (600)	wet toluene	71	53	(+)-**2** (80%)	(-)-**1** (91%)	29
7	(±)-**1**	PFL (400)	$CHCl_3$	95.5	18	(+)-**2** (93%)	(-)-**1** (20%)	32
8	(±)-**1**	PFL (400)	Et_2O	95.5	60	(+)-**2** (67%)	(-)-**1** (> **99%**)	30
9	(±)-**1**	PFL (400)	vinyl acetate[f)]	23.5	22	(+)-**2** (87%)	(-)-**1** (24%)	18
10	(±)-**1**	PPL (10000)	toluene	66	7	(+)-**2** (95%)	(-)-**1** (7%)	41
11	(±)-**1**	PPL (6000)	wet $CHCl_3$	45.5	9	(+)-**2** (48%)	(-)-**1** (5%)	3
12	(±)-**1**	PPL (6000)	$^iPr_2O$	45.5	29	(+)-**2** (94%)	(-)-**1** (38%)	43
13	(±)-**1**	PPL (10000)	Et_2O	71.5	44	(+)-**2** (**95%**)	(-)-**1** (75%)	**93**
14	(±)-**1**	PPL (14000)	Et_2O	98	50	(+)-**2** (**93%**)	(-)-**1** (**92%**)	**93**
15	(±)-**1**	PPL (14000)	Et_2O	107	53	(+)-**2** (89%)	(-)-**1** (**99%**)	**86**
16	(±)-**1**	PPL (10000)	wet Et_2O	90.5	2	(+)-**2** (76%)	(-)-**1** (2%)	8
17	(±)-**1**	PPL (10000)	THF-hexane (1:1)	117	30	(+)-**2** (94%)	(-)-**1** (40%)	50
18	(±)-**1**	PPL (6000)	vinyl acetate[f)]	163.5	35	(+)-**2** (94%)	(-)-**1** (50%)	56
19	(±)-**8**	PPL (6000)	vinyl acetate[f)]	119.5	n. d.[g)]	**9**[h)] (2%)	**8**[h)] (3%)	*ca* 1
20	(±)-**8**	PPL (10000)	Et_2O	119.5	n. d.[g)]	**9**[h)] (2%)	**8**[h)] (6%)	*ca* 1
21	(±)-**10**	PPL (10000)	vinyl acetate[f)]	42	23[i)]	**11**[h)] (n. d.)[k)]	**10**[h)] (9%)	2[l)]
22	(±)-**10**	PPL (10000)	Et_2O	89.5	30[i)]	**11**[h)] (n. d.)[k)]	**10**[h)] (24%)	5[l)]

a) Unless otherwise indicated all the reactions were carried out at room temperature using 2 mol equiv of vinyl acetate. For a typical experimental procedure, see ref 13. b) The enzymes used were Acylase I from *Aspergillus* species (AA-I), lipase from *Pseudomonas fluorescenses* (PFL) and lipase from pig pancreas (PPL) purchase from Aldrich, Fluka, and Sigma, respectively. The specific activities of AA-I is 0.5 U/mg (as defined in Aldrich catalogue: one unit hydrolyze 1.0 mmol of N-acetyl-L-methionine per hr at pH 7.0 at 25°C). The specific activity of PFL is 31.5 u/mg [as defined in Fluka catalogue: one unit corresponds to the quantity of enzyme that liberates 1 µmol of oleic acid per min at pH 8.0 and 40°C (using triolein as substrate)]. The specific activity of PPL is 17.5 U/mg (as defined in Sigma catalogue: one unit is the amount of enzyme which hydrolyzes 1 µmol of triacetin per hour at pH 7.4 at 37°C). The amount refers to the units of enzyme per mmol of (±)-**1**, (±)-**8**, or (±)-**10**. c) All the solvents were of the highest quality available (water content < 0.5%). "Wet solvent" means water-saturated solvent. d) The conversion degree c and the enantioselectivity E were calculated according to ref 11. e) The enantiomeric excesses were determined by capillary gas-liquid chromatography using a cyclodextrin-based chiral stationary phase (ref 14). The absolute configurations of (+)-**2** and (-)-**1** were determined by comparison with values of the literature (ref 8-10). f) *ca* 50 mol equiv of vinyl acetate were used. g) Due to the low enantiomeric excesses of **8** and **9**, the value of c could not be calculated accurately. h) The absolute configurations of **8**-**11** were not determined, although they were assumed to be as indicated in Scheme 1 by comparison with the result of the resolution of (±)-**1**. i) The conversion degree was determined by ^{1}H-NMR spectroscopy. k) The ee of **11** was not determined. l) The value of E was determined based on the ee of **10** and the conversion determined by ^{1}H-NMR spectroscopy.

alcohols are acetylated non-selectively.[18] These results show a critical dependence of the enantioselectivity on the structure of the substrate, which agrees with previous findings.[19]

Summarizing, a highly efficient procedure for the kinetic resolution of 2-bromo-2-cyclohexenol has been achieved. This method compares favourably with those previously reported.[8-10] Some useful characteristics of the present process are the use of a very cheap enzyme, and the results are reproducible on scaling-up,[13] the enzyme can be recovered and reused. Additionally, some of the advantages of the use of enzymes in organic solvents[6] have also been observed. Work is in progress in order to realize some of the synthetic applications of the chiral building blocks (-)-**1** and (+)-**2** indicated above,[1,7] as well as to learn further on the influence of organic solvents and substituents on the selectivity of enzyme-mediated transformations.[20]

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(6) The advantages of this methodology have been previously discussed, see: Herradón, B.; Valverde, S. *Tetrahedron: Asymmetry* **1994**, *5*, 1479-1500.
(7) Compounds of class **6** would be accesible through: a) sequential halogen-lithium exchange and reaction with electrophiles; b) transition-metal catalyzed nucleophilic substitution of the bromine atom; c) Pd(0) catalyzed carbonylation or alkoxycarbonylation. Cyclohexene derivatives of type **7** might be prepared through: d) Claisen rearrangement; e) Wittig rearrangement; and f) Pd(0)-catalyzed allylic substitution. g) Other synthetically useful applications would be the reactions of the double bond, including hydrogenation, hydroboration, hydrosilylation, and bis-hydroxylation, among others. Due to the steric and electronic bias of these molecules, these reactions are expected to be highly regio- and stereo-selective. For selected references on all these subjects, see *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon Press: Oxford; 1991.
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(11) (±)-**1** and (±)-**14** have been prepared from cyclopentene and cyclohexene, respectively, as reported in: Sandler, S. S. *J. Org. Chem.* **1967**, *32*, 3876-3881).
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(13) Most of the indicated experiments were carried out at low scale (0.25-0.5 mmol of substrate). The products of any reaction were not individually isolated, but the crude reaction product was directly analyzed by capillary glc on chiral column.[14] The mass balance, the conversion, and the g.l.c. analysis of the crude reaction product indicated that the overall yield of any reaction was > 90%. In some cases, the reactions were carried out at larger scale, the results being reproducible and the isolated overall yield were excellent. A representative experimental procedure is as follows. PPL (18.4 g) was added to a magnetically stirred solution of (±)-**1** (4.0 g, 22.6 mmol) in dry Et_2O (210 mL). After stirring for 5 minutes, vinyl acetate (5.3 mL, 48 mmol) was added. The mixture was stirred at room temperature for 102 hours. After this time, the enzyme was filtered-off[15] and thoroughly washed with CH_2Cl_2. The solvent was removed, and the residue was flash-chromatographed to give the acetate (+)-**2** [2.15 g, 44% yield, 89% ee; $[\alpha]_D$ +103, (CH_2Cl_2, c = 1.0); lit.[10]: +102.5 for a sample reported to be > 95%ee] and the alcohol (+)-**1** [1.88 g, 47% yield, 99% ee; $[\alpha]_D$ -74, (CH_2Cl_2, c = 1.0); lit.[10]: -71.8 ($CHCl_3$, c = 0.87) for a sample reported to be 87%ee].
(14) These stationary phases have been prepared by Mrs. Mª Isabel Jiménez (Instituto de Química Orgánica General, C. S. I. C.), to whom we thank for her assistance on the glc analysis.
(15) The recovered enzyme can be reused; although the activity is slightly lower than in the original experiment, the selectivity is of the same order.
(16) Approximately the same selectivity and velocity are observed using wet toluene or vinyl acetate as solvents.
(17) Similar selectivity has been achieved when the reaction has been carried out with 6000 units of PPL per mmol.
(18) When PFL or AA-I are the biocatalyst, low enantioselective transformations are also observed.
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