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Enzyme catalyzed reverse enantiomeric separation of methyl (±)-3-cyclohexene-1-carboxylate

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Abstract—We describe the differences of hydrolase-type enzymes pig liver esterase (PLE), horse liver esterase (HLE), and porcine pancreatic lipase (PPL) on the hydrolysis of methyl (±)-3-cyclohexene-1-carboxylate to afford both enantiomers with 89% to >99% ee. The resultant enantiomerically pure (*S*)-(−)-3-cyclohexene-1-carboxylic acid was transformed into (1*S*,5*S*)-(−)-5-(hydroxymethyl)-2-cyclohexen-1-ol via iodolactonization, subsequent elimination of iodine with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and reduction with lithium aluminum hydride (LAH).

1. Introduction

Experimental studies on the syntheses of complex targets have resulted in the development of reactions, which emphasize chemo-, regio-, and stereoselectivity.[1] In a wide variety of asymmetric methods, enzymatic asymmetric hydrolysis has been a powerful method in the development of strategies for the asymmetric synthesis of various natural products.[2] The nucleoside analogues have been widely explored as potential antiviral and antitumoral chemotherapeutic agents. In particular, six-membered carbocyclic nucleosides show the resistance to hydrolysis since glycosidic bond cleavage may occur in the degradative pathway of nucleoside type antivirals that is 2′,3′-dideoxynucleosides.[3] Moreover, a cyclohexene ring has structural properties that distinguish this ring system from common six-membered rings such as cyclohexane, cyclohexadiene, and benzene, and that allow us to categorize the cyclohexene ring as a bioisotere of a saturated furanose ring.[4] In the literature, there is only one example for the racemic synthesis of cyclohexenyl and cyclohexanyl nucleosides.[5]

The unique immunosuppressive properties of the 21-membered macrolactam FK-506,[6] isolated from *Streptomyces tsukubaensis*,[7] prompted a great deal of work on the total synthesis of this compound.[8] Since in the syntheses of both carbocyclic nucleosides and FK-506 and its fragments, optically active 3-cyclohexene-1-carboxylic acid **1** is the valuable starting compound,[5,8a,9] there are great efforts on the asymmetric synthesis of **1** with the maximum 95% ee.[8a,9a,c,10]

In connection with our studies in the field of enzymatic resolution of valuable chiral synthons, we tried to resolve methyl (±)-3-cyclohexen-1-carboxylate **2** to afford enantiomerically enriched **1** and **2** with high ee. During the course of our studies on the biotransformations of methyl (±)-3-cyclohexene-1-carboxylate **2**, the screening reactions were first completed with various lipases (i.e., pig liver esterase PLE, lipase from *Candida rugosa* CCL, horse liver esterase HLE, porcine pancreatic lipase PPL, and *Candida antarctica* lipase CAL) using substrate:enzyme ratio from 1:1 to 1:0.5. Among the lipases studied, PLE, HLE, and PPL proved suitable for the enantioselective hydrolysis of this substrate. In particular, PPL showed an interesting enzyme-depending reversal of enantioselectivity. The observed promising preliminary results directed us toward catalytic studies on this subject. Thus, PLE and HLE catalyzed reactions afforded (*S*)-configured carboxylic acid. In contrast to this, PPL yielded (*R*)-configured carboxylic acid. The enzymes used in catalytic level exhibited high ee values and showed unusual versatility and diversity of the enantioselective hydrolysis of this substrate.

We describe herein the highly efficient enantioselective resolution of the racemic substrate (±)-**2** with PLE,

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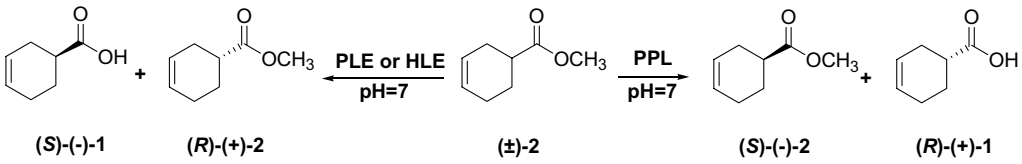


Scheme 1.

HLE, and PPL to afford (*S*)-(−)-**1** with the former two, while (*R*)-(+)-**1** was obtained with the latter enzyme (Scheme 1).

2. Results and discussions

Racemic **2** was obtained from commercially available racemic 3-cyclohexene-1-carboxylic acid using a slightly modified literature procedure. The first bioconversion was performed by PLE according to the following general procedure. To a stirred solution of 500 mg (±)-**2** in 50 mL pH 7.00 phosphate buffer, 100 μL PLE was added in one portion and the reaction mixture was stirred at 20 °C in a pH stat unit. The conversion was monitored by TLC and HPLC. After 4 h, 49% conversion was observed. The products were separated using flash column chromatography and compound (−)-**1** and was isolated in 43% yield and in >99% ee. The absolute configuration of compound (−)-**1** was assigned as (*S*) by comparison of its specific rotation with the literature data.[11] The second resolution was done on the same substrate using HLE under the same conditions as above, in which 10 mg of enzyme was used. After 6 h, 48% conversion was observed. (*S*)-(−)-**1** was obtained in 41% yield and 97% ee. The last attempt was done on the racemic substrate (±)-**2** using PPL (10 mg). When the hydrolysis of the above substrate catalyzed by PPL was allowed to proceed to 49% conversion (12 h at 20 °C), in contrast to the PLE and HLE hydrolysis results, the hydrolyzed acid of (*R*) absolute configuration, (+)-**1**, was obtained in 43% yield and 91% ee. The results are given in Table 1 and show that the enantiomeric excess (ee) varied from 91% to 99%.

In connection with our work on the development of novel procedures for the synthesis of cyclohexenyl type nucleosides in enantiomerically pure form, we transformed (*S*)-(−)-3-cyclohexene-1-carboxylic acid into the (−)-5-hydroxymethyl-2-cyclohexen-1-ol **5** using slightly modified literature procedures[9b,c] (Scheme 2). Iodolactonization of (*S*)-(−)-**1** followed by elimination of the iodide of (−)-**3** with DBU afforded the unsaturated lactone (−)-**4**. Reduction of (−)-**4** with lithium aluminum hydride quantitatively provided (−)-5-hydroxymethyl-2-cyclohexen-1-ol **5**. Since during the course of this transformation, the configuration at all stereogenic carbon atoms is preserved, the absolute configurations of the products are assessed by comparison of their specific rotations with the literature data.[9,10b,11]

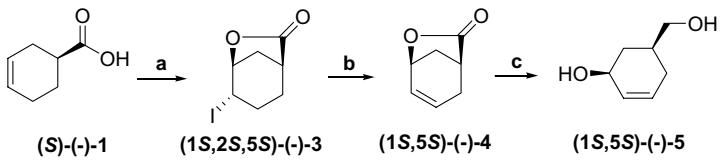
3. Conclusion

Herein, an enzyme-dependent reversal of enantioselectivity is demonstrated. Commercially available and inexpensive enzymes, PLE, HLE, and PPL, have shown specificity for the (*S*)- and (*R*)-enantiomer, respectively, of the ester **2** tested thus providing access to optically active acids of high enantiomeric purity and with opposite configurations. The availability and very low cost of the lipases, used in catalytic levels, renders the process very attractive for large-scale preparations. Transformation of acid (*S*)-(−)-**1** (>99% ee) into iodolactone (1*S*,2*S*,5*S*)-(−)-**3** followed by elimination and subsequent reduction afforded cyclohexenyl nucleoside precursor (1*S*,5*S*)-(−)-**5** with >99% ee and high chemical yield.

Table 1. Results of the enzyme catalyzed hydrolysis of (±)-**2**

Enzyme	Time (h)	Acids	Yield (%)[a]	$[\alpha]_D^{20}$	Ee (%)[b]	Esters	Yield (%)[a]	$[\alpha]_D^{20}$	Ee (%)[b]
PLE	4	(*S*)-(−)-**1**	43	−95.5	>99	(*R*)-(+)-**2**	38	+91.5	98
HLE	6	(*S*)-(−)-**1**	41	−92.4	97	(*R*)-(+)-**2**	43	+86.5	95
PPL	12	(*R*)-(+)-**1**	43	+85.8	91	(*S*)-(−)-**2**	41	−80.3	89

[a] Yields (%) are given as the isolated yields.
[b] Enantiomeric excess values are determined by the Phenomenex Chirex (*S*)-LEU and (*R*)-NEA (250×4.60 mm) chiral column HPLC analysis. Solvent system: hexane/*i*-propanol/MeOH = 85:15:5, flow rate: 0.5 mL/min. Retention times are 7.74 and 12.05 min.



Scheme 2. Reagents and conditions: (a) KI, I_2, $NaHCO_3$ (quantitative); (b) DBU, THF (91%); (c) $LiAlH_4$, THF (quantitative).

4. Experimental

^{1}H NMR spectra were recorded in $CDCl_3$ on Bruker Spectrospin Avance DPX 400 spectrometer. Chemical shifts are given in ppm downfield from tetramethylsilane. IR spectra were obtained from a Perkin–Elmer Model 1600 series FT-IR spectrometer and are reported in cm^{-1}. HPLC measurements were performed with ThermoFinnigan Spectra System instrument. Separations were carried out on Phenomenex Chirex (*S*)-LEU and (*R*)-NEA analytical column (250×4.60 mm) with hexane/isopropyl alcohol/methanol as eluent. All enzymatic transformations were carried out in Schott Gerate Titronic T200 pH stat unit. Optical rotations were measured in $CHCl_3$ solution in a 1 dm cell using a Bellingham and Stanley P20 polarimeter at 20 °C. Commercially available reagents and solvents, unless otherwise stated, were used without further purification. PLE (pig liver esterase) and HLE (horse liver esterase) were purchased from Sigma as a suspension in ammonium sulfate solution (3.2 mol/L) and as a powder, respectively. PPL (lipase, type II, from porcine pancreas) was purchased from Aldrich. (±)-3-Cyclohexene-1-carboxylic acid, DBU, and LAH were purchased from Acros.

4.1. Methyl (±)-3-cyclohexene-1-carboxylate 2

(±)-3-Cyclohexene-1-carboxylic acid (0.78 g, 6.24 mmol), MeI (3.73 mL, 60.00 mmol), Ag_2O (1.78 g, 7.68 mmol), and $CaSO_4$ (1.2 g, 8.80 mmol) were mixed and broken glass (0.60 g) was added to catalyze the reaction. The mixture was mixed for 24 h with the exclusion of moisture by the help of $CaCl_2$ tube. Excess MeI was recovered in vacuo and the residue was then diluted with $CHCl_3$ (50 mL). The solution was filtered, washed with $CHCl_3$ (50 mL), and dried over $MgSO_4$. The solvent was evaporated in vacuo to afford product **2**. (0.88 g, quantitative chemical yield) as a yellowish colored oil; R_f (EtOAc/hexane 1:1) 0.71; ν_{max} (neat) 3027, 2951, 1736 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 5.61–5.63 (2H, m, C*H*=C*H*), 3.61 (3H, s, O*Me*), 2.46–2.59 (1H, m, C*H*COOMe), 2.18–2.25 (2H, m, =CHCH_2CH), 1.98–2.09 (2H, m, =CHCH_2CH_2), 1.89–1.97 (1H, m, $CH_2CH_aH_bCH$), 1.57–1.68 (1H, m, $CH_2CH_aH_bCH$); δ_C (100.6 MHz, $CDCl_3$) 176.7, 127.0, 125.5, 51.9, 39.6, 27.8, 25.4, 24.8.

4.2. PLE hydrolysis of (±)-2

To a stirred solution of 500 mg *rac*-**2** in 50 mL pH 7.00 phosphate buffer, 100 μL PLE was added in one portion and the reaction mixture was stirred at 20 °C in a pH stat unit. The conversion was monitored by TLC and HPLC. The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$, and concentrated under reduced pressure. The product was purified by flash column chromatography (EtOAc–hexane, 1:1).

4.2.1. (*S*)-(−)-3-Cyclohexene-1-carboxylic acid (*S*)-(−)-1. (0.22 g, 43%) as a colorless oil; >99% ee $[\alpha]_D^{20} = -95.5$ (*c* 7.0, MeOH), lit.[11] $[\alpha]_D^{20} = -95.0$ (*c* 7.0, MeOH), lit.[10b] $[\alpha]_D^{20} = -92.35$ (*c* 4.5, MeOH).

4.2.2. (*R*)-(+)-Methyl 3-cyclohexene-1-carboxylate (*R*)-(+)-2. (0.19 g, 38%) as a yellowish colored oil; 98% ee $[\alpha]_D^{20} = +91.5$ (*c* 1.0, $CHCl_3$), lit.[12] $[\alpha]_D^{20} = +80.4$ (*c* 1.06, $CHCl_3$).

4.3. General procedure for HLE and PPL hydrolyses of 2

To a stirred solution of 500 mg *rac*-**2** in 50 mL pH 7.00 phosphate buffer, 100 mg HLE (or PPL) was added in one portion and the reaction mixture was stirred at 20 °C in a pH stat unit. The conversion was monitored by TLC and HPLC. The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$, and concentrated under reduced pressure. The product was purified by flash column chromatography (EtOAc–hexane, 1:1).

4.3.1. (*S*)-(−)-3-Cyclohexene-1-carboxylic acid (*S*)-(−)-1 (as a result of HLE hydrolysis). (0.21 g, 41%) as a colorless oil; 97% ee $[\alpha]_D^{20} = -92.4$ (*c* 7.0, MeOH), lit.[11] $[\alpha]_D^{20} = -95.0$ (*c* 7.0, MeOH), lit.[10b] $[\alpha]_D^{20} = -92.35$ (*c* 4.5, MeOH).

4.3.2. (*R*)-(+)-Methyl 3-cyclohexene-1-carboxylate (*R*)-(+)-2 (as a result of HLE hydrolysis). (0.22 g, 43%) as a yellowish colored oil; 95% ee $[\alpha]_D^{20} = +86.5$ (*c* 1.0, $CHCl_3$), lit.[12] $[\alpha]_D^{20} = +80.4$ (*c* 1.06, $CHCl_3$).

4.3.3. (*R*)-(+)-3-Cyclohexene-1-carboxylic acid (*R*)-(+)-1 (as a result of PPL hydrolysis). (0.22 g, 43%) as a colorless oil; 91% ee $[\alpha]_D^{20} = +85.8$ (*c* 7.0, MeOH), lit.[9c] $[\alpha]_D^{20} = +89.6$ (*c* 6.45, MeOH).

4.3.4. (*S*)-(−)-Methyl 3-cyclohexene-1-carboxylate (*S*)-(−)-2 (as a result of PPL hydrolysis). (0.21 g, 41%) as a yellowish colored oil; 89% ee $[\alpha]_D^{20} = -80.3$ (*c* 1.0, $CHCl_3$), lit.[12] $[\alpha]_D^{20} = -82.2$ (*c* 0.94, $CHCl_3$).

4.4. Synthesis of (1*S*,2*S*,5*S*)-(−)-2-iodo-7-oxabicyclo-[3.2.1]octan-6-one (1*S*,2*S*,5*S*)-(−)-3

A solution of $NaHCO_3$ (1.52 g, 18.1 mmol) in 25 mL of water was added to the (*S*)-(−)-3-cyclohexene-1-carboxylic acid (0.76 g, 6.02 mmol) with ice cooling. After the suspension dissolved, a solution of KI (6.0 g, 36.1 mmol) and I_2 (1.61 g, 6.33 mmol) in 15 mL of water was added. The resulting suspension was treated with $CHCl_3$ (30 mL), and the aqueous layer was extracted with $CHCl_3$ (2×50 mL). The combined extracts were washed with half-saturated $Na_2S_2O_3$ (2×50 mL), dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo to afford product **3**. (1.52 g, quantitative chemical yield) as a white solid; R_f (EtOAc/hexane 1:2) 0.68; mp 132 °C (lit. mp 135–136 °C);[9a] >99% ee $[\alpha]_D^{20} = -43.2$ (*c* 2.0 in $CHCl_3$), lit.[10b] $[\alpha]_D^{20} = -37.81$ (*c* 1.92 in $CHCl_3$). All spectroscopic data are in accordance with the literature values.[9c]

4.5. Synthesis of (1*S*,5*S*)-(−)-7-oxabicyclo[3.2.1]oct-2-en-6-one (1*S*,5*S*)-(−)-4

To a solution of (1*S*,2*S*,5*S*)-(−)-2-iodo-7-oxabicyclo[3.2.1]octan-6-one **3** (1.01 g, 4.01 mmol) in 28 mL THF, DBU (0.90 mL, 6.0 mmol) was added and the mixture stirred at reflux for 8 h. Upon being cooled to room temperature, the mixture was poured into 0.5 M HCl and extracted with ether (3 × 50 mL). The organic phase was washed with brine (50 mL), dried over anhydrous $MgSO_4$, concentrated in vacuo. The crude product was purified by flash column chromatography to afford product (−)-**4**. (0.45 g, 91%) as a colorless oil; R_f (EtOAc/hexane 1:3) 0.26; >99% ee $[\alpha]_D^{20} = -201.2$ (*c* 4.0 in $CHCl_3$), lit.[10b] $[\alpha]_D^{20} = -182.1$ (*c* 4.8 in $CHCl_3$). All spectroscopic data are in accordance with the literature values.[9c]

4.6. Synthesis of (1*S*,5*S*)-(−)-5-(hydroxymethyl)-2-cyclohexen-1-ol (1*S*,5*S*)-(−)-5

(1*S*,5*S*)-(−)-7-Oxabicyclo[3.2.1]oct-2-en-6-one **4** (0.40 g, 3.13 mmol) was dissolved in THF (10 mL) and added dropwise to $LiAlH_4$ (0.16 g, 3.13 mmol) dissolved in THF (30 mL) at 0 °C and stirred for 1 h. H_2O (0.1 mL), aq NaOH (0.13 mL) (15%, w/w), and H_2O (0.1 mL) were added sequentially, and the mixture was allowed to reach room temperature with stirring for 1 h. The salts were removed by filtration, and the solution was dried over $MgSO_4$ and concentrated in vacuo to give product **5**. (0.40 g, quantitative chemical yield) as a colorless semi-solid; $[\alpha]_D^{20} = -20.4$ (*c* 1.5 in MeOH), lit.[9b] for (1*R*,5*R*)-(+)-**5** $[\alpha]_D^{20} = -20.3$ (*c* 1.46 in MeOH); ν_{max} (neat) 3590, 3040, 1390, 1090; δ_H (400 MHz, $CDCl_3$): 5.64–5.73 (1H, m, CHOHC*H*=CH), 5.58–5.63 (1H, m, CH=C*H*CH_2), 4.29–4.39 (1H, m, C*H*OH), 3.40–3.53 (2H, m, CH_2OH), 2.65–3.05 (2H, br s, CHO*H* and CH_2O*H*), 1.96–2.11 (2H, m, CHOHCH_2), 1.77–1.88 (1H, m, =CHC$H_a H_b$), 1.65–1.77 (1H, m, =CHC$H_a H_b$), 1.15–1.27 (1H, m, C*H*CH_2OH); δ_C (100.6 MHz, $CDCl_3$): 131.4, 128.5, 67.4, 67.5, 35.56, 35.53, 28.4.

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