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Lipase-catalyzed synthesis of a tri-substituted cyclopropyl chiral synthon: a practical method for preparation of chiral 1-alkoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane

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Abstract

An efficient method for the preparation of optically active enantiomers of 1-ethoxycarbonyl-2-oxo-3-oxa-bicyclo[3.1.0]hexane **1** has been developed. Treatment of **1** with lipase Amano PS gave (1*S*,5*R*)-1-carboxy-2-oxo-3-oxabicyclo[3.1.0]hexane **4a** which was converted to (1*S*,5*R*)-1-ethoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane **1a** with high enantiomeric purity (98.0% *ee*, 75% yield), while the (1*R*,5*S*)-lactone ester **1b** remained intact. A simple procedure for the recovery of **4a** from the reaction mixture was also established. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

1-Alkoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexanes such as **1** are useful chiral synthons with a trisubstituted cyclopropane ring, which have been used for the synthesis of conformationally constrained amino acids[1–4] and, recently, for the synthesis of nucleoside analogues[5] with potent antiherpetic activity. Several synthetic methods for the enantiomers of 1-alkoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane have been reported. Cycloalkylation of dimethyl malonate with chiral epichlorohydrin gave the methyl ester of **1** in 93% *ee*.[3] Chiral glycidol triflate[4] and chiral cyclic sulfate of glycerol[6] were also used for the same purpose. The disadvantage of these methods is the use of expensive chiral sources, though these methods gave good enantiomeric excesses.[6] An alternative method was reported starting with prochiral 2-(tetrahydropyranyl)hydroxymethyl dimethyl cyclopropane-1,1-dicarboxylate utilizing enantioselective hydrolysis by an industrial esterase followed by lactonization. About 90% *ee* was achieved by this method after two crystallizations; however, the overall yield is quite low (<20%).[7] In the course of research using an enantiomer of **1** as a key intermediate, we attempted to develop an economical enzymatic process

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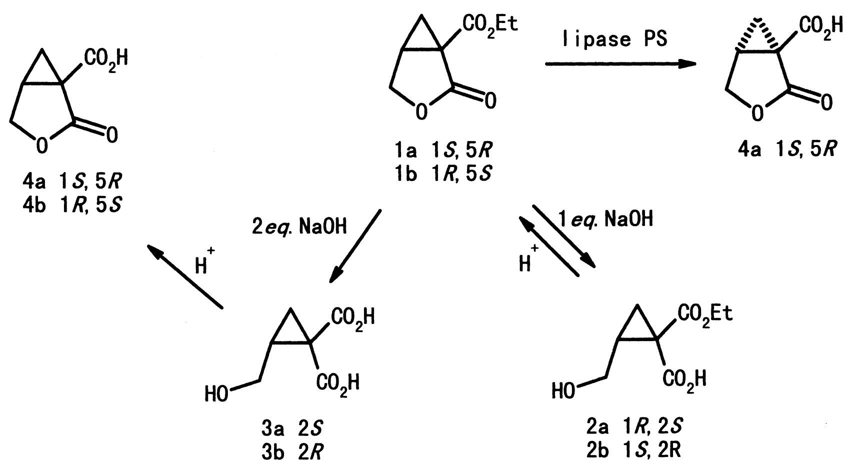
starting with racemic **1** which is easily prepared from epichlorohydrin and diethyl malonate in a single step.

2. Results and discussion

2.1. Screening of enzymes

To obtain an enzyme to achieve enantioselective hydrolysis, commercial lipases and esterases of microbial and mammalian origins were screened. From preliminary experiments in an aqueous medium, some lipases showed slow, but enantioselective hydrolysis of **1**. Esterases showed higher hydrolysis rates, but no stereoselectivity. It was also observed that hydrolysis by lipases was accelerated in the presence of organic solvents and a two-phase system consisting of phosphate buffer and diisobutylether was employed for screening. Compound **1** was treated with each enzyme, the reaction was terminated by addition of ethyl acetate, and the amount of remaining **1a** and **1b** in the organic layer was quantified by chiral HPLC. Under these conditions, consumption of some of **1** was observed in the non-enzymatic control, indicating that **1** was slowly hydrolyzed non-enzymatically. As described later, the lactone moiety of **1** is easily hydrolyzed under basic conditions and hydroxycarboxylic acid ester **2** was possibly formed under these conditions (Scheme 1). Thus, the amount of each enantiomer consumed in the enzymatic reaction was estimated by subtracting that in the non-enzymatic control as a reference, and the enantiomeric value (E)[8] was calculated as an index of selectivity of the enzymatic reaction as shown in Table 1. Among the lipases and esterases, lipases from *Pseudomonas* sp. such as lipase PS and lipase CES showed preferred consumption of (1S,5R)-1-ethoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane **1a** over (1R,5S)-lactone ester **1b**. Other lipases from yeast, fungi, and pancreas showed no stereoselective hydrolysis. Pig liver esterase showed some preference for **1a**. From these results, lipase PS was selected for the further optimization of reaction conditions.



Scheme 1.

2.2. Identification of the reaction product

There are two possible hydrolysis sites in **1**, namely the lactone ring and the ethyl ester. Since the hydroxycarboxylic acid formed by hydrolysis of the lactone moiety easily forms a lactone ring

Table 1
Enzymatic hydrolysis of **1** by lipases and esterase[a]

enzyme	origin	hydrolysis (%)[b] 1a	1b	E[c]
lipase CES	Pseudomonas sp.	89	6	34
lipase PS	Pseudomonas sp.	78	2	62
lipase AK	Pseudomonas sp.	47	5	13
lipase AY	Candida rugosa	43	17	3.0
lipase L10	Candida lipolytica	15	15	1.0
lipase type VII	Candida cyclindracea	34	19	1.9
lipase M	Mucor javanicus	19	12	1.7
lipase CE10	Humicola lanuginosa	14	11	1.3
lipase A	Aspergillus niger	12	9	1.3
lipase R	Penicillium roqueforti	12	12	1.0
lipase GC	Geotrichum candium	7	8	0.8
lipase D	Rhizopus delemer	14	10	1.4
lipase N	Rhizopus niveus	11	12	1.0
lipase type II	porcine pancreas	11	13	0.8
phospholipase C	Bacillus sp.	13	12	1.1
pig liver esterase A	pig liver	53	22	3.1

[a]25 mg of **1** was treated with 5 mg of enzyme in 1 ml of 0.1 M phosphate buffer (pH 7.0) and 3 ml diiodobutylether, and the mixture was stirred for 18 h at 37°C. Compounds **1a** and **1b** were extracted with 3 ml of ethyl acetate and analyzed by chiral HPLC. See Section 3.2 for details. [b]% of hydrolysis of **1a** is calculated as follows: 100×[(**1a** in non-enzymatic control)–(**1a** in enzymatic reaction)]/(**1a** in non-enzymatic control). % of hydrolysis of **1b** is determined in the same way. [c]E is calculated from the extent of enzymatic conversion of the substrate and the enantiomeric excess of the remaining substrate as described.[8]

during isolation, the reference compounds were prepared in situ to assign the structure of the enzymatic hydrolysis product of **1a**. Hydroxycarboxylic acid **2** was prepared by treatment of **1** with an equimolar amount of NaOH. When **1** was treated with an equimolar amount of NaOD in C_2D_5OD in an NMR tube, complete disappearance of NMR signals of **1** and appearance of new signals of **2** were observed within 15 minutes at room temperature.[9] Compound **1** was treated with two equimolar amounts of alkali to form dicarboxylic acid **3**, which was then cyclized to form lactone carboxylic acid **4** as shown in Scheme 1. These reference compounds are separable by reversed-phase HPLC and the major hydrolysis product of **1** by lipase PS was proved to be identical to **4** by reversed-phase HPLC analysis of the products in the aqueous phase. Small amounts of **2** and **3** were also formed; however, **2** was also formed in the non-enzymatic control in the same magnitude. On the other hand, neither **3** nor **4** was formed in the non-enzymatic control. Thus, it was proved that the lipases hydrolyzed the ethyl ester moiety of **1a** while keeping the more reactive lactone moiety intact.

2.3. Optimization of the reaction conditions

Since it was observed that the presence of organic solvents enhanced the hydrolytic activity of lipases and it is known that the choice of solvents affects the enantiomeric ratio,[10,11] further optimization of the reaction conditions was carried out using two-phase systems. The enzymatic reaction was carried out in a 1:1 mixture of phosphate buffer (pH 7.0) and various solvents. The reaction was terminated by addition of $CHCl_3$ and the remaining **1a** and **1b** in the organic phase was analyzed by chiral HPLC. In this case, the amount of **1a** and **1b** hydrolyzed was calculated from a difference between the initial and

remaining amounts as shown in Table 2. The ratio of hydrolyzed **1a** and **1b** is an index of selectivity of the enzymatic (the ethyl ester moiety) and non-enzymatic (the lactone moiety) hydrolysis. Use of water immiscible aliphatic hydrocarbons and higher dialkylethers resulted in high conversion and selectivity. Aromatic hydrocarbons such as toluene, which is often used as a co-solvent in lipase reactions,[12,13] gave inferior conversion.

Table 2
Total hydrolysis of **1** in various solvents[a]

solvent	hydrolysis (%)[b)] 1a	1b	1a/1b
toluene	43	14	3.1
cumene	70	19	3.7
petroleum benzin	91	23	4.0
n-hexane	87	36	2.4
methylisobutylketone	37	9	4.1
isopropylether	37	23	1.6
n-butylether	84	27	3.1
isobutylether	93	30	3.1
n-propylether	62	37	1.7

[a]25 mg of **1** was treated with 5 mg of lipase PS in 1 ml each of 0.1 M phosphate buffer (pH 7.0) and organic solvent, and the mixture was stirred for 21 h at 30°C. Compounds **1a** and **1b** were extracted with 2 ml of $CHCl_3$ and analyzed by chiral HPLC. [b]% of total hydrolysis of **1a** is calculated as follows: 100×[(**1a** added)–(**1a** in enzymatic reaction)]/(**1a** added). % of total hydrolysis of **1b** is determined in the same way.

The hydrolysis of **1b** is mainly caused by a non-enzymatic reaction at the lactone moiety, since the enzymatic reaction is highly stereospecific as shown in Table 1. The lactone moiety of **1** is slowly hydrolyzed in buffer and this spontaneous hydrolysis causes a decrease in selectivity. The selectivity is affected by the rate of the enzymatic hydrolysis at the ethyl ester moiety and the rate of the spontaneous hydrolysis at the lactone moiety. The latter may depend on the solubility of the substrate and water in the co-solvents. Generally, a water immiscible solvent is suitable as a co-solvent in lipase reactions.[14] In this case, with the presence of the lactone moiety in the substrate, such co-solvents are effective not only for the acceleration of the lipase reaction but also for protection from spontaneous hydrolysis. Once **2** is formed it may be possible that **2a** is a substrate of lipase; however, from separate experiments it was proved that **2** is not a substrate for hydrolysis by lipase and the formation of **2** disturbs the progress of the enzymatic reaction resulting in poor optical purity and yield. On the other hand, **3a** is recovered as **4a** by acid treatment. Thus, the key to achieve high efficiency is to accelerate the enzymatic reaction while minimizing spontaneous hydrolysis of **1**. As estimated, hydrolysis of the lactone ring is pH dependent, and the pH should be kept as low as possible. There is no difference in the rate of enzymatic reaction between pH 6 and 7 (data not shown). Since the progress of hydrolysis generates acid and makes the reaction conditions acidic, control of pH is required by using concd buffer or pH control in the actual process.

*2.4. Process for the preparation of **1a***

After further studies on several factors including effects of buffer/solvent ratio, reaction pH, ways of controlling pH, concentration of substrate and buffer, etc., the following process was established. Two grams of **1** were treated with 50 mg of lipase PS in 10 ml of 1:1 mixture of 0.5 M phosphate buffer (pH 7.0) and petroleum benzin for 23 h at 30°C with pH maintained over 5 by addition of 0.5N NaOH. In this process, starting the reaction at pH 7 resulted in better enantiomeric excess than starting at lower pH. Most of the reaction proceeds in the early stages of the reaction time, and the pH is kept as low as 5 in the later stage of the reaction to avoid hydrolysis of the lactone moiety.

After the enzymatic reaction, intact **1b** was recovered by $CHCl_3$ extraction (49.6% recovery, 83% *ee*) and **4a** was recovered from the acidified aqueous layer by absorption onto a column of hydrophobic absorbent SP-207 at pH 1 followed by elution with 20% methanol. Compound **3a** was converted to **4a** by acidification and also recovered in the same fraction. Compound **1** regenerated from **2** by acid treatment was eluted with 100% MeOH. Compound **4a** was then re-esterified with $SOCl_2$ and EtOH to give **1a** in 37.7% yield (75.4% based on **1a**) with 98.0% *ee*. The absolute stereochemistry of **1a** was supported by the previous report[3] as well as by X-ray crystallography of the 4-bromophenacyl ester of **4a** (data not shown).

This procedure gave **1a** with a higher enantiomeric excess than that expected from the small scale experiments such as those shown in Table 2. Under these reaction conditions, **1** shows limited solubility both in water and light petroleum. Most of the substrate remains insoluble and the substrate at the water/organic solvent interface might be more preferable for lipase hydrolysis as shown in the other experiments,[14] and the release of the product into the aqueous layer manipulates the equilibrium to promote hydrolysis. A high concentration of the substrate gives better results, minimizing spontaneous hydrolysis of the lactone moiety.

3. Experimental

3.1. Materials and methods

Reagents and solvents used were the highest quality available commercially. (*R*)- and (*S*)-Epichlorohydrin (>98% *ee*) were obtained from Daiso (Osaka). Lipase types II and VII were obtained from Sigma. Other enzymes were obtained from Amano. SEPABEADS SP-207 was purchased from Mitsubishi Chemicals. Racemic 1-ethoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane **1** was prepared from racemic epichlorohydrin and diethyl malonate as described previously.[5] The enantiomers were prepared from (*R*)- and (*S*)-epichlorohydrin.[5] ^{1}H NMR spectra were recorded with a Varian XL-300 300 MHz spectrometer, using tetramethylsilane as an internal standard. Mass spectra were recorded on a Jeol JMS-D300 spectrophotometer. Optical rotations were determined with a Jasco DIP370 polarimeter.

3.2. Analysis of hydrolysis products

The enantiomers of 1-ethoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane **1** were separated in a Chiralpack AD column (250 mm×4.6 mm Φ, Daicel) using *n*-hexane and ethanol (80:20) as eluent at a flow rate of 1 ml/min, monitoring UV absorbance at 230 nm. The enantiomeric excess of **1** was determined by this method. For the screening of enzymes, 5 mg of enzymes and 25 mg of **1** in 50 µl acetonitrile were incubated at 37°C for 18 h in a mixture of 1 ml of 0.1 M phosphate buffer and 3 ml of diisobutylether.

The reaction was terminated by an addition of 3 ml of ethyl acetate, and 5 μl each of the organic layer was subjected for the analysis of the enantiomers of **1**.

The reaction products in the aqueous phase were analyzed by reversed-phase HPLC using YMC-Pack ODS-AM302 (150 mm×4.6 mm Φ, YMC) with a gradient elution of 0.1% trifluoroacetic acid and acetonitrile at a flow rate of 1 ml/min, monitoring UV absorbance at 230 nm. Compound **2** was prepared by an addition of an equimolar amount of NaOH to **1** in EtOH. Compound **3** was prepared by treating **1** with two equimolar amount of NaOH. Since isolation of **2** and **3** was unsuccessful because of spontaneous formation of the lactone ring, their structures were confirmed by conversion to **1** and **4**, respectively, under acidic conditions. Purification of **2** and **3** by reversed-phase HPLC under the condition as above gave **1** and **4** quantitatively.

3.3. *Enzymatic process for preparation of **1a** and **1b***

Two grams of **1** and 50 mg of lipase Amano PS were suspended in 5 ml of 0.5 M phosphate buffer (pH 7.0) and 5 ml of petroleum benzin. The mixture was stirred for 23 h at 30°C with pH controlled at 5.0 by addition of 0.5N NaOH. The pH of the reaction mixture was adjusted to 7.0 by adding saturated $NaHCO_3$, and the products were extracted twice with 30 ml and 15 ml of $CHCl_3$. The organic layer was concentrated in vacuo to give 992 mg (49.6%) of **1b** with 82.9% *ee*.

The aqueous layer was acidified to pH 1.0 by addition of 3 ml of concd HCl and then applied onto a column containing 80 ml of SEPABEADS SP-207 previously treated with MeOH overnight, washed with water, and equilibrated with 0.1N HCl. The column was washed with 120 ml of 0.1N HCl and 80 ml of water, and **4a** was eluted with 300 ml of 20% MeOH. The fractions containing **4a** were concentrated in vacuo and the residue was dissolved in 20 ml of EtOH and treated with 1.0 ml of thionyl chloride at −10°C, and the mixture was held overnight at room temperature. After concentration in vacuo, the residue was dissolved in 20 ml of $CHCl_3$, washed with saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give 754 mg (37.7%) of **1a** with 98.0% *ee*.

Compound **1** derived from **2** in the reaction mixture was eluted from the column with 100% MeOH. After concentration in vacuo, 91.1 mg (4.6%) of **1b** (48.1% *ee*) was recovered.

Compound **1a**: colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J=7.1 Hz, 3H, CH_3CH_2), 1.37 (dd, J=4.8, 5.4 Hz, 1H, CH_2 of cyclopropane), 2.08 (dd, J=4.8, 8.0 Hz, 1H, CH_2 of cyclopropane), 2.72 (m, 1H, C*H* of cyclopropane), 4.18 (d, J=9.6 Hz, 1H, CH_2 of lactone), 4.27 (q, J=7.1 Hz, 2H, CH_3CH_2), 4.36 (dd, J=4.5, 9.6 Hz, 1H, CH_2 of lactone); FAB MS 170 (M^+); $[\alpha]_D^{25}$=−147.6 (c=1.20, EtOH) (lit.[5] $[\alpha]^{25}_D$=−146.6 (c=1.22, EtOH)).

Compound **4a**: waxy oil; ^{1}H NMR ($CDCl_3$) δ 1.56 (dd, J=4.5, 5.6 Hz, 1H, CH_2 of cyclopropane), 2.15 (dd, J=4.5, 7.8 Hz, 1H, CH_2 of cyclopropane), 2.96–3.30 (m, 1H, C*H* of cyclopropane), 4.31 (d, J=9.6 Hz, 1H, CH_2 of lactone), 4.47 (dd, J=5.0, 9.6 Hz, 1H, CH_2 of lactone); FAB MS 143 (MH^+); $[\alpha]_D^{25}$=−159.3 (c=1.22, MeOH). Anal. calcd for $C_6H_6O_4$: C, 50.71; H, 4.26; found: C, 50.35; H, 4.50.

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9. ^{1}H NMR (C_2D_5OD) of sodium salt of **2** is as follows: δ 1.19–1.38 (m, 2H, CH_2 of cyclopropane), 1.27 (t, J=7.2 Hz, 3H, CH_3CH_2), 1.91–2.02 (m, 1H, C*H* of cyclopropane), 3.44 (dd, J=9.4, 12.3 Hz, 1H, CH_2OD), 3.86 (dd, J=5.8, 12.3 Hz, 1H, CH_2OD), 4.16 (q, J=7.2 Hz, 2H, CH_3CH_2).
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