Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 2703–2712

TETRAHEDRON: *ASYMMETRY*

Chemoenzymatic synthesis of an α-substituted serine derivative

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Received 26 May 1999; accepted 16 June 1999

Abstract

An asymmetric synthesis of an α-substituted serine derivative was achieved by employing PLE-catalyzed hydrolysis of a prochiral malonate derivative, and the absolute configuration of the hydrolyzed product was unambiguously determined by the X-ray analysis of a camphorsulfonic acid derivative.

1. Introduction

Development of synthetic procedures for chiral α,α-disubstituted α-amino acids are of continuing interest,[1] since these compounds are needed for the synthesis of enzyme inhibitors and peptidomimetics. Among these amino acids, an α-substituted serine structure is often observed as a subunit of many biologically active natural products, such as conagenin **1**, lactacystin **2**, myriocins **3**, and mycestericins **4** (Fig. 1).[2]

Recently we have been involved in the asymmetric synthesis of biologically active natural products by utilization of PLE-catalyzed asymmetric hydrolysis of the pro-chiral malonate derivatives, and reported the preparation of the key intermediates for aphanorphine[3] and furanosesquiterpenes.[4] As an extension of this work, we are interested in the synthesis of an α-substituted serine derivative for the preparation of myriocin and its congeners.

As a means of investigating the scope and limitation of the PLE-catalyzed asymmetric hydrolysis of pro-chiral malonates, we chose the structurally simple cyclohexene derivatives as the starting materials, in which the discriminative sites for the enzyme lay between the sp^2- and sp^3-carbons. To the best of our knowledge for PLE-catalyzed asymmetric hydrolyses of malonate derivatives, no applications of compounds having such a small functional difference have appeared. In this paper, we would like to report our results for the PLE-catalyzed asymmetric hydrolysis of a malonate bearing very similar substituents.

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0957-4166/99/$ - see front matter
PII: S0957-4166(99)00270-0

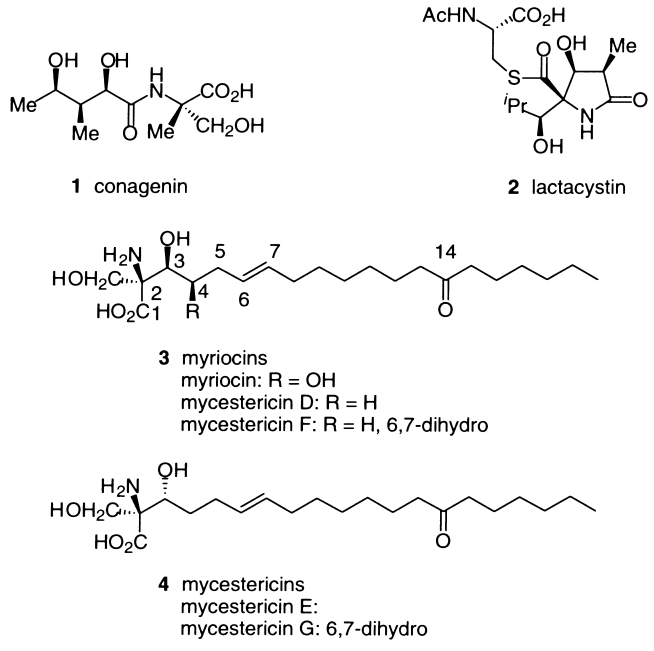
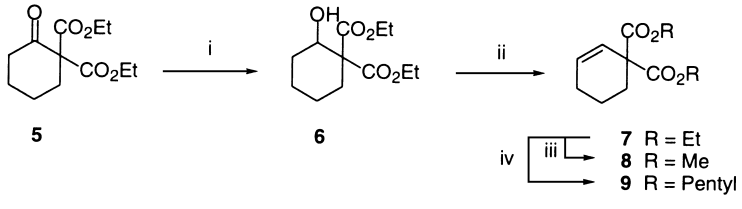


Figure 1.

2. Results and discussion

The starting pro-chiral diethyl malonate **7** was prepared from 2,2-dicarboethoxycyclohexanone **5**[4] in two steps involving reduction of the carbonyl group and dehydration of the alcohol **6** as described in Scheme 1, in 82% overall yield. The corresponding dimethyl malonate **8** was prepared by treatment of **7** with sodium methoxide, whereas dipentyl malonate **9** was obtained by treatment of **7** with pentyl alcohol in refluxing toluene in the presence of *p*-toluenesulfonic acid.



Scheme 1. *Reagents and conditions*: (i) $NaBH_4$, MeOH, room temp.; (ii) TFMSA, pyridine, −15°C to room temp.; (iii) NaOMe, MeOH, 0°C; (iv) *p*-TSA, 1-pentanol, 80°C

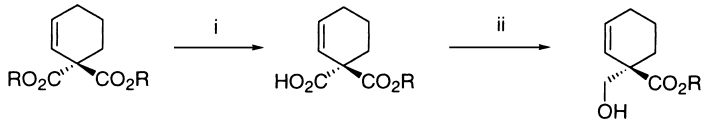
Incubation of the diethyl malonate derivative **7** in a 0.2 M phosphate buffer solution with 5% acetone between 15–20°C resulted in the desired acid-ester **10** in 92% yield (Table 1). Although the absolute configuration at the newly generated stereogenic center of **10** could not be determined at this stage, it was assumed to be *R* based on the proposed models for PLE hydrolysis of malonate derivatives[5] and also on our earlier work[3,4] (Scheme 2). Because of the relative instability of **10** at room temperature, the enantiomeric excess from the enzymatic hydrolysis was determined after reduction of **10** to the corresponding alcohol-ester **13**. Thus, reduction of the acid **10** was carried out using sodium borohydride via the mixed anhydride, prepared from the acid with ethyl chloroformate, to give the (*S*)-alcohol-ester **13**, $[\alpha]_D$ +50.4 (*c* 0.4, $CHCl_3$), in 65% yield. The enantiomeric excess of **13** was determined to be 64% by HPLC analysis [Chiralcel OD (Daicel Chemical Industries) (solvent: 5% isopropanol–hexane; retention

Table 1
Chemoenzymatic hydrolysis of the diester with PLE

run	diester	half-ester { $[\alpha]_D$ and (yield[1])}	primary alcohol { $[\alpha]_D$ and (yield[1])}	e.e. (%)[2]
1	**7**: diethyl	**10**: +9.4 (92)	**13**: +50.4 (65)	64
2	**8**: dimethyl	**11**: +5.7 (99)	**14**: +50.5 (62)	59
3	**9**: dipentyl	**12**: +3.6 (65)	**15**: +15.3 (63)	25

(1) isolated yield; (2) enantiomeric excess was determined by HPLC analysis using Chiralcel OD.

time of the major isomer: 45.07 min and minor isomer: 53.17 min; detection: UV 210 nm)]. Similarly, the enzymatic hydrolysis of the dimethyl ester **8** and dipentyl ester **9** afforded the corresponding acids (**11** and **12**) in 99 and 65% yields, respectively, which were also converted into the acid-esters (**14** and **15**) by adopting the same procedure as described for the preparation of **13**. HPLC analysis [Chiralcel OD (Daicel Chemical Industries)] indicated that the enantiomeric excess of alcohols **14** and **15** were 59 and 25%, respectively. Based on these results, it was found that the bulky ester group (run 3) did not seem to be suitable for the enzymatic hydrolysis of malonate derivatives in terms of yield and enantioselectivity, probably due to the presence of steric hindrance, and the mode of enantioselectivity seemed to be the same in the hydrolysis of these esters as expected. Since we presumed that further modifications of the ester groups would not improve the enantiomeric excess of the products, we focused our attention on the conversion of the hydrolyzed compound to an α-substituted serine derivative.



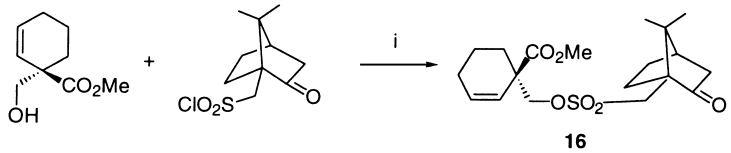
7 R = Et
8 R = Me
9 R = Pentyl

10 R = Et
11 R = Me
12 R = Pentyl

13 R = Et
14 R = Me
15 R = Pentyl

Scheme 2. *Reagents and conditions*: (i) PLE, 5% acetone–phosphate buffer, 20°C; (ii), $ClCO_2Et$, NEt_3, THF, 0°C then $NaBH_4$, EtOH, 0°C

In order to determine the absolute configuration, the alcohol-ester **14** was treated with (+)-10-camphorsulfonyl chloride to give the sulfonate ester **16**, which, after purification by silica gel column chromatography, was recrystallized from ethyl acetate to provide the fine crystals (Scheme 3). The X-ray analysis of the sulfonate **16** unambiguously demonstrated that the newly generated stereogenic center of the enzymatic hydrolysis product was *R* as expected (Fig. 2).



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Scheme 3. *Reagents and conditions*: (i) NEt_3, DMAP, CH_2Cl_2

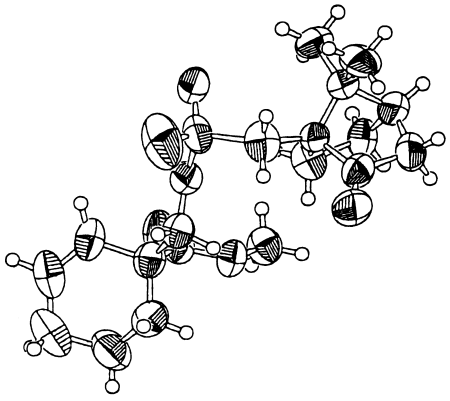
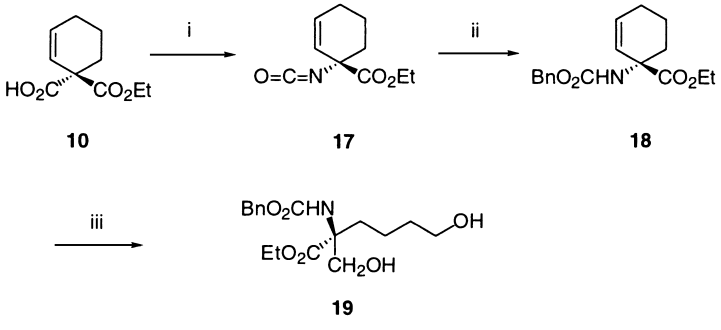


Figure 2. The ORTEP drawing of the sulfonate **16**

Although the absolute configuration of the half-ester was opposite to the natural myriocins, we attempted to establish the route for the conversion of the carboxyl function into an amino group with the retention of configuration, and decided to employ the Curtius rearrangement.[6] Thus, the reaction of the acid **10** with diphenylphosphoryl azide (DPPA) afforded the relatively stable isocyanate **17**, in 64% yield, which on treatment with benzyl alcohol in refluxing benzene gave the *N*-benzyloxycarbonyl derivative **18** in 49% yield (Scheme 4). Finally, ozonolysis of the olefin **18**, followed by reduction with sodium borohydride furnished the desired α-substituted serine derivative **19**, in 64% yield, in the correct enantiomeric series for the myriocins.



Scheme 4. *Reagents and conditions*: (i) DPPA, NEt_3, CH_2Cl_2, reflux; (ii) $PhCH_2OH$, benzene, reflux; (iii) O_3, CH_2Cl_2, −78°C then $NaBH_4$, EtOH, 0°C

In summary, we have disclosed an alternative procedure for the preparation of an α-substituted serine derivative in relatively few steps via asymmetric enzymatic hydrolysis of a malonate derivative, in which the difference between the sp^2 and sp^3 carbons was discriminated by the enzyme. The enantiomeric excess of the hydrolyzed product obtained here was not high enough for the synthesis of natural compounds; however, the skeletal modification, such as the functionalization of the starting material at the 2- and 6-positions of the cyclohexene ring, would be able to increase the enantioselectivity. Moreover, the synthesis of chiral compounds having either *R*- or *S*-configuration at the quaternary stereogenic center might also be possible by employing the functional exchange reaction, although attempting such transformation under various reaction conditions could not be achieved at the present time.

3. Experimental

3.1. General procedures

Melting points were measured with a Yanagimoto MP apparatus and are uncorrected. IR spectra were recorded for thin films on a JASCO FT/IR-200 Fourier transform infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained for solutions in $CDCl_3$ on a JEOL PMX 270 instrument (270 MHz), and chemical shifts are reported in ppm on the δ-scale from internal Me_4Si. Mass spectra were measured with a JEOL JMS D-300 spectrometer. Optical rotations were taken with a JASCO DIP-360 polarimeter. All new compounds described in the Experimental section were homogeneous on TLC. Enantiomeric excess (ee) determinations were carried out using a 5% isopropanol in *n*-hexane mobile phase with a Chiralcel OD column (Daicel Chemical Industries) on a Hitachi HPLC instrument.

3.2. Diethyl 2-hydroxycyclohexane-1,1-dicarboxylate ***6***

To a stirred solution of the ketone **5** (100 mg, 0. 41 mmol) in methanol (2.5 ml) was added sodium borohydride (15.6 mg, 0.41 mmol) portionwise at −15°C, and the resulting mixture was stirred for a further 5 h at the same temperature. After quenching the reaction by addition of a saturated ammonium chloride solution, the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (6:1, v/v) afforded the alcohol **6** (96.3 mg, 96%) as a colorless oil; ν_{max} (thin film)/cm^{-1} 3525, 2943, 1730; δ_H: 1.27 and 1.30 (each 3H, each t, *J*=7.1, 2×CH_3), 1.49–2.00 (7H, m, methylene protons), 2.18–2.36 (1H, m, methylene proton), 3.69 (1H, d, *J*=9.1, OH), 4.01 (1H, dt, *J*=3.7 and 9.1, 2-H), 4.14–4.34 (4H, m, 2×OCH_2); δ_C: 13.4, 21.5, 22.1, 29.3, 30.7, 59.5, 60.8, 71.6 and 170.7; (found: C, 59.00; H, 8.35; calcd for $C_{12}H_{20}O_5$: C, 59.00; H, 8.25%). [Found: (M^+), 244.1335; calcd for $C_{12}H_{20}O_5$: (M^+), 244.1311].

3.3. Diethyl 2-cyclohexene-1,1-dicarboxylate ***7***

To a stirred solution of the alcohol **6** (5.16 g, 21.1 mmol) in pyridine (50 ml) was added portionwise trifluoromethanesulfonic anhydride (TFMSA) (10.67 ml, 63.3 mmol) at −15°C, and the resulting mixture was allowed to warm up to room temperature over a period of 20 h. The solution was cooled to 0°C and treated with 10% potassium hydrogen sulfate solution, and extracted with ethyl acetate. The extract was washed with a saturated sodium hydrogen carbonate solution and brine, and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (20:1, v/v) afforded the olefin **7** (4.09 g, 86%) as a colorless oil; ν_{max} (thin film)/cm^{-1} 2980, 1732, 1257; δ_H: 1.18 (6H, t, *J*=7.1, 2×CH_3), 1.58–1.71 (2H, m, methylene protons), 1.90–2.10 (4H, m, methylene protons), 4.12 (4H, q, *J*=7.1, 2×OCH_2), 5.81 (1H, dt, *J*=1.8 and 10.1, 2-H), 5.92 (1H, dt, *J*=3.6 and 10.1, 3-H); δ_C: 13.7, 18.9, 24.1, 28.5, 54.3, 61.0, 123.9, 130.9 and 170.7; (found: C, 63.50; H, 8.05; calcd for $C_{12}H_{18}O_4$: C, 63.70; H, 8.00%). [Found: (M^+), 226.1207; calcd for $C_{12}H_{18}O_4$: (M^+), 226.1205].

3.4. Dimethyl 2-cyclohexene-1,1-dicarboxylate ***8***

To a stirred solution of the diethyl ester **7** (293 mg, 1.30 mmol) in methanol (10 ml) was slowly added sodium methoxide (175 mg, 3.24 mmol) at 0°C and the resulting mixture was stirred for 5 min at the

same temperature and for 15 h at room temperature. After treatment with a saturated ammonium chloride solution, the organic solvent was removed and the residue was extracted with ethyl acetate. The extract was washed with a saturated sodium hydrogen carbonate solution and brine, and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (20:1, v/v) afforded the dimethyl ester **8** (189 mg, 74%) as a colorless oil; ν_{max} (thin film)/cm^{-1} 2955, 1738, 1436; δ_H: 1.58–1.71 (2H, m, methylene protons), 1.92–2.11 (4H, m, methylene protons), 3.67 (6H, s, 2×Me), 5.81 (1H, dt, *J*=1.8 and 10.1, 2-H), 5.93 (1H, dt, *J*=3.6 and 10.1, 3-H); δ_C: 19.6, 24.2, 28.7, 52.5, 54.4, 123.8, 131.2 and 171.4; (found: C, 60.90; H, 7.40; calcd for $C_{10}H_{14}O_4$: C, 60.60; H, 7.10%). [Found: (M^+), 198.0881; calcd for $C_{10}H_{14}O_4$: (M^+), 198.0892].

3.5. *Dipentyl 2-cyclohexene-1,1-dicarboxylate* ***9***

A solution of the diethyl ester **7** (230 mg, 1.02 mmol) and *p*-tolenesulfonic acid (40 mg, 0.21 mmol) in 1-pentanol (5 ml) was heated at 80°C for 2 days. After the addition of a saturated sodium hydrogen carbonate solution, the mixture was extracted with ethyl acetate. The extract was washed with a saturated sodium hydrogen carbonate solution and brine, and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (100:1, v/v) afforded the dipentyl ester **9** (265 mg, 84%) as a colorless oil; ν_{max} (thin film)/cm^{-1} 2958, 1750, 1735, 1467; δ_H: 0.83 (6H, t, *J*=6.8, 2×CH_3), 1.16–1.34 (8H, m, methylene protons), 1.47–1.71 (6H, m, methylene protons), 1.91–2.10 (4H, m, methylene protons), 4.04 (2H, t, *J*=6.6, OCH_2), 4.05 (2H, t, *J*=6.6, OCH_2), 5.81 (1H, d, *J*=10.1, 2-H), 5.91 (1H, dt, *J*=3.5 and 10.1, 3-H); δ_C: 13.7, 19.1, 22.0, 24.2, 27.8, 28.0, 28.7, 54.5, 65.3, 124.0, 131.0 and 170.9; (found: C, 69.45; H, 9.70; calcd for $C_{18}H_{30}O_4$: C, 69.65; H, 9.75%). [Found: (M^+), 310.2141; calcd for $C_{18}H_{30}O_4$: (M^+), 310.2144].

3.6. *(*R*)-1-Ethoxycarbonyl-2-cyclohexene-1-carboxylic acid* ***10***

The diethyl ester **7** (600 mg, 2.65 mmol) was incubated with PLE (Amano, 120 mg) in a solution of 5% acetone in phosphate buffer (0.2 M, pH 7.2, 20 ml) at 20°C. The mixture was slowly stirred for 2 days and was treated with 1 M potassium hydrogen sulfate solution. After addition of ethyl acetate, the mixture was filtered through a pad of Celite to remove insoluble materials. The filtrate was extracted with ethyl acetate and the extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (8:1, v/v) afforded the half-ester **10** (482 mg, 92%) as a colorless oil; $[\alpha]_D$ +9.4 (*c* 0.5, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3500, 3184, 2942, 1715; δ_H: 1.27 (3H, t, *J*=7.1, CH_3), 1.66–1.80 (2H, m, methylene protons), 1.99–2.19 (4H, m, methylene protons), 4.22 (4H, q, *J*=7.1, OCH_2), 5.89 (1H, dt, *J*=1.9 and 10.1, 2-H), 6.03 (1H, dt, *J*=3.6 and 10.1, 3-H), 9.25–10.27 (1H, br s, COOH); δ_C: 13.9, 19.1, 24.3, 28.7, 54.6, 61.8, 123.4, 131.9, 170.7 and 177.2; (found: C, 60.40; H, 7.20; calcd for $C_{10}H_{14}O_4$: C, 60.60; H, 7.10%). [Found: (M^+), 198.0889; calcd for $C_{10}H_{14}O_4$: (M^+), 198.0892].

3.7. *(*R*)-1-Methoxycarbonyl-2-cyclohexene-1-carboxylic acid* ***11***

Enzymatic hydrolysis of the dimethyl ester **8** (227 mg, 1.15 mmol) was carried out under essentially the same reaction conditions as for the preparation of the mono-ethyl ester to give the mono-methyl ester **11** (209 mg, 99%) as a colorless oil; $[\alpha]_D$ +5.7 (*c* 0.8, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3510, 3180, 2952, 2617, 1725, 1438; δ_H: 1.59–1.92 (2H, m, methylene protons), 1.92–2.14 (4H, m, methylene protons), 3.69 (3H, s, Me), 5.82 (1H, dt, *J*=2.0 and 10.1, 2-H), 5.96 (1H, dt, *J*=3.7 and 10.1, 3-H), 8.44–9.42 (1H,

br s, COOH); δ_C: 19.1, 24.3, 28.8, 52.9, 54.5, 123.4, 131.9, 171.2 and 176.9; (found: C, 58.65; H, 6.85; calcd for $C_9H_{12}O_4$: C, 58.70; H, 6.55%). [Found: (M^+), 184.0750; calcd for $C_9H_{12}O_4$: (M^+), 184.0735].

3.8. (R)-1-Pentyloxycarbonyl-2-cyclohexene-1-carboxylic acid **12**

Enzymatic hydrolysis of the dipentyl ester **9** (143 mg, 0.46 mmol) was carried out under essentially the same reaction conditions as for the preparation of the mono-ethyl ester to give the mono-pentyl ester **12** (72 mg, 65%) as a colorless oil; $[\alpha]_D$ +3.6 (*c* 0.7, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3520 (sh), 3300 (sh), 2958, 2650, 1740, 1710; δ_H: 0.89 (3H, t, *J*=6.7, CH_3), 1.23–1.40 (4H, m, methylene protons), 1.54–1.82 (4H, m, methylene protons), 1.96–2.24 (4H, m, methylene protons), 4.15 (2H, t, *J*=6.6, OCH_2), 5.89 (1H, d, *J*=10.1, 2-H), 6.02 (1H, dt, *J*=3.6 and 10.1, 3-H), 7.49–8.90 (1H, br s, COOH); (found: C, 64.80; H, 8.55; calcd for $C_{13}H_{20}O_4$: C, 65.00; H, 8.40%). [Found: (M^+), 240.1353; calcd for $C_{13}H_{20}O_4$: (M^+), 240.1361].

3.9. (R)-Ethyl 1-hydroxymethyl-2-cyclohexene-1-carboxylate **13**

Ethyl chloroformate (0.05 ml, 0.52 mmol) was added dropwise to a solution of the half-ester **10** (80 mg, 0.40 mmol) and triethylamine (0.08 ml, 0.57 mmol) in dry THF (3 ml) at 0°C under argon, and the resulting mixture was stirred for a further 30 min at the same temperature. The solution was filtered to remove the precipitated triethylamine hydrochloride and the filtrate was diluted with ethanol (1 ml). To this solution was added dropwise sodium borohydride (15 mg, 0.40 mmol) and the mixture was stirred at 0°C for a further 12 h. The reaction was quenched by the addition of a saturated ammonium chloride solution and extracted with dichloromethane. The extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (4:1, v/v) afforded the alcohol **13** (60 mg, 65%) as a colorless oil; $[\alpha]_D$ +50.4 (*c* 0.4, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3458, 2940, 1721; δ_H: 1.27 (3H, t, *J*=7.1, CH_3), 1.60–1.75 (2H, m, methylene protons), 1.60–1.75 (3H, m, methylene protons), 1.92–2.16 (3H, m, methylene protons), 2.34 (1H, t, *J*=6.3, OH), 3.61 (1H, dd, *J*=6.1 and 10.9, C*H*HOH), 3.70 (1H, dd, *J*=6.1 and 10.9, C*H*HOH), 4.18 (2H, q, *J*=7.1, OCH_2), 5.64 (1H, dt, *J*=2.0 and 10.1, 2-H), 5.93 (1H, dt, *J*=3.8 and 10.1, 3-H); δ_C: 14.2, 18.8, 24.8, 27.6, 49.1, 60.9, 68.2, 125.6, 130.9 and 175.8; (found: C, 64.90; H, 8.80; calcd for $C_{10}H_{16}O_3$: C, 65.20; H, 8.75%). [Found: (M^+), 184.1075; calcd for $C_{10}H_{16}O_3$: (M^+), 184.1099].

3.10. (R)-Methyl 1-hydroxymethyl-2-cyclohexene-1-carboxylate **14**

The half-ester **11** (209 mg, 1.14 mmol) was reduced with sodium borohydride (43 mg, 1.14 mmol) via the corresponding mixed anhydride by the same procedure as for the preparation of the alcohol **13** to give the primary alcohol **14** (120 mg, 62%) as a colorless oil: $[\alpha]_D$ +50.5 (*c* 1.1, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3450, 2948, 1725, 1434; δ_H: 1.56–1.75 (3H, m, methylene protons), 1.92–2.20 (3H, m, methylene protons), 2.63 (1H, br s, OH), 3.62 (1H, dd, *J*=4.8 and 10.7, C*H*HOH), 3.69 (1H, dd, *J*=4.8 and 10.7, C*H*HOH), 3.72 (3H, s, Me), 5.65 (1H, dt, *J*=1.8 and 10.1, 2-H), 5.93 (1H, dt, *J*=3.8 and 10.1, 3-H); δ_C: 18.8, 24.8, 27.6, 49.2, 52.1, 125.6, 130.9 and 176.2; (found: C, 63.20; H, 8.55; calcd for $C_9H_{14}O_3$: C, 63.50; H, 8.30%). [Found: (M^++1), 171.1030; calcd for $C_9H_{14}O_3$: (M^++1), 171.1021].

3.11. (R)-Pentyl 1-hydroxymethyl-2-cyclohexene-1-carboxylate **15**

The half-ester **12** (770 mg, 3.21 mmol) was reduced with sodium borohydride (122 mg, 3.22 mmol) via the corresponding mixed anhydride by the same procedure as for the preparation of the alcohol **13** to give the primary alcohol **15** (453 mg, 63%) as a colorless oil: $[\alpha]_D$ +15.3 (*c* 0.4, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3460, 2958, 1720; δ_H: 0.91 (3H, t, *J*=6.8, Me), 1.20–1.44 (4H, m, methylene protons), 1.53–1.75 (5H, m, methylene protons), 1.90–2.15 (3H, m, methylene protons), 2.68 (1H, br s, OH), 3.61 (1H, d, *J*=10.8, C*H*HOH), 3.69 (1H, d, *J*=10.7, C*H*HOH), 4.11 (2H, t, *J*=6.7, OCH_2), 5.64 (1H, d, *J*=10.1, 2-H), 5.92 (1H, dt, *J*=3.8 and 10.1, 3-H); δ_C: 13.8, 18.8, 22.1, 24.8, 27.5, 27.9, 28.1, 49.2, 64.9, 68.2, 125.7, 130.6 and 175.7. [Found: (M^++1), 227.1660; calcd for $C_{13}H_{23}O_3$: (M^++1), 227.1647].

3.12. (R)-Methyl 1-(+)-10-camphorsulfonyloxymethyl-2-cyclohexene-1-carboxylate **16**

A solution of the alcohol **14** (50 mg, 0.29 mmol), triethylamine (0.15 ml, 1.08 mmol), a catalytic amount of 4,4-dimethylaminopyridine and (+)-10-camphorsulfonyl chloride in dichloromethane (3 ml) was heated at reflux for 5 days. The mixture was diluted with dichloromethane and washed with brine, and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (5:1, v/v) afforded the sulfonate **16** (80 mg, 71%) as colorless prisms; mp 82.5–83.0°C (ethyl acetate); δ_H: 0.89 (3H, s, Me), 1.12 (3H, s, Me), 1.37–1.52 (1H, m, methylene protons), 1.57–1.76 (4H, m, methylene protons), 1.90–2.23 (6H, m, methylene protons), 2.33–2.54 (2H, m, methylene protons), 3.00 (1H, d, *J*=15.1, SO_2CHH), 3.60 (1H, d, *J*=15.1, SO_2CHH), 3.74 (3H, s, OMe), 4.29 (1H, d, *J*=9.4, C*H*HO), 4.37 (1H, d, *J*=9.4, C*H*HO), 5.62 (1H, dt, *J*=2.1 and 10.1, 2-H), 6.00 (1H, dt, *J*=3.8 and 10.1, 3-H).

3.13. X-Ray analysis of the sulfonate **16**

Compound **16** was recrystallized from ethyl acetate as monoclinic crystals, mp 82.5–83.0°C; $C_{19}H_{28}O_6S$, space group $P2_1$, with *a*=9.005(3), *b*=20.244(4), *c*=11.371(3) Å, β=110.28(2)°, *Z*=2, *Dc*=1.36 g cm^{-3}. Intensity measurements were made with Cu*K*α radiation (λ=1.5418 Å; graphite monochromate) on a Rigaku AFC7R diffractometer in the ω–2θ mode with 4.36°<2θ<130.20°. A total 3425 unique reflections were collected. Of those, 2486 with $I>3\sigma(I)$ were judged as observed. Accurate cell parameters were obtained by least-squares techniques from the diffractometer setting for 24 reflections. The structure was solved using MITHRIL84, and refined by full matrix least-squares with TEXSAN. Convergence, with anisotropic thermal parameters for all non-hydrogen atoms, was reached at *R* 0.069 (*Rw* 0.066) using all the observed reflections.

3.14. (S)-1-Ethoxycarbonyl-2-cyclohexene-1-isocyanate **17**

To a stirred solution of the half-ester **13** (1.46 g, 7.37 mmol) in dry dichloromethane (15 ml) were added successively diphenylphosphoryl azide (2.38 ml, 11.1 mmol) and triethylamine (1.54 ml, 11.1 mmol) at ambient temperature under argon and the resulting mixture was stirred for a further 11 h at the same temperature. The mixture was cooled to 0°C and treated with a saturated ammonium chloride solution, and then extracted with dichloromethane. The extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (20:1, v/v) afforded the isocyanate **17** (925 mg, 64%) as a colorless oil; ν_{max} (thin film)/cm^{-1} 2943, 2250, 2143, 1747, 1718; δ_H: 1.27 (3H, t, *J*=7.2, CH_3), 1.61–1.83 (2H,

m, methylene protons), 1.99–2.17 (4H, m, methylene protons), 4.21 (4H, q, J=7.2, OCH_2), 5.83 (1H, dt, J=2.1 and 10.1, 2-H), 6.04 (1H, dt, J=3.8 and 10.1, 3-H); δ_C: 14.0, 19.1, 24.3, 28.7, 56.1, 61.9, 123.0, 132.4, 170.2 and 178.3. [Found: (M^+), 195.0896; calcd for $C_{10}H_{13}NO_3$: (M^+), 195.0896].

3.15. (S)-Ethyl 1-benzyloxycarbonylamino-2-cyclohexene-1-carboxylate **18**

A stirred solution of the isocyanate **17** (120 mg, 0.62 mmol) and benzyl alcohol (0.08 ml, 0.77 mmol) in dry benzene (5 ml) was heated at reflux for 1 h. After evaporation of the solvent, the residue was purified by column chromatography on silica gel using hexane:ethyl acetate (10:1, v/v) as eluent to give the carbamate **18** (92 mg, 49%) as a colorless oil; $[\alpha]_D$ +10.7 (*c* 1.1, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3350, 2938, 1738, 1722, 1516, 1265; δ_H: 1.21 (3H, t, J=6.7, CH_3), 1.55–1.86 (2H, m, methylene protons), 1.95–2.26 (4H, m, methylene protons), 4.17 (1H, distorted br s, OCH_2Me), 5.09 (2H, s, OCH_2Ph), 5.76 (1H, d J=9.9, 2-H), 6.00 (1H, dt, J=3.8 and 9.9, 3-H), 7.28–7.39 (5H, m, aromatic protons); δ_C: 14.0, 18.2, 24.6, 30.7, 58.0, 61.5, 66.7, 125.7, 128.1, 128.4, 133.0, 154.8 and 173.0; (found: C, 67.25; H, 7.15; N, 4.50; calcd for $C_{17}H_{21}NO_4$: C, 67.30; H, 7.00; N, 4.60%). [Found: (M^+), 303.1465; calcd for $C_{17}H_{21}NO_4$: (M^+), 303.1470].

3.16. (S)-Ethyl 2-benzyloxycarbonylamino-6-hydroxy-2-hydroxymethylhexanoate **19**

A solution of the cyclohexene derivative **18** (316 mg, 1.04 mmol) in dichloromethane (5 ml) was saturated with ozone at −78°C. The solution was stirred for 30 min at the same temperature and the ozone was removed by exchange with argon. Ethanol (1 ml) was added to the solution and the mixture was treated with sodium borohydride (40 mg, 1.06 mmol) at −78°C and then warmed up to 0°C. After the stirring had been continued for 12 h at the same temperature, the reaction was quenched by addition of saturated ammonium chloride solution, and the mixture was extracted with dichloromethane. The extract was washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave a residue, which was subjected to column chromatography on silica gel. Elution with hexane:ethyl acetate (1:1, v/v) afforded the diol **19** (224 mg, 64%) as a colorless oil; $[\alpha]_D$ −1.4 (*c* 0.8, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3418, 2940, 2870, 1715, 1506; δ_H: 1.28 (3H, t, J=7.1, CH_3), 1.12–1.43 (3H, m, methylene protons), 1.45–1.60 (1H, m, methylene proton), 1.65 (1H, br s, OH), 1.66–1.84 (1H, m, methylene proton), 2.06–2.25 (1H, m, methylene proton), 2.85 (1H, br s, OH), 3.59 (2H, t, J=6.3, 6-H_2), 3.83 (1H, d, J=11.4, C*H*HOH), 4.17 (1H, d, J=11.4, C*H*HOH), 4.25 (4H, q, J=7.1, OCH_2Me), 5.09 (2H, s, OCH_2Ph), 5.89 (1H, s, NH), 7.27–7.42 (5H, m, aromatic protons); δ_C: 14.0, 19.6, 31.5, 32.0, 62.0, 65.2, 65.5, 66.7, 128.0, 128.1, 128.5, 136.1, 155.1 and 172.3; (found: C, 60.15; H, 7.55; N, 4.00; calcd for $C_{17}H_{25}NO_6$: C, 60.15; H, 7.45; N, 4.15%). [Found: (M^+), 339.1694; calcd for $C_{17}H_{25}NO_6$: (M^+), 339.1682].

Acknowledgements

We are grateful to the Amano Pharmaceutical Co. Ltd of Japan for the donation of enzymes. This work was supported by the Ministry of Education, Science, Sports, and Culture of Japan.

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