Chiral Synthon Obtained with Pig Liver Esterase: Introduction of Chiral Centers into Cyclohexene Skeleton

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A versatile chiral synthon, (1*R*,6*S*)-6-methoxycarbonyl-3-cyclohexene-1-carboxylic acid, was obtained by an enantioselective hydrolysis of the corresponding *meso* diester with pig liver esterase. This enzymatic hydrolysis can easily be carried out on a multi-hundred gram scale. The chiral monoester thus obtained can be further converted into all stereoisomers of 1-amino-2-alkoxycarbonyl-4-cyclohexene derivatives in an enantio- and stereocontrolled manner. These derivatives are considered as potential key intermediates for synthesizing a variety of biologically interesting compounds such as aminocyclitol and carbapenem antibiotics.

Keywords enantioselective hydrolysis; *meso* diester; pig liver esterase; chiral cyclohexene skeleton

In the course of our synthetic studies on the fortimicin group of deoxyaminoglycoside antibiotics,[1] we were particularly interested in the enantioselective synthesis of fortamine **1**, the 1,4-diaminocyclitol moiety of fortimicin A.

In fortamine, all carbons of the cyclohexane ring are asymmetric, being substituted with oxygen or nitrogen functional groups. The construction of such a polyfunctional cyclohexane derivative under stereo- and regiochemical control seemed quite interesting and challenging from a synthetic point of view. Indeed, the synthesis of the fortamine skeleton has already been reported by several groups,[2] but enantiomerically pure fortamine has been obtained only by resolution of racemic intermediates.[2c] These facts clearly show that suitable chiral synthons having a cyclohexane skeleton are not easily available from natural sources. Therefore, asymmetric Diels–Alder reactions using a chiral dienophile[3] or by the use of a chiral catalyst[4] have been extensively studied to afford some useful chiral cyclohexene derivatives. In addition to these intermolecular Diels–Alder reactions, intramolecular Diels–Alder reaction involving a chiral *Z*-diene was also developed during the total synthesis of actinobolin.[5] In this paper, we wish to describe the development of a chiral cyclohexene synthon by a chemicoenzymatic approach, and the preparation of all stereoisomers of 1-amino-2-alkoxycarbonyl-4-cyclohexene derivatives in an enantio- and stereocontrolled manner.[6]

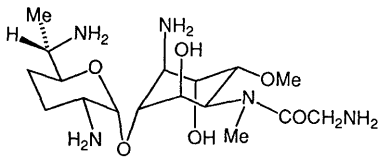
Introduction of Chiral Centers into Cyclohexene Skeleton A. Design of Symmetric Diester During the past several years, we have shown that the enantioselective total synthesis of some antibiotics can be efficiently achieved by a reasonable combination of enzymatic and non-enzymatic procedures.[7] This approach, which we call now a chemicoenzymatic approach, is particularly useful when we need a chiral starting material not readily available from conventional chiral pools. We have also demonstrated that only pig liver esterase can accommodate a wide variety of structures as good substrates.

The basic considerations can be summarized as follows; (1) the starting material should be a symmetric diester for enantioselective hydrolysis by pig liver esterase, (2) the starting material should be prepared easily in large quantity, (3) both ester groups should be easily transformable into other functional groups, and finally (4) the starting material should be as simple as possible in order to be applicable to a wide variety of compounds besides 1,4-diaminocyclitols.

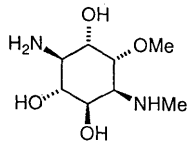
Based on these considerations, we became interested in the symmetric diester **2** as a substrate.

It might also be possible to start from symmetric diacetates such as **3** instead of the symmetric diester **2**. The diacetates seem to be more straightforward for the synthesis of fortamine skeleton, but they are not considered attractive because of the difficulty in preparation of the substrate **3**. More importantly, the enzymatic hydrolysis of such a diacetate is usually accompanied with formation of the diol. Although further hydrolysis of the monoacetate into the diol is characteristic of such a strategy in attaining high enantiomeric excess of the monoacetate,[8] control to obtain maximum chemical yield with maximum enantiomeric excess is difficult or tedious.

In contrast, when the substrate is a diester, the reaction generally terminates at the monoester stage, probably because the initially formed monoester exists as a carboxylate anion in weakly alkaline buffer-media, and may be too polar to be incorporated into the hydrophobic binding

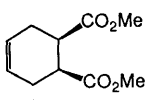


fortimicin A

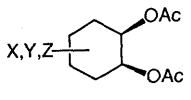


1 fortamine

Chart 1

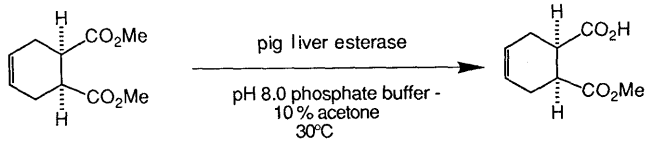


2



3

Chart 2



2 4

Chart 3

site of the enzyme. Therefore, the enzymatic reaction can be continued until all the diester is consumed. We observed no formation of a diacid in our cases (1,1-, 1,2-, 1,3-, and 1,5-dicarboxylates).[9] This is synthetically very important.

B. Enzymatic Hydrolysis of the Symmetric Diester 2 The diester **2** was easily prepared from a commercially available anhydride (*cis*-1,2,3,6-tetrahydrophthalic anhydride) with thionyl chloride in methanol.

The symmetric diester **2** (509 mg, 2.57 mmol) was treated with PLE[10] (436 units, 170 units/mmol **2**) in 0.05 M pH 8.0 phosphate buffer (90 ml) and acetone (10 ml) at 30 °C. The hydrolysis proceeded very smoothly, and after 3 h, the monoester **4** was isolated in 98% yield. No formation of the monoester **4** (racemic) by chemical hydrolysis was detected in the same buffer solution without PLE after 2 d.

The monoester **4** isolated by chromatography on silica gel showed an $[\alpha]_D$ value of $+15.8°$ ($c=0.20$, EtOH), close to that of the purified material recrystallized from ether–hexane (mp 65.5—66.0 °C; $[\alpha]_D^{20}$ $+17.7°$ ($c=1.00$, EtOH)), suggesting a high enantioselectivity in the present enzymatic hydrolysis.

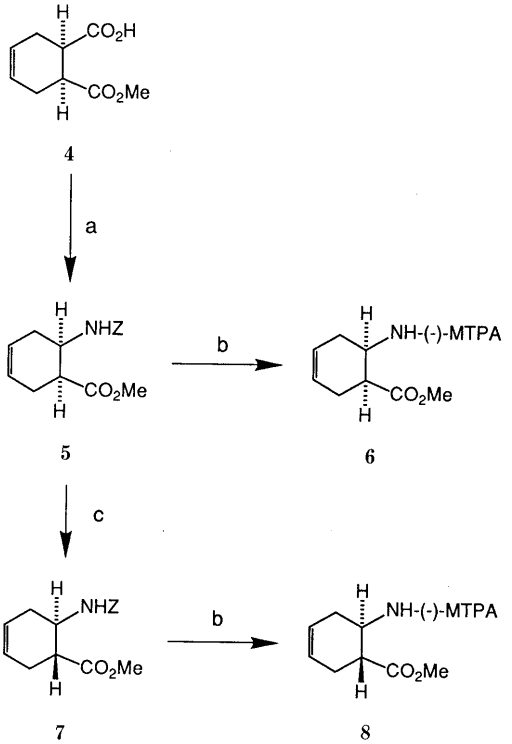
Enzymatic reaction on a large scale (*ca.* 3 mol of **2**) is described in Experimental.

C. Determination of the Enantiomeric Excess and the Absolute Configuration of the Chiral Monoester 4 Since our target compound was a diaminocyclitol, it was necessary to determine the enantiomeric excess and the absolute configuration of the chiral monoester **4** after transforming the carboxyl group into the amino group. Thus, the crude monoester **4** was reacted with ethyl chloroformate and triethylamine in acetone, and then sodium azide in H_2O was added to the reaction mixture. The resulting acid azide solution was subjected to thermal rearrangement in benzene and treated with benzyl alcohol in the presence of a catalytic amount of *p*-toluenesulfonic acid to afford the β-aminoester derivative **5**. The crude β-aminoester **5** was subjected to hydrogenation of the carbon–carbon double bond and hydrogenolysis of the benzyloxycarbonylamino group by treatment with catalytic Pd/C under a hydrogen atmosphere. The resulting β-aminoester was then reacted with (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride[11] ((−)-MTPACl) and pyridine to afford a mixture of (−)-MTPA amide, **6** and its diastereomer. In the proton nuclear magnetic resonance (^{1}H-NMR) spectra, two ester methyl groups appeared at δ 3.58 and δ 3.69, respectively, and the diastereomeric ratio was estimated to be over 50 to 1.

In order to reconfirm the enantiomeric excess, the crude β-aminoester **5** was epimerized to the *trans* isomer **7** with NaOMe in refluxing MeOH. The *trans* β-aminoester **7** was transformed to the mixture of the (−)-MTPA amide, **8** and its diastereomer, in a similar manner. The signals of the two ester methyl groups appeared at δ 3.47 and δ 3.65 also in a ratio of over 50 to 1. From these results, the enantiomeric excess of **4** was concluded to be over 96%. Enantiomerically pure monoester **4** could easily be obtained by recrystallization from EtOAc–hexane or Et_2O–hexane, and subsequent synthetic transformations were carried out starting from the enantiomerically pure monoester **4**.

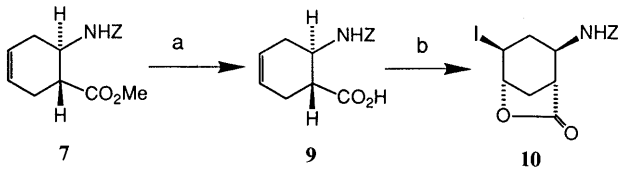
The absolute structure of **4** was unambigously verified by X-ray crystallographic analysis of the iodolactone **10** derived from the *trans* β-aminoester **5**.

The iodolactone was shown to be (1*R*,2*R*,4*S*,5*S*)-2-benzyloxycarbonylamino-4-iodo-7-oxo-6-oxabicyclo[3.2.1]-octane. A stereoview of the iodolactone **10** is shown in Chart 6. Therefore, the absolute structure of **4** is assigned as 1-methyl hydrogen (1*S*,2*R*)-1,2-cyclohex-4-enedicarboxylate.



a; (1) $ClCO_2Et$, Et_3N, (2) NaN_3, (3) C_6H_6, reflux, (4) BzlOH, *p*-TsOH.
b; (1) H_2, Pd/C, (2) (−)-MTPACl, Py. c; NaOMe

Chart 4



a; $NaOH/H_2O$–MeOH, quant.
b; I_2, KI, $NaHCO_3/H_2O$–CH_2Cl_2

Chart 5

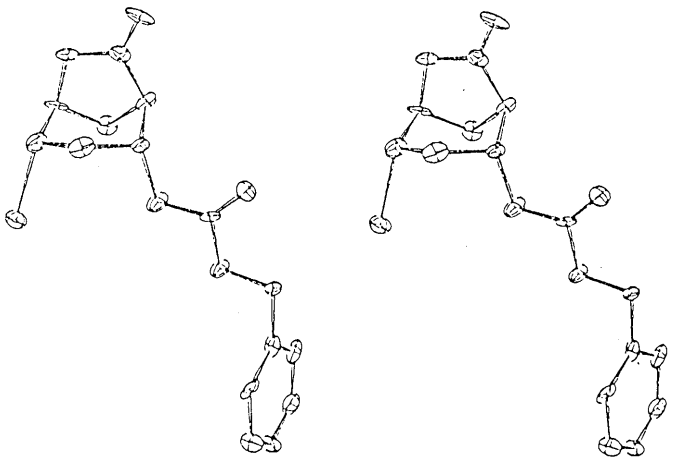


Chart 6

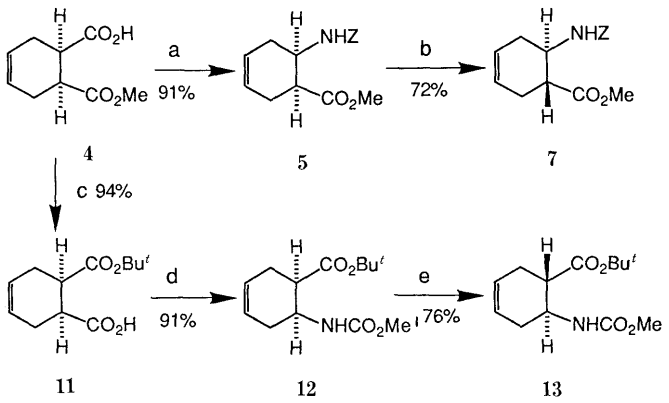
Independently, the same enzymatic hydrolysis was also reported by other three groups.[12] Related chiral cyclohexene derivatives having 1,2-dicarboxyl functional groups have also been obtained by chemical processes. The enantioselective alcoholysis of *meso* anhydride with chiral boronate leading to the enantiomer of **4** (in three steps) was reported by Mukaiyama.[13] Asymmetric Diels–Alder reactions leading to cyclohex-4-ene-1,2-*trans*-dicarboxylate derivatives were reported by Narasaka[4] (using 10 mol% chiral titanium catalyst modified by a tartaric acid-derived diol) and Yamamoto[14] (employing bis-*l*-menthyl fumarate as a chiral dienophile). The resolution of racemic **4** with cinchonidine was also reported by Tamura.[15]

The present enzymatic reaction can be carried out on a multihundred gram scale and enantiomerically pure **4** can be obtained by simple recrystallization (for details, see Experimental). In addition to the ready availability, the relatively simple structure, having a methoxycarbonyl group, carboxyl group and carbon–carbon double bond, makes the chiral monoester **4** a versatile synthon for the synthesis of various biologically significant compounds.

Preparation of All Stereoisomers of β-Aminoester, 5,7,12 and 13 The chiral monoester **4** was first converted to all the stereoisomers of β-aminoester derivatives. The preparation of **5** and **7**, two of the four stereoisomers, has already been achieved during the course of the determination of enantiomeric excess and absolute configuration, as described above. The overall yields of **5** and **7** from **4** were 91% and 66% (72% yields for the epimerization step), respectively. The 1*R*,6*S*- and 1*S*,6*S*-isomer, **12** and **13**, respectively, were prepared through the *tert*-butyl monoester **11** as shown in Chart 7. Thus, the chiral monoester **4** was treated with isobutene and catalytic H_2SO_4 in CH_2Cl_2, followed by 2 N NaOH to afford the *tert*-butyl monoester **11** in 94% yield. This process is one of the formal enantiomer conversions characteristic of our chemicoenzymatic approach,[7] and we observed no detectable racemization during the transformation. The *tert*-butyl monoester **11** was then subjected to Curtius rearrangement. Thus, acid azide was prepared by the mixed anhydride method in a similar manner to that described in the case of the methyl ester **4**. In this case, however, the rearrangement proceeded at a higher temperature (140 °C in xylene) and the intermediary isocyanate did not react with benzyl alcohol even under forcing conditions. Addition of methanol, on the other hand, to the isocyanate afforded very smoothly the methyl carbamate **12** in 91% overall yield. The epimerization of **12** with potassium *tert*-butoxide in *tert*-butanol gave the *trans* β-aminoester **13** in 58% isolated yield and 76% yield based on the recovered **12**.

Although the protecting groups are not the same, we could thus establish stereo- and enantioselective routes to all the isomers of 1-amino-2-alkoxycarbonyl-4-cyclohexene derivatives.

The monoester **4** is considered to be one of the most important optically active synthons in the synthesis of biologically active natural products because of its facile preparation (two steps from a commercially available anhydride in a large-scale operation). The following illustrations show the versatile utility of the chiral synthon **4**. The monoester **4** could be converted to the key intermediates of compactin (ML-236B) and mevinolin,[16] and the A ring moiety of 1α-VD_3.[17] Construction of a quaternary chiral center is also possible by the stereo- and regioselective introduction of a methyl group α to the alkoxycarbonyl group of **4** and **11**.[18] The synthesis of brefeldin A from **4** was reported by Gais.[12c,19]



a; (1) $ClCO_2Et$, Et_3N/acetone, −25 °C, then NaN_3/acetone–H_2O, −10 °C—r.t., (2) C_6H_6, reflux, then $PhCH_2OH$, *p*-TsOH/C_6H_6, reflux.
b; NaOMe/MeOH, reflux.
c; (1) isobutene, cat. H_2SO_4/CH_2Cl_2, r.t., (2) 2 N NaOH/MeOH–H_2O, r.t.
d; (1) $ClCO_2Et$, Et_3N/acetone, −25 °C, then NaN_3/acetone–H_2O, r.t., (2) xylene, 140 °C, then MeOH, *p*-TsOH/xylene, 45 °C.
e; *tert*-BuOK/*tert*-BuOH, r.t.

Chart 7

The β-aminoesters, **5**, **7**, **12** and **13**, derived from the chiral monoester **4** and **11** are also considered to be useful starting materials for the synthesis of various 1,4-diaminocyclitols. It should be mentioned here that the first enantioselective synthesis of fortamine **1** was achieved starting from the β-aminoester **12**.[20] Furthermore, another β-aminoester **5** was also utilized in the synthesis of carbapenem antibiotics such as *cis*-carbapenem,[21] thienamycin,[22] and a 1β-methylthienamycin intermediate.[23]

Experimental

General Methods Reagents and solvents were purchased from usual commercial sources, and were used as received or purified by distillation from appropriate drying agents. Reactions requiring anhydrous conditions were run under an atmosphere of dry argon. Silica gel (Wakogel C-200, C-300 or Fujigel BW 200) was used for column chromatography and silica gel (Kiesel gel 60 F_{254}, Merck) for analytical thin layer chromatography (TLC). Melting points were measured on a Yanagimoto micro melting apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL FX-100 (100 MHz) or JEOL GX-400 (400 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal reference, unless otherwise stated. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. Infrared (IR) spectra were obtained on a JASCO A-102 spectrometer. Mass spectra (MS) were obtained on a JEOL JMS-01 SG-2 mass spectrometer. Optical rotations were measured with a JASCO DIP-140 digital polarimeter.

Dimethyl *cis*-1,2-Cyclohex-4-enedicarboxylate (2) *cis*-1,2,3,6-Tetrahydrophthalic anhydride (152 g, 1.00 mol) was suspended in 200 ml of methanol, and the mixture was stirred overnight at room temperature. Thionyl chloride (72.9 ml, 119 g, 1.00 mol) was added dropwise to the resulting homogeneous solution at 0 °C. The cooling bath was removed and the stirring was continued for 3 h. Most of the methanol was removed under reduced pressure, and the residue was neutralized with saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2. The organic phase was washed (H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by distillation to give **2** (173 g, 87%)

as a colorless oil. **2**: bp 108.5—109.5 °C (3.0 mmHg): MS *m/z*: 167 ($M^+ - OCH_3$), 138. IR ($CHCl_3$) 3040, 3000, 1730 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 2.14—2.94 (m, 4H), 2.98—3.12 (m, 2H), 3.70 (s, 6H), 5.66 (m, 2H).

(1*R*,6*S*)-6-Methoxycarbonyl-3-cyclohexene-1-carboxylic Acid (4) PLE (250 μ, suspended in pH 6.8 buffer solution, 436 units, 170 units/mmol **2**) was added to a heterogeneous solution of **2** (509 mg, 2.57 mmol) in 0.05 M pH 8.0 phosphate buffer (90 ml) and acetone (10 ml) at 30 °C. The mixture was stirred at 30 °C for 3 h. The reaction mixture was acidified to pH 3.0 with 2 N HCl, and was extracted with AcOEt. The organic phase was washed (H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (eluted with Et_2O : hexane = 1 : 2) to give the monoester **4** (462 mg, 98%) as a white crystalline solid. **4**: mp 65.5—66.0 °C (Et_2O–hexane). *Anal.* Calcd for $C_9H_{12}O_4$: C, 58.69; H, 6.57. Found: C, 58.41; H, 6.67. MS *m/z*: 184 (M^+), 166, 153, 138, 124. $[\alpha]_D^{20}$ +2.52° (*c* = 4.33, $CHCl_3$) and +17.7° (*c* = 1.00, EtOH). IR (KBr): 3300—2500, 1725, 1690, 1440 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 2.14—2.48 (m, 4H), 2.95—3.18 (m, 2H), 3.70 (s, 3H), 5.67 (m, 2H), 9.43 (br s, 1H, CO_2H).

Enzymatic Hydrolysis of 2 on a Large Scale Phosphate buffer solution (pH 8.0) was prepared by adding KH_2PO_4 (544 g, 4.00 mol) and NaOH (147 g, 3.68 mol) to distilled H_2O (40 l). Then, the diester **2** (584.6 g, 2.95 mol) in acetone (2.0 l), and PLE (*ca.* 8.4 ml) were added to the above buffer solution. The amount of PLE used was about 5 units/mmol **2**. The whole mixture was gently stirred with a mechanical stirrer at ambient temperature (*ca.* 20—25 °C). An aqueous solution of NaOH was occasionally added to maintain the pH of the reaction mixture between 7 and 8. The reaction mixture became homogeneous. After 1 week, the solution was saturated with NaCl and acidified to pH 3.0 with HCl solution. The mixture was extracted with AcOEt (5 l × 3). Work-up as above afforded the crude monoester as a white crystalline solid. Recrystallization from AcOEt–hexane gave the enantiomerically pure monoester **4** (1st crop 459.2 g, 2nd crop 33.4 g, total 492.6 g, 91%).

Methyl (1*S*,6*R*)-6-Benzyloxycarbonylaminocyclohex-3-enecarboxylate (5) Ethyl chloroformate (8.6 ml, 9.8 g, 90 mmol) was added to a mixture of the monoester **4** (11.05 g, 60.0 mmol) and Et_3N (16.7 ml, 12.1 g, 120 mmol) in acetone (80 ml) at −25 °C. An aqueous solution of NaN_3 (9.75 g, 150 mmol) was added at −10 °C. The temperature was gradually raised to room temperature, and stirring was continued for 1 h. The reaction mixture was diluted with H_2O and the product was extracted with benzene. The organic phase was washed (H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated to leave the crude acid azide. The crude acid azide was dissolved in anhydrous benzene (80 ml) and heated under reflux for 2 h. Then, benzyl alcohol (6.48 g, 60.0 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added to the resulting solution of isocyanate in benzene. The mixture was heated under reflux for 1 h. The reaction mixture was cooled to ambient temperature, and diluted with benzene. The mixture was washed (H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (eluted with Et_2O : hexane = 1 : 3) to give the β-aminoester **5** (15.82 g, 91%) as a white crystalline solid. **5**: mp 50.0—51.0 °C (Et_2O–hexane). *Anal.* Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.47; H, 6.67; N, 4.72. MS *m/z*: 289 (M^+), 257, 245, 228, 198. $[\alpha]_D^{20}$ +21.3° (*c* = 1.00, $CHCl_3$). IR (KBr): 3360, 1730, 1690, 1510 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 2.16—2.56 (m, 4H), 2.83 (m, 1H), 3.68 (s, 3H), 4.25 (m, 1H), 5.08 (s, 2H), 5.40 (br s, 1H, NH), 5.64 (m, 2H), 7.33 (s, 5H).

Methyl (1*R*,6*R*)-6-Benzyloxycarbonylaminocyclohex-3-enecarboxylate (7) The *cis* β-aminoester **5** (325.6 mg, 1.13 mmol) was dissolved in dry MeOH (15 ml). NaOMe (95 mg, 1.8 mmol) was added, and the mixture was heated under reflux for 6 h. The reaction mixture was diluted with H_2O and neutralized with 1 N HCl solution. The mixture was extracted with CH_2Cl_2, and the organic phase was washed (H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by thin layer chromatography on silica gel (Merck, Kieselgel 60PF_{254}, developed with Et_2O : hexane = 1 : 1) to give the *trans* β-aminoester **7** (178.3 mg, 55%) as a white crystalline solid and the unchanged *cis* β-aminoester **5** (78.0 mg, 24%). **7**: mp 57.0—57.5 °C (Et_2O–hexane). *Anal.* Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.14; H, 6.61; N, 4.56. MS *m/z*: 289 (M^+), 257, 245, 228, 198. $[\alpha]_D^{20}$ −33.5° (*c* = 1.00, $CHCl_3$). IR (KBr): 3320, 1725, 1700, 1525, 1440 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.88—2.88 (m, 5H), 3.62 (s, 3H), 4.08 (m, 1H), 4.92 (br s, 1H, NH), 5.08 (s, 2H), 5.60 (s, 2H), 7.31 (s, 5H).

(−)-MTPA Amide 8 The crude *trans* β-aminoester **7** (104 mg, 0.36 mmol) was dissolved in MeOH (5 ml) and a catalytic amount of 10% Pd/C was added to the MeOH solution. The mixture was stirred under a hydrogen atmosphere at room temperature for 4 h. The catalyst was filtered off on Celite, and the filtrate was concentrated. The residue was dissolved in CCl_4 (1 ml), and (−)-MTPA chloride (106 mg, 0.41 mmol) and pyridine (0.1 ml) were added to the solution. The mixture was stirred at room temperature for 4 h. Water was added and the reaction mixture was acidified with 1 N HCl solution. The mixture was extracted with CH_2Cl_2, and the organic phase was washed (saturated $KHSO_4$, $NaHCO_3$, H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by TLC on silica gel (Merck, Kieselgel 60PF_{254}, developed with Et_2O : hexane = 1 : 1) to give a mixture (57.7 mg) of **8** and its isomer as a colorless oil.

In a similar manner, the crude *cis* β-aminoester **5** was transformed to a mixture of (−)-MTPA amide **6** and its isomer.

(1*R*,6*R*)-6-Benzyloxycarbonylaminocyclohex-3-enecarboxylic Acid (9) A 1 N NaOH solution (4.15 ml, 4.15 mmol) was added to the *trans* β-aminoester **7** (228.0 mg, 0.79 mmol) in MeOH (10 ml) at 0 °C, and the reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with H_2O and acidified with 1 N HCl solution. The mixture was extracted with CH_2Cl_2, and the organic phase was washed (saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by TLC on silica gel (Merck, Kieselgel 60PF_{254}, developed with CH_2Cl_2) to give the β-amino acid **9** (216.9 mg, quantitative yield) as a white crystalline solid. **9**: mp 108.5—109.5 °C (Et_2O–hexane). *Anal.* Calcd for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.29; H, 6.25; N, 4.82. MS *m/z*: 275 (M^+), 257, 231, 184; $[\alpha]_D^{20}$ −36.3° (*c* = 1.00, $CHCl_3$). IR (KBr): 3320, 3300—2600, 1725, 1690, 1650, 1550 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.90—2.92 (m, 5H), 4.06 (m, 1H), 5.04 (br s, 1H, NH), 5.08 (s, 2H), 5.48 (br s, 1H, CO_2H), 5.60 (s, 2H), 7.32 (s, 5H).

(1*R*,2*R*,4*S*,5*S*)-2-Benzyloxycarbonylamino-4-iodo-7-oxo-6-oxabicyclo-[3.2.1]octane (10) The β-amino acid **9** (110.1 mg, 0.4 mmol) was added to 0.5 N $NaHCO_3$ solution (2.4 ml), then KI (398.4 mg, 2.4 mmol), I_2 (203.0 mg, 0.8 mmol) and CH_2Cl_2 (1 ml) were added at 0 °C, and the reaction mixture was stirred overnight at room temperature. Th reaction mixture was diluted with CH_2Cl_2, and the organic phase was washed ($Na_2S_2O_3$, H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by recrystallization from benzene–ether to give the iodolactone **10** (134.3 mg, 84%) as white needles. **10**: mp 116.0—117.0 °C (benzene–Et_2O). *Anal.* Calcd for $C_{15}H_{16}INO_4$: C, 44.91; H, 4.02; N, 3.49. Found: C, 44.92; H, 3.97; N, 3.31. MS *m/z*: 401 (M^+), 310, 274, 266, 230. $[\alpha]_D^{20}$ −15.4° (*c* = 1.00, $CHCl_3$). IR (KBr): 3390, 1770, 1705, 1510 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 2.10—2.50 (m, 2H), 2.68—3.16 (m, 3H), 4.12—4.45 (m, 2H), 4.88 (m, 1H), 5.13 (s, 2H), 5.58 (br s, 1H, NH), 7.36 (s, 5H). Crystal data for **10**: $C_{15}H_{16}INO_4$; M_r = 401.2; space group $P2_1$; *a* = 22.029(11) Å; *b* = 6.520(4) Å; *c* = 10.704(6) Å; β = 91.43(5)°; *V* = 1536.9 Å^3; *Z* = 4 (2 molecules/a.u.); D_{cal} = 1.735 g cm^{-3}.

(1*S*,6*R*)-6-(*tert*-Butoxycarbonyl)-3-cyclohexene-1-carboxylic Acid (11) A mixture of the monoester **4** (61.4 g, 0.333 mol), isobutene (240 ml), concentrated H_2SO_4 (4.0 ml) and CH_2Cl_2 (100 ml) in a sealed tube was stirred at room temperature for 3 d. The mixture was poured into saturated $NaHCO_3$ solution, and extracted with hexane–Et_2O. The organic phase was washed (H_2O, saturated NaCl), dried ($MgSO_4$), filtered, and concentrated. The resulting crude diester was dissolved in MeOH (200 ml), and to this solution was added 2 N NaOH solution (400 ml, 0.8 mol) at 0 °C. The mixture was stirred at room temperature for 2 d. The mixture was acidified with 2 N HCl, and was extracted with Et_2O. The organic phase was washed (H_2O, saturated NaCl), dried (Mg_2SO_4), filtered, and concentrated. The residue was purified by recrystallization from petroleum ether to give **11** (71.0 g, 94%) as white needles. **11**: mp 80.0—81.0 °C (petroleum ether). *Anal.* Calcd for $C_{12}H_{18}O_4$: C, 63.70; H, 8.02. Found: C, 63.42; H, 8.08. MS *m/z* 227 ($M^+ + 1$), 211, 181, 169, 152, 125, 108. $[\alpha]_D^{20}$ +7.68° (*c* = 2.00, $CHCl_3$). IR (KBr): 3300—2500, 1725, 1695 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.44 (s, 9H), 2.28—2.34 (m, 4H), 2.88—3.08 (m, 2H), 5.62—5.70 (br s, 2H).

***tert*-Butyl (1*R*,6*S*)-6-Methoxycarbonylaminocyclohex-3-enecarboxylate (12)** The mixture of the *tert*-butyl monoester **11** (4.53 g, 20.0 mmol) and Et_3N (3.04 g, 30.0 mmol) in acetone (40 ml) was cooled to −25 °C. Ethyl chloroformate (2.61 g, 24.0 mmol) was added to the above solution at −25 °C. Then, a solution of NaN_3 (2.60 g, 40.0 mmol) in H_2O was added, and the mixture was stirred at room temperature for 1 h. The mixture was diluted with H_2O and extracted with benzene. The organic phase was washed (H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was dissolved in xylene (50 ml), and the mixture was heated at 140 °C for 50 min. MeOH (20 ml) and a catalytic amount of *p*-toluenesulfonic acid were added to the cooled solution, and the mixture

was heated at 45 °C for 45 min. The mixture was poured into H_2O, and neutralized with NaOH solution. The mixture was extracted with benzene, and the organic phase was washed (H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (eluted with Et_2O : hexane = 1 : 3) to give the methyl carbamate **12** (4.65 g, 91%) as a colorless syrup. **12**: MS *m/z*: 256 ($M^+ + 1$), 224, 199. $[\alpha]_D^{20}$ −19.4° (*c* = 1.07, $CHCl_3$). IR (KBr): 3440, 1720, 1510 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.44 (s, 9H), 2.12—2.48 (m, 4H), 2.68 (m, 1H), 3.65 (s, 3H), 4.18 (m, 1H), 5.40 (br s, 1H, NH), 5.54—5.75 (m, 2H).

***tert*-Butyl (1*S*,6*S*)-6-Methoxycarbonylaminocyclohex-3-enecarboxylate (13)** Potassium *tert*-butoxide in *tert*-butanol was prepared from K (105 mg, 2.7 mmol) and *tert*-BuOH (10 ml). To this solution, the *cis* β-aminoester **12** (700.0 mg, 2.74 mmol) in *tert*-BuOH (4 ml) was added. The mixture was stirred at room temperature for 1.5 h, diluted with H_2O, and neutralized with 2 N HCl solution. The mixture was extracted with Et_2O. The organic phase was washed (H_2O, saturated NaCl), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (eluted with Et_2O : hexane = 1 : 3) to give the *trans* β-aminoester **13** (404.5 mg, 58%) and the unchanged *cis* β-aminoester **12** (167.5 mg, 24%) as a white crystalline solid. **13**: mp 57.0—58.5 °C (petroleum ether). *Anal.* Calcd for $C_{13}H_{21}NO_4$: C, 61.16; H, 8.29; N, 5.49. Found: C, 61.16; H, 8.43; N, 5.27. MS *m/z*: 256 ($M^+ + 1$), 224, 199, 182. $[\alpha]_D^{20}$ +27.6° (*c* = 1.01, $CHCl_3$). IR (KBr): 3300, 1725, 1690, 1540 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.44 (s, 9H), 1.88—2.64 (m, 5H), 3.64 (s, 3H), 3.97 (m, 1H), 4.80 (br d, *J* = 8 Hz, NH), 5.61 (m, 2H).

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References and Notes

1) Fortimicin A: *a*) T. Nara, M. Yamamoto, I. Kawamoto, K. Takayama, R. Okachi, R. Takasawa, T. Sato, and S. Sato, *J. Antibiot.*, **30**, 533 (1977); *b*) R. Okachi, S. Takayama, T. Sato, S. Sato, M. Yamamoto, I. Kawamoto, and T. Nara, *ibid.*, **30**, 541 (1977); *c*) R. Egan, R. S. Stanaszek, M. Cirovic, S. L. Mueller, J. Tadanier, J. R. Martin, P. Collum, A. W. Goldstein, R. L. DeVault, A. C. Sinclair, E. E. Fager, and L. A. Mitscher, *ibid.*, **30**, 552 (1977). Sporaricin A: *d*) T. Deushi, A. Iwasaki, K. Kamiya, T. Kunieda, T. Mizoguchi, M. Nakayama, H. Itoh, T. Mori, and T. Oda, *ibid.*, **32**, 173 (1979); *e*) A. Iwasaki, H. Itoh, and T. Mori, *ibid.*, **32**, 180 (1979); *f*) T. Deushi, M. Nakayama, I. Watanabe, T. Mori, H. Naganawa, and H. Umezawa, *ibid.*, **32**, 187 (1979). Istamycin A and B: *g*) Y. Okami, K. Hotta, M. Yoshida, D. Ikeda, S. Kondo, and H. Umezawa, *ibid.*, **32**, 964 (1979); *h*) K. Hotta, N. Saito, and Y. Okami, *ibid.*, **33**, 1502 (1980). Sannamycin A: *i*) T. Deushi, J. Iwasaki, K. Kamiya, T. Mizoguchi, M. Nakayama, H. Itoh, and T. Mori, *ibid.*, **32**, 1061 (1979); *j*) I. Watanabe, T. Deushi, T. Yamaguchi, K. Kamiya, M. Nakayama, and T. Mori, *ibid.*, **32**, 1066 (1979); *k*) A. Iwasaki, H. Itoh, and T. Mori, *Int. J. System. Bacteriol.*, **31**, 280 (1981).

2) *a*) Fortimicin B from *myo*-inositol; Y. Honda and T. Suami, *Bull. Chem. Soc. Jpn.*, **55**, 1156 (1982); *b*) (±)-Fortamine and (±)-2-deoxyfortamine from 1,3-cyclohexadiene; S. Knapp, M. J. Sebastian, and H. Ramanathan, *J. Org. Chem.*, **43**, 4786 (1983); *c*) Resolved (−)-fortamine from 1,3-cyclohexadiene; S. Knapp, M. J. Sebastian, H. Ramanathan, P. Bharadwaj, and J. A. Potenza, *Tetrahedron*, **42**, 3405 (1986); *d*) Resolved (−)-3-de-*O*-methylfortamine from 1,2;4,5-dianhydro-*epi*-inositol; J. Schubert, R. Schwesinger, and H. Prinzbach, *Angew. Chem., Int. Ed. Engl.*, **23**, 167 (1984); J. Schubert, R. Schwesinger, L. Knothe, and H. Prinzbach, *Justus Liebigs Ann. Chem.*, **1986**, 2009; *e*) Resolved (−)-2-deoxyfortamine from dianhydro-deoxy-*epi*-inositol; R. Kühlmeyer, R. Schwesinger, and H. Prinzbach, *Tetrahedron Lett.*, **25**, 3429 (1984); *f*) (±)-Fortamine from *trans*-1,3-cyclohexadiene-5,6-diol diacetate; C. H. Kuo and N. L. Wendler, *ibid.*, **25**, 2291 (1984).

3) *a*) E. J. Corey and H. E. Ensley, *J. Am. Chem. Soc.*, **97**, 6708 (1975); *b*) S. Masamune, *Heterocycles*, **21**, 107 (1984).

4) K. Narasaka, M. Inoue, T. Yamada, J. Sugimori, and N. Iwasawa, *Chem. Lett.*, **1987**, 2409.

5) M. Yoshioka, H. Nakai, and M. Ohno, *J. Am. Chem. Soc.*, **106**, 1133 (1984).

6) Preliminary communication; S. Kobayashi, K. Kamiyama, T. Iimori, and M. Ohno, *Tetrahedron Lett.*, **25**, 2557 (1984).

7) *a*) M. Ohno, S. Kobayashi, and K. Adachi, "Enzymes as Catalysts in Organic Synthesis," ed. by M. P. Schneider, D. Reidel Publishing Company, 1986; *b*) K. Adachi, S. Kobayashi, and M. Ohno, *Chimia*, **40**, 311 (1986).

8) Y. F. Wang, C. S. Chen, G. Girdaukas, and C. J. Sih, *J. Am. Chem. Soc.*, **106**, 3695 (1984).

9) However, we observed that about 20% of dicarboxylic acid was obtained by treatment of azelaic acid dimethyl ester (1,7-dicarboxylate) with pig liver esterase. Unpublished result, Reiji Ohashi from our laboratory.

10) PLE was purchased from Sigma Co., Ltd. (E 3128), and used as received.

11) J. A. Dale, D. L. Dull, and H. S. Mosher, *J. Org. Chem.*, **34**, 2543 (1969).

12) Our enzymatic step described here was first presented at the 27th Meeting of the Kanto Branch of the Pharmaceutical Society of Japan, Tokyo, Nov. 1983: *a*) P. Mohr, N. Waespe-Sarcevic, C. Tamm, K. Gawronska, and J. K. Gawronski, *Helv. Chim. Acta*, **66**, 2501 (1983); *b*) M. Schneider, N. Engel, P. Honiocke, G. Heinemann, and H. Gorish, *Angew. Chem., Int. Ed. Engl.*, **23**, 67 (1984); *c*) H.-J. Gais and K. L. Lukas, *ibid.*, **23**, 142 (1984).

13) M. Ohshima and T. Mukaiyama, *Chem. Lett.*, **1987**, 377.

14) K. Furuta, K. Iwanaga, and H. Yamamoto, *Tetrahedron Lett.*, **27**, 4507 (1986). See also H. M. Walborsky, L. Barash, and T. C. Davis, *J. Org. Chem.*, **26**, 4778 (1961); *idem*, *Tetrahedron*, **19**, 2333 (1963).

15) N. Tamura, H. Natsugari, Y. Kawano, Y. Matsushita, K. Yoshioka, and M. Ochiai, *Chem. Pharm. Bull.*, **35**, 996 (1987).

16) S. Kobayashi, Y. Eguchi, M. Shimada, and M. Ohno, *Chem. Pharm. Bull.*, in press.

17) S. Kobayashi, J. Shibata, M. Shimada, and M. Ohno, *Tetrahedron Lett.*, in press.

18) M. Shimada, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, **29**, 6961 (1988).

19) *a*) H.-J. Gais, *Angew. Chem., Int. Ed. Engl.*, **23**, 143 (1984); *b*) H.-J. Gais and T. Lied, *ibid.*, **23**, 145 (1984).

20) *a*) K. Kamiyama, S. Kobayashi, and M. Ohno, *Chem. Lett.*, **1987**, 29; *b*) S. Kobayashi, K. Kamiyama, and M. Ohno, *J. Org. Chem.*, in press.

21) M. Kurihara, K. Kamiyama, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, **26**, 5831 (1985).

22) H. Kaga, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, **29**, 1057 (1988).

23) H. Kaga, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, **30**, 113 (1989).