Formal Total Synthesis of (−)-Indolmycin

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(+)-Indolmycenic ester 4, a key intermediate for the synthesis of (−)-indolmycin (1), was prepared from (2*S*,3*R*)-epoxybutyrate 5 *via* (2*S*,3*S*)-2-hydroxy-3-chlorobutyrate 6 or (2*R*,3*R*)-2-mesyloxy-3-hydroxybutyrate 12, which were obtained by lipase-catalyzed kinetic resolution of the corresponding 2-acetates.

Keywords (−)-indolmycin; (+)-indolmycenic ester; asymmetric hydrolysis; α-acetoxy ester; lipase; epoxide-ring opening

Indolmycin (**1**), an antibiotic isolated from an African strain of *Streptomyces albus*, exhibits an antibacterial activity against *Staphylococci*.[1] Other indolymcin congeners (**2** and **3**) having a methoxyl or a hydroxyl group in the 5′-position of the indole skeleton have been obtained by the addition of indole precursors to growing cultures of *Streptomyces griseus* ATCC 12648.[2] These congeners display a moderate increase in antimicrobial activity compared to **1**. The relative and absolute configurations of two chiral centers in indolmycin have already been determined, as demonstrated in **1**.[3] Synthesis of the natural (−)-**1** has been achieved by Mukaiyama's group,[4] while synthesis of (±)-**1** has been reported by three other groups.[3a,b,5] The key intermediate employed in Mukaiyama's synthesis is the (+)-indolmycenic ester **4**[6] having (2*S*,3*R*) absolute configurations. In this paper, we report the synthesis of (+)-indolmycenic ester **4** using a lipase-catalyzed kinetic resolution at a key step, which means that the total synthesis

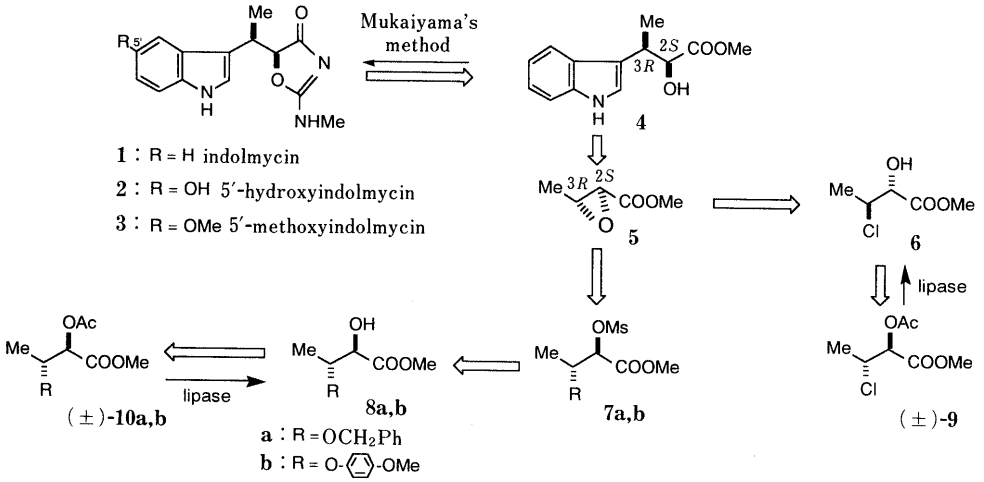


Chart 1

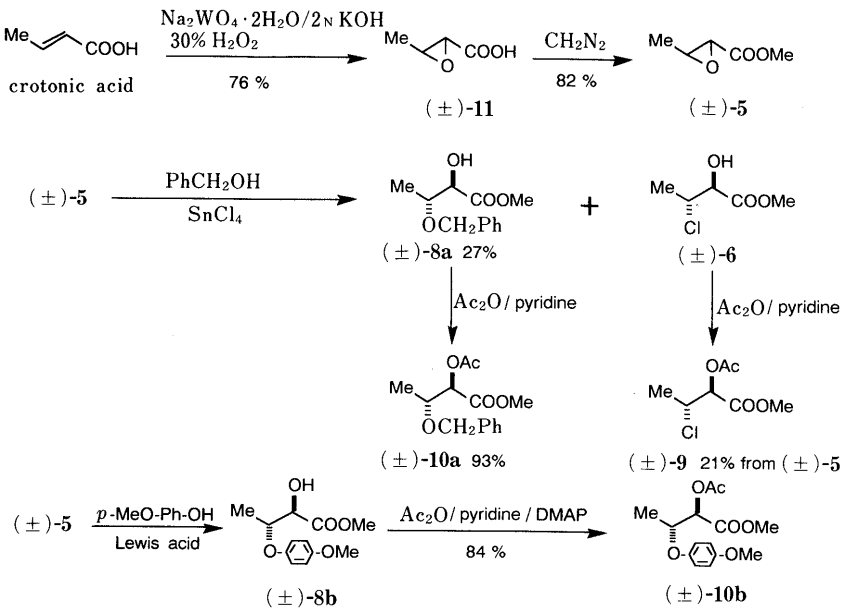


Chart 2

of (−)-indolmycin was formally accomplished.

Retrosynthetic analysis of optically active **4** (Chart 1) reveals that (2*S*,3*R*)-*trans*-2,3-epoxybutyrate **5** could be a precursor, since the $SnCl_4$ catalyzed reaction of (±)-**5** and indole has been reported to yield only (±)-4.[3a,b] (2*S*,3*R*)-**5** would be obtained from either optically active chlorohydrin **6** or mesylate **7**, which could be derived from enantioselective hydrolysis of (±)-**9** or (±)-**10a**, **b**, respectively, with commercially available lipase.

Synthesis of (±)-9-and (±)-10a, b, Substrates for Lipase-Catalyzed Hydrolysis Epoxidation of crotonic acid[7] followed by esterification (82%) with CH_2N_2 afforded (±)-*trans*-2,3-epoxybutyrate **5**, which was subjected to reaction with benzyl alcohol in the presence of $SnCl_4$ gaving β-benzyloxy ester (±)-**8a** (27%) and β-chloro ester (±)-**6**. After separation, they were converted to the corresponding acetates (±)-**10a** (93%) and (±)-**9** [21% from (±)-**5**], respectively (Chart 2).

The structure of (±)-**8a** and (±)-**6** were determined as follows. The C_2-protons in (±)-**8a** (δ 4.346, br s, 1H) and (±)-**6** (δ 4.378, dd, $J_{2,3}=3.2$ Hz, $J_{2,OH}=3.2$ Hz, 1H) shifted to a lower field on acetylation [(±)-**10a**; δ 5.271, d, $J_{2,3}=3.4$ Hz, 1H: (±)-**9**; δ 5.256, d, $J_{2,3}=3.7$ Hz, 1H], whereas the C_3-protons [(±)-**8a**; δ 3.860, dq, $J_{2,3}=3.2$ Hz, $J_{3,Me}=6.3$ Hz, 1H: (±)-**10a**; δ 3.963, dq, $J_{2,3}=3.4$ Hz, $J_{3,Me}=6.5$ Hz, 1H: (±)-**6**; δ4.325, dq, $J_{2,3}=3.2$ Hz, $J_{3,Me}=6.8$ Hz, 1H: (±)-**9**; δ 4.428, dq, $J_{2,3}=3.7$ Hz, $J_{3,Me}=6.8$ Hz, 1H] appeared in almost the same region. In a similar manner, the structure of α-hydroxy-β-phenoxy ester (±)-**8b** was confirmed based on the nuclear magnetic resonance (NMR) data (δ 5.300, d, $J_{2,3}=3.4$ Hz, 1H, C_2-H) for the corresponding acetate (±)-**10b**, which was obtained in a 84% yield from **8b** by acetylation. These NMR data clearly show that benzyloxy, phenoxy, and chloro anions attacked the C-3 position of (±)-**5**. Since nucleophilic epoxide-ring opening is known to take place with inversion of the configuration, (±)-**10a** and (±)-**9** should have *anti*-configurations.

For the purpose of improving the yield of the α-hydroxy ester (±)-**8a**, ring-opening of (±)-**5** was carried out changing the Lewis acid and/or oxygen nucleophile. The results are shown in Table I.

When $BF_3\cdot Et_2O$ was used, the yield was considerably decreased (entry 2). In the absence of $PhCH_2OH$, chlorohydrin (±)-**6** was obtained as a single product (entry 3). The best result (46%) was achieved when a *p*-methoxyphenol was employed as an oxygen nucleophile instead of $PhCH_2OH$ in the presence of $BF_3\cdot Et_2O$ at 0 °C (entry 6).

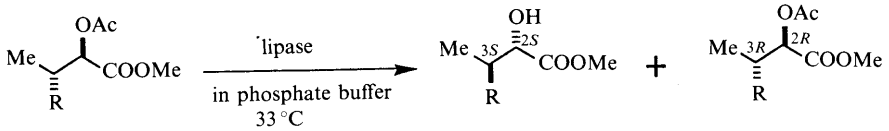
Lipase-Catalyzed Enantioselective Hydrolysis of (±)-9 and (±)-10a, b Asymmetric hydrolyses of three kinds of α-acetoxy esters were then carried out. Enzymatic hydrolyses of (±)-**9** and (±)-**10a**, **b** were performed in 0.1 M phosphate buffer solution (pH 7.25) at 33 °C, and selected data are shown in Table II.

The lipases used in the present study were as follows: "Amano-M-10" from *Mucol javanicus*, "OF-360" from *Candida cylindracea*, "Amano F-AP-15" from *Rhizopus javanicus*, "Toyo" from *Chromobacterium viscosum*, Saiken "Lusepase" from *Rhizopus japonicus*, "Amano P" from *Pseudomonas* sp., "Newlase" from *Rhizopus niveus*, and "Amano GC-4" from *Geotrichum candidum*. In all cases, the configurations of the C_2-OH groups liberated by lipase-catalyzed hydrolysis of the corresponding acetates were found to be "*S*" and those of the recovered C_2-OAc groups were "*R*". The unhydrolyzed substrates (2*R*,3*R*)-**9** and (2*R*,3*R*)-**10a**, **b** were obtained with a much higher optical purity than the hydrolyzed compounds in most cases. In

TABLE I. Reaction of (±)-**5** and Nucleophile in the Presence of Lewis Acid

Entry	Nucleophile	Lewis acid	Products (yield, %)	
1	$PhCH_2OH$	$SnCl_4$	(±)-**8a** (27),	(±)-**6** (21)
2	$PhCH_2OH$	$BF_3\cdot Et_2O$	(±)-**8a** (19)	
3	None	$SnCl_4$		(±)-**6** (39)
4	$PhCH_2OH$	Et_2AlCl	Recovery	
5	*p*-MeO–ArOH	$SnCl_4$	(±)-**8b** (26)	
6	*p*-MeO–ArOH	$BF_3\cdot Et_2O$	(±)-**8b** (46)	

TABLE II.



R=Cl	(±)-**9**	(2*S*,3*S*)-**6**	(2*R*,3*R*)-**9**
R=OCH_2Ph	(±)-**10a**	(2*S*,3*S*)-**8a**	(2*R*,3*R*)-**10a**
R=O-⟨⟩-OMe	(±)-**10b**	(2*S*,3*S*)-**8b**	(2*R*,3*R*)-**10b**

Entry	Substrate	Lipase	Time (h)	Product (yield, %) (optical purity, % ee)	
1	(±)-**9**	Amano P	19	(2*S*,3*S*)-**6** (12) (48)	(2*R*,3*R*)-**9** (24) (>99)
2	(±)-**9**	Amano F-AP-15	19	(2*S*,3*S*)-**6** (31) (24)	(2*R*,3*R*)-**9** (12) (82)
3	(±)-**10a**	Amano M-10	62	(2*S*,3*S*)-**8a** (47) (81)	(2*R*,3*R*)-**10a** (50) (79)
4	(±)-**10a**	OF-360	62	(2*S*,3*S*)-**8a** (63) (28)	(2*R*,3*R*)-**10a** (28) (95)
5	(±)-**10a**	Amano F-AP-15	62	(2*S*,3*S*)-**8a** (63) (49)	(2*R*,3*R*)-**10a** (34) (86)
6	(±)-**10a**	Toyo	62	(2*S*,3*S*)-**8a** (16) (64)	(2*R*,3*R*)-**10a** (50) (87)
7	(±)-**10a**	Lusepase	62	(2*S*,3*S*)-**8a** (52) (81)	(2*R*,3*R*)-**10a** (43) (90)
8	(±)-**10b**	Lusepase	40	(2*S*,3*S*)-**8b** (30) (88)	(2*R*,3*R*)-**10b** (65) (44)
9	(±)-**10b**	Amano F-AP-15	40	(2*S*,3*S*)-**8b** (32) (86)	(2*R*,3*R*)-**10b** (37) (76)
10	(±)-**10b**	Newlase	40	(2*S*,3*S*)-**8b** (23) (86)	(2*R*,3*R*)-**10b** (65) (33)
11	(±)-**10b**	Amano GC-4	40	(2*S*,3*S*)-**8b** (53) (58)	(2*R*,3*R*)-**10b** (43) (72)
12	(±)-**10b**	Amano P	40	(2*S*,3*S*)-**8b** (42) (70)	(2*R*,3*R*)-**10b** (43) (95)

particular, when (±)-**10b** was treated with "Amano P" (entry 12), both the recovery and the optical yield of the unchanged acetate (2*R*,3*R*)-**10b** were found to be considerably high. In the case of entry 1, the optical purity of (2*S*,3*S*)-**6** with the desired absolute configuration was unfortunately very poor, although that of the recovered (2*R*,3*R*)-**9** was extremely high. For the purpose of determining the optical purity of the product, the racemates ((±)-**6** and (±)-**8a, b**) were converted into the corresponding (+)-MTPA esters by treatment with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride ((+)-MTPACl)[8] in pyridine. In every case, the signals due to ester methyl protons appeared in distinctly different fields [(+)-MTPA ester from (±)-**6**; δ 3.836, 3.785: (+)-MTPA ester from (±)-**8a**; δ 3.805, 3.763: (+)-MTPA ester from (±)-**8b**; δ 3.765, 3.772] of the 400 MHz NMR spectrum without using any shift reagent. In order to correlate the chemical shift and the absolute structure, authentic samples were prepared.

The known optically pure (2*R*,3*S*)-epoxybutyrate **5**[9] was treated with $PhCH_2OH$ in the presence of $SnCl_4$ to afford the optically active α-hydroxy esters [(2*R*,3*R*)-**8a** $[\alpha]_D^{23}$ −28.71° (*c*=2.95, $CHCl_3$) and (2*R*,3*R*)-**6**] which were converted into the corresponding (+)-MTPA esters [(2*R*,3*R*)-**8a**-(+)-MTPA; δ 3.805 and (2*R*,3*R*)-**6**-(+)-MTPA; δ 3.836], respectively. Thus, by correlating these chemical shifts of the authentic (+)-MTPA esters [(2*R*,3*R*)-**8a**-(+)-MTPA and (2*R*,3*R*)-**6**-(+)-MTPA] with those of the (+)-MTPA ester of the reaction products,[10] estimation of their optical purity as well as their stereostructure can be achieved with high accuracy for (2*S*,3*S*)-**6**, (2*S*,3*S*)-**8a**, (2*R*,3*R*)-**9** and (2*R*,3*R*)-**10a**. The absolute structure of the recovered acetate **10b** was determined as "2*R*,3*R*" by converting it into 2-mesyloxy-3-hydroxy butyrate **12** and by correlation with (2*R*,3*R*)-**12** of known absolute configuration. The conversion experiments will be described in the next part of this article. Once the absolute structure of the unchanged **10b** is established, the optical purity can be estimated in the same way as mentioned above.

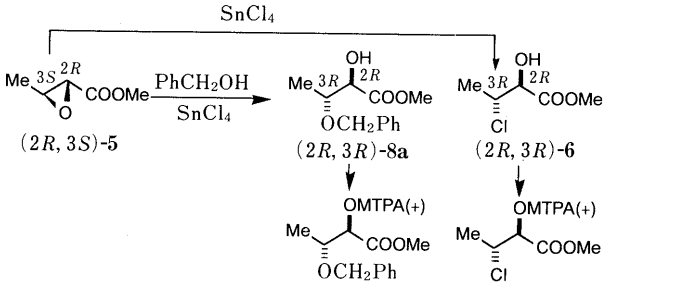


Chart 3

Synthesis of (+)-Indolmycenic Ester 4 Optically active (2*R*,3*R*)-**10a** (entry 4, 12; 95% ee) was used as starting material for the synthesis of indolmycenic ester **4**.

Alkaline hydrolysis of (2*R*,3*R*)-**10a** with K_2CO_3 in MeOH afforded (2*R*,3*R*)-**8a** which was treated with methanesulfonyl chloride (MsCl) to give the mesylate (2*R*,3*R*)-**7a** $[[\alpha]_D^{24}$ +32.43° (*c*=2.3, $CHCl_3$)] in a 92% yield. Hydrogenolysis of (2*R*,3*R*)-**7a** afforded (2*R*,3*R*)-**12** $[[\alpha]_D^{24}$ +32.54° (*c*=2.2, $CHCl_3$)] in a 94% yield. Alkaline hydrolysis of (2*R*,3*R*)-**10b** (entry 12; 95% ee) gave (2*R*,3*R*)-**8b** $[[\alpha]_D^{27}$ −38.5° (*c*=5.0, $CHCl_3$)] in a 78% yield, which was treated with MsCl to afford the mesylate (2*R*,3*R*)-**7b** $[[\alpha]_D^{26}$ +6.64° (*c*=2.5, $CHCl_3$)] in a 84% yield. Removal of the *p*-methoxyphenoxy group of (2*R*,3*R*)-**7b** was effectively achieved with ceric ammonium nitrate (CAN)[11] giving (2*R*,3*R*)-**12** $[[\alpha]_D^{26}$ +32.55° (*c*=1.1, $CHCl_3$)] in a 93% yield, which was identical [infrared (IR), NMR, $[\alpha]_D$, 32.55° (*c*=1.1, $CHCl_3$) and thin-layer chromatography (TLC)] with the above-mentioned (2*R*,3*R*)-**12**. The (2*R*,3*R*)-mesylate **12** was treated with NaOMe in MeOH and then hydrolyzed, providing the desired (2*S*,3*R*)-epoxybutyric acid **11** $[[\alpha]_D^{22}$ +80.13° (*c*=1.5, PhH)] in quantitative yield. The sign of the $[\alpha]_D$ value of **11** was opposite to that of the known (2*R*,3*S*)-**11**[12] $[[\alpha]_D^{25}$ −82.5° (*c*=0.59, PhH)]. The absolute structure of **11** was thus unambiguously confirmed as 2*S*, 3*R*. Esterification of (2*S*,3*R*)-**11** with CH_2N_2 and subsequent condensation of (2*S*,3*R*)-**5** with indole yielded the (2*S*,3*R*)-indolmycenic ester **4** $[[\alpha]_D^{23}$ +4.53° (*c*=0.53, MeOH)] whose physical data ($[\alpha]_D$, NMR and IR) were identical with the reported values $[[\alpha]_D^{24}$ +4.3° (*c*=0.93, MeOH); corresponds to 93% ee].[4]

Experimental

IR spectra (CCl_4) were measured on a JASCO A-3 spectrophotometer. NMR spectra were measured on a JEOL GX-400 instrument. Spectra were taken with 5—10% (w/v) solutions in $CDCl_3$ with Me_4Si as an internal reference. High-resolution mass spectra (HRMS) were obtained with a

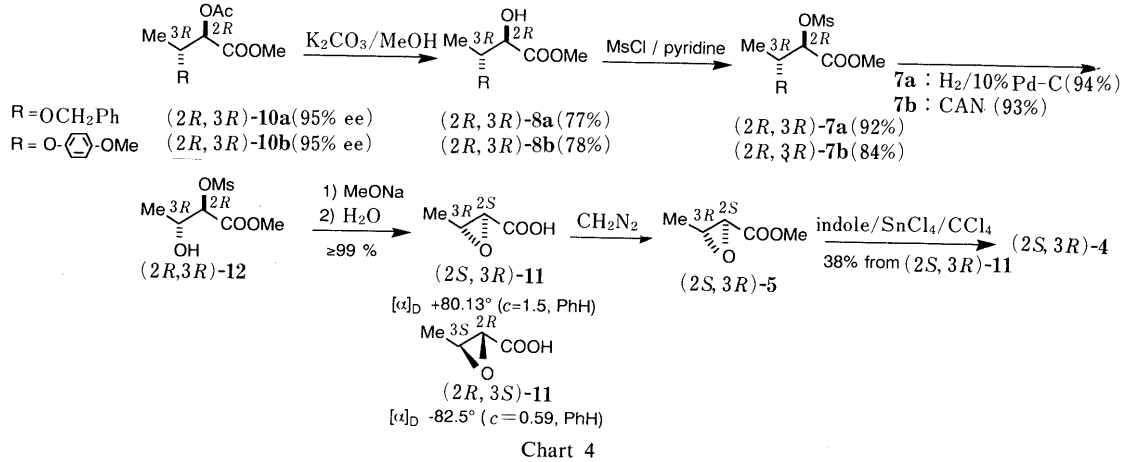


Chart 4

JEOL JMS-D 300 spectrometer. Optical rotations were measured on a Perkin-Elmer model 241 MC polarimeter. For column chromatography, silica gel (Wakogel C-200) was employed. All organic solvent extracts were washed with satd. brine and dried over anhydrous magnesium sulfate.

Methyl *trans*-2,3-Epoxybutyrate ((±)-5) Esterification of 2,3-epoxybutanoic acid (±)-**11**[7] (5.03 g) with excess CH_2N_2/ether solution gave a crude ester which was distilled to afford a colorless oil (±)-**5** (bp 65—78 °C (29 mmHg), 4.72 g; 82%). (±)-**5**: *Anal.* HRMS Calcd for $C_5H_8O_3$ (M^+; *m/z*): 116.016. Found: 116.019. NMR δ: 1.405 (3H, d, J=5.1 Hz, 3-Me), 3.205 (1H, d, J=1.8 Hz, 2-H), 3.242 (1H, dq, J=1.8, 5.1 Hz, 3-H), 3.780 (3H, s, COOMe).

Methyl 3-Benzyloxy-2-hydroxybutyrate ((+)-8a), Methyl 3-Chloro-2-hydroxybutyrate ((±)-6), Methyl 2-Acetoxy-3-chlorobutyrate ((±)-9) and Methyl 2-Hydroxy-3-(*p*-methoxyphenoxy)butyrate ((±)-8b) i) Reaction with $PhCH_2OH$ in the Presence of $SnCl_4$: A solution of $SnCl_4$ (2 ml) in CCl_4 (3 ml) was added dropwise with stirring to a mixture of (±)-**5** (4.16 g) and $PhCH_2OH$ (3.9 g) at −20 °C. After 0.5 h, the reaction mixture was stirred for 1.5 h at room temperature. The reaction mixture was diluted with H_2O, and extracted with ether. The ether extract was washed with saturated aqueous $NaHCO_3$ and dried. The solvent was removed *in vacuo* to afford an oily residue, which was chromatographed on silica gel (300 g). The fraction eluted with 10% AcOEt in hexane (v/v) gave a mixture (3.028 g) of chlorohydrin (±)-**6** and $PhCH_2OH$. The second fraction eluted with 20% AcOEt in hexane afforded a colorless oil (±)-**8a** (2.202 g; 27%). (±)-**8a**: *Anal.* HRMS Calcd for $C_{12}H_{16}O_4$ (M^+; *m/z*): 224.105. Found: 224.105. IR: 1740, 3550 cm^{-1}. NMR δ: 1.216 (3H, d, J=6.3 Hz, 3-Me), 3.797 (3H, s, COOMe), 3.860 (1H, dq, J=3.2, 6.3 Hz, 3-H), 4.346 (1H, br s, 2-H), 4.604 (2H, q, J=12 Hz OCH_2Ph). The above-mentioned mixture (3.028 g) was acetylated with Ac_2O (3 ml) in pyridine (10 ml). After usual work-up, the crude residue was chromatographed on silica gel (100 g) to give a colorless oil (±)-**9** (1.459 g; 21% yield from (±)-**5**) from 2% AcOEt in hexane eluate. (±)-**9**: *Anal.* HRMS Calcd for $C_7H_{11}ClO_4$ (M^+; *m/z*): 194.035. Found: 194.043. IR: 1760 cm^{-1}. NMR δ: 1.562 (3H, d, J=6.8 Hz, 3-Me), 2.208 (3H, s, $OCOCH_3$), 3.794 (3H, s, COOMe), 4.428 (1H, dq, J=3.7, 6.8 Hz, 3-H), 5.256 (1H, d, J=3.7 Hz, 2-H).

ii) Reaction with $PhCH_2OH$ in the Presence of $BF_3 \cdot Et_2O$: $BF_3 \cdot Et_2O$ (0.1 ml) was added dropwise with stirring to a mixture of (±)-**5** (119 mg) and $PhCH_2OH$ (118 mg) in CCl_4 (5 ml) at 0 °C. After the reaction mixture had been stirred for 1 h at room temperature, it was worked-up and purified in the same manner as in the case of i) to afford (±)-**8a** (44 mg; 19%). The spectral data were identical with those of the above-mentioned (±)-**8a**.

iii) Reaction with None of the Oxygen Nucleophiles in the Presence of $SnCl_4$: A solution of $SnCl_4$ (1 ml) in CCl_4 (2 ml) was added dropwise with stirring to a solution of (±)-**5** (1.843 g) in CCl_4 (10 ml) at −20 °C. After the reaction mixture had been stirred for 2 h, it was worked-up in the same manner as in the case of i). The residue was chromatographed on silica gel (42 g) to give an oily (±)-**6** (0.941 g; 39%) from 10% AcOEt in the hexane eluate. (±)-**6**: *Anal.* HRMS Calcd for $C_5H_9ClO_3$ (M^+; *m/z*): 152.023. Found: 151.996. IR: 1745, 3550 cm^{-1}. NMR δ: 1.535 (3H, d, J=6.8 Hz, 3-Me), 3.850 (3H, s, COOMe), 4.325 (1H, dq, J=3.2, 6.8 Hz, 3-H), 4.378 (1H, dd, J=3.2, 3.2 Hz, 2-H).

iv) Reaction with *p*-Methoxyphenol in the Presence of $SnCl_4$: $SnCl_4$ (0.5 ml) was added with stirring to a mixture of (±)-**5** (0.58 g) and *p*-methoxyphenol (0.62 g) in CCl_4 (10 ml) at 0 °C. After the reaction mixture had been stirred for 1 h at 0 °C, it was worked-up in the same manner as in the case of i). The residue was chromatographed on silica gel (50 g) to afford an oily product (±)-**8b** (0.313 g; 26%) from 10% AcOEt in hexane eluate. (±)-**8b**: *Anal.* HRMS Calcd for $C_{12}H_{16}O_5$ (M^+; *m/z*): 240.100. Found: 240.099. IR: 1723, 3480 cm^{-1}. NMR δ: 1.308 (3H, d, J=6.4 Hz, 3-Me), 3.768 (3H, s, COOMe), 3.834 (3H, s, OMe), 4.439 (1H, dd, J=3.1, 6.1 Hz, 2-H), 4.542 (1H, dq, J=3.1, 6.4 Hz, 3-H).

v) Reaction with *p*-Methoxyphenol in the Presence of $BF_3 \cdot Et_2O$: $BF_3 \cdot Et_2O$ (0.5 ml) was added with stirring to a mixture of (±)-**5** (0.58 g) and *p*-methoxyphenol (0.62 g) in CCl_4 (10 ml) at 0 °C. After the reaction mixture had been stirred for 1 h at room temperature, it was worked-up and purified in the same manner as in the case of i) to give an oily product (±)-**8b** (0.557 g; 46%). The spectral data were identical with those of the above-mentioned (±)-**8b**.

Methyl 2-Acetoxy-3-benzyloxybutyrate (+)-10a A mixture of 3-benzyloxy-2-hydroxy ester (±)-**8a** (1.984 g), Ac_2O (5 ml) and pyridine (5 ml) was stirred for 24 h at room temperature. The reaction mixture was diluted with H_2O and evaporated *in vacuo*. The ether extract from the residue was washed with 10% aqueous HCl and saturated aqueous $NaHCO_3$, and dried. The solvent was removed *in vacuo* to afford an oily residue, which was chromatographed on silica gel (80 g) to provide an oily acetate (±)-**10a** (2.195 g; 93%) from 5% AcOEt in the hexane eluate. (±)-**10a**: *Anal.* HRMS Calcd for $C_{14}H_{18}O_5$ (M^+; *m/z*): 266.115. Found: 266.114. IR: 1755 cm^{-1}. NMR δ: 1.281 (3H, d, J=6.5 Hz, 3-Me), 2.180 (3H, s, $OCOCH_3$), 3.756 (3H, s, COOMe), 3.963 (1H, dq, J=3.4, 6.5 Hz, 3-H), 4.595 (2H, q, J=11.8 Hz, OCH_2Ph), 5.271 (1H, d, J=3.4 Hz, 2-H).

Methyl 2-Acetoxy-3-(*p*-methoxyphenoxy)butyrate ((±)-10b) A mixture of 3-(*p*-methoxy)-phenoxy-2-hydroxy ester (±)-**8b** (2.25 g), Ac_2O (5 ml) and pyridine (5 ml) in the presence of 4-dimethylaminopyridine (DMAP; 0.5 g) was stirred for 24 h at room temperature. The reaction mixture was worked-up in the same manner as described for the synthesis of (±)-**10a**. The residue was chromatographed on silica gel (60 g) to give an oily acetate (±)-**10b** (2.223 g; 84%) from 10% AcOEt in hexane eluate. (±)-**10b**: *Anal.* HRMS Calcd for $C_{14}H_{18}O_6$ (M^+; *m/z*): 282.110. Found: 282.110. IR: 1735 cm^{-1}. NMR δ: 1.387 (3H, d, J=6.4 Hz, 3-Me), 2.190 (3H, s, $OCOCH_3$), 3.765 (3H, s, COOMe), 3.771 (3H, s, OMe), 4.641 (1H, dq, J=3.4, 6.4 Hz, 3-H), 5.300 (1H, d, J=3.4 Hz, 2-H).

General Procedure for Enzyme-Catalyzed Hydrolysis of (±)-9 and (±)-10a, b A solution of each substrate (*ca.* 100 mg) in 0.1 M phosphate buffer (pH 7.25, 20 ml) was incubated with lipase (*ca.* 50 mg) at 33 °C. Progress of the reaction was monitored by TLC and when the spots due to acetates and alcohols became the same size, the hydrolysis was terminated by extracting the mixture with AcOEt. The AcOEt extract was washed and dried. After removal of the solvent *in vacuo*, the crude product was purified by column chromatography on silica gel (30 g). The results are summarized in Table II. The specific rotations of (2*S*,3*S*)-**8b** and (2*R*,3*R*)-**10b** obtained by the use of lipase "Amano P" (entry 12 in Table II) were as follows. (2*S*,3*S*)-**8b**: $[\alpha]_D^{21}$ +28.80° (c=1.76, $CHCl_3$: corresponds to 70% ee) (2*R*,3*R*)-**10b**: $[\alpha]_D^{27}$ −0.8° (c=4.0, $CHCl_3$; corresponds to 95% ee).

Preparation of (+)-MTPA Esters [a Mixture of (2*S*,3*S*)-6-(+)-MTPA and (2*R*,3*R*)-6-(+)-MTPA] from (±)-6 Pyridine (0.3 ml) was added to a mixture of (±)-**6** (23 mg) and (+)-MTPACl (30 mg), and the reaction mixture was stirred for 26 h at room temperature. After addition of H_2O, the reaction mixture was extracted with ether. The ether extract was washed and dried. Removal of the solvent afforded an oily residue, which was subjected to preparative thin layer chromatography (prep. TLC) (Kieselgel 60 F_{254}, 200 × 200 × 0.5 mm, solvent, AcOEt–hexane (3 : 1)) to give (+)-MTPA esters (40 mg) as a homogeneous oil. NMR δ: 3.836 (3H, s, COOMe), 3.785 (3H, s, COOMe), 1.504 (3H, d, J=6.8 Hz, 3-Me), 1.474 (3H, d, J=6.8 Hz, 3-Me).

Preparation of (+)-MTPA Esters [a Mixture of (2*S*,3*S*)-8a-(+)-MTPA and (2*R*,3*R*)-8a-(+)-MTPA] from (±)-8a Pyridine (0.3 ml) was added to a mixture of (±)-**8a** (18 mg) and (+)-MTPACl (34 mg), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was worked-up and purified in the same manner as described for the (+)-MTPA esterification of (±)-**6** to afford (+)-MTPA esters (36 mg) as a homogeneous oil. NMR δ: 3.805 (3H, s, COOMe), 3.763 (3H, s, COOMe), 1.267 (3H, d, J=6.6 Hz, 3-Me), 1.209 (3H, d, J=6.4 Hz, 3-Me).

Preparation of (+)-MTPA Esters [a Mixture of (2*S*,3*S*)-8b-(+)-MTPA and (2*R*,3*R*)-8b-(+)-MTPA] from (±)-8b Pyridine (0.3 ml) was added to a mixture of (±)-**8b** (16 mg) and (+)-MTPACl (38 mg) in the presence of DMAP (5 mg), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was worked-up and purified in the same manner as described for the (+)-MTPA esterification of (+)-**6** to give (+)-MTPA esters (25 mg) as a homogeneous oil. NMR δ: 3.765 (3H, s, COOMe), 3.772 (3H, s, COOMe), 1.296 (3H, d, J=6.4 Hz, 3-Me), 1.349 (3H, d, J=6.4 Hz, 3-Me).

Preparation of Authentic (2*R*,3*R*)-6-(+)-MTPA A solution of $SnCl_4$ (0.2 ml) in CCl_4 (0.3 ml) was added dropwise with stirring to a solution of (2*R*,3*S*)-**5**[9] (198 mg) in CCl_4 (2 ml) at −20 °C. After the reaction mixture had been stirred for 1 h, it was worked-up and purified in the same manner as described for the preparation of (±)-**6** to afford (2*R*,3*R*)-**6** (100 mg; 38%) as a homogeneous oil. The spectral data of (2*R*,3*R*)-**6** were identical with those of (±)-**6**. Pyridine (0.3 ml) was added to a mixture of (2*R*,3*R*)-**6** (20 mg) and (+)-MTPACl (51 mg), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was worked-up and purified in the same manner as described for the preparation of (+)-**6**-(+)-MTPA to give (2*R*,3*R*)-**6**-(+)-MTPA (46 mg) as a homogeneous oil. (2*R*,3*R*)-**6**-(+)-MTPA: NMR δ: 3.836 (3H, s, COOMe), 1.474 (3H, d, J=6.8 Hz, 3-Me).

Preparation of Authentic Samples [(2*R*,3*R*)-8a, (2*R*,3*R*)-9 and (2*R*,3*R*)-8a-(+)-MTPA] A solution of $SnCl_4$ (3 ml) in CCl_4 (3 ml) was added dropwise with stirring to a mixture of (2*R*,3*S*)-**5** (3.018 g) and $PhCH_2OH$ (2.8 g) in CCl_4 (15 ml) at −20 °C. After the reaction mixture had been stirred for 1 h at room temperature, it was worked-up and chromato-

graphed on silica gel (100 g) in the same manner as described for the preparation of (±)-**8a**. The less polar fraction eluted with 10% AcOEt in hexane was a mixture of (2*R*,3*R*)-**6** and $PhCH_2OH$, which was acetylated and then purified by column chromatography to provide (2*R*,3*R*)-**9** (1.618 g; 32% yield from (2*R*,3*S*)-**5**) as a homogeneous oil from 2% AcOEt in the hexane eluate. $[\alpha]_D^{23}$ +8.3° (*c*=3.0, $CHCl_3$). The spectral data of (2*R*,3*R*)-**9** were identical with those of (±)-**9**. The more polar fraction eluted with 20% AcOEt in hexane was a colorless oil (2*R*,3*R*)-**8a** (1.081 g; 19%). (2*R*,3*R*)-**8a**: $[\alpha]_D^{23}$ −28.71° (*c*=2.95, $CHCl_3$). The spectral data of (2*R*,3*R*)-**8a** were identical with those of (±)-**8a**. Pyridine (0.3 ml) was added to a mixture of (2*R*,3*R*)-**8a** (15 mg) and (+)-MTPACl (40 mg), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was worked-up and purified in the same manner as described for the preparation of (±)-**8a**-(+)-MTPA to give (2*R*,3*R*)-**8a**-(+)-MTPA (27 mg) as a homogeneous oil. (2*R*,3*R*)-**8a**-(+)-MTPA: NMR δ: 3.805 (3H, s, COOMe), 1.209 (3H, d, *J*=6.4 Hz, 3-Me).

Determination of the Absolute Structure and Optical Purity of the Hydrolyzed Products Pyridine (0.3 ml) was added to a mixture of each hydrolyzed product (10—20 mg) and (+)-MTPACl (30—50 mg), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was worked-up and purified in the same manner as described for the preparation of (±)-**6**-(+)-MAPA to give the corresponding (+)-MTPA esters. The absolute structure and the optical purity were determined by 400 MHz NMR analysis of each (+)-MTPA ester, and the results are shown in Table II.

Determination of the Absolute Structure and Optical Purity of the Unchanged Products i) In the Case of (2*R*,3*R*)-**9**: A mixture of (2*R*,3*R*)-**9** (20—30 mg) and 30% HBr–AcOH (3 drops) in MeOH (0.3 ml) was stirred for 20 h at room temperature. The reaction mixture was diluted with H_2O and extracted with ether. The ether extract was washed with saturated aqueous $NaHCO_3$ and dried. The solvent was removed *in vacuo* to afford crude (2*R*,3*R*)-**6**, which was converted to the corresponding (+)-MTPA esters in the same manner as described for the preparation of (±)-**6**-(+)-MTPA. The absolute structure and the optical purity were determined by 400 MHz NMR analysis of each (+)-MTPA ester, and the results are shown in Table II.

ii) In the Case of (2*R*,3*R*)-**10a**, **b**: A mixture of (2*R*,3*R*)-**10a**, **b** (20—30 mg) and K_2CO_3 (*ca.* 15 mg) in MeOH (0.5 ml) was stirred for 1 h at room temperature. The reaction mixture was diluted with H_2O, and extracted with ether. The ether extract was washed and dried. The solvent was removed *in vacuo* to afford crude (2*R*,3*R*)-**8a**, **b**, which was converted to the corresponding (+)-MTPA esters in the same manner as described for the preparation of (±)-**8b** (+)-MTPA ester. The absolute structure and the optical purity were determined by 400 MHz NMR analysis of each (+)-MTPA ester, and the results are shown in Table II.

Methyl (2*R*,3*R*)-2-Hydroxy-3-benzyloxybutyrate (8a) A mixture of (2*R*,3*R*)-**10a** (345 mg, 95% ee, entry 4 in Table II) and K_2CO_3 (220 mg) in MeOH (4 ml) was stirred for 1 h at room temperature. The reaction mixture was worked-up and purified by column chromatography in the same manner as described for the preparation of authentic (2*R*,3*R*)-**8a** to give (2*R*,3*R*)-**8a** (225 mg; 77%) as a homogeneous oil. The spectral data of (2*R*,3*R*)-**8a** were identical with those of (±)-**8a**.

Methyl (2*R*,3*R*)-2-Mesyloxy-3-benzyloxybutyrate (7a) MsCl (0.72 g) was added with stirring to a solution of (2*R*,3*R*)-**8a** (1.072 g) in pyridine (2 ml) at 0 °C, and the reaction mixture was stirred for 2 h at room temperature. The mixture was then diluted with H_2O, and extracted with ether. The ether extract was washed and dried. The solvent was removed *in vacuo* to afford an oily residue, which was chromatographed on silica gel (40 g) to give a colorless oil (2*R*,3*R*)-**7a** (1.322 g; 92%) from 20% AcOEt in the hexane eluate. (2*R*,3*R*)-**7a**: *Anal.* HRMS Clacd for $C_{13}H_{18}O_6S$ (M^+; *m/z*): 302.082. Found: 302.080. $[\alpha]_D^{24}$ +32.43° (*c*=2.3, $CHCl_3$). IR: 1180, 1370, 1720 (sh), 1750 (sh), 1760 cm^{-1}. NMR δ: 1.272 (3H, d, *J*=6.5 Hz, 3-Me), 3.125 (3H, s, OSO_2Me), 3.814 (3H, s, COOMe), 4.049 (1H, dq, *J*=3, 6.5 Hz, 3-H), 4.614 (2H q, *J*=11.5 Hz, OCH_2Ph), 5.257 (1H, d, *J*=3 Hz, 2-H).

Methyl (2*R*,3*R*)-2-Mesyloxy-3-hydroxybutyrate (12) from (2*R*,3*R*)-7a A solution of (2*R*,3*R*)-**7a** (1.31 g) in MeOH (10 ml) was hydrogenated at ordinary temperature and pressure over 10% Pd–C (0.2 g). After hydrogen absorption had ceased, the catalyst was filtered off and the filtrate was evaporated to give a residue, which was chromatographed on silica gel (40 g) to provide a colorless oil (2*R*,3*R*)-**12** (0.862 g; 94%) from 50% AcOEt in the hexane eluate. (2*R*,3*R*)-**12**: MS *m/z*: 213 (M^++1). $[\alpha]_D^{24}$ +32.54° (*c*=2.2, $CHCl_3$). IR: 1180, 1365, 1760, 3530 cm^{-1}. NMR δ: 1.287 (3H, d, *J*=6.5 Hz, 3-Me), 3.200 (3H, s, OSO_2Me), 3.841 (3H, s, COOMe), 4.306 (1H, br s, 3-H), 5.079 (1H, d, *J*=3.7 Hz, 2-H).

Methyl (2*R*,3*R*)-2-Hydroxy-3-(*p*-methoxyphenoxy)butyrate (8b) A mixture of (2*R*,3*R*)-**10b** (242 mg, 95% ee, entry 12 in Table II and K_2CO_3 (117 mg) in MeOH (2 ml) was stirred for 1 h at room temperature. The reaction mixture was worked-up and purified by column chromatography in the same manner as described for the preparation of (±)-**8b** to give (2*R*,3*R*)-**8b** (161 mg; 78%) as a homogeneous oil. $[\alpha]_D^{27}$ −38.5° (*c*=5.0, $CHCl_3$). The spectral data of (2*R*,3*R*)-**8b** were identical with those of (±)-**8b**.

Methyl (2*R*,3*R*)-2-Mesyloxy-3-(*p*-methoxyphenoxy)butyrate (7b) MsCl (200 mg) was added with stirring to a solution of (2*R*,3*R*)-**8b** (147 mg) in pyridine (1 ml) at 0 °C, and the reaction mixture was stirred for 12 h at room temperature. The mixture was then worked-up and purified in the same manner as described for the preparation of (2*R*,3*R*)-**7a** to afford (2*R*,3*R*)-**7b** (163 mg; 84%) as a homogeneous oil. (2*R*,3*R*)-**7b**: $[\alpha]_D^{26}$ +6.64° (*c*=2.5, $CHCl_3$). NMR δ: 1.374 (3H, d, *J*=6.4 Hz, 3-Me), 3.151 (3H, s, OSO_2Me), 3.774 (3H, s, COOMe), 3.840 (3H, s, OMe), 4.727 (1H, dq, *J*=3, 6.4 Hz, 3-H), 5.275 (1H, d, *J*=3 Hz, 2-H).

Methyl (2*R*,3*R*)-2-Mesyloxy-3-hydroxybutyrate (12) from (2*R*,3*R*)-7b A mixture of (2*R*,3*R*)-**7b** (75 mg) and ceric ammonium nitrate (CAN, 400 mg) in CH_3CN (2.5 ml)/H_2O (1 ml) solution was stirred for 3 h at 0 °C. The reaction mixture was diluted with H_2O, and extracted with ether. The ether extract was washed and dried. The solvent was removed *in vacuo* to give an oily residue, which was subjected to prep. TLC (Kieselgel 60 F_{254}, 200×200×0.5 mm; solvent, AcOEt–hexane (1:1) to afford (2*R*,3*R*)-**12** (46 mg; 93%) as a homogeneous oil. (2*R*,3*R*)-**12**: $[\alpha]_D^{26}$ +32.55° (*c*=1.1, $CHCl_3$). The spectral data of (2*R*,3*R*)-**12** were identical with those of the above-mentioned (2*R*, 3*R*)-**12** from (2*R*, 3*R*)-**7a**.

(2*S*,3*R*)-Epoxybutanoic Acid (11) A sodium methoxide/MeOH solution (prepared from Na (190 mg) in absolute MeOH (4ml)) was added with stirring to a solution of (2*R*,3*R*)-**12** (840 mg) in absolute MeOH (2 ml) at 0 °C, and the reaction mixture was stirred for 1 h at room temperature. The mixture was then diluted with H_2O and extracted with ether. The ether extract was discarded. The water layer was acidified with 10% aqueous HCl and extracted with AcOEt (5 times) after saturation with $(NH_4)_2SO_4$. Removal of the solvent gave (2*S*,3*R*)-**11** (413 mg; quantitative yield). (2*S*,3*R*)-**11**: $[\alpha]_D^{22}$ +80.13° (*c*=1.5, PhH). NMR δ: 1.436 (3H, d, *J*=5.2 Hz, 3-Me), 3.240 (1H, d, *J*=1.9 Hz, 2-H), 3.284 (1H, dq, *J*=1.9, 5.2 Hz, 3-H).

Methyl (2*S*,3*R*)-Epoxybutyrate (5) and Methyl (2*S*,3*R*)-Indolmycenate (4) Esterification of (2*S*,3*R*)-**11** (398 mg) with an excess of CH_2N_2/ether solution yielded the corresponding methyl ester (2*S*,3*R*)-**5**, whose NMR spectra were identical with those of (±)-**5**. A solution of $SnCl_4$ (0.5 ml) in CCl_4 (3 ml) was added with stirring to a solution of the above (2*S*,3*R*)-**5** and indole (600 mg) in CCl_4 (5 ml) at 0 °C. The reaction mixture was stirred for 1 h at room temperature. The mixture was then diluted with saturated aqueous $NaHCO_3$ and extracted with chloroform. The chloroform layer was washed and dried. The solvent was removed *in vacuo* to give an oily residue, which was chromatographed on silica gel (40 g) to afford (2*S*,3*R*)-**4** (350 mg; 38% yield from (2*S*,3*R*)-**11**) as a homogeneous oil from 25% AcOEt in the hexane eluate. (2*S*,3*R*)-**4**: *Anal.* HRMS Calcd for $C_{13}H_{15}NO_3$ (M^+; *m/z*): 233.106. Found 233.109. $[\alpha]_D^{23}$ +4.53° (*c*=0.53, MeOH). IR: 1736, 3500 cm^{-1}. NMR δ: 1.326 (3H, d, *J*=7.1 Hz, 3-Me), 2.755 (1H, d, *J*=5.1 Hz, 2-OH), 3.636 (1H, dq, *J*=3.2, 7.1 Hz, 3-H), 3.788 (3H, s, COOMe), 4.509 (1H, dd, *J*=3.2, 5.1 Hz, 2-H).

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