Synthesis of Ozonolysis Products of Myxothiazol

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By applying the Hantzsch thiazole procedure, the synthesis of bithiazole derivatives (2 and (±)-3) corresponding to degradation products of myxothiazol was achieved. Optically active (*S*)-3 (87—88% ee) and its analog (*S*)-16 (91% ee) were obtained through enantioselective hydrolysis of the corresponding acetates ((±)-18, (±)-20 and (±)-21) using lipase in water-saturated organic solvent.

Keywords myxothiazol; antibiotic; bithiazole; lipase; enantioselective hydrolysis

Myxothiazol (**1**) is an antibiotic isolated by Trowitzsch and his co-workers from the myxobacterium *Myxococcus fulvus*; it has high antifungal activity, which is caused by inhibition of the ubiquinol: cytochrome c reductase system.[1] Many kinds of natural products possess the bithiazole skeleton and some have important biological activities, such as antitumor, anthelminthic, antifungal, and antibacterial activities.[2] The structure of myxothiazol was established by a combination of chemical methods and X-ray analysis of a degradation product.[3] Ozonolysis of myxothiazol followed by oxidative work-up yielded the acetyl-bithiazole derivative **2**, while reductive work-up after ozonolysis afforded the bithiazole-diol **3**. The absolute configuration of **3** was established by X-ray structural analysis as *S*. A total synthesis of myxothiazol as a diastereomeric mixture has been reported.[4] We describe here the syntheses of the acetyl-bithiazole **2** and the optically active (*S*) bithiazole-diol **3** *via* enzymatic resolution in an organic solvent.

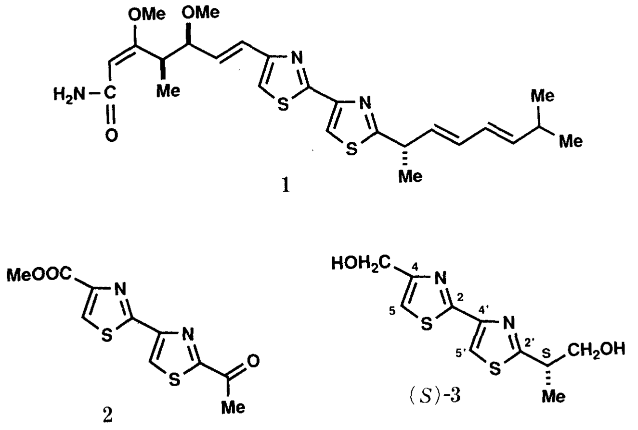
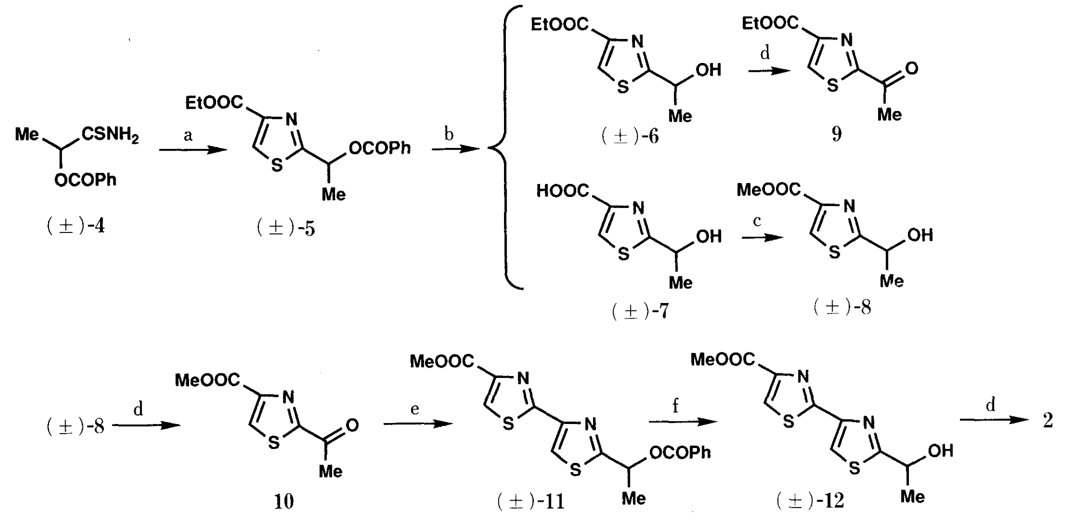


Chart 1

Synthesis of the Acetyl-bithiazole 2 Application of the Hantzsch thiazole synthesis to 2-benzoyloxy propionthioamide (±)-**4** and commercially available ethyl bromopyruvate was reported to give the (±)-monothiazole ester **5**.[5] Deprotection of the benzoyl group of (±)-**5** by alkaline hydrolysis afforded the (±)-hydroxy ethyl ester **6** (59% yield) and the (±)-hydroxy acid **7**. The latter was esterified with CH_2N_2 to give the known (±)-hydroxy methyl ester **8**[6] (37% yield). Jones oxidation of both hydroxy esters (±)-**6** and (±)-**8** provided the acetyl esters **9** (96% yield) and the known **10**[6] (88% yield), respectively. Bromination of **10** followed by reaction with (±)-**4** gave the (±)-bithiazole benzoate **11** in 43% yield along



a, $BrCH_2COCOOEt$ b, i) 1% NaOH/EtOH ii) H^+ c, CH_2N_2
d, CrO_3/H^+ e, i) Br_2/HBr ii) (±)-4/MeOH f, 1% NaOH/MeOH

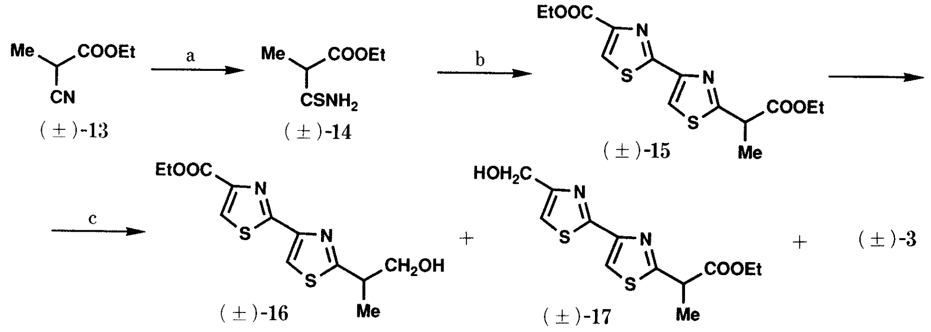
Chart 2

with the recovered (±)-**10** (22% yield). Alkaline hydrolysis of (±)-**11** yielded the (±)-hydroxy bithiazole **12** in 92% yield, and this was oxidized with Jones reagent to provide the acetyl-bithiazole **2** (85% yield). The spectral data (infrared (IR) spectra, nuclear magnetic resonance (NMR) spectra) of the synthetic **2** were identical with those reported for **2**.[1b)]

Synthesis of (±)-Bithiazole Diol 3 The commercially available (±)-2-cyano propionate **13** was treated with hydrogen sulfide gas in the presence of triethanolamine to afford the (±)-thioamide **14** in 45% yield.[7)] Bromination of **9** followed by treatment of (±)-**14** provided the (±)-bithiazole diester **15** in 47% yield along with the recovered **9** (44% yield).[7)] Reduction of (±)-**15** with $LiBH_4$ afforded the (±)-hydroxy esters **16** (8% yield) and **17** (5% yield) and the (±)-bithiazole diol **3** (80% yield). The NMR spectra of the synthetic (±)-**3** thus obtained were identical with those of the reported (*S*)-**3**.[3)] The structures of (±)-**16** and (±)-**17** were determined by NMR analysis. The signal due to the methine proton of (±)-**16** appeared as a multiplet (δ 3.36—3.44), so the propanol moiety was found to exist at the 2′-position of the bithiazole ring. On the other hand, the structure of (±)-**17** was confirmed by the fact that the signals due to the methine proton and hydroxylmethyl group of (±)-**17** appeared at δ 4.45 as a quartet ($J=7.3$ Hz) and at δ 4.81 as a singlet, respectively.

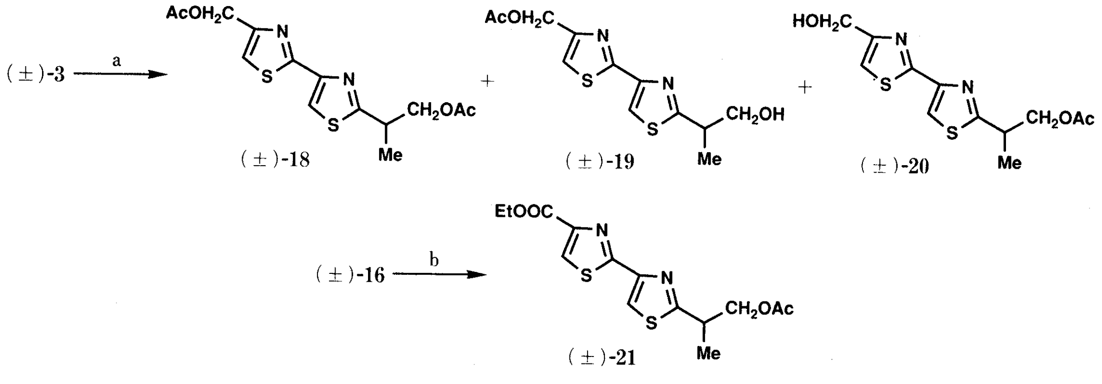
Synthesis of Substrates for Lipase-Catalyzed Hydrolysis Acetylation of (±)-**3** using 4 eq of Ac_2O gave the (±)-diacetate **18** in 94% yield. When 1.1 eq of Ac_2O was employed, two monoacetates (±)-**19** (31% yield), (±)-**20** (20% yield) along with (±)-**18** (14% yield) were obtained. The structures of (±)-**19** and (±)-**20** were determined by NMR analysis. The signal due to the methylene proton adjacent to an acetoxyl group of (±)-**19** appeared as a singlet (δ 5.24), so the acetoxylmethyl group was concluded to exist at the 4-position of the bithiazole ring. On the other hand, the structure of (±)-**20** was confirmed by the fact that the signal due to the hydroxylmethyl group appeared at δ 4.81 as a singlet. Acetylation of (±)-**16** afforded the corresponding (±)-acetate **21** in 98% yield.

Lipase-Catalyzed Enantioselective Hydrolysis In a preliminary experiment, it was found that three racemates ((±)-**3**, (±)-**16** and (±)-**21**) were well separated by high-performance liquid chromatographic (HPLC) analysis with a chiral column (Chiralcel OD (4.6 × 250 mm)). Then enantioselective hydrolysis of four kinds of acetates ((±)-**18**, (±)-**19**, (±)-**20** and (±)-**21**) using nine kinds of commercial lipases (Amano M-10 from *Mucol javanicus*, MY-30 from *Candida cylindracea*, OF-360 from *Candida cylindracea*, Sigma type VII from *Candida cylindracea*, Nagase P from *Pseudomonas* sp., Godo E-1 from *Pseudomonas cepacia*, Godo E-160 from *Pseudomonas* sp., Godo E-180 from *Pseudomanas* sp., Immobilized Amano P[8)] from *Pseudomonas* sp.) was carried out in water-saturated isopropyl ether at 33 °C. Selected data are given in Table I. The used lipases can not be recovered after the reaction because they include large amounts of



a, $H_2S/N(CH_2CH_2OH)_3$ b, $9/Br_2/HBr$ c, $LiBH_4$

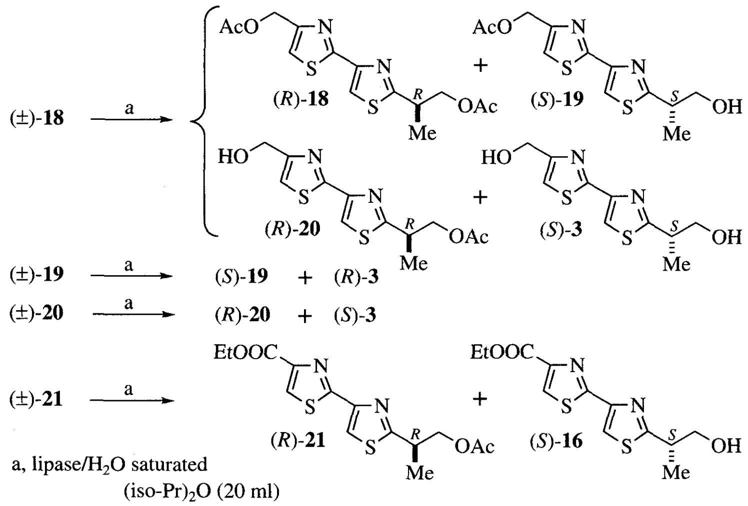
Chart 3



a, Ac_2O(1.1 eq)/pyridine b, Ac_2O (excess)/pyridine

Chart 4

Table I.



a, lipase/H_2O saturated (iso-$Pr)_2O$ (20 ml)

Entry	Substrate (mg)	Lipase (mg)	Time (h)	Product % (% ee)			
1	(±)-**18** (100)	Amano M-10 (100)	48	(*R*)-**18**	41 (48)	(*S*)-**19**	22 (75)
				(*R*)-**20**	16 (74)	(*S*)- **3**	19 (73)
2	(±)-**18** (100)	Nagase P (100)	48	(*R*)-**18**	60 (32)	(*S*)-**19**	17 (70)
				(*R*)-**20**	8 (42)	(*S*)- **3**	13 (40)
3	(±)-**18** (100)	Godo E-1 (100)	48	(*R*)-**18**	24 (34)	(*S*)-**19**	6 (73)
				(*R*)-**20**	39 (60)	(*S*)- **3**	26 (87)
4	(±)-**18** (100)	Immobilized amano P	4	(*R*)-**18**	8 (72)	(*S*)-**19**	12 (26)
				(*R*)-**20**	27 (85)	(*S*)- **3**	41 (37)
5	(±)-**18** (100)	Godo E-160 (100)	48			(*S*)-**19**	4 (44)
				(*R*)-**20**	35 (75)	(*S*)- **3**	57 (41)
6	(±)-**18** (100)	Sigma type VII (100)	48	(*S*)-**18**	10 (21)	(*R*)-**19**	8 (17)
				(*S*)-**20**	30 (22)	(*R*)- **3**	49 (15)
7	(±)-**19** (100)	Godo E-1 (100)	24	(*S*)-**19**	17 (2)	(*R*)- **3**	75 (10)
8	(±)-**20** (100)	Godo E-1 (100)	8	(*R*)-**20**	55 (62)	(*S*)- **3**	42 (88)
9	(±)-**20** (100)	Amano M-10 (100)	55	(*R*)-**20**	51 (36)	(*S*)- **3**	27 (83)
10	(±)-**20** (83)	Amano P (100)	26	(*R*)-**20**	20 (52)	(*S*)- **3**	74 (7)
11	(±)-**21** (100)	Amano M-10 (100)	72	(*R*)-**21**	51 (32)	(*S*)-**16**	21 (85)
12	(±)-**21** (100)	Godo E-1 (100)	72	(*R*)-**21**	51 (81)	(*S*)-**16**	47 (91)

inorganic material as a support for the enzymes. On the other hand, when immobilized lipase is used, it can be recovered by means of simple filtration and repeatedly used.[8] In the present case, repeated runs were not carried out using the immobilized lipase Amano P with ENTP-4000 prepolymer, but this immobilized lipase showed the same efficiency as the native lipase.[8]

When a mixture of (±)-**18** and lipase Godo E-1 was incubated, the (*S*)-diol **3** (26% yield, 87% ee), two monoacetates (*S*)-**19** (6% yield, 73% ee) and (*R*)-**20** (39% yield, 60% ee), and the unchanged diacetate (*R*)-**18** (24% yield, 34% ee) were obtained (entry 3). The absolute stereochemistry of the diol **3** was confirmed to be *S* because the $[\alpha]_D$ value ($[\alpha]_D$ +4.74° ($c=0.59$, $CHCl_3$); corresponds to 87% ee) of the present **3** was consistent with that of the authentic diol **3** ($[\alpha]_D$ +5.4°, $CHCl_3$)[3] derived from natural myxothiazol. The absolute stereochemistry of the unidentified monoacetates **19**, **20** and diacetate **18** were confirmed to be *S*-, *R*- and *R*-configuration, respectively, as shown in Table I, by HPLC analysis after conversion of each acetate by treatment with K_2CO_3 in MeOH into the corresponding diol **3**. This lipase Godo E-1 was also found to function effectively for the enantioselective hydrolysis of (±)-monoacetates **20** and **21** to provide the (*S*)-alcohols **3** (42% yield, 88% ee; entry 8) and **16** (47% yield, 91% ee; entry 12), respectively. The absolute stereochemistry of the hydroxy ester **16** was confirmed to be *S* by means of HPLC analysis after conversion of the hydroxy ester **16** into the diol **3** using $LiBH_4$.

In conclusion, by applying the Hantzsch thiazole procedure, the synthesis of bithiazole derivatives (**2** and (±)-**3**) corresponding to the degradation products was achieved. Enantioselective hydrolysis of three types of acetates ((±)-**18**, (±)-**20** and (±)-**21**) was carried out using lipase in water-saturated isopropyl ether to afford the (*S*)-alcohols ((*S*)-**3** and (*S*)-**16**) possessing high optical purity (87—91% ee).

Experimental

All melting points were measured on a Yanaco MP-S3 micro melting point apparatus and are uncorrected. IR spectra were measured on a JASCO A-3 spectrophotometer. NMR spectra were measured on a JEOL EX 4000 instrument. Spectra were taken with 5—10% (w/v) solution in $CDCl_3$ with Me_4Si as an internal reference. High-resolution mass spectra (HRMS) were obtained with a JEOL JMS-D-300 spectrometer. The HPLC system was composed of two SSC instruments (ultraviolet (UV) detector 3000B and flow system 3100). All organic solvent extracts were washed with saturated brine and dried over anhydrous magnesium sulfate

($\mathrm{MgSO_4}$). All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

(±) 4-Ethoxycarbonyl-2-(1-hydroxyethyl)thiazole 6 and (±) 4-Methoxycarbonyl-2-(1-hydroxyethyl)thiazole (8) A solution of (±)-**5** (24.77g) in 1% (w/v) NaOH/EtOH solution (322 ml) was stirred for 1 h at room temperature. After neutralization of the reaction mixture with 10% HCl aqueous solution, it was concentrated under reduced pressure to give a residue. The resulting residue was diluted with $\mathrm{H_2O}$ and extracted with AcOEt. Evaporation of the AcOEt layer gave an oily product. The water layer was again acidified with 10% HCl aqueous solution and the whole was concentrated under reduced pressure to afford a solid crude hydroxy acid (±)-**7**,[9] which was esterified with $\mathrm{CH_2N_2}$ ether solution after dissolving with a small amount of acetone. Evaporation of the $\mathrm{Et_2O}$ layer gave an oily product. Both products were individually chromatographed on silica gel (200 g) to yield (±)-**6** (9.64 g, 59% yield) and the known (±)-**8**[6] (5.63 g, 37% yield), respectively, from the 20% AcOEt in *n*-hexane eluate. (±)-**6**: crystallization from AcOEt–*n*-hexane afforded colorless needles, mp 90—90.5 °C. IR($\mathrm{CHCl_3}$): 3350, 1700 cm^{1}. NMR δ: 1.40 (3H, t, $\mathrm{COOCH_2C\underline{H}_3}$), 1.66 (3H, d, $J=6.5$ Hz, *sec*-Me), 4.41 (2H, q, $\mathrm{COOC\underline{H}_2CH_3}$), 5.24 (1H, q, $J=6.5$ Hz, methine), 8.12 (1H, s, thiazole-H).

2-Acetyl-4-ethoxycarbonylthiazole (9) and 2-Acetyl-4-methoxycarbonylthiazole (10) Jones reagent (60 ml) was added to a solution of (±)-**6** (20.47 g) in acetone (100 ml) under ice-water cooling and the whole was stirred for 50 min. After addition of isopropyl alcohol (40 ml), the whole was stirred for 30 min and evaporated under reduced pressure to give a residue. The resulting residue was diluted with $\mathrm{H_2O}$ and extracted with $\mathrm{CHCl_3}$. Evaporation of the organic solvent gave an oily product, which was crystallized from AcOEt in *n*-hexane to provide colorless prisms **9** (19.53 g, 96% yield). **9**: mp 60—60.5 °C. *Anal.* Calcd for $\mathrm{C_8H_9NO_3S}$: C, 48.23; H, 4.55; N, 7.03. Found: C, 48.67; H, 4.66; N, 6.92. IR($\mathrm{CCl_4}$): 1692, 1720, 1740(sh) cm^{-1}. NMR δ: 1.44 (3H, t, $\mathrm{COOCH_2C\underline{H}_3}$), 2.79 (3H, s, $\mathrm{COCH_3}$), 4.46 (2H, q, $\mathrm{COOC\underline{H}_2CH_3}$), 8.45 (1H, s, thiazole-H). Jones oxidation of (±)-**8** (15.89 g) gave a crude acetyl compound **10** in the same way as described for the preparation of (±)-**9**. Crystallization of crude **10** from AcOEt in *n*-hexane afforded colorless plates **10** (13.81 g, 88% yield), whose physical data were identical with those reported for **10**.[6]

(±) 2′-(1-Benzoyloxyethyl)-4-methoxycarbonyl-2,4′-bithiazole (11) A mixture of **10** (4.7 g), $\mathrm{Br_2}$ (4.1 g) and 30% HBr/AcOH (5 drops) in $\mathrm{CCl_4}$ (24 ml) was stirred for 30 min at room temperature. The reaction mixture was diluted with $\mathrm{H_2O}$ and extracted with ether. The ether layer was washed with saturated $\mathrm{NaHCO_3}$ aqueous solution and evaporated to provide a crude bromo compound, which was reacted with (±)-**4** (3.72 g) in MeOH (15 ml) for 90 min under reflux. The reaction mixture was evaporated to give a residue, which was diluted with saturated $\mathrm{NaHCO_3}$ aqueous solution and extracted with $\mathrm{CHCl_3}$. Evaporation of the chloroform provided a crude oily product, which was chromatographed on silica gel (200 g) to afford the recovered **10** (1.05 g, 22% yield) from the 10% AcOEt in *n*-hexane eluate and a pale yellow oil (±)-**11** (4.12 g, 43% yield) from the 20% AcOEt in *n*-hexane eluate. (±)-**11**: MS *m/z*: 374 ($\mathrm{M^+}$), 343 ($\mathrm{M^+}-\mathrm{OMe}$), 269 ($\mathrm{M^+}-\mathrm{COPh}$). IR($\mathrm{CCl_4}$): 1725 cm^{-1}. NMR δ: 1.87 (3H, d, $J=6.5$ Hz, *sec*-Me), 3.94 (3H, s, COOMe), 6.40 (1H, q, $J=6.5$ Hz, methine), 8.10, 8.15 (each 1H, s, 5- and 5′-H).

(±) 2′-(1-Hydroxyethyl)-4-methoxycarbonyl-2,4′-bithiazole (12) A solution of (±)-**11** (11.2 g) in 10% (w/v) NaOH/MeOH solution (120 ml) was stirred for 50 min at room temperature. The reaction mixture was worked up in the same way as described for the preparation of (±)-**6** to give an oily product, which was chromatographed on silica gel (300 g) to afford (±)-**12** (7.41 g, 92% yield) from the 20% AcOEt in *n*-hexane eluate. Recrystallization of (±)-**12** from AcOEt provided colorless needles. (±)-**12**: mp 158.5—159 °C. MS *m/z*: 270 ($\mathrm{M^+}$), 255 ($\mathrm{M^+}-\mathrm{Me}$), 239 ($\mathrm{M^+}-\mathrm{OMe}$). IR ($\mathrm{CCl_4}$): 3400, 1725, 1740 (sh) cm^{-1}. NMR δ: 1.69 (3H, d, $J=6.5$ Hz, *sec*-Me), 3.98 (3H, s, COOMe), 5.24 (1H, q, $J=6.5$ Hz, methine), 8.11, 8.18 (each 1H, s, 5- and 5′-H).

2′-Acetyl-4-methoxycarbonyl-2,4′-bithiazole (2) Jones reagent (3 ml) was added to a solution of (±)-**12** (336 mg) in acetone (10 ml) under ice-water cooling and the whole was stirred for 45 min. The reaction mixture was worked up in the same way as described for the preparation of **9** to afford a product, which was recrystallized from AcOEt to give **2** as colorless needles (284 mg, 85% yield). **2**: mp 213—214 °C.[10] *Anal.* Calcd for $\mathrm{C_{10}H_8N_2O_3S_2}$: C, 44.76; H, 3.01; N, 10.44. Found: C, 44.76; H, 3.12; N, 10.33. MS *m/z*: 268 ($\mathrm{M^+}$). IR ($\mathrm{CHCl_3}$): 1690, 1720 cm^{-1}. NMR δ: 2.78 (3H, s, $\mathrm{COCH_3}$), 3.99 (3H, s, COOMe), 8.25, 8.46 (each 1H, s, 5- and 5′-H).

(±) 2-Ethoxycarbonyl Propionthioamide (14) Hydrogen sulfide gas ($\mathrm{H_2S}$) was bubbled into a mixture of (±)-**13** (43 g) and triethanolamine (16.5 g) in EtOH (50 ml) for 7 h at 60 °C. The reaction mixture was diluted with $\mathrm{H_2O}$ and extracted with ether. Evaporation of the organic solvent gave an oily product, which was chromatographed on silica gel (500 g) to provide (±)-**14** (24.76 g, 45% yield) from the 20% AcOEt in *n*-hexane eluate. Recrystallization of (±)-**14** afforded colorless plates. (±)-**14**: mp 68—69 °C. *Anal.* Calcd for $\mathrm{C_6H_{11}NO_2S}$: C, 44.70; H, 6.88; N, 8.69. Found: C, 44.50; H, 7.06; N, 8.62. IR($\mathrm{CCl_4}$): 1720, 3320, 3470 cm^{-1}. NMR δ: 1.28 (3H, t, $\mathrm{COOCH_2C\underline{H}_3}$), 1.57 (3H, d, $J=7.2$ Hz, *sec*-Me), 3.83 (1H, q, $J=7.2$ Hz, methine), 4.18 (2H, q, $\mathrm{COOC\underline{H}_2CH_3}$).

(±) 2′-(1-Ethoxycarbonylethyl)-4-ethoxyethyl-2,4′-bithiazole (15) A mixture of **9** (*ca.* 4 g), $\mathrm{Br_2}$ (4 g) and 30% HBr/AcOH (10 drops) in $\mathrm{CHCl_3}$ (30 ml) was stirred for 22 h at room temperature. This preparation was carried out five times on the same scale (total amount of **9** used; 19.45 g). The reaction mixture was worked up in the same way as described for the bromination of **10** to give a crude bromo compound, which was reacted with (±)-**14** (15.74 g) in EtOH (100 ml) for 1 h under reflux. The reaction mixture was worked up in the same way as described for the preparation of (±)-**11** to provide a crude oily product, which was chromatographed on silica gel (800 g) to afford the recovered **9** (8.52 g, 44% yield) and a pale yellow oil (±)-**15** (15.59 g, 47% yield) in order of elution with 20% AcOEt in *n*-hexane. (±)-**15**: MS *m/z*: 340 ($\mathrm{M^+}$). *Anal.* HRMS Calcd for $\mathrm{C_{14}H_{16}N_2O_4S_2}$ ($\mathrm{M^+}$) *m/z*: 340.0552. Found: 340.0527. IR($\mathrm{CCl_4}$): 1720, 1740 cm^{-1}. NMR δ: 1.29, 1.43 (each 3H, t, $\mathrm{COOCH_2C\underline{H}_3}\times 2$), 1.71 (3H, d, $J=7.1$ Hz, *sec*-Me), 4.24, 4.45 (each 2H, q, $\mathrm{COOC\underline{H}_2CH_3}\times 2$), 8.12, 8.17 (each 1H, s, 5- and 5′-H).

(±) 4-Ethoxycarbonyl-2′-(1-hydroxymethyethyl)-2,4′-bithiazole (16), (±) 2′-(1-Ethoxycarbonylethyl)-4-hydroxymethyl-2,4′-bithiazole (17) and (±) 4-Hydroxymethyl-2′-(1-hydroxymethylethyl)-2,4′-bithiazole (3) $\mathrm{LiBH_4}$ (1 g) was added to a solution of (±)-**15** (*ca.* 7.8 g) in dry tetrahydrofuran (THF) (40 ml) under ice-water cooling and the whole was stirred for 90 min at room temperature. This preparation was carried out twice on the same scale (total amount of (±)-**15** used 15.59 g). The reaction mixture was diluted with saturated brine and extracted with AcOEt. Evaporation of the organic solvent gave an oily residue, which was crystallized from $\mathrm{Et_2O}$–AcOEt to provide (±)-**3** (4.99 g). The mother liquid was chromatographed on silica gel (100 g) to afford the less polar product (±)-**16** (1.10 g, 8% yield) and the more polar product (±)-**17** (0.63 g, 5% yield) from the 20% AcOEt in *n*-hexane eluate. The product eluted with AcOEt was crystallized from $\mathrm{Et_2O}$–AcOEt to give (±)-**3** as colorless needles (4.37 g, total amount 9.36 g, 80% yield). (±)-**16**: recrystallization from AcOEt afforded pale yellow needles, mp 76—76.5 °C. *Anal.* Calcd for $\mathrm{C_{12}H_{14}N_2O_3S_2}$: C, 48.30; H, 4.73; N, 9.39. Found: C, 48.01; H, 4.87; N, 9.24. IR (Nujol): 3400, 1715 cm^{-1}. NMR δ: 1.43 (3H, t, $\mathrm{COOCH_2C\underline{H}_3}$), 1.45 (3H, d, $J=7.1$ Hz, *sec*-Me), 3.36—3.44 (1H, m, methine), 3.88—3.93 (2H, m, $\mathrm{C\underline{H}_2OH}$), 4.45 (2H, q, $\mathrm{COOC\underline{H}_2CH_3}$), 8.09, 8.17 (each 1H, s, 5- and 5′-H). (±)-**17**: pale yellow oil. *Anal.* HRMS Calcd for $\mathrm{C_{12}H_{14}N_2O_3S_2}$ ($\mathrm{M^+}$) *m/z*: 298.0446. Found: 298.0436. IR($\mathrm{CHCl_3}$): 3360, 1715 cm^{-1}. NMR δ: 1.29 (3H, t, $\mathrm{COOCH_2C\underline{H}_3}$), 1.70 (3H, d, $J=7.3$ Hz, *sec*-Me), 4.23 (2H, q, $\mathrm{COOC\underline{H}_2CH_3}$), 4.45 (1H, q, $J=7.3$ Hz, methine), 4.81 (2H, s, $\mathrm{C\underline{H}_2OH}$), 7.21, 7.79 (each 1H, s, 5- and 5′-H). (±)-**3**: recrystallization from AcOEt–*n*-hexane afforded colorless needles, mp 98—98.5 °C. *Anal.* Calcd for $\mathrm{C_{10}H_{12}N_2O_2S_2}$: C, 46.85; H, 4.72; N, 10.93. Found: C, 46.67; H, 4.74; N, 10.86. IR (Nujol): 3330 cm^{-1}. NMR δ: 1.44 (3H, d, $J=7.1$ Hz, *sec*-Me), 3.35—3.43 (1H, m, methine), 3.86—3.96 (2H, m, $\mathrm{C\underline{H}_2OH}$), 4.81 (2H, q, 4-$\mathrm{C\underline{H}_2OH}$), 7.20, 7.86 (each 1H, s, 5- and 5′-H).

(±) 4-Acetoxymethyl-2′-(1-acetoxymethylethyl)-2,4′-bithiazole (18) A mixture of (±)-**3** (1.03 g) and $\mathrm{Ac_2O}$ (1.7 g, 4 eq) in pyridine (10 ml) was stirred for 2 h at room temperature. The reaction mixture was diluted with $\mathrm{H_2O}$ and extracted with AcOEt. The AcOEt layer was washed with 10% HCl aqueous solution and then saturated $\mathrm{NaHCO_3}$ aqueous solution. Evaporation of the organic solvent gave an oily product, which was chromatographed on silica gel (50 g) to afford (±)-**18** (1.294 g, 94% yield) from the 30% AcOEt in *n*-hexane eluate. A part of (±)-**18** was recrystallized from AcOEt-*n*-hexane to afford colorless needles. (±)-**18**: mp 59.5—60.5 °C. *Anal.* Calcd for $\mathrm{C_{14}H_{16}N_2O_4S_2}$: C, 49.40; H, 4.74; N, 8.23. Found: C, 49.38; H, 4.85; N, 8.12. IR (Nujol): 1725 cm^{-1}. NMR ($\mathrm{CD_3OD}$) δ: 1.47 (3H, d, $J=7.1$ Hz, *sec*-Me), 2.02, 2.11 (each 3H, s, $\mathrm{OAc}\times 2$), 3.57—3.64 (1H, m, methine), 4.36 (2H, dq, $\mathrm{C\underline{H}_2OAc}$), 5.21 (2H, s, 4-$\mathrm{C\underline{H}_2OAc}$), 7.55, 8.05 (each 1H, s, 5- and 5′-H).

(±) 4-Acetoxymethyl-2′-(1-hydroxymethylethyl)-2,4′-bithiazole (19)

and (±) 2′-(1-Acetoxymethylethyl)-4-hydroxymethyl-2,4′-bithiazole (20) A mixture of (±)-**3** (1.495 g) and Ac_2O (0.6 g, 1.1 eq) in pyridine (20 ml) was stirred for 90 min at room temperature. The reaction mixture was worked up in the same way as described for the preparation of (±)-**18** to give an oily product, which was chromatographed on silica gel (30 g) to provide, in order of elution, the diacetate (±)-**18** (278 mg, 14% yield) from the 30% AcOEt in *n*-hexane eluate, the monoacetate (±)-**19** (539 mg, 31% yield) from the 40% AcOEt in *n*-hexane eluate, the monoacetate (±)-**20** (348 mg, 20% yield) from the AcOEt eluate, and the recovered diol (±)-**3** (508 mg, 34% yield) from the 50% AcOEt in MeOH eluate. (±)-**19**: crystallization from AcOEt–*n*-hexane afforded colorless needles, mp 97.5—98 °C. *Anal.* Calcd for $C_{12}H_{14}N_2O_3S_2$: C, 48.30; H, 4.73; N, 9.39. Found: C, 48.21; H, 4.68; N, 9.35. IR (Nujol): 3380, 1730 cm^{-1}. NMR δ: 1.45 (3H, d, J=7.3 Hz, *sec*-Me), 2.14 (3H, s, OAc), 3.38—3.42 (1H, m, methine), 3.85—3.98 (2H, m, $C\underline{H}_2OH$), 5.24 (2H, s, 4-$C\underline{H}_2OAc$), 7.31, 7.90 (each 1H, s, 5- and 5′-H). (±)-**20**: crystallization from AcOEt–*n*-hexane afforded colorless needles, mp 76—76.5 °C. *Anal.* Calcd for $C_{12}H_{14}N_2O_3S_2$: C, 48.30; H, 4.73; N, 9.39. Found: C, 48.22; H, 4.80; N, 9.34. NMR δ: 1.48 (3H, d, J=7.3 Hz, *sec*-Me), 2.06 (3H, s, OAc), 3.55—3.63 (1H, m, methine), 4.36 (2H, dq, J=6.1, 6.8, 10.9 Hz, $C\underline{H}_2OAc$), 4.81 (2H, s, 4-$C\underline{H}_2OH$), 7.21, 7.90 (each 1H, s, 5- and 5′-H).

(±) 2′-(1-Acetoxymethylethyl)-4-ethoxycarbonyl-2,4′-bithiazole (21) A mixture of (±)-**16** (1.48 g) and Ac_2O (2.3 g, 4 eq) in pyridine (7 ml) was stirred for 12 h at room temperature. The reaction mixture was worked up in the same way as described for the preparation of (±)-**18** to give an oily product, which was chromatographed on silica gel (50 g) to afford a pale yellow oil (±)-**21** (1.682 g, 98% yield) from the 30% AcOEt in *n*-hexane eluate. (±)-**21**: *Anal.* HRMS Calcd for $C_{14}H_{16}N_2O_4S_2$: 340.0552. Found: 340.0553. IR (Nujol): 1715 cm^{-1}. NMR δ: 1.43 (3H, t, $COOCH_2C\underline{H}_3$), 1.49 (3H, d, J=6.8 Hz, *sec*-Me), 2.07 (3H, s, OAc), 3.54—3.63 (1H, m, methine), 4.37 (2H, dq, J=7, 6, 11 Hz, $C\underline{H}_2OAc$), 4.48 (2H, q, $COOC\underline{H}_2CH_3$), 8.08, 8.17 (each 1H, s, 5- and 5′-H).

HPLC Analysis of Three Racemates ((±)-3, (±)-16 and (±)-21) by Using a Chiral Column Three kinds of racemates were analyzed individually to provide well separated peaks ((±)-**3**, 29.82 and 33.61 min; (±)-**16**, 30.04 and 44.46 min; (±)-**21**; 29.08 and 37.13 min) of each enantiomer under the following analytical conditions (eluent, *n*-hexane–EtOH (10 : 1); detection, UV at 296 nm; flow rate, 1.0 ml/min). The assignment of these peaks was achieved by comparison with those of the reported sample (*S*)-**3**.[3] Namely, the peak with shorter retention time (t_R=29.82 min) was found to correspond to that of the (*S*)-**3** enantiomer and the peak with longer retention time (t_R=33.61 min) to that of the (*R*)-**3** enantiomer.

General Procedure of Enantioselective Hydrolysis A mixture of the substrate (*ca.* 100 mg) and lipase (100 mg) in water-saturated (iso-Pr)$_2$O (20 ml) was shaken at 33 °C for an adequate time. When spots of the starting material and product on thin-layer chromatography (TLC) showed an approximately 1 : 1 ratio, the reaction mixture was filtered with the aid of Celite. The filter bed was washed with AcOEt and the combined filtrate was evaporated to give a residue. Separation of the residue by column chromatography on silica gel afforded the recovered acetates and alcohols, respectively. For the purpose of determining the optical purity and the stereochemistry, a small amount of the acetates (*ca.* 5 mg) was deacetylated with K_2CO_3 (*ca.* 5 mg) in MeOH (0.1 ml) at room temperature to provide the corresponding alcohols. The hydrolyzed products and alcohols derived from acetates were individually analyzed by HPLC. In the case of the determination of the stereochemistry of (*S*)-**16**, a solution of (*S*)-**16** (19 mg) in MeOH (0.5 ml) was treated with $LiBH_4$ (10 mg) to give the (*S*)-diol **3** (16 mg), which was analyzed by HPLC. The results are summarized in Table I. (*R*)-**18**: $[\alpha]_D^{24}$ +3.25° (c=1.23, $CHCl_3$); corresponds to 48% ee (entry 1), (*S*)-**19**: $[\alpha]_D^{24}$ +6.54° (c=1.07, $CHCl_3$); corresponds to 73% ee (entry 3), (*R*)-**20**: $[\alpha]_D^{24}$ −1.58° (c=1.64, $CHCl_3$); corresponds to 60% ee (entry 3), (*S*)-**3**: $[\alpha]_D^{22}$ +4.74° (c=0.59, $CHCl_3$); corresponds to 87% ee (entry 3), (*R*)-**21**: $[\alpha]_D^{21}$ +5.0° (c=2.0, $CHCl_3$); corresponds to 81% ee (entry 12), (*S*)-16: $[\alpha]_D^{21}$ +4.36° (c=1.1, $CHCl_3$); corresponds to 91% ee (entry 12).

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References and Notes

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