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Total synthesis of (4*R*,5*S*,6*E*,14*S*)- and (4*R*,5*S*,6*E*,14*R*)-cystothiazoles F

Hiroyuki Akita,[a,*] Yuki Iwaki,[a,b] Keisuke Kato,[a] Jianhua Qi[c] and Makoto Ojika[c]

[a]*School of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan*
[b]*Research Institute, Novartis Pharma K.K., 8 Ohkubo, Tsukuba, Ibaraki 300-2611, Japan*
[c]*Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan*

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Abstract—Total synthesis of (4*R*,5*S*,6*E*,14*S*)- and (4*R*,5*S*,6*E*,14*R*)-cystothiazoles F **3** was achieved from the chiral bithiazole-type primary alcohols [(*S*)- and (*R*)-4-ethoxycarbonyl-2′-(1-hydroxymethylethyl)-2,4′-bithiazoles **8**], which were obtained based on the enzymatic resolution of racemic alcohol **8** and its acetate **9**. From a direct comparison by means of chiral HPLC between natural cystothiazole F **3** and synthetic compounds [(4*R*,5*S*,6*E*,14*S*)- and (4*R*,5*S*,6*E*,14*R*)-cystothiazoles **3**], natural cystothiazole F **3** was found to be a 33:67 diastereomeric mixture [(4*R*,5*S*,6*E*,14*S*)-**3**:(4*R*,5*S*,6*E*,14*R*)-**3** = 33:67].

1. Introduction

Antifungal substances known as cystothiazoles A **1** and B **2** were isolated from the myxobacterium *Cystobacter fuscus* strain AJ-13278 by using an inhibition assay against the phytopathogenic fungus *Phytophthora capsici*.[1] A further study of the large-scale culture of this strain resulted in the isolation of additional bithiazole-type antibiotics, cystothiazole F **3**[2] and melithiazol B **4**.[3] The synthesis of cystothiazoles A **1**,[4] B **2**,[4f,5] and melithiazol B **3**[3b] has already been reported by some research groups including our own. The structure of cystothiazole F **3** was deduced to be 15-hydroxycystothiazole A based on the spectral analysis. The relative stereochemistry (4*R**,5*S**) could be the same as that of **1** because of the similar ^{1}H NMR data (H-4 and H-5),[2] while the absolute configuration of the C(14)-carbon of **3** was not determined. Herein we report the determination of the absolute structure of cystothiazole F **3** based on the total synthesis of **3** (Scheme 1).

2. Results and discussion

Retrosynthetically, the synthesis of **3** can be achieved by Wittig condensation of the left-half aldehyde **A**, **5**, and the right-half chiral phosphonium iodide **B**. The synthesis of chiral aldehyde (+)-**5** was achieved in the total synthesis of cystothiazole A **1**.[4b,d] For the synthesis of **B**, (*S*)-**6** or (*R*)-**6**, the lipase-catalysed optical resolution of alcohol (±)-**8** or acetate (±)-**9** is thought to be the most effective method. The synthesis of substrate (±)-**8** or (±)-**9** for the enzymatic reaction is shown in Scheme 2.

Hydroboration of the reported *exo*-olefin **7**[3b] gave a primary alcohol (±)-**8** (82% yield), which was subjected to acetylation to afford the corresponding acetate (±)-**9** (93% yield). Initially, (±)-**8** was subjected to screening experiments using several types of commercially available lipases. Among them, lipase 'Godo E-1' from *Pseudomonas* sp. was found to be effective. When (±)-**8** was subjected to enantioselective acetylation using 'Godo E-1' in the presence of isopropenyl acetate as acylating reagent for 1 day, acetate (*S*)-**9** {47%, $[\alpha]_D^{25} = -4.6$ (*c* 1.28, $CHCl_3$); corresponding to 83% ee} and unchanged alcohol (*R*)-**8** {50%, $[\alpha]_D^{25} = -3.7$ (*c* 1.03, $CHCl_3$); corresponding to 74% ee} were obtained (Table 1, entry 1). The enantiomeric excess (ee) was calculated by means of HPLC analysis. The *E*-value[6] of this enzymatic reaction was estimated to be 23.8. The absolute structures of enzymatic reaction products (*S*)-**9** and (*R*)-**8** were determined by the direct comparison of the reported samples[7] (*R*)-**9** {$[\alpha]_D^{21} = +5.0$ (*c* 2.0, $CHCl_3$); corresponding to 81% ee} and (*S*)-**8** {$[\alpha]_D^{21} = +4.4$ (*c* 1.1, $CHCl_3$); corresponding to 91% ee}. When this reaction was carried out for a prolonged period (2 days), (*S*)-**9** (70%, 37% ee) and (*R*)-**8** (28%, 99% ee) were

* Corresponding author. Tel.: +81 047 472 1805; fax: +81 047 476 6195; e-mail: akita@phar.toho-u.ac.jp

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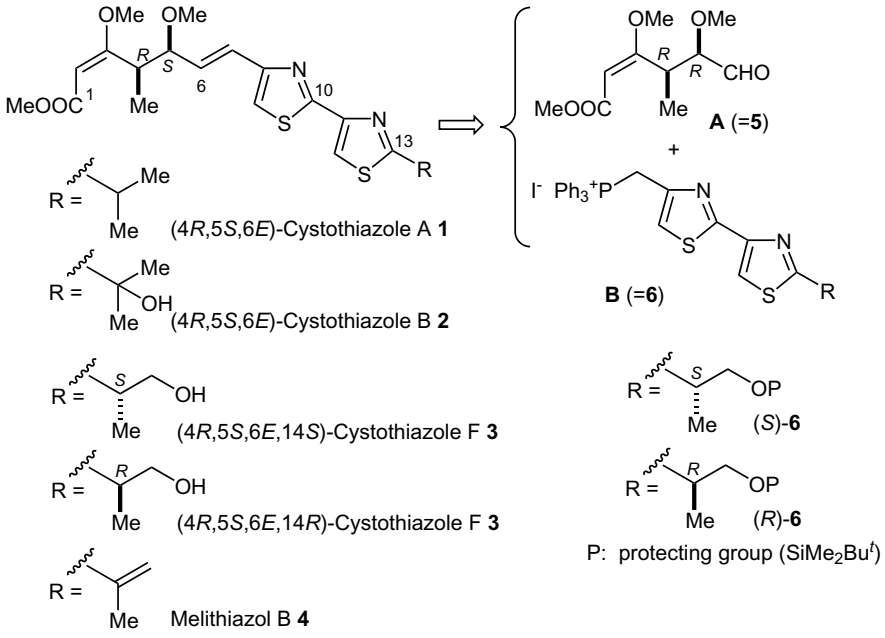
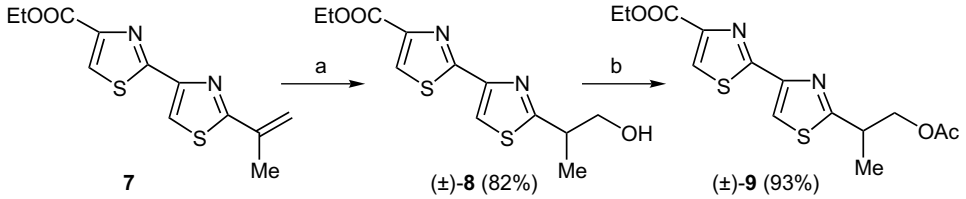
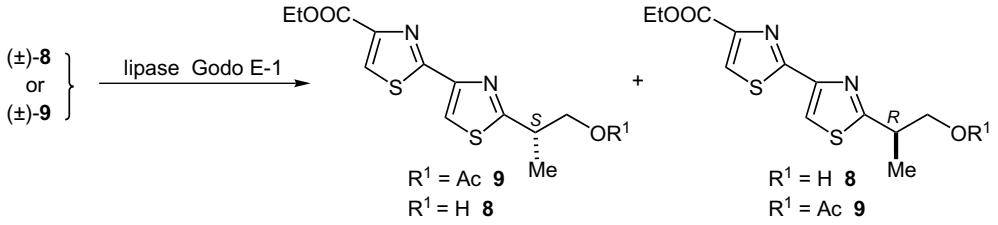


Scheme 1.



Scheme 2. Reagents and conditions: (a) (1) BH_3/THF, (2) 30% H_2O_2/NaOH/H_2O_2; (b) Ac_2O/pyridine.

Table 1.

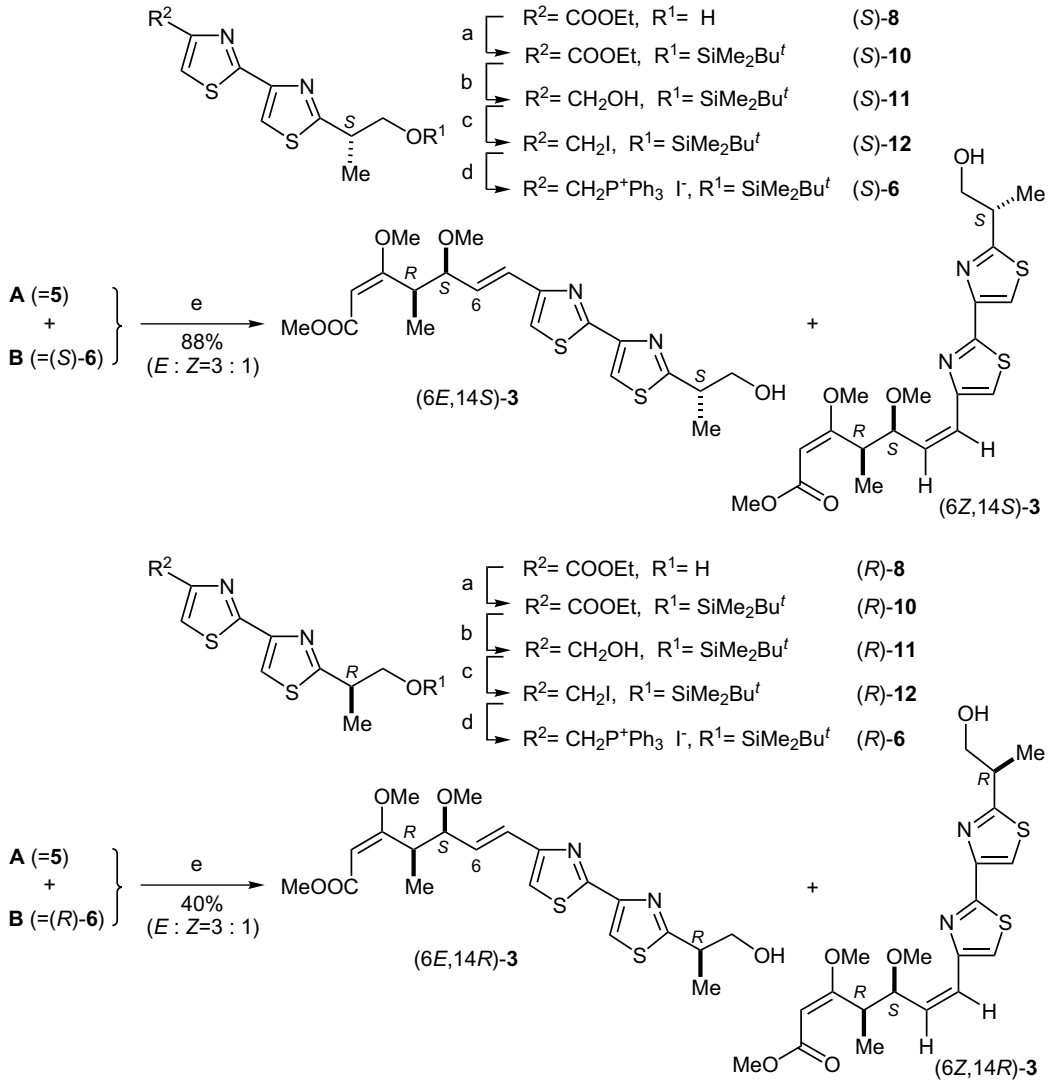


Entry	Substrate (g)	Acylating reagent	Solvent	Time (d)	Products % (% ee)	
1	(±)-**8** (0.894)	Isopropenyl acetate	*i*-Pr_2O	1	(*S*)-**9** 47 (83)	(*R*)-**8** 50 (74)
2	(±)-**8** (1.500)	Isopropenyl acetate	*i*-Pr_2O	2	(*S*)-**9** 70 (37)	(*R*)-**8** 28 (99)
3	(±)-**9** (1.299)		*i*-Pr_2O/H_2O	1	(*R*)-**9** 62 (59)	(*S*)-**8** 37 (99)
4	(±)-**9** (1.198, 37% ee)		*i*-Pr_2O/H_2O	1	(*R*)-**9** 44 (16)	(*S*)-**8** 50 (98)

obtained (Table 1, entry 2). On the other hand, asymmetric hydrolysis of (±)-**9** for 1 day gave (*S*)-**8** (37%, 99% ee) and (*R*)-**9** (62%, 59% ee) (Table 1, entry 3). Acetate (*S*)-**9** with 37% ee was again subjected to enantioselective hydrolysis to afford (*S*)-**8** (50%, 98% ee) and (*R*)-**9** (44%, 16% ee) (Table 1, entry 4).

The synthesis of (4*R*,5*S*,6*E*,14*S*)- and (4*R*,5*S*,6*E*,14*R*)-cystothiazoles **3** from the enzymatic productions, (*S*)- and (*R*)-**8**, respectively, is shown in Scheme 3.

Protection of the hydroxyl group of (*S*)-**8** as a silyl ether group followed by reduction with $LiBH_4$ gave an (*S*)-alcohol **11** in 90% overall yield. Treatment of (*S*)-**11** with I_2/triphenylphosphine/imidazole provided iodide (*S*)-**12** (89% yield), which was treated with Ph_3P to give phosphonium salt (*S*)-**6** in 85% yield. Condensation of (*S*)-**6** with (+)-**5** in the presence of lithium bis(trimethylsilyl)amide in THF afforded (4*R*,5*S*,6*Z*,14*S*)-**3** {0.065 g, 22%, $[\alpha]_D^{26} = +206.0$ (*c* 1.36, $CHCl_3$)} and (4*R*,5*S*,6*E*,14*S*)-**3** {0.196 g, 66%, $[\alpha]_D^{26} = +86.2$ (*c* 1.05, $CHCl_3$)}. The synthesis of (4*R*,5*S*,



Scheme 3. Reagents and conditions: (a) *t*-BuMe$_2$SiCl/imidazole/DMF; (b) LiBH$_4$/THF; (c) I$_2$/Ph$_3$P/imidazole; (d) Ph$_3$P/benzene; (e) (1) $Li^+N^-(SiMe_3)_2$/THF, (2) $Bu_4N^+F^-$/THF.

6*Z*,14*R*)-**3** {$[\alpha]_D^{27} = +208.8$ (*c* 0.3, $CHCl_3$)} and (4*R*,5*S*, 6*E*,14*R*)-**3** {$[\alpha]_D^{26} = +94.4$ (*c* 0.78, $CHCl_3$)} from (*R*)-**8** was carried out in the same way as for the synthesis of (4*R*,5*S*,6*Z*,14*S*)-**3** and (4*R*,5*S*,6*E*,14*S*)-**3**. The NMR data (^{1}H and ^{13}C NMR) of the synthetic (4*R*,5*S*,6*E*,14*S*)- and (4*R*,5*S*,6*E*,14*R*)-cystothiazoles F **3** were found to be quite similar to those of natural product cystothiazole F **3**.[2] Meanwhile, the sign of the specific rotation of natural **3** {$[\alpha]_D^{23} = +77.0$ (*c* 0.074, $CHCl_3$)}[2] was the same as that of the synthetic (4*R*,5*S*,6*E*,14*S*)-**3** and (4*R*,5*S*,6*E*,14*R*)-**3**, respectively. At this stage, identification of natural cystothiazole F **3** and synthetic **3** seemed to be difficult and direct comparison by means of chiral HPLC analysis was carried out. The result is shown in Figure 1, and natural cystothiazole F **3** was found to be a 33:67 diastereomeric mixture [(4*R*,5*S*,6*E*,14*S*)-**3**:(4*R*,5*S*,6*E*,14*R*)-**3** = 33:67].

The antifungal activities of the synthetic (4*R*,5*S*,6*E*,14*S*)-**3** and (4*R*,5*S*,6*E*,14*R*)-**3** against the phytopathogenic fungus *P. capsici* were evaluated by using a paper disc assay method as reported previously.[2] The minimum dose applied on a paper disc to inhibit the fungal growth was 1 μg/disc. The synthetic (4*R*,5*S*,6*E*,14*S*)-**3** and (4*R*,5*S*,6*E*,14*R*)-**3** also showed the activities at a similar level of dosage in comparison to that of natural **3**. On the other hand, 6(*Z*)-isomers [(4*R*,5*S*,6*Z*,14*S*)-**3** and (4*R*,5*S*,6*Z*,14*R*)-**3**] did not indicate antifungal activity. The generation of cystothiazoles B **2** and F **3** might be explained by the enzyme-assisted and non-selective oxidation of the isopropyl group of cystothiazole A **1**. Actually, the bioconversion of **1** by the antibiotic producer, *C. fuscus*, without the absorbent resin gave a number of polar metabolic derivatives, which correspond to the oxidative products including cystothiazole B **2** and 14,15-dihydroxy cystothiazole A analogues. In the case of 14,15-dihydroxy cystothiazole A analogues, the major isomer was accompanied by a minor isomer, suggesting that one of the two methyl groups preferentially hydroxylated.[8]

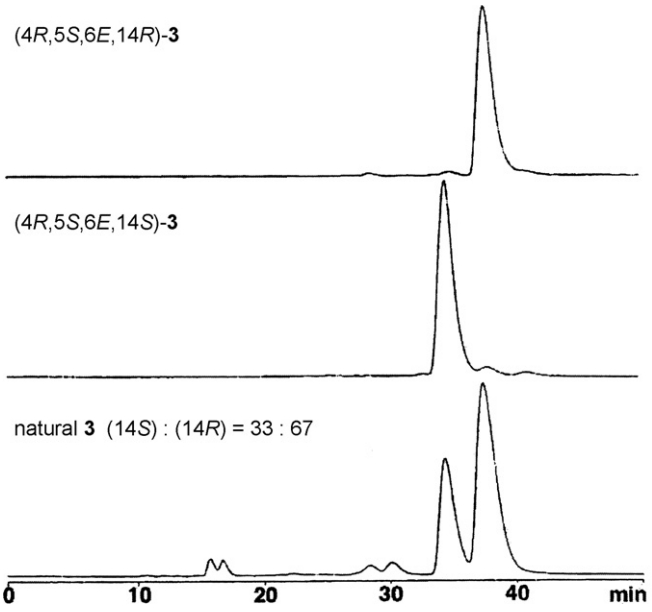


Figure 1. Chiral HPLC analysis of cystothiazole F (**3**). Conditions: column: CHIRALCEL OD (4.6 × 250 mm); solvent: hexane–*i*-PrOH (9:1); flow rate: 0.5 ml/min; detection: 310 nm, sample: 10 μg.

3. Conclusion

In conclusion, the total synthesis of (4*R*,5*S*,6*E*,14*S*)- and (4*R*,5*S*,6*E*,14*R*)-cystothiazoles F **3** was achieved from the chiral bithiazole-type primary alcohols (*S*)- and (*R*)-**8**, which were obtained based on the enzymatic resolution of racemic alcohol **8** and its acetate **9**. From a direct comparison by means of chiral HPLC between natural cystothiazole F **3** and synthetic compounds, [(4*R*,5*S*,6*E*,14*S*)- and (4*R*,5*S*,6*E*,14*R*)-cystothiazoles **3**], natural cystothiazole F **3** was found to be a 33:67 diastereomeric mixture [(4*R*,5*S*,6*E*,14*S*)-**3**:(4*R*,5*S*,6*E*,14*R*)-**3** = 33:67].

4. Experimental

4.1. General

All melting points were measured on a Yanaco MP-3S micro-melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL AL 400 spectrometer in $CDCl_3$. High-resolution mass spectra (HRMS) and the fast atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS 600H spectrometer. IR spectra were recorded with a JASCO FT/IR-300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

4.2. (±)-4-Ethoxycarbonyl-2′-(1-hydroxymethylethyl)-2,4′-bithiazole 8

To a solution of **7** (0.2 g, 0.71 mmol) in THF (10 ml) was added 1 M BH_3·THF (2.1 ml, 2.1 mmol) at 0 °C under an argon atmosphere and the whole mixture was stirred for 4 h at the same temperature. Aqueous NaOH (5%, 1 ml) and 30% H_2O_2 solution were added to the reaction mixture and the whole mixture was stirred for 15 min at the same temperature. The reaction mixture was diluted with brine and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was chromatographed on silica gel (5 g, *n*-hexane–AcOEt = 1:1) to afford (±)-**8** (0.175 g, 82%). Recrystallisation of (±)-**8** from AcOEt provided pale yellow needles. (±)-**8**: mp 76–76.5 °C; IR (Nujol): 3400, 1715 cm^{-1}; ^{1}H NMR (400 MHz): δ 1.43 (3H, t, $J = 7.2$ Hz), 1.45 (3H, d, $J = 7.1$ Hz), 3.36–3.44 (1H, m), 3.88–3.93 (2H, m), 4.45 (2H, q, $J = 7.2$ Hz), 8.09 (1H, s), 8.17 (1H, s). Anal. Calcd for $C_{12}H_{14}N_2O_3S_2$: C, 48.30; H, 4.73; N, 9.39. Found: C, 48.01; H, 4.87; N, 9.24.

4.3. (±)-2′-(1-Acetoxymethylethyl)-4-ethoxycarbonyl-2,4′-bithiazole 9

A mixture of (±)-**8** (0.99 g, 3.3 mmol) and Ac_2O (1.35 g, 13.2 mmol) in pyridine (5 ml) was stirred for 15 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The AcOEt layer was washed with 10% HCl aqueous solution and then saturated $NaHCO_3$ aqueous solution. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was chromatographed on silica gel (20 g, *n*-hexane–AcOEt = 6:1) to afford (±)-**9** (1.12 g, 99%) as a pale yellow oil. Compound (±)-**9**: IR (Nujol): 1715 cm^{-1}; ^{1}H NMR (400 MHz): δ 1.43 (3H, t), 1.49 (3H, d, $J = 6.8$ Hz), 2.07 (3H, s), 3.54–3.63 (1H, m), 4.37 (2H, ddd, $J = 7$, 6, 11 Hz), 4.48 (2H, q), 8.08 (1H, s), 8.17 (1H, s). HRMS (*m*/*z*): calcd for $C_{14}H_{16}N_2O_4S_2$: 340.0552 (M^+). Found: 340.0553.

4.4. HPLC analysis of the racemic alcohol (±)-8 and acetate (±)-9 by using a chiral column

Two racemates (±)-**8** and (±)-**9** individually gave two well separated peaks. Compound (±)-**8**; 25.2 and 38.4 min corresponding to each enantiomer under the following analytical conditions (column, CHIRALCEL OD (250 × 4.6 mm); eluent, *n*-hexane–EtOH = 10:1; detection, UV at 296 nm; flow rate, 1 ml/min). Compound (±)-**9**; 24.4 and 32.0 min corresponding to each enantiomer under the following analytical conditions (column, CHIRALCEL OD (250 × 4.6 mm); eluent, *n*-hexane–EtOH = 10:1; detection, UV at 296 nm; flow rate, 1 ml/min).

4.5. Enzymatic resolution

(1) Table 1, entry 1: A mixture of (±)-**8** (0.894 g, 3.0 mmol), isopropenyl acetate (1.5 g, 15 mmol) and lipase 'Godo-1' (0.9 g) in diisopropyl ether (180 ml) was stirred at 33 °C for 1 d. The reaction mixture was filtered with the aid of Celite and the precipitate was washed with EtOAc. The combined organic solvent was evaporated to give a residue, which was chromatographed on silica gel (10 g, *n*-hexane–EtOAc = 3:1) to afford (*S*)-**9** [0.479 g, 47%, $[\alpha]_D^{25} = -4.6$ (*c* 1.28, $CHCl_3$); corresponding to 83% ee, (*S*)-**9** ($t_R = 24.4$ min): (*R*)-**9** ($t_R = 32.0$ min) = 91.5:7.5] and (*R*)-**8** [0.447 g, 50%, $[\alpha]_D^{25} = -3.7$ (*c* 1.03, $CHCl_3$); corre-

sponding to 74% ee, (*R*)-**8** (t_R = 38.4 min): (*S*)-**8** (t_R = 25.2 min) = 87:13] in elution order. Enantiomeric excess (ee) of (*R*)-**8** and (*S*)-**9** was analysed by HPLC. (2) Table 1, entry 2: A mixture of (±)-**8** (1.5 g, 5.0 mmol), isopropenyl acetate (2.5 g, 25 mmol) and lipase 'Godo-1' (1.5 g) in diisopropyl ether (300 ml) was stirred at 33 °C for 2 days. The reaction mixture was worked up in the same way as for (1) to give (*S*)-**9** (1.198 g, 70%, 37% ee) and (*R*)-**7** (0.420 g, 28%, 99% ee). (3) Table 1, entry 3: A mixture of (±)-**9** (1.299 g, 3.8 mmol) and lipase 'Godo-1' (1.3 g) in H_2O–saturated diisopropyl ether (240 ml) was stirred at 33 °C for 1 d. The reaction mixture was worked up in the same way as for (1) to give (*R*)-**9** (0.805 g, 62%, 59% ee) and (*S*)-**8** (0.421 g, 37%, 99% ee). (4) Table 1, entry 4: A mixture of 37% ee of (*S*)-**9** (1.198 g, 3.5 mmol) and lipase 'Godo-1' (1.2 g) in H_2O–saturated diisopropyl ether (240 ml) was stirred at 33 °C for 1 day. The reaction mixture was worked up in the same way as for (1) to give (*R*)-**9** (0.527 g, 44%, 16% ee) and (*S*)-**8** (0.525 g, 50%, 98% ee).

4.6. (*S*)-2′-(1-^{t}Butyldimethylsiloxymethylethyl)-4-hydroxymethyl-2,4′-bithiazole 11

(i) A solution of (*S*)-**8** (0.796 g, 2.27 mmol), imidazole (0.326 g, 5.32 mmol) and ^{t}butyldimethylsilyl chloride (TBDMSCl, 0.603 g, 4.0 mmol) in DMF (4 ml) was stirred for 2 h at room temperature. The reaction mixture was diluted with brine and extracted with *n*-hexane. The organic layer was dried over $MgSO_4$ and evaporated to give crude (*S*)-**10**, which was used for the next reaction without further purification. (ii) A mixture of crude (*S*)-**10** and $LiBH_4$ (0.232 g, 10.6 mmol) in THF (5 ml) was stirred for 1 h at room temperature. The reaction mixture was diluted with brine and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give a residue, which was chromatographed on silica gel (30 g, *n*-hexane–AcOEt = 5:1) to afford (*S*)-**11** (0.891 g, 90% from (*S*)-**8**) as a pale yellow oil. Compound (*S*)-**11**: $[\alpha]_D^{28}$ = +3.45 (*c* 1.05, $CHCl_3$); IR (neat): 3322 cm^{-1}; ^{1}H NMR (400 MHz): δ 0.012 (3H, s), 0.016 (3H, s), 0.88 (9H, s), 1.43 (3H, d, *J* = 6.8 Hz), 3.01 (1H, s), 3.41 (1H, dq, *J* = 6.8, 5.6 Hz), 3.85 (2H, dq, *J* = 9.6, 5.6 Hz), 4.82 (2H, s), 7.19 (1H, s), 7.85 (1H, s). ^{13}C NMR (100 MHz): δ −5.50 (2C), 17.34, 18.26, 25.86 (3C), 41.17, 60.99, 67.22, 115.10, 115.59, 148.23, 157.09, 163.79, 174.60. MS (FAB) *m*/*z*: 371 (M^++1).

4.7. (*S*)-2′-(1-^{t}Butyldimethylsiloxymethylethyl)-4-iodomethyl-2,4′-bithiazole 12

To a mixture of (*S*)-**11** (0.852 g, 2.3 mmol), triphenylphosphine (0.905 g, 3.45 mmol) and imidazole (0.313 g, 4.6 mmol) in THF (5 ml) was added I_2 (0.876 g, 3.45 mmol) under an argon atmosphere at 0 °C and the whole mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 1% aqueous $Na_2S_2O_3$ and brine, and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (20 g, *n*-hexane–AcOEt = 10:1) to afford (*S*)-**12** (0.985 g, 89%) as a pale yellow oil. Compound (*S*)-**12**: $[\alpha]_D^{23}$ = +2.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz): δ 0.02 (6H, s), 0.88 (9H, s), 1.43 (3H, d, *J* = 6.4 Hz), 3.40 (1H, dq, *J* = 6.4, 6.0 Hz), 3.84 (2H, dq, *J* = 9.6, 6.0 Hz), 4.56 (2H, s), 7.25 (1H, s), 7.87 (1H, s). ^{13}C NMR (100 MHz): δ −5.53 (2C), −1.44, 17.32, 18.23, 25.83 (3C), 41.12, 67.17, 115.86, 116.61, 148.04, 153.89, 163.34, 174.51. MS (FAB) *m*/*z*: 353 (M^+−I).

4.8. (*S*)-2′-(1-^{t}Butyldimethylsiloxymethylethyl)-2,4′-bithiazolyl-4-methylenetriphenyl phosphonium iodide 6

A mixture of (*S*)-**12** (0.934 g, 1.95 mmol) and triphenylphosphine (0.612 g, 2.33 mmol) in benzene (15 ml) was stirred for 20 h at reflux. After cooling, the resulting colorless powder (*S*)-**6** (1.23 g, 85%) was obtained by filtration. Compound (*S*)-**6**: ^{1}H NMR (400 MHz): δ −0.002 (6H, s), 0.87 (9H, s), 1.43 (3H, d, *J* = 7.2 Hz), 3.35 (1H, dq, *J* = 6.8, 6.0 Hz), 3.81 (2H, dq, *J* = 9.6, 6.0 Hz), 5.54 (2H, d, *J* = 13.6 Hz), 7.36 (1H, s), 7.62–7.86 (15H, m), 8.11 (1H, s). MS (FAB) *m*/*z*: 615 (M^+−I).

4.9. (6*E*,14*S*)- and (6*Z*,14*S*)-Cystothiazoles F 3

(i) To a solution of (*S*)-**6** (1.0 g, 1.35 mmol) in THF (5 ml) was added lithium bis(trimethylisilyl)amide (1 M solution in THF, 1.35 ml, 1.35 mmol) at 0 °C under an argon atmosphere and the whole mixture was stirred for 20 min at the same temperature. A solution of (+)-**5** (0.145 g, 0.67 mmol) in THF (2 ml) was added to the above reaction mixture at 0 °C and the whole mixture was stirred for 15 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to afford a crude 3:1 diastereomeric mixture, which was used for the next reaction without further purification. (ii) To a solution of the above mixture in THF (4 ml) was added 1 M tetrabutylammonium fluoride ($Bu_4N^+F^-$) solution (0.68 ml, 0.68 mmol) at 0 °C and the whole mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to afford a crude product, which was chromatographed on silica gel (10 g, *n*-hexane–AcOEt = 10:1) to give (6*Z*,14*S*)-**3** (0.065 g, 22%, colorless oil) and (6*E*,14*S*)-**3** (0.196 g, 66%, colorless oil) in elution order. Compound (6*E*,14*S*)-**3**: $[\alpha]_D^{26}$ = +86.2 (*c* 1.05, $CHCl_3$); IR (neat): 3436, 1706, 1622 cm^{-1}; ^{1}H NMR (400 MHz): δ 1.21 (3H, d, *J* = 7.2 Hz), 1.43 (3H, d, *J* = 6.8 Hz), 3.32 (3H, s), 3.38 (1H, dq, *J* = 7.2, 6.8 Hz), 3.59 (3H, s), 3.65 (3H, s), 3.80 (1H, dd, *J* = 8.0, 7.6 Hz), 3.87 (1H, dd, *J* = 10.8, 7.2 Hz), 3.92 (1H, dd, *J* = 10.8, 6.8 Hz), 4.16 (1H, dq, *J* = 8.0, 7.2 Hz), 4.96 (1H, s), 6.41 (1H, dd, *J* = 15.8, 7.6 Hz), 6.56 (1H, d, *J* = 15.8 Hz), 7.08 (1H, s), 7.89 (1H, s). ^{13}C NMR (100 MHz): δ 14.04, 17.31, 39.78, 40.18, 50.77, 55.49, 56.98, 66.83, 84.33, 91.06, 115.12, 115.15, 125.37, 131.80, 148.72, 154.51, 161.96, 167.70, 175.34, 176.68. HRMS (*m*/*z*): calcd for $C_{20}H_{27}N_2O_5S_2$: 439.1362 (M^++1). Found: 439.1350. Compound (6*Z*,14*S*)-**3**: $[\alpha]_D^{26}$ = +206.0 (*c* 1.36, $CHCl_3$); IR (neat): 3427, 1707, 1622 cm^{-1}; ^{1}H NMR (400 MHz): δ 1.25 (3H, d, *J* = 6.8 Hz), 1.45 (3H, d, *J* = 7.2 Hz), 3.32 (3H, s), 3.34 (3H, s), 3.40 (1H, dq, *J* = 7.2, 4.4 Hz), 3.66 (3H, s), 3.89 (1H, dd, *J* = 10.8, 7.2 Hz), 3.94 (1H, dd, *J* = 10.8, 4.4 Hz), 4.22 (1H, dq, *J* = 9.0, 6.8 Hz), 4.92 (1H,

s), 5.07 (1H, t, $J = 9.0$ Hz), 5.60 (1H, dd, $J = 12.0$, 10.0 Hz), 6.57 (1H, d, $J = 12.0$ Hz), 7.23 (1H, s), 7.87 (1H, s). ^{13}C NMR (100 MHz): δ 14.72, 17.34, 39.22, 40.21, 50.77, 55.05, 56.23, 66.86, 78.50, 91.08, 114.89, 117.94, 125.26, 132.79, 148.85, 153.64, 161.04, 167.77, 175.55, 176.51. HRMS (*m*/*z*): calcd for $C_{20}H_{27}N_2O_5S_2$: 439.1362 (M^++1). Found: 439.1345.

4.10. (*R*)-2′-(1-^{t}Butyldimethylsiloxymethylethyl)-4-hydroxymethyl-2,4′-bithiazole 11

(i) A solution of (*R*)-**8** (0.452 g, 1.52 mmol), imidazole (0.206 g, 3.03 mmol) and ^{t}butyldimethylsilyl chloride (TBDMSCl, 0.0.343 g, 2.28 mmol) in DMF (2 ml) was stirred for 2 h at room temperature. The reaction mixture was diluted with brine and extracted with *n*-hexane. The organic layer was dried over $MgSO_4$ and evaporated to give crude (*R*)-**10**, which was used for the next reaction without further purification. (ii) A mixture of crude (*R*)-**10** and $LiBH_4$ (0.132 g, 6.06 mmol) in THF (5 ml) was stirred for 1 h at room temperature. The reaction mixture was diluted with brine and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give a residue, which was chromatographed on silica gel (10 g, *n*-hexane–AcOEt = 5:1) to afford (*R*)-**11** (0.527 g, 94% from (*R*)-**8**) as a pale yellow oil. Compound (*R*)-**11**: $[\alpha]_D^{28} = -2.72$ (*c* 1.29, $CHCl_3$). Spectral data (^{1}H and ^{13}C NMR) of (*R*)-**11** were identical with those of (*S*)-**11**. MS (FAB) *m*/*z*: 371 (M^++1).

4.11. (*R*)-2′-(1-^{t}Butyldimethylsiloxymethylethyl)-4-iodomethyl-2,4′-bithiazole 12

To a mixture of (*R*)-**11** (0.502 g, 1.36 mmol), triphenylphosphine (0.533 g, 2.03 mmol) and imidazole (0.184 g, 2.71 mmol) in THF (5 ml) was added I_2 (0.516 g, 2.03 mmol) under an argon atmosphere at 0 °C and the whole mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 1% aqueous $Na_2S_2O_3$ and brine, and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (10 g, *n*-hexane–AcOEt = 10:1) to afford (*R*)-**12** (0.551 g, 84%) as a pale yellow oil. Compound (*R*)-**12**: $[\alpha]_D^{29} = -3.3$ (*c* 1.2, $CHCl_3$). Spectral data (^{1}H and ^{13}C NMR) of (*R*)-**12** were identical with those of (*S*)-**12**. MS (FAB) *m*/*z*: 353 (M^+−I).

4.12. (*R*)-2′-(1-^{t}Butyldimethylsiloxymethylethyl)-2,4′-bithiazolyl-4-methylenetriphenyl phosphonium iodide 6

A mixture of (*R*)-**12** (0.527 g, 1.1 mmol) and triphenylphosphine (0.346 g, 1.32 mmol) in benzene (7 ml) was stirred for 20 h at reflux. After cooling, the resulting colorless powder (*R*)-**6** (0.575 g, 70%) was obtained by filtration. Compound (*R*)-**6**: ^{1}H NMR data of (*R*)-**6** were identical with those of (*S*)-**6**. MS (FAB) *m*/*z*: 615 (M^+−I).

4.13. (6*E*,14*R*)- and (6*Z*,14*R*)-Cystothiazoles F 3

(i) To a solution of (*R*)-**6** (0.50 g, 0.67 mmol) in THF (5 ml) was added lithium bis(trimethylisilyl)amide (1 M solution in THF, 0.67 ml, 0.67 mmol) at 0 °C under an argon atmosphere and the whole mixture stirred for 20 min at the same temperature. A solution of (+)-**5** (0.072 g, 0.34 mmol) in THF (2 ml) was added to the above reaction mixture at 0 °C and the whole mixture was stirred for 50 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to afford a crude 3:1 diastereomeric mixture, which was used for the next reaction without further purification. (ii) To a solution of the above mixture in THF (4 ml) was added 1 M tetrabutylammonium fluoride ($Bu_4N^+F^-$) solution (0.21 ml, 0.21 mmol) at 0 °C and the whole mixture was stirred for 30 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to afford a crude product, which was chromatographed on silica gel (8 g, *n*-hexane–AcOEt = 10:1) to give a mixture of (6*Z*,14*R*)-**3** and (6*E*,14*S*)-**3**. This mixture was subjected to preparative thin-layer chromatography (silica gel, *n*-hexane–AcOEt = 2:1) to afford (6*E*,14*R*)-**3** (0.045 g, 30%, colorless oil) and (6*Z*,14*R*)-**3** (0.015 g, 10%, colorless oil). Compound (6*E*,14*R*)-**3**: $[\alpha]_D^{26} = +94.6$ (*c* 0.78, $CHCl_3$); IR (neat): 3456, 1705, 1621 cm^{-1}; ^{1}H NMR (400 MHz): δ 1.21 (3H, d, $J = 6.8$ Hz), 1.44 (3H, d, $J = 7.2$ Hz), 3.33 (3H, s), 3.39 (1H, dq, $J = 7.2$, 4.0 Hz), 3.61 (3H, s), 3.66 (3H, s), 3.81 (1H, dd, $J = 8.0$, 7.6 Hz), 3.88 (1H, dd, $J = 10.8$, 7.2 Hz), 3.93 (1H, dd, $J = 10.8$, 4.0 Hz), 4.17 (1H, dq, $J = 8.0$, 6.8 Hz), 4.96 (1H, s), 6.42 (1H, dd, $J = 15.6$, 7.6 Hz), 6.57 (1H, d, $J = 15.6$ Hz), 7.09 (1H, s), 7.90 (1H, s). ^{13}C NMR (100 MHz): δ 14.06, 17.33, 39.81, 40.17, 50.80, 55.51, 57.00, 66.87, 84.36, 91.08, 115.15, 115.19, 125.39, 131.84, 148.75, 154.54, 161.98, 167.74, 175.39, 176.71. HRMS (*m*/*z*): calcd for $C_{20}H_{27}N_2O_5S_2$: 439.1362 (M^++1). Found: 439.1366. Compound (6*Z*,14*R*)-**3**: $[\alpha]_D^{27} = +208.8$ (*c* 0.3, $CHCl_3$); IR (neat): 3458, 1705, 1621 cm^{-1}; ^{1}H NMR (400 MHz): δ 1.25 (3H, d, $J = 6.8$ Hz), 1.45 (3H, d, $J = 7.2$ Hz), 3.32 (3H, s), 3.34 (3H, s), 3.39 (1H, dq, $J = 7.2$, 4.4 Hz), 3.66 (3H, s), 3.89 (1H, dd, $J = 10.8$, 7.2 Hz), 3.94 (1H, dd, $J = 10.8$, 4.4 Hz), 4.22 (1H, dq, $J = 9.2$, 6.8 Hz), 4.92 (1H, s), 5.09 (1H, dd, $J = 10.0$, 9.2 Hz), 5.61 (1H, dd, $J = 12.0$, 10.0 Hz), 6.58 (1H, d, $J = 12.0$ Hz), 7.23 (1H, s), 7.88 (1H, s). ^{13}C NMR (100MHz): δ 14.55, 17.60, 39.37, 40.19, 50.84, 55.17, 56.22, 66.87, 84.90, 91.33, 114.86, 118.02, 125.39, 132.53, 148.80, 153.72, 161.09, 167.65, 175.84, 176.66. HRMS (*m*/*z*): calcd for $C_{20}H_{27}N_2O_5S_2$: 439.1362 (M^++1). Found: 439.1366.

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References

1. Ojika, M.; Suzuki, Y.; Tsukamoto, A.; Sakagami, Y.; Fudou, R.; Yoshimura, T.; Yamanaka, S. *J. Antibiotics* **1998**, *51*, 275–281.

2. Suzuki, Y.; Ojika, M.; Sakagami, Y.; Fudou, R.; Yamanaka, S. *Tetrahedron* **1998**, *54*, 11399–11404.
3. (a) Böhlendrof, B.; Herrmann, M.; Hecht, H.-J.; Sasse, F.; Forche, E.; Kunze, B.; Reichenbach, H.; Höfle, G. *Eur. J. Org. Chem.* **1999**, 2601–2608; (b) Akita, H.; Sasaki, T.; Takayama, H.; Kato, K. *Heterocycles* **2005**, *66*, 219–220.
4. (a) Williams, D. R.; Patnaik, S.; Clark, M. P. *J. Org. Chem.* **2001**, *66*, 8463–8469; (b) Kato, K.; Nishimura, A.; Yamamoto, Y.; Akita, H. *Tetrahedron Lett.* **2002**, *43*, 643–645; (c) Bach, T.; Heuser, S. *Chem. Eur. J.* **2002**, *8*, 5585–5592; (d) Kato, K.; Sasaki, T.; Takayama, H.; Akita, H. *Tetrahedron* **2003**, *59*, 2679–2685; (e) Patrick, L. D.; Andre, B. C. *Org. Lett.* **2003**, *5*, 4163–4165; (f) Shao, J.; Panek, J. S. *Org. Lett.* **2004**, *6*, 3083–3085; (g) Ojika, M.; Watanabe, T.; Qi, J.; Tanino, T.; Sakagami, Y. *Tetrahedron* **2004**, *60*, 187–194; (h) Akita, H.; Sutou, N.; Sasaki, T.; Kato, K. *Tetrahedron* **2006**, *62*, 11592–11598.
5. Sasaki, T.; Kato, K.; Akita, H. *Chem. Pharm. Bull.* **2004**, *52*, 770–771.
6. Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695–707.
7. Akita, H.; Nozawa, M.; Nagumo, S. *Chem. Pharm. Bull.* **1994**, *42*, 1208–1212.
8. Suzuki, Y.; Ojika, M.; Sakagami, Y. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 390–396.