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Tetrahedron 60 (2004) 187–194
Tetrahedron

Syntheses of cystothiazole A and its stereoisomers: importance of stereochemistry for antifungal activity

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Received 24 September 2003; accepted 22 October 2003

Abstract—The enantiocontrolled total syntheses of all the stereoisomers of a myxobacterial antibiotic, cystothiazole A, are described. The natural *syn* stereochemistry at the C4–C5 position was controlled by the asymmetric Evans aldol process, whereas the *anti* relationship was introduced by a modified Evans aldol methodology. Starting with a known aldehyde, the common substrate of the aldol reactions, cystothiazole A and its three stereoisomers were synthesized in 9 steps. All three stereoisomers did not show antifungal activity even at a dosage 2500-fold that of cystothiazole A.

1. Introduction

Cystothiazole A (**1**) is an antifungal and cytotoxic antibiotic isolated from the myxobacterium *Cystobacter fuscus* by the authors.[1] The structural features of this antibiotic are the presence of β-methoxyacrylate and bithiazole moieties. This metabolite might be biosynthetically derived via a hybrid process by polyketide synthetases and non-ribosomal peptide synthetases, as supported by incorporation experiments.[2] A number of such natural products has recently been discovered from myxobacteria, e.g. melithiazoles[3,4] and myxothiazol A.[5,6] This family of β-methoxyacrylate type molecules is thought to bind to the cytochrome bc_1 complex of the mitochondrial respiratory chain system to exhibit fungicidal activity, but not inhibit the bacterial system.[7] The mode of action of cystothiazole A (**1**) was demonstrated by an NADH oxidase inhibition experiment using sub-mitochondrial membrane.[1] The derivatives named cystothiazoles B–F were also isolated as minor components from a fermentation broth.[1,8] Cystothiazole A (**1**) was the major and most active principal among the cystothiazole family. Based on the structure-activity relationships (SAR) within the natural cystothiazoles, it was found that the β-methoxyacrylate moiety was essential and the lipophilicity of the terminal alkyl group (isopropyl) is important for the antifungal activity.[8] Although these SAR are informative to some extent, more detailed information should be accumulated prior to developing artificial candidates for agrochemicals or antitumor agents based on **1**. In particular, the importance of the stereochemistry at the C4–C5 position of **1** is of interest for developing achiral simple derivatives.

Two research groups have accomplished the enantiocontrolled total synthesis of cystothiazole A (**1**). Williams and co-workers carried out the first chiral syntheses of **1** and cystothiazole C via the asymmetric Evans aldol process.[9] The second total synthesis of **1** was accomplished by Akita and co-workers.[10,11] The formal syntheses of **1** and cystothiazole C were also reported.[12] The absolute stereochemistry of cystothiazole E, the natural derivative lacking the β-methoxyacrylate moiety, was determined by the total syntheses of both mirror image compounds.[12,13] The syntheses of myxothiazol A, the earliest member of the bithiazole-type β-methoxyacrylate family, were reported in racemic forms.[14,15] We herein describe the first syntheses of all the stereoisomers of cystothiazole A (**1**) to clarify the significance of the C4–C5 stereochemistry of **1** for antifungal activity.

2. Results and discussion

Since the C4–C5 stereogenic centers of cystothiazole A (**1**) is an aldol-type one, the generally applicable methodology toward the stereoisomers of **1** might be the asymmetric Evans aldol condensation[16] and the combined use of the asymmetric *anti* aldol methodology that was a modified version of the Evans aldol process devised by Heathcock and co-workers.[17] The retrosynthetic routes to the stereoisomers as well as the mother compound **1** itself are shown in Scheme 1. The natural **1** and its enantiomer *ent*-**1** are thus obtained by the original Evans aldol process via *syn* aldols from the common aldehyde **2**, whereas, two diastereomers,

Keywords: Cystothiazole A; Stereoisomers; Antifungal.
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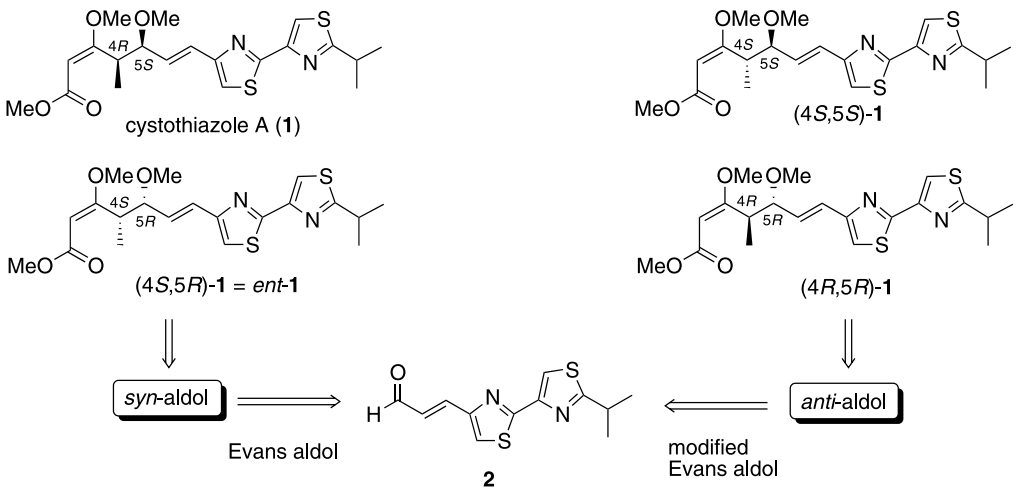
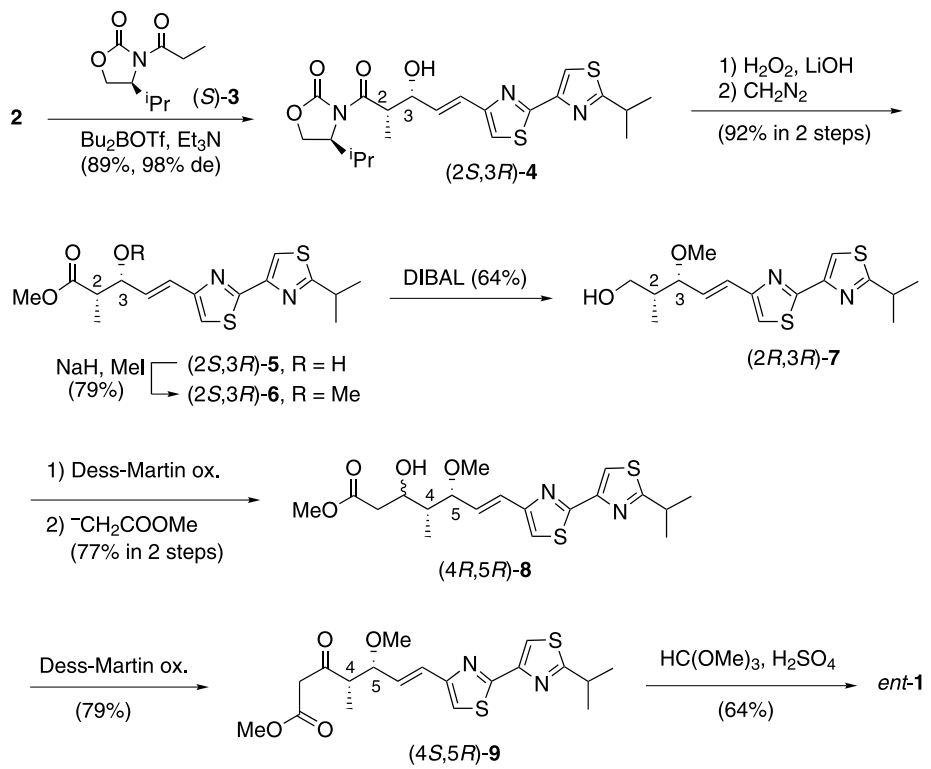
doi:10.1016/j.tet.2003.10.078



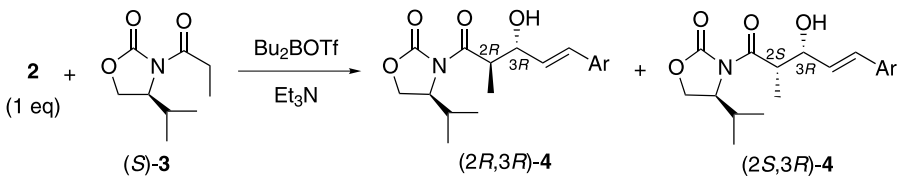
Scheme 1. Synthetic strategy toward all the stereoisomers of cystothiazole A (**1**).

(4*S*,5*S*)-**1** and (4*R*,5*R*)-**1**, could be prepared by the modified Evans aldol methodology via *anti* aldols from **2**. The route for the total synthesis of *ent*-**1** is summarized in Scheme 2. The stereocontrolled formation of (2*S*,3*R*)-*syn*-aldol **4** was performed by the asymmetric Evans aldol reaction[16] with the known aldehyde **2**[9] and (*S*)-oxazolidinone (*S*)-**3** in a high yield and a high diastereoselectivity (Fig. 1, entry 1). Removal of the chiral auxiliary by alkaline hydrogen peroxide followed by diazomethane treatment provided methyl ester **5**, which was then converted to the methyl ether **6**. The yields of **6** varied somewhat due to unknown side products that might arise from β-elimination, although the epimerization at the C-2 position of the isolated product was not observed. Reduction of **6** with DIBAL led to primary alcohol **7**, which, after oxidation to an aldehyde, was subjected to an addition reaction with the carbanion of methyl acetate to afford β-hydroxy ester **8** as a diastereomeric mixture. Dess–Martin periodinane oxidation of **8** gave a β-ketoester, which was finally converted to (−)-**1** (*ent*-**1**) by acid catalyzed enol formation. The total yield of *ent*-**1** from **2** was 16% in 9 steps. The melting point and spectroscopic data of the synthetic *ent*-**1** were identical to those for natural **1** except for the specific rotation: $[\alpha]_D^{25}=-113$ (*c* 0.246, $CHCl_3$) (lit.[1] $[\alpha]_D^{23}=+109$ (*c* 0.24, $CHCl_3$)). Cystothiazole A (**1**) itself was also synthesized in the same manner via aldol (2*R*,3*S*)-**4** that was prepared with the common aldehyde **2** and oxazolidinone (*R*)-**3** (Fig. 1).

The diasteromeric isomers of cystothiazole A, (4*S*,5*S*)-**1** and (4*R*,5*R*)-**1**, were next synthesized from aldehyde **2** by the



Scheme 2. Synthesis of *ent*-cystothiazole A (*ent*-**1**).



entry	(*S*)-**3** (eq)	Et_3N (eq)	Bu_2BOTf (eq)	yield (%)	(2*R*,3*R*)-**4** : (2*S*,3*R*)-**4**[a]
1	2.2	2.8	2.0	90	1:99
2	1.1	2.5	2.2	55	75:25
3	1.0	1.1	2.0	27	99: 1
4	1.0	1.2	1.4	37	95: 5
5	2.0	2.4	2.6	48	21:79

[a] Calculated from isolated yields

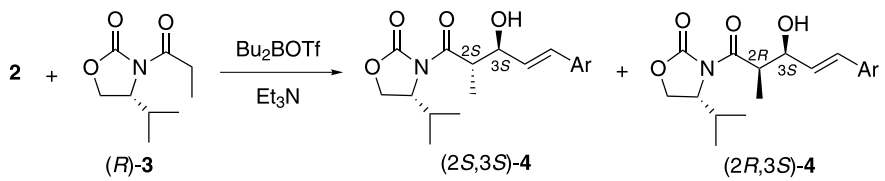
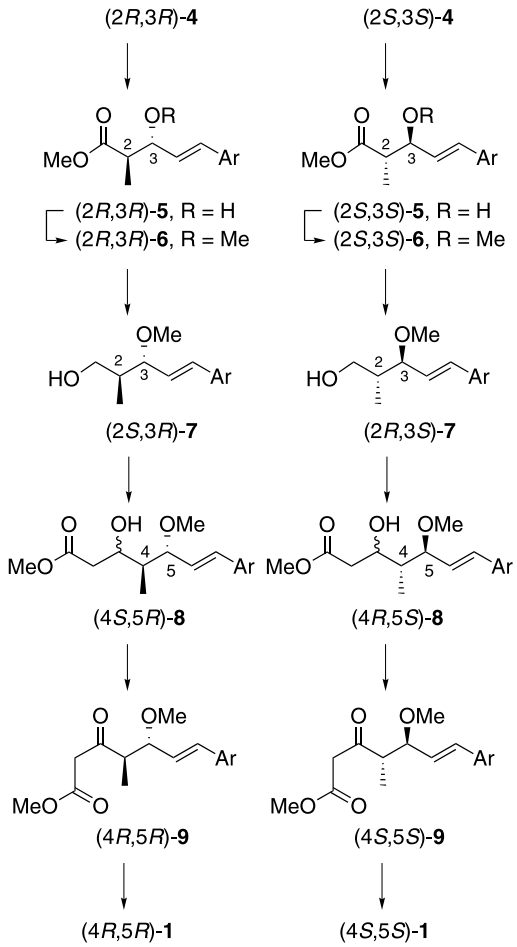


Figure 1. Asymmetric aldol addition reactions under the Evans' conditions (entry 1) and the modified *anti*-selective conditions toward the *anti* aldol (2*R*,3*R*)-**4** (entry 2–5). The second equation represents the aldol reactions to afford the antipodes.

asymmetric *anti* aldol process, although it was reported that the variation of substrate aldehydes was limited.[17] The significant feature of this method is the use of an excess (2 equiv.) of the Lewis acid to oxazolidinone **3**. An open transition state was suggested to account for such *anti* selectivity. An example of the original Evans aldol process conducted for the synthesis of *ent*-**1** (vide supra) is shown in Fig. 1 (entry 1), indicating a high *syn* selectivity as well as a satisfactory chemical yield. On the other hand, when the *anti* aldol conditions were applied to our system by using 2 equiv. of dibutylboron triflate against (*S*)-**3**, the chemical yield and selectivity of the desired *anti* aldol (2*R*,3*R*)-**4** were both low (Fig. 1, entry 2). Reduction of number of equivalents of the base increased the *anti* selectivity, but its yield was much lower than the previous case (entry 3). The low yield of the product might be due to decomposition of the bithiazole moiety of the substrate **2** by the excess Lewis acid. A small excess of the Lewis acid against the base slightly increased the yield and kept a moderate *anti* selectivity (entry 4). A trial was unsuccessful when the amounts of all the reagents were increased to approximately twice those used in entry 4 in order to obtain a higher yield of the *anti* isomer (entry 5). We finally chose the conditions of entry 4 in Fig. 1 to prepare the *anti* aldols. Starting with the *anti* aldols, (2*R*,3*R*)-**4** and (2*S*,3*S*)-**4**, the two diastereomers of cystothiazole A, (4*R*,5*R*)-**1** and (4*S*,5*S*)-**1**, respectively, were synthesized by a series of reactions (Scheme 3), which is the same as that for *ent*-**1** (Scheme 2). Although the *anti* aldols, (2*R*,3*R*)-**4** and (2*S*,3*S*)-**4**, were obviously enantiomers each other from the spectroscopical evidences, their absolute stereochemistry was not very reliable because of the low yields of the *anti* selective Evans aldol process. To confirm the absolute stereochemistry of the *anti* aldols the modified Mosher method[18] was applied to the methyl ester (2*S*,3*S*)-**5** obtained from an *anti* aldol, (2*S*,3*S*)-**4**. Thus, the methyl ester (2*S*,3*S*)-**5** was converted to



Scheme 3. Reaction sequences toward (2*R*,3*R*)-cystothiazole A ((2*R*,3*R*)-**1**) and (2*S*,3*S*)-**1**. The reaction conditions were the same as those for the corresponding steps in Scheme 2.

(*S*)-MTPA ester (**10s**) and (*R*)-MTPA ester (**10r**), and the 5*S* configuration of this compound was demonstrated from the chemical shift differences between these MTPA esters ($\Delta\delta=\delta_{10s}-\delta_{10r}$) as shown in Figure 2.

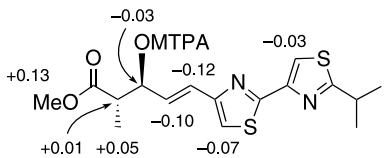


Figure 2. $\Delta\delta$ ($=\delta_{10s}-\delta_{10r}$) values for the MTPA esters (**10r** and **10s**) of (2*S*,3*S*)-**5**.

In antifungal tests using a phytopathogenic fungus *Phytophthora capsici*, synthetic cystothiazole A (**1**) showed an activity until the dose was 0.04 μg/disk, which was the same activity as that of the natural one.[1] However, not only the enantiomer but also the two diastereomers did not show any antifungal activity up to 100 μg/disk. This result was not expected at all, because all the stereoisomers possess the β-methoxyacrylate unit that is regarded as the binding site to the target molecule. The importance of the β-methoxyacrylate moiety in **1** was actually demonstrated by the fact that the synthetic intermediate (4*R*,5*S*)-**9** was inactive, despite its natural stereochemistry. Furthermore, several non-chiral β-methoxyacrylate-type compounds have been developed to the market as pesticides.[19] These findings suggest that unsuitable directions of the substituents at the C4–C5 position completely interfere in the binding of the β-methoxyacrylate moiety to the target molecule.

3. Experimental

3.1. General

Melting points were measured on a Yanaco MP-J3 micro melting point apparatus. Fuji Silysia silica gel BW-300 was employed for open column chromatography. Pre-coated silica gel 60 F_{254} plates (Merck) were used for thin-layer chromatography (TLC). HPLC was performed on a JASCO high-pressure gradient system equipped with PU-980 pumps. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-7000S spectrometer. UV spectra were recorded on a JASCO Ubest-50 UV/VIS spectrophotometer. NMR spectra were recorded on a Bruker ARX 400 (400 MHz) spectrometer. NMR chemical shifts were referenced to the solvent peak of δ_H 7.26 (residual $CHCl_3$) for protons and δ_C 77.0 ($CDCl_3$) for ^{13}C. FAB MS (positive) measurements were performed on a JEOL Mstation JMS-700 mass spectrometer using *m*-nitrobenzyl alcohol as a matrix. High-resolution MS were recorded on an Applied Biosystems Mariner Biospectrometry Workstation in the positive ESI mode using an angiotensin I/neurotensin mixture as the internal calibration standard. Antifungal tests were carried out by the previously reported method.[1]

3.1.1. (*S*)-4-Isopropyl-3-[(*E*)-(2*S*,3*R*)-3-hydroxy-5-(2′-isopropyl[2,4′]bithiazol-4-yl)-2-methyl-4-pentenoyl]oxazolidin-2-one ((2*S*,3*R*)-4) and (2*R*,3*S*)-4. To a cooled solution of (*S*)-4-isopropyl-3-propionyl-2-oxazolidinone ((*S*)-**3**) (1.31 g, 7.11 mmol) in dry dichloromethane (14.5 ml) at 0 °C were added a 1 M solution of dibutylboron triflate (6.4 ml) in dichloromethane and then dry triethylamine (1.3 ml, 9.0 mmol). After being stirred for 30 min and then cooled to −78 °C, to the resulting solution was added a solution of **2** (853 mg, 3.25 mmol) in dry dichloromethane (25 ml), and the mixture was stirred at −78 °C for 1 h 40 min and then at 0 °C for 40 min. The reaction mixture was diluted with 0.5 M phosphate buffer (pH 7) (8.6 ml), MeOH (50 ml), and 30% H_2O_2 (8.6 ml), and stirred for an additional 40 min. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with 5% sodium bicarbonate and then with brine. The solution was dried and concentrated to afford an oil, which was chromatographed on silica gel (4:1 hexane EtOAc) to give (2*S*,3*R*)-**4** (1.29 g, 89% yield, 98% de) and its diastereomer (13 mg, 0.9%). The same procedure with (*R*)-4-isopropyl-3-propionyl-2-oxazolidinone ((*R*)-**3**) gave the enantiomer (2*R*,3*S*)-**4** in 98% yield (99% de based on the yield of a diastereomer).

Compound (2*S*,3*R*)-**4**. Colorless oil; $[\alpha]_D^{24}=+35$ (*c* 0.09, $CHCl_3$); UV (MeOH) 216 (ε 26,400), 249 (20,100), 312 (10,100) nm; IR (film) 3504 (br), 3113, 2967, 2875, 1779, 1698, 1498, 1458, 1386, 1204, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, *J*=6.9 Hz, 3H), 0.92 (d, *J*=6.9 Hz, 3H), 1.31 (d, *J*=7.1 Hz, 3H), 1.44 (d, *J*=6.8 Hz, 6H), 2.36 (m, 1H), 3.12 (brs, 1H, OH), 3.37 (sept, *J*=6.8 Hz, 1H), 4.00 (dq, *J*=3.4, 7.1 Hz, 1H), 4.21 (dd, *J*=9.1, 3.1 Hz, 1H), 4.27 (dd, *J*=9.1, 8.3 Hz, 1H), 4.47 (dt, *J*=8.2, 3.5 Hz, 1H), 4.72 (brt, *J*=4.1 Hz, 1H), 6.62 (dd, *J*=15.5, 5.0 Hz, 1H), 6.73 (dd, *J*=15.5, 1.4 Hz, 1H), 7.08 (s, 1H), 7.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.5 (q), 14.8 (q), 17.9 (q), 23.1 (q, 2C), 28.4 (d), 33.3 (d), 42.7 (d), 58.3 (d), 63.4 (d), 71.7 (d), 115.0 (d), 115.8 (d), 124.1 (d), 131.7 (d), 148.7 (s), 153.5 (s), 154.2 (s), 162.8 (s), 177.1 (s), 178.6 (s); MS (FAB) *m/z* (rel. int.) 472 ($[M+Na]^+$, 3), 450 ($[M+H]^+$, 73) 432 (43), 406 (2), 364 (3), 340 (8), 303 (12), 275 (72), 265 (60), 237 (65), 219 (13), 176 (4), 136 (85), 77 (43). Anal. found: C, 56.11%; H, 6.00%; N, 9.17%, calcd for $C_{21}H_{27}N_3O_4S_2$: C, 56.10%; H, 6.05%; N, 9.35%.

Compound (2*R*,3*S*)-**4**. $[\alpha]_D^{24}=-38$ (*c* 0.31, $CHCl_3$); HRMS (ESI) *m/z* 450.1531 $(M+H)^+$, calcd for $C_{21}H_{28}N_3O_4S_2$, 450.1516.

3.1.2. (*S*)-4-Isopropyl-3-[(*E*)-(2*R*,3*R*)-3-hydroxy-5-(2′-isopropyl[2,4′]bithiazol-4-yl)-2-methyl-4-pentenoyl]oxazolidin-2-one ((2*R*,3*R*)-4) and (2*S*,3*S*)-4. To a cooled solution of (*S*)-**3** (42 mg, 0.23 mmol) in dry dichloromethane (1 ml) at 0 °C were added a 1 M solution of dibutylboron triflate (0.32 ml) in dichloromethane and then dry triethylamine (0.06 ml, 0.28 mmol). After being stirred for 30 min and then cooled to −78 °C, to the resulting solution was added a solution of **2** (60 mg, 0.23 mmol) in dry dichloromethane (1.1 ml), and the mixture was stirred at −78 °C for 1 h 30 min and then at 0 °C for 90 min. The reaction mixture was diluted with 0.5 M phosphate buffer (pH 7) (0.4 ml), MeOH (2.5 ml), and 30% H_2O_2 (0.4 ml), and stirred for an additional 40 min. Similar procedures for work-up and separation to those for (2*S*,3*R*)-**4** afforded

(2*R*,3*R*)-**4** (35.7 mg, 35%) and its diastereomer (1.9 mg, 2%). (2*S*,3*S*)-**4** was obtained in 23% yield by the same method as that for (2*R*,3*R*)-**4** by using **2** and (*R*)-**3**.

Compound (2*R*,3*R*)-**4**. Colorless oil; $[\alpha]_D^{24}=+3.6$ (*c* 0.14, $CHCl_3$); UV (MeOH) 216 (ε 25,900), 248 (19,000), 311 (10,400) nm; IR (film) 3480 (br), 3111, 2964, 2875, 1773, 1700, 1684, 1199, 966 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.85 (d, *J*=6.9 Hz, 3H), 0.90 (d, *J*=6.9 Hz, 3H), 1.23 (d, *J*=6.9 Hz, 3H), 1.43 (d, *J*=6.9 Hz, 6H), 2.35 (dq, *J*=3.1, 6.9 Hz, 1H), 3.36 (sept, *J*=6.9 Hz, 1H), 4.10 (dq, *J*=3.8, 6.9 Hz, 1H), 4.21 (dd, *J*=9.1, 3.1 Hz, 1H), 4.28 (dd, *J*=9.1, 8.3 Hz, 1H), 4.47 (dt, *J*=8.3, 3.7 Hz, 1H), 4.74 (t, *J*=4.0 Hz, 1H), 6.65 (dd, *J*=15.7, 4.9 Hz, 1H), 6.73 (d, *J*=15.7 Hz, 1H), 7.08 (s, 1H), 7.89 (s, 1H). Anal. found: C, 56.03%; H, 6.33%; N, 9.30%, calcd for $C_{21}H_{28}N_3O_4S_2$: C, 56.10%; H, 6.05%; N, 9.35%.

Compound (2*S*,3*S*)-**4**. $[\alpha]_D^{24}=-3.7$ (*c* 0.10, $CHCl_3$), HRMS (ESI) *m*/*z* 450.1534, calcd for calcd for $C_{21}H_{28}N_3O_4S_2$ 450.1516.

3.1.3. Methyl (*E*)-(2*S*,3*R*)-3-hydroxy-5-(2′-isopropyl[2,4′]bithiazol-4-yl)-2-methyl-4-pentenoate ((2*S*,3*R*)-5), (2*R*,3*S*)-5, (2*R*,3*R*)-5, and (2*S*,3*S*)-5. To a cooled solution of (2*S*,3*R*)-**4** (1.29 g, 2.87 mmol) in 80% aqueous THF (70 ml) at 0 °C was added 30% H_2O_2 (5.9 ml, 59 mmol) and 0.8 M LiOH (30 ml, 24 mmol), and the mixture was stirred for 1 h 15 min. The reaction mixture was diluted with 1.3 M $Ne_2S_2O_3$ (45 ml), acidified with 6 M HCl to pH 1, and extracted with EtOAc (30 ml) five times. The combined extracts were concentrated and the residual oil was treated with diazomethane in ether. The reaction mixture was concentrated, and the residual oil was chromatographed on silica gel (4:1 hexane–EtOAc) to give (2*S*,3*R*)-**5** (932 mg, 92% in 2 steps). The enantiomer (2*R*,3*S*)-**5** and two diastereomers, (2*R*,3*R*)-**5** and (2*S*,3*S*)-**5**, were obtained in 95%, 91%, and 85% yields, respectively, by the same procedure.

Compound (2*S*,3*R*)-**5**. Colorless needles; mp 45.5–46.5 °C (hexane–EtOAc); $[\alpha]_D^{24}=-17$ (*c* 0.31, $CHCl_3$); UV (MeOH) 220 (ε 24,500), 248 (21,100), 313 (10,500) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (d, *J*=7.2 Hz, 3H), 1.44 (d, *J*=6.9 Hz, 6H), 2.76 (dq, *J*=3.9, 7.2 Hz, 1H), 2.83 (d, *J*=4.0 Hz, 1H, OH), 3.37 (sept, *J*=6.9 Hz, 1H), 3.73 (s, 3H), 4.67 (m, 1H), 6.61 (dd, *J*=15.5, 5.2 Hz, 1H), 6.71 (dd, *J*=15.5, 1.2 Hz, 1H), 7.08 (s, 1H), 7.85 (s, 1H). Anal. found: C, 54.52%; H, 5.85%; N, 7.90%, calcd for $C_{16}H_{20}N_2O_3S_2$: C, 54.52%; H, 5.72%; N, 7.95%.

Compound (2*R*,3*S*)-**5**. Colorless oil, $[\alpha]_D^{25}=+15$ (*c* 0.70, $CHCl_3$) (reported data:[9] $[\alpha]_D^{28}=+13.2$ (*c* 0.5, $CHCl_3$)).

Compound (2*R*,3*R*)-**5**. Colorless oil; $[\alpha]_D^{25}=-15$ (*c* 0.40, $CHCl_3$); UV (MeOH) 220 (ε 23,400), 248 (20,300), 312 (10,600) nm; IR ($CHCl_3$) 3120, 2984, 1732, 1174, 1012, 970 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.25 (d, *J*=7.0 Hz, 3H), 1.44 (d, *J*=7.0 Hz, 6H), 2.71 (quint, *J*=7.2 Hz, 1H), 2.84 (br, 1H, OH), 3.37 (sept, *J*=7.0 Hz, 1H), 3.73 (s, 3H), 4.41 (dd, *J*=6.0, 7.0 Hz, 1H), 6.62 (dd, *J*=15.5, 6.0 Hz, 1H), 6.68 (d, *J*=15.5 Hz, 1H), 7.10 (s, 1H), 7.86 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (q), 23.1 (q, 2C), 33.3 (d), 45.6 (d), 51.9 (d), 74.3 (d), 115.0 (d), 116.2 (d), 125.0 (d), 132.1 (d), 148.6 (s), 153.9 (s), 163.0 (s), 175.9 (s), 178.7 (s); MS (FAB) *m*/*z* (rel. int.) 375 ($[M+Na]^+$, 5), 353 ($[M+H]^+$, 100), 335 (70), 321 (4), 293 (9), 275 (41), 265 (89), 237 (91), 235 (12), 170 (31), 136 (26), 77 (20). Anal. found: C, 54.79%; H, 5.81%; N, 7.92%, calcd for $C_{16}H_{20}N_2O_3S_2$: C, 54.52%; H, 5.72%; N, 7.95%.

Compound (2*S*,3*S*)-**5**. Colorless oil; $[\alpha]_D^{25}=+18$ (*c* 0.41, $CHCl_3$); HRMS (ESI) *m*/*z* 353.0989 $(M+H)^+$, calcd for $C_{16}H_{21}N_2O_3S_2$ 353.0988.

3.1.4. Methyl (*E*)-(2*S*,3*R*)-5-(2′-isopropyl[2,4′]bithiazol-4-yl)-3-methoxy-2-methyl-4-pentenoate ((2*S*,3*R*)-6), (2*R*,3*S*)-6, (2*R*,3*R*)-6, and (2*S*,3*S*)-6. To a cooled solution of (2*S*,3*R*)-**5** (384 mg, 1.09 mmol) in dry DMF (13.6 ml) at 0 °C were added MeI (0.69 ml, 11 mmol) and NaH (60% in mineral oil, 46.0 mg, 1.16 mmol), and the mixture was stirred for 2 h. An additional NaH (45.4 mg, 1.16 mmol) was added, and the mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with water (20 ml). The mixture was extracted with ether (30 ml) five times. The combined ethereal extracts were washed with brine, dried, and concentrated. The residual oil was chromatographed on silica gel (4:1 hexane–EtOAc) to give (2*S*,3*R*)-**6** (314 mg, 78%). The enantiomer (2*R*,3*S*)-**6** and two diastereomers, (2*R*,3*R*)-**6** and (2*S*,3*S*)-**6**, were obtained in 92, 92, and 82% yields, respectively, by the same procedure.

Compound (2*S*,3*R*)-**6**. Colorless oil; $[\alpha]_D^{24}=-10$ (*c* 0.18, $CHCl_3$); UV (MeOH) 220 (ε 26,000), 248 (21,800), 312 (11,300) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 1.25 (d, *J*=7.1 Hz, 3H), 1.44 (d, *J*=6.8 Hz, 6H), 2.72 (dq, *J*=6.0, 7.1 Hz, 1H), 3.35 (s, 1H), 3.37 (sept, *J*=6.8 Hz, 1H), 3.68 (s, 3H), 4.04 (dd, *J*=6.0, 7.2 Hz, 1H), 6.50 (dd, *J*=15.8, 7.2 Hz, 1H), 6.63 (d, *J*=15.8 Hz, 1H), 7.12 (s, 1H), 7.86 (s, 1H). Anal. found: C, 55.71%; H, 6.13%; N, 7.66%, calcd for $C_{17}H_{22}N_2O_3S_2$: C, 55.71%; H, 6.05%; N, 7.64%.

Compound (2*R*,3*S*)-**6**. Colorless oil; $[\alpha]_D^{25}=+10.3$ (*c* 0.44, $CHCl_3$) (reported data:[9] $[\alpha]_D^{25}=+9.7$ (*c* 2.25, $CHCl_3$)); HRMS (ESI) *m*/*z* 367.1121 $(M+H)^+$, calcd for $C_{17}H_{23}N_2O_3S_2$ 367.1145.

Compound (2*R*,3*R*)-**6**. Mp 65–66 °C; $[\alpha]_D^{25}=-16$ (*c* 0.20, $CHCl_3$); UV (MeOH) 220 (ε 22,300), 249 (19,100), 310 (10,800) nm; IR (KBr) 3127, 1737, 1530, 1221, 1167, 1104, 1092, 978, 763 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.12 (d, *J*=7.1 Hz, 3H), 1.43 (d, *J*=6.9 Hz, 6H), 2.70 (dq, *J*=9.0, 7.1 Hz, 1H), 3.31 (s, 1H), 3.36 (sept, *J*=6.9 Hz, 1H), 3.72 (s, 3H), 3.90 (t, *J*=8.6 Hz, 1H), 6.38 (dd, *J*=15.6, 8.3 Hz, 1H), 6.64 (d, *J*=15.6 Hz, 1H), 7.13 (s, 1H), 7.86 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7 (q), 23.1 (q, 2C), 33.3 (d), 45.1 (d), 51.7 (q), 56.8 (q), 84.2 (d), 115.1 (d), 116.1 (d), 127.5 (d), 129.7 (d), 148.6 (s), 153.7 (s), 162.9 (s), 175.4 (s), 178.7 (s); MS (FAB) *m*/*z* (rel. int.) 389 ($[M+Na]^+$, 5), 367 ($[M+H]^+$, 70), 335 (90), 307 (10), 279 (100), 275 (35), 249 (15), 237 (13), 154 (50), 136 (42), 107 (21), 69 (47), 57 (55). Anal. found: C, 55.83%; H, 6.15%; N, 7.84%, calcd for $C_{17}H_{22}N_2O_3S_2$: C, 55.71%; H, 6.05%; N, 7.64%.

Compound (2*S*,3*S*)-**6**. Colorless needles, mp 65–66 °C (hexane–EtOAc); $[\alpha]_D^{25}=+17$ (*c* 0.10, $CHCl_3$); HRMS

(ESI) *m/z* 367.1124 $(M+H)^+$, calcd for $C_{17}H_{23}N_2O_3S_2$ 367.1145.

3.1.5. (*E*)-(2*R*,3*R*)-5-(2′-Isopropyl[2,4′]bithiazolyl-4-yl)-3-methoxy-2-methyl-4-penten-1-ol ((2*R*,3*R*)-7), (2*S*,3*S*)-7, (2*S*,3*R*)-7, and (2*R*,3*S*)-7. To a cooled solution of (2*S*,3*R*)-**6** (176.0 mg, 0.48 mmol) in dry dichloromethane (2.8 ml) at −78 °C was added a 1 M toluene solution of DIBAL (1.0 ml), and the mixture was stirred at −78 °C for 1 h and then 0 °C for 20 min. The reaction was quenched with MeOH (0.3 ml) and the mixture was stirred for 30 min. The mixture was diluted with saturated NH_4Cl (6.5 ml) and water (6.5 ml), and transferred into a separatory funnel with 0.9 M H_2SO_4 and dichloromethane. The organic layer was separated and the aqueous layer was extracted with ether (20 ml) 4 times. The combined organic layers were combined, washed with saturated $NaHCO_3$ and brine, dried, and concentrated. The residual oil was chromatographed on silica gel (4:1 hexane–EtOAc) to give alcohol (2*R*,3*R*)-**7** (104 mg, 64%). The enantiomer (2*S*,3*S*)-**7** and two diastereomers, (2*S*,3*R*)-**7** and (2*R*,3*S*)-**7**, were obtained in 65, 69, and 85% yields, respectively, by the same procedure.

Compound (2*R*,3*R*)-**7**. Colorless oil; $[\alpha]_D^{24}=-42.3$ (*c* 0.23, $CHCl_3$), UV (MeOH) 221 (ε 22,900), 247 (19,700), 313 (10,400) nm; IR (film) 3422 (br), 3105, 2968, 2931, 2874, 2823, 1684, 1618, 1538, 1498, 1464, 1081, 1035, 974 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.96 (d, *J*=7.1 Hz, 3H), 1.44 (d, *J*=7.0 Hz, 6H), 2.10 (m, 1H), 2.59 (br, 1H, OH), 3.36 (s, 3H), 3.38 (sept, *J*=7.0 Hz, 1H), 3.60 (brd, *J*=10.7 Hz, 1H), 3.75 (dd, *J*=10.7, 7.7 Hz, 1H), 3.92 (dd, *J*=6.9, 4.2 Hz, 1H), 6.56 (dd, *J*=15.7, 6.9 Hz, 1H), 6.63 (d, *J*=15.7 Hz, 1H), 7.11 (s, 1H), 7.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2 (q), 23.1 (q, 2C), 33.6 (d), 39.8 (d), 57.0 (q), 66.0 (t), 85.7 (d), 115.0 (d), 115.7 (d), 126.3 (d), 129.9 (d), 148.6 (s), 154.0 (s), 162.9 (s), 178.7 (s); MS (FAB) *m/z* (rel. int.) 361 ($[M+Na]^+$, 4), 339 ($[M+H]^+$, 90), 307 (55), 279 (100), 277 (48), 265 (25), 249 (20), 237 (15), 224 (13), 170 (30), 136 (77), 115 (15), 91 (46), 69 (56), 55 (68). Anal. found: C, 56.79%; H, 6.68%; N, 8.12%, calcd. for $C_{16}H_{22}N_2O_2S_2$: C, 56.77%; H, 6.55%; N, 8.28%.

Compound (2*S*,3*S*)-**7**. $[\alpha]_D^{25}=+41.5$ (*c* 0.31, $CHCl_3$); HRMS (ESI) *m/z* 339.1213 $(M+H)^+$, calcd for $C_{16}H_{23}N_2O_2S_2$ 339.1196.

Compound (2*S*,3*R*)-**7**. Colorless oil; $[\alpha]_D^{25}=-4$ (*c* 0.18, $CHCl_3$); UV (MeOH) 222 (ε 16,700), 248 (17,500), 313 (9400) nm; IR (film) 3422 (br), 3104, 2822, 1654, 1538, 1183, 1082, 1034, 974, 801, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, *J*=7.0 Hz, 3H), 1.44 (d, *J*=7.0 Hz, 6H), 1.97 (dq, *J*=3.9, 7.0 Hz, 1H), 3.06 (br, 1H, OH), 3.35 (s, 3H), 3.37 (sept, *J*=7.0 Hz, 1H), 3.65 (m, 3H), 6.45 (dd, *J*=15.7, 8.1 Hz, 1H), 6.59 (d, *J*=15.7 Hz, 1H), 7.12 (s, 1H), 7.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8 (q), 23.1 (q, 2C), 33.3 (d), 40.1 (d), 56.7 (q), 67.4 (t), 88.3 (d), 115.1 (d), 115.8 (d), 126.6 (d), 131.1 (d), 148.6 (s), 153.9 (s), 162.9 (s), 178.7 (s); MS (FAB) *m/z* (rel. int.) 361 ($[M+Na]^+$, 3), 339 ($[M+H]^+$, 92), 307 (55), 279 (100), 277 (49), 265 (26), 248 (18), 237 (12), 224 (13), 170 (30), 154 (92), 136 (80), 107 (33),95 (26), 91 (46), 55 (68). Anal. found: C, 56.78%; H, 6.43%; N, 8.00%, calcd. for $C_{16}H_{22}N_2O_2S_2$: C, 56.77%; H, 6.55%; N, 8.28%.

Compound (2*R*,3*S*)-**7**. $[\alpha]_D^{25}=+5$ (*c* 0.09, $CHCl_3$); HRMS (ESI) *m/z* 339.1183 $(M+H)^+$, calcd for $C_{16}H_{23}N_2O_2S_2$ 339.1196.

3.1.6. Methyl (*E*)-(3*SR*,4*R*,5*R*)-7-(2′-isopropyl-[2,4′]bithiazolyl-4-yl)-3-hydroxy-5-methoxy-4-methyl-6-pentenoate ((4*R*,5*R*)-8), (4*S*,5*S*)-8, (4*S*,5*R*)-8, and (4*R*,5*S*)-8. To a solution of alcohol (2*R*,3*R*)-**7** (104 mg, 0.31 mmol) in dry dichloromethane (4.4 ml) was added dry pyridine (0.40 ml, 4.9 mmol) and Dess–Martin periodinane (223 mg, 0.52 mmol), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with ether (2 ml), saturated $NaHCO_3$ (2 ml), and saturated $Na_2S_2O_3$ (2 ml), and then stirred at room temperature for 1 h. The reaction mixture was extracted with ether (5 ml) five times. The combined ethereal layers were washed with saturated $NaHCO_3$, water, and brine, successively, dried, and concentrated to give crude aldehyde as an yellow oil.

To a cooled solution of dry diisopropylamine (0.18 ml, 1.33 mmol) in dry THF (1.8 ml) at −78 °C was added a 1.7 M hexane solution of butyllithium (0.74 ml, 1.25 mmol), and the mixture was stirred for 20 min. To the resulting solution was added dry methyl acetate (0.11 ml, 1.4 mmol), and the mixture was stirred at −78 °C for 20 min. To the resulting solution was added a solution of the above crude aldehyde in dry THF (2.3 ml), and the mixture was stirred at −78 °C for 80 min. The reaction mixture was diluted with 10% aqueous citric acid (2.9 ml). The organic layer was separated and the aqueous layer was extracted with ether (6 ml) three times. The combined organic layers were washed with brine, dried, and concentrated. The residual oil was chromatographed on silica gel (4:1 hexane–EtOAc) to give (4*R*,5*R*)-**8** (97.3 mg, 77%) as a diastereomeric mixture. The enantiomer (4*S*,5*S*)-**8** and two diastereomers, (4*S*,5*R*)-**8** and (4*R*,5*S*)-**8**, were obtained in a diastereomeric mixture in 74, 52, and 84% yields, respectively, by the same procedure.

Compound (4*R*,5*R*)-**8**. Colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (d, *J*=7.1 Hz, 2.4H), 1.04 (d, *J*=7.1 Hz, 0.6H), 1.44 (d, *J*=7.0 Hz, 6H), 1.92 (m, 1H), 2.47 (dd, *J*=15.4, 8.9 Hz, 1H), 2.61 (dd, *J*=15.4, 3.6 Hz, 1H), 3.35 (s, 0.6H), 3.36 (sept, *J*=7.0 Hz, 1H), 3.36 (s, 2.4H), 3.70 (s, 0.6H), 3.72 (s, 2.4H), 3.79 (d, *J*=4.1 Hz, 1H, OH), 3.98 (m, 1H), 4.11 (m, 1H), 4.33 (m, 1H), 6.59 (m, 2H), 7.11 (s, 1H), 7.86 (s, 0.2H), 7.87 (s, 0.8H); ^{13}C NMR (100 MHz, $CDCl_3$, data due to major diastereomer) δ 11.6 (q), 23.1 (q, 2C), 33.6 (d), 39.9 (d), 42.6 (d), 51.7 (q), 57.1 (q), 70.6 (d), 83.6 (d), 115.0 (d), 115.8 (d), 126.0 (d), 130.0 (d), 148.6 (s), 154.0 (s), 162.9 (s), 173.0 (s), 178.6 (s). Anal. found: C, 55.59%; H, 6.49%; N, 6.74%, calcd for $C_{19}H_{26}N_2O_4S_2$: C, 55.58%; H, 6.38%; N, 6.82%.

Compound (4*S*,5*S*)-**8**. The NMR data were the same as those for (4*S*,5*R*)-**8** except for the diastereomeric ratio.

Compound (4*S*,5*R*)-**8** and (4*R*,5*S*)-**8**. Colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (d, *J*=6.8 Hz, 1.2H), 0.95 (d, *J*=6.8 Hz, 1.8H), 1.44 (d, *J*=6.8 Hz, 6H), 1.85 (m, 1H), 1.95 (m, 1H), 2.41 (dd, *J*=15.4, 3.6 Hz, 0.6H), 2.47 (dd, *J*=16.4, 8.8 Hz, 0.4H), 2.56 (dd, *J*=15.4, 10.0 Hz, 0.6H), 2.61 (dd, *J*=16.4, 2.8 Hz, 0.4H), 3.33 (s, 1.2H), 3.35 (s, 1.8H), 3.36

(septet, 1H), 3.71 (s, 1.8H), 3.72 (s, 1.2H), 3.73 (t, J=8.0 Hz, 0.6H), 3.83 (t, J=8.5 Hz, 0.4H), 4.06 (dt, J=2.0, 8.0 Hz, 0.4H), 4.44 (brd, J=9.6 Hz, 0.6H), 6.43 (dd, J=15.6, 8.2 Hz, 0.4H), 6.47 (dd, J=15.6, 7.6 Hz, 0.6H), 6.61 (d, J=15.6 Hz, 1H), 7.12 (s, 1H), 7.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.4 (q, 0.6C), 12.4 (q, 0.4C), 23.1 (q, 2C), 33.3 (d), 39.1 (d, 0.6C), 39.3 (d, 0.4C), 42.3 (d, 0.6C), 42.9 (d, 0.4C), 51.7 (q), 56.5 (q, 0.4C), 56.9 (q, 0.6C), 68.7 (d, 0.6C), 71.3 (d, 0.4C), 85.6 (d, 0.6C), 85.8 (d, 0.4C), 115.1 (d), 115.9 (d), 126.6 (d, 0.6C), 127.0 (d, 0.4C), 130.4 (d, 0.4C), 131.1 (d, 0.6C), 148.6 (s), 153.8 (s), 162.9 (s), 173.0 (s, 0.6C), 173.3 (s, 0.4C), 178.7 (s).

3.1.7. Methyl (*E*)-(4*S*,5*R*)-7-(2′-isopropyl[2,4′]-bithiazolyl-4-yl)-5-methoxy-4-methyl-3-oxo-6-pentenoate ((4*S*,5*R*)-9), (4*R*,5*S*)-9, (4*R*,5*R*)-9, and (4*S*,5*S*)-9. To a cooled solution of (4*R*,5*R*)-**8** (101 mg, 0.25 mmol) in dry dichloromethane (4.3 ml) was added Dess–Martin periodinane (188 mg, 0.45 mmol), and the mixture was stirred at 0 °C for 2 h and then at room temperature for 2 h. The reaction mixture was diluted with ether (1.8 ml), saturated $NaHCO_3$ (1.8 ml), and saturated $Na_2S_2O_3$ (1.8 ml), and then stirred at room temperature for 1 h. The reaction mixture was extracted with ether (8 ml) three times. The combined ethereal layers were washed with brine, dried, and concentrated. The residual oil was chromatographed on silica gel (4:1 hexane–EtOAc) to give ketoester (4*S*,5*R*)-**9** (79.5 mg, 79%). The enantiomer (4*R*,5*S*)-**9** and two diastereomers, (4*R*,5*R*)-**9** and (4*S*,5*S*)-**9**, were obtained from (4*S*,5*S*)-**8**, (4*S*,5*R*)-**8**, and (4*R*,5*S*)-**8** in 62, 70, and 71% yields, respectively, by the same procedure.

Compound (4*S*,5*R*)-**9**. Pale yellow oil; $[\alpha]_D^{24}=-6.8$ (c 0.60, $CHCl_3$); UV (MeOH) 221 (ε 25,100), 249 (22,000), 312 (11,200) nm; IR (film) 3109, 2874, 2825, 1749, 1715, 1653, 1628, 1088, 975, 801, 759 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (d, J=7.0 Hz, 3H), 1.44 (d, J=6.8 Hz, 6H), 2.99 (dq, J=5.4, 7.0 Hz, 1H), 3.33 (s, 3H), 3.37 (sept, J=6.9 Hz, 1H), 3.56 (d, J=15.7 Hz, 1H), 3.62 (d, J=15.7 Hz, 1H), 3.71 (s, 3H), 4.00 (dd, J=7.4, 5.4 Hz, 1H), 6.42 (dd, J=15.6, 7.4 Hz, 1H), 6.61 (d, J=15.6 Hz, 1H), 7.12 (s, 1H), 7.86 (s, 1H); MS (FAB) m/z (rel. int.) 431 ([M+Na]$^+$, 2), 409 ([M+H]$^+$, 39), 377 (16), 345 (9), 340 (4), 307 (12), 279 (85), 277 (22), 263 (11), 248 (10), 219 (9), 136 (60), 95 (40). Anal. found: C, 55.92%; H, 6.04%; N, 6.90%, calcd for $C_{19}H_{24}N_2O_4S_2$: C, 55.86%; H, 5.92%; N, 6.86%.

Compound (4*R*,5*S*)-**9**. Colorless oil; $[\alpha]_D^{25}=+6.1$ (c 0.51, $CHCl_3$) (reported data:[9] $[\alpha]_D^{30}=+3.8$ (c 0.71, $CHCl_3$); HRMS (ESI) m/z 409.1225 (M+H)$^+$, calcd for $C_{19}H_{25}N_2O_4S_2$ 409.1250.

Compound (4*R*,5*R*)-**9**. Colorless needles; mp 91–92 °C (hexane–EtOAc); $[\alpha]_D^{25}=-51$ (c 0.08, $CHCl_3$); UV (MeOH) 222 (ε 13,400), 250 (18,600), 311 (11,200) nm; IR (KBr) 3090, 2820, 1745, 1711, 1262, 1088, 1008, 962, 799, 756 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (d, J=6.8 Hz, 3H), 1.45 (d, J=6.8 Hz, 6H), 2.94 (dq, J=9.0, 6.8 Hz, 1H), 3.25 (s, 3H), 3.38 (sept, J=6.8 Hz, 1H), 3.58 (d, J=15.6 Hz, 1H), 3.64 (d, J=15.6 Hz, 1H), 3.74 (s, 3H), 3.79 (t, J=9.0 Hz, 1H), 6.39 (dd, J=15.6, 8.4 Hz, 1H), 6.63 (d, J=15.6 Hz, 1H), 7.14 (s, 1H), 7.89 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.3 (q), 23.1 (q, 2C), 33.4 (d), 50.3 (d), 50.7 (t), 52.2 (q), 56.7 (q), 85.3 (d), 115.1 (d), 116.3 (d), 127.6 (d), 129.9 (d), 148.6 (s), 153.6 (s), 163.0 (s), 167.7 (s), 178.7 (s), 205.9 (s); MS (FAB) m/z (rel. int.) 431 ([M+Na]$^+$, 2), 409 ([M+H]$^+$, 29), 377 (25), 345 (7), 340 (6), 307 (12), 279 (60), 277 (17), 219 (10), 136 (62), 55 (85). Anal. found: C, 55.87%; H, 6.02%; N, 6.82%, calcd for $C_{19}H_{24}N_2O_4S_2$: C, 55.86%; H, 5.92%; N, 6.86%.

Compound (4*S*,5*S*)-**9**. Colorless needles; mp 91–92 °C (hexane–EtOAc); $[\alpha]_D^{25}=+52$ (c 0.10, $CHCl_3$); HRMS (ESI) m/z 409.1230 (M+H)$^+$, calcd for $C_{19}H_{25}N_2O_4S_2$ 409.1250.

3.1.8. *ent*-Cystothiazole A (*ent*-1), cystothiazole A (1), (2*S*,3*S*)-1, and (2*R*,3*R*)-1. To a cooled solution of (4*S*,5*R*)-**9** (54.8 mg, 0.15 ml) in trimethyl orthoformate (11 ml) at 0 °C was added concentrated sulfuric acid, and the mixture was stirred at 0 °C for 5 h. The reaction mixture was diluted with saturated $NaHCO_3$ (3 ml) and extracted with ether (7 ml) four times. The combined extracts were washed with brine, dried, and concentrated. The residual oil was chromatographed on silica gel (4:1 hexane–EtOAc) to give *ent*-**1** (39.7 mg, 64%). Cystothiazole A (**1**) and two diastereomers, (4*S*,5*S*)-**1** and (4*R*,5*R*)-**1**, were obtained from (4*R*,5*S*)-**9**, (4*S*,5*S*)-**9**, and (4*R*,5*R*)-**9** in 66, 78, and 75% yields, respectively, by the same procedure.

ent-Cystothiazole A (*ent*-**1**). Colorless needles, mp 112–113 °C (hexane–EtOAc); $[\alpha]_D^{25}=-113$ (c 0.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (d, J=6.8 Hz, 3H), 1.44 (d, J=6.9 Hz, 6H), 3.33 (s, 3H), 3.37 (sept, J=6.9 Hz, 1H), 3.60 (s, 3H), 3.66 (s, 3H), 3.81 (t, J=7.7 Hz, 1H), 4.17 (dq, J=7.7, 6.8 Hz, 1H), 4.96 (s, 1H), 6.41 (dd, J=15.8, 7.7 Hz, 1H), 6.57 (d, J=15.8 Hz, 1H), 7.08 (s, 1H), 7.86 (s, 1H); HRMS (ESI) m/z 423.1426 (M+H)$^+$, calcd for $C_{20}H_{27}N_2O_4S_2$ 423.1407.

Cystothiazole A (**1**). Colorless needles, mp 111–112 °C; $[\alpha]_D^{25}=+111$ (c 0.24, $CHCl_3$) (reported data:[1] $[\alpha]_D^{25}=+109$ (c 0.24, $CHCl_3$).

(4*R*,5*R*)-Cystothiazole A ((4*R*,5*R*)-**1**). Colorless needles; mp 103–104 °C (hexane–EtOAc); $[\alpha]_D^{25}=+21$ (c 0.09, $CHCl_3$); UV (MeOH) 222 (ε 25,100), 247 (22,000), 313 (11,200) nm; IR (film) 3009, 1716, 1628, 1150, 1096, 977, 821, 761 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (d, J=7.2 Hz, 3H), 1.44 (d, J=6.8 Hz, 6H), 3.28 (s, 3H), 3.38 (sept, J=6.8 Hz, 1H), 3.67 (s, 3H), 3.68 (s, 3H), 3.85 (dd, J=9.2, 8.4 Hz, 1H), 4.22 (dq, J=9.2, 7.2 Hz, 1H), 5.08 (s, 1H), 6.46 (dd, J=15.7, 8.4 Hz, 1H), 6.63 (d, J=15.7 Hz, 1H), 7.11 (s, 1H), 7.88 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.7 (q), 23.1 (q, 2C), 33.4 (d), 39.4 (d), 50.8 (q), 55.5 (q), 56.7 (q), 83.8 (d), 91.1 (d), 115.0 (d), 115.3 (d), 122.1 (d), 126.7 (d), 131.5 (d), 148.7 (s), 154.2 (s), 167.9 (s), 177.3 (s), 178.6 (s); MS (FAB) m/z (rel. int.) 445 ([M+Na]$^+$, 2), 423 ([M+H]$^+$, 16), 391 (32), 375 (5), 359 (72), 331 (15), 317 (4), 279 (100), 265 (21), 248 (19), 237 (8), 207 (5), 170 (14), 155 (17), 136 (10), 95 (9), 91 (10), 57 (22). Anal. found: C, 56.77%; H, 6.20%; N, 6.58%, calcd. for $C_{20}H_{26}N_2O_4S_2$: C, 56.85%; H, 6.20%; N, 6.63%.

(4*S*,5*S*)-Cystothiazole A ((4*S*,5*S*)-**1**). Colorless needles; mp 103–104 °C (hexane–EtOAc); $[\alpha]_D^{25}=-22$ (*c* 0.05, $CHCl_3$); HRMS (ESI) *m*/*z* 423.1393 $(M+H)^+$, calcd for $C_{20}H_{27}N_2O_4S_2$ 423.1407.

3.1.9. (*R*)- and (*S*)-MTPA esters (10r and 10s) from (2*S*,3*S*)-5. To a solution containing (2*S*,3*S*)-**5** (0.5 mg, 0.0014 mmol) and (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid ((*R*)-MTPA acid, 6.5 mg, 0.028 mmol) in dry dichloromethane (0.5 ml) were added successively 4-(dimethylamino)pyridine (DMAP, 1.7 mg, 0.014 mmol), dicyclohexylcarbodiimide (DCC, 11.5 mg, 0.056 mmol) and 10-camphorsulfonic acid (CSA, 1.9 mg, 0.0084 mmol). After being stirred at room temperature for 12 h, the reaction was quenched by stirring with water (0.5 ml) for 10 min. The mixture was concentrated, the residue was suspended in water (1.5 ml) and extracted with EtOAc (1.5 ml) 3 times. The combined EtOAc extracts were washed successively with saturated aqueous $NaHCO_3$ and H_2O. After evaporation of the solvent, the residue was separated by TLC (silica gel, 3:1 hexane/EtOAc) to give (*R*)-MTPA ester **10r** (0.5 mg). The corresponding (*S*)-MTPA ester (**10s**, 0.6 mg) was prepared from (2*S*,3*S*)-**5** (0.5 mg) by the same way with (*S*)-MTPA acid.

Compound **10r**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (d, *J*=7.16 Hz, 3H), 1.45 (d, *J*=6.92 Hz, 6H), 2.916 (dq, *J*=8.6, 7.2 Hz, 1H), 3.38 (sept, *J*=6.9 Hz, 1H), 3.52 (s, 3H), 3.55 (s, 3H), 5.84 (t, *J*=8.61 Hz, 1H), 6.53 (dd, *J*=15.48, 8.61 Hz, 1H), 6.79 (d, *J*=15.48 Hz, 1H), 7.15 (s, 1H), 7.34–7.51 (m, 5H), 7.87 (s, 1H).

Compound **10s**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (d, *J*=7.20 Hz, 3H), 1.45 (d, *J*=6.88 Hz, 6H), 2.924 (dq, *J*=8.1, 7.2 Hz, 1H), 3.38 (sept, *J*=6.9 Hz, 1H), 3.52 (s, 3H), 3.68 (s, 3H), 5.81 (t, *J*=8.06 Hz, 1H), 6.43 (dd, *J*=15.46, 8.06 Hz, 1H), 6.67 (d, *J*=15.46 Hz, 1H), 7.08 (s, 1H), 7.31–7.48 (m, 5H), 7.84 (s, 1H).

Acknowledgements

This work was financially supported by Research for the Future Program from JSPS and by the Fujisawa foundation.

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