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Alternative synthesis of cystothiazole A

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Abstract—Palladium-catalyzed methoxycarbonylation of (−)-(2*R*,3*S*)-1-*tert*-butyldimethylsiloxy-3-methyl-2-methoxypenta-4-yne **9** derived from (2*R*,3*S*)-epoxy butanoate **5** gave the acetylenic ester **10**, which was treated with MeOH in the presence of Bu_3P to afford selectively (*Z*)-β-methoxy acrylate congener **11** in 86% yield. Treatment of (*Z*)-**11** with 99.8% enrichment of $CDCl_3$ followed by consecutive desilylation and oxidation afforded the left-half aldehyde (+)-**2**. The overall yield (10 steps from **5**; 23%) of (+)-**2** via the present route was improved in comparison to that (10 steps from **5**; 10%) of the previously reported route. By applying the modified Julia's coupling method, selectivity (*E*/*Z*=14:1) of the (*E*)-form (cystothiazole A **1**) against the (*Z*)-form was improved in comparison to the Wittig method (*E*/*Z*=4:1 to 6.9:1).

1. Introduction

Antifungal substances, myxothiazole A[1] and cystothiazole A **1**,[2] were isolated from different strains of myxobacterium *Myxococcus fulvus* and *Cystobacter fuscus*, respectively. These antibiotics possessing a bis-thiazole skeleton as well as a β-methoxy acrylate moiety and cystothiazole A **1** have indicated potent antifungal activity against the phytopathogenic fungus, *Phytophthora capsici* (2 μg/disk), and have shown activity against a broad range of additional fungi with no effect on bacterial growth.[2] Furthermore, cystothiazole A **1** was examined for in vitro cytotoxicity using human colon carcinoma HCT-116 and human leukemia K562 cells. The IC_{50} values of cystothiazole A **1** were 110–130 ng/ml, which were significantly higher than those of myxothiazole A. The fungicidal activity of these β-methoxy acrylate (MOA) inhibitors has been shown to be due to their ability to inhibit mitochondrial respiration by blocking electron transfer between cytochrome *b* and cytochrome *c*.[3] The absolute structure of cystothiazole A **1** was established by a combination of spectroscopic analysis and chemical degradation of the natural product.[2] The left-half structure of cystothiazole A **1** has been reported to be responsible for antifungal activity.[4] In future, for the purpose of structure–biological activity relationship study of cystothiazole A congeners, efficient synthesis of the left-half aldehyde **2** is considered to be highly important.

The first enantiocontrolled synthesis of cystothiazole A **1** was achieved based on the preparation of a bis-thiazole core for the application of the asymmetric Evans aldol methodology for the development of the C(4)/C(5) vicinal stereochemistry.[5] After our synthesis of cystothiazoles A **1**[6a,b] and B[6c] was reported, syntheses of cystothiazoles A **1**,[4,7–9] B,[9] C,[5,7] and E[10] were reported. In this paper, we describe a new chiral synthesis of cystothiazole A **1** for the purpose of improvement of the overall yield and the (*E*)-selectivity of the C(6)/C(7) double bond. Our retrosynthetic strategy of cystothiazole A **1** is illustrated in Scheme 1. Retrosynthetically, the synthesis of **1** can be achieved by the modified Julia's coupling[11] of the left-half aldehyde **2** and the right-half sulfone **3** or **4**. The synthesis of the left-half aldehyde **2** is shown in Scheme 2.

By applying the previously reported procedure,[6b] the reaction of (2*R*,3*S*)-epoxy butanoate **5**[12] and lithium silyl-acetylide in the presence of Et_2AlCl gave **6** in 71% yield. Methylation of **6** followed by consecutive reduction and silylation afforded the silyl ether **9** possessing the terminal acetylene group in 52% overall yield from **6**. By applying Tsuji's procedure,[13] the acetylenic compound **9** was converted to acetylenecarboxylate **10** in 87% yield under atmospheric pressure (balloon) of carbon monoxide at room temperature using 5 mol % of $PdCl_2$ and a stoichiometric amount of $CuCl_2$ in MeOH. By applying the reported procedures,[14] conjugate addition of MeOH to acetylenecarboxylate **10** in the presence of a catalytic amount of Bu_3P afforded the corresponding (*Z*)-β-methoxy-α,β-unsaturated ester **11** in 86% yield. The (*Z*)-geometry of **11** was confirmed by the NOE enhancement for the olefinic proton and the methine proton (8.6%). Isomerization of (*Z*)-**11** to (*E*)-**12** was carried out by the following procedure. When a solution of (*Z*)-**11** in $CDCl_3$ (chloroform-*d*+1% v/v TMS (D, 99.8%)+silver foil) from Cambridge Isotope Laboratories, Inc.) was stood for 3 days at room temperature, (*E*)-**12** was

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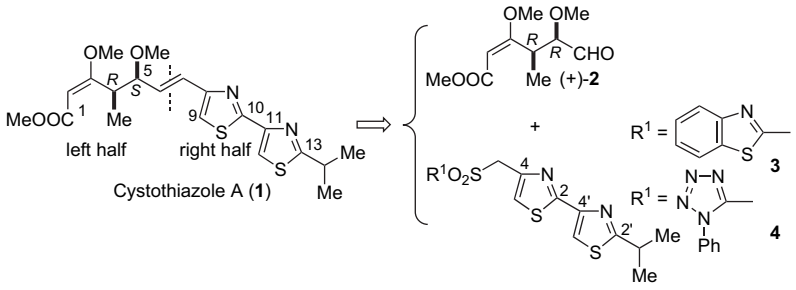
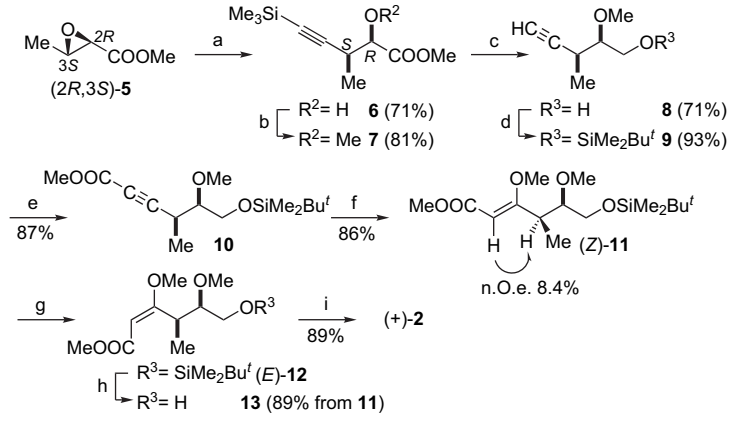


Scheme 1.

exclusively obtained. The crude (*E*)-**12** was treated with $Et_3N\cdot(HF)_3$ in CH_2Cl_2 to give the primary alcohol (+)-**13** ($[\alpha]_D$ +91.4 (*c* 0.64, $CHCl_3$)) in 85% overall yield from (*Z*)-**11**, which was identical with the reported (+)-**13** ($[\alpha]_D$ +76.1 (*c* 0.7, $CHCl_3$)).[6b] Another $CDCl_3$ (99.8% atom % D from Aldrich) was also effective for this selective isomerization. On the other hand, the distillated $CHCl_3$, CH_2Cl_2, $(CF_3SO_3)Yb/CH_2Cl_2$, and silica gel/MeOH were inactive for this selective isomerization. Treatment of (*Z*)-**11** with pyridinium *p*-toluenesulfonate (PPTS)/MeOH/PhH or $(CF_3SO_3)Sc/CH_2Cl_2$ or $InCl_3/CH_2Cl_2$ gave a complex mixture including a mixture of (*Z*)-**12** and (*E*)-**12**. The same type of isomerization as conversion of (*Z*)-**11** to (*E*)-**12** in 99.8% $CDCl_3$ was observed in our previous case.[15] In the case of β-methoxy acrylate, it was reported that a trace amount of acidic impurities often was sufficient to bring about rapid equilibration toward the (*E*)-form from the (*Z*)-form at ordinary temperatures.[16] The thus-obtained (+)-**13** was subjected to Dess–Martin periodinane oxidation to provide the desired aldehyde (+)-**2** ($[\alpha]_D$ +104.7 (*c* 0.55, $CHCl_3$)) in 89% yield. Then, the synthesis of the right-half sulfone **3** or **4** is carried out as shown in Scheme 3.

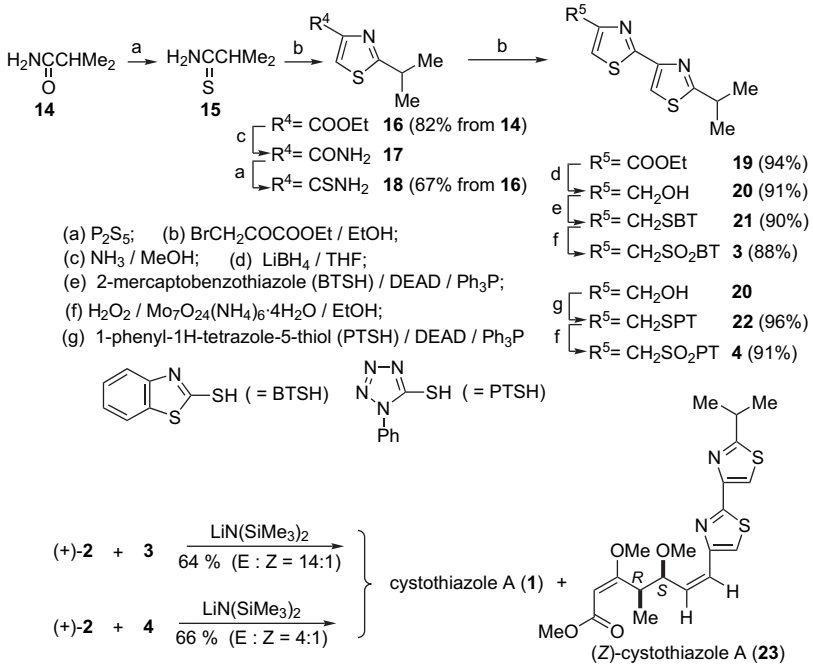
Treatment of isopropylamide **14** with P_4S_{10} gave an isopropylthioamide **15**, which was reacted with α-bromopyruvate to afford a mono-thiazole ester **16** in 82% overall yield from **14**. Treatment of **16** with NH_3/MeOH followed by thioamidation with P_4S_{10} yielded a thioamide **18** in 67% overall yield from **16**. The reaction of **18** with α-bromopyruvate afforded a bithiazole ester **19** in 94% yield. $LiBH_4$ reduction (**20**, 91% yield) of **19** followed by treatment with 2-mercaptobenzothiazole (BTSH)/Ph_3P/diethylazodicarboxylate (DEAD) provided a sulfide **21** in 90% yield. The sulfide **21** was subjected to oxidation with 30% H_2O_2 in the presence of $Mo_7O_{24}(NH_4)_6\cdot 4H_2O$ to give the corresponding sulfone **3** in 88% yield. Moreover, an alcohol **20** was treated with 1-phenyl-1*H*-tetrazole-5-thiol (PTSH)/Ph_3P/diethylazodicarboxylate (DEAD) to provide a sulfide **22** in 96% yield. The sulfide **22** was subjected to oxidation with 30% H_2O_2 in the presence of $Mo_7O_{24}(NH_4)_6\cdot 4H_2O$ to give the corresponding sulfone **4** in 91% yield. Then, the modified Julia's coupling of the aldehyde **2** with sulfones (**3** or **4**) was carried out. The reaction of **2** and **3** in the presence of lithium bis(trimethylsilyl)amide in THF gave a mixture (*E*/*Z*=14:1) of cystothiazole A **1** and (*Z*)-cystothiazole A **23** in 64% yield. A part of this mixture was again subjected to chromatography to provide (*E*)-**1**, whose spectral data (^1H and ^{13}C NMR) were identical with those of the reported data.[6b] The reaction of **2** and **4** in the presence of lithium bis(trimethylsilyl)amide in THF gave a mixture (*E*/*Z*=4:1) of cystothiazole A **1** and (*Z*)-cystothiazole A **23** in 66% yield.

In conclusion, palladium-catalyzed methoxycarbonylation of (–)-(2*R*,3*S*)-1-*tert*-butyldimethylsiloxy-3-methyl-2-methoxypenta-4-yne **9** derived from (2*R*,3*S*)-epoxy butanoate **5** gave the acetylenic ester **10**, which was treated with MeOH in the presence of Bu_3P to afford selectively



(a) $Li^+ {}^-C{\equiv}C{-}SiMe_3$ / Et_2AlCl; (b) MeI / Ag_2O; (c) (1) $Bu_4N^+F^-$, (2) $LiBH_4$;
(d) $^tBuMe_2SiCl$ / imidazole / DMF; (e) $PdCl_2$ (5 mol %) / $CuCl_2$ / NaOAc / CO (balloon) / CH_2Cl_2;
(f) Bu_3P/MeOH; (g) $CDCl_3$ (D; 99.8%); (h) $Et_3N\cdot(HF)_3$ / CH_2Cl_2; (i) Dess-Martin periodinane

Scheme 2.



Scheme 3.

(*Z*)-β-methoxy acrylate congener **11** in 86% yield. Treatment of (*Z*)-**11** with 99.8% enrichment of $CDCl_3$ followed by consecutive desilylation and oxidation gave the left-half aldehyde (+)-**2**. The overall yield (10 steps from **5**, 23%) of (+)-**2** via the present route was improved in comparison to that (10 steps from **5**, 10%) of the previously reported route.[6a,b] By applying the modified Julia's coupling method, selectivity (*E*/*Z*=14:1) of the (*E*)-form (cystothiazole A **1**) against the (*Z*)-form was improved in comparison to the Wittig method (*E*/*Z*=4:1[6b] to 6.9:1[8]).

2. Experimental

2.1. General

All melting points were measured on a Yanaco MP-3S micro melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on JEOL AL 400 spectrometer in $CDCl_3$. Carbon substitution degrees were established by DEPT pulse sequence. High-resolution mass spectra (HRMS) and the fast atom bombardment mass spectra (FABMS) 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.

2.2. (−)-(2*R*,3*S*)-1-*tert*-Butyldimethylsiloxy-3-methyl-2-methoxypenta-4-yne (9)

n-Butyllithium (*n*-BuLi, 1.6 M in hexane, 24.5 ml, 39.2 mmol) was added to a stirred solution of trimethylsilylacetylene (3.8 g, 38.7 mmol) in toluene (50 ml) at −40 °C under an argon atmosphere and the mixture was stirred for 1 h at 0 °C. A solution of (2*R*,3*S*)-**5** (3.0 g, 25.8 mmol) in toluene (10 ml) was added to the above reaction mixture and the whole mixture was stirred for 3 h at 5–10 °C. The reaction mixture was diluted with H_2O (20 ml) at 0 °C and the generated white precipitate was filtered off with the aid of Celite. The precipitate was washed with AcOEt and the washing and filtrate were combined. The extracted organic layer was dried over $MgSO_4$ and evaporated to give an oily product, which was chromatographed on silica gel (60 g, *n*-hexane–AcOEt 30:1) to afford methyl (2*R*,3*S*)-2-hydroxy-3-methyl-5-trimethylsilyl-4-pentynoate **6** (3.91 g, 71% yield) as a colorless oil. Compound (−)-**6**: $[\alpha]_D^{25}$ −24.1 (*c* 1.07, $CHCl_3$); IR (neat): 3482, 2168, 1741 cm^{-1}; ^1H NMR: δ 0.15 (9H, s), 1.21 (3H, d, *J*=7.2 Hz), 2.86–2.93 (1H, m), 3.81 (3H, s), 4.21 (1H, d, *J*=4.4 Hz); ^{13}C NMR: δ −0.07, 15.8, 32.3, 52.4, 73.5, 86.9, 106.1, 173.1; HRMS (FAB) calcd for $C_{10}H_{19}O_3Si$ (M^++1): 215.1104. Found: *m*/*z*: 215.1106.

A mixture of **6** (2.105 g, 9.8 mmol), methyl iodide (18 g, 127 mmol), and Ag_2O (3.2 g, 13.8 mmol) in DMF (6 ml) was stirred for 48 h at room temperature. The reaction mixture was diluted with AcOEt (30 ml) and filtered off with the aid of Celite. The filtrate was diluted with H_2O and extracted with *n*-hexane. The organic layer was dried over $MgSO_4$. Concentration of the organic layer gave a crude residue, which was chromatographed on silica gel (50 g, *n*-hexane–AcOEt 60:1) to provide methyl (2*R*,3*S*)-2-methoxy-3-methyl-5-trimethylsilyl-4-pentynoate **7** (1.819 g, 81% yield) as a colorless oil. Compound (−)-**7**: $[\alpha]_D^{23}$ −0.32 (*c* 1.225, $CHCl_3$); IR (neat): 1752, 2170 cm^{-1}; ^1H NMR: δ 0.14 (9H, s), 1.23 (3H, d, *J*=6.8 Hz), 2.89 (1H, dq, *J*=6.4, 6.8 Hz), 3.43 (3H, s), 3.71 (1H, d, *J*=6.4 Hz), 3.77 (3H, s); ^{13}C NMR: δ −0.02, 16.4, 30.5, 51.8, 58.9, 84.0, 86.6, 106.3, 171.4; HRMS (FAB) calcd for $C_{11}H_{21}O_3Si$ (M^++1): 229.1260. Found: 229.1289.

To a solution of (2*R*,3*S*)-**7** (1.66 g, 7.3 mmol) in THF (20 ml) was added 1 M tetrabutylammonium fluoride (TBAF) in THF solution (3.7 ml, 3.7 mmol) at 0 °C and the whole mixture was stirred for 10 min at 0 °C. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give a crude residue, which was used for the next reaction without further purification. To a solution of the above 2-methoxy ester in THF (20 ml) was added $LiBH_4$ (0.641 g, 29.4 mmol) at 0 °C and the whole mixture was stirred for 60 min at room temperature. The reaction mixture was diluted with MeOH (2 ml), H_2O (100 ml), and Et_2O (100 ml), and the whole mixture was stirred for 12 h at the same temperature. The generated precipitate was filtered off with the aid of Celite to afford the filtrate. The filtrate was extracted with Et_2O. The organic layer was dried over $MgSO_4$. Evaporation of the filtrate gave a crude residue, which was chromatographed on silica gel (20 g, *n*-hexane–AcOEt 5:1) to provide (–)-**8** as a colorless oil (0.66 g, 71%). Compound (–)-**8**: $[\alpha]_D^{27}$ −27.6 (*c* 1.05, $CHCl_3$); IR (neat): 3429, 3296, 2113 cm^{-1}; ^1H NMR: δ 1.25 (3H, d, *J*=6.8 Hz), 2.11 (1H, d, *J*=2.4 Hz), 2.38 (1H, br s), 2.76 (1H, ddq, *J*=2.4, 6.8, 6.8 Hz), 3.16 (1H, ddd, *J*=3.2, 5.6, 6.8 Hz), 3.80 (2H, dq, *J*=12, 5.6 Hz); ^{13}C NMR: δ 17.1, 27.3, 58.4, 61.9, 70.3, 84.5, 85.5; HRMS (FAB) calcd for $C_7H_{13}O_2$ (M^++1): 129.0916. Found: 129.0919.

A solution of (–)-**8** (0.452 g, 3.5 mmol), imidazole (0.492 g, 7.1 mmol), and *tert*-butyldimethylsilyl chloride (TBDMSCl, 0.797 g, 5.3 mmol) in DMF (2 ml) was stirred for 1 h at room temperature. The reaction mixture was diluted with brine and extracted with AcOEt/*n*-hexane (1:1). The organic layer was dried over $MgSO_4$ and evaporated to give a crude **9**, which was chromatographed on silica gel (30 g, *n*-hexane/AcOEt 30:1) to afford **9** (0.801 g, 93%) as a colorless oil. Compound (–)-**9**: $[\alpha]_D^{25}$ −4.74 (*c* 1.06, $CHCl_3$); IR (neat): 2115 cm^{-1}; ^1H NMR: δ 0.06 (6H, s), 0.89 (9H, s), 1.21 (3H, d, *J*=7.2 Hz), 2.05 (1H, d, *J*=2.4 Hz), 2.68 (1H, ddq, *J*=2.4, 7.2, 6.4 Hz), 3.16 (1H, ddq, *J*=4.4, 5.6, 6.4 Hz), 3.71 (1H, dd, *J*=5.6, 10.8 Hz), 3.82 (1H, dd, *J*=4.4, 10.8 Hz); ^{13}C NMR: δ −5.46, −5.41, 16.4, 18.3, 25.9 (3C), 27.4, 59.0, 63.3, 69.4, 84.6, 86.5; HRMS (FAB) calcd for $C_{13}H_{27}O_2Si$ (M^++1): 243.1781. Found: 243.1785.

2.3. (–)-Methyl (4*S*,5*R*)-6-*tert*-butyldimethylsiloxy-4-methyl-5-methoxy-2-hexynoate (10)

A 100-ml two-necked round-bottomed flask, containing a magnetic stirring bar, $PdCl_2$ (47 mg, 0.27 mmol), anhydrous $CuCl_2$ (1.79 g, 13.2 mmol), and anhydrous NaOAc (1.09 g, 13.3 mmol) in MeOH (25 ml), was fitted with a rubber septum and three-way stopcock connected to a balloon filled with carbon monoxide. The apparatus was purged with carbon monoxide by pumping–filling via the three-way stopcock. A solution of (–)-**9** (1.289 g, 5.33 mmol) in MeOH (3.5 ml) was added to the above stirred mixture via a syringe at 0 °C. After being stirred for 4 h, the reaction mixture was diluted with AcOEt (100 ml)/H_2O (100 ml). The organic layer was dried over $MgSO_4$. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica gel (30 g, *n*-hexane/AcOEt 50:1) to provide (–)-**10** as a homogeneous oil (1.399 g, 87%). $[\alpha]_D^{24}$ −15.1 (*c* 0.86, $CHCl_3$); IR (neat): 2236, 1717 cm^{-1}; ^1H NMR: δ 0.06 (6H, s), 0.89 (9H, s), 1.25 (3H, d, *J*=7.2 Hz), 2.86 (1H, dq, *J*=7.2, 6.8 Hz), 3.23–3.27 (1H, m), 3.47 (3H, s), 3.68–3.77 (2H, m), 3.74 (3H, s); ^{13}C NMR: δ −5.52, −5.47, 15.0, 18.2, 25.8 (3C), 27.5, 52.5, 59.0, 62.7, 73.7, 83.6, 91.3, 154.1; HRMS (FAB) calcd for $C_{15}H_{29}O_4Si$ (M^++1): 301.1836. Found: 301.1837.

2.4. (–)-Methyl (4*R*,5*R*)-6-*tert*-butyldimethylsiloxy-3,5-dimethoxy-4-methyl-2(*Z*)-hexenoate (11)

A mixture of (–)-**10** (1.25 g, 4.2 mmol), Bu_3P (0.254 g, 1.25 mmol), and MeOH (0.335 g, 10.5 mmol) in CH_2Cl_2 (25 ml) was stirred for 4 h at room temperature. The reaction mixture was evaporated to give a crude residue, which was chromatographed on silica gel (30 g, *n*-hexane/AcOEt 50:1) to afford (–)-**11** (1.90 g, 86%) as a colorless oil. Compound (–)-**11**: $[\alpha]_D^{24}$ −15.6 (*c* 0.96, $CHCl_3$); IR (neat): 1717, 1630 cm^{-1}; ^1H NMR: δ 0.05 (6H, s), 0.89 (9H, s), 1.11 (3H, d, *J*=7.2 Hz), 2.59 (1H, dq, *J*=6.8, 6.8 Hz), 3.30 (1H, dd, *J*=5.6, 6.8 Hz), 3.41 (3H, s), 3.61 (2H, d, *J*=5.6 Hz), 3.66 (3H, s), 3.91 (3H, s), 5.07 (1H, s); ^{13}C NMR: δ −5.45, −5.38, 13.4, 18.3, 25.9 (3C), 40.5, 50.9, 59.3, 60.2, 62.9, 83.1, 95.8, 165.9, 174.7; HRMS (FAB) calcd for $C_{16}H_{33}O_5Si$ (M^++1): 333.2097. Found: 332.2120.

2.5. (+)-Methyl (4*R*,5*R*)-6-hydroxy-3,5-dimethoxy-4-methyl-2(*E*)-hexenoate (13)

A solution of (–)-**11** (0.103 g, 0.45 mmol) in $CDCl_3$ (2 ml; D, 99.8% including 1% v/v TMS, silver foil, available from Cambridge Isotope Laboratories Inc.) was stood for 2 weeks at room temperature. Evaporation of the solvent gave **12** as a colorless oil, which was used for the next reaction without further purification. Compound **12**: ^1H NMR: δ 0.03 (6H, s), 0.87 (9H, s), 1.15 (3H, d, *J*=7.2 Hz), 3.33 (1H, dq, *J*=3.0, 7.2 Hz), 3.46 (3H, s), 3.54 (1H, dd, *J*=7.2, 11.2 Hz), 3.61 (3H, s), 3.61–3.65 (1H, m), 3.65 (3H, s), 3.97–4.04 (1H, m), 4.96 (1H, s).

A mixture of **12** and $Et_3N\cdot(HF)_3$ (0.6 g, 3.7 mmol) in CH_2Cl_2 (4 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica gel (5 g, *n*-hexane/AcOEt 3:1) to afford (+)-**13** (0.06 g, 89% from (–)-**11**) as a colorless oil. Compound (+)-**13**: $[\alpha]_D^{23}$ +91.4 (*c* 0.64, $CHCl_3$); IR (KBr): 3450, 1711, 1623, 1150 cm^{-1}; ^1H NMR: δ 1.19 (3H, d, *J*=6.8 Hz), 3.24–3.32 (1H, m), 3.44–3.48 (1H, m), 3.45 (3H, s), 3.63 (3H, s), 3.67–3.73 (1H, m), 3.69 (3H, s), 4.07–4.15 (1H, m), 5.05 (1H, s); ^{13}C NMR: δ 14.5, 36.0, 51.2, 55.6, 58.1, 61.4, 83.6, 91.4, 168.6, 176.6; HRMS (FAB) calcd for $C_{10}H_{17}O_5$ (M^++1): 217.1076. Found: 217.1079.

2.6. (+)-Methyl (4*R*,5*R*)-3,5-dimethoxy-4-methyl-2(*E*)-hexenoate (2)

A mixture of (+)-**14** (0.142 g, 0.65 mmol) and Dess–Martin reagent (0.708 g, 1.67 mmol) in CH_2Cl_2 (6 ml) was stirred for 2 h at room temperature. The reaction mixture was diluted with ether. The organic layer was washed with 7% aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of

the organic solvent gave a crude residue, which was chromatographed on silica gel (5 g, *n*-hexane/AcOEt 9:1) to provide (+)-**2** as a colorless oil (0.125 g, 89%). Compound (+)-**2**: $[\alpha]_D^{23}$ +104.7 (*c* 0.55, $CHCl_3$); IR (KBr): 1712, 1628, 1441, 1149, 1095 cm^{-1}; ^1H NMR: δ 1.20 (3H, d, *J*=7.2 Hz), 3.43 (3H, s), 3.56 (1H, dd, *J*=2.4, 6.8 Hz), 3.64 (3H, s), 3.68 (3H, s), 4.48 (1H, dq, *J*=6.8, 7.2 Hz), 5.07 (1H, s), 9.59 (1H, q, *J*=2.4 Hz); ^{13}C NMR: δ 13.9, 36.5, 51.0, 55.7, 58.6, 87.4, 91.9, 167.6, 174.4, 202.1; HRMS (FAB) calcd for $C_{10}H_{17}O_5$ (M^++1): 217.1076. Found: 217.1079.

2.7. Ethyl 2-isopropylthiazole-4-carboxylate (16)

To a solution of phosphorus pentasulfide (P_4S_{10}; 5.1 g, 11.47 mmol) in ether (40 ml) was added isobutylamide **14** (10 g, 114.7 mmol) and the whole mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with brine and extracted with ether. The organic layer was dried over $MgSO_4$ and evaporated to give a crude **15**. A mixture of the crude **15** and ethyl α-bromopyruvate (22.39 g, 114.5 mmol) in EtOH (100 ml) was stirred at reflux for 15 min. The reaction mixture was evaporated, diluted with AcOEt, and washed with 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude oil, which was chromatographed on silica gel (200 g, *n*-hexane/AcOEt 20:1) to afford **16** as a colorless compound (18.92 g, 82% overall yield from **14**). Compound **16**: IR (neat): 1726 cm^{-1}; ^1H NMR: δ 1.40 (3H, t, *J*=7.2 Hz), 1.42 (6H, t, *J*=7.0 Hz), 3.43 (1H, sept, *J*=7.0 Hz), 4.42 (2H, q, *J*=7.2 Hz), 8.06 (1H, s); ^{13}C NMR: δ 14.4, 23.3 (C2), 33.6, 61.4, 126.4, 146.6, 161.6, 179.0; MS (FAB) *m/z*: 200 (M^++1).

2.8. 2-Isopropylthiazole-4-thioamide (18)

The thiazole ester **16** (20.01 g, 100.1 mmol) was treated with saturated NH_3 in MeOH (10 ml) in a sealed tube and the whole mixture was left to stand for 3 days at room temperature. After cooling, the reaction mixture was concentrated to afford a crude amide **17**. To a solution of crude **17** in benzene (300 ml) was added phosphorus pentasulfide (P_4S_{10}; 36.5 g, 82.1 mmol) and the whole mixture was stirred for 2 h at reflux. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (300 g, *n*-hexane/AcOEt 10:1) to afford **18** as a pale yellow compound (12.53 g, 67% from **16**). Recrystallization of **18** from *n*-hexane afforded **18** as pale yellow needles. Compound **18**: mp 83–85 °C; IR (KBr): 3290, 3155, 1634 cm^{-1}; ^1H NMR: δ 1.40 (6H, d, *J*=6.9 Hz), 3.28 (1H, sept, *J*=6.9 Hz), 7.79 (1H, br s), 8.32 (1H, s), 8.70 (1H, br s); ^{13}C NMR: δ 22.9, 33.3, 85.1, 126.4, 152.5, 177.7, 190.9. Anal. Calcd for $C_7H_{10}N_2S_2$: C, 45.13; H, 5.41; N, 15.04%. Found: C, 45.13; H, 5.36; N, 15.01. FABMS *m/z*: 187 (M^++1).

2.9. Ethyl 2′-isopropyl[2,4′]bithiazolyl-4-carboxylate (19)

A solution of **19** (0.99 g, 5.3 mmol) and ethyl bromopyruvate (1.14 g, 5.8 mmol) in absolute EtOH (30 ml) was stirred for 1 h at reflux. The reaction mixture was diluted with 7% aqueous $NaHCO_3$ and extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (30 g, *n*-hexane/AcOEt 30:1) to afford **19** (1.41 g, 94%). Recrystallization of **19** from *n*-hexane provided **19** as colorless needles. Compound **19**: mp 76–77 °C; IR (KBr): 1729 cm^{-1}; ^1H NMR: δ 1.43 (3H, d, *J*=7.1 Hz), 1.44 (6H, d, *J*=6.9 Hz), 3.36 (1H, sept, *J*=6.9 Hz), 4.44 (2H, q, *J*=7.1 Hz), 8.02 (1H, s), 8.16 (1H, s); ^{13}C NMR: δ 14.4, 23.1, 33.4, 61.5, 116.1, 127.4, 147.5, 147.6, 161.3, 163.5, 178.4. Anal. Calcd for $C_{12}H_{14}N_2O_2S_2$: C, 51.02; H, 5.00; N, 9.92%. Found: C, 50.89; H, 5.02; N, 9.96. FABMS *m/z*: 283 (M^++1).

2.10. 2′-Isopropyl[2,4′]bithiazolyl-4-methyl alcohol (20)

A mixture of **19** (1.787 g, 6.3 mmol) and $LiBH_4$ (0.69 g, 31.6 mmol) in THF (20 ml) was stirred for 2 h at room temperature. The reaction mixture was diluted with H_2O (10 ml) and the whole was stirred for 5 h at the same temperature. The reaction mixture was extracted with AcOEt and washed with 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 5:1) to afford **20** (1.506 g, 91%). Recrystallization of **20** from *n*-hexane provided **20** as colorless needles. Compound **20**: mp 56–58 °C; IR (KBr): 3247, 1536, 1447 cm^{-1}; ^1H NMR: δ 1.44 (6H, d, *J*=7.2 Hz), 3.25–3.40 (1H, br s), 3.37 (1H, sept, *J*=7.2 Hz), 4.81 (2H, s), 7.19 (1H, t, *J*=0.8 Hz), 7.84 (1H, s); ^{13}C NMR: δ 23.1, 23.1, 33.4, 60.9, 115.0, 115.2, 148.4, 157.2, 163.7, 178.8. Anal. Calcd for $C_{10}H_{12}N_2OS_2$: C, 49.97; H, 5.03; N, 11.66%. Found: C, 50.27; H, 5.19; N, 11.43. FABMS *m/z*: 241 (M^++1).

2.11. 2′-Isopropyl[2,4′]bithiazolyl-4-methylenethio-2-benzothiazole (21) and 2′-isopropyl[2,4′]bithiazolyl-4-methylenesulfonyl-2-benzothiazole (3)

To a mixture of **20** (0.1 g, 0.42 mmol), triphenylphosphine (0.218 g, 0.83 mmol), and diethylazocarboxylate (0.27 g, 0.62 mmol) in THF (5 ml) was added 2-mercaptobenzothiazole (0.14 g, 0.84 mmol) under argon atmosphere and the whole mixture was stirred for 60 min at room temperature. The reaction mixture was diluted with 2 M aqueous NaOH and extracted with Et_2O. The organic layer was washed with 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 30:1) to afford **22** (0.146 g, 90%). Recrystallization of **21** from *n*-hexane provided **21** as colorless needles. Compound **21**: mp 74–75 °C; IR (KBr): 3119, 2962, 1430, 997 cm^{-1}; ^1H NMR: δ 1.42 (6H, d, *J*=7.0 Hz), 3.36 (1H, sept, *J*=7.0 Hz), 4.76 (2H, s), 7.27–7.31 (1H, m), 7.33 (1H, s), 7.40–7.44 (1H, m), 7.73–7.75 (1H, m), 7.82 (1H, s), 7.89–7.91 (1H, m); ^{13}C NMR: δ 23.1, 23.1, 33.0, 33.3, 115.0, 117.5, 121.0, 121.6, 124.3, 126.0, 135.4, 148.4, 152.1, 153.1, 163.2, 166.0, 178.7. Anal. Calcd for $C_{17}H_{15}N_3S_4$: C, 52.41; H, 3.88; N, 10.79%. Found: C, 52.44; H, 3.96; N, 10.33. FABMS *m/z*: 390 (M^++1).

To a solution of **21** (0.094 g, 0.24 mmol) in EtOH (2 ml) were added $Mo_7O_{24}(NH_4)_6 \cdot 4H_2O$ (0.03 g, 0.024 mmol) and 30% H_2O_2 (0.5 ml) at 0 °C, and the whole mixture was stirred for 60 min at room temperature. The reaction mixture

was diluted with brine and extracted with AcOEt. The organic layer was dried over $MgSO_4$. The organic layer was evaporated to give a residue, which was chromatographed on silica gel (15 g, *n*-hexane/AcOEt 3:1) to afford **3** (0.089 g, 88%). Recrystallization of **3** from *n*-hexane/AcOEt provided **3** as colorless needles. Compound **3**: mp 129–130 °C; IR (KBr): 1332, 1150 cm^{-1}; ^1H NMR: δ 1.38 (6H, d, J=6.8 Hz), 3.31 (1H, sept, J=6.8 Hz), 5.15 (2H, s), 7.19 (1H, s), 7.68 (1H, s), 7.67–7.71 (1H, m), 7.74–7.78 (1H, m), 8.21–8.23 (1H, m), 8.29–8.31 (1H, m); ^{13}C NMR (acetone-d_6): δ 23.1, 23.1, 33.8, 57.4, 115.7, 122.9, 123.8, 126.1, 128.7, 128.9, 138.2, 144.7, 148.9, 153.8, 163.6, 167.1, 179.4. Anal. Calcd for $C_{17}H_{15}N_3O_2S_4$: C, 48.43; H, 3.59; N, 9.97%. Found: C, 48.30; H, 3.61; N, 9.80. FABMS *m/z*: 422 (M^++1).

2.12. 2′-Isopropyl[2,4′]bithiazolyl-4-methylenethio-5-(1-phenyl-1*H*)-tetrazole (22) and 2′-isopropyl[2,4′]-bithiazolyl-4-methylenesulfonyl-5-(1-phenyl-1*H*)-tetrazole (4)

To a mixture of **20** (0.1 g, 0.42 mmol), triphenylphosphine (0.218 g, 0.83 mmol), and diethylazocarboxylate (0.27 g, 0.62 mmol) in THF (5 ml) was added 1-phenyl-1*H*-tetrazole-5-thiol (0.148 g, 0.83 mmol) under argon atmosphere and the whole mixture was stirred for 60 min at room temperature. The reaction mixture was diluted with 2 M aqueous NaOH and extracted with Et_2O. The organic layer was washed with 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 6:1) to afford **22** (0.16 g, 96%). Recrystallization of **22** from *n*-hexane provided **22** as colorless needles. Compound **22**: mp 102–104 °C; IR (KBr): 3114, 2957, 1498, 1393 cm^{-1}; ^1H NMR: δ 1.43 (6H, d, J=6.8 Hz), 3.36 (1H, sept, J=6.8 Hz), 4.77 (2H, s), 7.43 (1H, s), 7.51–7.55 (5H, m), 7.79 (1H, s); ^{13}C NMR: δ 23.1, 23.1, 33.0, 33.3, 115.1, 118.4, 123.9, 123.9, 129.8, 129.8, 130.1, 133.6, 148.3, 150.9, 153.8, 163.5, 178.8. Anal. Calcd for $C_{17}H_{16}N_6S_3$: C, 50.98; H, 4.03; N, 20.98%. Found: C, 51.00; H, 4.00; N, 20.89. FABMS *m/z*: 401 (M^++1).

To a solution of **22** (0.34 g, 0.85 mmol) in EtOH (1 ml) were added $Mo_7O_{24}(NH_4)_6\cdot 4H_2O$ (0.105 g, 0.085 mmol) and 30% H_2O_2 (1 ml) at 0 °C, and the whole mixture was stirred for 60 min at room temperature. The reaction mixture was diluted with brine and extracted with AcOEt. The organic layer was 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 8:1) to afford **4** (0.333 g, 91%). Recrystallization of **4** from *n*-hexane/AcOEt provided **4** as colorless needles. Compound **4**: mp 121–122 °C; IR (KBr): 1338, 1160 cm^{-1}; ^1H NMR: δ 1.43 (6H, d, J=6.8 Hz), 3.35 (1H, sept, J=6.8 Hz), 5.02 (2H, s), 7.44 (1H, s), 7.50–7.60 (5H, m), 7.66 (1H, s); ^{13}C NMR: δ 23.1, 23.1, 33.3, 58.1, 115.7, 122.4, 125.6, 125.6, 129.5, 129.5, 131.5, 132.9, 141.3, 147.7, 153.2, 164.2, 179.1. Anal. Calcd for $C_{17}H_{16}N_6O_2S_3$: C, 47.21; H, 3.73; N, 19.43%. Found: C, 47.24; H, 3.77; N, 19.17. FABMS *m/z*: 433 (M^++1).

2.13. Modified Julia's coupling of (+)-2 and 3 (synthesis of cystothiazole A (*E*)-1)

To a solution of **3** (0.17 g, 0.40 mmol) in THF (3 ml) was added lithium bis(trimethylsilyl)amide (1 M solution in THF, 0.4 ml, 0.4 mmol) at 0 °C under argon atmosphere and the whole mixture was stirred for 20 min at the same temperature. A solution of (+)-**2** (0.073 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 20 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 (12 g, *n*-hexane/AcOEt 20:1) to give a 14:1 mixture (0.091 g, 64%) of (*E*)-**1** and (*Z*)-cystothiazole **23**. A part of this mixture was again subjected to chromatography on silica gel (5 g, *n*-hexane/AcOEt 20:1) to afford (*E*)-**1** (0.005 g). ^1H and ^{13}C NMR data of the present (*E*)-**1** were identical with those of the reported data.[2,5]

2.14. Modified Julia's coupling of (+)-2 and 4 (synthesis of cystothiazole A (*E*)-1)

To a solution of **4** (0.216 g, 0.50 mmol) in THF (5 ml) was added lithium bis(trimethylsilyl)amide (1 M solution in THF, 0.5 ml, 0.5 mmol) at 0 °C under argon atmosphere and the whole mixture was stirred for 20 min at the same temperature. A solution of (+)-**2** (0.09 g, 0.42 mmol) in THF (2 ml) was added to the above reaction mixture at 0 °C and the whole mixture was stirred for 20 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 (15 g, *n*-hexane/AcOEt 20:1) to give a 4:1 mixture (0.116 g, 66%) of (*E*)-**1** and (*Z*)-cystothiazole **23**.

References and notes

1. (a) Gerth, K.; Irschik, H.; Reichenbach, H.; Trowitzsch, W. *J. Antibiot.* **1980**, *33*, 1474–1479; (b) Trowitzsch, W.; Reifenstahl, G.; Wray, V.; Gerth, K. *J. Antibiot.* **1980**, *33*, 1480–1490; (c) Trowitzsch, W.; Höfle, G.; Sheldrick, W. S. *Tetrahedron Lett.* **1981**, *22*, 3829–3832.
2. Ojika, M.; Suzuki, Y.; Tsukamoto, A.; Sakagami, Y.; Fudou, R.; Yoshimura, T.; Yamanaka, S. *J. Antibiot.* **1998**, *51*, 275–281.
3. Thierbach, G.; Reichenbach, H. *Biochim. Biophys. Acta* **1981**, *638*, 282–289.
4. Ojika, M.; Watanabe, T.; Qi, J.; Tanino, T.; Sakagami, Y. *Tetrahedron* **2004**, *60*, 187–194.
5. Williams, D. R.; Patnaik, S.; Clark, M. C. *J. Org. Chem.* **2001**, *66*, 8463–8469.
6. (a) Kato, K.; Nishimura, A.; Yamamoto, Y.; Akita, H. *Tetrahedron Lett.* **2002**, *43*, 643–645; (b) Kato, K.; Sasaki, T.; Takayama, H.; Akita, H. *Tetrahedron* **2003**, *59*, 2679–2685; (c) Sasaki, T.; Kato, K.; Akita, H. *Chem. Pharm. Bull.* **2004**, *52*, 770–771.
7. Bach, T.; Heuser, S. *Chem.—Eur. J.* **2002**, *8*, 5585–5592.
8. DeRoy, P. L.; Charette, A. B. *Org. Lett.* **2003**, *5*, 4163–4165.
9. Shao, J.; Panek, J. S. *Org. Lett.* **2004**, *6*, 3083–3085.
10. Bach, T.; Heuser, S. *Angew. Chem., Int. Ed.* **2001**, *40*, 3184–3185.
11. Blakemore, P. R. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2563–2585.

12. (a) Akita, H.; Kawaguchi, T.; Enoki, Y.; Oishi, T. *Chem. Pharm. Bull.* **1990**, *38*, 323–328; (b) Akita, H.; Todoroki, R.; Endo, H.; Ikari, Y.; Oishi, T. *Synthesis* **1993**, 513–516; (c) Kato, K.; Ono, M.; Akita, H. *Tetrahedron: Asymmetry* **1997**, *8*, 2295–2298. Now, *n*-butyl (2*R*,3*S*)-epoxy butyrate corresponding to (2*R*,3*S*)-**5** is commercially available from Osaka Yuki Kagaku Kogyo Co., Ltd (Japan). Japan Kokai Tokkyo Koho JP 3095539.
13. Tsuji, J.; Takahashi, M.; Takahashi, T. *Tetrahedron Lett.* **1980**, *21*, 849–850.
14. Inanaga, J.; Baba, Y.; Hanamoto, T. *Chem. Lett.* **1993**, 241–244.
15. Kato, K.; Nishimura, A.; Yamamoto, Y.; Akita, H. *Tetrahedron Lett.* **2001**, *42*, 4203–4205.
16. Rhoads, S. J.; Chattopadhyay, J. K.; Waali, E. E. *J. Org. Chem.* **1970**, *35*, 3352–3358.