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Total Synthesis of Cystothiazole A by Microwave-Assisted Olefin Cross-Metathesis

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A short and convergent synthesis of the myxobacterial antibiotic cystothiazole A by a microwave-assisted olefin cross-metathesis reaction and a sequential microwave-assisted cross-metathesis/Stille coupling is described.

Introduction

Cystothiazole A (**1**) is the major and most active member of a family of bithiazole metabolites which have been isolated from the myxobacterium *Cystobacter fuscus*.[1] Possessing a highly pharmacophoric β-methoxyacrylate (MOA) moiety, the cystothiazoles belong to the class of complex III inhibitors which were found to selectively bind to the cytochrome bc_1 complex of the mitochondrial respiratory chain (Figure 1).[2]

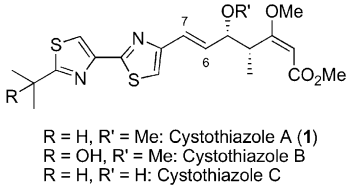
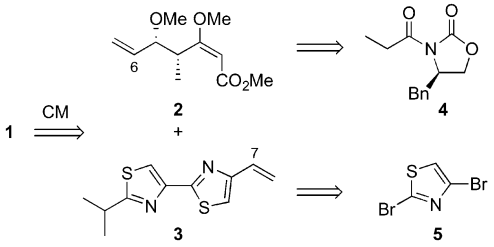


Figure 1. Selected cystothiazoles.

Moreover, further structure-activity relationship studies revealed that the natural (4*R*,5*S*) stereochemistry[3] and the (*E*) configuration of the C6–C7 double bond[4] are necessary for the antifungal activity which is increasing with the lipophilicity of the terminal side chain.[5] In addition to its potent activity against a broad range of fungi including *Phytophthora capsici* and *Candida albicans*, cystothiazole A (**1**) also displays in vitro cytotoxicity towards human colon carcinoma HCT-116 and leukemia K562 cells with an IC_{50} value of 130 and 110 ng/mL, respectively,[1a] making it an attractive target for the development of agrochemical and antitumor agents. Due to these intriguing biological properties and its interesting chemical structure, several enantioselective but rather laborious total syntheses of **1** have been reported.[6] Encouraged by our recent results on the cross-metathesis (CM) of vinyl-substituted thiazoles,[7a] which have already led to the total synthesis of melithiazole C,[7b] we set out to apply the same strategy to the synthesis of **1** which was thus fragmented into the right-half β-methoxyacrylate **2** and the vinylbithiazole **3** (Scheme 1).



Scheme 1. Retrosynthetic analysis of **1**.

Results and Discussion

While **2** could be prepared in five steps and 50% overall yield from the Evans propionate **4**,[7b] the required vinylbithiazole **3** was prepared in three steps starting from commercially available 2,4-dibromothiazole (**5**). Accordingly, the synthesis of cystothiazole A (**1**) began with the preparation of the bithiazolyl bromide **7** which can be obtained from **5** by two consecutive Negishi cross-coupling reactions as described by Bach et al.[8] However, in our approach, an alternative Stille cross-coupling reaction between isopropylthiazole **6**[9] and **5** furnished the known bromide **7** in a slightly improved yield of 78% (Scheme 2).[10]

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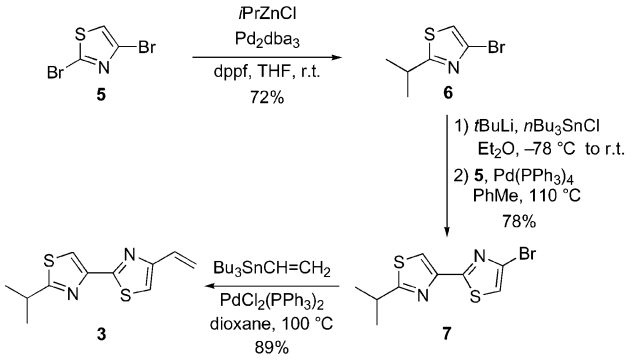
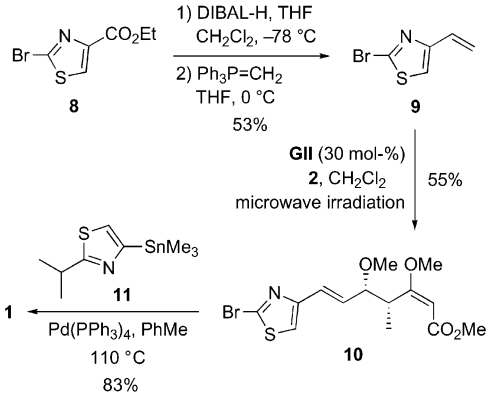


Scheme 2. Synthesis of the vinylbithiazole **3**.

A further Stille coupling with tributyl(vinyl)tin (1.1 equiv.) under standard conditions [5 mol-% $PdCl_2(PPh_3)_2$, dioxane, 100 °C][11] then provided the desired vinylbithiazole **3** in 50% overall yield. To our surprise, attempted CM of **3** (1–2 equiv.) with the β-methoxyacrylate **2** by using either 30 mol-% of Grubbs 2nd generation catalyst (**GII**) or the Hoveyda-Grubbs catalyst (**HG**) under various conditions (including addition of catalytic or stoichiometric amounts of a complexating Lewis acid), resulted in less than 5% conversion to cystothiazole A (**1**), and mainly homodimerization and decomposition of the vinylbithiazole were observed (Scheme 3). Since dramatic improvements of yields and reaction rates in microwave-assisted CM reactions have been reported,[12] we were prompted to study this CM reaction under microwave irradiation (Scheme 3).[13] Much to our delight, **1** could be obtained in 25% isolated yield together with an unidentified side-product and both homodimers when the reaction was conducted by sequential addition of **3** (5× 0.2 equiv.) to a solution of **2** and 30 mol-% (20 + 10 mol-%) of **GII** in CD_2Cl_2 over 5 h (Scheme 3).

In order to further improve the overall efficiency of our CM approach, we additionally investigated the cross-coupling reaction of **2** with 2-bromo-4-vinylthiazole (**9**) since it would enable access to **1** as well as related natural products and heterocyclic analogues by subsequent Pd-catalyzed C-2 arylation.[14] Thus, our alternative synthesis of **1** began with DIBAL reduction[15] of the readily available ethyl ester **8**,[16] followed by a Wittig olefination of the crude aldehyde (Ph_3PCH_3Br, *t*BuOK, THF, 0 °C) to give rise to **9** in 53% yield over two steps (Scheme 4). CM of **9** with **2** under our optimized conditions then furnished the key intermediate **10** in an acceptable yield of 55%.

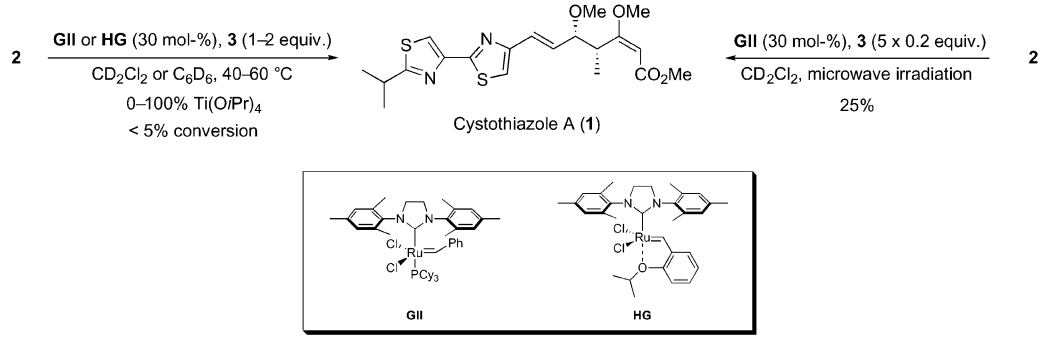


Scheme 4. Synthesis of **1** by sequential CM/Stille coupling.

As expected, final C-2 arylation of **10** with the known stannane **11**[8] [$Pd(PPh_3)_4$, PhMe, reflux] proceeded smoothly and afforded cystothiazole A (**1**) as the only product in 83% yield. In both cases the spectroscopic and physical data of **1** are identical with those reported for the natural product {$[a]_D^{20}$ = +110 (*c* = 0.5, $CHCl_3$); ref.[1a] $[a]_D^{25}$ = +109 (*c* = 0.24, $CHCl_3$)}.

Conclusions

We have described a short and efficient synthesis of cystothiazole A (**1**) which was obtained in six steps and 12.5% overall yield or seven steps and 23% overall yield starting from the commercial Evans propionate **4**. The key feature in our convergent approach consists either of the microwave-assisted CM reaction of a vinyl-functionalized bithiazole or a sequential microwave-assisted CM/Stille coupling, the latter allowing easy access to heterocyclic analogues and related natural products.



Scheme 3. Synthesis of **1** by CM of **2** and **3**.

Experimental Section

General: All reactions were performed under argon in anhydrous solvents (CH_2Cl_2 and toluene were distilled from CaH_2, THF and Et_2O were distilled from sodium/benzophenone). TLC was performed on Merck $60F_{254}$ silica gel plates and visualized with a UV lamp (254 nm) and a $KMnO_4/K_2CO_3$/AcOH solution in H_2O followed by heating. Flash chromatography was performed with Merck Geduran Si60 silica gel (40–63 μM). Optical rotations were determined with a Perkin–Elmer 343 polarimeter. Infrared (IR) spectra were recorded with a Bruker TENSOR™ 27 (IRFT) spectrometer, and wavenumbers are indicated in cm^{-1}. ^1H NMR spectra were recorded with a Bruker AVANCE 400 spectrometer at 400 MHz, and data are reported as follows: chemical shift in ppm relative to the residual solvent peak, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet or overlap of non-equivalent resonances), integral. ^{13}C NMR spectra were recorded with a Bruker AVANCE 400 spectrometer at 100 MHz, and data are reported as follows: chemical shift in ppm relative to the residual solvent peak, multiplicity with respect to proton (deduced from DEPT experiments, s = C_q, d = CH, t = CH_2, q = CH_3). Mass spectra with electronic impact (MS) were recorded with a Hewlett–Packard tandem 5890A/5971 GC-MS (70 eV) instrument. High-resolution mass spectra (HRMS) were recorded by the Groupe de Spectrométrie de Masse de l'Université Pierre et Marie Curie (Paris).

4-Bromo-2′-isopropyl-2,4′-bithiazole (7):[8] To a solution of **6** (206 mg, 1 mmol) in Et_2O (5 mL) was added *t*BuLi (1.3 mL, 1.5 M in pentane, 2.1 mmol) dropwise at –78 °C. After 15 min at –78 °C, Bu_3SnCl (326 mg, 1 mmol) was added slowly, and the mixture was stirred at room temp. for 1.5 h. The reaction was quenched with H_2O (5 mL), and the aqueous phase was extracted with Et_2O (3× 5 mL). Drying of the combined organic phases and evaporation of the solvent gave the crude stannane which was dissolved in toluene (10 mL) and treated with **5** (243 mg, 1 mmol) and $Pd(PPh_3)_4$ (58 mg, 0.05 mmol). After refluxing for 16 h, H_2O (10 mL) was added, and the aqueous phase was extracted with Et_2O (3× 10 mL). Drying of the combined organic phases, evaporation of the solvent and purification of the residue by flash chromatography (SiO_2; hexane → 5% Et_2O in hexane) gave **7** (225 mg, 78%) as a white solid. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.82 (s, 1 H), 7.15 (s, 1 H), 3.29 (sept, J = 7 Hz, 1 H), 1.37 (d, J = 7 Hz, 6 H) ppm.

2′-Isopropyl-4-vinyl-2,4′-bithiazole (3): To a solution of bromide **7** (250 mg, 0.86 mmol) and $PdCl_2(PPh_3)_2$ (30 mg, 0.04 mmol) in dioxane (5 mL) was added tributyl(vinyl)tin (317 mg, 1 mmol) dropwise, and the mixture was stirred at 100 °C for 20 h. After dilution with Et_2O (80 mL), the organic phase was washed with H_2O (2× 15 mL), dried with $MgSO_4$ and concentrated. Purification of the residue by flash chromatography (SiO_2; hexane → 5% Et_2O in hexane) gave **3** (180 mg, 89%) as a yellow oil. ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.80 (s, 1 H), 7.05 (s, 1 H), 6.68 (dd, J = 10.8 and 17 Hz, 1 H), 6.05 (dd, J = 1.5 and 17.0 Hz, 1 H), 5.32 (dd, J = 1.5 and 10.8 Hz, 1 H), 3.31 (sept, J = 7 Hz, 1 H), 1.37 (d, J = 7 Hz, 6 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 178.7 (s), 162.8 (s), 155.0 (s), 148.6 (s), 129.9 (d), 116.7 (t), 115.6 (d), 115.1 (d), 33.4 (d), 23.2 (2× q) ppm. IR: $\tilde{\nu}$ = 3098, 2964, 2330, 1628, 1537, 1497, 1441, 1383, 1311, 1178, 1036, 983, 914, 796, 762 cm^{-1}. HRMS (ESI): calcd. for $C_{11}H_{12}N_2NaS_2$ $[M + Na]^+$ 259.0334; found 259.0330.

2-Bromo-4-vinylthiazole (9): To a solution of **8** (472 mg, 2 mmol) in THF/CH_2Cl_2 (1:1, 30 mL) was added DIBAL-H (6 mL, 1 M solution in CH_2Cl_2, 6 mmol) dropwise to maintain the temperature < –70 °C. After 5 h at –78 °C, the reaction was quenched by addition of MeOH (5 mL) at the same temperature, and the mixture was poured into cold 1 M HCl (20 mL). The aqueous phase was extracted with Et_2O (3× 20 mL), and the combined organic phases were dried with $MgSO_4$. Evaporation of the solvent gave the crude aldehyde (330 mg) which was dissolved in THF (5 mL) and added dropwise to a cooled (0 °C) solution of methylenetriphenylphosphorane [prepared from Ph_3PCH_3Br (1.23 g, 3.4 mmol) and *t*BuOK (390 mg, 3.4 mmol) at 0 °C in THF (10 mL)]. After 1 h at 0 °C, the reaction was quenched with saturated aqueous NH_4Cl (20 mL), and the aqueous phase was extracted with Et_2O (3× 20 mL). Drying of the combined organic phases with $MgSO_4$, evaporation of the solvent and purification of the residue by flash chromatography (SiO_2; pentane → 5% Et_2O in pentane) gave **9** (200 mg, 53%) as a yellow oil. ^1H NMR ($CDCl_3$, 400 MHz): δ = 6.96 (s, 1 H), 6.56 (dd, J = 10.8 and 17.5 Hz, 1 H), 6.00 (dd, J = 1.2 and 17.5 Hz, 1 H), 5.32 (dd, J = 1.2 and 10.8 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 154.6 (s), 136.1 (s), 128.6 (d), 118.4 (d), 117.8 (t) ppm. IR: $\tilde{\nu}$ = 3100, 1629, 1489, 1435, 1396, 1309, 1005, 981, 917, 843, 763 cm^{-1}. HRMS (ESI): calcd. for C_5H_5BrNS $[M + H]^+$ 191.9299; found 191.9297.

Methyl 7-(2-Bromothiazol-4-yl)-3,5-dimethoxy-4-methylhepta-2,6-dienoate (10): A solution of **9** (23 mg, 0.12 mmol) in CH_2Cl_2 (0.5 mL) was added in 5 portions (5× 0.1 mL) to a solution of **2** (25 mg, 0.12 mmol) and **GII** (17 mg, 20%) in CH_2Cl_2 (0.6 mL), and the mixture was irradiated for 1 h after each addition (400 W, 100 °C with cooling). Another portion of **GII** (9 mg, 10%) was then added, and the mixture was irradiated for further 30 min before the solvent was evaporated. Purification of the residue by flash chromatography (SiO_2; Et_2O/hexane, 1:3) gave **10** (25 mg, 55%) as a colourless viscous oil. $[\alpha]_D^{20}$ = +120.3 (c = 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 6.92 (s, 1 H), 6.40 (d, J = 16.0 Hz, 1 H), 6.31 (dd, J = 7.0 and 16 Hz, 1 H), 4.89 (s, 1 H), 4.06 (dq, J = 7.0 and 7.5 Hz, 1 H), 3.73 (t_{app}, J = 7.5 Hz, 1 H), 3.59 (s, 3 H), 3.53 (s, 3 H), 3.24 (s, 3 H), 1.12 (d, J = 7.0 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 176.5 (s), 167.6 (s), 154.0 (s), 135.7 (s), 132.9 (d), 124.0 (d), 117.7 (d), 91.1 (d), 84.0 (d), 57.1 (q), 55.5 (q), 50.8 (q), 39.9 (d), 14.1 (q) ppm. IR: $\tilde{\nu}$ = 2924, 1709, 1623, 1436, 1382, 1147, 1094, 1011 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{18}BrNO_4NaS$ $[M + Na]^+$ 398.0032; found 398.0033.

Cystothiazole A (1).[1a] **From 2:** A solution of **3** (11 mg, 0.047 mmol) in CD_2Cl_2 (0.5 mL) was added in 5 portions (5× 0.1 mL) to a solution of **2** (10 mg, 0.047 mmol) and **GII** (8 mg, 20%) in CD_2Cl_2 (0.2 mL), and the mixture was irradiated for 1 h after each addition (400 W, 100 °C with cooling). Another portion of **GII** (4 mg, 10%) was then added, and the mixture was irradiated for further 30 min before the solvent was evaporated. Purification of the residue by flash chromatography (SiO_2; Et_2O/hexane, 1:2) gave **1** (5 mg, 25%) as a colourless viscous oil. **From 10:** A solution of **10** (8 mg, 0.02 mmol), stannane **11** (8 mg, 0.02 mmol) and $Pd(PPh_3)_4$ (1.2 mg, 5%) in toluene (0.2 mL) was stirred at 110 °C for 16 h. Evaporation of the solvent and purification of the residue by preparative TLC (SiO_2; Et_2O/hexane, 1:2) gave **1** (7 mg, 83%) as a colourless viscous oil. $[\alpha]_D^{20}$ = +110 (c = 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.78 (s, 1 H), 7.02 (s, 1 H), 6.50 (d, J = 15.8 Hz, 1 H), 6.34 (dd, J = 7.8 and 15.8 Hz, 1 H), 4.89 (s, 1 H), 4.11 (dq, J = 7.0 and 7.8 Hz, 1 H), 3.74 (t_{app}, J = 7.8 Hz, 1 H), 3.59 (s, 3 H), 3.53 (s, 3 H), 3.30 (sept, J = 7.0 Hz, 1 H), 3.26 (s, 3 H), 1.37 (d, J = 7.0 Hz, 6 H), 1.14 (d, J = 7.0 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 178.6 (s), 176.7 (s), 167.7 (s), 162.6 (s), 154.3 (s), 148.6 (s), 131.6 (d), 125.5 (d), 115.0 (d), 114.9 (d), 91.1 (d), 84.4 (d), 57.0 (q), 55.5 (q), 50.8 (q), 39.8 (d), 33.3 (d), 23.1 (2q), 14.1 (q) ppm. IR: $\tilde{\nu}$ = 3105, 2968, 1710, 1624, 1150, 1086 cm^{-1}. HRMS (ESI): calcd. for $C_{20}H_{26}N_2O_4NaS_2$ $[M + Na]^+$ 445.1226; found 445.1216.

Acknowledgments

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[10] The reported yield is 75% based on **6** (see ref.[8]).

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