

HETEROCYCLES, Vol. 71, No. 5, 2007, pp. 1203 - 1209. © The Japan Institute of Heterocyclic Chemistry
Received, 21st February, 2007, Accepted, 29th March, 2007, Published online, 29th March, 2007. COM-07-11032

SYNTHESIS OF A DIASTEREOMERIC MIXTURE OF (4*R*,5*S*,6*E*,14*R*)- AND (4*R*,5*S*,6*E*,14*S*)-MELITHIAZOLS G

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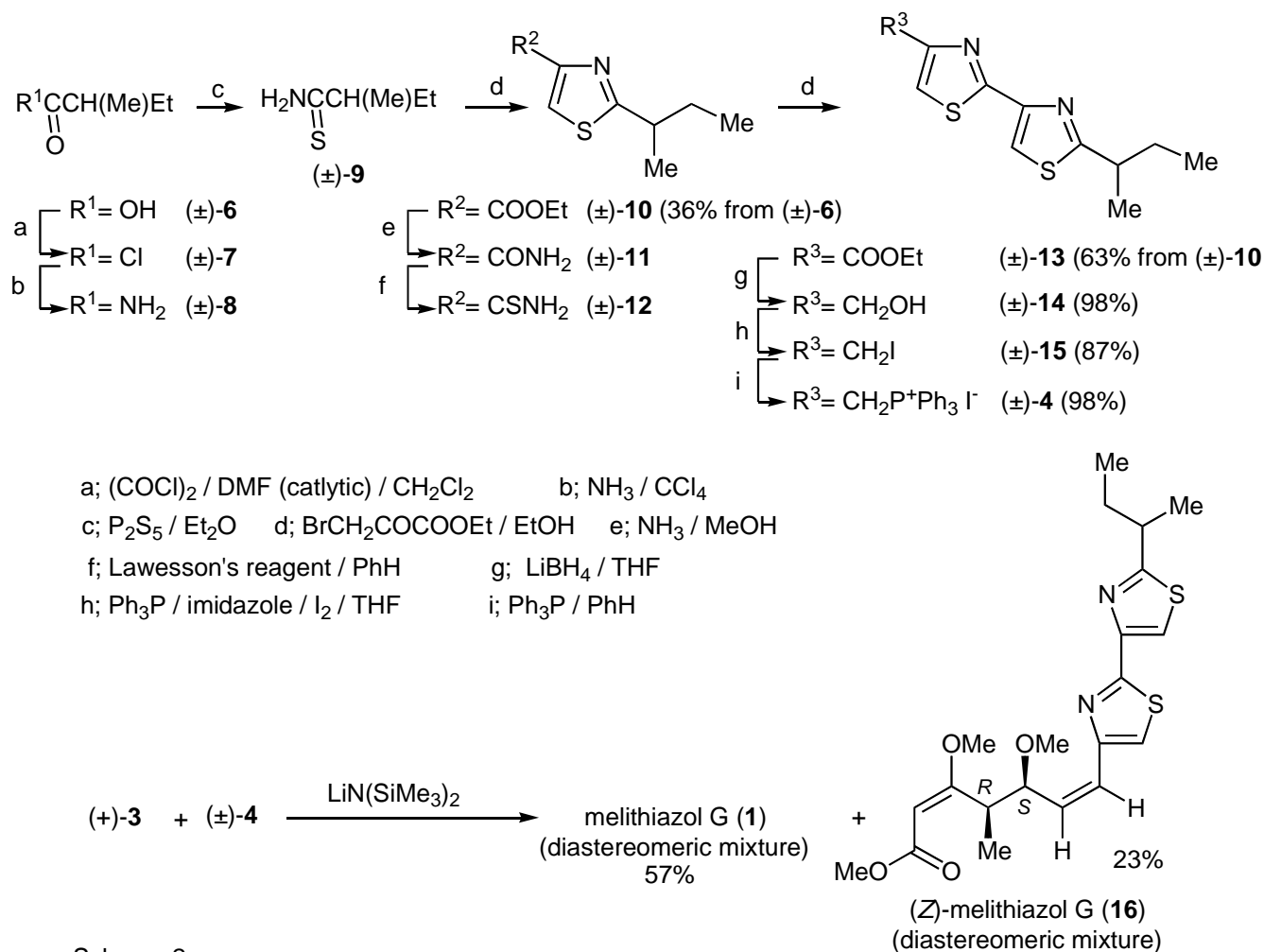
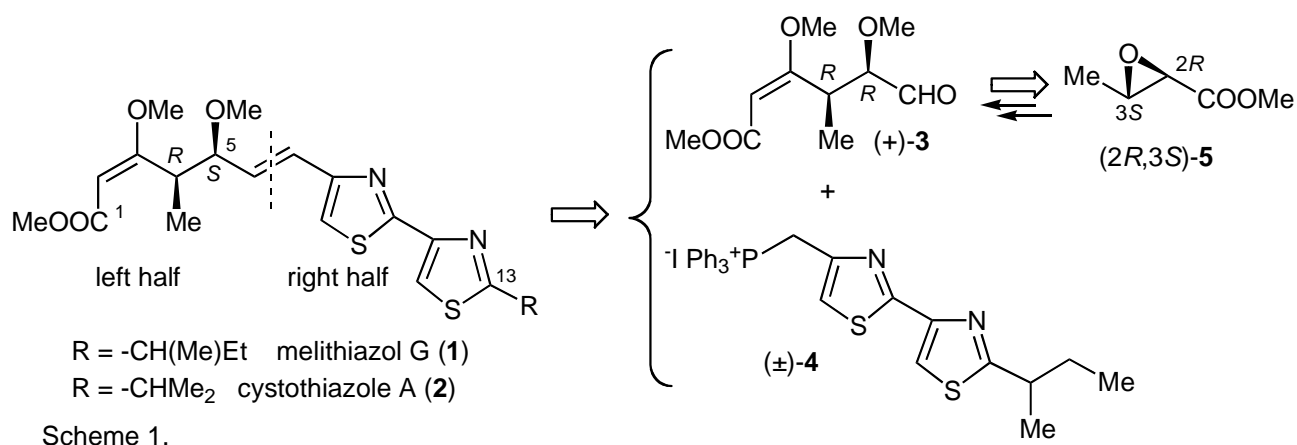
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Abstract- A Wittig reaction between (+)-**3** and the phosphoranylide derived from the bithiazole-type phosphonium iodide [(±)-**4**] using lithium bis(trimethylsilyl)amide afforded a diastereomeric mixture of the (+)-(4*R*,5*S*,6*E*,14*R*)- and (4*R*,5*S*,6*E*,14*S*)-melithiazols G (**1**), whose NMR spectral data were identical with those of the natural product (**1**). The antifungal activity of the synthetic diastereomeric mixture of melithiazols G (**1**) against the phytopathogenic fungus, *Phytophthora capsici*, was evaluated by using a paper disc assay method.

Melithiazol G (**1**) has been isolated from myxobacterium, *Myxococcus stipitatus*, strain Mx s64, and exhibit antifungal, cytotoxic activities and inhibition of NADH oxidation.¹ The structure of **1** was established on the basis of spectroscopic analysis and the absolute configurations of **1** was deduced as (4*R*,5*S*) by the structural similarity with the same type compounds as melithiazol E,¹ which was identical with an antifungal substance named cystothiazole A (**2**)¹ from the myxobacterium *Cystobacter fuscus* strain AJ-13278 by using an inhibition assay against the phytopathogenic fungus, *Phytophthora capsici*.² Information such as a specific rotation for the purpose of confirmation of the absolute structure of **1** was not reported and the absolute configuration of C(14)-carbon was not mentioned. Meanwhile, we already reported the total synthesis of cystothiazole A (**2**) based on a chemoenzymatic method.³ This paper describes the synthesis of a diastereomeric mixture of (4*R*,5*S*,6*E*,14*R*)- and (4*R*,5*S*,6*E*,14*S*)-melithiazols G (**1**) and determination of absolute configurations of C(4) and C(5)-carbons in the natural melithiazol G (**1**) based on the examination of antifungal activity of the synthetic diastereomeric mixture of melithiazols G (**1**). (Scheme 1)

Retrosynthetically, the synthesis of **1** can be achieved by Wittig condensation of the left-half aldehyde [(+)-**3**] and the right-half phosphonium iodide [(±)-**4**]. The synthesis of chiral aldehyde (+)-**3** from



(2*R*,3*S*)-epoxy ester (**5**) was achieved in the total synthesis of cystothiazole A (**2**).³ The synthesis of the right part [(±)-**4**] is shown in Scheme 2.

Treatment of commercially available (±)-2-methylbutyric acid (**6**) with oxalyl chloride gave the corresponding acid chloride [(±)-**7**], which was treated with NH₃ / CCl₄ to afford the corresponding amide [(±)-**8**]. Treatment of (±)-**8** with P₂S₅ gave the corresponding thioamide [(±)-**9**], which was reacted with α-bromopyruvate to provide a mono-thiazole ester [(±)-**10**] in 36% overall yield from (±)-**6**. Treatment of (±)-**10** with NH₃ / MeOH followed by thioamidation with Lawesson's reagent

yielded a thioamide [(±)-**12**], which was reacted with α -bromopyruvate to afford a bithiazole ester [(±)-**13**] in 63% overall yield from (±)-**10**. LiBH₄ reduction (alcohol [(±)-**14**]: 98% yield) of (±)-**13** followed by treatment with I₂/Ph₃P/imidazole provided an iodide [(±)-**15**] in 87% yield. The reaction of (±)-**15** and triphenylphosphine gave a phosphonium salt [(±)-**4**] in 98% yield, which was condensed with (+)-**3** in the presence of lithium bis(trimethylsilyl)amide in THF to afford a mixture [(+)-(6*E*)-**1** / (+)-(6*Z*)-**16** = ca. 2.5:1] of olefins in 80% yield. Both isomers were isolated by means of preparative HPLC to provide (+)-**1** as colorless needles ([α]_D +100.0 (c=0.945, CHCl₃)) and (+)-**16** as a colorless oil ([α]_D +253.6 (c=0.565, CHCl₃)). Although (+)-(6*E*)-**1** and (+)-(6*Z*)-**16** were diastereomeric mixture concerning C(14)-chiral center, ¹H- and ¹³C-NMR spectra seem not to be complex, respectively. The NMR data of the diastereomeric mixture of (+)-**1** were identical with those (¹H-NMR) of the reported melithiazol G (**1**).¹ The (*Z*)-geometry of (+)-**16** was confirmed by the coupling constant (*J*=12.0 Hz) due to the olefinic protons.

The antifungal activity of the synthetic diastereomeric mixture (**1**) against the phytopathogenic fungus, *Phytophthora capsici*, was evaluated by using a paper disc assay method as reported previously.⁴ The minimum dose applied on a paper disc to inhibit the fungal growth was 1 μ g/disc. The synthetic mixture (**1**) also showed the activities at a similar level of dosage (0.2 μ g/disc) in comparison to that (0.2 μ g/disc) of cystothiazole A (**2**).⁴ On the other hand, 6(*Z*)-isomer (**16**) did not indicate antifungal activity. According to the recent studies on antifungal tests using the phytopathogenic fungus, *Phytophthora capsici*, synthetic cystothiazole A (**2**) ((4*R*, 5*S*)-**2**) showed activity up to a dose of 0.04 μ g/disc. However, not only the enantiomer ((4*S*, 5*R*)-**2**) but also the two diastereomers ((4*S*, 5*S*)-**2**) and (4*R*, 5*R*)-**2**) showed no antifungal activity up to 100 μ g/disc.⁵ These results indicate the β -methoxyacrylate unit possessing (4*R*,5*S*,6*E*)-chemical structure is essential for antifungal activity. Therefore, the absolute structure of natural melithiazol G (**1**) might be confirmed as (4*R*,5*S*)-configuration because both natural product and synthetic product indicate antifungal activity, although the tested microorganisms were different.

CONCLUSION

A Wittig reaction between (+)-**3** and the phosphoranylidene derived from the bithiazole-type phosphonium iodide [(±)-**4**] using lithium bis(trimethylsilyl)amide afforded a diastereomeric mixture of (+)-(4*R*,5*S*,6*E*,14*R*)- and (4*R*,5*S*,6*E*,14*S*)-melithiazols G (**1**), whose NMR spectral data were identical with those of the natural product (**1**). The absolute structure of natural melithiazol G (**1**) might be confirmed as (4*R*,5*S*)-configuration because both natural product and synthetic product indicate antifungal activity.

EXPERIMENTAL

All melting points were measured on a Yanaco MP-3S micro melting point apparatus and are uncorrected. ¹H- and ¹³C-NMR spectra were recorded on JEOL AL 400 spectrometer in CDCl₃. HRMS spectra and the FAB spectra were obtained with a JEOL JMS 600H spectrometer. IR spectra

were recorded with a JASCO FT/IR-300 spectrophotometer. The preparative HPLC system was composed of a detector (Shodex RI-1) and a pump (JASCO PU-2080 Plus). HPLC analysis conditions were as follows; column: YMC-Pack ProC₁₈ [150x20 mm and Precolumn (50x20 mm)]. Solvent: MeOH/H₂O (80:20), flow rate: 5 mL/min. 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-sec-Butylthiazole-4-carboxylic Acid Ethyl Ester (10)

i) To a solution of (±)-**6** (1.0 g, 9.8 mmol) and DMF (0.2 mL) in CH₂Cl₂ (15 mL) was added oxalyl chloride (1.7 mL, 19.8 mmol) under argon atmosphere at 0°C and the whole mixture was stirred for 10 min at 0°C. The reaction mixture was evaporated to give a crude (±)-**7**, which was used for the next reaction without further purification. ii) NH₃ gas was poured into the crude (±)-**7** in CCl₄ (10 mL) and the reaction mixture was evaporated to give the crude (±)-**8**, which was used for the next reaction without further purification. iii) To a solution of crude (±)-**8** in Et₂O (20 mL) was added phosphorus pentasulfide (P₄S₁₀; 0.436 g, 0.98 mmol) and the whole mixture was stirred for 2 h at rt. The reaction mixture was diluted with brine and extracted with ether. The organic layer was dried over MgSO₄ and evaporated to give the crude (±)-**9**, which was used for the next reaction without further purification. iv) A mixture of the crude (±)-**9** and ethyl α-bromopyruvate (1.91 g, 9.8 mmol) in EtOH (30 mL) was stirred at reflux for 2 h. The reaction mixture was evaporated, diluted with AcOEt, and washed with 7% aqueous NaHCO₃. The organic layer was dried over MgSO₄ and evaporated to give a crude oil, which was chromatographed on silica gel (40 g, *n*-hexane:AcOEt=10:1) to afford (±)-**10** (0.436 g, 36% overall yield from (±)-**6**) as a pale yellow oil. (±)-**10**: IR (KBr): 1727, 1205 cm⁻¹; ¹H-NMR: 0.94 (3H, t, *J*=7.4 Hz), 1.39 (3H, d, *J*=6.8 Hz), 1.40 (3H, t, *J*=7.2 Hz), 1.65-1.76 (1H, m), 1.79-1.90 (1H, m), 3.15-3.25 (1H, m), 4.42 (2H, q, *J*=7.2 Hz), 8.07 (1H, s). ¹³C-NMR: 11.7, 14.4, 20.9, 30.9, 40.4, 61.3, 126.5, 146.5, 161.6, 178.3. MS (FAB) *m/z*: 214 (M⁺+1).

(±) 2'-sec-Butyl[2,4']bithiazolyl-4-carboxylic Acid Ethyl Ester (13)

i) A mixture of (±)-**10** (2.6 g, 12.2 mmol) and NH₃ saturated MeOH (10 mL) in a sealed tube was stood for 2 d at rt. After cooling, the reaction mixture was evaporated to afford a crude amide (±)-**11**. ii) To a solution of crude (±)-**11** in benzene (40 mL) was added Lawesson's reagent (2.47 g, 6.1 mmol) and the whole mixture was stirred for 20 min at reflux. The reaction mixture was diluted with H₂O and extracted with AcOEt. The organic layer was washed with brine and dried over MgSO₄. The organic layer was evaporated to give the crude thioamide (±)-**12**. iii) To a solution of the crude thioamide (±)-**12** and ethyl α-bromopyruvate (2.38 g, 12.2 mmol) in absolute EtOH (40 mL) was stirred for 1 h at reflux. The reaction mixture was evaporated, diluted with 7% aqueous NaHCO₃ and extracted with AcOEt. The organic layer was washed with brine and dried over MgSO₄. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (60 g, *n*-hexane:AcOEt=10:1) to afford (±)-**13** (2.26 g, 63% from (±)-**10**). Recrystallization of (±)-**13** from

n-hexane gave pale yellow needles. (±)-**13**: mp 61-62 °C; IR (KBr): 1726, 1202 cm⁻¹; ¹H-NMR: 0.97 (3H, t, *J*=7.2 Hz), 1.42 (3H, d, *J*=6.8 Hz), 1.43 (3H, t, *J*=7.2 Hz), 1.68-1.80 (1H, m), 1.82-1.93 (1H, m), 3.11-3.20 (1H, m), 4.45 (2H, q, *J*=7.2 Hz), 8.04 (1H, s), 8.16 (1H, s). ¹³C-NMR: 11.7, 14.4, 20.7, 30.8, 40.1, 61.5, 116.2, 127.6, 147.7, 147.9, 161.5, 163.8, 177.9. Anal. Calcd for C₁₆H₁₄N₂O₂S₂: C, 52.68; H, 5.44; N, 9.45. Found: C, 52.59; H, 5.39; N, 9.22. MS (FAB) *m/z*: 297 (M⁺+1).

(±) **2'-sec-Butyl[2,4']bithiazolyl-4-methanol (14)**

A mixture of (±)-**13** (2.0 g, 6.75 mmol) and LiBH₄ (0.59 g, 27 mmol) in THF (60 mL) was stirred for 3 h at rt. The reaction mixture was diluted with H₂O (20 mL) and the whole was stirred for 15 h at the same temperature. The reaction mixture was extracted with AcOEt and washed with brine, and dried over MgSO₄. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (50 g, *n*-hexane:AcOEt=1:1) to afford (±)-**14** (1.674 g, 98%) as a colorless oil. (±)-**14**: IR (KBr): 3349 cm⁻¹; ¹H-NMR: 0.96 (3H, t, *J*=7.4 Hz), 1.40 (3H, d, *J*=6.8 Hz), 1.66-1.76 (1H, m), 1.80-1.91 (1H, m), 3.11-3.20 (1H, m), 3.97 (1H, br.s), 4.81 (2H, s), 7.19 (1H, s), 7.86 (1H, s). ¹³C-NMR: 11.7, 20.8, 30.7, 40.1, 60.7, 115.1, 115.3, 148.2, 157.2, 163.8, 178.0. Anal. Calcd for C₁₆H₁₄N₂OS₂: C, 51.94; H, 5.55; N, 11.01. Found: C, 51.61; H, 5.61; N, 10.84. MS (FAB) *m/z*: 255 (M⁺+1).

(±) **2'-sec-Butyl[2,4']bithiazolyl-4-methyleneiodide (15)**

To a mixture of (±)-**14** (1.42 g, 5.59 mmol), triphenylphosphine (1.61 g, 6.15 mmol) and imidazole (0.57 g, 8.4 mmol) in THF (15 mL) was added I₂ (1.56 g, 6.15 mmol) under argon atmosphere and the whole mixture was stirred for 10 min at rt. The reaction mixture was diluted with H₂O and extracted with AcOEt. The organic layer was washed with brine and dried over MgSO₄. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (30 g, *n*-hexane:AcOEt=5:1) to afford (±)-**15** (1.77 g, 87%) as pale yellow needles. (±)-**15**: IR (KBr): 2962, 1695, 1499 cm⁻¹; ¹H-NMR: 0.96 (3H, t, *J*=7.4 Hz), 1.40 (3H, d, *J*=6.8 Hz), 1.67-1.77 (1H, m), 1.81-1.91 (1H, m), 3.11-3.20 (1H, m), 4.56 (2H, s), 7.26 (1H, s), 7.87 (1H, s). ¹³C-NMR: -1.40, 11.7, 20.8, 30.7, 40.1, 115.3, 116.7, 148.3, 153.4, 163.3, 177.9. Anal. Calcd for C₁₁H₁₃IN₂S₂: C, 36.27; H, 3.60; N, 7.69. Found: C, 36.52; H, 3.72; N, 7.25. MS (FAB) *m/z*: 365 (M⁺+1).

(±) **2'-sec-Butyl[2,4']bithiazolyl-4-methylenetriphenylphosphonium Iodide (4)**

A mixture of (±)-**15** (1.53 g, 4.20 mmol) and triphenylphosphine (1.21 g, 4.6 mmol) in benzene (30 mL) was stirred for 20 h at reflux. After cooling, the resulting colorless powder (±)-**4** (2.58 g, 98%) was obtained by filtration. (±)-**4**: mp 258-259°C; ¹H-NMR: 0.95 (3H, t, *J*=7.2 Hz), 1.37 (3H, d, *J*=6.8 Hz), 1.64-1.74 (1H, m), 1.78-1.87 (1H, m), 3.10-3.20 (1H, m), 5.46 (2H, q, *J*=14 Hz), 7.27 (1H, s), 7.61-7.68 (6H, m), 7.7-7.84 (9H, m), 8.06 (1H, s). Anal. Calcd for C₂₉H₂₈IN₂PS₂: C, 55.59; H, 4.50; N, 4.47. Found: C, 55.53; H, 4.52; N, 4.36. MS (FAB) *m/z*: 499 (M⁺-I).

Wittig condensation of (+)-3 and (±)-4

To a solution of (±)-**4** (0.695 g, 1.11 mmol) in THF (5 mL) was added lithium

bis(trimethylsilyl)amide (1M solution in THF, 1.11 mL, 1.11 mmol) at 0 °C under argon atmosphere and the whole mixture was stirred for 20 min at the same temperature. A solution of (+)-**3** (0.12 g, 0.55 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 H₂O and extracted with AcOEt. The organic layer was dried over MgSO₄ and evaporated to afford a crude product which was chromatographed on silica gel (10 g, *n*-hexane:AcOEt=20:1) to give a mixture ((*E*) : (*Z*)= ca. 2.5:1) of **1**. This mixture was subjected to preparative HPLC to afford (+)-**1** (0.135 g, 57%) as colorless needles and (+)-**16** (0.057g, 23%) as a colorless oil. (+)-**1** (as a diastereomeric mixture): [α]_D²⁵ +100.0 (c=0.945, CHCl₃); IR (KBr): 3112, 2928, 1712, 1619, 1456, 1377, 1261, 1137, 1080 cm⁻¹; ¹H-NMR (CDCl₃): 0.96 (3H, t, *J*=7.2 Hz), 1.22 (3H, d, *J*=6.8 Hz), 1.40 (3H, t, *J*=7.2 Hz), 1.67-1.77 (1.72) (1H, m), 1.82-1.92 (1.85) (1H, m), 3.13-3.21 (3.16) (1H, m), 3.33 (3H, s), 3.60 (3H, s), 3.67 (3H, s), 3.81(1H, t, *J*=7.6 Hz), 4.17 (1H, dq, *J*=7.6, 6.8 Hz), 4.97 (1H, s), 6.41 (1H, dd, *J*=15.8, 7.6 Hz), 6.57 (1H, d, *J*=15.8 Hz), 7.09 (1H, s), 7.86 (1H, s). ¹³C-NMR (CDCl₃): 11.7, 14.1, 20.8, 30.7, 39.8, 40.1, 50.8, 55.5, 57.0, 84.4, 91.1, 114.8, 115.0, 125.6, 131.6, 148.6, 154.4, 162.6, 167.7, 176.7, 177.8. HRMS (FAB) (m/z): Calcd for C₂₁H₂₈N₂O₄S₂ (M⁺+1): 437.1569. Found: 437.1558. (+)-**16** (as a diastereomeric mixture): [α]_D²⁵ +253.6 (c=0.565, CHCl₃); IR (KBr): 2927, 1711, 1621, 1449, 1379, 1267, 1146, 1090 cm⁻¹; ¹H-NMR (CDCl₃): 0.98 (3H, t, *J*=7.2 Hz), 1.26 (3H, d, *J*=6.8 Hz), 1.41 (3H, t, *J*=6.8 Hz), 1.68-1.78 (1H, m), 1.82-1.93 (1H, m), 3.12-3.22 (1H, m), 3.33 (3H, s), 3.34 (3H, s), 3.67 (3H, s), 4.23 (1H, dq, *J*=9.2, 6.8 Hz), 4.92 (1H, s), 5.10 (1H, t, *J*=9.2 Hz), 5.59 (1H, dd, *J*=12.0, 9.6 Hz), 6.58 (1H, d, *J*=12.0 Hz), 7.22 (1H, s), 7.83 (1H, s). ¹³C-NMR (CDCl₃): 11.7, 14.8, 20.8, 30.7, 39.3, 40.2, 50.8, 55.1, 56.3, 78.6, 91.2, 114.6, 117.8, 125.5, 132.6, 148.8, 153.5, 161.7, 167.8, 176.6, 178.0. HRMS (FAB) (m/z): Calcd for C₂₁H₂₈N₂O₄S₂ (M⁺+1): 437.1569. Found: 437.1586.

ACKNOWLEDGEMENTS

The authors are grateful to Professor Makoto Ojika at Nagaya University in Japan for performance of a biological experiment using the synthetic products. We also thank Professor Kazuo Koike at Toho University in Japan for preparative HPLC separation of the synthetic products performed in his laboratory.

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