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

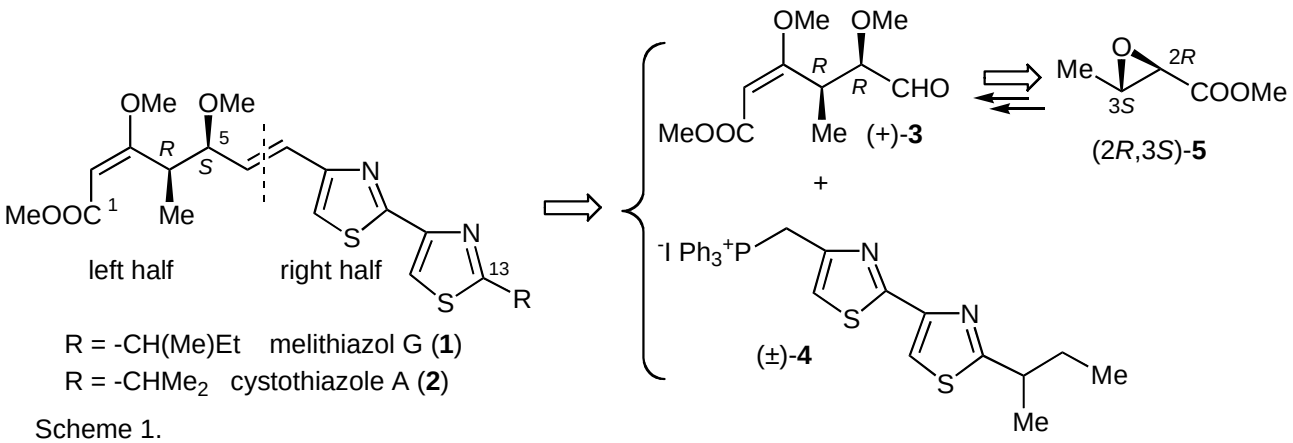
Hiroyuki Takayama,[a] Keisuke Kato,[b] Masayuki Kimura,[a] and Hiroyuki Akita[b]*

[a]School of Pharmacy, Nihon Pharmaceutical University, 10281, Komuro, Inamachi, Kita-Adachigun, Saitama 362-0806, Japan
[b]School of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan

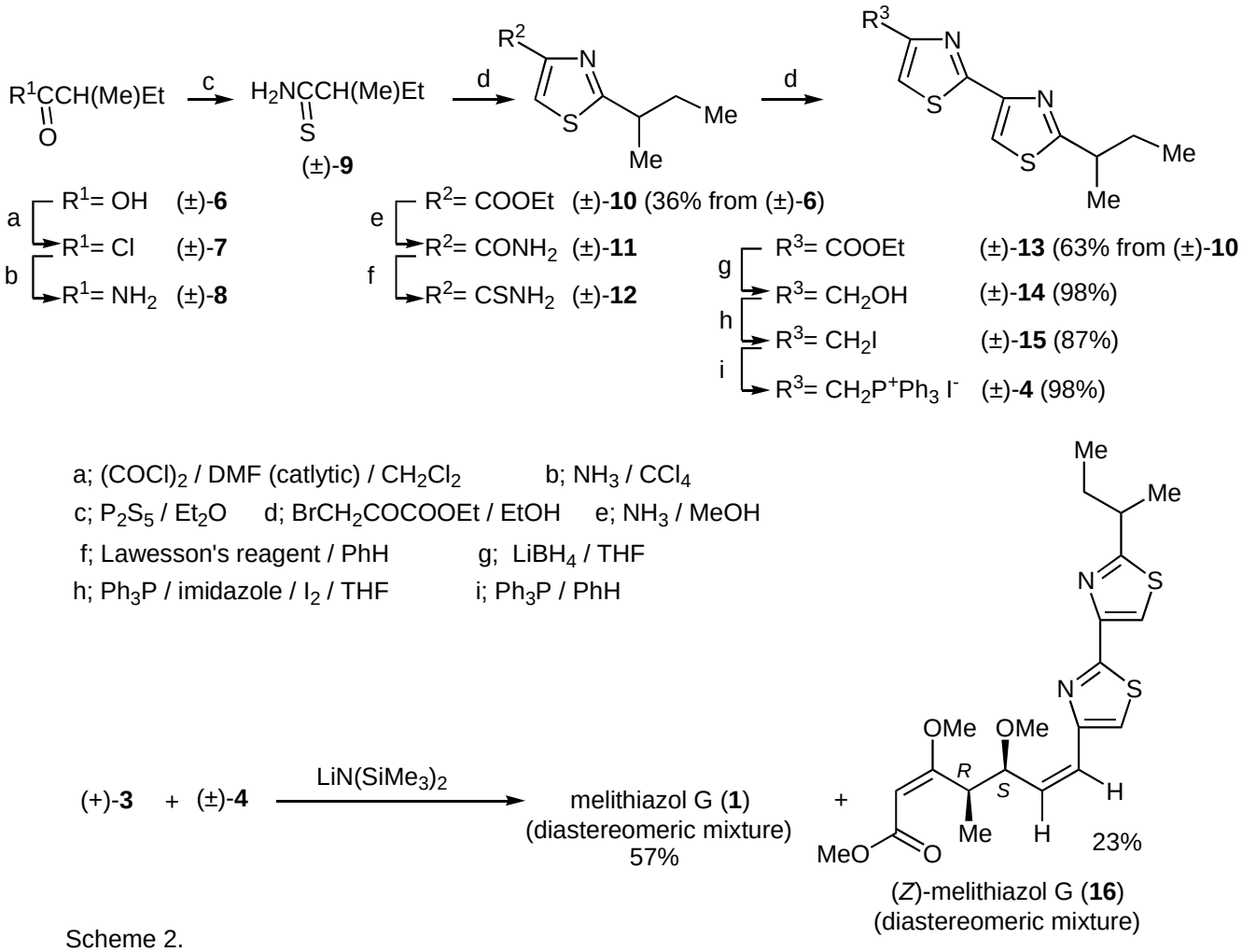
Abstract- A Wittig reaction between (+)-**3** and the phosphoranylide derived from the bithiazole-type phosphonium iodide [(±)-**4**] using lithium bis(trimethylsilyl)amide afforded a diastereomeric mixture of the (+)-(4*R*,5*S*,6*E*,14*R*)- and (4*R*,5*S*,6*E*,14*S*)-melithiazols G (**1**), whose NMR 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*,5S,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



Scheme 1.



Scheme 2.

(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 NH_3 / CCl_4 to afford the corresponding amide [(±)-**8**]. Treatment of (±)-**8** with P_2S_5 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 NH_3 / 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**. $LiBH_4$ reduction (alcohol [(±)-**14**]: 98% yield) of (±)-**13** followed by treatment with I_2/Ph_3P/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 ($[\alpha]_D$ +100.0 (c=0.945, $CHCl_3$)) and (+)-**16** as a colorless oil ($[\alpha]_D$ +253.6 (c=0.565, $CHCl_3$)). Although (+)-(6*E*)-**1** and (+)-(6*Z*)-**16** were diastereomeric mixture concerning C(14)-chiral center, ^{1}H- and ^{13}C-NMR spectra seem not to be complex, respectively. The NMR data of the diastereomeric mixture of (+)-**1** were identical with those (^{1}H-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 μg/disc. The synthetic mixture (**1**) also showed the activities at a similar level of dosage (0.2 μg/disc) in comparison to that (0.2 μg/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 phosphoranylide 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. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL AL 400 spectrometer in $CDCl_3$. 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 ProC_{18} [150x20 mm and Precolumn (50x20 mm)]. Solvent: MeOH/H_2O (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 CH_2Cl_2 (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) NH_3 gas was poured into the crude (±)-**7** in CCl_4 (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 Et_2O (20 mL) was added phosphorus pentasulfide (P_4S_{10}; 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 $MgSO_4$ 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 $NaHCO_3$. The organic layer was dried over $MgSO_4$ 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}; ^{1}H-NMR: δ 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). ^{13}C-NMR: δ 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 NH_3 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 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 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 $NaHCO_3$ 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 (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}; ^{1}H-NMR: δ 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). ^{13}C-NMR: δ 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 $C_{16}H_{14}N_2O_2S_2$: 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 $LiBH_4$ (0.59 g, 27 mmol) in THF (60 mL) was stirred for 3 h at rt. The reaction mixture was diluted with H_2O (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 $MgSO_4$. 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}; ^{1}H-NMR: δ 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). ^{13}C-NMR: δ 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 $C_{16}H_{14}N_2OS_2$: 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 I_2 (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 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 (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}; ^{1}H-NMR: δ 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). ^{13}C-NMR: δ -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 $C_{11}H_{13}IN_2S_2$: 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; ^{1}H-NMR: δ 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 $C_{29}H_{28}IN_2PS_2$: 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(trimethylisilyl)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 H_2O and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to afford a crude product which was chromatographed on silica gel (10 g, *n*-hexane:AcOEt=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 diasteromeric mixture): $[\alpha]_D^{25}$ +100.0 (c=0.945, $CHCl_3$); IR (KBr): 3112, 2928, 1712, 1619, 1456, 1377, 1261, 1137, 1080 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 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). ^{13}C-NMR ($CDCl_3$): δ 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 $C_{21}H_{28}N_2O_4S_2$ (M^++1): 437.1569. Found: 437.1558. (+)-**16** (as a diastereomeric mixture): $[\alpha]_D^{25}$ +253.6 (c=0.565, $CHCl_3$); IR (KBr): 2927, 1711, 1621, 1449, 1379, 1267, 1146, 1090 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 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). ^{13}C-NMR ($CDCl_3$): δ 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 $C_{21}H_{28}N_2O_4S_2$ (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.

REFERENCES AND NOTE

1 B. Böhlendrof, M. Herrmann, H. -J. Hecht, F. Sasse, E. Forche, B. Kunze, H. Reichenbach, and G. Höfle, *Eur. J. Org. Chem*., 1999, 2601.

2 Y. Suzuki, M. Ojika, Y. Sakagami, R. Fudou, and S. Yamanaka, *Tetrahedron*, 1998, **54**, 11399.

3 a) K. Kato, K, A. Nishimura, Y. Yamamoto, and H. Akita, *Tetrahedron Lett.*, 2002, **43**, 643.
b) K. Kato, T. Sasaki, H. Takayama, and H. Akita, *Tetrahedron*, 2003, **59**, 2679.
c) H. Akita, N. Sutou, T. Sasaki, and K. Kato, *Tetrahedron*, 2006, **62**, 11592.

4 M. Ojika, Y. Suzuki, A. Tsukamoto, Y. Sakagami, R. Fudou, T. Yoshimura, and S. Yamanaka, *J. Antibiot.*, 1998, **51**, 275.

5 M. Ojika, T. Watanabe, J. Qi, T. Tanino, and Y. Sakagami, *Tetrahedron*, 2004, **60**, 187.