Synthesis of (–)-Actinonin

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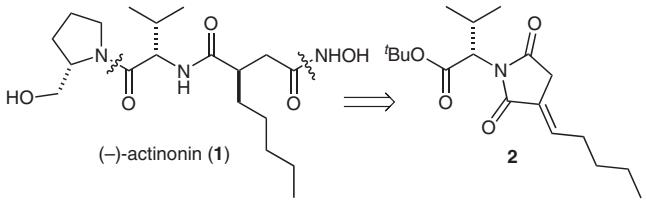
Abstract: Synthesis of (–)-actinonin in 17% overall yield was accomplished in seven steps via the formation of an isoimide derivative as the key intermediate. The synthesis was carried out using commercially available dimethyl maleate without the use of a highly expensive reagent.

Key words: actinonin, PDF inhibitor, antibacterial agent, total synthesis, catalytic hydrogenation

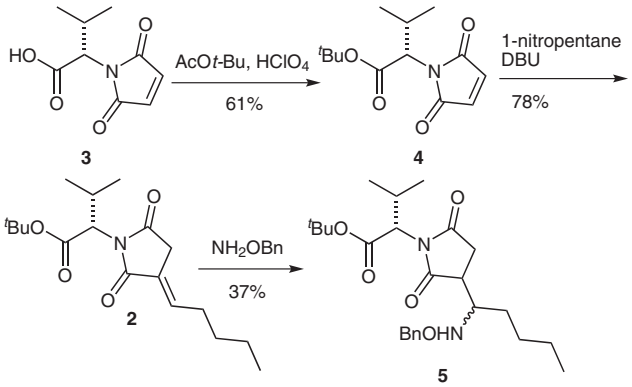
(–)-Actinonin (**1**) is a naturally occurring antibacterial agent that exhibits potent peptide deformylase (PDF) inhibitory activity.[1] As yet, two total synthetic methods for **1** have been reported;[2] however, the total yield of **1** in these methods was low because of the difficulty involved in the separation of the isomers[2a] and chiral induction.[3b] In this paper, we report a total synthesis of **1** in 17% overall yield.

The first synthetic strategy of **1** involved a key reaction of succinimide with *O*-benzylhydoxylamine (Scheme 1).



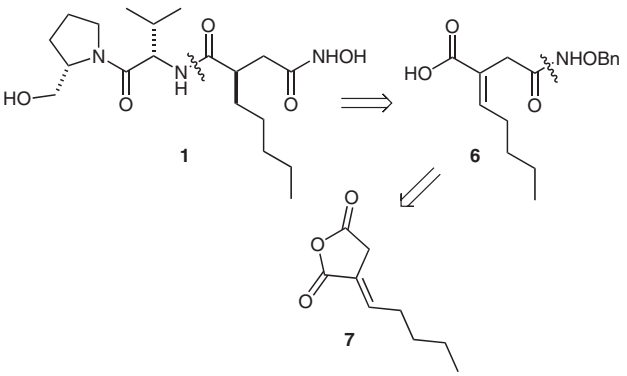
Scheme 1

The key intermediate **2** was obtained by the addition–elimination reaction[3] of a valine maleimide ester **4**,[4a,b] which was obtained by the esterification of a known carboxylic acid **3**,[5] with 1-nitropentane in the presence of a base (Scheme 2). The *E*-configuration of the pentylidene group of **2** was established from the chemical shift value (δ = 6.83 ppm) of a deshielded methine proton in the ^{1}H NMR spectrum. The reaction of **2** with NH_2OBn in refluxing methanol for three days did not afford a ring-opened product, but instead yielded a 1,4-addition product **5**. Thus, the desired compound was not obtained; however, we could find that the N-substituted succinimide derivative **2** does not easily react with NH_2OBn.



Scheme 2

The next synthetic strategy of **1** involved a key reaction of succinic anhydride derivative **7** with NH_2OBn to produce the hydroxamate **6** (Scheme 3). We expected that the conversion from succinimide into the succinic anhydride derivative would accomplish the synthesis of **1**.



Scheme 3

Intermediate **7** was derived in three steps from dimethyl maleate (**8**) (Scheme 4). The *E*-configuration of the pentylidene group of **7** was established from the chemical shift value (δ = 7.04 ppm) of a deshielded methine proton in the ^{1}H NMR spectrum. In contrast to the reaction between **2** and NH_2OBn, the reaction of **7** with NH_2OBn under mild, room-temperature conditions for 30 minutes successfully afforded a ring-opened product in high yield. At this point, it was not possible to determine whether the structure of the product corresponds to **6** or **6′**. We expected that the hydroxylamine would attack the less hindered and more electrophilic carbonyl carbon atom of the succinic anhydride moiety **7** to afford **6**.

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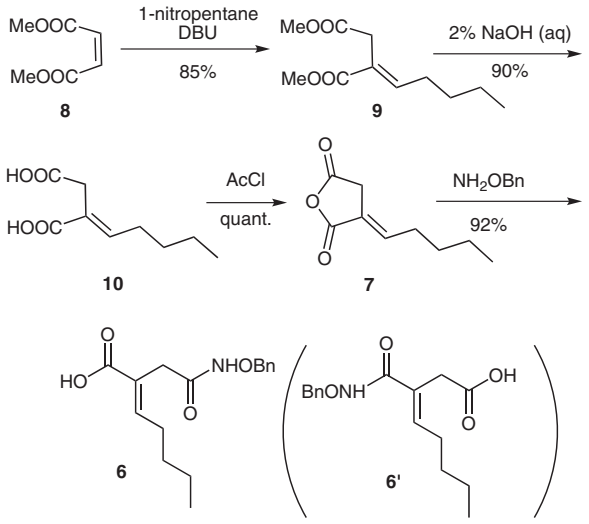
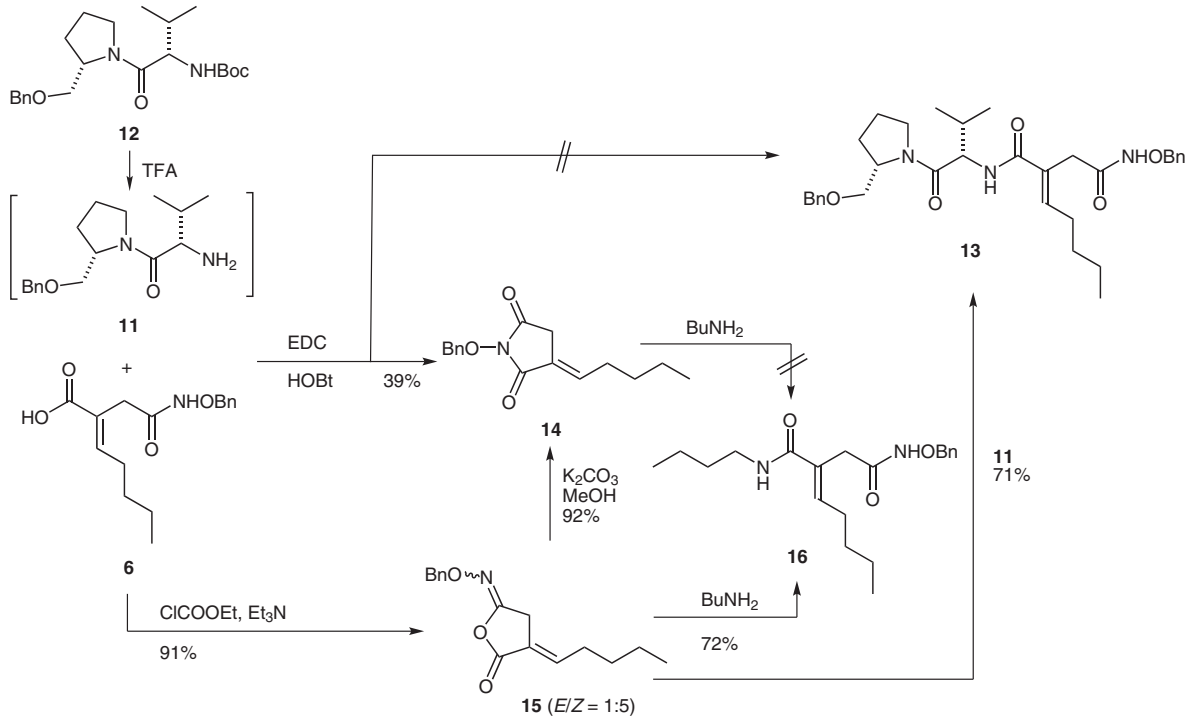


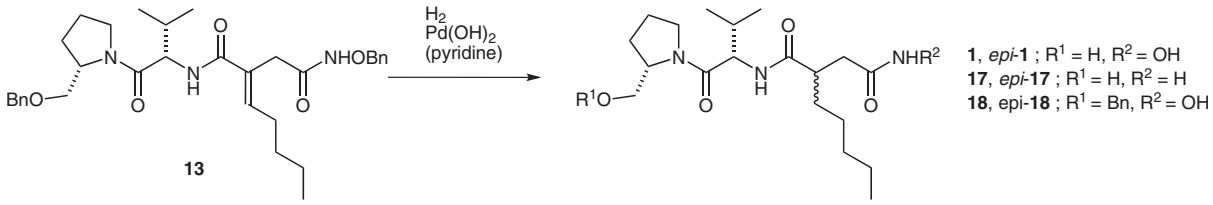
Scheme 4

Condensation of **6** with amine **11**, which was obtained by the deprotection of reported prolinol derivative **12**,[2b] using EDC/HOBt did not afford the desired amide **13**, but instead afforded the succinimide **14**. The product **14** was subjected to intramolecular dehydration; however, it did not react with butylamine (Scheme 5). In contrast, treatment of **6** with ethyl chloroformate afforded isoimide **15**[6] as an *E*/*Z* mixture (isolated ratio = 1:5) based on oxime double bond (C=N) in excellent yield. We determined the configuration of each product by the chemical shift value of the methylene protons [(*E*)-**15**: 3.56–3.58 ppm; (*Z*)-**15**: 3.44–3.46 ppm] in the isoimide ring in the ^{1}H NMR spectrum. Based on our finding that **15** can react with butylamine to afford the ring-opened product **16**, we successfully synthesized **13** in satisfactory yield from the coupling reaction of **15** with **11**. Treatment of **15** with potassium carbonate in methanol at room temperature afforded **14** in 92% yield over 15 minutes.

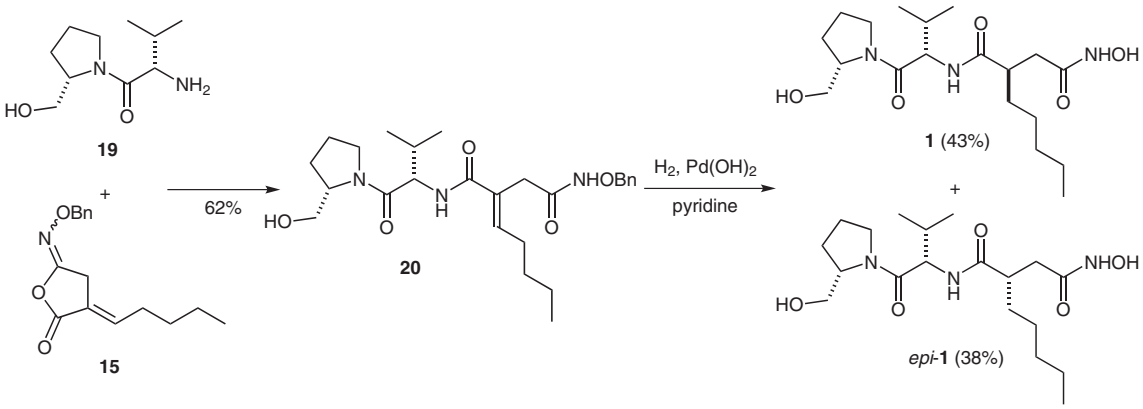
While the catalytic hydrogenation of **13** afforded **1** along with its epimer (*epi*-**1**), the yield of **1** decreased considerably because of the production of over-reduction product **17** and *epi*-**17** (Scheme 6). It is known that pyridine on catalytic hydrogenation can inhibit over-reduction of N–O bond cleavage.[7] Therefore, catalytic hydrogenation of **13** was attempted in the presence of pyridine; however, debenzylation of the benzyl group protected prolinol did not occur, giving an inseparable mixture of **18** and its epimer *epi*-**18**. From these experimental results, we conclude that the ease of reduction of the functional group of



Scheme 5



Scheme 6



Scheme 7

13 in the presence of pyridine is of the order OBn (hydroxamate) > double bond > NOH (hydroxamic acid) > OBn (prolinol).

Based on these observations, a synthetic method starting from the unprotected prolinol derivative **19** (Scheme 7) was developed. The coupling reaction of isoimide **15** with valinylprolinol **19**[2a] afforded **20**, in which the hydroxy group of prolinol was free, in moderate yield. The catalytic hydrogenation of **20** in the presence of $Pd(OH)_2$ and pyridine proceeded as expected to afford only **1** and its epimer *epi*-**1** in 43 and 38% yield, respectively. Thus, the synthesis of actinonin (**1**) in 17% overall yield was accomplished in seven steps from commercially available dimethyl maleate (**8**) without using highly expensive reagents.

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR 350 spectrometer. Mass spectra (MS) were recorded on an AutoSpec spectrometer. ^{1}H NMR and ^{13}C NMR spectra were run on a Varian Mercury 300, a Varian VXR500, or a Varian Unity INOVA AS600 spectrometer. Optical rotations were measured on a Perkin-Elmer 2400II polarimeter.

(*S*)-*N*-[(1-*tert*-Butoxycarbonyl-2-methyl)propyl]maleimide (4)

To a solution of carboxylic acid **3** (443 mg, 2.33 mmol)[7] in *t*-BuOAc (5.60 mL) was added dropwise 60% $HClO_4$ (26.0 μL, 0.239 mmol) at 0 °C and the mixture was stirred for 3 h under argon atmosphere. The reaction mixture was poured into EtOAc (5 mL) at the same temperature. The organic layer was washed with H_2O (10 mL), sat. aq $NaHCO_3$ (10.5 mL), and brine (5 mL), dried over ($MgSO_4$), filtered, and concentrated to give **4** as a pale yellow oil; yield: 358 mg (61%); $[\alpha]_D^{24}$ –39.6 (*c* = 1.0, MeOH).

IR (neat): 1715, 1740, 1775 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.86 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.07 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.42 [s, 9 H, $(CH_3)_3C$], 2.56–2.70 [m, 1 H, $(CH_3)_2CH$], 4.44 (d, *J* = 8.1 Hz, 1 H, $CHCO_2t$-Bu), 6.73 (s, 2 H, CH=CH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 19.38, 20.74, 27.74, 28.29, 58.41, 82.06, 134.00, 167.53, 170.23 (CO).

Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.60; H, 7.80; N, 5.64.

(*S*)-*N*-[(1-*tert*-Butoxycarbonyl-2-methyl)propyl]-2-{(*E*)-pentylidene}succinimide (2)

To a solution of **4** (8.44 g, 33.3 mmol) and 1-nitropentane (3.92 g, 33.5 mmol) in anhyd THF (170 mL) was added DBU (4.80 mL, 32.2 mmol) at r.t. and the mixture was stirred for 20 min at the same temperature under argon atmosphere. To the reaction mixture was added silica gel (Merck silica gel 60, 6.0 g). After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 7.0 cm, *l* = 12 cm; EtOAc–hexane, 1:8–1:6) to give **2** as a colorless oil; yield: 8.45 g (78%); $[\alpha]_D^{24}$ –44.4 (*c* = 1.0, MeOH).

IR (neat): 1715, 1740, 1775 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.83 [d, *J* = 6.9 Hz, 3 H, $(CH_3)_2CH$], 0.92 (t, *J* = 7.2 Hz, 3 H, CH_3CH_2), 1.11 [d, *J* = 6.9 Hz, 3 H, $(CH_3)_2CH$], 1.29–1.54 (m, 4 H, $CH_3CH_2CH_2$), 1.42 [s, 9 H, $(CH_3)_3C$], 2.19 (q, *J* = 7.5 Hz, 2 H, CH_2CH=C), 2.58–2.70 [m, 1 H, $(CH_3)_2CH$], 3.24 (t, *J* = 1.2 Hz, 2 H, CH_2CO), 4.38 (d, *J* = 8.4 Hz, 1 H, $CHCO_2t$-Bu), 6.83 (tt, *J* = 7.5, 2.1 Hz, 1 H, CH=C).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.71, 19.45, 21.11, 22.28, 27.78, 27.84, 29.50, 30.11, 31.64, 58.75, 81.97, 124.93, 139.34, 167.34, 169.31, 173.49.

Anal. Calcd for $C_{18}H_{29}NO_4$: C, 66.84; H, 9.04; N, 4.33. Found: C, 66.74; H, 9.04; N, 4.47.

Reaction of 2 with *O*-Benzylhydroxylamine; (2*RS*,1′*RS*,1″*RS*)-*N*-(1-*tert*-Butoxycarbonyl-2-methylpropyl)-2-[(1-benzyloxyamino)pentyl]succinimide (5)

To a solution of **2** (217 mg, 0.671 mmol) and *O*-benzylhydroxylamine hydrochloride (325 mg, 2.04 mmol) in MeOH (6 mL) was added *N*-methylmorpholine (225 μL, 2.05 mmol) at r.t. and the mixture was stirred at reflux for 70 h under argon atmosphere. The mixture was poured into sat. aq $NaHCO_3$ (5 mL) and extracted with EtOAc (10 mL). The organic layer was washed with brine (5 mL) and dried ($MgSO_4$). After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 7.0 cm, *l* = 11 cm; EtOAc–hexane, 1:6) to give **5** as a diastereomeric mixture; yield: 114 mg (37%); pale yellow oil.

IR (neat): 1705, 1740, 1778 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.77–0.91 [m, 6 H, $(CH_3)_2CH$ and CH_3CH_2], 1.08 [d, *J* = 6.6 Hz, 3 H, $(CH_3)_2CH$], 1.20–1.43 [1m, 5 H, $(CH_3)_3C$ and $CH_3(CH_2)_3CH_2$], 2.42–2.78 and 3.06–3.50 [m, 5 H, CH_2CO, CHCON, CHNHOBn and $(CH_3)_2CH$], 4.25–4.33 (m, 1 H, $CHCO_2t$-Bu), 4.56–4.68 (m, 2 H, CH_2Ph), 5.65 (br s, 1 H, NH), 7.28–7.39 (m, 5 H, ArH).

Anal. Calcd for $C_{25}H_{38}N_2O_5$: C, 67.24; H, 8.58; N, 6.27. Found: C, 67.40; H, 8.88; N, 6.32.

(*E*)-Dimethyl Pentylidenebutanedioate (9)

To a solution of dimethyl maleate (**8**; 5.00 mL, 40.0 mmol) and 1-nitropentane (4.68 g, 40.0 mmol) in anhyd THF (150 mL) was added DBU (5.95 mL, 40.0 mmol) at r.t. and the mixture was stirred at the same temperature for 5 h under argon atmosphere. After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 7.0 cm, *l* = 11 cm; EtOAc–hexane, 1:6–1:4) to give **9** as colorless oil; yield: 7.29 g (85%).

IR (neat): 1655, 1720, 1742 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.90 (t, *J* = 6.9 Hz, 3 H, CH_3CH_2), 1.28–1.49 [m, 4 H, $CH_3(CH_2)_2$], 2.18 [q, *J* = 7.5 Hz, 2 H, $CH_3(CH_2)_2CH_2$], 3.36 (s, 2 H, $CH_2CO_2CH_3$), 3.68 and 3.74 (s, 6 H, 2 × CH_3O), 6.97 (t, *J* = 7.5 Hz, 1 H, CH=C).

(*E*)-Pentylidenesuccinic Acid (10)

To a solution of **9** (3.80 g, 17.7 mmol) in EtOH (70 mL) was added aq 2% NaOH (70 mL) at r.t. and the mixture was stirred at reflux for 5 h. The mixture was poured into H_2O (600 mL) and washed with EtOAc (2 × 120 mL). The aqueous layer was acidified with aq 5 M HCl (100 mL) and extracted with EtOAc (3 × 120 mL). The combined organic layers were washed with brine (160 mL), dried ($MgSO_4$), filtered, and concentrated. Recrystallization (EtOAc–hexane) from the residue gave **10** as colorless prisms; yield: 2.97 g (90%); mp 148–149 °C (EtOAc–hexane).

IR (KBr): 1640, 1700, 2930 cm^{-1}.

^1H NMR (300 MHz, DMSO-d_6): δ = 0.84 (t, *J* = 6.9 Hz, 3 H, CH_3), 1.20–1.40 [m, 4 H, $CH_3(CH_2)_2$], 2.09 [q, *J* = 7.5 Hz, 2 H, $CH_3(CH_2)_2CH_2$], 3.17 (s, 2 H, CH_2CO_2H), 6.75 (t, *J* = 7.5 Hz, 1 H, CH=C), 12.18 (s, 2 H, 2 × CO_2H).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 13.84, 21.95, 27.98, 30.33, 32.09, 126.85, 144.16, 168.23, 172.15.

Anal. Calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58. Found: C, 58.05; H, 7.29.

(*E*)-Pentylidenesuccinic Anhydride (7)

A mixture of **10** (6.00 g, 32.2 mmol) and acetyl chloride (17.2 mL) was stirred at reflux for 3 h. After removal of the solvent, EtOAc (100 mL) was added to the residue. The organic layer was washed with sat. aq $NaHCO_3$ (60 mL) and brine (60 mL), dried ($MgSO_4$), filtered, and concentrated to give **7** as a pale yellow oil; yield: 5.50 g (quant).

IR (neat): 1680, 1780, 1840 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 0.93 (t, *J* = 7.2 Hz, 3 H, CH_3), 1.36 (qt, *J* = 7.5, 7.5 Hz, 2 H, CH_3CH_2), 1.47–1.54 (m, 2 H, $CH_3CH_2CH_2$), 2.23 [qt, *J* = 7.5, 1.5 Hz, 2 H, $CH_3(CH_2)_2CH_2$], 3.48 (td, *J* = 2.5, 1.5 Hz, 2 H, CH_2CO), 7.04 (tt, *J* = 7.5, 1.5 Hz, 1 H, CH=C).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.60, 22.22, 26.68, 30.34, 31.69, 122.35, 146.04, 164.92, 168.61.

Anal. Calcd for $C_9H_{12}O_3$: C, 64.27; H, 7.19. Found: C, 64.06; H, 7.37.

(*E*)-*N*-Benzyloxy-2-pentylidenesuccinamic Acid (6)

To a solution of **7** (2.52 g, 15.0 mmol) and *O*-benzylhydroxylamine hydrochloride (2.63 g, 16.5 mmol) in $CHCl_3$ (6 mL) was added *N*-methylmorpholine (1.81 mL, 16.5 mmol) at r.t. and the mixture was stirred at the same temperature for 30 min under argon atmosphere. The mixture was washed with 1 M aq HCl solution (90 mL) and brine (60 mL), and dried ($MgSO_4$). After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 5.5 cm, *l* = 6.0 cm; EtOAc) to give **6** as colorless needles; yield: 4.03 g (92%); mp 86.2–88.4 °C (EtOAc–hexane).

IR (KBr): 1658, 1688, 3235 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.92 (t, *J* = 7.2 Hz, 3 H, CH_3), 1.30–1.51 [m, 4 H, $CH_3(CH_2)_2$], 2.30–2.44 [m, 2 H, $CH_3(CH_2)_2CH_2$], 3.12 (s, 2 H, $CH_2CONHOBn$), 4.88 (s, 2 H, CH_2Ph), 7.11 (t, *J* = 7.5 Hz, 1 H, CH=C), 7.36 (s, 5 H, ArH), 8.58 (br s, 1 H, NH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.85, 22.42, 29.10, 30.44, 32.02, 78.01, 124.15, 128.56, 128.73, 129.26, 135.07, 150.55, 167.76, 172.09.

Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 65.79; H, 7.20; N, 4.78.

Reaction of 6 with 11; (*E*)-*N*-Benzyloxypentylidenesuccinimide (14)

To a solution of 2-benzyloxymethyl-*N*-(*N*-*tert*-butoxycarbonyl-L-valinyl)pyrrolidine (**12**; 82.7 mg, 0.204 mmol) in anhyd CH_2CH_2 (0.4 mL) was added TFA (0.224 mL) at r.t. and the mixture was stirred at the same temperature for 35 min under argon atmosphere. The mixture was poured into EtOAc (10 mL) and the organic layer was washed with sat. aq $NaHCO_3$ (7 mL), dried ($MgSO_4$), and concentrated to give the amine **11** as a pale colorless oil. To a solution of **11** and **6** (64.9 mg, 0.223 mmol) in anhyd DMF (1.0 mL) was added EDC (42.0 mg, 0.220 mmol) at r.t. and the mixture was stirred at the same temperature for 2.5 h under argon atmosphere. The mixture was poured into EtOAc (10 mL) and the organic layer was washed with sat. aq $NaHCO_3$ (7 mL), H_2O (7 mL), and brine (7 mL), and dried ($MgSO_4$). After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 2.0 cm, *l* = 11 cm; EtOAc–hexane, 1:4) to give **14** as colorless prisms; yield: 24.0 mg (39%); mp 74.2–75.2 °C (hexane).

IR (neat): 1675, 1715, 1775 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.91 (t, *J* = 7.2 Hz, 3 H, CH_3), 1.25–1.52 [m, 4 H, $CH_3(CH_2)_2$], 2.16 [q, *J* = 7.5 Hz, 2 H, $CH_3(CH_2)_2CH_2$], 3.16 (t, *J* = 1.2 Hz, 2 H, CH_2CO), 5.14 (s, 2 H, CH_2Ph), 6.84 (tt, *J* = 7.5, 2.4 Hz, 1 H, CH=C), 7.34–7.42 and 7.47–7.54 (m, 5 H, ArH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.75, 22.31, 29.07, 29.98, 30.06, 78.82, 121.75, 128.47, 129.31, 129.83, 133.39, 140.47, 164.78, 168.33.

Anal. Calcd for $C_{16}H_{21}NO_4$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.21; H, 6.77; N, 5.10.

FAB-MS: *m*/*z* = 274 (M^+ + 1).

(*E*)-*N*-Benzyloxy-2-pentylidenesuccinimidic Anhydride (15)

To a solution of **6** (1.47 g, 5.05 mmol) and Et_3N (766 μL, 5.50 mmol) in anhyd THF (30 mL) was added ethyl chlorocarbonate (522 μL, 5.50 mmol) at r.t. and the mixture was stirred at the same temperature for 1 h under argon atmosphere. The mixture was poured into EtOAc (225 mL). The organic layer was washed with 1 M aq HCl (100 mL), sat. aq $NaHCO_3$ (100 mL), H_2O (100 mL), and brine (70 mL), and dried ($MgSO_4$). After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 4.0 cm, *l* = 13 cm; EtOAc–hexane, 1:8–1:6) to give **15**; yield: 1.25 g (91%). (*E*)- and (*Z*)-**15** (1:5) were separated by repeated column chromatography.

(*Z*)-15 (Major Product)

Colorless oil.

IR (neat): 1670, 1690, 1808 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 0.92 (t, *J* = 7.5 Hz, 3 H, CH_3), 1.31–1.38 (m, 2 H, CH_3CH_2), 1.45–1.51 (m, 2 H, $CH_3CH_2CH_2$), 2.18–2.23 [m, 2 H, $CH_3(CH_2)_2CH_2$], 3.44–3.46 (m, 2 H, $CH_2C{=}N$), 5.06 (s, 2 H, CH_2Ph), 6.95 (tt, *J* = 7.5, 3.0 Hz, 1 H, CH=C), 7.29–7.41 (m, 5 H, ArH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.66, 22.25, 26.99, 29.75, 30.17, 76.60, 121.76, 127.90, 128.17, 128.29, 137.23, 145.22, 147.84, 165.19.

Anal. Calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.05; H, 7.23; N, 5.04.

FAB-MS: *m*/*z* = 274 (M^+ + 1).

(*E*)-15 (Minor Product)
Colorless oil.

IR (Nujol): 1672, 1692, 1808 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (t, *J* = 7.2 Hz, 3 H, CH_3), 1.25–1.53 [m, 4 H, $CH_3(CH_2)_2$], 2.16–2.27 [m, 2 H, $CH_3(CH_2)_2CH_2$], 3.56–3.58 (m, 2 H, CH_2C=N), 5.05 (s, 2 H, CH_2Ph), 6.93 (tt, *J* = 7.8, 3.0 Hz, 1 H, CH=C), 7.30–7.42 (m, 5 H, ArH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.74, 22.36, 26.34, 29.84, 30.27, 76.89, 121.72, 127.97, 128.43, 128.59, 136.88, 145.25, 159.27, 165.69.

Reaction of 15 with Base; (*E*)-*N*-Benzyloxypentylidenesuccinimide (14)
To a solution of **15** (27.5 mg, 0.101 mol) in MeOH (0.5 mL) was added K_2CO_3 (15.3 mg, 0.111 mmol) at r.t. and the mixture was stirred at the same temperature under argon atmosphere. The mixture was poured into EtOAc (10 mL), and the organic layer was washed with brine (8 mL) and dried ($MgSO_4$). The solvent was removed to give **14** (27 mg, 92%), which was identified with compound obtained from the reaction of **6** with **11** by ^{1}H NMR data.

Reaction of 15 with Butylamine; (*E*)-*N*-Benzyloxy-*N*′-butyl-3-pentylidenesuccinimide (16)
To a solution of **15** (47.0 mg, 0.172 mmol) in CH_2Cl_2 (1.2 mL) was added butylamine (20.0 μL, 0.202 mmol) at r.t. and the mixture was stirred at the same temperature for 6 h under argon atmosphere. The mixture was poured into EtOAc (10 mL) and the organic layer was washed with aq 1 M aq HCl (5 mL), sat. aq $NaHCO_3$ (5 mL), H_2O (5 mL), and brine (5 mL), and dried ($MgSO_4$). After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 2.0 cm, *l* = 8 cm; EtOAc–hexane, 1:1–2:1) to give **16** as colorless needles; yield: 43.0 mg (72%); mp 90.8–93.2 °C (EtOAc–hexane).

IR (Nujol): 1642, 1692, 1702, 3200, 3300 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.90 (t, *J* = 7.2 Hz, 3 H, CH_3), 0.94 (t, *J* = 7.2 Hz, 3 H, CH_3), 1.28–1.57 [m, 8 H, 2 $CH_3(CH_2)_2$], 2.25 [td, *J* = 7.2, 7.2 Hz, 2 H, $CH_3(CH_2)_2CH_2CH$], 3.09 (s, 2 H, CH_2CONHOBn), 3.26 [td, *J* = 7.2, 6.0 Hz, 2 H, $CH_3(CH_2)_2CH_2NH$], 4.88 (s, 2 H, CH_2Ph), 6.07 (br s, 1 H, NH), 6.17 (t, *J* = 7.2 Hz, 1 H, CH=C), 7.30–7.44 (m, 5 H, ArH), 9.91 (br s, 1 H, NH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.71, 13.85, 20.07, 22.36, 28.27, 30.81, 31.45, 33.59, 39.64, 77.95, 128.37, 128.44, 129.02, 129.58, 135.37, 138.82, 168.15, 170.10.

Anal Calcd for $C_{20}H_{30}N_2O_3$: C, 69.33; H, 8.73; N, 8.09. Found: C, 69.30; H, 8.61; N, 8.18.

(1′*S*, 2″*S*)-*N*-Benzyloxy-*N*′-{1-[(2-benzyloxymethyl)pyrrolidinylcarbonyl]-2-methylpropyl}-3-[(*E*)-pentylidene]succinamide (13)
To 2-benzyloxymethyl-*N*-(*N*-*tert*-butoxycarbonyl-L-valinyl)pyrrolidine (**12**; 1.56 g, 3.86 mmol) was added dropwise TFA (4.2 mL) at r.t. over 5 min and the mixture was stirred at the same temperature for 30 min under argon atmosphere. After removal of the solvent, EtOAc (200 mL) was added to the residue and the organic layer was washed with sat. aq $NaHCO_3$ (140 mL), dried ($MgSO_4$), and concentrated to give **11** as a pale colorless oil. To a solution of **11** in anhyd $CHCl_3$ (28 mL) was added **15** (996 mg, 3.65 mmol) at r.t. and the mixture was stirred at the same temperature for 4 d under argon atmosphere. The mixture was poured into EtOAc (200 mL) and the organic layer was washed with sat. aq $NaHCO_3$ (140 mL), H_2O (140 mL), and brine (100 mL), and dried ($MgSO_4$). After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 4.0 cm, *l* = 12 cm; EtOAc–hexane, 2:1–4:1) to give **13** as a colorless oil; yield: 1.46 g (71%); $[\alpha]_D^{24}$ –42.5 (*c* = 1.0, MeOH).

IR (neat): 1630, 3240 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.87–0.97 (m, 9 H, 3 CH_3), 1.30–1.44 [m, 4 H, $CH_3(CH_2)_2$], 1.86–2.11 [m, 5 H, $(CH_3)_2CH$ and $(CH_2)_2CH_2N$], 2.25–2.33 [m, 2 H, $CH_3(CH_2)_2CH_2$], 3.10 (s, 2 H, CH_2CONHOBn), 3.43–3.70 (m, 4 H, CH_2OH and CH_2N), 4.29–4.32 (m, 1 H, CH_2CHN), 4.50 (d, *J* = 3.0 Hz, 2 H, OCH_2Ph), 4.59 (dd, *J* = 8.5, 8.5 Hz, 1 H, CHCO), 4.88 (s, 2 H, $NHOCH_2$Ph), 6.28 (t, *J* = 7.0 Hz, 1 H, CH=C), 6.71 (d, *J* = 8.5 Hz, 1 H, NH), 7.24–7.40 (m, 10 H, ArH), 9.94 (br s, 1 H, NHOBn).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.85, 17.62, 19.43, 22.41, 24.46, 27.25, 28.46, 30.72, 31.71, 33.81, 47.70, 55.76, 56.82, 69.90, 73.16, 77.95, 127.36, 127.51, 127.55, 128.30, 128.34, 129.02, 129.13, 135.53, 138.25, 139.67, 168.09, 169.96, 170.16.

FAB-MS: *m*/*z* = 564 (M^+ + 1).

FAB-HRMS: *m*/*z* calcd for $C_{33}H_{46}N_3O_5$: 564.3437; found: 564.3415.

Catalytic Hydrogenation of 13
To **13** (240 mg, 0.426 mmol) in MeOH (6 mL) was added 10% $Pd(OH)_2$/C (69.7 mg) at r.t. and the mixture was stirred at the same temperature for 95 min under H_2 atmosphere. After filtration and removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 1.8 cm, *l* = 11 cm; *i*-PrOH–EtOAc, 1:7–1:1) to give **1** (38.3 mg) and a mixture (52.8 mg) of *epi*-**1**, **17**, and *epi*-**17**. The products were purified by repeated column chromatography.

1 [(–)-Actinonin]
Colorless powder; mp 141.2–145.1 °C (Et_2O–MeOH) [Lit.[2] mp 148 °C (Et_2O–MeOH)]; $[\alpha]_D^{21}$ –60.0 (*c* = 1.0, MeOH) {Lit.[2] $[\alpha]_D^{22}$ –48 (*c* = 1.0, MeOH)}.

IR (Nujol): 1615, 1645, 3300, 3370 cm^{-1}.

^{1}H NMR (500 MHz, CD_3OD): δ = 0.88 (t, *J* = 7.0 Hz, 3 H, CH_3CH_2), 0.96 [d, *J* = 7.0 Hz, 3 H, $(CH_3)_2CH$], 1.00 [d, *J* = 7.0 Hz, 3 H, $(CH_3)_2CH$], 1.19–1.36 [m, 6 H, $CH_3(CH_2)_2$], 1.36–1.45 [m, 1 H, $CH_3(CH_2)_2CHH'$], 1.50–1.60 [m, 1 H, $CH_3(CH_2)_2CHH'$], 1.86–2.13 [m, 5 H, $(CH_3)_2CH$ and $(CH_2)_2CH_2N$], 2.18 (dd, *J* = 14.5, 7.0 Hz, 1 H, CHH′CONHOH), 2.34 (dd, *J* = 14.5, 8.0 Hz, 1 H, CHH′CONHOH), 2.75–2.84 (m, 1 H, $CHCH_2CONHOH$), 3.50 (dd, *J* = 10.5, 6.0 Hz, 1 H, CHH′OH), 3.54–3.60 (m, 1 H, CHH′N), 3.66 (dd, *J* = 10.5, 4.5 Hz, 1 H, CHH′OH), 3.88 (ddd, *J* = 10.0, 7.5, 7.5 Hz, 1 H, CHH′N), 4.08–4.16 (m, 1 H, CH_2CHN), 4.38 (d, *J* = 8.5 Hz, 1 H, CHCO).

Anal. Calcd for $C_{19}H_{35}N_3O_5$: C, 59.20; H, 9.15; N, 10.90. Found: C, 59.36; H, 9.17; N, 11.09.

FAB-MS: *m*/*z* = 386 (M^+ + 1).

***epi*-1 [(–)-Epiactinonin]**
Colorless amorphous powder; $[\alpha]_D^{22}$ –61.9 (*c* = 1.4, MeOH).

IR ($CHCl_3$): 1618, 1665, 3260, 3410 cm^{-1}.

^{1}H NMR (500 MHz, CD_3OD): δ = 0.91 (t, *J* = 7.0 Hz, 3 H, CH_3CH_2), 0.95 [d, *J* = 7.0 Hz, 3 H, $(CH_3)_2CH$], 1.00 [d, *J* = 7.0 Hz, 3 H, $(CH_3)_2CH$], 1.22–1.36 [m, 6 H, $CH_3(CH_2)_2$], 1.40–1.50 [m, 1

H, $CH_3(CH_2)_2CHH'$], 1.55–1.64 [m, 1 H, $CH_3(CH_2)_2CHH'$], 1.88–2.12 [m, 5 H, $(CH_3)_2CH$ and $(CH_2)_2CH_2N$], 2.15 (dd, J = 14.5, 7.0 Hz, 1 H, $CHH'CONHOH$), 2.31 (dd, J = 14.5, 7.0 Hz, 1 H, $CHH'CONHOH$), 2.75–2.84 (m, 1 H, $CHCH_2CONHOH$), 3.50 (dd, J = 11.0, 6.0 Hz, 1 H, $CHH'OH$), 3.53–3.62 (m, 1 H, $CHH'N$), 3.64 (dd, J = 11.0, 5.0 Hz, 1 H, $CHH'OH$), 3.82–3.90 (m, 1 H, $CHH'N$), 4.08–4.18 (m, 1 H, CH_2CHN), 4.38 (d, J = 8.5 Hz, 1 H, CHCO), 8.11 (d, J = 8.0 Hz, 1 H, NH or OH).

FAB-HRMS: m/z calcd for $C_{19}H_{36}N_3O_5$: 386.2655; found: 386.2632.

17

This compound was identified to be the same as that obtained by catalytic hydrogenation (H_2, Pd/C) of **1**; colorless amorphous powder; $[\alpha]_D^{22}$ –49.0 (c = 2.2, MeOH).

IR ($CHCl_3$): 1622, 1680, 3340 cm^{-1}.

^1H NMR (500 MHz, CD_3OD): δ = 0.88 (t, J = 7.0 Hz, 3 H, CH_3CH_2), 0.96 [d, J = 7.0 Hz, 3 H, $(CH_3)_2CH$], 0.99 [d, J = 7.0 Hz, 3 H, $(CH_3)_2CH$], 1.19–1.38 [m, 6 H, $CH_3(CH_2)_2$], 1.38–1.46 [m, 1 H, $CH_3(CH_2)_2CHH'$], 1.50–1.64 [m, 1 H, $CH_3(CH_2)_2CHH'$], 1.86–2.13 [m, 5 H, $(CH_3)_2CH$ and $(CH_2)_2CH_2N$], 2.30 (dd, J = 15.0, 6.0 Hz, 1 H, $CHH'CONH_2$), 2.48 (dd, J = 15.0, 8.5 Hz, 1 H, $CHH'CONH_2$), 2.73–2.82 (m, 1 H, $CHCH_2CONH_2$), 3.50 (dd, J = 10.5, 6.0 Hz, 1 H, $CHH'OH$), 3.54–3.62 (m, 1 H, $CHH'N$), 3.66 (dd, J = 10.5, 4.5 Hz, 1 H, $CHH'OH$), 3.88 (ddd, J = 10.0, 7.0, 7.0 Hz, 1 H, $CHH'N$), 4.07–4.16 (m, 1 H, CH_2CHN), 4.39 (d, J = 8.5 Hz, 1 H, CHCO), 8.03 (d, J = 8.0 Hz, 1 H, NH or OH).

FAB-MS: m/z = 370 (M^+ + 1).

FAB-HRMS: m/z calcd for $C_{19}H_{36}N_3O_4$: 370.2706; found: 370.2712.

***epi*-17**

This compound was identified with the same as that one obtained by catalytic hydrogenation (H_2, Pd/C) of *epi*-**1**; colorless amorphous powder; $[\alpha]_D^{22}$ –66.6 (c = 2.0, MeOH).

IR ($CHCl_3$): 1628, 1670, 3335 cm^{-1}.

^1H NMR (500 MHz, CD_3OD): δ = 0.90 (t, J = 7.0 Hz, 3 H, CH_3CH_2), 0.97 [d, J = 7.0 Hz, 3 H, $(CH_3)_2CH$], 1.00 [d, J = 7.0 Hz, 3 H, $(CH_3)_2CH$], 1.22–1.36 [m, 6 H, $CH_3(CH_2)_2$], 1.42–1.51 [m, 1 H, $CH_3(CH_2)_3CHH'$], 1.53–1.66 [m, 1 H, $CH_3(CH_2)_2CHH'$], 1.86–2.12 [m, 5 H, $(CH_3)_2CH$ and $(CH_2)_2CH_2N$], 2.30 (dd, J = 15.0, 7.0 Hz, 1 H, $CHH'CONH_2$), 2.48 (dd, J = 15.0, 7.5 Hz, 1 H, $CHH'CONH_2$), 2.72–2.80 (m, 1 H, $CHCH_2CONH_2$), 3.44–3.61 (m, 2 H, $CHH'OH$ and $CHH'N$), 3.66 (dd, J = 10.5, 4.5 Hz, 1 H, $CHH'OH$), 3.82–3.92 (m, 1 H, $CHH'N$), 4.07–4.18 (m, 1 H, CH_2CHN), 4.40 (d, J = 8.5 Hz, 1 H, CHCO).

FAB-HRMS: m/z calcd for $C_{19}H_{36}N_3O_4$: 370.2706; found: 370.2704.

(1′*S*,2″*S*)-*N*-Benzyloxy-*N*′-{1-[(2-hydroxymethyl)pyrrolidinylcarbonyl]-2-methylpropyl}-3-[(*E*)-pentylidene]succinamide) (20)

To a solution of **19**[2a] (708 mg, 3.54 mmol) in anhyd $CHCl_3$ (25 mL) was added **15** (1.25 g, 4.58 mmol) at r.t. and the mixture was stirred at the same temperature for 4 d under an argon atmosphere. The mixture was poured into EtOAc (180 mL) and the organic layer was washed with sat. aq $NaHCO_3$ (60 mL), H_2O (60 mL), and brine (40 mL), and dried ($MgSO_4$). After removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 4.0 cm, l = 10 cm; EtOAc) to give **20** as a colorless amorphous powder; yield: 1.04 g (62%); $[\alpha]_D^{24}$ –20.3 (c = 1.2, MeOH).

IR (neat): 1620, 1650, 1670, 3240, 3400 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 0.91 (t, J = 7.0 Hz, 3 H, CH_3CH_2), 0.94 [t, J = 7.0 Hz, 3 H, $(CH_3)_2CH$], 0.99 [t, J = 7.0 Hz, 3 H, $(CH_3)_2CH$], 1.30–1.45 [m, 4 H, $CH_3(CH_2)_2$], 1.56–1.65 [m, 1 H, $(CH_3)_2CH$], 1.84–2.12 [m, 4 H, $(CH_2)_2CH_2N$], 2.22–2.32 [m, 2 H, $CH_3(CH_2)_2CH_2$], 3.10 (s, 2 H, $CH_2CONHOBn$), 3.50 (dt, J = 10.0, 7.5 Hz, 1 H, $CHH'N$), 3.58 (dd, J = 11.0, 7.5 Hz, 1 H, $CHH'OH$), 3.69 (dd, J = 11.0, 3.0 Hz, 1 H, $CHH'OH$), 3.80–3.87 (m, 1 H, $CHH'N$), 4.26 (dddd, J = 7.5, 7.5, 7.5, 3.0 Hz, 1 H, CH_2CHN), 4.59 (dd, J = 8.0, 8.0 Hz, 1 H, CHCO), 4.88 (s, 2 H, $NHOCH_2Ph$), 6.30 (t, J = 6.5 Hz, 1 H, CH=C), 6.71 (d, J = 8.0 Hz, 1 H, NH), 7.25–7.40 (m, 5 H, ArH), 9.81 (br s, 1 H, $NHOBn$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.79, 17.84, 19.28, 22.34, 24.31, 27.72, 28.37, 30.63, 31.45, 33.36, 48.15, 56.07, 61.05, 66.43, 77.88, 128.28, 128.76, 128.97, 135.40, 140.06, 168.04, 19.78, 172.53.

FAB-MS: m/z = 474 (M^+ + 1).

FAB-HRMS: m/z calcd for $C_{26}H_{40}N_3O_5$: 474.2968; found: 474.2951.

Actinonin (1) and Epiactinonin (*epi*-1)

To **20** (91.4 mg, 193 μmol) and pyridine (50 μL) in EtOH (2.5 mL) was added 10% Pd/C (52.6 mg) at r.t. and the mixture was stirred at the same temperature for 70 min under H_2 atmosphere. After filtration and removal of the solvent, the residue was subjected to column chromatography (Wako gel C = 200, ϕ = 1.8 cm, l = 9.5 cm; *i*-PrOH–EtOAc, 1:7–1:3) to give **1** (32.1 mg, 43%) and *epi*-**1** (28.2 mg, 38%).

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