Synthesis and antifouling activity of 3-isocyanotheonellin and its analogues

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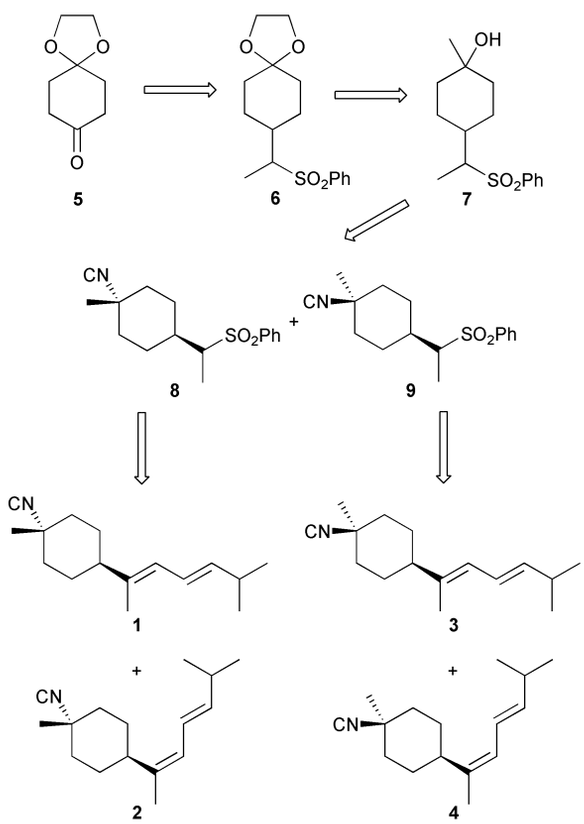
A new synthesis of 3-isocyanotheonellin, a marine sesquiterpene with potent antifouling activity against the larvae of the barnacle *Balanus amphirite*, and its analogues is described. The highlight of the synthetic strategy is the one-step construction of a tertiary isocyanide from an alcohol using trimethylsilyl cyanide and a silver salt. The antifouling activities of 3-isocyanotheonellin and its analogues are also reported.

Introduction

Many benthic organisms are known to usually prevent settling by other fouling organisms. This is considered to be because they have developed a chemical defense system against predators whose larvae settle on and become attached to prey. Therefore, the metabolites of these benthic organisms are potential non-toxic antifouling agents.[1–5] A variety of natural products with antifouling activity have been isolated from marine organisms,[6] and these may be expected to be lead compounds in non-toxic antifouling agents. 3-Isocyanotheonellin **1**, isolated from a nudibranch species, is a sesquiterpene of the bisabolene class with an isocyano functional group at the C-3 position.[7,8] In spite of its simple structure, this natural product exhibits potent antifouling activity against the larvae of the barnacle *Balanus amphirite* (EC_{50} 0.13 μg mL^{-1}).[9,10] However, its toxicity in high concentrations and structure–activity relationships with its analogues have not yet been demonstrated. A total synthesis of **1** has previously been reported by Ichikawa.[11,12] We thought that a more efficient synthetic route to this natural product, as well as various synthetic analogues, and their antifouling activities would contribute to the field of antifouling.

Recently, we reported an efficient direct method for the preparation of isocyanides from *tert*-alcohols.[13,14] This method would be convenient for the preparation of isocyano compounds. For this reason, we envisioned that this method could be used to prepare an isocyano group of **1** and its analogues. We describe herein a short total synthesis of 3-isocyanotheonellin **1** and its analogues and report on their antifouling activities.

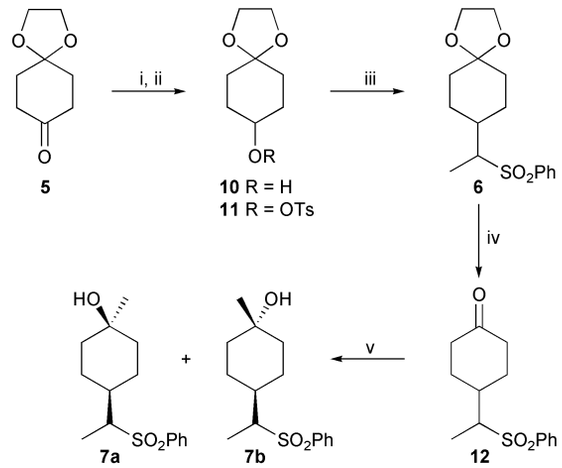


Scheme 1 Synthetic plan.

Results and discussion

Our synthetic plan starting with 1,4-cyclohexanedione monoethylene ketal **5** is illustrated in Scheme 1. We envisioned that analogues **2**, **3**, and **4** as well as 3-isocyanotheonellin **1** could be obtained in the same manner. The key step is the direct isocyanation of *tert*-alcohol **7** to afford isocyanosulfones **8** and **9**. Alcohol **7** could be prepared from **5** through the introduction of an ethyl phenyl sulfonyl group followed by methylation. The conjugated diene system of **1** and its geometrical isomer **2** could be constructed by a Julia olefination[15] of **8** with 4-methylpent-2-enal at the final stage. In addition, stereoisomer **3** and its geometrical isomer **4** could also be prepared from **9** in the same way.

The synthesis of *tert*-alcohol **7**, the substrate of the key step, is shown in Scheme 2. Ketone **5** was first reduced to alcohol **10** with $NaBH_4$, which was then treated with TsCl and pyridine

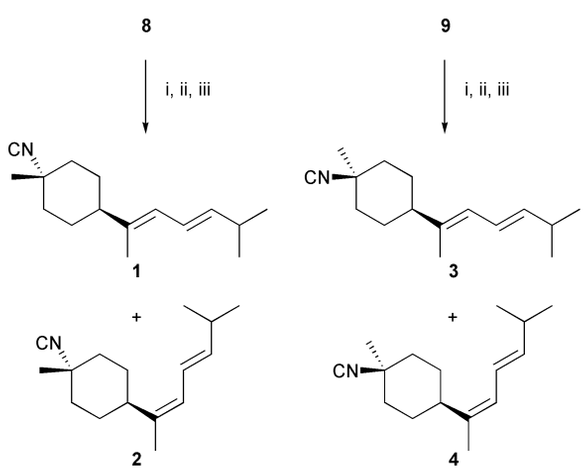


Scheme 2 *Reagents and conditions*: i, $NaBH_4$, MeOH, 0 °C, quant.; ii, TsCl, Pyr, rt, 98%; iii, *n*-BuLi, $EtSO_2Ph$, 0 °C⟶rt, 70%; iv, *p*-TsOH, 10% aq. acetone, 82%; v, MeLi, THF, 0 °C, 85% (**7a** : **7b**, 2 : 3).

to give the tosylate† **11** in 98% yield over two steps. The nucleophilic substitution of tosylate **11** by ethyl phenyl sulfone, lithiated with *n*-BuLi, yielded the desired sulfone **6** in 70% yield. Sulfone **6** was converted to ketone **12** by deprotection of the acetal with *p*-TsOH in 82% yield. Methylation of ketone **12** with MeLi led to the diastereomers of *tert*-alcohol **7a** and **7b** (2 : 3) in 85% yield.

Our preliminary work had indicated that the direct isocyanation of *tert*-alcohol **7** would produce mainly isocyanide **9**, which is the precursor of stereoisomer **3**. Therefore, we examined this step in the construction of isocyanides **8** and **9** from *tert*-alcohol **7** with TMSCN under various conditions to obtain information on stereoselectivity. As indicated in Table 1, excellent yields were obtained when the reactions of **7** were performed with $AgClO_4$ in nitromethane and dichloromethane. Although **9** was the major product in all cases the stereoselectivity was moderately changed when $AgBF_4$ was used in dichloromethane, with the yield of **9** being reduced (entry 3).

Synthesis of 3-isocyanotheonellin **1** and its analogues **2**, **3**, and **4** was accomplished by the Julia olefination,[15] which is shown in Scheme 3, as the final stage of the reaction. Treatment

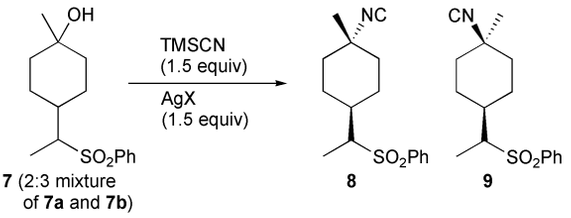


Scheme 3 *Reagents and conditions:* i, LDA, THF, then 4-methylpent-2-enal, −78 °C; ii, Ac_2O, pyr, rt; iii, 5% Na–Hg, Na_2HPO_4, EtOAc–MeOH, −10 °C, (**1** : **2**, 2 : 1, 50%; **3** : **4**, 2 : 1, 55%).

of isocyanosulfone **8** with LDA followed by entrapment of the generated anion with 4-methylpent-2-enal provided the coupling product, which was converted to the corresponding β-acetoxysulfone with acetic anhydride and pyridine. Reductive elimination of the β-acetoxysulfone by a sodium amalgam

† The IUPAC name for tosylate is toluene-*p*-sulfonate.

Table 1 Reactions of *tert*-alcohol **7** with TMSCN and AgX



Entry	AgX	Solvent	Yield (%)	Ratio (**8** : **9**)[a]
1	$AgClO_4$	$MeNO_2$	90	8 : 92
2	$AgClO_4$	CH_2Cl_2	88	11 : 89
3[b]	$AgBF_4$	CH_2Cl_2	48	30 : 70

[a] Determined by ^{1}H-NMR. [b] 2.0 equiv. of TMSCN was used.

Table 2 Antifouling activities of compounds **1**, **2**, **3**, and **4**[a]

Compound	EC_{50} (μg mL^{-1})	LD_{50} (μg mL^{-1})
1	0.19	>100
(Natural product)[b]	(0.13)	—
2	0.29	>100
3	0.18	>100
4	0.41	>100
$CuSO_4$	0.27	2.7

[a] The antifouling assay was repeated five times. [b] Refs. 9 and 10.

gave the geometrical 2 : 1 mixture of **1** and (*Z*,*E*)-diene **2** in 50% yield from **8**. The mixture of **1** and **2** was separated into pure compounds by HPLC. The spectroscopic data of **1** were identical to those reported in the literature. The analogues **3** and **4** were synthesized in 58% yield from isocyanosulfone **9** using the same method.

We next explored the structure–activity relationship of 3-isocyanotheonellin **1**. The antifouling activities of **1** and its analogues **2**, **3**, and **4** were evaluated against larvae of the barnacle *Balanus amphirite*, and the activity of $CuSO_4$ was also evaluated as a positive control. Antifouling tests were carried out using cyprid larvae cultured as follows. Adult barnacles, *Balanus amphirite*, attached to bamboo poles were procured from oyster farms in Lake Hamana, Shizuoka, and maintained in an aquarium at 20 °C by feeding on *Artemia salina* nauplii. Broods released I-II stage nauplii upon immersion in seawater after drying for 1 day. Nauplii thus obtained were cultured in 80%-filtered seawater at 25 °C by feeding with the diatom *Chaetoceros gracilis* at concentrations of 2.5×10^5 cells mL^{-1}. During days 1–4, larvae were washed in 80%-filtered seawater and were transferred to fresh vessels containing the *C. gracilis* suspension. Larvae reached the cyprid stage in 5 days. The cyprids were stored at 5 °C until used. Test samples were dissolved in ethanol; aliquots of the solution were added to the wells in 24-well polystyrene tissue culture plates and were air-dried. To each well was added 2 mL of 80%-filtered seawater and six two-day-old cyprids. Four wells were used for each experiment. The plates were kept in the dark for 48 h and 120 h at 25 °C, and the number of larvae which attached, metamorphosed, died, or did not settle were counted under a microscope.

The 50% settlement inhibitory effect (EC_{50}) and 50% lethal dose (LD_{50}) are shown in Table 2. The activity exhibited by the synthesized 3-isocyanotheonellin **1** was almost equivalent to that of the natural product. Indeed, the activity exhibited by stereoisomer **3** was almost as high as that of **1**. Furthermore, (*Z*,*E*)-dienes **2** and **4** also exhibited potent activity, although this was slightly lower than for **1**. In addition, these compounds showed a low mortality rate (LD_{50} > 100 μg mL^{-1}) in high concentrations, and the antifouling activities of **1** and **11** were

stronger than those observed for $CuSO_4$. From these results, we determined that the isocyano function was an important factor in the exhibition of potent antifouling activity.

Conclusion

In conclusion, we have achieved a short total synthesis of 3-isocyanotheonellin **1** using a one-step construction of isocyanide from a *tert*-alcohol as a key step. Moreover, three analogues were synthesized in the same manner, and their antifouling activities were evaluated against the larvae of the barnacle *Balanus amphirite*. The synthesized **1** and its analogues exhibited high antifouling activity without significant toxicity. These results will allow us to investigate the structure–activity relationships in more detail by using various derivatives of 3-isocyanotheonellin **1** in the future.

Experimental

Mps were determined on a MEL-TEMP (Laboratory Device) and are uncorrected. NMR spectra were obtained in $CDCl_3$ on a JEOL Alpha-600 spectrometer. All ^{1}H NMR spectra are reported in ppm relative to TMS. All ^{13}C NMR spectra are reported in ppm relative to the central line of the triplet for $CDCl_3$ at 77.03 ppm. IR spectra were recorded on a JEOL WINSPEC-50 spectrometer. Low- and high-resolution mass spectra were recorded on a JEOL SX-102A spectrometer under ionization conditions (70 eV). Chromatographic separations were carried out on a silica gel column (Fuji Silysia Chemical BW-127ZH; 100–270 mesh). HPLC was performed on a HITACHI L-6000 Pump instrument equipped with an L-4000 UV Detector (254 nm). The stereochemistry of compounds **7a** and **7b** were established from NOE experiments.

4,4-Ethylenedioxycyclohexan-1-ol 10

To a solution of ketone **5** in methanol (50 mL) was added sodium borohydride (1.46 g, 38.4 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. After removal of the solvent under reduced pressure, brine (30 mL) was added to the residue, and the resultant mixture was then extracted with EtOAc (300 mL). The combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The obtained crude alcohol **10** (5.06 g, quant.) was used in the next experiment without purification. ^{1}H NMR δ 3.98–3.92 (4H, m), 3.83–3.78 (1H, m), 1.91–1.86 (2H, m), 1.84–1.79 (2H, m), 1.70–1.55 (4H, m), 1.37 (1H, br s); ^{13}C NMR δ 108.26, 68.20, 64.33, 64.30, 32.06, 31.58; IR (neat) 3500 (br), 2940, 2886, 1234, 1143, 1105 cm^{-1}; LR-EIMS: *m*/*z* (%) 158 (M^+, 31), 140 (7), 111 (19), 99 (100), 86 (94); HR-EIMS: calcd for $C_8H_{14}O_3$ 158.0943, found 158.0945.

4,4-Ethylenedioxycyclohexyl toluene-*p*-sulfonate 11

To a solution of alcohol **10** (5.0 g, 31.6 mmol) in dry pyridine (30 mL) was added toluene-*p*-sulfonyl chloride (7.24 g, 38 mmol). After the reaction mixture was stirred at ambient temperature for 18 h, the reaction was quenched with brine (30 mL), and the resultant mixture was then extracted with EtOAc (300 mL). The combined extracts were washed with 3 M HCl, aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc : hexane = 1 : 2) to give a white solid of tosylate **11** (9.67 g, 98%). Recrystallization from EtOAc–hexane provided an analytically pure crystalline sample of tosylate **11**; white crystals, mp 67–68 °C (from EtOAc–hexane); ^{1}H NMR δ 7.80 (2H, d, J = 8 Hz), 7.33 (2H, d, J = 8 Hz), 4.67–4.62 (1H, m), 3.95–3.88 (4H, m), 2.45 (3H, s), 1.90–1.75 (6H, m), 1.59–1.51 (2H, m); ^{13}C NMR δ 144.50, 134.55, 129.80, 127.59, 107.40, 78.90, 64.38, 64.35, 30.67, 29.13, 21.65; IR (KBr) 3043, 2958, 2881, 1598, 1448, 1351, 1189, 1095 cm^{-1}; LR-EIMS: *m*/*z* (%) 312 (M^+, 15), 140 (39), 99 (100), 86 (11); HR-EIMS: calcd for $C_{15}H_{20}O_5S$ 312.1031, found 312.1020.

4-[(1-Phenylsulfonyl)ethyl]cyclohexan-1-one ethylene ketal 6

To a solution of ethyl phenyl sulfone (5.31 g, 31.2 mmol) in THF (100 mL) cooled at 0 °C was added *n*-BuLi (1.6 M in hexane, 19.5 mL, 31.2 mmol) under an argon atmosphere. The resulting yellow solution was stirred for 30 min, and then a solution of tosylate **11** (6.5 g, 20.8 mmol) in THF (40 mL) was added over 10 min at 0 °C under an argon atmosphere. The reaction mixture was warmed to room temperature and stirred at ambient temperature for an additional 40 h. After the reaction was quenched by addition of aqueous NH_4Cl (20 mL), the mixture was extracted with EtOAc (300 mL). The combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc–hexane = 1 : 3–1 : 1) to give a white solid of **6** (4.5 g, 70%). Recrystallization from EtOAc–hexane provided an analytically pure crystalline sample of sulfone **11**; white crystals, mp 104–105 °C (from EtOAc–hexane); ^{1}H NMR δ 7.90–7.87 (2H, m), 7.67–7.63 (1H, m), 7.59–7.54 (2H, m), 3.96–3.90 (4H, m), 3.01–2.92 (1H, dq, J = 7 and 2.5 Hz), 2.30–2.23 (1H, m), 2.04–1.98 (1H, m), 1.81–1.75 (2H, m), 1.67–1.51 (4H, m), 1.50–1.41 (1H, m), 1.22 (3H, d, J = 7.0 Hz); ^{13}C NMR δ 138.51, 133.53, 129.15, 128.65, 108.07, 64.31, 64.27, 63.97, 34.95, 34.82, 34.22, 29.46, 24.17, 9.31; IR (KBr) 3064, 2985, 2931, 2875, 1448, 1303, 1159, 1139, 1103 cm^{-1}; LR-EIMS: *m*/*z* (%) 310 (M^+, 3), 169 (20), 141 (33), 99 (100), 77 (9); HR-EIMS: calcd for $C_{16}H_{22}O_4S$ 310.1239, found 310.1239.

4-[(1-Phenylsulfonyl)ethyl]cyclohexan-1-one 12

To a solution of acetal **11** (4.4 g, 14.17 mmol) in acetone–water (100 mL, 9 : 1) was added $TsOH\cdot H_2O$ (266 mg, 1.4 mmol), and the resulting solution was refluxed for 12 h. After removal of the solvent under reduced pressure, the residue was diluted with EtOAc (200 mL). The organic layers were then washed with aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by recrystallization from EtOAc–hexane to give white crystals of ketone **12** (3.1 g, 82%); white crystals, mp 124–126 °C (from EtOAc–hexane); ^{1}H NMR δ 7.93–7.89 (2H, m), 7.70–7.66 (1H, m), 7.62–7.57 (2H, m), 3.08 (1H, dq, J = 7 and 3 Hz), 2.79–2.72 (1H, m), 2.47–2.35 (5H, m), 1.98–1.92 (1H, m), 1.78–1.67 (1H, m), 1.66–1.56 (1H, m), 1.21 (3H, d, J = 7 Hz); ^{13}C NMR δ 210.13, 138.19, 133.81, 129.31, 128.68, 63.26, 41.06, 40.48, 34.58, 31.80, 26.91, 9.52; IR (KBr) 3083, 2970, 2929, 2867, 1714, 1583, 1479, 1313, 1265, 1174 cm^{-1}; LR-EIMS: *m*/*z* (%) 266 (M^+, 3), 124 (100), 97 (12), 77 (19); HR-EIMS: calcd for $C_{14}H_{18}O_3S$ 266.0977, found 266.0976.

trans-1-Methyl-4-[(1-phenylsulfonyl)ethyl]cyclohexan-1-ol 7a and *cis*-1-methyl-4-[(1-phenylsulfonyl)ethyl]cyclohexan-1-ol 7b

To a solution of MeLi (1.14 M in Et_2O, 25.0 mL, 28.5 mmol) in THF (30 mL) cooled at 0 °C was added a solution of ketone **12** (2.48 g, 9.31 mmol) in THF (20 mL) under an argon atmosphere. After the reaction mixture was stirred at 0 °C for 1 h, the reaction was quenched and neutralized with 3M HCl (9.5 mL), and the resultant mixture was then extracted with EtOAc (200 mL). The combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc–hexane = 1 : 2–1 : 1) to give white crystals of **7a** and **7b** (922 mg and 1.31 g, 85%). Recrystallization from EtOAc–hexane provided analytically pure crystalline samples of alcohol **7a** and **7b**.

7a; White crystals, mp 94–96 °C (from EtOAc–hexane); ^{1}H NMR δ 7.91–7.86 (2H, m), 7.68–7.63 (1H, m), 7.60–7.54 (2H, m), 2.99 (1H, dq, J = 7 and 3 Hz), 2.23–2.13 (1H, m), 2.01–1.92 (1H, m), 1.78–1.70 (2H, m), 1.60–1.45 (3H, m), 1.44–1.34 (2H, m), 1.33–1.24 (1H, m), 1.22 (3H, s), 1.21 (3H, d, J = 7 Hz); ^{13}C NMR δ 138.49, 133.55, 129.16, 128.62, 70.25, 63.79, 40.16, 39.71, 35.87, 29.33, 25.41, 24.60, 9.62; IR (KBr) 3502, 3372, 3062, 2977, 2942, 2854, 1448, 1303, 1145, 1085 cm^{-1}; LR-EIMS: *m*/*z* (%) 282 (M^+, 2), 267 (8), 212 (14), 170 (4), 143 (23), 123 (100), 81 (74); HR-EIMS: calcd for $C_{15}H_{22}O_3S$ 282.1290, found 282.1290.

7b; White crystals, mp 119–121 °C; ^{1}H NMR δ 7.91–7.86 (2H, m), 7.67–7.63 (1H, m), 7.59–7.54 (2H, m), 2.96 (1H, dq, J = 7 and 2.5 Hz), 2.24–2.16 (1H, m), 1.85–1.79 (1H, m), 1.75–1.33 (7H, m), 1.23 (3H, d, J = 7 Hz), 1.21 (3H, s), 0.99 (1H, br s); ^{13}C NMR δ 138.66, 133.47, 129.12, 128.64, 68.65, 64.44, 38.67, 38.25, 35.34, 31.56, 27.32, 22.23, 9.45; IR (KBr) 3504, 3426, 3068, 2958, 2927, 2877, 2854, 1446, 1295, 1137, 1087 cm^{-1}; LR-EIMS: *m*/*z* (%) 282 (M^+, 0.7), 267 (5), 212 (4), 170 (4), 143 (18), 123 (100), 81 (62); HR-EIMS: calcd for $C_{15}H_{22}O_3S$ 282.1290, found 282.1291.

trans-1-Methyl-4-[(1-phenylsulfonyl)ethyl]cyclohexyl isocyanide 8 and *cis*-1-methyl-4-[(1-phenylsulfonyl)ethyl]cyclohexyl isocyanide 9

To a solution of alcohol **7** (2 : 3 mixture of **7a** and **7b**, 141 mg, 0.5 mmol) in dichloromethane (1 mL) were added TMSCN (135 μL, 1.0 mmol) and then $AgBF_4$ (194 mg, 0.5 mmol) under an argon atmosphere. The reaction mixture was stirred at ambient temperature for 2 h, and the reaction was then quenched with aqueous $NaHCO_3$ (1 mL). After being stirred for an additional 10 min, the mixture was filtered with celite and washed with EtOAc (100 mL). The combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was passed through a short silica gel column (EtOAc–hexane = 1 : 1) to give a 3 : 7 mixture of isocyanides **8** and **9** (70 mg, 48%). Pure isocyanides **8** and **9** were obtained by collecting the products of a repeated reaction and then using silica gel column chromatography (EtOAc–hexane = 1 : 3) and preparative TLC. Recrystallization from ethyl acetate–hexane provided analytically pure crystalline samples of isocyanide **8** and **9**.

8; White crystals, mp 115–116 °C (from EtOAc–hexane); ^{1}H NMR δ 7.90–7.85 (2H, m), 7.69–7.64 (1H, m), 7.60–7.55 (2H, m), 2.97 (1H, dq, J = 7 and 3.5 Hz), 2.23–2.16 (1H, m), 2.00–1.80 (5H, m), 1.64–1.57 (1H, m), 1.52–1.44 (4H, m), 1.43 (3H, t, J = 2 Hz), 1.39–1.31 (1H, m), 1.20 (3H, d, J = 7.5 Hz); ^{13}C NMR δ 152.74 (t, J = 5 Hz), 138.27, 133.73, 129.25, 128.63, 63.19, 56.15 (t, J = 5 Hz), 38.26, 37.86, 35.19, 26.82, 24.72, 23.01, 10.15; IR (KBr) 3068, 2985, 2946, 2865, 2134, 1448, 1303, 1141, 1087 cm^{-1}; LR-EIMS: *m*/*z* (%) 291 (M^+, 1), 265 (1), 167 (4), 150 (7), 123 (60), 122 (100), 107 (30), 93 (49); HR-EIMS: calcd for $C_{16}H_{21}O_2NS$ 291.1293, found 291.1294.

9; White crystals, mp 105–106 °C (from EtOAc–hexane); ^{1}H NMR δ 7.91–7.86 (2H, m), 7.69–7.64 (1H, m), 7.61–7.55 (2H, m), 3.00–2.94 (1H, dq, J = 7 and 2.5 Hz), 2.33–2.25 (1H, m), 2.03–1.91 (3H, m), 1.73–1.51 (3H, m), 1.50–1.37 (5H, m) including 1.43 (3H, br s), 1.24 (3H, d, J = 7.5 Hz); ^{13}C NMR δ 154.33 (t, J = 5 Hz), 138.37, 133.63, 129.20, 128.60, 63.94, 57.61 (t, J = 5 Hz), 38.10, 37.52, 34.42, 29.77, 27.25, 22.39, 9.50; IR (KBr) 3062, 2985, 2929, 2859, 2132, 1446, 1301, 1143, 1089 cm^{-1}; LR-EIMS: *m*/*z* (%) 291 (M^+, 1), 264 (2), 167 (2), 150 (12), 123 (81), 122 (100), 107 (30), 93 (49); HR-EIMS: calcd for $C_{16}H_{21}O_2NS$ 291.1293, found 291.1291.

3-Isocyanotheonellin 1 and *trans*-4-[(*Z*,*E*)-1,5-dimethylhexan-1,3-dienyl]-1-methylcyclohexyl isocyanide 2

To a solution of LDA [prepared from diisopropylamine (0.12 mL, 0.84 mmol) and *n*-BuLi (1.6 M in hexane, 0.5 mL, 0.8 mmol)] in THF (5 mL) cooled at −78 °C was added a solution of isocyanide **8** (115 mg, 0.395 mmol) in THF (5 mL) under an argon atmosphere. After the mixture was stirred at −78 °C for 20 min, 4-methylpent-2-enal (10 μL, 0.86 mmol) was added and stirring was continued for a further 30 min under an argon atmosphere. After the reaction was quenched by addition of aqueous NH_4Cl (20 mL), the mixture was extracted with EtOAc (150 mL). The combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was roughly purified by column chromatography on silica gel (EtOAc–hexane = 1 : 3–1 : 2) to give a crude β-hydroxysulfone (135 mg). This was dissolved in a mixture of acetic anhydride (2 mL) and pyridine (2 mL). After the mixture was stirred at ambient temperature for 10 h, the reaction was quenched with brine (10 mL), and the resultant mixture was then extracted with EtOAc (150 mL). The combined extracts were washed with 1 M HCl, aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was roughly purified by column chromatography on silica gel (EtOAc–hexane = 1 : 2) to give a crude β-acetoxysulfone (140 mg). This, dissolved in MeOH–EtOAc (15 mL, 2 : 1), was treated with Na_2HPO_4 (150 mg, 1.05 mmol) followed by 5% sodium amalgam (1.3 g) at −10 °C under an argon atmosphere. After the reaction mixture was stirred at ambient temperature for 12 h, the reaction was quenched with water and the resultant mixture was then extracted with Et_2O (150 mL). The combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc–hexane = 1 : 20) to give a mixture of 3-isocyanoteonellin **1** and **2** (2 : 1, 45 mg, 50%). A part of the mixture was further purified by reversed-phase HPLC (Mightysil RP-18, 250–50 mm; 80% MeOH) to give pure **1** (24 mg) and **2** (12 mg).

1; Colorless oil; ^{1}H NMR δ 6.21 (1H, ddd, J = 15, 10.5 and 1 Hz), 5.80 (1H, dd, J = 10.5 and 1 Hz), 5.59 (1H, dd, J = 15 and 7 Hz), 2.39–2.31 (1H, m), 2.00–1.88 (3H, m), 1.86–1.78 (2H, m), 1.75–1.67 (5H, m) including 1.72 (3H, br s), 1.47–1.37 (5H, m) including 1.44 (3H, t, J = 2 Hz), 1.01 (6H, d, J = 6.5 Hz) [lit.[12] (300 MHz, $CDCl_3$) δ 6.20 (1H, ddd, J = 15, 10.8, 1 Hz), 5.79 (1H, d, J = 10.8 Hz), 5.58 (1H, dd, J = 15, 6.8 Hz), 2.33 (1H, m), 1.70 (3H, br s), 1.42 (3H, t, J = 2 Hz), 0.99 (6H, d, J = 6.8 Hz); lit.[7] (200 MHz, $CDCl_3$) δ 6.21 (1H, dd, J = 15, 10 Hz), 5.80 (1H, d, J = 10 Hz), 5.60 (1H, dd, J = 15, 7 Hz), 2.35 (1H, m), 1.72 (3H, s), 1.43 (3H, br s), 1.01 (6H, d, J = 7 Hz)]; ^{13}C NMR δ 152.14 (t, J = 5 Hz), 140.75, 140.75, 138.70, 123.87, 123.42, 56.77 (t, J = 5 Hz), 44.81, 38.27, 31.42, 26.48, 25.12, 22.54, 15.24 [lit.[12] (75 MHz, $CDCl_3$) δ 152.2, 140.5, 138.4, 123.7, 123.3, 56.6, 44.6, 38.1, 31.5, 26.3, 22.4, 15.1; lit.[7] (67.80 MHz, $CDCl_3$) δ 152.2 (br t, J = 4 Hz), 140.6, 138.6, 123.8, 123.4, 56.7, 44.8, 38.2 (t, J = 5 Hz), 31.4, 26.4, 25.1, 22.5, 15.2]; IR (neat) 3033, 2954, 2923, 2852, 2129, 1463, 1380, 1259, 1126, 964 cm^{-1} [lit.[12] (neat) 3040, 2970, 2880, 2130, 1470, 1385, 1128, 965 cm^{-1}; lit.[7] ($CHCl_3$) 2120 (NC), 1460, 1370, 1120 cm^{-1}]; LR-EIMS: *m*/*z* (%) 231 (M^+, 46), 216 (8), 204 (21), 189 (25), 188 (14), 161 (54), 121 (49), 105 (88), 95 (73), 93 (100); HR-EIMS: calcd. for $C_{16}H_{25}N$ 231.1987, found 231.2004 [lit.[12] found 231.1975; lit.[7] 231.1970].

2; Colorless oil; ^{1}H NMR δ 6.19 (1H, dd, J = 15 and 11 Hz), 5.76 (1H, d, J = 11 Hz), 5.56 (1H, dd, J = 15 and 7.5 Hz), 2.61 (1H, tt, J = 12 and 4 Hz), 2.39–2.30 (1H, m), 2.03–1.97 (2H, m), 1.95–1.88 (2H, m), 1.67 (3H, br s), 1.54–1.40 (6H, s) including 1.47 (3H, t, J = 2 Hz), 1.33–1.25 (1H, m), 1.01 (6H, d, J = 7 Hz); ^{13}C-NMR δ 152.00 (t, J = 5 Hz), 140.79, 138.60, 126.10, 122.23, 56.33 (t, J = 5 Hz), 38.80, 31.47, 26.21, 24.36, 22.62, 19.73, 14.14; IR (neat) 3033, 2958, 2923, 2854, 2129, 1456, 1380, 1255, 1120, 964 cm^{-1}; LR-EIMS: *m*/*z* (%) 231 (M^+, 60), 216 (11), 204 (26), 189 (35), 188 (19), 161 (48), 121 (65), 105 (89), 95 (84), 93 (100); HR-EIMS: calcd for $C_{16}H_{25}N$ 231.1987, found 231.1981.

cis-4-[(_E_,_E_)-1,5-Dimethylhexan-1,3-dienyl]-1-methylcyclohexyl isocyanide 3 and _cis_-4-[(_Z_,_E_)-1,5-dimethylhexan-1,3-dienyl]-1-methylcyclohexyl isocyanide 4

These compounds were obtained from isocyanide **9** (314 mg, 1.07 mmol) by the same procedure described for its isomer to give a mixture of **3** and **4** (2 : 1, 146 mg, 59%). A part of the mixture was also further purified by the same procedure to give pure **3** (40 mg) and **4** (20 mg).

3; Colorless oil; ^{1}H NMR δ 6.22 (1H, ddd, J = 15, 10.5 and 1 Hz), 5.84 (1H, d, J = 10.5 Hz), 5.60 (1H, dd, J = 15 and 7 Hz), 2.40–2.31 (1H, m), 1.98–1.93 (2H, m), 1.88–1.81 (1H, m), 1.75 (3H, br s), 1.74–1.61 (4H, m), 1.43 (3H, t, J = 2 Hz), 1.41–1.33 (2H, m), 1.02 (6H, d, J = 6.5 Hz); ^{13}C NMR δ 153.93 (t, J = 4 Hz), 140.49, 139.60, 123.84, 123.44, 57.75 (t, J = 5 Hz), 46.02, 38.31, 31.40, 30.06, 26.71, 22.54, 14.77 [lit.;[11] ($CDCl_3$) δ 153.9, 140.2, 139.2, 123.7, 123.3, 57.6, 46.0, 38.3, 31.3, 30.0, 26.7, 22.5, 14.7]; IR (neat) 3033, 2958, 2931, 2854, 2129, 1456, 1380, 1249, 1124, 964 cm^{-1}; LR-EIMS: _m/z_ (%) 231 (M^+, 73), 216 (33), 204 (35), 189 (100), 188 (78), 136 (63), 121 (61), 95 (89), 93 (83); HR-EIMS: calcd for $C_{16}H_{25}N$ 231.1987, found 231.1975.

4; Colorless oil; ^{1}H NMR δ 6.19 (1H, dd, J = 15 and 11 Hz), 5.77 (1H, d, J = 11 Hz), 5.55 (1H, dd, J = 15 and 7 Hz), 2.53 (1H, tt, J = 12 and 3 Hz), 2.38–2.28 (1H, m), 1.99–1.92 (2H, m), 1.84–1.75 (2H, m), 1.74 (3H, br s), 1.54–1.39 (7H, m) including 1.45 (3H, t, J = 2 Hz), 1.00 (6H, d, J = 7 Hz); ^{13}C NMR δ 154.00 (t, J = 5 Hz), 140.36, 139.61, 125.61, 122.35, 57.67 (t, J = 5 Hz), 38.53, 38.18, 31.47, 26.01, 22.64, 19.81; IR (neat) 3033, 2962, 2935, 2865, 2127, 1446, 1380, 1245, 1128, 966 cm^{-1}; LR-EIMS: _m/z_ (%) 231 (M^+, 78), 216 (43), 204 (19), 189 (88), 188 (100), 136 (75), 121 (66), 95 (93), 93 (83); HR-EIMS: calcd for $C_{16}H_{25}N$ 231.1987, found 231.1968.

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