This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This Accepted Manuscript will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Taurospongins B and C, new acetylenic fatty acid derivatives possessing a taurine amide residue from a marine sponge of the family Spongiidae

Takaaki Kubota, Haruna Suzuki, Azusa Takahashi-Nakaguchi, Jane Fromont, Tohru Gonoi and Jun’ichi Kobayashi

Introduction

Marine sponges of the family Spongiidae have been demonstrated to be a rich source of unique bioactive meroterpenoids and acetylenic fatty acid derivatives possessing a taurine amide residue, taurospongins B (1) and C (2), have been isolated from an Okinawan marine sponge of the family Spongiidae. The absolute configurations for 1 and 2 were elucidated on the basis of their spectral data, especially 2D NMR and ESIMS/MS data. Here we describe the isolation and structure elucidation of 1 and 2.

Fig. 1 Taurospongins B (1), C (2), and A (3).

Results and discussion

The sponge family Spongiidae collected at Okinawa, was extracted with MeOH. After evaporation, the MeOH extract was partitioned stepwise between organic solvents (EtOAc and n-BuOH) and H₂O. A part of n-BuOH soluble materials was fractionated by gel filtration (Sephadex LH-20, MeOH). A fraction eluted in a relatively early stage was purified by C₁₈ column chromatography (MeOH/H₂O) and SiO₂ column chromatography (CH₃Cl/MeOH) to afford taurospongins B (I, 1.7 mg, 0.00048%, wet weight) and C (2, 4.2 mg, 0.0012%). Taurospongin B (1) was obtained as an optically active colorless amorphous solid. The molecular formula of I was revealed to be C₁₆H₁₈NO₅S by HRESIMS data [m/z 682.47036 (M⁺)], Δ-1.84 ppm]. IR absorptions indicated the existence of hydroxy (3421 cm⁻¹), ester carbonyl (1732 cm⁻¹), and amide carbonyl (1646 cm⁻¹) functionalities. The inspection of the HMOC and HMBC spectra with ¹H and ¹³C NMR data disclosed that I consists of an oxygenated quaternary carbon (Table 1). The geometry of a double bond between C-8’ and C-9’ was assigned as Z by the vicinal coupling constant (J₈’₉’=3.8 Hz). HMBC correlations of H₂-1”/C-1 and H₂-2’/C-1’ clarified that N-bearing carbon C-1” (δC 37.3) and a carbonyl-bearing carbon C-2 (δC 48.6) were connected via an amide bond containing a carbonyl carbon C-1 (δC 174.6). Linkings of C-2, a methylene carbon C-4, and a methyl carbon C-11 through an oxygenated quaternary carbon C-3 (δC 73.4) were inferred from HMBC correlations of H₃-11/C-2, H₂-4/C-3, and H₃-11/C-4. An HMBC correlation of H₂-2’/C-1’ revealed that a carbonyl-bearing carbon C-2’ (δC 36.2) was attached to a carbonyl carbon C-1’ (δC 174.6).
The structure of Fig. 2

Selected 2D NMR correlations for taurospangins B (1) and C (2) in CD$_3$OD.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>position</td>
<td>$\delta^h$ multi (J in Hz)</td>
<td>$\delta^b$ multi (J in Hz)</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>174.6</td>
</tr>
<tr>
<td>2</td>
<td>2.40 d (14.0)</td>
<td>48.6</td>
</tr>
<tr>
<td>3</td>
<td>2.34 d (14.0)</td>
<td>73.4</td>
</tr>
<tr>
<td>4</td>
<td>1.59 m$^g$</td>
<td>43.8</td>
</tr>
<tr>
<td>5</td>
<td>1.53 m$^g$</td>
<td>21.6</td>
</tr>
<tr>
<td>6</td>
<td>1.44 m$^g$</td>
<td>36.6</td>
</tr>
<tr>
<td>7</td>
<td>4.98 m</td>
<td>76.2</td>
</tr>
<tr>
<td>8</td>
<td>1.59 m$^g$</td>
<td>38.3</td>
</tr>
<tr>
<td>9</td>
<td>1.43 m$^g$</td>
<td>20.5$^k$</td>
</tr>
<tr>
<td>10</td>
<td>0.96 m (7.4)</td>
<td>15.1$^l$</td>
</tr>
<tr>
<td>11</td>
<td>1.25 s</td>
<td>27.6</td>
</tr>
<tr>
<td>1'</td>
<td>-</td>
<td>174.6</td>
</tr>
<tr>
<td>2'</td>
<td>2.51 m</td>
<td>36.2</td>
</tr>
<tr>
<td>3'</td>
<td>2.48 m$^g$</td>
<td>16.5</td>
</tr>
<tr>
<td>4'</td>
<td>-</td>
<td>80.2</td>
</tr>
<tr>
<td>5'</td>
<td>-</td>
<td>82.2</td>
</tr>
<tr>
<td>6'</td>
<td>2.18 m</td>
<td>20.7$^h$</td>
</tr>
<tr>
<td>7'</td>
<td>2.23 m</td>
<td>28.8$^h$</td>
</tr>
<tr>
<td>8'</td>
<td>5.44 dt (10.3, 5.6)</td>
<td>130.0</td>
</tr>
<tr>
<td>9'</td>
<td>5.45 dt (10.3, 5.6)</td>
<td>132.8</td>
</tr>
<tr>
<td>10'</td>
<td>2.09 m</td>
<td>29.0$^h$</td>
</tr>
<tr>
<td>11'~22'</td>
<td>1.52~1.15 m$^g$</td>
<td>31.7~31.1$^f$</td>
</tr>
<tr>
<td>23'</td>
<td>1.33 m$^g$</td>
<td>33.9</td>
</tr>
<tr>
<td>24'</td>
<td>1.38 m$^g$</td>
<td>24.5</td>
</tr>
<tr>
<td>25'</td>
<td>0.94 m (7.1)</td>
<td>15.2$^l$</td>
</tr>
<tr>
<td>1''</td>
<td>3.66 t (6.5)</td>
<td>37.3</td>
</tr>
<tr>
<td>2''</td>
<td>3.02 t (6.5)</td>
<td>52.2</td>
</tr>
</tbody>
</table>

$^a$Recorded at 600 MHz. $^b$Recorded at 150 MHz. $^c$2H. $^d$3H. $^e$24H. $^f$12C. $^g$J-values were not determined because of overlapping with other signals. $^h,i,j,k,l$ These signals might be exchange.

Linkage of C-3' and C-6' by a triple bond between acetylenic carbons C-4' ($\delta^c$ 80.2) and C-5' ($\delta^c$ 82.2) was uncovered by HMBC correlations of H$_2$-3'/C-4' and H$_2$-6'/C-5'. The chemical shift of a proton H-7 ($\delta^c$ 4.98) of an oxygenated carbon C-7 ($\delta^c$ 76.2) implied that C-7 was esterified to C-1'. These data and the molecular formula of 1 indicated an attachment of a sulfo group to C-2' and the connection of C-10' and C-23' by a methylene chain.

**Fig. 2** Selected 2D NMR correlations for taurospangins B (1).

The structure of 1 elucidated from the NMR data was also confirmed by a charge-remote fragmentation pattern induced by a sulfo group observed in the FABMS/MS spectrum of 1 (Fig. 3). Thus, the gross structure of taurospangin B (1) was elucidated as shown.

**Fig. 3** Fragmentation patterns observed in FABMS/MS spectrum of taurospangin B (1) [precursor ion, m/z 682 (M-H)$. The m/z values were indicated in italics.

The relative stereochemistry of taurospangin B (1) was established by comparison of the NMR data of taurineamide part (4) of 1 with its two possible diastereomers (6a and 6b). The taurineamide part (4) was obtained by methanolsysis of an ester.
Scheme 1 Methanalysis of taurospong B (1).

The taurine amides 6a and 6b were synthesized from D-mevalono lactone as follows (Schemes 2 and 3). A hydroxy group of D-mevalono lactone was protected by a TES group and then treated with DIBAL to give lactol (8), which was coupled with phosphorane by Wittig reaction. After protection of hydroxy group by a BOM group, the resulting chloro-olefin (10) was subjected to a coupling reaction with n-butylaldehyde to afford a mixture of acetylenic alcohols (11a and 11b), which was converted into an acetylenic ketone (12) by oxidation using AZADO.

Scheme 2 Synthesis of 6a and 6b (part 1).

The acetylenic ketone (12) was derived to chiral acetylenic alcohol (11a) by a Noyori asymmetric transfer hydrogenation using Ru[(S,S)-Tsdpen](p-cymene). The absolute stereochemistry at C-7 of 11a was verified by modified Mosher’s method. Treatment of taurineamide part (4) with (R)-(-) and (S)(+)-MTPA gave (S)- and (R)-MTPA esters of 4, respectively. Δδ values obtained from 1H NMR data of MTPA esters of 4 (Fig. 5) indicated that 4 was an enantiomer of 6a and the absolute configuration at C-7 of 4 was R. Therefore, the absolute configurations at C-3 and C-7 of taurospong B (1) were assigned as both R.

Scheme 3 Synthesis of 6a and 6b (part 2).

were uncovered to be syn relationship.

Taurospong C (2) was obtained as an optically active colorless amorphous solid. The molecular formula of 2 was elucidated as C_{13}H_{16}NO_{3}S by HRESIMS data [m/z 698.46739 (M-H), Δ+0.28 mmu]. IR absorptions suggested that 2 possesses hydroxy (3369 cm\(^{-1}\)), ester carbonyl (1730 cm\(^{-1}\)), amide carbonyl (1636 cm\(^{-1}\)) functionalities. The \(^1\)H and \(^13\)C NMR data of 2 were almost superimposable to those of taurospong A (3)\(^6,12,13\), except for disappearance of signals derived from an acetyl group. In addition, the difference of the molecular formula between 2 and 3 implied that 2 was a 9-O-desacetyl form of 3. To verify the prediction, 2 was derived to 9-O-acetyl form by treatment of 2 with acetic anhydride and pyridine. Since the spectral data of 9-O-acetyl form of 2 was identical with those of 3, 2 was assigned as 9-O-desacetyl taurospong A (3).

Antimicrobial assay\(^1\) of taurospongs B (1), C (2), and A (3) revealed that 2 and 3 exhibited mild and good antifungal activity,
respectively, against Cryptococcus neoformans (MIC, 32 and 1 µg/mL), while 1 did not show such activity (MIC, >32.0 µg/mL). Taurospirngs B (1), C (2), and A (3) were not active against other fungi Aspergillus niger, Trichophyton mentagrophytes, and Candida albicans (MIC, >32 µg/mL), and bacteria Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Micrococcus luteus (IC_{50} > 32 µg/mL). Taurospirngs B (1), C (2), and A (3) did not show cytotoxicity (IC_{50} > 10 µg/mL) against murine lymphoma L1210 and human epidermoid carcinoma KB cells *in vitro*.

**Conclusions**

Taurospirngs B (1) and C (2), two new acetylenic fatty acid derivatives possessing a tuarine amide residue, have been isolated from an Okinawan marine sponge of the family Spongidae. The absolute structures of 1 and 2 were established by combination of spectroscopic analysis and synthetic chemistry. Taurospirngs C (2) and A (3) showed mild and good antifungal activity, respectively, against *Cryptococcus neoformans* (MIC, 32 and 1 µg/mL).

**Experimental section**

**General experimental procedures**

Optical rotations were recorded on a JASCO P-1030 polarimeter. IR spectra were recorded on JASCO FT/IR-230 spectrometer. ^1^H and ^13^C NMR spectra were recorded on JEOL ECA400 and JEOL ECA500 spectrometers using 5 mm cell and a Bruker AMX-600 spectrometer using 2.5 mm micro cell. The 7.20 and 77.0 ppm resonances of residual CHCl, and CDCl, and 3.35 and 49.8 ppm resonances of residual CD, and CD, were used as internal references for ^1^H and ^13^C NMR spectra, respectively. EIMS spectra were recorded on a JEOL JMS-700TZ mass spectrometer. ESIIMS spectra were recorded on JEOL JMS-700TZ and Thermo Scientific Exactive mass spectrometers.

**Sponge description**

The sponge (SS-1202, family Spongidae) was collected at Unten Port, Okinawa, and kept frozen until used. The sponge was medium brown mound with a spiky, conoalose surface, hispid with a fine adherent membrane, and alcohol brown stains. Dermis was unarmored. The sponge has a reticulate fibre skeleton with some pilling of fibres centrally. The reticulation is irregular and all fibres are uncoiled. Primary fibres are ~50 µm wide. Secondary fibres are ~40 µm wide with finer fibres between as a tertiary skeleton, 10 µm wide. The voucher specimen was deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

**Extraction and isolation**

The sponge of the family Spongidae (SS-1202, 1.25 kg, wet weight) collected at Okinawa, was extracted with MeOH (2L x 2) to afford the extract (73.3 g), which was partitioned stepwise between organic solvents [EtOAc (500 mL x 3) and n-BuOH (500 mL x 3)] and H₂O (500 mL) to give EtOAc-soluble materials (11.8 g) and n-BuOH-soluble materials (3.6 g). A part (1.0 g) of n-BuOH soluble materials was fractionated by gel filtration (Sephadex LH-20, GE Healthcare; eluent, MeOH). A fraction was purified by C_{18} column chromatography (Cosmosil 140 C_{18} PREP, Nakarai Tesque Inc.; eluent, MeOH/H₂O, 70:30 to 100:0) and SiO₂ column chromatography (Wakosil C-300, Wako Pure Chemical Industries, Ltd.; eluent, CH₃CH₂MeOH, 95:5 to 1:00) to afford taurospirngs B (1, 1.7 mg, 0.0048%, wet weight) and C (2, 4.2 mg, 0.0012%).

**Taurospirng B (1).** colorless amorphous solid; [α]D²⁵ + 3.4 (c 1.0, MeOH); IR (film) 3400, 1732, 1646, 1456, 1172, 1046 cm⁻¹; ^1^H and ^13^C NMR data see Table 1; HRESIMS (neg) m/z 682.47036 [calced for C_{38}H_{42}NO_{5}s (M-H)] Δ=1.84 mmu.

**Taurospirng C (2).** colorless amorphous solid; [α]D²⁵ + 0.5 (c 1.0, MeOH); IR (film) 3369, 1730, 1636, 1465, 1171, 1044 cm⁻¹; ^1^H and ^13^C NMR data see Table 1; HRESIMS (neg) m/z 698.46739 [calced for C_{39}H_{44}NO_{5}s (M-H)] Δ=0.28 mmu.

**Solvency of taurospirng B (1).** Taurospirng B (1, 1.0 mg, 1.46 µmol) was dissolved in MeOH/1M HCl (10:1, 500 µL). After stirring for 12 h at 80 °C, the mixture was concentrated by Ar blowing. The residue was extracted with CHCl₃, which was concentrated by Ar blowing to afford acetylenic fatty acid part (5, 0.5 mg, 1.28 µmol, 88%). The remaining CHCl₃ insoluble material was tuarineamide part (4, 0.4 mg, 1.23 µmol, 84%).

**Taurineamide part (4).** ^1^H NMR (600 MHz, CD,OD) δ 3.67 (t, J = 6.6 Hz, 2H), 3.58 (m, 1H), 3.03 (t, J = 6.6 Hz, 2H), 2.43 (d, J = 14.0 Hz, 1H), 2.36 (d, J = 14.0 Hz, 1H), 1.66–1.32 (m, 10H), 1.27 (s, 3H), 0.97 (t, J = 7.1 Hz, 9H); HRESIMS (neg) m/z 324.14876 [calced for C_{23}H_{24}NO_{5}s (M-H)] Δ=0.13 mmu.

(S)-MTPAester of 4. (R)-(-)-MTPACI (1.23 µl) was added to a solution of 4 (0.4 mg, 1.23 µmol) in pyridine (50 µl). After stirring for 1h at rt, (R)-(-)-MTPACI (1.25 µl) was added to the mixture additionally. After stirring for 1h at rt, the reaction mixture was concentrated in vacuo. The residue was dissolved with MeOH and passed through an ion-exchange column (Amberlite IR-120 H⁺ form, eluent, MeOH) and concentrated in vacuo. Then, the residue was purified by C18 HPLC (Mightyss RP-18 GP, Kanto Chemical Co., Inc., 4.6 x 250 mm; eluent, CH₃CN/H₂O, 70–100%; flow rate, 1.0 mL/min; UV detection at 230 nm) to afford (S)-MTPA ester of 4 (0.4 mg, 0.74 mmol, 60%); ^1^H NMR (600 MHz, CD,OD) δ 7.75 (m, 1H), 7.48 (m, 1H), 5.16 (m, 1H), 3.65 (t, J = 6.6 Hz, 2H), 3.59 (s, 3H), 3.02 (t, J = 6.6 Hz, 2H), 2.30 (d, J = 14.3 Hz, 1H), 2.24 (d, J = 14.3 Hz, 1H), 1.59 (m, 2H), 1.24 (m, 2H), 1.80–1.10 (m, 6H), 1.14 (s, 3H), 0.99 (t, J = 7.3 Hz, 3H); HRESIMS (neg) m/z 540.18929 [calced for C_{29}H_{33}NO_{5}sF₆S (M-H)] Δ=0.84 mmu.

(R)-MTPAester of 4. The (R)-MTPA ester of 4 (0.4 mg, 0.74 mmol) was obtained from 4 (0.4 mg, 1.23 µmol) in 60% by using (S)-(-)-MTPACI through the same procedure as described for preparation of the (S)-MTPA ester of 4; ^1^H NMR (600 MHz, CD,OD) δ 7.57 (m, 1H), 7.47 (m, 4H), 5.17 (m, 1H), 3.66 (t, J = 6.6 Hz, 2H), 3.60 (s, 3H), 3.02 (t, J = 6.6 Hz, 2H), 2.38 (d, J = 14.3 Hz, 1H), 2.32 (d, J = 14.3 Hz, 1H), 1.69 (m, 2H), 1.42 (m, 2H), 1.80–1.10 (m, 6H), 1.22 (s, 3H), 0.90 (t, J = 7.3 Hz, 3H); HRESIMS (neg) m/z 540.18851 [calced for C_{29}H_{33}NO_{5}sF₆S (M-H)] Δ=0.06 mmu.

(R)-4-methyl-4-((triethyloxycarbonyl)oxy)tetrahydro-2H-pyran-2-one (7). Imidazole (1.12 g, 16.45 mmol), DMAP (100.4 mg, 0.82 mmol), and TESCI (2.06 ml, 12.27 mmol) were added to a solution of D-mevalonolactone (1.07 g, 8.22 mmol) in DMF (20 mL) at 0 °C. After stirring for 24 h at rt, H₂O was added to the mixture, which was extracted with EtOAc. The organic layer was washed with saturated aqueous CuSO₄, H₂O, and brine, dried with MgSO₄, and concentrated in vacuo. The residue was purified by a SiO₂ column (n-hexane/EtOAc, 100:0 to 85:15) to afford 7.
(1.36 g, 4.23 mmol) and AZADO (42.9 mg, 0.28 mmol) were added to a solution of 11a and 11b (1.24 g, 2.82 mmol) in CH₂Cl₂ (4 mL). After stirring for 4 h at rt, CH₂Cl₂ (35 mL) and a mixture of saturated aqueous NaHCO₃ and saturated aqueous Na₂SO₃ (1:1, 30 mol) were added to the mixture, which was extracted with CH₂Cl₂. The organic layer was washed with brine, dried with MgSO₄, and concentrated in vacuo. The residue was purified by a SiO₂ column (n-hexane/Me₂CO, 100:0 to 90:10) to afford 12 (1.16 g, 888 mmol, 95%; pale yellow oil). [δ] = 17.1 Hz (20 ml) at 78 °C. After stirring for 3 h at rt, the reaction mixture was concentrated in vacuo. The residue was purified by a SiO₂ column (n-hexane/Me₂CO, 100:0 to 80:20) to afford 12a (563 mg, 1.30 mmol, 97%, 97.3 d); pale brown oil; [δ] = 17.1 Hz (20 ml) at 78 °C. After stirring for 3 h at rt, the reaction mixture was concentrated in vacuo. The residue was purified by a SiO₂ column (n-hexane/Me₂CO, 100:0 to 90:10) to afford 10 (1.29 g, 4.63 mmol, 62%), which was directly used in the next step; pale yellow oil; HRESIMS (pos.) m/z 344.1360 [calcd for C₁₅H₂₁O₅Si] (M+Na) = 0.10 mmu.

(5)-7-(3-Chloroallyl)-9,9-diethyl-7-methyl-1-phenyl-4,8-dioxo-9-silaundecane (10). N,N-diisopropylphenylamine (3.15 ml, 18.56 mmol) and benzylchloromethyl ether (1.91 ml, 13.92 mmol) were added dropwise to a solution of 9 (1.29 g, 4.63 mmol) in CH₂Cl₂ (32 ml) at 0 °C. After stirring for 12 h at 0 °C, saturated aqueous NaHCO₃ was added to the mixture, which was extracted with EtOAc. The organic layer was washed with H₂O and brine, dried with MgSO₄, and concentrated in vacuo. The residue was purified by a SiO₂ column (n-hexane/Me₂CO, 100:0 to 90:10) to afford 10 (1.56 g, 3.91 mmol, 84%), which was directly used in the next step; pale yellow oil; HRESIMS (pos.) m/z 421.9366 [calcd for C₁₅H₂₁O₅Si] (M+Na) = 0.04 mmu.

(8S)-10-((benzoxymethoxy)-8-methyl-8-((triethylsilyloxy)dec-5-yn-4-ol (11a). A solution of 12 (580 mg, 1.34 mmol) in iPrOH (11.6 ml) was added to a solution of [1S,2S]-N-(p-toluenesulfonyl)-1,2-diphenylethanesiamine [(p-cymene) ruthenium](II) (68.4 mg, 0.11 mmol) in iPrOH (8.2 ml). After stirring for 3 h at rt, the reaction mixture was concentrated in vacuo. The residue was purified by a SiO₂ column (n-hexane/Me₂CO, 100:0 to 80:20) to afford 11a (563 mg, 1.30 mmol, 97%, 97.3 d); pale brown oil; [δ] = 17.1 Hz (20 ml) at 78 °C. After stirring for 3 h at rt, the reaction mixture was concentrated in vacuo. The residue was purified by a SiO₂ column (n-hexane/Me₂CO, 100:0 to 90:10) to afford 11b (1.29 g, 4.63 mmol, 62%), which was directly used in the next step; pale yellow oil; HRESIMS (pos.) m/z 425.7512 [calcd for C₁₅H₂₁O₅Si] (M+Na) = 0.66 mmu.

(R)-MTPA ester of 11a. DMAP (0.2 mg, Et₃N (0.65 µL), and (R)-(-)-MTPACl (0.33 mmol) were added to a solution of 11a (0.5 mg, 1.15 mmol) in CH₂Cl₂ (30 µl). After stirring for 3 h at rt, the mixture was added dropwise to a solution of 1 (1.41 g, 3.53 mmol) in THF (15 ml) at 78 °C. After stirring for 2 h at 78 °C, n-BuLi (1.60 M in hexane, 2.21 ml, 3.54 mmol) was added dropwise at 78 °C additionally. Then a solution of butyraldehyde (0.80 ml, 8.86 mmol) in THF (15 ml) was added dropwise to the mixture. After stirring for 1 h at -78 °C, saturated aqueous NH₄Cl was added to the mixture, which was extracted with EtOAc. The organic layer was washed with H₂O and brine, dried with MgSO₄, and concentrated in vacuo. The residue was purified by a SiO₂ column (n-hexane/Me₂CO, 100:0 to 90:10) to afford mixture of 11a and 11b (1.21 g, 2.78 mmol, 79%), which was directly used in the next step; pale yellow oil; HRESIMS (pos.) m/z 457.2474 [calcd for C₂₅H₂₃O₅Si] (M+Na) = 0.03 mmu.

(S)-10-((benzoxymethoxy)-8-methyl-8-((triethylsilyloxy)dec-5-yn-4-one (12). Iodobenzene diacetate
(4R,8S)-10-(benzoxylmethoxy)-8-methyl-7-
(triethylsilyloxy)dec-5-en-1-yl (11b). 11b (555 mg, 1.27 mmol) was obtained from 12 (580 mg, 1.34 mmol) in 96% (79.3 dr) by using (1R,2R)-N-(p-toluenesulfonyl)-1,2-
diphenylethanediamine-[p-cymene]ruthenium(II) through the same procedure as described for preparation of 11a; pale brown oil; [α]D 10 +8.4 (c 1.77, CHCl3); IR (neat) νmax 3447, 2926, 2875, 1545, 1376, 1150, 1102, 1041, 741 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 7.38-7.26 (m, 5H), 4.76 (s, 2H), 4.61 (s, 2H), 4.35 (brs, 1H), 3.74 (t, J = 7.3 Hz, 2H), 2.44 (dd, J = 16.4 and 1.8 Hz, 1H), 2.36 (dd, J = 16.4 and 1.8 Hz, 1H), 1.98 (m, 1H), 1.87 (m, 2H), 1.63 (m, 2H), 1.46 (m, 2H), 1.33 (s, 3H), 0.94 (t, J = 7.8 Hz, 9H), 0.93 (t, J = 7.3 Hz, 4H), 0.59 (q, J = 7.8 Hz, 6H); 13C NMR (100 MHz, CDCl3) δ 137.8, 128.4, 127.9, 127.4, 93.4, 83.4, 82.3, 74.0, 69.2, 64.4, 62.4, 41.2, 40.1, 33.4, 28.0, 18.5, 13.7; 1H; HRESIMS (pos. m/z 571.36052 [calcd for C25H22O3NaSi (M+Na) − 0.41 mmu].

(35,7S)-7-(tert-butyldimethylsilyloxy)-3-methyl-3-
(triethylsilyloxy)dec-1-en-1-ol (14a). 20% Pd(OH)2/C (13.5 mg) was added to a solution of 13a (27 mg, 0.049 mmol) in EtOAc and 20% Pd(OH)2/C (2.5 ml) were added to the mixture every 1 h. After stirring under H2 for 5 h at rt, the mixture was filtered through a cotton plug, and the filtrate was concentrated in vacuo. The residue was purified by a SiO2 column (CHCl3) to afford 14a (10.9 mg, 0.025 mmol, 51%); colorless oil; [α]D 10 +4.2 (c 1.87, CHCl3); IR (neat) νmax 3363, 2952, 2935, 2873, 1455, 1253, 1040, 773 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 7.28-7.18 (m, 2H), 1.73 (s, 3H), 0.89 (t, J = 7.2 Hz, 3H), 0.86 (q, J = 7.9 Hz, 6H); 13C NMR (100 MHz, CDCl3) δ 77.8, 72.0, 60.0, 43.2, 42.0, 39.4, 37.6, 27.7, 25.9, 20.6, 18.5, 18.1, 14.3, 7.0, 6.7, -4.5; HRESIMS (pos. m/z 455.33448 [calcd for C25H22O3NaSi (M+Na) − 0.18 mmu].

(35R,7R)-7-(tert-butyldimethylsilyloxy)-3-methyl-3-
(triethylsilyloxy)dec-1-en-1-ol (14b). 8.4 (mg, 0.019 mmol) was obtained from 13b (15 mg, 0.027 mmol) in 70% through the same procedure as described for preparation of 14a; colorless oil; [α]D 10 −2.2 (c 1.39, CHCl3); IR (neat) νmax 3368, 2929, 2917, 2261, 1614, 1261, 1041, 775 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 7.39 (2H, brt, J = 5.0 Hz), 3.63 (brq, J = 5.6 Hz), 3.18 (brs, 1H), 1.75 (dt, J = 14.4, 5.5 Hz, 1H), 1.70-1.17 (m, 12H), 0.96 (OH, t, J = 7.9 Hz, TES-Me), 0.89 (t, J = 7.3 Hz, 1H), 0.88 (s, 9H), 0.61 (q, J = 7.9 Hz, 6H), 0.04 (s, 3H), 0.03 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 77.9, 71.9, 60.3, 43.1, 43.9, 39.4, 37.7, 27.7, 25.9, 20.5, 18.6, 18.1, 14.3, 7.0, 6.8, -4.5; HRESIMS (pos. m/z 455.33448 [calcd for C25H22O3NaSi (M+Na) − 0.64 mmu].

(35S,7S)-3,7-dihydroxy-3-methyldecanoic acid (15a). DMSO (70.0 µl) and Et3N (24.0 µl) were added to a solution of 14a (5.0 mg, 0.012 mmol) in CH2Cl2 (212.0 µl). After stirring for 5 min at 0 °C, SO2-pyr (19.2 mg) was added to the mixture. After stirring for 1 h at rt, the mixture was treated with water and extracted with EtOAc. The organic layer was washed with H2O and brine, dried with MgSO4, and concentrated in vacuo to afford crude aldehyde. Then t-BuOH (0.73 ml), NaH/PtO2 (8.2 mg in 182.0 µl of H2O), and 2-methyl-2-butenone (2M in THF, 50.4 µl) were added to the crude aldehyde. After stirring for 5 min at rt, NaClO3 (7.3 mg) was added to the mixture. After stirring for 15 min at 0 °C, saturated aqueous NaHSO3 was added to the mixture, which was extracted with EtOAc and brine, dried with MgSO4, and concentrated in vacuo. The residue was purified by a SiO2 column (n-hexane/ EtOAc, 100:0 to 90:10) to afford 15a (2.0 mg, 0.0092 mmol, 77%); colorless oil; [α]D 10 −5.9 (c 2.02, CHCl3); IR (neat) νmax 3410, 2923, 2853, 1717, 1562, 1456, 1240, 1129, 774 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 5.05 (brs, 1H), 3.64 (m, 1H), 2.56 (d, J = 5.6 Hz, 1H), 2.47 (d, J = 15.6 Hz, 1H), 1.61-1.55 (m, 10H), 1.28 (s, 3H), 0.92 (t, J = 6.8 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 178.5, 71.8, 71.4, 44.6, 41.3, 39.6, 37.2, 26.7, 19.9.

Page 6 of 8
Cytotoxic assays.

Antimicrobial assay.

Acknowledgments

We thank Mr. Z. Nagahama and Mr. K. Uehara, Okinawa, for their help with sponge collection, and Ms. S. Oka, Instrumental Analysis Division, Equipment Management Center, Creative Research Institute, Hokkaido University, for measurements of mass spectrometry. We specially thank ADEKA corporation for supplying D-mevalonolactone, and Prof. Y. Iwabuchi, Graduate School of Pharmaceutical Sciences, Tohoku University, for supplying AZADO. This work was supported by The Naito Foundation, Cooperative Research Program of Medical Mycology Research Center, Chiba University, and Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Notes and references
Graphical Abstract

taurospongin B