Marine Natural Products. XXXII.[1] Absolute Configurations of C-4 of the Manoalide Family, Biologically Active Sesterterpenes from the Marine Sponge *Hyrtios erecta*

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Received July 14, 1993; accepted August 31, 1993

Cytotoxic sesterterpenes, manoalide 25-acetals (1a, 1b), *seco*-manoalide (2), (*E*)-neomanoalide (3), (*Z*)-neomanoalide (4), and heteronemin (6), were isolated from the marine sponge *Hyrtios erecta* (collected at Amami Island, Kagoshima Prefecture, Japan) by bioassay-guided separation and the absolute configurations of these manoalide family members have been determined. Manoalide 25-acetals (1a, 1b) were shown to exhibit *in vivo* antitumor activity and to inhibit the DNA-relaxing activity of mouse DNA topoisomerase I and the DNA-unknotting activity of calf thymus DNA topoisomerase II.

Keywords marine sponge; *Hyrtios erecta*; sesterterpene; manoalide; neomanoalide; antitumor activity

Some years ago, de Silva and Scheuer isolated four sesterterpenes with antibiotic activity, *i.e.*, manoalide (**1**),[2] *seco*-manoalide (**2**),[3] and (*E*)- and (*Z*)-neomanoalides (**3** and **4**),[3] from the Palauan marine sponge *Luffariella variabilis* and elucidated their plane structures. Afterwards, it was found that manoalide inhibits phospholipase A_2 and exhibits topical anti-inflammatory activity,[4] while *seco*-manoalide inhibits aldose reductase.[5] Since then, over twenty papers concerning biological activities of manoalide have been reported, and manoalide is currently a candidate for a nonsteroidal anti-inflammatory medicine. Because of these desirable biological activities, several synthetic investigations of manoalide have been carried out and racemic manoalide has been synthesized by several groups.[6]

As part of our continuing study of marine natural products, seeking new biologically active compounds,[1,7] we have investigated the chemical constituents of the marine sponge *Hyrtios erecta* by means of bioassay-guided fractionation and separation. Examination of cytotoxicity against L1210 and KB cell lines led us to the isolation of four sesterterpenes, manoalide (**1**) [as a mixture of 25-epimers (**1a, 1b**) [*vide infra*)], *seco*-manoalide (**2**), (*E*)-neomanoalide (**3**), and (*Z*)-neomanoalide (**4**), together with two scalarane-type sesterterpenes, scalarafuran (**5**) and heteronemin (**6**), as shown in Chart 1. In this paper, we report the isolation of these cytotoxic sesterterpenes and the absolute configurations of the manoalide family.[8,9]

A fresh whole marine sponge *Hyrtios erecta*, collected in July at Amami Island, Kagoshima Prefecture, Japan, was immersed in acetone, and the acetone solution was concentrated under reduced pressure at below 30 °C. The acetone extract thus prepared was found to have a growth-inhibitory effect against L1210 and KB cells (89.0 and 94.3% inhibitions at 5 μg/ml concentration, respectively). The acetone extract was partitioned into an ethyl acetate (AcOEt)–water mixture and the water phase was further partitioned with 1-butanol. The AcOEt-soluble portion, which contained cytotoxic constituents [97.3% (L1210) and 94.9% (KB) inhibitions at 1 μg/ml], was then subjected to silica gel column chromatography (SiO_2 column) to provide four fractions: fr. 1 [97% (L1210), 73.9% (KB) at 1 μg/ml], fr. 2 [97.3% (L1210), 86.0% (KB) at 1 μg/ml], fr. 3 [96.0% (L1210), 20.6% (KB) at 1 μg/ml], and fr. 4 [92.3% (L1210), 95.9% (KB) at 1 μg/ml], in order of elution.

Fraction 1 was further separated repeatedly by SiO_2 column chromatography to give scalarafuran (**5**) [IC_{50}: 2.9 μg/ml (L1210) and 4.0 μg/ml (KB)] and heteronemin (**6**) [IC_{50}: 0.058 μg/ml (L1210) and 0.23 μg/ml (KB)]. Detailed analyses of the proton and carbon-13 nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra demonstrated the identity of these two furano-sesterterpenoids with scalarafuran[10] and heteronemin,[11] which were previously isolated from two marine sponges, *Spongia idia* and *Heteronema erecta*, respectively.

Repeated chromatographic separation of fr. 3 provided a linear sesterterpene, *seco*-manoalide (**2**) [IC_{50}: 0.033 μg/ml (L1210) and 0.42 μg/ml (KB)], together with 5-hydroxyindole-3-aldehyde (**7**) (without cytotoxicity).[12] The physicochemical properties of **2** were identical with those of an authentic sample of *seco*-manoalide.[3,8] Fraction 4 was further separated repeatedly by SiO_2 column chromatography to provide (*E*)-neomanoalide (**3**) and (*Z*)-neomanoalide (**4**) [IC_{50} of the mixture of **3** and **4**: 2.8 μg/ml (L1210) and 3.6 μg/ml (KB)]. Here again, the physicochemical properties of **3** and **4** confirmed their identity with (*E*)-neomanoalide[3] and (*Z*)-neomanoalide,[3,8] respectively.

Chromatographic separation of fr. 2 afforded manoalide [IC_{50}: 0.022 μg/ml (L1210) and 0.26 μg/ml (KB)], which was one of the major components of the AcOEt-soluble portion (Chart 1), and was obtained as a mixture (*ca.* 3 : 2) of the C-25 epimers (designated as manoalide 25-acetals hereafter in this paper). Manoalide 25-acetals (**1a, 1b**) obtained here behaved as a single compound on TLC and HPLC (ordinary-phase and reversed-phase) analyses. However, the ^{1}H-NMR and ^{13}C-NMR spectra of manoalide 25-acetals (**1a, 1b**) showed a complicated signal

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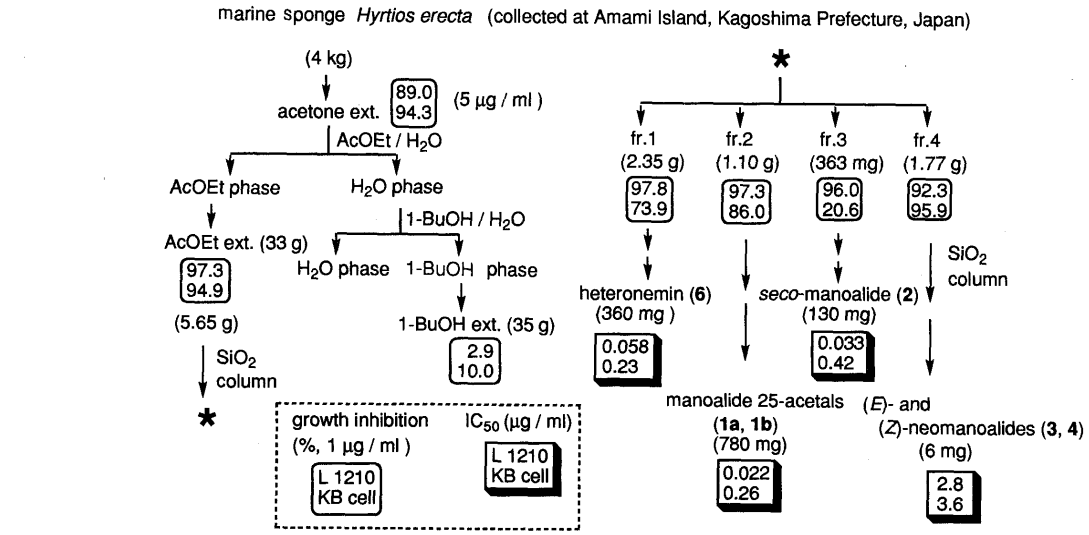

Chart 1. Cytotoxicity-Directed Separation Procedure for Compounds of the Manoalide Family

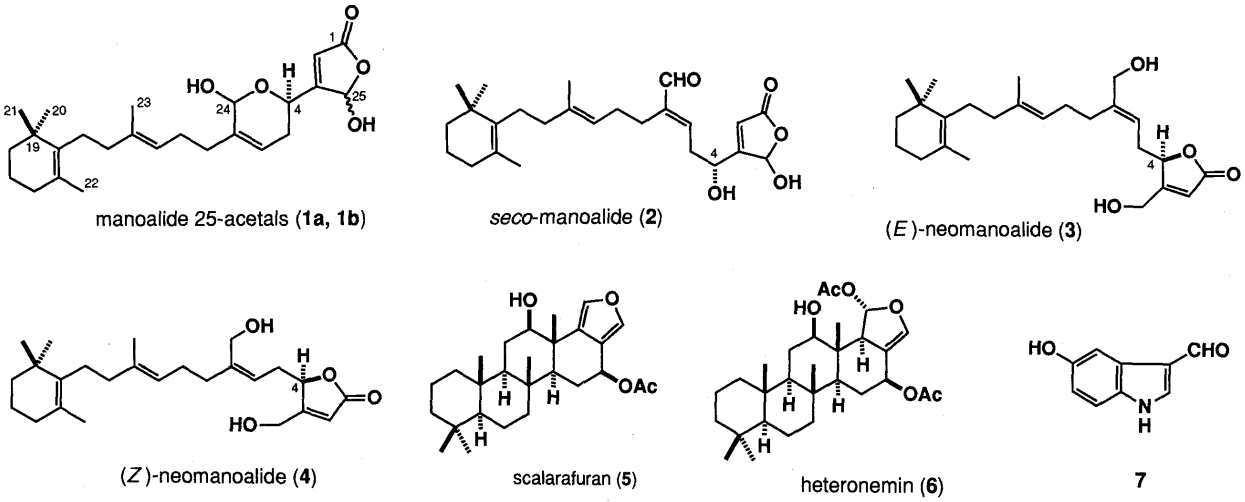

Chart 2

pattern (Table I). The major signals observed in the ^{1}H- and ^{13}C-NMR spectra of manoalide 25-acetals (**1a, 1b**) were identical with those of manoalide (**1**),[2,8,9] while the minor signals ascribable to both protons and carbons attached to the butenolide ring were observed separately. The correlations between those major proton and carbon signals, as well as minor signals, were observed by means of ^{1}H–^{13}C shift correlation spectroscopy (^{1}H–^{13}C COSY). Consequently, manoalide 25-acetals (**1a, 1b**) obtained here appear to be a mixture of manoalide and its epimer at the 25-acetal moiety, although the absolute configurations of both are undetermined.

Acetylation of manoalide 25-acetals (**1a, 1b**) furnished two diacetates, which were separated by HPLC to provide **8a** and **8b**. Compounds **8a** and **8b** were identical with the products of acetylation of authentic manoalide (**1**).[2,8,9] Furthermore, acetylation under mild conditions of manoalide 25-acetals (**1a, 1b**) afforded a 25-monoacetate mixture (**9a, 9b**), which was identical with the product of mild acetylation of authentic manoalide (**1**).[2,8,9] Consequently, it has been elucidated that manoalide 25-acetals (**1a, 1b**) consist of a mixture of manoalide and its 25-acetal epimer.[13]

Manoalide and related sesterterpenes contain one asymmetric carbon at C-4 except for the C-24 and C-25 in the two acetal moieties. In 1988, the C-4 absolute configuration of manoalide (**1**) was shown to be *R* on the basis of a synthesis of the enantiomer of manoalide diol (**10**), which was obtainable by sodium borohydride ($NaBH_4$) reduction of manoalide.[14] As for the absolute configurations of the other members of the manoalide family, a chemical conversion has been carried out. Thus, *seco*-manoalide (**2**) was converted to manoalide (**1**) by ultraviolet (UV) irradiation in benzene solution.[3] In regard to the structural relationship between manoalide (**1**) and (*E*)- and (*Z*)-neomanoalide (**3, 4**), we may presume that the C-4 hydroxyl group of manoalide-acid, which is supposedly formed by opening of two acetal moieties of manoalide (**1**), cyclizes with the C-1 carboxyl group to form a butenolide having two aldehyde groups at C-24 and C-25. Reduction of the aldehydic residues would give two neomanoalides (**3, 4**).

In order to confirm the absolute configuration at C-4 of the manoalide family, we have prepared several

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derivatives of these manoalides suitable for applying Horeau's method[15] and the modified Mosher's method.[16] First, manoalide diol (**10**), prepared by $NaBH_4$ reduction of manoalide 25-acetals (**1a**, **1b**), was converted to the C-24 monoacetate **11** by treatment with acetic anhydride in pyridine at 0 °C. The monoacetate **11** was subjected to Horeau's method.[15] The recovered α-phenylbutyric acid was treated with (+)-α-phenylethylamine and the resulting mixture was analyzed by gas-liquid chromatography (GLC), revealing the *R* configuration of C-4 of **11**. Consequently, the C-4 absolute configuration of manoalide (**1**) has been reconfirmed to be *R*.[17]

Next, *seco*-manoalide (**2**) was subjected to UV-irradiation in the presence of Rose Bengal to provide manoalide (**1**), which was found to be identical with an authentic sample by comparison of physical properties including the $[\alpha]_D$ values. Thus, the C-4 absolute configuration of *seco*-manoalide (**2**) has been confirmed to be the same as that of manoalide (**1**).

Finally, in order to determine the C-4 absolute configuration of (*E*)-neomanoalide (**3**), a modified Mosher's method[16] was applied to a 4-hydroxyl derivative of **3**. Compound **3** was first treated with *tert*-butyldimethylsilyl (TBDMS) chloride to furnish the 24,25-diTBDMS derivative **12**, which was further treated with methyl lithium to give the butenolide-opened derivative **13**, having a free 4-hydroxyl residue. The tetraol-24,25-diTBDMS derivative **13** was then converted to (+)-(*R*)- and (−)-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetates (MTPA esters) (**14a** and **14b**) which were subjected to ^{1}H-NMR analysis. Due to the anisotropic effect of the benzene ring, negative $\Delta\delta$ ($=\delta(-)-\delta(+)$) values were obtained for the signals ascribable to protons attached to the carbons at C-2 (−0.06) and C-25 (−0.14, −0.23) of **14a** and **14b**, while positive $\Delta\delta$ values were obtained for the signals due to protons at C-5 (+0.04, +0.05), C-6 (+0.08), and C-24 (2H, singlet, +0.05). Consequently, the C-4 absolute configuration of (*E*)-neomanoalide (**3**) has been clarified to be *R*, being the same as that of manoalide (**1**). Furthermore, the circular dichroism (CD) spectra of both (*E*)-neomanoalide (**3**) and (*Z*)-neomanoalide (**4**) showed negative maxima, with $[\theta]_{211}$ −31000 for **3** and $[\theta]_{212}$ −54000 for **4**, due to the transition of the butenolide moiety. In conclusion, it has been clarified that *seco*-manoalide (**2**), (*E*)-neomanoalide (**3**), and (*Z*)-neomanoalide (**4**) have the 4*R* absolute configuration, like manoalide (**1**).

As mentioned above, manoalide 25-acetals (**1a**, **1b**) and heteronemin (**6**) were found to exhibit cytotoxic activity. We next examined the *in vivo* antitumor activity of manoalide 25-acetals (**1a**, **1b**). It was found that manoalide 25-acetals (**1a**, **1b**) showed antitumor activity in P388 leukemia-inoculated CDF_1 mice (*T*/*C* 150% at 1 mg/kg). In addition, manoalide 25-acetals (**1a**, **1b**) were shown to inhibit the DNA-relaxing activity of mouse DNA topoisomerase I and the DNA-unknotting activity of calf

TABLE I. ^{1}H- and ^{13}C-NMR Data for Manoalide 25-Acetals (**1a**, **1b**)

Carbon	^{1}H (major)	^{1}H (minor)	^{13}C (major)	^{13}C (minor)
1	—	—	172.3 (s)	172.0 (s)
2	6.08 (br s)	6.04 (s)	116.8 (d)	117.9 (d)
3	—	—	169.2 (s)	168.4 (s)
4	4.91 (dd)	4.86 (m)	63.3 (d)	62.4 (d)
5	2.30 (2H, m)	*a)*	32.7 (t)	*a)*
6	5.73 (m)	5.72 (m)	120.7 (d)	121.1 (d)
7	—	—	137.4 (s)	*a)*
8	*ca.* 2.0 (2H, m)	*a)*	40.3 (t)	*a)*
9	*ca.* 2.0 (2H, m)	*a)*	27.9 (t)	*a)*
10	5.16 (m)	5.14 (m)	122.8 (d)	*a)*
11	—	—	137.0 (s)	*a)*
12	*ca.* 2.1 (2H, m)	*a)*	39.8 (t)	*a)*
13	*ca.* 2.1 (2H, m)	*a)*	25.9 (t)	*a)*
14	—	—	136.9 (s)	*a)*
15	—	—	126.9 (s)	*a)*
16	1.90 (2H, m)	*a)*	32.5 (t)	*a)*
17	1.57 (2H, m)	*a)*	19.5 (t)	*a)*
18	1.41 (2H, m)	*a)*	39.8 (t)	*a)*
19	—	—	34.9 (s)	*a)*
20	1.00 (3H, s)	*a)*	28.6 (q)	*a)*
21	1.00 (3H, s)	*a)*	28.6 (q)	*a)*
22	1.60 (3H, s)	*a)*	19.8 (q)	*a)*
23	1.65 (3H, s)	*a)*	16.1 (q)	*a)*
24	5.36 (s)	5.33 (s)	91.2 (d)	91.4 (d)
25	6.17 (s)	6.28 (s)	98.0 (d)	98.4 (d)

a) These signals overlapped with the signals observed for an authentic sample of manoalide (**1**).[8]

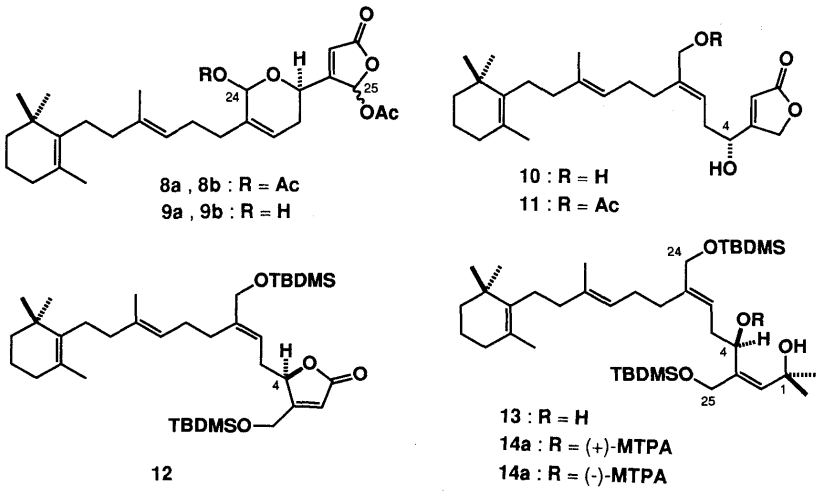

Chart 3

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thymus DNA topoisomerase II, IC_{50} being around 25 μM for both enzymes. Heteronemin (**6**) showed no inhibitory effect.

Experimental

The instruments used to obtain physical data and experimental conditions for chromatography were the same as described in our preceding paper.[1)]

Isolation of Manoalide 25-Acetals (1), *seco*-Manoalide (2), (*E*)-Neomanoalide (3), (*Z*)-Neomanoalide (4), Scalarafuran (5), and Heteronemine (6) The marine sponge *Hyrtios erecta* (4 kg, wet) was collected at Amami Island, Kagoshima Prefecture, Japan in July. The frozen sample was steeped in acetone, and the filtrate was concentrated under reduced pressure below 30 °C to give an aqueous suspension, which was partitioned with AcOEt. The AcOEt-soluble portion was evaporated under reduced pressure to give the AcOEt extract (33 g). The H_2O phase was further partitioned with 1-butanol to give the 1-butanol-soluble portion (35 g). The cytotoxicity of each fraction towards L1210 and KB cell lines was examined as described below to find the cytotoxic constituents.

The AcOEt extract (5.65 g) was subjected to silica gel column chromatography (SiO_2 column) (1-hexane : AcOEt = 5 : 1 → 1 : 3) to furnish four fractions, fr. 1 (2.35 g), fr. 2 (1.10 g), fr. 3 (365 mg), and fr. 4 (1.77 g). Fraction 1 (2.35 g) was further subjected to SiO_2 column chromatography [a) 1-hexane : AcOEt = 10 : 1 → 6 : 1; b) 1-hexane : AcOEt = 10 : 1] to give scalarafuran (**5**) (114 mg) and heteronemin (**6**) (360 mg). Fraction 2 (1.10 g) was separated on an SiO_2 column [a) 1-hexane : AcOEt = 3 : 1 → 1 : 1; b) $CHCl_3$: MeOH = 60 : 1] to give manoalide 25-acetals (**1a, 1b**) (780 mg). Fraction 3 (363 mg) was separated on an SiO_2 column [a) 1-hexane : AcOEt = 1 : 1; b) $CHCl_3$: MeOH = 50 : 1 → 20 : 1] to give *seco*-manoalide (**2**) (130 mg) and 5-hydroxyindole-3-aldehyde (**7**) (26 mg). Fraction 3 (1.77 g) was separated on an SiO_2 column [a) $CHCl_3$: MeOH : H_2O = 100 : 3 : 1 (lower phase) → 5 : 5 : 1; b) 1-hexane : AcOEt = 1 : 1; c) $CHCl_3$: MeOH = 50 : 1] to give (*E*)-neomanoalide (**3**) (3 mg) and (*Z*)-neomanoalide (**4**) (3 mg).

Manoalide 25-Acetals (**1a, 1b**): An amorphous solid, $[\alpha]_D$ +80° (c = 0.2, MeOH, 25 °C). UV λ_{max}^{MeOH} nm (ε): 227 (4000). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3311, 2928, 1770. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): as given in Table I. ^{13}C-NMR (67.8 MHz, $CDCl_3$, δ_C): as given in Table I. High-resolution (HR)-MS Obsd. m/z: 416.256. Calcd for $C_{25}H_{36}O_5$: 416.256 (M^+).

seco-Manoalide (**2**): An amorphous solid, $[\alpha]_D$ +16° (c = 0.02, $CHCl_3$, 25 °C). UV λ_{max}^{MeOH} nm (ε): 229 (18000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3324, 2944, 1766, 1688. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 9.42 (1H, s, 24-H), 6.57 (1H, t-like, J = *ca.* 7 Hz, 6-H), 6.10 (2H, br s, 2,25-H), 5.12 (1H, t-like, J = *ca.* 7 Hz, 10-H), 4.83 (1H, m, 4-H), 2.84 (2H, m, 5-H), 2.32 (2H, t-like, J = *ca.* 7.5 Hz, 13-H), 2.08 (2H, t-like, J = *ca.* 7.5 Hz, 12-H), 2.01 (4H, m, 8,9-H), 1.91 (2H, t-like, J = *ca.* 6.5 Hz, 16-H), 1.61 (3H, s, 23-H), 1.60 (3H, s, 22-H), 1.56 (2H, m, 17-H), 1.41 (2H, t-like, J = *ca.* 6 Hz, 18-H), 0.99 (6H, s, 20,21-H). ^{13}C-NMR (67.8 MHz, $CDCl_3$, δ_C): 195.5 (d, C-24), 171.3 (s, C-1), 170.6 (s, C-3), 148.5 (d, C-6), 145.9 (s, C-7), 137.6 (s, C-11), 137.0 (s, C-14), 127.1 (s, C-15), 122.3 (d, C-10), 118.5 (d, C-2), 98.3 (d, C-25), 66.8 (d, C-4), 40.3 (t, C-12), 39.9 (t, C-18), 35.1 (s, C-19), 34.6 (t, C-5), 32.8 (t, C-16), 29.8 (2C, q, C-20, 21), 28.7 (t, C-9), 26.8 (t, C-13), 24.6 (t, C-8), 19.7 (q, C-22), 19.6 (t, C-17), 16.1 (q, C-23). HR-MS Obsd. m/z: 416.256. Calcd for $C_{25}H_{36}O_5$: 416.256 (M^+).

(*E*)-Neomanoalide (**3**): An amorphous solid, $[\alpha]_D$ −26° (c = 0.5, CH_2Cl_2, 25 °C). UV λ_{max}^{MeOH} nm (ε): 220 (8400). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390, 2920, 1750. CD (c = 0.1, MeOH): $[\theta]_{211}$ −31000 (neg. max.). ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 6.06 (1H, br s, 2-H), 5.41 (1H, t-like, J = *ca.* 7 Hz, 6-H), 5.14 (1H, t-like, J = *ca.* 6.5 Hz, 10-H), 5.08 (1H, t-like, J = *ca.* 5 Hz, 4-H), 4.56 (1H, d, J = *ca.* 16 Hz, 25-H_a), 4.47 (1H, d, J = *ca.* 16 Hz, 25-H_b), 4.08 (2H, s, 24-H), 2.74 (1H, ddd, J = 15.3, 7.3, 5.2 Hz, 5-H_a), 2.54 (1H, ddd, J = 15.3, 6.7, 6.7 Hz, 5-H_b), 1.65 (3H, s, 23-H), 1.61 (3H, s, 22-H), 1.00 (6H, s, 20, 21-H). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 173.3 (s, C-3), 172.8 (s, C-1), 143.4 (d, C-7), 137.1 (2C, s, C-11, 14), 127.0 (s, C-15), 122.7 (d, C-10), 117.0 (d, C-6), 115.8 (d, C-2), 81.9 (d, C-4), 66.0 (t, C-24), 58.5 (t, C-25), 40.3 (t, C-18), 39.9 (t, C-12), 35.7 (s, C-19), 32.8 (t, C-16), 30.2 (t, C-5), 28.7 (2C, q, C-20, 21), 28.5 (t, C-8), 27.9 (t, C-9), 26.7 (t, C-13), 19.8 (q, C-22), 19.6 (t, C-17), 16.1 (q, C-23). MS m/z (%): 402 (2.5, M^+), 137 (100).

(*Z*)-Neomanoalide (**4**): An amorphous solid, $[\alpha]_D$ −28° (c = 0.8, CH_2Cl_2, 25 °C). UV λ_{max}^{MeOH} nm (ε): 222 (8100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 2930, 1750. CD (c = 0.1, MeOH): $[\theta]_{212}$ −54000 (neg. max.). ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 6.02 (1H, br s, 2-H), 5.25 (1H, t-like, J = *ca.* 7.5 Hz, 6-H), 5.12 (1H, t-like, J = *ca.* 6.5 Hz, 10-H), 5.11 (1H, t-like, J = *ca.* 5 Hz, 4-H), 4.57 (1H, dd, J = 16.5, 1.2 Hz, 25-H_a), 4.50 (1H, dd, J = 16.5, 1.2 Hz, 25-H_b), 4.16 (1H, d, J = 11.9 Hz, 24-H_a), 4.12 (1H, d, J = 11.9 Hz, 24-H_b), 2.81 (1H, ddd, J = 14.7, 7.3, 4.9 Hz, 5-H_a). 2.74 (1H, ddd, J = 14.3, 7.9, 5.5 Hz, 5-H_b), 1.65 (3H, s, 23-H), 1.61 (3H, s, 22-H), 1.00 (6H, s, 20,21-H). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 173.3 (s, C-3), 172.6 (s, C-1), 143.4 (d, C-7), 137.0 (s, C-14), 136.8 (s, C-11), 127.0 (s, C-15), 123.0 (d, C-10), 120.0 (d, C-6), 115.7 (d, C-2), 82.1 (d, C-4), 60.0 (t, C-24), 58.5 (t, C-25), 40.3 (t, C-18), 39.9 (t, C-12), 35.6 (t, C-8), 35.0 (s, C-19), 32.8 (t, C-16), 30.3 (t, C-5), 28.7 (2C, q, C-20, 21), 27.9 (t, C-9), 26.7 (t, C-13), 19.9 (q, C-22), 19.6 (t, C-17), 16.1 (q, C-23). MS m/z (%): 402 (0.8, M^+), 137 (100).

Acetylation of Manoalide 25-Acetals (1a, 1b), Giving the Diacetates 8a and 8b A solution of **1a, 1b** (35 mg) in pyridine (1 ml) was treated with acetic anhydride (0.03 ml) and the mixture was stirred at 25 °C for 2 h. Then it was poured into water, and the whole was extracted with AcOEt. The AcOEt extract was washed with brine and dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt solution furnished a crude product, which was purified by HPLC (Zorbax SIL, 1-hexane : AcOEt = 3 : 1) to give **8a** (20 mg) and **8b** (8 mg).

8a: A glassy solid, $[\alpha]_D$ +81° (c = 1.0, $CHCl_3$, 25 °C). ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 6.85 (1H, br s, 25-H), 6.60 (1H, br s, 24-H), 5.85 (1H, br s, 2-H), 5.45 (1H, br d, J = *ca.* 5 Hz, 6-H), 5.27 (1H, t-like, J = *ca.* 7 Hz, 10-H), 4.52 (1H, br d, J = *ca.* 9 Hz, 4-H), 2.25 (4H, br s), 2.17 (4H, m), 1.99 (2H, m), 1.94 (2H, m, 16-H), 1.73, 1.69 (both 3H, s, OAc), 1.68 (3H, s, 23-H), 1.62 (2H, m, 17-H), 1.52 (3H, s, 22-H), 1.49 (2H, m, H-18), 1.11 (6H, s, 20,21-H). ^{13}C-NMR (67.8 MHz, C_6D_6, δ_C): 169.3 (s, C-1), 168.6, 168.5 (both s, OAc), 164.0 (s, C-3), 137.3 (2C, s, C-11, 14), 135.4 (s, C-7), 128.2 (s, C-15), 122.9 (d, C-10), 122.4 (d, C-6), 119.1 (d, C-2), 92.4 (d, C-25), 89.6 (d, C-24), 65.0 (d, C-4), 40.8 (t, C-12), 40.1 (t, C-18), 35.2 (s, C-19), 33.0 (t, C-16), 28.8 (2C, q, C-20, 21), 28.6 (2C, t, C-5, 9), 28.3 (t, C-8), 26.3 (t, C-13), 20.6 (q, OAc), 20.0 (t, C-17), 19.9 (2C, q, C-22, OAc), 16.2 (q, C-23). FAB-MS m/z (%): 523 [$(M+Na)^+$, 9], 441 [$(MH-AcOH)^+$, 30], 381 [$(MH-2AcOH)^+$, 33].

8b: A glassy solid, $[\alpha]_D$ +32° (c = 0.7, $CHCl_3$, 25 °C). ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 6.97 (1H, br s, 25-H), 6.47 (1H, br s, 24-H), 5.55 (1H, br s, 2-H), 5.43 (1H, br d, J = *ca.* 5 Hz, 6-H), 5.29 (1H, t-like, J = *ca.* 6.5 Hz, 10-H), 4.34 (1H, br d, J = *ca.* 9 Hz, 4-H), 2.27 (4H, br s), 2.18 (4H, m), 1.99 (2H, m), 1.94 (2H, m, 16-H), 1.73, 1.70 (both 3H, s, OAc), 1.69 (3H, s, 23-H), 1.61 (2H, m, 17-H), 1.61 (3H, s, 22-H), 1.47 (2H, m, 18-H), 1.12 (6H, s, 20,21-H). ^{13}C-NMR (67.8 MHz, C_6D_6, δ_C): 168.9 (s, C-1), 168.7, 167.9 (both s, OAc), 164.1 (s, C-3), 137.3, 137.2 (both s, C-11, 14), 135.2 (s, C-7), 128.2 (s, C-15), 122.9 (d, C-10), 122.4 (d, C-6), 119.2 (d, C-2), 92.6 (d, C-25), 89.9 (d, C-24), 64.3 (d, C-4), 40.8 (t, C-12), 40.1 (t, C-18), 35.2 (s, C-19), 33.0 (t, C-16), 28.8 (2C, q, C-20, 21), 28.4 (2C, t, C-5, 9), 28.3 (t, C-8), 26.3 (t, C-13), 20.6 (q, OAc), 20.3 (t, C-17), 20.0, 19.9 (both q, C-22, OAc), 16.2 (q, C-23). FAB-MS m/z (%): 523 [$(M+Na)^+$, 7], 441 [$(MH-AcOH)^+$, 42]. 381 [$(MH-2AcOH)^+$, 39].

Acetylation of Manoalide (1), Giving the Diacetates 8a and 8b Compound **1** (34 mg), which was obtained previously from a Palauan marine sponge *Luffariella* sp.,[8,9)] was acetylated under the same conditions as described above to give **8a** (19 mg) and **8b** (9 mg). These products were identified by comparisons of ^{1}H- and ^{13}C-NMR and $[\alpha]_D$ data with those of the above acetylation products of manoalide 25-acetals (**1a, 1b**).

Acetylation of Manoalide 25-Acetals (1a, 1b), Giving the Monoacetates 9a and 9b A solution of **1a, 1b** (10 mg) in pyridine (0.1 ml) was treated with acetic anhydride (0.003 ml) and the whole was stirred at 25 °C for 30 min. The reaction mixture was worked up as described above and the crude product (10 mg) was purified by SiO_2 column chromatography (1-hexane : AcOEt = 4 : 1) to give a monoacetate mixture (**9a** : **9b** = 54 : 46) (7 mg).

9a, 9b: An amorphous solid. ^{1}H-NMR (270 MHz, C_6D_6, **9a**: δ and **9b**: $\underline{\delta}$): 7.10 (1H, br s, 25-H), 6.94 (1H, br s, 25-H), 5.68 (1H, br d, J = *ca.* 1 Hz, 2-H), 6.09 (1H, br d, J = *ca.* 1 Hz, 2-H), 5.36[a)] (2H, m, 6, 10-H), 5.08[a)] (1H, br d, J = *ca.* 4.5 Hz, 24-H), 4.75 (1H, br dd, J = *ca.* 11.5, 2 Hz, 4-H), 4.53 (1H, br d, J = *ca.* 10 Hz, 4-H), 2.27 (3H, s, OAc), 2.25 (3H, s, OAc), 1.94[a)] (2H, t-like, J = *ca.* 6 Hz, 16-H), 1.70 (3H, s, 23-H), 1.69 (3H, s, 23-H), 1.63[a)] (2H, m, 17-H), 1.60 (3H, s, 22-H), 1.49[a)] (2H, m, 18-H), 1.45 (3H, s, 22-H), 1.12 (6H, s, 20,21-H), 1.11 (6H, s, 20,21-H). ^{13}C-NMR (67.8 MHz, C_6D_6, **9a**: δ_C and **9b**: $\underline{\delta_C}$): 168.7[a)] (2C, s, C-1 and OAc), 166.4[a)] (s, C-3), 138.0 (s, C-11), 137.9 (s, C-11), 137.3 (2C, s, C-7, 14), 136.9 (2C, s, C-7, 14), 127.3[a)] (s, C-15), 123.5[a)] (d, C-10), 120.7 (d, C-6), 120.5 (d, C-6), 118.8 (d, C-2), 118.0 (d, C-2), 92.9 (d, C-25), 92.4 (d, C-25), 91.8 (d, C-24), 91.6 (d, C-24), 63.0 (d, C-4), 61.9 (d, C-4),

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40.8[a] (t, C-18), 40.1[a] (2C, t, C-8, 12), 35.2[a] (s, C-19), 33.0[a] (2C, t, C-5, 16), 28.8[a] (2C, q, C-20, 21), 28.4[a] (t, C-9), 26.4[a] (t, C-13), 20.0[a] (t, C-17), 20.0[a] (q, OAc), 19.8[a] (q, C-22), 16.2[a] (q, C-23). *a*) Indicates overlapping with the signals of **9b**.

Acetylation of Manoalide (1), Giving the Monoacetates 9a and 9b A solution of **1** (17 mg)[8,9] in pyridine (0.17 ml) was treated with acetic anhydride (0.005 ml) and the whole was stirred at 25 °C for 30 min. The reaction mixture was worked up as described above and the crude product (13 mg) was purified by SiO_2 column chromatography (1-hexane : AcOEt = 4 : 1) to give a monoacetate mixture (**9a** : **9b** = 54 : 46) (11 mg). The monoacetate mixture (**9a, 9b**) thus obtained was identified by comparisons of the ^{1}H- and ^{13}C-NMR data with those of the monoacetate mixture obtained above from manoalide 25-acetals (**1a, 1b**).

Photoisomerization of *seco*-Manoalide (2), Giving 1 A solution of **2** (32 mg) and Rose Bengal (5 mg) in benzene (7 ml) in a Pyrex tube was irradiated with a low-pressure Hg lamp at 25 °C for 12 h. The product, obtained by removal of the solvent under reduced pressure, was purified by SiO_2 column chromatography (1-hexane : AcOEt = 2 : 1) to give **1** (13 mg). **1** thus obtained was identified by comparisons of the ^{1}H-, ^{13}C-NMR, IR, and $[\alpha]_D$ data with those of authentic manoalide.

Reduction of Manoalide 25-Acetals (1a, 1b), Giving 10 A solution of **1a, 1b** (600 mg) in MeOH (4 ml) was treated with $NaBH_4$ (20 mg) and the whole mixture was stirred under an N_2 atmosphere at 30 °C for 2 h. The reaction mixture was partitioned into a 1-BuOH–AcOEt/H_2O mixture and the organic phase was separated, washed with brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was purified by SiO_2 column chromatography ($CHCl_3$: MeOH : H_2O = 7 : 3 : 1, lower phase) to furnish manoalide diol (**10**) (500 mg).[14]

10: An amorphous solid, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3409, 2931, 2863, 1780, 1747, 1634. CD (c = 0.01, MeOH): $[\theta]_{235}$ +1200 (pos. max.), $[\theta]_{215}$ −3600 (neg. max.). ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 5.98 (1H, br s, 2-H), 5.38 (1H, t-like, J = *ca.* 8 Hz, 6-H), 5.13 (1H, m, 10-H), 4.88 (2H, br s, 25-H), 4.64 (1H, t-like, J = *ca.* 5.5 Hz, 4-H), 4.56 (1H, d, J = 16.2 Hz, 25-H$_a$), 4.47 (1H, d, J = 16.2 Hz, 25-H$_b$), 4.18 (1H, d, J = 11.6 Hz, 24-H$_a$), 4.10 (1H, d, J = 11.6 Hz, 24-H$_b$), 2.54 (2H, m, 5-H), 2.02—2.15 (8H), 1.65 (3H, s, 23-H), 1.61 (3H, s, 22-H), 1.00 (6H, s, 20,21-H). MS m/z (%): 402 (1.9, M$^+$), 137 (100).

Acetylation of Manoalide Diol (10), Giving 11 A solution of **10** (55 mg) in pyridine (1.5 ml) was treated with acetic anhydride (0.02 ml) and the whole mixture was stirred at 0 °C for 30 min. The reaction mixture was worked up as described above to furnish a crude product (100 mg), which was purified by SiO_2 column chromatography (1-hexane : AcOEt = 3 : 1) and reversed-phase HPLC (Cosmosil 5C_{18}, MeOH : H_2O = 10 : 1) to give the monoacetate **11** (17 mg).

11: A glassy solid. $[\alpha]_D$ +69° (c = 1.3, MeOH, 25 °C). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490, 3437, 1780, 1746, 1643, 1600. ^{1}H-NMR (90 MHz, $CDCl_3$, δ): 5.98 (1H, br s, 2-H), 5.43 (1H, t-like, J = *ca.* 7.5 Hz, 6-H), 5.11 (1H, m, 10-H), 4.89 (2H, br s, H-25), 4.71 (1H, d, J = 12.5 Hz, 24-H$_a$), 4.46 (1H, d, J = 12.5 Hz, 24-H$_b$), 2.55 (2H, m, 5-H), 2.15 (4H, m, 12,13-H), 2.08 (3H, s, OAc), 1.91 (2H, m, 16-H), 1.65 (3H, s, 23-H), 1.60 (3H, s, 22-H), 1.51 (2H, m, 17-H), 1.45 (2H, m, 18-H), 0.99 (6H, s, 20,21-H).

Application of Horeau's Method to 11 A solution of **11** (7 mg) in pyridine (7 μl) was treated with a 50% (±)-α-phenylbutyric anhydride–benzene solution (8 μl) and the whole mixture was warmed in a sealed vial at 40 °C for 1.5 h. (+)-(*R*)-α-Phenylethylamine (7 μl) was then added to the reaction mixture and the whole was mixed thoroughly by agitation for 5 min, then diluted with AcOEt (300 μl) and analyzed by GLC [column: OV-17 FFS (SCOT) capillary column 0.32 mm × 50 m; column temperature, 150 °C]. A parallel reaction was carried out with cyclohexanol (18 μmol) in a similar manner. The relative proportions of the amides of (−)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid obtained were measured and the corresponding values obtained from the reaction with cyclohexanol were subtracted. The increment due to the amide of (−)-(*R*)-α-phenylbutyric acid was 39%.

Silylation of (*E*)-Neomanoalide (3) Followed by Methylation, Giving 13 A solution of **3** (64 mg) in DMF (1.2 ml) was treated with *tert*-butylchlorodimethylsilane (53 mg) and imidazole (24 mg), and the whole mixture was stirred under an N_2 atmosphere at 25 °C for 30 min. The reaction mixture was poured into water and the whole was extracted with AcOEt. The AcOEt phase was separated and washed with water, then evaporated under reduced pressure to give the di-TBDMS derivative (**12**, 100 mg). A solution of **12** (41 mg) in benzene (5 ml) was then treated with methyl lithium (1.6 M solution in diethyl ether, 0.1 ml) and the whole was stirred at 0 °C for 30 min under an N_2 atmosphere. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The organic phase was separated and washed with water, then evaporated under reduced pressure to give a crude product (41 mg), which was purified by SiO_2 column chromatography (1-hexane : AcOEt = 4 : 1) to give **13** (34 mg).

13: A colorless oil. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3433, 2940, 2865, 1464, 1255. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 5.58 (1H, br s, 2-H), 5.47 (1H, br dd, J = *ca.* 8, 7.5 Hz, 6-H), 5.15 (1H, t-like, J = *ca.* 5.5 Hz, 10-H), 4.62 (1H, dd, J = 7.3, 5.9 Hz, 4-H), 4.20 (1H, dd, J = 12.9, 1.3 Hz, 25-H$_a$), 4.07 (1H, dd, J = 12.9, 1.3 Hz, 25-H$_b$), 4.09 (2H, br s, 24-H), 2.60 (1H, ddd, J = 14.3, 7.9, 7.3 Hz, 5-H$_a$), 2.43 (1H, ddd, J = 14.3, 7.3, 5.9 Hz, 5-H$_b$), 2.10 (4H, m), 2.02 (4H, m), 1.91 (2H, t-like, J = *ca.* 6 Hz, 16-H), 1.64 (3H, s, 23-H), 1.61 (3H, s, 22-H), 1.56 (2H, m, 17-H), 1.42 (2H, m, 18-H), 1.38, 1.37 (both 3H, s, 1-CH_3), 1.00 (6H, s, 20,21-H), 0.91 (18H, s, TBDMS), 0.08, 0.07 (both 6H, s, TBDMS). ^{13}C-NMR (67.8 MHz, $CDCl_3$, δ_C): 141.4 (s, C-7), 138.5 (s, C-3), 137.2 (s, C-11), 136.5 (s, C-14), 134.6 (d, C-2), 127.0 (s, C-15), 123.4 (d, C-10), 121.3 (d, C-6), 71.1 (s, C-1), 70.3 (d, C-4), 66.9, 66.8 (both t, C-24, 25), 40.3 (t, C-18), 39.9 (t, C-12), 35.1 (s, C-19), 34.6 (t, C-5), 32.8 (t, C-16), 31.9, 31.7 (both q), 28.7 (2C, q, C-20, 21), 28.4 (t, C-8), 27.9 (t, C-9), 27.1 (t, C-13), 26.1, 26.0 (both 3C, q, TBDMS), 19.9 (q, C-22), 19.6 (t, C-17), 18.5, 18.4 (both s, TBDMS), 16.2 (q, C-23), −5.2 (4C, q, TBDMS). FAB-MS m/z (%): 685 [(M + Na)$^+$, 0.3], 645 [(MH − H_2O)$^+$, 1.1]. HR-FAB-MS Obsd. m/z: 685.5089. Calcd for $C_{39}H_{94}O_4NaSi_2$: 685.5023.

Preparation of (+)-(*R*)-MTPA Ester (14a) and (−)-(*S*)-MTPA Ester (14b) A solution of **13** (4 mg) in CH_2Cl_2 (0.5 ml) was treated with (+)-(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride (17 mg), dicyclohexylcarbodiimide (DCC) (17 mg), and dimethylaminopyridine (DMAP) (6 mg), and the whole mixture was stirred at 25 °C for 20 min under an N_2 atmosphere. The reaction mixture was then partitioned into water and extracted with AcOEt. The AcOEt phase was separated, washed with brine, dried over $MgSO_4$, and then evaporated under reduced pressure. The crude product was purified by SiO_2 column chromatography (1-hexane : CH_2Cl_2 = 1 : 10) to give (+)-(*R*)-MTPA ester (**14a**, 4.9 mg). (−)-(*S*)-MTPA ester (**14b**, 6 mg) was prepared from **13** (5 mg) under the same conditions as described above.

14a: A colorless oil. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3524, 2929, 2862, 1714, 1493, 1450, 1254. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 7.57—7.36 (5H, m, MTPA), 6.80 (1H, dd, J = 7.9, 6.6 Hz, 4-H), 5.81 (1H, br s, 2-H), 5.32 (1H, dd, J = 7.9, 7.3 Hz, 6-H), 5.13 (1H, m, 10-H), 4.20 (1H, dd, J = 14.2, 1.3 Hz, 25-H$_a$), 4.05 (1H, dd, J = 14.2, 1.3 Hz, 25-H$_b$), 3.99 (2H, br s, 24-H), 3.50 (3H, s, OMe), 2.67 (1H, ddd, J = 14.5, 7.9, 7.3 Hz, 5-H$_a$), 2.38 (1H, ddd, J = 14.5, 7.9, 6.6 Hz, 5-H$_b$), 2.09—1.95 (8H, m), 1.91 (2H, t-like, J = *ca.* 6 Hz, 16-H), 1.63 (3H, s, 23-H), 1.60 (3H, s, 22-H), 1.57 (2H, m, 17-H), 1.42 (2H, m, 18-H), 1.39, 1.35 (both 3H, s, 1-CH_3), 1.00 (6H, s, 20,21-H), 0.90, 0.89 (both 9H, s, TBDMS), 0.04 (9H, s, TBDMS), 0.03 (3H, s, TBDMS).

14b: A colorless oil. IR $\nu_{max}^{CCl_4}$ cm^{-1}. 3534, 2938, 2860, 1640, 1495, 1465, 1255. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 7.57—7.38 (5H, m, MTPA), 6.78 (1H, dd, J = 8.7, 5.8 Hz, 4-H), 5.76 (1H, br s, 2-H), 5.40 (1H, dd, J = 7.4, 7.1 Hz, 6-H), 5.13 (1H, br s, 10-H), 4.06 (1H, d, J = 12.5 Hz, 25-H$_a$), 4.04 (2H, br s, 24-H), 3.82 (1H, d, J = 12.5 Hz, 25-H$_b$), 3.54 (3H, s, OMe), 2.71 (1H, ddd, J = 14.5, 8.7, 7.4 Hz, 5-H$_a$), 2.43 (1H, ddd, J = 14.5, 7.1, 5.8 Hz, 5-H$_b$), 2.11—1.96 (8H, m), 1.91 (2H, t-like, J = *ca.* 6 Hz, 16-H), 1.62 (3H, s, 23-H), 1.60 (3H, s, 22-H), 1.58 (2H, m, 17-H), 1.43 (2H, m, 18-H), 1.41, 1.32 (both 3H, s, 1-CH_3), 1.00 (6H, s, 20,21-H), 0.91, 0.88 (both 9H, s, TBDMS), 0.06 (9H, s, TBDMS), −0.01, −0.02 (3H, s, TBDMS).

Acknowledgement The authors are grateful to Dr. M. Endo, Suntory Institute for Biomedical Research, for his generous gift of a marine sponge specimen of *Luffariella* sp., which was collected at the Palau Islands. The authors are also grateful to Prof. T. Ando, Research Institute, Aichi Cancer Center, for the examination of topoisomerase-inhibitory activity and to the Ministry of Education, Science and Culture of Japan and The Tokyo Biochemical Research Foundation for financial support.

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