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Odoamide, a cytotoxic cyclodepsipeptide from the marine cyanobacterium *Okeania* sp.

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ABSTRACT

The bioassay-guided fractionation of the Okinawan marine cyanobacterium *Okeania* sp. led to the isolation of the 26-membered cyclodepsipeptide odoamide (**1**). The gross structure of **1** was determined by 1D and 2D NMR analyses, whereas its absolute stereochemistry was determined using a variety of different methods, including synthesis and chemical degradation followed by chiral HPLC analysis. Notably, odoamide (**1**) showed potent cytotoxicity against HeLa S3 human cervical cancer cells with an IC_{50} value of 26.3 nM.

1. Introduction

Cyanobacteria are well known as prolific producers of structurally unique and biologically active secondary metabolites.[1,2] Furthermore, some of these metabolites have been reported to exhibit interesting biological properties, including antimicrobial, neurotoxic and cytotoxic activities. Many of these compounds, including curacin A,[3] apratoxin A,[4] jamaicamides[5] and bisebromoamide,[6] are also produced by cyanobacteria belonging to the genus of *Lyngbya*, which has been studied extensively. *Okeania* sp. has recently been identified as a new genus of cyanobacteria[7] and a significant source of structurally interesting compounds such as bastimolide A,[8] which is a polyhydroxylated macrolide with a 40-membered ring; polycavernoside D,[9] which is a glycosidic macrolide and an analog of the polycavernosides; and kurahyne B,[10] which is an acetylene-containing lipopeptide.

As part of our ongoing effort to identify novel bioactive secondary metabolites from marine cyanobacteria, we recently isolated odoamide (**1**) from an Okinawan cyanobacterium belonging to the genus *Okeania* sp (Fig. 1). Herein, we report the isolation, structural determination and biological evaluation of compound **1**.

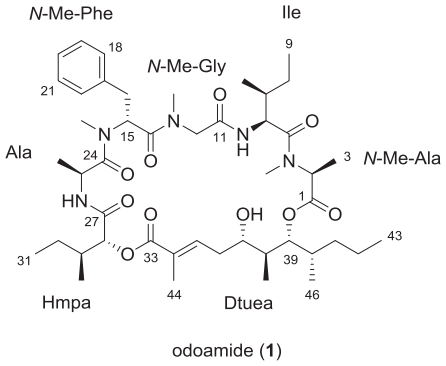

Fig. 1. Structure of odoamide (**1**).

2. Results and discussion

The cyanobacterium *Okeania* sp. (1.2 kg, wet weight), which was collected from Odo, Okinawa Prefecture, Japan, was extracted with methanol. The extract was fractionated based on its toxicity against brine shrimp to give odoamide (**1**) as a colorless oil. The molecular formula of **1** was determined to be $C_{46}H_{73}N_5O_{10}$ by HRESIMS, which gave a mass ion with an *m/z* value of 878.5254 for $[M+Na]^+$ (calcd 878.5250). The NMR data for **1** is summarized in Table 1. The ^1H NMR spectrum of compound **1** revealed the presence of three

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N-methylamide groups (δ_H 2.89, 3.04 and 3.30), a methyl group connected to a non-protonated sp_2 carbon (δ_H 1.91) and nine high-field methyl groups (δ_H 0.85–1.42). The ^{13}C NMR spectrum indicated the presence of seven carbonyl carbons (δ_C 170.4, 171.3, 172.6, 172.7, 172.8, 173.1 and 174.9) and two olefinic carbons (δ_C 128.7 and 146.8).

Table 1
NMR spectral data of odoamide (**1**) in CD_3OD

Unit	No.	δ_H (*J* in Hz)[a]	δ_C[b]
N-Me-Ala	1		172.7
	2	3.94, q (7.0)	60.3
	3	1.42, d (7.0)	14.0
	4	3.30, s	37.6
Ile	5		173.1
	6	5.05, d (6.2)	54.8
	7	1.82–1.84, m	39.5
	8a	1.62–1.68, m	24.8
	8b	1.30–1.34, m	
	9	0.92–0.94, m	11.7
	10	1.05, d (6.9)	16.1
N-Me-Gly	11		171.3
	12a	4.19, d (18.3)	52.7
	12b	3.57, d (18.3)	
	13	2.89, s	36.7
N-Me-Phe	14		172.6
	15	5.46, dd (10.4, 5.2)	55.1
	16a	3.01–3.07, m	35.9
	16b	2.94, dd (14.4, 5.2)	
	17		138.5
	18/22	7.15–7.17, m	130.7
	19/21	7.17–7.19, m	129.1
	20	7.13–7.15, m	127.5
	23	3.04, s	30.5
Ala	24		174.9
	25	4.50, q (7.0)	46.5
	26	0.85, d (7.0)	15.8
Hmpa	27		172.8
	28	4.86–4.87, m	77.7
	29	1.86–1.89, m	38.6
	30a	1.46–1.56, m	27.5
	30b	1.32–1.36, m	
	31	0.94, t (7.4)	12.0
	32	0.96, d (6.7)	14.7
Dtuea	33		170.4
	34		128.7
	35	7.30–7.34, m	146.8
	36a	2.20–2.30, m	30.6
	36b	2.00–2.07, m	
	37	3.72–3.78, m	71.5
	38	2.12–2.18, m	41.4
	39	4.87–4.89, m	79.3
	40	1.78–1.82, m	36.0
	41a	1.19–1.27, m	37.9
	41b	1.05–1.14, m	
	42	1.35–1.41, m	21.6
	43	0.88, t (7.3)	14.6
	44	1.91, s	12.6
	45	0.89–0.91, m	10.2
	46	0.91–0.93, m	13.3

[a] Recorded at 400 MHz.
[b] Recorded at 100 MHz.

Two-dimensional NMR analyses of COSY, HSQC and HMBC spectra in CD_3OD suggested the presence of *N*-methylalanine (*N*-Me-Ala), isoleucine (Ile), *N*-methylglycine (*N*-Me-Gly), *N*-methylphenylalanine (*N*-Me-Phe) and alanine (Ala). COSY correlations between H-28/H-29, H-29/H-30, H-30/H-31 and H-29/H-32 suggested the presence of an isoleucine type spin system. Furthermore, the HSQC spectrum of **1** indicated that H-28 was attached to an oxymethine carbon (δ_C 77.7), and this residue was therefore characterized as 2-hydroxy-3-methylpentanoic acid (Hmpa). The sequence of these residues was deduced by HMBC analysis, which revealed correlations between H-4/C-2, H-4/C-5, H-6/C-5, H-6/C-11, H-12/C-11, H-13/C-12, H-13/C-14, H-15/C-14, H-23/C-15, H-23/C-24, H-25/C-24 and H-25/C-27 (Fig. 2).

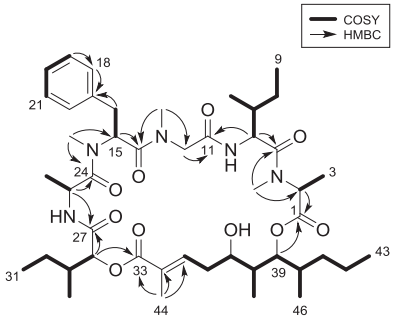


Fig. 2. Key 2D NMR correlations for **1**.

The structure of the remaining $C_{14}H_{24}O_3$ unit was determined as follows. COSY analysis connected the proton signals of the olefinic proton H-35 to the methine proton at H-38, via the allylic methylene protons and oxymethine proton at H_2-36 and H-37, respectively. Furthermore, H-38 showed correlations to the methyl group at C-45 and the oxymethine proton at H-39. Additional COSY correlations between H-39/H-40, H-40/H_2-41, H_2-41/H_2-42, H_2-42/H_3-43 and H-40/H_3-46 and HMBC correlations between H_3-44/C-33, H_3-44/C-34 and H_3-44/C-35 led to this group being identified as 5,7-dihydroxy-2,6,8-trimethyl-undec-2-enoic acid (Dtuea) (Fig. 2). The characteristic chemical shifts of H-37 (δ_H 3.75) and C-37 (δ_C 71.5) were also consistent with the presence of a hydroxyl group at C-37. The *E*-geometry of the $\Delta^{34(35)}$ double bond was assigned based on the NOESY correlation between H-36/H-44 and the ^{13}C NMR chemical shifts of the methyl group at C-44 (δ_C 12.6).[11]

Amino acid-derived units and Dtuea were connected on the basis of HMBC data. The α-proton (H-28) of Hmpa showed a cross peak to the C-33 carbonyl carbon of Dtuea, and H-39 of Dtuea correlated with the C-1 carbonyl carbon of *N*-Me-Ala to complete a 26-membered ring in odoamide (**1**) (Fig. 2).

Several chemical techniques were used to determine the stereochemistry of **1**. To assign the absolute configurations of the amino acids, peptide **1** was hydrolyzed under the acidic conditions and separated by HPLC to yield the different amino acid components and Hmpa. The stereochemistries of *N*-Me-Ala, Ile, *N*-Me-Phe and Ala were determined to be L, L, D and L, respectively, following their derivatization with Marfey's reagent and subsequent analysis of by HPLC.[12] The different isomers of Hmpa were synthesized by the diazotization of the corresponding amino acids.[13] The resulting isomers were derivatized with Mosher's reagent before being analyzed on HPLC. The results of these experiments revealed the absolute configuration of Hmpa to be D-*allo*.

The absolute configuration of C-37 was determined by the synthesis of the corresponding *S*- and *R*-MTPA esters of the hydroxy group at C-37 in **1**. The $\Delta\delta_{(S-R)}$ values (Fig. 3) revealed for these esters that C-37 existed as the *S*-configuration.[14] Given that it was not possible to determine the relative stereochemistry of the Dtuea moiety by NOESY experiments involving **1**, we turned our attention to the structure of the triol (**2**), which was obtained from the reduction of **1** with lithium aluminum hydride (Scheme 1). In the acetonide (**3**) derived from **2** (Scheme 1), the axial methyl group and the methyl group at C-45 showed NOESY correlations to the axial proton H-37 and H-39 (Fig. 4). In addition to these results, the relative stereochemistry of C-38 and C-39 was determined based on the ^{13}C chemical shifts of the methyl groups of the acetonide moiety (δ_C 19.7 and 30.4) of **3**.[15] Thus, the relative configuration of **2**

was revealed to be as shown in Fig. 4, and the absolute configuration of the Dtuea moiety in **1** was therefore determined to be 38*S*, 39*R*.

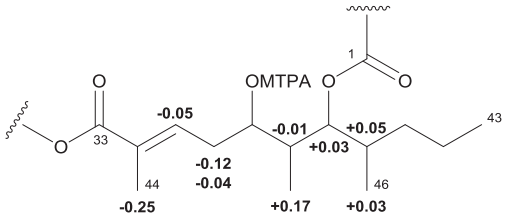
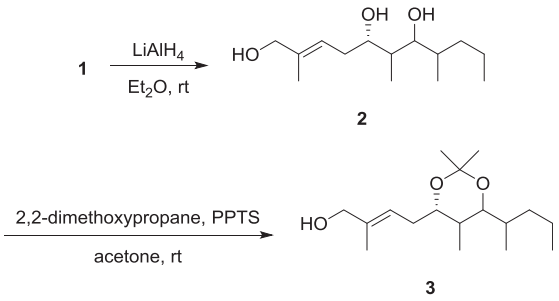


Fig. 3. $\Delta\delta$ values (δ_S–δ_R) in ppm for the MTPA esters of **1**.



Scheme 1. Preparation of acetonide **3**.

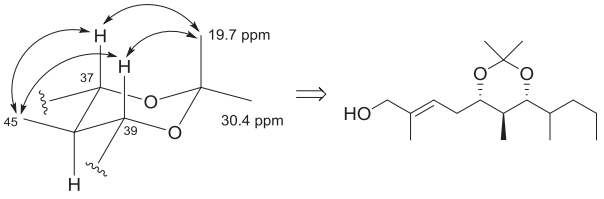
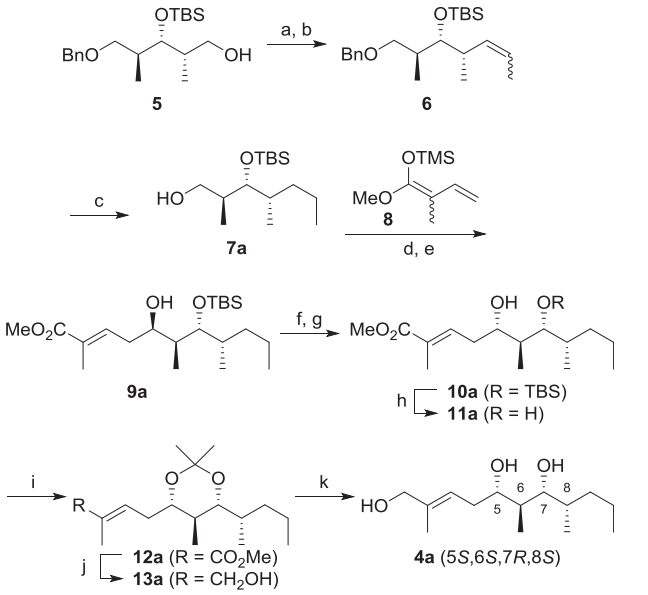


Fig. 4. Structure of acetonide **3** with relevant ^{13}C NMR data and selected NOESY correlations.

To confirm the absolute configuration of C-40, we prepared the two possible diastereoisomers of the triol, **4a** (Scheme 2) and **4b** (see Supplementary data) according to the synthetic procedure in the previous report.[16] A comparison of the ^{1}H NMR spectra of the synthetic triols **4a** and **4b** with **2** revealed that the absolute stereochemistry of the Dtuea moiety in odoamide (**1**) is 37*S*, 38*S*, 39*R*, 40*S* (Fig. 5).

Structurally, odoamide (**1**) is a 26-membered cyclodepsipeptide belonging to aurilide class, and is therefore related to the aurilide[17] class of compounds, including aurilides B and C,[18] kulokekahilide-2,[19] palau'amide[20] and lagunamides A and B.[21] Furthermore, compound **1** is a close analog of lagunamide A, with these two compounds differing by a single methylene group in their polyketide moiety. Although, absolute stereochemistry of langunamide A was determined using a variety of different methods, chemical degradation followed by chiral HPLC analysis and $^3J_{H-H}$ coupling constant values, the absolute configurations of the two chiral centers at C7 and C39 were revised[22] and the absolute configurations of these two chiral centers are the same as in odoamide (**1**). Furthermore, the molecular formula of **1** was identical to that of lagunamide C,[23] though the positions of the functional groups attached to the polyketide moieties of these two compounds were different. For example, odoamide (**1**) has a 5,7-dihydroxy-2,6,8-trimethyl-undec-2-enoic acid moiety, whereas lagunamide C has a 5,8-dihydroxy-2,6,9-trimethylundec-2-enoic acid group (Fig. 6).



Scheme 2. Synthesis of triol **4a**. Reagents and conditions: (a) $(COCl)_2$, DMSO, DIPEA, CH_2Cl_2, −78 °C to 0 °C; (b) ethyltriphenylphosphonium bromide, *n*-BuLi, THF, rt, 72% (2 steps, dr=7:1); (c) 10% Pd/C, H_2, EtOH, rt, 85%; (d) $(COCl)_2$, DMSO, DIPEA, CH_2Cl_2, −78 °C to 0 °C; (e) **8**, BF_3 Et_2O, CH_2Cl_2, Et_2O, −78 °C, 85% (2 steps); (f) Dess–Martin periodinane, CH_2Cl_2, rt; (g) $NaBH_4$, MeOH, −78 °C, 83% (2 steps, dr>97:3); (h) TBAF, THF, rt, 81%; (i) 2,2-dimethoxypropane, PPTS, CH_2Cl_2, rt, 90%; (j) DIBAL, toluene, THF, −78 °C, 90%; (k) HCl, MeOH, H_2O, rt, 51%.

The biological activity of odoamide (**1**) was evaluated using assays for brine shrimp toxicity[24] and in vitro cytotoxicity[25] against HeLa S3 cells. Odoamide (**1**) showed moderate toxicity against brine shrimp with an LD_{50} value of 1.2 μM and exhibited potent cytotoxic activity against HeLa S3 cells with an IC_{50} value of 26.3 nM. Further biological and pharmacological studies involving **1** are currently underway in our laboratory.

3. Conclusion

The novel cyclodepsipeptide odoamide (**1**) has been isolated from the Okinawan marine cyanobacterium *Okeania* sp. The structure of this compound was established by spectroscopic analysis, HPLC analysis and synthetic methods. Odoamide (**1**) represents a new addition to the aurilide class of natural products, bearing an additional methylene group on its polyketide moiety compared with lagunamide A. Odoamide (**1**) displayed cytotoxic activity against HeLa S3 cells at nanomolar level.

4. Experimental section

4.1. General experimental procedures

Optical rotation was measured on a JASCO P-1010 polarimeter or a JASCO P-1020 polarimeter. UV and IR spectra were measured on a JASCO V-660 UV visible spectrophotometer, and a JASCO FT/IR-6100 spectrometer or a JASCO FT/IR-4100 spectrometer, respectively. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AVANCE Nanobay400 MHz NMR spectrometer or a JEOL ECA-500 spectrometer. The chemical shifts were reported in relative to the residual solvent signals (δ_H 3.31, δ_C 49.1) in CD_3OD, or to Me_4Si signal for ^{1}H NMR and the residual solvent signal for ^{13}C NMR in $CDCl_3$ as internal standards. HRMS data were obtained using a Thermo SCIENTIFIC Exactive mass spectrometer, a Shimadzu LC-ESI-IT-TOF-MS equipment or a JMS-HX/HX 110A mass spectrometer. HPLC isolation of odoamide (**1**) was conducted on a JASCO

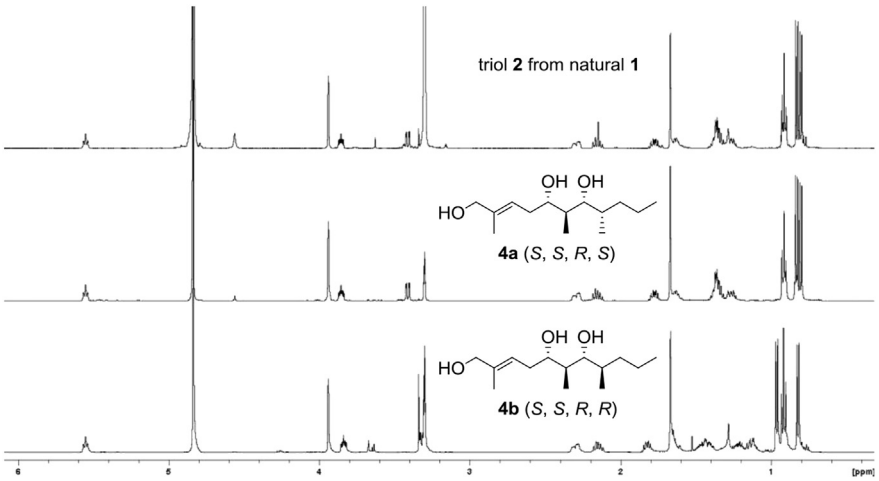


Fig. 5. Comparison of ^{1}H NMR spectra of triols.

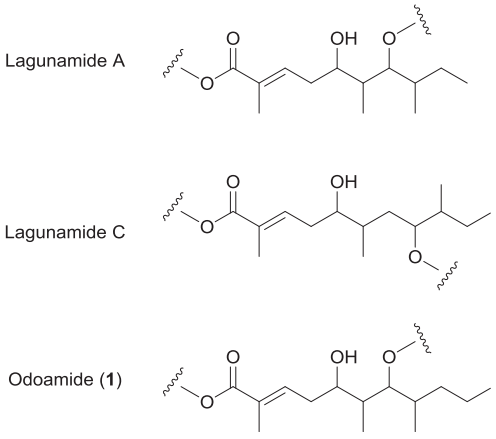


Fig. 6. Polyketide moiety in lagumanides A and C, and odoamide (**1**).

PU-2080 Plus Intelligent HPLC pump and a JASCO UV-2075 Plus Intelligent UV/VIS detector. Cell viability in 96-well plates was measured using a BioTek ELx800 absorbance microplate reader.

4.2. Marine cyanobacterial samples

Samples of the marine cyanobacterium, *Okeania* sp. were collected by hand from the coast of Odo, Okinawa Prefecture, Japan in May 2009. The cyanobacterium was identified by 16S rRNA sequence analysis.

4.3. Extraction and isolation

Approximately 1.2 kg (wet weight) of the cyanobacterial samples were extracted with MeOH (2.0 L). The extract was concentrated, and the residue was partitioned between H_2O-EtOAc (1:1). The material obtained from the organic layer was further partitioned between MeOH/H_2O (90:10) and *n*-hexane. The aqueous MeOH fraction (3.0 g) was separated by column chromatography on ODS (30.0 g) using 40% aqueous MeOH, 60% aqueous MeOH, 80% aqueous MeOH and MeOH. The fraction (976.4 mg) eluted with 80% aqueous MeOH was subjected to reversed-phase HPLC [Develosil ODS-HG-5 (20×250 mm), 85% MeOH at 5.0 mL/min, UV detection at 215 nm] to give seven fractions. Fraction six was subjected to further HPLC [Develosil ODS-HG-5 (20×250 mm), 70% MeCN at 5.0 mL/min, UV detection at 215 nm] to yield odoamide (**1**, 64.4 mg, t_R=40.0 min). The purity of **1** was determined as >95% by HPLC analysis.

Odoamide (**1**): colorless oil; $[\alpha]_D^{26}$ −19.6 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 207 nm (4.47); IR (neat) 3333, 2962, 1646, 1540, 1204, 1096 cm^{-1}; For ^{1}H NMR (400.13 MHz, CD_3OD) and ^{13}C NMR (100.61 MHz, CD_3OD) data, see Table 1; HRESIMS *m/z* $[M+Na]^+$ 878.5254 (calcd for $C_{46}H_{73}N_5O_{10}Na$, 878.5250).

4.4. Absolute configurations of amino acid residues in 1

Odoamide (**1**, 2.5 mg) was treated with 5N HCl (0.5 mL) at 105 °C for 12 h. The hydrolyzate was concentrated to dryness and partitioned between H_2O-EtOAc (1:1). The aqueous layer was subjected to HPLC [Cosmosil HILIC (4.6×250 mm), MeCN/10 mM $AcONH_4$=85:15 at 1.0 mL/min, UV detection at 215 nm] to yield *N*-Me-Phe, Ile, *N*-Me-Ala, Ala. Each amino acid was added with 0.1% solution of Nα-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (L-FDAA, Marfey's reagent, 200 μL) in acetone and 0.5M $NaHCO_3$ (100 μL) followed by heating at 40 °C for 90 min. After cooling to room temperature, the reaction mixture was neutralized with 2N HCl (25 μL) and diluted with MeOH (300 μL). The solution was subjected to reversed-phase HPLC [Cosmosil $5C_{18}$-AR-II (4.6×250 mm), MeOH/20 mM AcONa=55:45 (solvent A) or 45:55 (solvent B) at 1.0 mL/min, UV detection at 340 nm]. The L-FDAA derivatives of standard amino acids were prepared by the same procedure. The retention times (min) of the authentic standards were as follows: L-*N*-Me-Phe (7.4) and D-*N*-Me-Phe (9.5) in solvent A, L-*N*-Me-Ala (6.6), D-*N*-Me-Ala (9.5), L-Ala (5.5) and D-Ala (10.9) in solvent B. The retention time and ESIMS product ion (*m/z* $[M+Na]^+$) of the L-FDAA derivatives of *N*-Me-Phe from the hydrolyzate were 9.5 min (440.1) in solvent A, proving the configuration of *N*-Me-Phe was D. The retention times and ESIMS product ions (*m/z* $[M+Na]^+$) of the L-FDAA derivatives of *N*-Me-Ala and Ala from the hydrolyzate were 6.6 min (378.1) and 5.5 min (364.1) in solvent B, respectively, proving the configurations of *N*-Me-Ala and Ala were L.

The configuration of Ile from the hydrolyzate could not be determined by ODS HPLC, so it was analyzed by chiral HPLC [DAICEL CHIRALPAK QN-AX (4.6×150 mm), MeOH/MeCN/H_2O=49:49:2 (50 mM HCOOH, 25 mM DEA) at 1.0 mL/min, UV detection at 340 nm]. The retention times of the authentic standards were as follows: L-allo-Ile (9.4), L-Ile (10.2), D-Ile (14.8) and D-allo-Ile (15.2). The retention time and ESIMS product ion (*m/z* $[M+Na]^+$) of the L-FDAA derivative of Ile from the hydrolyzate was 10.2 min (406.1), proving the configuration of Ile was L.

4.5. Absolute configuration of 2-hydroxy-3-methylpentanoic acid (Hmpa) unit in 1

The absolute configuration of the Hmpa unit in **1** was determined by derivatization with Mosher's reagent and HPLC analyses of the derivatives. Odoamide (**1**, 2.5 mg) was treated with 5N HCl (0.5 mL) at 105 °C for 12 h. The hydrolyzate was concentrated to dryness and partitioned between H_2O-EtOAc (1:1). The EtOAc layer was subjected to reversed-phase HPLC [Cosmosil $5C_{18}$-MS-II (10×250 mm), 15% MeCN with 0.05% TFA at 4.0 mL/min, UV detection at 215 nm] to yield pure Hmpa. Natural Hmpa and authentic standards were subjected to reversed-phase HPLC [Cosmosil $5C_{18}$-MS-II (4.6×250 mm), 15% MeCN with 0.05% TFA at 1.0 mL/min, UV detection at 215 nm]. The retention time of Hmpa in **1** corresponded to that of 2*S**, 3*R**-Hmpa at 12.7 min (2*S**, 3*S**-Hmpa; 12.2 min). Then, natural Hmpa, 2*S*, 3*R*-Hmpa and 2*R*, 3*S*-Hmpa were reacted with (*S*)-MTPACl (10 μL) and *N*,*N*-dimethyl-4-aminopyridine (DMAP, 2.0 mg) in pyridine (100 μL) with rapid stirring at room temperature for 28 h, followed by purification on reversed-phase HPLC [Cosmosil Cholester (10×250 mm), 60% MeCN with 0.05% TFA at 4.0 mL/min, UV detection at 215 nm] to yield the corresponding esters. These derivatized samples were subjected to reversed-phase HPLC [Cosmosil Cholester (4.6×250 mm), 60% MeCN with 0.05% TFA at 1.0 mL/min, UV detection at 215 nm] to establish the absolute configuration of Hmpa unit in **1** as 2*R*, 3*S* (11.2 min) but not 2*S*, 3*R* (10.5 min). ESIMS product ion of MTPA derivative of Hmpa from **1**: *m/z* $[M+Na]^+$ 371.1.

4.6. Preparation of MTPA esters of 1

Odoamide (**1**, 1.2 mg) was reacted with *R*-MTPACl (10 μL) and DMAP (0.9 mg) in pyridine (100 μL), and the mixture was stirred at room temperature for 23 h. The reaction mixture was concentrated, and the residue was partitioned between EtOAc-0.1M $NaHCO_3$ (1:1). The extract was subjected to reversed-phase HPLC [Cosmosil $5C_{18}$-AR-II (10×250 mm), 90% MeOH at 4.0 mL/min, UV detection at 215 nm] to yield *S*-MTPA ester (1.2 mg). Using the same procedure as described above, *R*-MTPA ester (1.4 mg) was obtained from **1** (1.3 mg).

S-MTPA ester: ^{1}H NMR (500 MHz, CD_3OD) δ 7.36 (H-35), 2.54 (H-36a), 2.23 (H-36b), 6.10 (H-37), 2.31 (H-38), 4.95 (H-39), 1.84 (H-40) 1.60 (H-44), 1.02 (H-45), 0.95 (H-46).

R-MTPA ester: ^{1}H NMR (500 MHz, CD_3OD) δ 7.41 (H-35), 2.66 (H-36a), 2.27 (H-36b), 6.07 (H-37), 2.32 (H-38), 4.92 (H-39), 1.79 (H-40) 1.85 (H-44), 0.85 (H-45), 0.92 (H-46).

4.7. Preparation of acetonide 3 via triol 2

To a stirred solution of odoamide (**1**, 4.5 mg) in diethyl ether (Et_2O, 1.5 mL) was added lithium aluminum hydride (LAH, 14.2 mg), and the mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of water (15 μL), 15% NaOH (15 μL) and water (45 μL), and the mixture was extracted with Et_2O. The extract was subjected to reversed-phase HPLC [Cosmosil $5C_{18}$-AR-II (10×250 mm), 40% MeCN with 0.1% TFA at 4.0 mL/min, UV detection at 215 nm] to yield triol **2** (0.8 mg).

To a solution of **2** (0.8 mg) in acetone (100 μL) were added 2,2-dimethoxypropane (10 μL) and pyridinium *p*-toluenesulfonate (PPTS, 1.0 mg), the mixture was stirred at room temperature for 10 h. The reaction was quenched by addition of $NaHCO_3$ (saturated solution), and the mixture was extracted with EtOAc, concentrated to yield acetonide **3** (0.5 mg).

4.8. Preparation of triol 4a

*4.8.1. (4S,5R,6S)-7-Benzyloxy-5-(tert-butyldimethylsilyloxy)-4,6-dimethylhept-2-ene (**6**).* To a stirred solution of oxalyl chloride (7.3 mL, 85.6 mmol) in CH_2Cl_2 (428 mL) under argon was added DMSO (12.2 mL, 171.2 mmol) in CH_2Cl_2 (29 mL) at −78 °C. After stirring for 30 min, a solution of **5**[26] (15.1 g, 42.8 mmol) in CH_2Cl_2 (148 mL) was added dropwise and stirred at −78 °C for 1.5 h *i*-Pr_2NEt (37.3 mL, 214.0 mmol) was added and the reaction mixture was stirred at 0 °C for 30 min. The mixture was quenched with aqueous saturated NH_4Cl. The whole was extracted with CH_2Cl_2 and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure to give the corresponding aldehyde, which was used without further purification. To a stirred suspension of ethyltriphenylphosphonium bromide (33.4 g, 89.9 mmol) in THF (360 mL) under argon was added *n*-BuLi (1.6 M in hexane; 55.2 mL, 85.6 mmol) at room temperature. After stirring for 30 min, a solution of the above aldehyde in THF (86 mL) was added and the reaction mixture was stirred for 1.5 h. The mixture was quenched with aqueous saturated $NaHCO_3$. The whole was extracted with EtOAc and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was filtrated through a short pad of silica gel with hexane-EtOAc (9:1). Further purification by flash chromatography over silica gel with hexane-$CHCl_3$ (8:1) gave compound **6** (diastereomixture, 11.2 g, 72%, dr=7:1) as a colorless oil: $[\alpha]_D^{26}$ +4.17 (*c* 1.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.02 (s, 0.8H), 0.04 (s, 2.6H), 0.04 (s, 2.6H), 0.88 (s, 1.2H), 0.89 (s, 7.8H), 0.92 (d, *J*=6.9 Hz, 2.6H), 0.94 (d, *J*=6.9 Hz, 0.4H), 0.97–0.99 (m, 3H), 1.57 (dd, *J*=6.9, 1.7 Hz, 2.6H), 1.62 (d, *J*=4.0 Hz, 0.4H), 1.97–2.06 (m, 1H), 2.27–2.31 (m, 0.1H), 2.63–2.70 (m, 0.9H), 3.24–3.28 (m, 1H), 3.43–3.45 (m, 1H), 3.55–3.58 (m, 1H), 4.47 (s, 2H), 5.20–5.25 (m, 1H), 5.31–5.37 (m, 1H), 7.25–7.33 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.1, −3.9, −3.8, 13.0, 14.9, 15.2, 15.9, 17.2, 18.0, 18.4, 26.1(3C), 26.2 (3C), 35.0, 37.8, 38.4, 40.4, 72.7, 72.9, 78.4, 122.6, 123.9, 127.3, 127.5 (2C), 128.2 (2C), 134.9, 135.5, 138.8; HRESIMS *m/z* $[M+Na]^+$ 385.2533 (calcd for $C_{22}H_{38}O_2SiNa$, 385.2533).

*4.8.2. (2S,3R,4S)-3-(tert-Butyldimethylsilyloxy)-2,4-dimethylheptan-1-ol (**7a**).* To a stirred solution of **6** (1.1 g, 3.1 mmol) in EtOH (30.9 mL) was added 10% Pd/C (657.7 mg, 0.6 mmol) at room temperature and the mixture was flushed with H_2 gas. After stirring for 1 h, the reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane-EtOAc (10:1) to give compound **7a** (722.8 mg, 85%) as a colorless oil: $[\alpha]_D^{27}$ −20.0 (*c* 1.05, $CHCl_3$); IR (neat) 3374 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.08 (s, 3H), 0.11 (s, 3H), 0.89–0.93 (m, 15H), 0.96 (d, *J*=6.9 Hz, 3H), 1.12–1.19 (m, 1H), 1.20–1.26 (m, 1H), 1.36–1.47 (m, 2H), 1.59–1.66 (m, 1H), 1.84–1.88 (m, 1H), 2.61 (dd, *J*=6.3, 5.2 Hz, 1H), 3.50 (dd, *J*=5.7, 4.0 Hz, 1H), 3.56–3.66 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.1, −4.0, 14.3, 15.1, 16.4, 18.3, 20.8, 26.1 (3C), 35.3, 37.5, 38.1, 66.3, 81.2; HRESIMS *m/z* $[M+Na]^+$ 297.2220 (calcd for $C_{15}H_{34}O_2SiNa$, 297.2220).

*4.8.3. Methyl (5R,6S,7R,8S,E)-7-(tert-butyldimethylsilyloxy)-5-hydroxy-2,6,8-trimethylundecen-2-oate (**9a**).* To a stirred solution of *i*-Pr_2NH (7.1 mL, 50.8 mmol) in THF (104 mL) under argon was added *n*-BuLi (2.6 M in hexane; 19.5 mL, 50.8 mmol) at 0 °C. After 20 min, methyl tiglate (5.6 mL, 46.2 mmol) and TMSCl (8.8 mL, 69.3 mmol) in THF (16 mL) were added successively at −78 °C. The reaction was continued for 1 h at this temperature and for additional 1.5 h at room temperature. Then, pentane and cold saturated $NaHCO_3$ were added to the reaction mixture. The whole was extracted with pentane and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure to give compound **8**, which was used without further purification.[27] To a stirred solution of oxalyl chloride (2.6 mL, 30.8 mmol) in CH_2Cl_2 (154 mL) under argon was added DMSO (4.4 mL, 61.6 mmol) in CH_2Cl_2 (10 mL) at −78 °C. After stirring for

30 min, a solution of **7a** (4.2 g, 15.4 mmol) in CH_2Cl_2 (53 mL) was added dropwise and stirred at −78 °C for 1.5 h *i*-Pr_2NEt (21.5 mL, 123.2 mmol) was added and the reaction mixture was stirred at 0 °C for 30 min. The mixture was quenched with aqueous saturated NH_4Cl. The whole was extracted with CH_2Cl_2 and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure to give the corresponding aldehyde, which was used without further purification. To a stirred solution of the above aldehyde in CH_2Cl_2 (118 mL) and Et_2O (11.8 mL) under argon were added diene **8** and $BF_3{\cdot}OEt_2$ (2.9 mL, 23.1 mmol) at −78 °C. After stirring for 2 h, a mixture of THF/H_2O/1N HCl (5:1:0.4 v/v, 77 mL) was added to the reaction mixture. The mixture was warmed to room temperature and stirred for 15 min. Then, aqueous saturated $NaHCO_3$ was added to the mixture at 0 °C. The whole was extracted with CH_2Cl_2 and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane-EtOAc (20:1 to 10:1) to give compound **9a** (5.1 g, 85%) as a colorless oil: $[\alpha]_D^{26}$ −0.35 (*c* 1.02, $CHCl_3$); IR (neat) 3503, 1716 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.12 (s, 3H), 0.88–0.92 (m, 15H), 1.02 (d, *J*=7.4 Hz, 3H), 1.04–1.12 (m, 1H), 1.15–1.26 (m, 1H), 1.33–1.41 (m, 1H), 1.42–1.49 (m, 1H), 1.66–1.70 (m, 1H), 1.71–1.77 (m, 1H), 1.87 (s, 3H), 2.20–2.26 (m, 1H), 2.39–2.45 (m, 1H), 3.54–3.57 (m, 2H), 3.73 (s, 3H), 4.21–4.24 (m, 1H), 6.78–6.81 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −3.9, −3.8, 12.2, 12.6, 14.3, 16.4, 18.3, 20.7, 26.1 (3C), 34.2, 35.4, 37.6, 37.8, 51.7, 70.4, 83.0, 129.0, 138.8, 168.4; HRESIMS *m/z* $[M+Na]^+$ 409.2744 (calcd for $C_{21}H_{42}O_4SiNa$, 409.2745).

*4.8.4. Methyl (5S,6S,7R,8S,E)-7-(tert-butyldimethylsilyloxy)-5-hydroxy-2,6,8-trimethylundec-2-enoate (**10a**).* To a stirred solution of **9a** (10.7 g, 27.8 mmol) in CH_2Cl_2 (199 mL) under argon was added Dess–Martin periodinane (17.7 g, 41.7 mmol) at room temperature. After stirring for 1 h, the reaction mixture was quenched with aqueous saturated $NaHCO_3$ and $Na_2S_2O_3$. The whole was extracted with CH_2Cl_2 and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane-EtOAc (25:1 to 15:1) to give the corresponding ketone, which was used without further purification. To a stirred solution of the above ketone in MeOH (263 mL) under argon was added $NaBH_4$ (6.0 g, 157.8 mmol) at −78 °C. After stirring for 20 h, the reaction mixture was quenched with aqueous saturated NH_4Cl and concentrated under reduced pressure. The residue was extracted with EtOAc and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane-EtOAc (10:1) to give compound **10a** (8.9 g, 83%, dr>97:3) as a colorless oil: $[\alpha]_D^{27}$ −9.16 (*c* 1.18, $CHCl_3$); IR (neat) 3522, 1716 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.12 (s, 3H), 0.85 (d, *J*=6.9 Hz, 3H), 0.88–0.93 (m, 15H), 1.11–1.17 (m, 1H), 1.22–1.29 (m, 1H), 1.33–1.41 (m, 2H), 1.58–1.64 (m, 1H), 1.73–1.77 (m, 1H), 1.86 (s, 3H), 2.26–2.32 (m, 1H), 2.42–2.44 (m, 1H), 3.49 (dd, *J*=5.4, 3.7 Hz, 1H), 3.52 (s, 1H), 3.68–3.71 (m, 1H), 3.73 (s, 3H), 6.94–6.97 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.2, −3.9, 12.7, 14.3, 14.8, 16.1, 18.2, 20.7, 26.0 (3C), 33.5, 35.5, 38.6, 41.9, 51.6, 73.0, 81.5, 128.7, 139.3, 168.5; HRESIMS *m/z* $[M+Na]^+$ 409.2743 (calcd for $C_{21}H_{42}O_4SiNa$, 409.2745).

*4.8.5. Methyl (5S,6S,7R,8S,E)-5,7-dihydroxy-2,6,8-trimethylundec-2-enoate (**11a**).* To a stirred solution of **10a** (229.4 mg, 0.59 mmol) in THF (5.9 mL) under argon was added TBAF (1 M in THF; 1.8 mL, 1.8 mmol) at room temperature. After stirring for 3 h, the reaction mixture was quenched with aqueous saturated NH_4Cl. The whole was extracted with Et_2O and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane-EtOAc (5:1 to 2:1) to give compound **11a** (130.6 mg, 81%) as a colorless oil: $[\alpha]_D^{26}$ −6.85 (*c* 0.95, $CHCl_3$); IR (neat) 3362, 1712 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.79 (d, *J*=6.9 Hz, 3H), 0.85 (d, *J*=6.9 Hz, 3H), 0.91 (t, *J*=7.2 Hz, 3H), 1.24–1.42 (m, 4H), 1.67–1.75 (m, 2H), 1.87 (s, 3H), 2.35–2.41 (m, 1H), 2.46–2.51 (m, 1H), 2.82 (br s, 1H), 3.54 (dd, *J*=9.5, 2.0 Hz, 1H), 3.74 (s, 3H), 3.82 (td, *J*=7.7, 3.4 Hz, 1H), 4.04 (br s, 1H), 6.91–6.95 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.8, 12.7, 12.9, 14.2, 20.4, 34.2, 34.6, 36.4, 40.7, 51.7, 75.9, 79.6, 129.4, 138.8, 168.5; HRESIMS *m/z* $[M+Na]^+$ 295.1870 (calcd for $C_{15}H_{28}O_4Na$, 295.1880).

*4.8.6. Methyl (E)-2-methyl-4-{(4S,5S,6R)-2,2,5-trimethyl-6-[(S)-pentan-2-yl]-1,3-dioxan-4-yl}but-2-enoate (**12a**).* To a stirred solution of **11a** (100.0 mg, 0.37 mmol) in CH_2Cl_2 (3.7 mL) under argon were added 2,2-dimethoxypropane (0.453 mL, 3.7 mmol) and PPTS (93.0 mg, 0.37 mmol) at room temperature. After stirring for 3 h, the reaction mixture was quenched with aqueous saturated $NaHCO_3$. The whole was extracted with CH_2Cl_2 and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane-EtOAc (15:1) to give compound **12a** (103.6 mg, 90%) as a colorless oil: $[\alpha]_D^{26}$ −2.27 (*c* 0.78, $CHCl_3$); IR (neat) 1715 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.75 (d, *J*=6.3 Hz, 3H), 0.83 (d, *J*=6.3 Hz, 3H), 0.88–0.91 (m, 3H), 1.23–1.33 (m, 4H), 1.34 (s, 3H), 1.39 (s, 3H), 1.45–1.52 (m, 1H), 1.64–1.68 (m, 1H), 1.84 (d, *J*=1.1 Hz, 3H), 2.26–2.32 (m, 1H), 2.46–2.51 (m, 1H), 3.42 (dd, *J*=10.0, 2.0 Hz, 1H), 3.57 (dt, *J*=12.8, 4.4 Hz, 1H), 3.74 (s, 3H), 6.85–6.88 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.8, 12.5, 12.6, 14.3, 19.4, 20.4, 30.0, 32.7, 32.9, 35.3, 36.2, 51.7, 74.0, 75.7, 97.8, 128.5, 139.2, 168.6; HRESIMS *m/z* $[M+Na]^+$ 335.2186 (calcd for $C_{18}H_{32}O_4Na$, 335.2193).

*4.8.7. (E)-2-Methyl-4-{(4S,5S,6R)-2,2,5-trimethyl-6-[(S)-pentan-2-yl]-1,3-dioxan-4-yl}but-2-en-1-ol (**13a**).* To a stirred solution of **12a** (87.0 mg, 0.28 mmol) in THF (2.8 mL) under argon was added DIBAL (1.0 M in toluene; 0.84 mL, 0.84 mmol) at −78 °C. After stirring for 3 h, a saturated aqueous solution of sodium potassium tartrate was added to the reaction mixture and the mixture was stirred at room temperature for 1 h. The whole was extracted with Et_2O and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane-EtOAc (7:1 to 4:1) to give compound **13a** (71.7 mg, 90%) as a colorless oil: $[\alpha]_D^{27}$ −1.60 (*c* 1.03, $CHCl_3$); IR (neat) 3317 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.74 (d, *J*=6.9 Hz, 3H), 0.82 (d, *J*=6.9 Hz, 3H), 0.88–0.90 (m, 3H), 1.18–1.34 (m, 4H), 1.34 (s, 3H), 1.38 (s, 3H), 1.43–1.51 (m, 1H), 1.64–1.67 (m, 5H), 2.12–2.17 (m, 1H), 2.36–2.40 (m, 1H), 3.40 (dd, *J*=10.3, 2.3 Hz, 1H), 3.47–3.52 (m, 1H), 4.01 (s, 2H), 5.52–5.55 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.8, 12.5, 13.9, 14.3, 19.5, 20.4, 30.0, 31.5, 32.7, 35.1, 36.2, 68.9, 74.6, 75.8, 97.7, 122.5, 135.9; HRESIMS *m/z* $[M+Na]^+$ 307.2247 (calcd for $C_{17}H_{32}O_3Na$, 307.2244).

*4.8.8. (5S,6S,7R,8S,E)-2,6,8-Trimethylundec-2-ene-1,5,7-triol (**4a**).* To a stirred solution of **13a** (50.6 mg, 0.18 mmol) in MeOH (3.2 mL) was added 1N HCl (0.54 mL, 0.54 mmol) at room temperature. After stirring overnight, the reaction mixture was quenched with aqueous saturated $NaHCO_3$. The whole was extracted with EtOAc and the extract was washed with brine, dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexane-EtOAc (3:1 to 1:2) to give compound **4a** (22.5 mg, 51%) as a colorless oil: $[\alpha]_D^{29}$ −2.82 (*c* 1.00, $CHCl_3$); IR (neat) 3348 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 0.80 (d, *J*=6.9 Hz, 3H), 0.83 (d, *J*=6.9 Hz, 3H), 0.90–0.93 (m, 3H), 1.24–1.29 (m, 1H), 1.32–1.40 (m, 3H), 1.60–1.67 (m, 1H), 1.67 (s, 3H), 1.74–1.82 (m, 1H), 2.12–2.18 (m, 1H), 2.27–2.31 (m, 1H), 3.41 (dd, *J*=9.7, 2.3 Hz, 1H),

3.85–3.88 (m, 1H), 3.94 (s, 2H), 5.54–5.57 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 12.0, 12.5, 14.1, 14.7, 21.5, 32.1, 35.7, 37.9, 42.3, 69.1, 75.4, 78.1, 123.9, 137.4; HRFABMS *m/z* $[M+H]^+$ 245.2115 (calcd for $C_{14}H_{29}O_3$, 245.2111).

4.9. Brine shrimp toxicity assay

The brine shrimp (*Artemia salina*) toxicity assay was performed using a slight modification of the original method. The samples were dissolved in MeOH and transferred to 0.7 cm squares of filter paper. The squares were dried and added to test vials of artificial seawater (2.0 mL). Approximately 10 hatched brine shrimp were transferred to the vials. After 24 h at room temperature, the brine shrimp were counted and the percentage of live verses total shrimp was calculated to determine the LD_{50} values.

4.10. Cytotoxicity assay

The cytotoxicity against human cell lines HeLa S3 was measured by means of the MTT assay. HeLa S3 cells were seeded at 4×10^3 cells/well in 96 well plates and cultured overnight at 37 °C with 5% CO_2. Various concentrations of compound were added and the culture plates were kept for 72 h. MTT solution (20 μL, 5 mg/mL in DMSO) was added to each well and the plate was further incubated for 4 h. After the incubation, all remaining supernatant were removed and DMSO (150 μL) was added to each well to dissolve the resultant formazan crystal. Absorbance was measured by using a microplate reader at a wavelength of 540 nm, using 630 nm as the reference wavelength. The IC_{50} values were calculated by curve fitting method.

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Supplementary data

Supplementary data (1D and 2D NMR spectra for odoamide preparation of triol and gene sequencing.) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.07.031.

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