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Tumescenamide C, an antimicrobial cyclic lipodepsipeptide from *Streptomyces* sp.

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ABSTRACT

Tumescenamide C, a new cyclic lipodepsipeptide, was isolated from a culture broth of an actinomycete *Streptomyces* sp. KUSC_F05. Tumescenamide C was a congener of tumescenamides A and B, representing a sixteen-membered ring system, consisting of two proteinogenic and three non-proteinogenic amino acids, to which a methyl-branched fatty acid was attached. The planar structure was determined by spectroscopic analysis, while its absolute stereochemistry was determined by chemical degradation and asymmetric synthesis. Tumescenamide C exhibited antimicrobial activity with high selectivity against *Streptomyces* species.

1. Introduction

Secondary metabolites produced by microbes continue to attract the attention of organic chemists, biological chemists and chemical biologists, due to their sophisticated chemical structures and highly specific biological activities. Cyclic depsipeptide is one of the important classes of such metabolites, e.g., daptomycin produced by *Streptomyces roseosporus* disturbs the bacterial cell membrane and is used as an antibacterial drug[1]; FK228 produced by *Chromobacterium violaceum* is a potent histone deacetylase inhibitor and used as a treatment for cutaneous T-cell lymphoma.[2] In spite of the huge number of natural products so far reported, it is an important task to discover novel secondary metabolites, as they will provide new insights into natural product chemistry and have the potential to be developed as research tools and pharmaceuticals. In our continuing search for bioactive metabolites from microbes, a culture broth of an actinomycete *Streptomyces* sp. KUSC_F05 was found to contain antitumor trieneansamycin compounds including cytotrienin A.[3] Analysis of the constituents in the same culture broth indicated the presence of another abundant metabolite **1**. Spectral analysis revealed that this metabolite was a new congener of tumescenamides produced by *Streptomyces tumescens* (Fig. 1).[4] To determine the stereochemistry of **1**, we applied advanced Marfey method for amino acid residues,[5,6] while the stereocenters in the methyl-branched fatty acid were determined by PGME method[7] and synthetic work. Herein we report the isolation, structure elucidation and biological activity of **1**.

2. Results and discussion

2.1. Isolation

A culture broth of *Streptomyces* sp. KUSC_F05 (3 L) was extracted with the same volume of *n*-BuOH. The extract was concentrated in vacuo and separated by silica gel column chromatography. Analysis of the obtained fractions by ODS HPLC revealed the presence of a variety of trieneansamycin compounds including cytotrienin A.[3] Meanwhile, a metabolite whose UV absorption pattern was different from those of trieneansamycin compounds was found. The corresponding fraction was subjected to reversed-phase HPLC to give tumescenamide C (**1**, 61.5 mg) as a colorless amorphous solid.

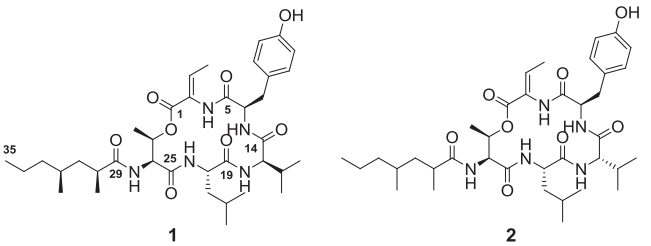

Fig. 1. Structures of tumescenamide C (**1**) and tumescenamide A (**2**).

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2.2. Structure determination

The molecular formula of tumescenamide C (**1**) was established as $C_{37}H_{57}N_5O_8$ on the basis of HR-FABMS (*m/z* 698.4129 $[M-H]^-$, Δmmu=+1.1) and NMR spectral data (Table 1). The ^{1}H NMR spectrum exhibited signals characteristic of a peptide, including exchangeable NH signals at δ 8.75 (1H, br s), δ 8.44 (1H, br s), δ 8.13 (1H, br s), δ 7.75 (1H, d, *J*=8.2 Hz), and 7.66 (1H, d, *J*=8.6 Hz), and four α-protons at δ 4.72 (1H, dd, *J*=8.6, 3.2 Hz), 4.50 (1H, ddd, *J*=12.3, 8.5, 3.5 Hz), 4.31 (1H, t, *J*=7.6 Hz) and 3.83 (1H, br d, *J*=3.0 Hz). Additionally, nine signals corresponding to methyl groups at δ 1.73 (3H, d, *J*=7.0 Hz), 1.42 (3H, d, *J*=6.3 Hz), 1.10 (3H, d, *J*=7.0 Hz), 1.00 (3H, d, *J*=6.6 Hz), 0.94 (3H, d, *J*=6.6 Hz), 0.81 (3H, d, *J*=6.8 Hz), 0.79 (3H, t, *J*=7.3 Hz), 0.70 (3H, d, *J*=6.9 Hz) and 0.61 (3H, d, *J*=6.8 Hz) were observed. Two aromatic doublets at δ 7.18 (2H, d, *J*=8.6 Hz) and 6.69 (2H, d, *J*=8.6 Hz) indicated the presence of a *para*-substituted phenyl ring. The ^{13}C NMR spectrum indicated the existence of a peptide backbone; six amide or ester carbonyl carbon signals at δ 180.1, 178.5, 173.7, 172.0, 171.4, and 165.3, and four α-carbons at δ 62.4, 57.2, 56.8, and 54.5 were observed. A detailed analysis of the 2D NMR data including DQF-COSY, HMQC and HMBC spectra revealed the presence of one residue each of leucine (Leu), threonine (Thr), valine (Val), tyrosine (Tyr), 2-amino-2-butenoic acid (Abu), and 2,4-dimethylheptanoic acid (Dmh) (Fig. 2). The connectivity of the five amino acid residues and one acyl moiety was determined based on the HMBC and ROESY correlations (Fig. 2). For example, HMBC correlations from the NH signal at δ 7.75 (6-NH, Tyr) and the α-proton at δ 3.83 (H-15, Val) to the amide carbon at δ 173.7 (C-14) connected Tyr and Val. The connectivity of Thr to Abu was established by an HMBC correlation from the protons at δ 5.15 (H-27, Thr) and δ 6.79 (H-3, Abu) to Abu carbonyl carbon at δ 165.3 (C-1). The planar structure of **1** was revealed to be same to that of tumescenamide A (**2**, Fig. 1).[4] Next, we conducted chemical degradation and asymmetric synthesis to determine the chirality for all stereogenic centers in **1**.

Table 1
^{1}H and ^{13}C NMR spectra of **1** in CD_3OH[a]

	Position	δ_H	Mult.(*J* in Hz)	δ_C
(*Z*)-Abu	1			165.3
	2			127.6
	3	6.79	q (7.2)	137.9
	4	1.73	d (7.0)	14.6
	2-NH	8.13	br s	
D-Tyr	5			172.0
	6	4.50	ddd (12.3, 8.5, 3.5)	57.2
	7	a 3.37	dd (13.9, 3.2)	36.6
		b 3.07	dd (14.2, 12.3)	
	8			129.7
	9	7.18	d (8.6)	131.3
	10	6.69	d (8.6)	116.3
	11			157.4
	12	6.69	d (8.6)	116.3
	13	7.18	d (8.6)	131.4
	6-NH	7.75	d (8.2)	
	11-OH			
D-Val	14			173.7
	15	3.83	br d (3.0)	62.4
	16	2.08	m	29.9
	17	0.81	d (6.8)	19.1
	18	0.61	d (6.8)	17.1
	15-NH	8.75	br s	
L-Leu	19			178.5
	20	4.31	t (7.6)	54.5
	21	a 1.56	m	40.9
		b 1.51	m	
	22	1.65	m	25.7
	23	1.00	d (6.6)	22.9
	24	0.94	d (6.6)	22.7
	20-NH	8.44	br s	
L-Thr	25			171.4
	26	4.72	dd (8.6, 3.2)	56.8
	27	5.15	qd (6.2, 3.4)	75.4
	28	1.42	d (6.3)	16.3
	26-NH	7.66	d (8.9)	
2*S*,4*S*-Dmh	29			180.1
	30	2.60	m	39.4
	31	a 1.67	m	42.4
		b 1.05	m	
	32	1.33	m	31.4
	33	a 1.15	m	40.2
		b 0.89	m	
	34	1.20	m	20.8
	35	0.79	t (7.3)	14.5
	30-Me	1.10	d (7.0)	19.1
	32-Me	0.70	d (6.9)	20.2

[a] ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively.

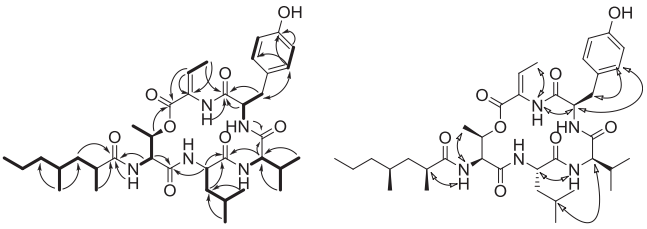


Fig. 2. Selected DQF-COSY (bold) and HMBC (arrow) correlations for **1** (left) and ROESY correlations for **1** (right).

The absolute configurations of the amino acids were determined by advanced Marfey method.[5,6] Acid hydrolysate of **1** was condensed with 1-fluoro-2,4-dinitro-phenyl-5-L/D-leucinamide (L- or D-FDLA), and analyzed by LC-MS to reveal the presence of L-Leu, D-Val and D-Tyr (Table 2, Fig. S43). The stereochemistry of Thr was determined to be L-Thr by comparing the retention times of FDLA derivatives of authentic standard amino acids L-Thr and L-*allo*-Thr (Table 2). The geometry of the double bond in Abu was determined to be *Z*, based on a ROESY correlation between protons at δ 1.73 (H-4) and δ 8.13 (2-NH) (Fig. 2). The absolute configuration of C-30 was determined by applying the PGME method.[7] 2,4-Dimethylheptanoic acid (**3**) was obtained from the acid hydrolysate of **1** and condensed with (*R*)- or (*S*)- phenylglycine methyl ester (PGME) to afford **3a** and **3b**, respectively. The ^{1}H NMR spectra of the obtained diastereomers were compared, and $\Delta\delta$ values revealed the 30*S* stereochemistry (Fig. 3a). The remaining chiral center at C-32 could not be determined by NMR analysis and required the synthesis of authentic standards for comparison purposes.

Table 2
Retention times of L- and D-FDLA derivatives of amino acids[a]

Amino acid	t_{RL}[b] (min)	t_{RD}[c] (min)	*m/z*	Absolute configuration
Tyr[d]	30.7	27.9	770.2740	D
Val	22.3	17.4	412.1827	D
Leu	19.5	25.0	426.1983	L
Thr	12.0	15.5	414.1619	L
L-Thr	12.0	15.5	414.1619	L
L-*allo*-Thr	12.7	14.0	414.1619	L

[a] Amino acid derivatives were analyzed by LC-ESI MS.
[b] Retention times of L-FDLA derivatives.
[c] Retention times of D-FDLA derivatives.
[d] Retention times for bis derivatives are shown.

To determine the absolute configuration of C-32, we planned to synthesize (2*S*,4*R*)-Dmh **4** and (2*S*,4*S*)-Dmh **5** and to compare their spectral data with that of the natural Dmh **3**. 2*S* configuration was expected to be obtained by the asymmetric alkylation introduced by Evans and co-workers,[8] while 4*S* and 4*R* could be derived from

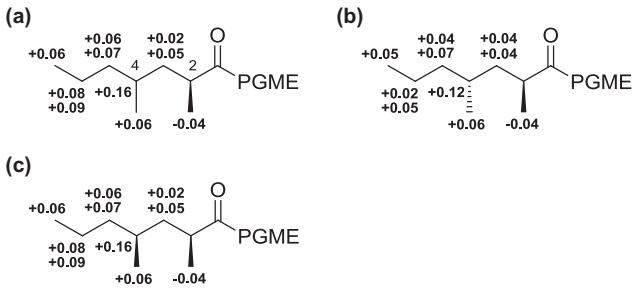


Fig. 3. $\Delta\delta$ ($\delta_{(S)\text{-PGME}}-\delta_{(R)\text{-PGME}}$) values for PGME derivatives. (a) **3a** and **3b**; (b) **4a** and **4b**; (c) **5a** and **5b**.

the commercially available starting materials (Scheme 1). We first synthesized (*R*)-2-methypentyl trifluoromethanesulfonate (**12**) from (*S*)-methyl 3-hydroxy-2-methypropanoate (**6**) in six steps with 23% yield in a similar way to that reported by Organ and coworkers.[9] Then, diastereoselective alkylation of (4*R*)-propionyloxazolidinone (**13**) with the chiral triflate **12** was carried out to obtain (2*S*,4*R*)-2,4-dimethylsubstituted oxazolidinone **14a** and its C-2 diastereomer **14b** in a ca. 8:1 ratio. These two diastereomers were easily separated by column chromatography to give **14a** in 24% yield. Hydrolytic cleavage of the chiral auxiliary from **14a** was done with alkaline hydrogen peroxide to yield **4**. Synthesis of **5**, a C-4 diastereomer of **4**, was started from commercially available racemic alcohol **15**. Alcohol **15** was converted to triflate **16**, followed by a reaction with oxazolidinone **13** to yield a *2S* mixture of **14a** and **14c** in 32% yield and a *2R* mixture of **14b** and **14d** in 4% yield. The major mixture was easily separated from the minor mixture by silica gel column chromatography. Although **14a** and **14c** exhibited similar elution properties on a reversed-phase HPLC column, we could successfully separate them on a small scale sufficient for the next two steps. Purified **14c** was subjected to oxidative hydrolysis with alkaline hydrogen peroxide to obtain **5**.

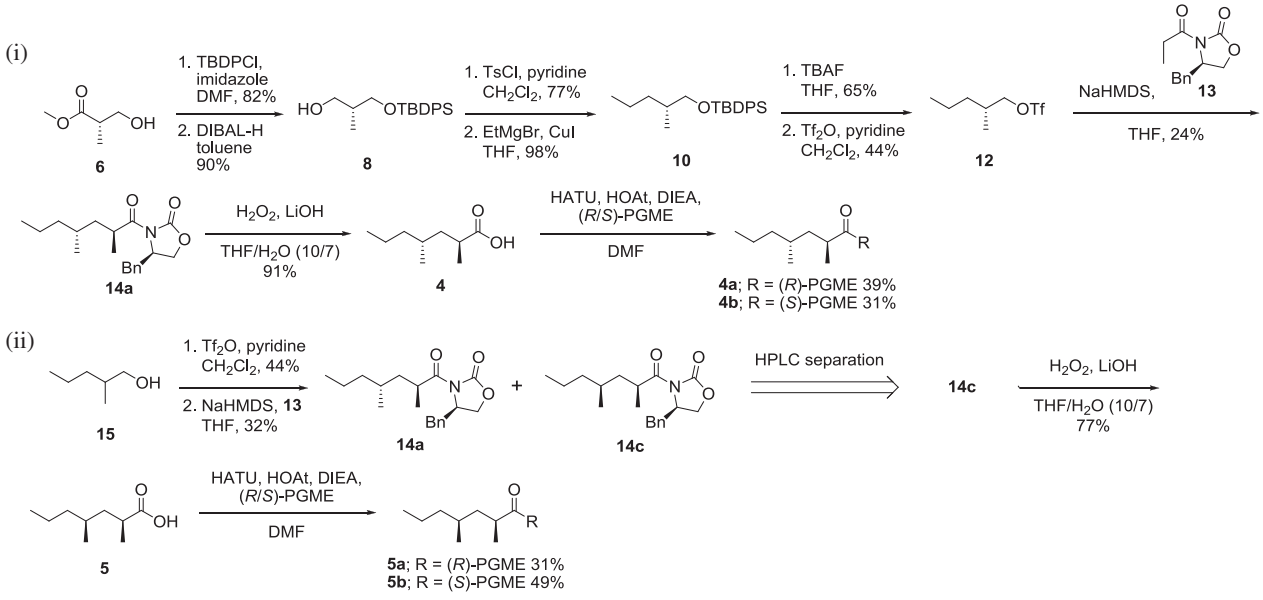
Synthesized carboxylic acids **4** and **5** exhibited apparently different ^{1}H NMR spectra, while ^{1}H NMR spectra of the natural acid **3** closely resembled that of **5**, suggesting that stereocenters in **3** were 2*S* and 4*S* (Fig. 4). Further analysis was carried out by using non-volatile PGME derivatives of **3**, **4**, and **5**. First, the PGME derivatives of **5**, **5a**, and **5b**, gave ^{1}H NMR spectra that were indistinguishable from those of **3a** and **3b**, respectively (Supplementary data, Fig. S42). Next, $\Delta\delta$ values of the ^{1}H NMR spectra for **5a** and **5b** completely matched to those obtained for **3a** and **3b** (Fig. 3). Finally, specific optical rotations of **3a** and **5a** ($[\alpha]_D^{26}=-143.3$ and -144.4, respectively) were smaller than **4a** ($[\alpha]_D^{26}=-134.4$). In conclusion, the side chain of **1** was determined to be (2*S*,4*S*)-2,4-dimethylheptanoic acid.

The chemical structure of tumescenamide C (**1**) was unambiguously determined, consisting of L-Leu, D-Val, D-Tyr, L-Thr, (*Z*)-Abu and (2*S*,4*S*)-Dmh. Tumescenamide C was revealed to be an isomer of tumescenamide A (**2**) since Val of **2** was reported to have an L-configuration. Dmh moieties in **1** and **2** had same relative stereochemistry although the absolute stereochemistry of Dmh in **2** is not known; the stereochemistry of Dmh in **2** was deduced to be 2*S**, 4*S** by *J*-based NMR analysis. Curiously, the ^{1}H and ^{13}C NMR spectra of **1** in acetone-d_6 were similar to those reported for **2** (Table S1).

2.3. Biological activities

Tumescenamide C (**1**) is composed of a sixteen-membered depsipeptide portion and a methyl-branched acyl group. The ring size is the same to those of actinomycin D and FK228, although the constituent amino acids differed.[2,10] The most characteristic feature of **1** is the acyl group. Although several natural products possessing fatty acids with branched methyl groups have been reported,[11,12] tumescenamides A (**2**) and C (**1**) are the only examples possessing 2,4-dimethylheptanoic acid. Tumescenamide A was reported to weakly upregulate the expression of a reporter gene under the control of the insulin degrading enzyme promoter. However, the physiological function of **2** remains to be understood.

We conducted an antimicrobial assay and found that tumescenamide C (**1**) inhibited the growth of streptomycetes cells including *Streptomyces coelicolor* A3(2) and *Streptomyces lividans* TK23; tumescenamide C exhibited growth inhibitory zone at 3.0 μg/paper disk (Table 3). Tumescenamide C also inhibited the growth of the producing strain *Streptomyces* sp. KUSC_F05, although a larger amount of the compound, 30 μg/paper disc, was required. Antimicrobial activity against gram positive bacteria including *Bacillus subtilis* and *Staphylococcus aureus* and gram negative bacteria including *Escherichia coli* and *Pseudomonas aeruginosa* was not



Scheme 1. Synthesis of (i) (2*S*,4*R*)-Dmh and (ii) (2*S*,4*S*)-Dmh.

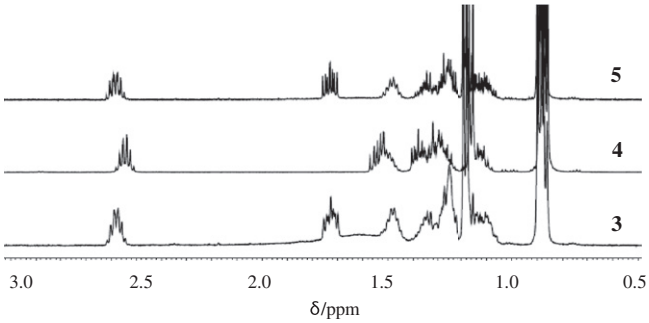


Fig. 4. Comparison of the ^{1}H NMR spectra for 2,4-dimethylheptanoic acids **3** (natural), **4** (synthesized 2*S*,4*R*) and **5** (synthesized 2*S*,4*S*) in $CDCl_3$ (500 MHz).

Table 3
Antimicrobial activity of tumescenamide C (**1**)

Organism	Relevant characteristics	Minimal amount for growth inhibition (μ g/disc)[a]
Streptomyces coelicolor A3(2)	Gram (+)	3
Streptomyces lividans TK23	Gram (+)	3
Streptomyces sp. KUSC_F05	Gram (+)	30
Staphylococcus aureus IFO13276	Gram (+)	>100
Bacillus subtilis IFO3134	Gram (+)	>100
Escherichia coli IFO3972	Gram (-)	>100
Pseudomonas aeruginosa IFO13275	Gram (-)	>100

Organism	Relevant characteristics	IC_{50} (μg/mL)[b]
Schizosaccharomyces pombe JY3	Yeast	>100
Saccharomyces cerevisiae BY4741	Yeast	>100
Candida albicans IFO1594	Yeast	>100

[a] Examined by paper disc diffusion assay.
[b] Examined in a liquid medium.

detected even when 100 μg of **1** was loaded onto paper discs. Yeast cells including *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Candida albicans* were tolerant to **1** at a concentration of 100 μg/mL. Growth of mammalian cells (HeLa, HT1080, ARPE19 and Mv1Lu cells) was not affected by **1** at a concentration of 100 μg/mL. Tumescenamide C seems to affect some cellular architecture or signaling pathways, which are conserved in streptomycetes.

3. Conclusion

In summary, a cyclic depsipeptide tumescenamide C (**1**), a new congener of tumescenamides, was isolated and its chemical structure was determined. The depsipeptide portion of **1** consists of L-Leu, D-Val, D-Tyr, L-Thr and (*Z*)-2-amino-2-butenoic acid, revealing that **1** and **2** are epimeric at the α carbon of the Val residue. The acyl groups, 2,4-dimethylheptanoic acid, had same relative stereochemistry. This is the first report to synthesize (2*S*,4*S*)-2,4-dimethylheptanoic acid and its (4*R*)-epimer in a diastereo-specific manner, by which we unambiguously determined the absolute stereochemistry of the acyl group found in **1**. Tumescenamide C exhibited unique biological properties; selective growth inhibition against streptomycetes cells was observed. The mechanism of action and biological role of tumescenamide C is of interest.

4. Experimental

4.1. General

All reagents and solvents were used as received from commercial suppliers. IR spectra were measured using an FTIR spectrometer equipped with a ZnSe ATR plate. Optical rotations were determined using the sodium D line (589 nm). NMR spectra were measured on a 500 MHz instrument. ^{1}H and ^{13}C chemical shifts are relative to the solvent; δ_H 3.31 and δ_C 49.00 for CD_3OD and δ_H 7.26 and δ_C 77.16 for $CDCl_3$. Mass spectral data were collected with FABMS or ESI MS.

4.2. Fermentation, extraction and isolation

A slant culture of *Streptomyces* sp. KUSC_F05 was inoculated into a 100 mL of culture medium A (1% of glucose, 2.5% of soluble starch, 0.5% of fish meal, 0.3% of NZ case, 0.3% of pharma media, 0.2% of yeast extract, and 0.2% of $CaCO_3$) in a 500 mL flask. The flasks were shaken on a rotary shaker (150 rpm) at 28 °C for 72 h. This preculture (2 mL) was transferred into a 100 mL of a culture medium B (2.0% of oatmeal, 1% of glucose, 2.5% of dextrin, 0.5% of fish meal, 0.5% of B. molasses, 1.0% of pharma media, 0.2% of ebios, and 0.2% of $CaCO_3$), and the fermentation was carried out at 28 °C for 72 h. To the culture broth (totally 3 L) was added the same volume of *n*-BuOH. The *n*-BuOH extract (10.96 g) was fractionated on a silica gel column (120×70 mm) with a stepwise elution of $CHCl_3$/MeOH (from 100:0 to 0:100). The fraction eluted with $CHCl_3$/MeOH (100:5) (1.4 g) was chromatographed on a silica gel column (360×46 mm) with another solvent system (*n*-hexane/EtOAc=50:50 to 0:100). A portion of the EtOAc fraction (110 mg of 714 mg) was subjected to ODS HPLC on Senshu Pak PEGASIL ODS SP100 (250×20 mm) with MeOH/H_2O (50:50) to afford tumescenamide C (**1**, 61.9 mg, t_R=40 min): colorless amorphous solid; mp 173–176 °C; $[\alpha]_D^{25}$+37.0 (*c* 2.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.24), 278 (3.09) nm; IR (neat) 3301 (br), 2959, 2933, 2872, 1651, 1517, 1257 cm^{-1}; HRMS (FAB) *m*/*z* 698.4129 [M−H]$^-$ calcd for $C_{37}H_{56}N_5O_8$, 698.4134; ^{1}H NMR and ^{13}C NMR, see Table 1.

4.3. Acid hydrolysis of 1 and advanced Marfey method

A portion of **1** (0.1 mg) was hydrolyzed with 6 N HCl (200 μL) for 42 h at 105 °C and then dried in vacuo. The obtained hydrolysate was dissolved in H_2O (100 μL), to which 1 M $NaHCO_3$ (20 μL) was added, and the solution was split into two. To a 60 μL aliquot of the hydrolysate was added L-FDLA (1% w/v in acetone, 50 μL) or D-FDLA (1% w/v in acetone, 50 μL), and the mixtures were stirred for 1 h at 70 °C. The solutions were cooled to room temperature, neutralized with 1 N HCl (10 μL), evaporated, and then dissolved in MeCN (200 μL). The derivatives were analyzed by LC-MS; LC separation was performed on a reversed-phase column (Shim-Pack XR-ODS, 75×2.0 mm) with a gradient elution system of H_2O/MeCN containing 0.1% formic acid (80:20 to 20:80 for 40 min, 0.2 mL/min). ESI MS was performed in a positive ionization mode.

4.4. PGME derivatives of the natural Dmh (3a and 3b)

Tumescenamide C (**1**, 10.4 mg, 1.49×10^{-2} mmol) was hydrolyzed with 6 N HCl (2 mL) for 33.5 h at 105 °C, which was subsequently extracted with $CHCl_3$ (5×0.8 mL). The combined extract was dried over anhydrous Na_2SO_4, concentrated in vacuo, and separated with preparative TLC ($CHCl_3$/MeOH=10:1) to afford 2,4-dimethylheptanoic acid (**2**, 0.8 mg, 5.1×10^{-3} mmol). A portion of **3** (0.4 mg, 2.5×10^{-3} mmol) was mixed with HATU (3.4 mg, 8.9×10^{-3} mmol), HOAt (2.1 mg, 1.5×10^{-2} mmol), (*R*)-PGME·HCl (1.4 mg, 6.9×10^{-3} mmol) and DIEA (1.5 mg, 1.2×10^{-2} mmol) in DMF (100 μL), which was allowed stirred at room temperature. After 3.5 h, 1.5 mL of EtOAc was added to the reaction mixture. The organic layer was washed with satd aq NH_4Cl (3×1.5 mL), concentrated, and subjected to preparative TLC ($CHCl_3$/MeOH=10:1), followed by purification by reversed-phase HPLC on Cosmosil 5C18-MS-II (250×10 mm) with H_2O/MeCN (40:60) to afford an (*R*)-PGME derivative of Dmh (**3a**, 0.4 mg, 1.3×10^{-3} mmol, t_R=21 min) as a colorless amorphous solid: $[\alpha]_D^{26}$−143.3 (*c* 0.03, $CHCl_3$); IR (neat) 3298 (br), 2957, 2930, 2871, 1748, 1652, 1531, 1456, 1211 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.29 (m, 5H), 6.40 (br d, *J*=6.8 Hz, 1H), 5.59 (d, *J*=7.2 Hz, 1H),

3.73 (s, 3H), 2.39 (m, 1H), 1.66 (m, 1H), 1.34–1.15 (m, 4H), 1.15 (d, J=6.8 Hz, 3H), 1.10 (m, 1H), 1.01 (m, 1H), 0.83 (d, J=6.6 z, 3H), 0.80 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.1, 171.7, 136.9, 129.1 (2C), 128.6, 127.4 (2C), 56.3, 52.9, 42.0, 39.5, 39.1, 30.4, 20.0, 19.7, 18.6, 14.4; HRMS (ESI) m/z 328.1842 $[M+Na]^+$ calcd for $C_{18}H_{27}NNaO_3$, 328.1883.

The remaining portion of **3** (0.4 mg, 2.5×10^{-3} mmol) was mixed with HATU (3.3 mg, 8.7×10^{-3} mmol), HOAt (1.0 mg, 7.4×10^{-3} mmol), (*S*)-PGME·HCl (1.5 mg, 7.4×10^{-3} mmol) and DIEA (1.5 mg, 1.2×10^{-2} mmol) in DMF (100 μL) and allowed stirred for 3.5 h at room temperature. The reaction mixture was separated as described above to afford (*S*)-PGME derivative of Dmh (**3b**, 0.3 mg, 9.8×10^{-4} mmol, t_R=21 min) as a colorless oil: $[\alpha]_D^{26}$+147.3 (*c* 0.02, $CHCl_3$); IR (neat) 3298, 2956, 2929, 2871, 1749, 1652, 1533, 1456, 1219 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.30 (m, 5H), 6.37 (br d, J=6.9 Hz, 1H), 5.58 (d, J=7.1 Hz, 1H), 3.73 (s, 3H), 2.40 (m, 1H), 1.71 (m, 1H), 1.45 (m, 1H), 1.38–1.20 (m, 3H), 1.16–1.04 (m, 2H), 1.11 (d, J=6.9 Hz, 3H), 0.89 (d, J=6.6 Hz, 3H), 0.86 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.1, 171.7, 136.9, 129.1 (2C), 128.6, 127.4 (2C), 56.3, 52.9, 41.9, 39.6, 39.1, 30.5, 20.1, 19.8, 18.7, 14.5; HRMS (ESI) m/z 328.1843 $[M+Na]^+$ calcd for $C_{18}H_{27}NNaO_3$, 328.1883.

4.5. Synthesis of (2*S*,4*R*)-2,4-dimethylheptanoic acid (4)

*4.5.1. (S)-Methyl 3-(tert-butyldiphenylsilyloxy)-2-methylpropanoate (**7**).* To a stirred solution of (*S*)-methyl 3-hydroxy-2-methylpropanoate (**6**; 916 mg, 7.75 mmol) in DMF (7.8 mL) under nitrogen atmosphere was added imidazole (817 mg, 12.0 mmol) and TBDPSCl (2.57 g, 9.34 mmol). After being stirred for 1.5 h at room temperature, the reaction was quenched by addition of satd aq NH_4Cl (40 mL). The mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed (SiO_2, *n*-hexane/EtOAc=50:1) to give **7** (2.26 g, 6.33 mmol, 81.7%) as a colorless oil: $[\alpha]_D^{25}$+16.8 (*c* 0.34, $CHCl_3$); IR (neat) 2956, 2934, 2857, 1742, 1595, 1112 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.66 (m, 4H), 7.46–7.37 (m, 6H), 3.84 (dd, J=9.6, 6.9 Hz, 1H), 3.74 (dd, J=9.6, 5.8 Hz, 1H), 3.69 (s, 3H), 2.73 (m, 1H), 1.17 (d, J=7.1 Hz, 3H), 1.04 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.5, 135.7 (4C), 133.7, 133.6, 129.8 (2C), 127.8 (4C), 66.1, 51.7, 42.5, 26.9 (3C), 19.4, 13.6; HRMS (ESI) m/z 379.1695 $[M+Na]^+$ calcd for $C_{21}H_{28}NaO_3Si$, 379.1700. Spectral data were in agreement with those reported previously.[13]

*4.5.2. (R)-3-(tert-Butyldiphenylsilyloxy)-2-methyl-1-propanol (**8**).* A solution of **7** (2.21 g, 6.20 mmol) in 40 mL of dry toluene was cooled to −78 °C under nitrogen atmosphere, to which DIBAL-H (15 mL, 15 mmol, 1 M solution in toluene) was added dropwise. After being stirred for 20 min at −78 °C, the mixture was warmed to 0 °C, and stirred for 10 min. The reaction was quenched with satd aq sodium potassium tartrate (40 mL) and the slurry was stirred at room temperature for 10 h. The mixture was extracted with EtOAc (2×40 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (SiO_2, *n*-hexane/EtOAc=15:1 to 5:1) to yield **8** (1.83 g, 5.57 mmol, 89.9%) as a colorless oil: $[\alpha]_D^{25}$+5.7 (*c* 0.98, $CHCl_3$); IR (neat) 3395 (br), 2959, 2930, 2858, 1472, 1428, 1111 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (m, 4H), 7.48–7.40 (m, 6H), 3.75 (dd, J=10.1, 4.6 Hz, 1H), 3.71–3.68 (m, 2H), 3.63 (dd, J=10.2, 7.6 Hz, 1H), 2.57 (s, 1H), 2.01 (m, 1H), 1.09 (s, 9H), 0.86 (d, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 135.7 (4C), 133.3, 133.3, 129.9 (2C), 127.9 (4C), 68.7, 67.7, 37.5, 27.0 (3C), 19.3, 13.3; HRMS (ESI) m/z 351.1738 $[M+Na]^+$ calcd for $C_{20}H_{28}NaO_2Si$, 351.1751. Spectral data were in agreement with those reported previously.[13]

*4.5.3. (S)-3-(tert-Butyldiphenylsilyloxy)-2-methylpropyl 4-methylbenzenesulfonate (**9**).* To a stirred solution of **8** (1.65 g, 5.02 mmol) in 18.5 mL of CH_2Cl_2 under nitrogen atmosphere were added pyridine (4 mL, 49.6 mmol) and TsCl (1.94 g, 10.2 mmol) at 0 °C. After being stirred at 0 °C for 10 h, satd aq NH_4Cl (20 mL) was added. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flush column chromatography (SiO_2, *n*-hexane/MeOH=50:1 and 20:1) to yield **9** (1.87 g, 3.86 mmol, 77.0%) as a colorless oil: $[\alpha]_D^{26}$+6.0 (*c* 0.74, $CHCl_3$); IR (neat) 2960, 2931, 2858, 1472, 1428, 1362, 1189, 1177, 1112 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79 (d, J=8.3 Hz 2H), 7.59 (m, 4H), 7.45–7.41 (m, 2H), 7.39–7.35 (m, 4H), 7.31 (d, J=8.2 Hz, 2H), 4.13 (dd, J=9.2, 5.7 Hz, 1H), 4.01 (dd, J=9.3, 6.0 Hz, 1H), 3.56 (dd, J=10.2, 4.8 Hz, 1H), 3.47 (dd, J=10.2, 6.6 Hz, 1H), 2.42 (s, 3H), 2.01 (m, 1H), 0.98 (s, 9H), 0.89 (d, J=6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.7, 135.7 (2C), 135.6 (2C), 133.5, 133.5, 133.2, 129.9 (2C), 129.8 (2C), 128.1 (2C), 127.8 (4C), 72.3, 64.7, 35.8, 26.9 (3C), 21.8, 19.3, 13.4; HRMS (ESI) m/z 505.1838 $[M+Na]^+$ calcd for $C_{27}H_{34}NaO_4SSi$, 505.1839. Spectral data were in agreement with those reported previously.[14]

*4.5.4. (R)-tert-Butyl(2-methylpentyloxy)diphenylsilane (**10**).* A mixture of **9** (349 mg, 0.723 mmol) and CuI (91.4 mg, 0.480 mmol) was cooled to 0 °C under nitrogen atmosphere, to which 8 mL of 1 M EtMgBr in THF (8 mmol) was added. After being stirred for 3 h at 0 °C, the reaction was quenched with satd aq NH_4Cl (8 mL). The aqueous layer was extracted with EtOAc (2×8 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (SiO_2, *n*-hexane/EtOAc=20:1) to give **10** (241 mg, 0.708 mmol, 98.0%) as a colorless oil: $[\alpha]_D^{25}$+1.5 (*c* 0.54, $CHCl_3$); IR (neat) 2957, 2930, 2858, 1471, 1427, 1111 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.67 (m, 4H), 7.44–7.35 (m, 6H), 3.52 (dd, J=9.7, 5.6 Hz, 1H), 3.43 (dd, J=9.7, 6.4 Hz, 1H), 1.66 (m, 1H), 1.45–1.17 (m, 3H), 1.09 (m, 1H), 1.06 (s, 9H), 0.91 (d, J=6.7 Hz, 3H), 0.87 (t, J=7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 135.8 (4C), 134.3 (2C), 129.6 (2C), 127.7 (4C), 69.2, 35.6 (2C), 27.0 (3C), 20.2, 19.5, 17.0, 14.5; HRMS (ESI) m/z 363.2091 $[M+Na]^+$ calcd for $C_{22}H_{32}NaOSi$, 363.2115.

*4.5.5. (R)-2-Methyl-1-pentanol (**11**).* To a stirred solution of **10** (237 mg, 0.695 mmol) in 5 mL of dry THF under nitrogen atmosphere was added 1.5 mL of 1 M TBAF in THF (1.5 mmol) at 0 °C. The mixture was stirred for 20 min at 0 °C, warmed to room temperature, and stirred for 5 h. The reaction was quenched with satd aq NH_4Cl (5 mL). The aqueous layer was extracted with EtOAc (2×5 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (SiO_2, *n*-hexane/EtOAc=3:1) to give **11** (46.1 mg, 0.451 mmol, 64.9%) as a colorless liquid: $[\alpha]_D^{24}$+13.1 (*c* 0.26, $CHCl_3$); IR (neat) 3318 (br), 2957, 2928, 2871, 1727, 1596 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.75 (dd, J=10.4, 5.7 Hz, 1H), 3.39 (dd, J=10.5, 6.6 Hz, 1H), 1.61 (m, 2H), 1.42–1.21 (m, 3H), 1.08 (m, 1H), 0.90 (d, J=6.7 Hz, 3H), 0.89 (t, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 68.5, 35.6, 35.5, 20.2, 16.7, 14.4. The ^{1}H NMR spectrum matched with that of the commercially available racemic 2-methyl-1-pentanol.

*4.5.6. (R)-2-Methylpentyl trifluoromethanesulfonate (**12**).* A stirred solution of **11** (69.6 mg, 0.681 mmol) in 3 mL of CH_2Cl_2 under nitrogen atmosphere was cooled to −78 °C, to which pyridine (100 μL, 98 mg, 1.24 mmol) and Tf_2O (200 μL, 344 mg, 1.22 mmol) were added. After being stirred for 50 min at −78 °C, the reaction was quenched with satd aq NH_4Cl (3 mL). The aqueous layer was extracted with $CHCl_3$ (3 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (SiO_2, *n*-hexane/EtOAc=50:1) to give **12** (70.1 mg, 0.299 mmol, 43.9%) as a colorless liquid: $[\alpha]_D^{24}$+0.1 (*c* 0.78, $CHCl_3$); IR (neat) 2966, 2936, 2877, 1415, 1247, 1205, 1146 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.40 (dd, J=9.6, 5.6 Hz, 1H), 4.32 (dd, J=9.6, 6.6 Hz, 1H), 1.97 (m, 1H), 1.45–1.27 (m, 3H), 1.22 (m,

1H), 1.02 (d, J=6.8 Hz, 3H), 0.92 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 118.8 (q, J=318.8 Hz), 82.1, 34.6, 33.2, 19.9, 16.2, 14.2.

*4.5.7. (R)-4-Benzyl-3-((2S,4R)-2,4-dimethylheptanoyl)-2-oxazolidinone (**14a**).* A stirred solution of (*R*)-4-benzyl-3-propionyl-2-oxazolidinone (**13**; 139 mg, 0.595 mmol) in dry THF (1.5 mL) under nitrogen atmosphere was cooled to −78 °C, to which 0.31 mL of 1.9 M NaHMDS in THF (0.589 mmol) was added. After being stirred for 30 min at −78 °C, **12** (64.1 mg, 0.274 mmol) in 0.5 mL of dry THF was added. The reaction mixture was stirred for 2.5 h at −78 °C, warmed to 0 °C and stirred for 1.5 h, and then quenched with satd aq NH_4Cl (1.5 mL). The aqueous layer was extracted with EtOAc (2×1.5 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (SiO_2, *n*-hexane/EtOAc=10:1) to give **14a** (20.5 mg, 0.0646 mmol, 23.6%) as a colorless oil: $[\alpha]_D^{25}$ −43.1 (*c* 0.50, $CHCl_3$); IR (neat) 2958, 2929, 2873, 1783, 1699, 1600, 1456, 1387, 1351, 1210, 1099 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35–7.31 (m, 2H), 7.28 (m, 1H), 7.24–7.20 (m, 2H), 4.68 (m, 1H), 4.19 (dd, J=9.0, 7.5 Hz, 1H), 4.15 (dd, J=9.1, 3.4 Hz, 1H), 3.87 (m, 1H), 3.30 (dd, J=13.3, 3.3 Hz, 1H), 2.72 (dd, J=13.3, 9.8 Hz, 1H), 1.60 (m, 1H), 1.53 (m, 1H), 1.40 (m, 1H), 1.38–1.25 (m, 3H), 1.16 (m, 1H), 1.15 (d, J=6.7 Hz, 3H), 0.91 (d, J=6.2 Hz, 3H), 0.89 (t, J=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.0, 153.2, 135.5, 129.6 (2C), 129.1 (2C), 127.5, 66.1, 55.5, 41.0, 39.8, 38.2, 35.5, 30.3, 20.2, 19.2, 16.8, 14.4; HRMS (ESI) m/z 340.1867 $[M+Na]^+$ calcd for $C_{19}H_{27}NNaO_3$, 340.1883.

*4.5.8. (2S,4R)-2,4-Dimethylheptanoic acid (**4**).* To a stirred solution of **14a** (10.1 mg, 0.0318 mmol) in a mixed solvent of THF (0.25 mL) and H_2O (0.15 mL) was added $LiOH\cdot H_2O$ (7.85 mg, 0.125 mmol) and 31% aq H_2O_2 (28.5 μL, 0.260 mmol) at 0 °C. The mixture was stirred for 1.5 h at 0 °C and concentrated in vacuo. The residue was diluted with H_2O (0.4 mL), washed with CH_2Cl_2 (2×0.4 mL), acidified with 6 N HCl until pH reached under 2, and extracted with CH_2Cl_2 (2×1 mL). The CH_2Cl_2 extracts of the acidified aqueous layer were concentrated in vacuo to give **4** (4.6 mg, 0.029 mmol, 91%) as a colorless liquid: $[\alpha]_D^{26}$ +11.2 (*c* 0.38, $CHCl_3$); IR (neat) 2959, 2931, 2871, 1708, 1465 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.2 (br, 1H), 2.55 (ddq, J=6.9, 6.9, 6.9 Hz, 1H), 1.58–1.46 (m, 2H), 1.38 (m, 1H), 1.36–1.24 (m, 2H), 1.20–1.08 (m, 2H), 1.16 (d, J=6.9 Hz, 3H), 0.88 (t, J=7.0 Hz, 3H), 0.86 (d, J=6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 183.2, 40.9, 39.4, 37.2, 30.3, 20.1, 19.4, 17.0, 14.4; HRMS (ESI) m/z 203.1007 $[M-H+2Na]^+$ calcd for $C_9H_{17}Na_2O_2$, 203.1018.

4.6. PGME derivatives of (2*S*,4*R*)-2,4-dimethylheptanoic acid (4a and 4b)

*4.6.1. (R)-Methyl 2-((2S,4R)-2,4-dimethylheptanamido)-2-phenylacetate (**4a**).* A solution of **4** (1.7 mg, 0.011 mmol), HATU (12.3 mg, 0.0324 mmol), HOAt (4.1 mg, 0.030 mmol), (*R*)-PGME·HCl (6.9 mg, 0.034 mmol) and DIEA (8.5 μL, 6.4 mg, 0.050 mmol) in 0.4 mL of DMF was allowed stirred for 7.5 h at room temperature. The reaction was quenched with satd aq NH_4Cl (1 mL) and diluted with EtOAc (1 mL). The organic layer was washed with satd aq NH_4Cl (2×1 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was separated by preparative TLC with $CHCl_3$/MeOH (10:1) followed by purification by reversed-phase HPLC (Cosmosil 5C18-MS-II, 250×10 mm) with H_2O/MeCN (40:60) to afford **4a** (1.3 mg, 0.0043 mmol, 39%, t_R=21 min) as a colorless amorphous solid: $[\alpha]_D^{25}$ −134.4 (*c* 0.15, $CHCl_3$); IR (neat) 3288 (br), 2955, 2928, 2872, 1746, 1650, 1520, 1455, 1200, 1158 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.30 (m, 5H), 6.39 (br d, J=6.6 Hz, 1H), 5.59 (d, J=7.1 Hz, 1H), 3.74 (s, 3H), 2.37 (ddq, J=6.9, 6.9, 6.9 Hz, 1H), 1.45 (m, 1H), 1.41–1.15 (m, 5H), 1.14 (d, J=6.8 Hz, 3H), 1.05 (m, 1H), 0.83 (t, J=6.9 Hz, 3H), 0.79 (d, J=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.3, 171.8, 136.9, 129.1 (2C), 128.6, 127.4 (2C), 56.3, 52.9, 41.6, 39.4, 39.0, 30.3, 20.1, 19.6, 17.8, 14.4; HRMS (ESI) m/z 328.1847 $[M+Na]^+$ calcd for $C_{18}H_{27}NNaO_3$, 328.1883.

*4.6.2. (S)-Methyl 2-((2S,4R)-2,4-dimethylheptanamido)-2-phenylacetate (**4b**).* A solution of **4** (1.9 mg, 0.012 mmol), HATU (14.9 mg, 0.0392 mmol), HOAt (4.8 mg, 0.035 mmol), (*S*)-PGME·HCl (7.2 mg, 0.036 mmol) and DIEA (9.0 μL, 6.8 mg, 0.052 mmol) in 0.4 mL of DMF was stirred for 7.5 h at room temperature. As described above for **4a**, **4b** (1.8 mg, 0.0059 mmol, 49%, t_R=21 min) was purified and obtained as a colorless amorphous solid: $[\alpha]_D^{26}$ +144.4 (*c* 0.15, $CHCl_3$); IR (neat) 3299 (br), 2957, 2930, 2873, 1749, 1652, 1533, 1456, 1219 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.30 (m, 5H), 6.37 (br d, J=6.3 Hz, 1H), 5.58 (d, J=7.0 Hz, 1H), 3.73 (s, 3H), 2.37 (ddq, J=6.9, 6.9, 6.9 Hz, 1H), 1.53–1.44 (m, 2H), 1.40–1.22 (m, 4H), 1.12 (m, 1H), 1.10 (d, J=6.8 Hz, 3H), 0.88 (t, J=6.8 Hz, 3H), 0.85 (d, J=5.8 z, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.3, 171.7, 136.8, 129.1 (2C), 128.6, 127.4 (2C), 56.3, 52.9, 41.4, 39.5, 38.9, 30.2, 20.1, 19.6, 17.6, 14.5; HRMS (ESI) m/z 328.1843 $[M+Na]^+$ calcd for $C_{18}H_{27}NNaO_3$, 328.1883.

4.7. Synthesis of (2*S*,4*S*)-2,4-dimethylheptanoic acid (5)

*4.7.1. (R)-4-benzyl-3-((2S,4S)-2,4-dimethylheptanoyl)-2-oxazolidinone (**14c**).* A stirred solution of racemic 2-methyl-1-pentanol (**15**, 414 mg, 4.05 mmol) in 20 mL of CH_2Cl_2 was cooled to −78 °C under nitrogen atmosphere, to which pyridine (500 μL, 490 mg, 6.19 mmol) and Tf_2O (1.00 mL, 1.72 g, 7.34 mmol) were added. The mixture was stirred for 20 min at −78 °C and for 40 min at 0 °C, and quenched with satd aq NH_4Cl (20 mL). The aqueous layer was extracted with $CHCl_3$ (20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (SiO_2, *n*-hexane/EtOAc=50:1) to give racemic 2-methylpentyl trifluoromethanesulfonate (**16**, 415 mg, 1.77 mmol, 43.7%) as a colorless liquid. ^{1}H NMR spectra of **16** was identical with that of **12**. To a stirred solution of **13** (169 mg, 0.726 mmol) in 3 mL of dry THF at −78 °C under nitrogen atmosphere was added 0.48 mL of 1.9 M NaHMDS in THF (0.912 mmol). The mixture was stirred for 30 min at −78 °C and then **16** (100 mg, 0.427 mmol) in 1.1 mL of dry THF was added. After being stirred for 2.5 h at −78 °C, the reaction mixture was allowed to slowly warm to 0 °C for 1 h, stirred for another 1 h at 0 °C and quenched with satd aq NH_4Cl (3 mL). The aqueous layer was extracted with EtOAc (3 mL), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed (SiO_2, *n*-hexane/EtOAc=10:1) to give a mixture of **14a** and **14c** (43.2 mg, 0.136 mmol, 31.9%, the ratio of **14a** and **14c** was 7:5 judged from the ^{1}H NMR spectrum) as a colorless oil. The mixture was subjected to reversed-phase HPLC (Cosmosil 5C18-AR-II, 250×20 mm, H_2O/MeOH (25:75)), to yield **14c**: $[\alpha]_D^{26}$ −33.0 (*c* 0.73, $CHCl_3$); IR (neat) 2958, 2929, 2872, 1783, 1698, 1456, 1387, 1350, 1235, 1207, 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31–7.35 (m, 2H), 7.28 (m, 1H), 7.24–7.20 (m, 2H), 4.69 (m, 1H), 4.18 (dd, J=9.0, 7.6 Hz, 1H), 4.15 (dd, J=9.0, 3.1 Hz, 1H), 3.94 (m, 1H), 3.30 (dd, J=13.3, 3.3 Hz, 1H), 2.73 (dd, J=13.3, 9.7 Hz, 1H), 1.87 (m, 1H), 1.47 (m, 1H), 1.42–1.24 (m, 3H), 1.19 (m, 1H), 1.17 (d, J=6.7 Hz, 3H), 1.11 (m, 1H), 0.91 (d, J=6.6 Hz, 3H), 0.89 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.9, 153.2, 135.5, 129.6 (2C), 129.1 (2C), 127.5, 66.1, 55.5, 41.5, 39.2, 38.2, 35.4, 30.7, 20.1, 20.1, 18.1, 14.4; HRMS (ESI) 340.1871 $[M+Na]^+$ calcd for $C_{19}H_{27}NNaO_3$, 340.1883.

*4.7.2. (2S,4S)-2,4-Dimethylheptanoic acid (**5**).* To a stirred solution of **14c** (12.4 mg, 0.0391 mmol) in a mixed solvent of THF (0.3 mL) and H_2O (0.2 mL) was added $LiOH\cdot H_2O$ (6.2 mg, 0.125 mmol) and 31% aq H_2O_2 (20 μL, 0.182 mmol) at 0 °C. The mixture was stirred for 20 min at 0 °C and concentrated in vacuo. The residue was diluted with H_2O (0.5 mL), washed with CH_2Cl_2 (4×0.5 mL), acidified with 6 N HCl until pH reached under 2, and extracted with CH_2Cl_2 (3×1 mL). The CH_2Cl_2 extracts of the acidified aqueous layer were

concentrated in vacuo to give **5** (4.8 mg, 0.030 mmol, 77%) as a colorless liquid: $[\alpha]_D^{25}$ +17.5 (*c* 0.08, $CHCl_3$); IR (neat) 2958, 2930, 2872, 1707, 1464, 1428, 1111 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.58 (m, 1H), 1.73 (m, 1H), 1.48 (m, 1H), 1.39–1.21 (m, 3H), 1.19 (d, *J*=7.0 Hz, 3H), 1.19–1.06 (m, 2H), 0.89 (d, *J*=6.6 Hz, 3H), 0.88 (t, *J*=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 182.4, 41.4, 39.4, 37.3, 30.6, 20.0, 19.7, 18.0, 14.4; HRMS (ESI) *m*/*z* 203.1004 $[M-H+2Na]^+$ calcd for $C_9H_{17}Na_2O_2$, 203.1018.

4.8. PGME derivatives of (2*S*,4*R*)-2,4-dimethylheptanoic acid (5a and 5b)

*4.8.1. (S)-Methyl 2-((2S,4R)-2,4-dimethylheptanamido)-2-phenylacetate (**5a**).* A solution of **5** (1.9 mg, 0.012 mmol), HATU (14.9 mg, 0.0392 mmol), HOAt (4.8 mg, 0.035 mmol), (*S*)-PGME·HCl (7.2 mg, 0.036 mmol) and DIEA (9.0 μL, 6.8 mg, 0.052 mmol) in 0.4 mL of DMF was allowed stirred for 7.5 h at room temperature. Following the isolation procedure described above for **4a**, **5a** (1.8 mg, 0.0059 mmol, 49%, t_R=21 min) was purified and obtained as a colorless amorphous solid: $[\alpha]_D^{26}$ −144.4 (*c* 0.15, $CHCl_3$); IR (neat) 3299 (br), 2957, 2930, 2873, 1749, 1652, 1533, 1456, 1219 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.36–7.29 (m, 5H), 6.39 (br d, *J*=6.6 Hz, 1H), 5.59 (d, *J*=7.2 Hz, 1H), 3.73 (s, 3H), 2.39 (m, 1H), 1.66 (m, 1H), 1.34–1.15 (m, 4H), 1.15 (d, *J*=6.9 Hz, 3H), 1.10 (m, 1H), 1.01 (m, 1H), 0.83 (d, *J*=6.6 z, 3H), 0.80 (t, *J*=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.1, 171.7, 136.9, 129.1 (2C), 128.6, 127.4 (2C), 56.3, 52.9, 42.0, 39.5, 39.1, 30.4, 20.0, 19.7, 18.6, 14.4; HRMS (ESI) *m*/*z* 328.1843 $[M+Na]^+$ calcd for $C_{18}H_{27}NNaO_3$, 328.1883.

*4.8.2. (S)-methyl 2-((2S,4S)-2,4-dimethylheptanamido)-2-phenylacetate (**5b**).* A solution of **5** (2.0 mg, 0.013 mmol), HATU (9.6 mg, 0.025 mmol), HOAt (4.0 mg, 0.029 mmol), (*S*)-PGME·HCl (11.5 mg, 0.057 mmol) and DIEA (15.0 μL, 11.3 mg, 0.0872 mmol) in 0.25 mL of DMF was stirred for 7.5 h at room temperature. **5b** (2.0 mg, 0.0066 mmol, 51%, t_R=21 min) was purified as described above and obtained as a colorless oil: $[\alpha]_D^{26}$ +155.7 (*c* 0.16, $CHCl_3$); IR (neat) 3296, 2957, 2928, 2872, 1749, 1649, 1527, 1456, 1206 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.30 (m, 5H), 6.37 (br d, *J*=6.8 Hz, 1H), 5.59 (d, *J*=7.1 Hz, 1H), 3.73 (s, 3H), 2.40 (m, 1H), 1.71 (m, 1H), 1.45 (m, 1H), 1.38–1.20 (m, 3H), 1.16–1.04 (m, 2H), 1.11 (d, *J*=6.9 Hz, 3H), 0.89 (d, *J*=6.6 Hz, 3H), 0.86 (t, *J*=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.0, 171.7, 136.9, 129.1 (2C), 128.6, 127.4 (2C), 56.3, 52.9, 41.9, 39.6, 39.1, 30.5, 20.1, 19.8, 18.7, 14.5; HRMS (ESI) *m*/*z* 328.1839 $[M+Na]^+$ calcd for $C_{18}H_{27}NNaO_3$, 328.1883.

4.9. Antimicrobial assay

Growth inhibitory activity of **1** against gram positive and gram negative bacteria was examined by paper disc diffusion assay. Spores of *S. coelicolor* A3(2), *S. lividans* TK23 or *Streptomyces* sp. KUSC_F05, or cells of *S. aureus* subsp. *aureus* IFO13276, *B. subtilis* IFO3134, *E. coli* IFO 0392 or *P. aeruginosa* IFO13275 were suspended in 1% agar medium and overlaid on solid medium plates. Methanol solutions of tumescenamide C were loaded onto paper disks (ϕ 6 mm), which were dried and placed on the plates. After incubating at 30 °C for 3 days, growth inhibitory zone was measured. Maltose-Bennett's medium (NBRC medium 231; 0.1% of yeast extract, 0.1% of fish extract, 0.2% of NZ amine and 1% of maltose·H_2O) was used to cultivate *S. coelicolor*, *S. lividans* and *Streptomyces* sp. KUSC_F05. NBRC medium 802 (1% of polypepton, 0.2% of yeast extract and 0.1% of $MgSO_4 \cdot 7H_2O$) was used to cultivate *S. aureus* subsp. *aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. Growth inhibition against yeast cells including *S. pombe* JY3, *S. cerevisiae* BY4741 and *C. albicans* IFO1594, was examined in a liquid medium as described before.[15]

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Supplementary data

1D and 2D NMR spectra for tumescenamide C, 1D NMR data for other synthetic compounds and LC-MS chromatogram of FDLA derivatives. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2012.04.075.

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