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Total synthesis and reassignment of stereochemistry of obyanamide

Wei Zhang, Zhen-Hua Ma, Duo Mei, Chun-Xia Li, Xiu-Li Zhang and Ying-Xia Li*

Key Laboratory of Marine Drugs, The Ministration of Education of China, School of Pharmacy, Ocean University of China, Qingdao 266003, China

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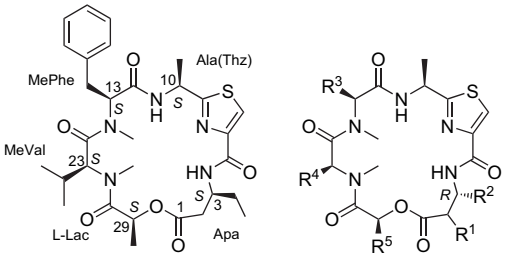
Abstract—The total synthesis of a marine cytotoxic cyclic depsipeptide obyanamide is reported. The synthesis has led to a reassignment of the C-3 configuration in β-amino acid residue. And this revision is also supported by biological test.

1. Introduction

Marine cyanobacteria are well known to be a rich source of bioactive peptides and depsipeptides,[1] many of which are commonly synthetic focus in the quest for new leads in pharmaceutical field. Structurally these molecules can be cyclic, contain modified amino acid units and polyketide portions, or be any combination thereof.

Obyanamide (**1**, Fig. 1) is a cyclic depsipeptide that was isolated from the marine cyanobacterium *Lyngbya confervoides* by Moore and co-workers.[2] Some other structural analogues (Fig. 1) have also been isolated, namely guineamides A and B[3] and ulongamides A–E.[4] The structures of obyanamide and its congeners were determined by a combination of NMR spectroscopy, MS, and chemical degradation. Structurally these compounds consist of similar five subunits: β-amino acid residues, Ala (Thz) units, two *N*-methylated α-amino acids (including aromatic amino acids, commonly Phe or Tyr), and α-hydroxy acids. Through in vitro studies on cytotoxicity against several tumor cells, all these compounds above were found to have low to moderate activities. Structure–activity relationship (SAR) studies on these compounds might help to find out more efficient agents. So we decided to explore an efficient synthesis of obyanamide and its analogues. Previous studies[5] suggested that the structure of the natural obyanamide should be revised. And now, we would like to amend its stereochemistry at C-3 position.



Obyanamide (**1**)

	R^1	R^2	R^3	R^4	R^5
Guineamide A	Me*	Et	Bn	*i*-Pr	Me
Guineamide B	Me#	Me#	Bn	*i*-Pr	*i*-Pr
Ulongamide A	Me**	*n*-Pr	*i*-Pr	Bn	Me
Ulongamide B	Me**	*n*-Pr	*i*-Pr	*p*-OH Bn	Me
Ulongamide C	Me**	*n*-Pr	Bn	*p*-OH Bn	Me
Ulongamide D	Me*	*n*-Pr	*i*-Pr	*p*-OH Bn	*i*-Pr
Ulongamide E	Me*	*n*-Pr	*i*-Pr	*p*-OH Bn	

Figure 1. Obyanamide and some structure related natural products. **S* configuration; #unknown; ***R* configuration.

2. Results and discussion

Our retrosynthetic analysis of **1** is displayed as Scheme 1. The route required, therefore, the preparation of two protected fragments before macrocyclization.

The starting material (*S*)-2-aminobutyric acid **5** (Scheme 2) was first converted to diazoketone via protection of amino

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* Corresponding author. Tel.: +86 53282032150; fax: +86 53282033054; e-mail: liyx417@ouc.edu.cn

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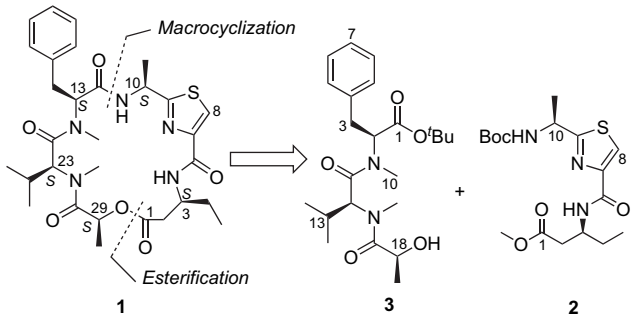
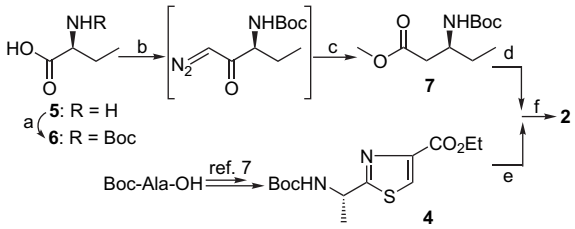


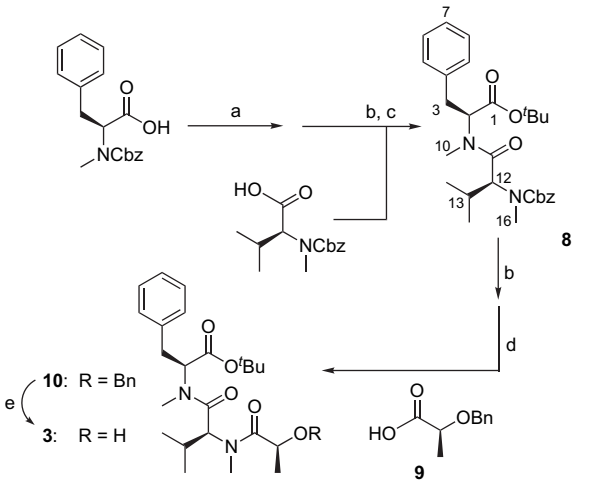
Scheme 1. Retrosynthetic analysis of **1**.



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, 91%; (b) $ClCO_2Et$, Et_3N, then CH_2N_2; (c) AgOAc, MeOH, 61% for two steps; (d) TFA; (e) LiOH, THF–MeOH–H_2O; (f) EDC, HOAt, DIPEA, 98% for three steps.

group with *tert*-butyloxycarbonyl (Boc), activation of carbonyl group with ethyl chloroformate, and treatment with diazomethane. Then this intermediate was directly subjected to Wolff's rearrangement in absolute methanol. Thus, the desired β-amino acid moiety **7** was obtained in three steps in a satisfied yield.[6] Removal of the Boc protection of **7** with trifluoroacetic acid (TFA) and coupling with the free acid from **4**[7] gave dipeptide **2** in 98% yield.

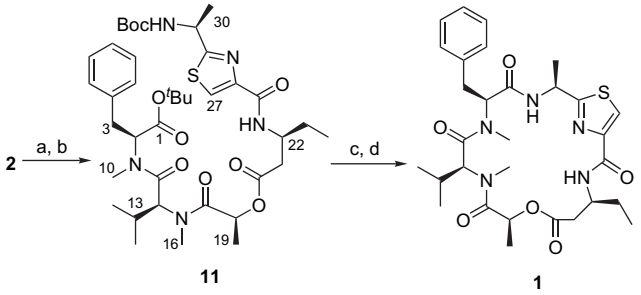
In order to construct fragment **3** (Scheme 3), two *N*-methylated amino acid units, Cbz-MePhe-OH and Cbz-MeVal-OH, were prepared with McDermott's method.[8] After protection of the carboxylic acid of Cbz-MePhe-OH with *tert*-butyl (^{t}Bu) group,[9] the two units were coupled. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and the efficient additive 1-hydroxy-7-azabenzotriazole (HOAt) were used as coupling reagents to form the hindered



Scheme 3. Reagents and conditions: (a) ^{t}BuOH, EDC, DMAP, 85%; (b) H_2, Pd–C, EtOAc, 2 h; (c) EDC, HOAt, DIPEA, CH_2Cl_2, 78% for two steps; (d) EDC, HOAt, DIPEA, 87% for two steps; (e) H_2, Pd–C, EtOAc, 3 h, 98%.

peptide. And this reaction gave moderate to high yield. However, incorporation of (*S*)-lactic acid with the amine H-MeVal-MePhe-O^{t}Bu from **8** in the presence of EDC–HOAt or 2-bromo-1-ethyl pyridinium tetrafluoroborate (BEP)[10] gave complex products. This might result from the nucleophilic competition between the amine and the α-hydroxyl group of the acid. Thus, the hydroxy acid was first treated with BnBr and sodium metal in liquid ammonia,[11] to give the corresponding (*S*)-2-benzyloxy lactic acid **9**, which was then used in the following coupling step instead of lactic acid, giving satisfied result. Removal of the benzyl group gave fragment **3** in 98% yield.

With two fragments in hand, we could now progress to the cyclic depsipeptide (Scheme 4). However, coupling of the alcohol **3** with the free acid from dipeptide **2** using EDC and 4-(dimethylamino) pyridine (DMAP) as coupling reagents in CH_2Cl_2 gave low yield, probably due to steric limitations. Thus, Yamaguchi's procedure[12] was adopted and worked well to produce the linear pentapeptide **11** in 94% yield. Treatment of **11** with TFA in CH_2Cl_2 and finally cyclization gave compound **1** in 59% yield over two steps.



Scheme 4. Reagents and conditions: (a) LiOH, THF–MeOH–H_2O; (b) 2,4,6-trichlorobenzoyl chloride, DIPEA, THF, then **3** in DMAP–toluene, 94%; (c) TFA; (d) HATU, DIPEA, THF, 59% for two steps.

To our surprise, the analytical data of **1** were inconsistent with those published for natural product, with some differences in both the ^{1}H and ^{13}C NMR spectra. What is more, the value and sign of optical rotation of the synthetic sample were also quite different from that of the natural product {$[\alpha]_D^{28}$ −96.3 (*c* 0.06, MeOH) while lit.[2] $[\alpha]_D^{27}$ +20 (*c* 0.04, MeOH)}. We were confident that the structure of the synthetic obyanamide is correct; we therefore turned our thoughts to the source of stereochemical assignment. All the compounds in Figure 1 are isolated from the species *Lyngbya*, and all of the amino groups in β-amino acid residues are *R* configuration except for compound **1**. So we doubt the correctness of stereochemistry at this position. To this end, we synthesized Boc-(*R*)-Apa-OMe following the same synthesis as for **7**, but starting with (*R*)-2-aminobutyric acid. This was readily achieved, and Boc-(*R*)-Apa-OMe was incorporated into the synthesis as previously performed to afford **1a** with no adverse consequences (Scheme 5). To our glad, it was indeed found that the data for the newly synthesized compound **1a** was an excellent match for the literature data on obyanamide (Fig. 2). Notably, the consequence of chemical shifts of five protons connecting to the chiral carbon atoms of **1a** ($\delta_{H\text{-}3}<\delta_{H\text{-}10}<\delta_{H\text{-}23}<\delta_{H\text{-}29}<\delta_{H\text{-}13}$) was consistent with the natural product, while that of compound **1** ($\delta_{H\text{-}3}<\delta_{H\text{-}23}<\delta_{H\text{-}13}<\delta_{H\text{-}10}<\delta_{H\text{-}29}$) was not.

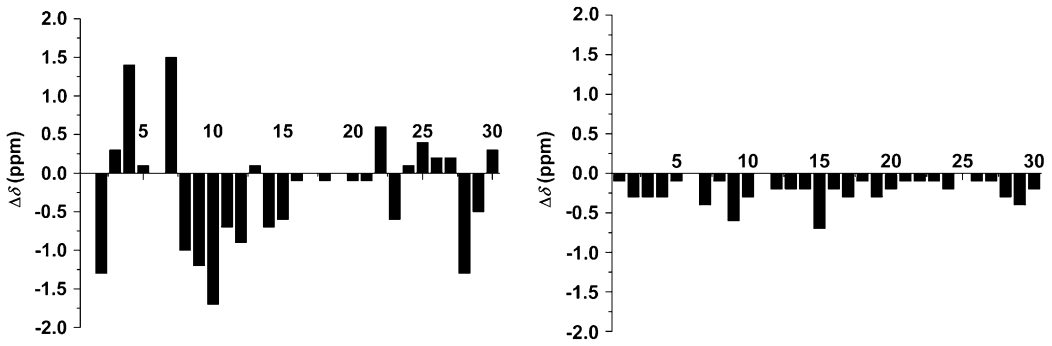
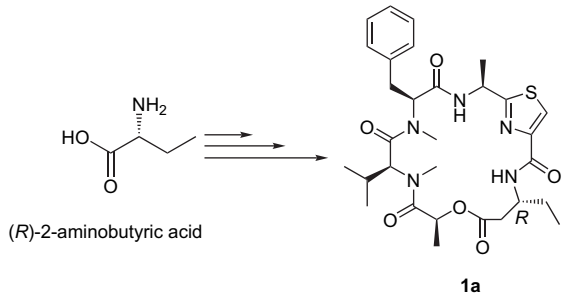


Figure 2. Differences in ^{13}C NMR shifts between natural obyanamide, compound **1** (left), and compound **1a** (right).



Scheme 5. Synthesis of compound **1a**.

Both of the two synthetic cyclic depsipeptides were subjected to biological test on several cancer cell lines. At a concentration of 10 μM, compound **1** showed no inhibition of KB, LoVo, HL-60, P388, A-549, or BEL-7402. While **1a** exhibited moderate cytotoxicity against KB, HL-60, and LoVo cells with IC_{50} values of 6.7, 6.8, and 19.0 μM, respectively.[13]

3. Conclusion

The marine cytotoxic cyclic depsipeptide obyanamide has been synthesized. As a result, the configuration at C-3 position has been amended as *R*.

4. Experimental

4.1. General information

Solvents were purified by standard methods. TLC was carried out on Merck 60 F_{254} silica gel plates and visualized by UV irradiation or by staining with iodine absorbed on silica gel, ninhydrin solution, or with aqueous acidic ammonium molybdate solution as appropriate. Flash column chromatography was performed on silica gel (200–300 mesh, Qingdao, China). Optical rotations were obtained using a JASCO P-1020 digital polarimeter. NMR spectra were recorded on JEOL JNM-ECP 600 MHz spectrometers. Chemical shifts are reported in parts per million (ppm), relative to the signals due to the solvent. Data are described as the followings: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constants (Hz), integration, and assignment. Mass spectra were obtained on a Q-Tof Ultima Global mass spectrometer.

4.1.1. Synthesis of compound 1.

4.1.1.1. (*S*)-2-*tert*-Butoxycarbonylaminobutyric acid (6). To a solution of (*S*)-2-aminobutyric acid **5** (1.55 g, 15.0 mmol) in 15 mL of 1 M NaOH and 10 mL MeOH was added $(Boc)_2O$ (4.14 mL, 18.0 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 12 h. After most of the methanol was evaporated, the solution was acidified to pH 2 with 1 M HCl and extracted with EtOAc (3×50 mL). The organic extracts were combined and washed with brine (2×10 mL). Evaporation of the solvent gave the title compound (2.77 g, 91%) as an oil: R_f=0.43 (*n*-hexane–EtOAc–HOAc=10:10:1); $[\alpha]_D^{29}$ −15.2 (*c* 1.0, MeOH); ^1H NMR (DMSO-d_6) δ 0.91 (t, *J*=7.3 Hz, 3H, H-4), 1.42 (s, 9H, ^tBu), 1.56–1.64 (m, 1H, H-3a), 1.68–1.75 (m, 1H, H-3b), 3.81–3.84 (m, 1H, H-2), 7.09 (d, *J*=8.0 Hz, 1H, N*H*), 12.46 (s, 1H, CO_2H); HRESIMS calcd for $C_9H_{17}NO_4Na$ $[M+Na]^+$ 226.1055, found 226.1046.

4.1.1.2. (*S*)-Methyl 3-(*tert*-butoxycarbonylamino)-pentanoate (7). Compound **6** (732 mg, 3.6 mmol) was dissolved in dry THF (20 mL) and cooled to −20 °C under argon. After addition of Et_3N (510 μL, 3.6 mmol) and $ClCO_2Et$ (345 μL, 3.6 mmol), the mixture was stirred for 20 min at that temperature. A very carefully dried, cooled etheral solution of CH_2N_2 (obtained from 1.55 g, 15.0 mmol of *N*-methyl-*N*-nitrosourea) was added. Stirring was continued for 4 h as the mixture was allowed to warm to room temperature. Excess CH_2N_2 was destroyed by the addition of a few drops of HOAc. The mixture was then diluted with Et_2O and washed with saturated $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc–petroleum ether=1:5 to 1:3) to give the diazoketone, which was dried under high vacuum for 4 h and then dissolved in dry methanol (20 mL). After addition of Et_3N (1.5 mL, 10.7 mmol) and AgOAc (66 mg, 0.4 mmol) at −20 °C, the resulting mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was then filtered through a pad of Celite and the resulting filtrate was evaporated in vacuo. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated to leave an oil. The oil was purified by silica gel chromatography using EtOAc–petroleum ether (1:9 to 1:6) as eluent to give the ester **7** (511 mg, 61% over two steps) as a white solid: R_f= 0.39 (EtOAc–petroleum ether=1:3); $[\alpha]_D^{20}$ −16.8 (*c* 1.0, MeOH); ^1H NMR ($CDCl_3$) δ 0.93 (t, *J*=7.7 Hz, 3H, H-5),

1.44 (s, 9H, ^tBu), 1.52–1.56 (m, 2H, H-4), 2.48–2.55 (m, 2H, H-2), 3.68 (s, 3H, CO_2CH_3), 3.83–3.84 (m, 1H, H-3), 4.90 (br, 1H, N*H*); ^{13}C NMR ($CDCl_3$) δ 10.6 (C-5), 27.6 (C-4), 28.3 ($(CH_3)_3COC$=O), 38.8 (C-2), 49.0 (C-3), 51.6 (CO_2CH_3), 79.2 ($(CH_3)_3COC$=O), 155.4 ($(CH_3)_3COC$=O), 172.2 (C-1); HRESIMS calcd for $C_{11}H_{21}NO_4Na$ $[M+Na]^+$ 254.1368, found 254.1364.

4.1.1.3. Boc-Ala(Thz)-(*S*)-Apa-OMe (2). LiOH monohydrate (126 mg, 3.0 mmol) was added to a solution of ester **4**[7] (451 mg, 1.5 mmol) in THF–MeOH–H_2O (4 mL/2 mL/1 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. After most of the solvent was evaporated, the solution was acidified to pH 2 with 1 M HCl and extracted with EtOAc (3×20 mL). The organic extracts were combined and washed with brine (2×5 mL). Evaporation of the solvent gave the corresponding acid, which was used directly in the next step.

TFA (3 mL) was added to a solution of **7** (347 mg, 1.5 mmol) in 3 mL CH_2Cl_2 at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. The solvent was evaporated and the residual oil was dissolved twice in CH_2Cl_2 (3 mL) with evaporation each time to give TFA salt, which was used directly in the next step.

EDC (288 mg, 1.5 mmol) was added to a suspension of the carboxylic acid, the TFA salt, and HOAt (204 mg, 1.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C followed by DIPEA (524 μL, 3.0 mmol). The reaction mixture was warmed to room temperature and stirred overnight. After diluted with EtOAc (80 mL), the whole mixture was washed with 10% citric acid (3×10 mL), 5% $NaHCO_3$ (3×10 mL), and brine (3×10 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–petroleum ether=1:4 to 1:2) to give dipeptide **2** (566 mg, 98%) as a colorless oil: R_f=0.30 (EtOAc–petroleum=1:1); $[\alpha]_D^{29}$ −21.3 (*c* 0.41, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.98 (t, *J*=7.3 Hz, 3H, H-5), 1.47 (s, 9H, ^tBu), 1.61 (d, *J*=6.5 Hz, 3H, H-11), 1.67–1.72 (m, 2H, H-4), 2.64 (dd, *J*=15.6, 5.4 Hz, 1H, H-2a), 2.66 (dd, *J*=15.6, 5.4 Hz, 1H, H-2b), 3.70 (s, 3H, CO_2CH_3), 4.32–4.38 (m, 1H, H-3), 5.06–5.08 (m, 1H, H-10), 5.13 (br, 1H, N*H*), 7.63 (d, *J*=9.2 Hz, 1H, N*H*), 8.00 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ 10.7 (C-5), 21.6 (C-11), 27.3 (C-4), 28.3 ($(CH_3)_3COC$=O), 38.5 (C-2), 47.6 (C-3), 48.7 (C-10), 51.7 (CO_2CH_3), 80.2 ($(CH_3)_3COC$=O), 123.0 (C-8), 150.0 (C-7), 155.0, 160.6, 171.9 and 174.0 (4 quat. C); HRESIMS calcd for $C_{17}H_{27}N_3O_5SNa$ $[M+Na]^+$ 408.1569, found 408.1581.

4.1.1.4. Cbz-MePhe-O^{t}Bu. To a solution of Cbz-MePhe-OH[8] (1.57 g, 5.0 mmol) and ^{t}BuOH (7.41 g, 100.0 mmol) in CH_2Cl_2 (25 mL) were added EDC (1.44 g, 7.5 mmol) and DMAP (307 mg, 2.5 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The solvent was evaporated and the residue was dissolved in EtOAc (200 mL) and washed with 10% citric acid (3×20 mL), 5% $NaHCO_3$ (3×20 mL), and brine (3×20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc–petroleum=1:12 to 1:8) to give the title compound (1.57 g, 85%) as a colorless oil: R_f=0.40 (EtOAc–petroleum ether=1:4); $[\alpha]_D^{20}$ −73.0 (*c* 0.2, MeOH); ^{1}H NMR ($CDCl_3$, one rotamer of two) δ 1.42 (s, 9H, ^tBu), 2.80 (s, 3H, N-CH_3), 2.95 (dd, *J*=14.5, 11.2 Hz, 1H, H-3a), 3.24 (dd, *J*=14.7, 5.0 Hz, 1H, H-3b), 4.74 (dd, *J*=10.6, 5.0 Hz, 1H, H-2), 4.95–5.11 (m, overlapped, 2H, $C_6H_5CH_2OC$=O), 7.11–7.33 (m, 10H, Ar *H*); HRESIMS calcd for $C_{22}H_{27}NO_4Na$ $[M+Na]^+$ 392.1838, found 392.1835.

4.1.1.5. Cbz-MeVal-MePhe-O^{t}Bu (8). Palladium on charcoal (75 mg, 10 wt %) was added to a solution of Cbz-MePhe-O^{t}Bu (739 mg, 2.0 mmol) in EtOAc (10 mL). The reaction mixture was purged with hydrogen three times and stirred for 2 h at room temperature. The suspension was filtered through a pad of Celite, washed with EtOAc (3×5 mL), and concentrated in vacuo. The amine was dried under high vacuum for 4 h and used directly in the next step.

DIPEA (542 μL, 3.0 mmol) and EDC (460 mg, 2.4 mmol) were added successively to a suspension of Cbz-MeVal-OH (531 mg, 2.0 mmol), amine, and HOAt (327 mg, 2.4 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was allowed to stir at that temperature for 2 h and at room temperature for another 18 h. After diluted with EtOAc (50 mL), the whole mixture was washed with 10% citric acid (3×5 mL), 5% $NaHCO_3$ (3×5 mL), and brine (3×5 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–petroleum ether=1:6 to 1:4) to give dipeptide **8** as a colorless oil (750 mg, 78%): R_f=0.35 (EtOAc–petroleum ether=1:3); $[\alpha]_D^{29}$ −102.3 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, one main rotamer of four) δ 0.79 (d, *J*=7.0 Hz, 3H, H-14), 0.89 (d, *J*=6.6 Hz, 3H, H-15), 1.45 (s, 9H, ^tBu), 2.09–2.12 (m, 1H, H-13), 2.86 (s, 3H, N-CH_3), 2.91 (s, 3H, N-CH_3), 3.30 (dd, *J*=10.3, 4.7 Hz, 1H, H-3a), 3.34 (dd, *J*=10.9, 5.1 Hz, 1H, H-3b), 4.58 (d, *J*=10.7 Hz, 1H, H-12), 5.00–5.41 (d and dd like, overlapped, 3H, H-2 and $C_6H_5CH_2OC$=O), 7.03–7.51 (m, 10H, Ar *H*); ^{13}C NMR ($CDCl_3$, one main rotamer of four) δ 17.6 (C-14), 19.4 (C-15), 26.3 (C-13), 27.9 ($C(CH_3)_3$), 28.6 (N-CH_3), 29.5 (N-CH_3), 34.3 (C-3), 58.3 (C-2), 60.0 (C-12), 67.1 ($C_6H_5CH_2OC$=O), 81.7 ($C(CH_3)_3$), 126.4, 126.9, 128.4, 128.9, 129.1 and 129.3 (Ar *C*H), 136.8 and 137.1 (Ar *C*), 156.6, 169.8 and 170.3 (*C*=O ×3); HRESIMS calcd for $C_{28}H_{38}N_2O_5Na$ $[M+Na]^+$ 505.2678, found 505.2695.

4.1.1.6. BnO-Lac-OH (9). Freshly cut sodium metal (575 mg, 25.0 mmol) was added to anhydrous ammonia (60 mL) at −70 °C, and 10 min later (*S*)-lactic acid (900 mg, 10.0 mmol) was added with vigorous stirring under argon. To the mixture was added BnBr (2.4 mL, 20.0 mmol) after another 10 min. The turbid solution was stirred for 1 h at −50 °C. The ammonia was then removed by slow evaporation. The residue was dissolved in distilled water (50 mL) and the solution was extracted with Et_2O (3×10 mL). The aqueous phase was chilled and acidified to pH 2 with 2 M HCl, and extracted with EtOAc (5×20 mL). The combined organic layers were washed with brine (2×10 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography ($CHCl_3$–MeOH=60:1 to 30:1) followed by recrystallization from *n*-hexane to give the acid (609 mg, 34%) as a colorless solid: R_f=0.40 ($CHCl_3$–MeOH=10:1); $[\alpha]_D^{26}$ −74.2 (*c* 4.6, C_6H_6) {lit.[14] $[\alpha]_D^{25}$ −53 (*c* 4.6, C_6H_6)}; ^{1}H NMR (DMSO-d_6) δ 1.32 (d, *J*=6.9 Hz, 3H, H-3), 4.00 (q, *J*=6.9 Hz, 1H,

H-2), 4.41 (d, J=11.9 Hz, 1H, $C_6H_5CH_aH_b$), 4.59 (d, J=11.9 Hz, 1H, $C_6H_5CH_aH_b$), 7.29–7.35 (m, 5H, Ar *H*), 12.72 (s, 1H, CO_2H); ^{13}C NMR (DMSO-d_6) δ 18.5 (C-3), 70.8 ($C_6H_5CH_2$), 73.4 (C-2), 127.5, 127.6, 128.2 (Ar *C*H), 138.1 (Ar *C*), 174.2 (CO_2H); HRESIMS calcd for $C_{10}H_{12}O_3Na$ $[M+Na]^+$ 203.0684, found 203.0692.

4.1.1.7. BnO-Lac-MeVal-MePhe-O^{t}Bu (10). To a solution of Cbz-MeVal-MePhe-O^{t}Bu **8** (965 mg, 2.0 mmol) in EtOAc (10 mL) was added palladium on charcoal (100 mg, 10 wt %). The reaction mixture was purged with hydrogen three times and stirred for 2 h at room temperature. The suspension was filtered through a pad of Celite, washed with EtOAc (3×5 mL), and concentrated in vacuo. The amine was dried under high vacuum for 4 h and used directly in the next step.

DIPEA (542 μL, 3.0 mmol) and EDC (460 mg, 2.4 mmol) were added successively to a suspension of BnO-Lac-OH **9** (531 mg, 2.0 mmol), amine, and HOAt (327 mg, 2.4 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was allowed to stir at that temperature for 2 h and at room temperature for another 12 h. After diluted with EtOAc (50 mL), the whole mixture was washed with 10% citric acid (3×5 mL), 5% $NaHCO_3$ (3×5 mL), and brine (3×5 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–petroleum ether=1:5 to 1:2) to give **10** as a colorless oil (950 mg, 93%): R_f=0.52 (EtOAc–petroleum ether=1:1); $[\alpha]_D^{20}$ −124.6 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, one main rotamer of four) δ 0.81 (d, J=6.4 Hz, 3H, H-14), 0.94 (d, J=6.4 Hz, 3H, H-15), 1.21 (d, J=6.4 Hz, 3H, H-19), 1.46 (s, 9H, ^tBu), 2.18–2.28 (m, 1H, H-13), 2.39 (s, 3H, N-CH_3), 2.85 (s, 3H, N-CH_3), 2.93–2.95 (dd like, overlapped, 1H, H-3a), 3.36 (dd, J=14.6, 4.1 Hz, 1H, H-3b), 4.15 (q, J=6.4 Hz, 1H, H-18), 4.25 (d, J=11.4 Hz, 1H, $C_6H_5CH_aH_bO$), 4.54 (d, J=11.9 Hz, 1H, $C_6H_5CH_aH_bO$), 5.09 (d, J=10.5 Hz, 1H, H-12), 5.47 (dd, J=11.4, 4.1 Hz, 1H, H-2), 7.16–7.32 (m, 10H, Ar *H*); ^{13}C NMR ($CDCl_3$, one main rotamer of four) δ 17.5 (C-19), 18.0 (C-14), 19.6 (C-15), 26.6 (C-13), 28.0 (C(CH_3)$_3$), 29.1 (N-CH_3), 31.8 (N-CH_3), 34.3 (C-3), 58.0 (C-2), 58.3 (C-12), 70.7 ($C_6H_5CH_2O$), 72.1 (C-18), 81.9 ($C(CH_3)_3$), 126.5, 126.9, 127.9, 128.4, 128.8 and 129.5 (Ar *C*H), 137.2 and 137.6 (Ar *C*), 169.7, 170.1 and 171.8 (*C*=O ×3); HRESIMS calcd for $C_{30}H_{42}N_2O_5Na$ $[M+Na]^+$ 533.2991, found 533.2974.

4.1.1.8. HO-Lac-MeVal-MePhe-O^{t}Bu (3). Palladium on charcoal (75 mg, 10 wt %) was added to a solution of BnO-Lac-MeVal-MePhe-O^{t}Bu **10** (709 mg, 1.4 mmol) in EtOAc (10 mL). The reaction mixture was purged with hydrogen three times and stirred for 3 h at room temperature. The suspension was filtered through a pad of Celite, washed with EtOAc (3×5 mL), and concentrated in vacuo to give the alcohol **3** (572 mg, 98%) as a wax solid: R_f=0.39 (EtOAc–petroleum ether=1:1); $[\alpha]_D^{20}$ −103.8 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.75 (d, J=6.8 Hz, 3H, H-14), 0.94 (d, J=6.8 Hz, 3H, H-15), 1.05 (d, J=6.4 Hz, 3H, H-19), 1.47 (s, 9H, ^tBu), 2.27–2.31 (m, 1H, H-13), 2.33 (s, 3H, N-CH_3), 2.79 (s, 3H, N-CH_3), 2.93 (dd, J=15.1, 12.4 Hz, 1H, H-3a), 3.39 (dd, J=15.1, 4.6 Hz, 1H, H-3b), 3.63 (br, 1H, Lac O*H*), 4.27–4.31 (m, 1H, H-18), 4.99 (d, J=10.5 Hz, 1H, H-12), 5.52 (dd, J=12.4, 4.6 Hz, 1H, H-2), 7.16–7.33 (m, 5H, Ar *H*); ^{13}C NMR ($CDCl_3$) δ 17.8 (C-14), 19.6 (C-15), 20.9 (C-19), 27.9 (C(CH_3)$_3$), 29.0 (C-13), 29.5 (N-CH_3), 31.4 (N-CH_3), 34.2 (C-3), 57.8 (C-2), 59.0 (C-12), 64.3 (C-18), 82.0 ($C(CH_3)_3$), 126.6 (C-7), 128.8 and 129.4 (C-5, C-6, C-8, and C-9), 137.1 (C-4), 169.5, 169.6 and 175.3 (*C*=O ×3); HRESIMS calcd for $C_{23}H_{36}N_2O_5Na$ $[M+Na]^+$ 443.2522, found 443.2520.

4.1.1.9. Pentapeptide (11). LiOH monohydrate (21 mg, 0.50 mmol) was added to a solution of ester **2** (88 mg, 0.23 mmol) in THF–MeOH–H_2O (1 mL/0.5 mL/0.3 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 2 h. After most of the solvent was evaporated, the solution was acidified to pH 2 with 1 M HCl and extracted with EtOAc (3×5 mL). The organic extracts were combined and washed with brine (2×2 mL). Evaporation of the solvent gave the corresponding acid, which was dried under high vacuum for 4 h and then dissolved in 2 mL dry THF followed by addition of DIPEA (156 μL, 0.89 mmol) and 2,4,6-trichlorobenzoyl chloride (105 μL, 0.67 mmol). The reaction mixture was allowed to stir at room temperature for 3 h before it was concentrated to dryness under argon. The residue was dissolved in dry toluene (3 mL), and alcohol **3** (84 mg, 0.20 mmol) and DMAP (110 mg) were added. The mixture was stirred for 3 h at room temperature, diluted with EtOAc (20 mL), and washed with 10% citric acid (3×2 mL), 5% $NaHCO_3$ (3×2 mL), and brine (3×2 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–petroleum=1:5 to 1:2) to give the pentapeptide **11** as a white foam (146 mg, 94%): R_f=0.31 (EtOAc–petroleum ether=2:1); $[\alpha]_D^{20}$ −87.4 (*c* 0.1, MeOH); ^{1}H NMR ($CDCl_3$, one main rotamer) δ 0.49 (d, J=6.4 Hz, 3H, H-14), 0.72 (d, J=6.9 Hz, 3H, H-15), 0.94–0.98 (t like, overlapped, 3H, H-24), 1.41 (d, J=6.8 Hz, 3H, H-19), 1.45 (s, 9H, ^tBu), 1.46 (s, 9H, *Boc*), 1.59 (d, J=6.9 Hz, 3H, H-30), 1.62–1.70 (m, 2H, H-23), 2.14–2.26 (m, 1H, H-13), 2.64–2.70 (dd and dd like, overlapped, 2H, H-21a and H-21b), 2.82 (s, 3H, N-CH_3), 2.94 (s, 3H, N-CH_3), 2.95–2.99 (dd like, overlapped, 1H, H-3a), 3.35 (dd, J=15.1, 5.0 Hz, 1H, H-3b), 4.38–4.39 (m, 1H, H-22), 4.95 (d, J=10.6 Hz, 1H, H-12), 5.07 (q, J=6.9 Hz, 1H, H-18), 5.27–5.30 (m, overlapped, 2H, H-2 and H-29), 7.15–7.25 (m, 5H, Ar *H*), 7.58 (d, J=9.1 Hz, 1H, 22-N*H*), 8.00 (s, 1H, H-27); ^{13}C NMR ($CDCl_3$, one main rotamer) δ 10.6 (C-24), 16.4 (C-19), 17.7 (C-15), 19.0 (C-14), 21.5 (C-30), 27.2 (C-13), 27.3 (C-23), 28.0 ((CH_3)$_3$C), 28.3 ((CH_3)$_3$COC=O), 29.7 (N-CH_3), 32.2 (N-CH_3), 34.3 (C-3), 38.4 (C-21), 47.5 (C-22), 48.7 (C-29), 58.2 (C-12), 58.8 (C-2), 67.3 (C-18), 81.7 ((CH_3)$_3C$), 81.9 ((CH_3)$_3C$OC=O), 123.0 (C-27), 126.6 (C-7), 128.4 and 128.6 (C-5, C-6, C-8, and C-9), 137.1 (C-4), 149.8 (C-26), 154.9, 160.6, 169.6, 170.0, 170.8 and 170.9 (quat. C); HRESIMS calcd for $C_{39}H_{59}N_5O_9SNa$ $[M+Na]^+$ 796.3931, found 796.3948.

4.1.1.10. Obyanamide (1). To a solution of pentapeptide **11** (65.0 mg, 0.084 mmol) in CH_2Cl_2 (0.5 mL) was added TFA (1 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h. Residual TFA was removed by successive addition and evaporation of CH_2Cl_2 (3×2 mL). The residue was dissolved in dry THF (170 mL), and then HATU (160 mg, 0.42 mmol) and DIPEA (117 μL, 0.67 mmol) were added successively. The resultant

mixture was stirred for 3 days at room temperature and concentrated in vacuo. The residue was dissolved in EtOAc (20 mL) and washed with 10% citric acid (3×2 mL), 5% $NaHCO_3$ (3×2 mL), and brine (3×2 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column ($CHCl_3$–MeOH=80:1 to 40:1) followed by Pharmadex LH-20 ($CHCl_3$–MeOH=1:1) to give compound **1** (29.3 mg, 59%) as a colorless solid: R_f=0.46 ($CHCl_3$–MeOH=20:1); $[\alpha]_D^{28}$ −96.3 (*c* 0.06, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.59 (d, *J*=6.4 Hz, 3H, H-25), 0.87 (d, *J*=6.4 Hz, 3H, H-26), 1.01 (t, *J*=7.4 Hz, 3H, H-5), 1.45 (d, *J*=6.4 Hz, 3H, H-11), 1.49 (d, *J*=6.2 Hz, 3H, H-30), 1.69–1.76 (m, 1H, H-4a), 1.94–2.01 (m, 1H, H-4b), 2.26–2.32 (m, 1H, H-24), 2.73 (dd, *J*=16.5, 4.1 Hz, 1H, H-2a), 2.79–2.84 (m, overlapped, 2H, H-2b and H-14a), 3.07 (s, 3H, H-21), 3.20 (s, 3H, H-27), 3.44 (dd, *J*=13.5, 8.7 Hz, 1H, H-14b), 4.09–4.14 (m, 1H, H-3), 5.02 (d, *J*=10.5 Hz, 1H, H-23), 5.15 (dd, *J*=8.7, 5.9 Hz, 1H, H-13), 5.30–5.34 (m, 1H, H-10), 5.41 (q, *J*=6.4 Hz, 1H, H-29), 7.16 (t like, *J*=7.3, 6.8 Hz, 1H, H-18), 7.18–7.24 (d and dd like, overlapped, 4H, H-16, H-17, H-19, and H-20), 7.95 (s, 1H, H-8), 7.99 (d, *J*=7.3 Hz, 1H, 10-N*H*), 8.81 (d, *J*=9.1 Hz, 1H, 3-N*H*); ^{13}C NMR ($CDCl_3$) δ 11.4 (C-5), 16.3 (C-30), 18.8 (C-26), 18.9 (C-25), 23.4 (C-11), 27.6 (C-4), 27.7 (C-24), 29.0 (C-21), 30.1 (C-27), 36.5 (C-14), 37.8 (C-2), 46.6 (C-10), 47.9 (C-3), 57.3 (C-23), 61.0 (C-13), 67.4 (C-29), 122.2 (C-8), 127.0 (C-18), 128.8 (C-16 and C-20), 129.4 (C-17 and C-19), 136.4 (C-15), 150.5 (C-7), 160.4 (C-6), 167.3 (C-12), 168.9 (C-9), 170.0 (C-1), 170.4 (C-22), 171.9 (C-28); HRESIMS calcd for $C_{30}H_{42}N_5O_6S$ $[M+H]^+$ 600.2856, found 600.2848.

4.1.2. Synthesis of compound 1a.

4.1.2.1. (*R*)-2-*tert*-Butoxycarbonylaminobutyric acid. Obtained from the (*R*)-2-aminobutyric acid according to the preparation of **6**. A colorless oil: R_f=0.43 (*n*-hexane–EtOAc–HOAc=10:10:1); $[\alpha]_D^{29}$ +17.3 (*c* 1.02, MeOH); ^{1}H NMR (DMSO-d_6) δ 0.87 (t, *J*=7.3 Hz, 3H, H-4), 1.38 (s, 9H, ^{t}*Bu*), 1.52–1.59 (m, 1H, H-3a), 1.64–1.71 (m, 1H, H-3b), 3.76–3.80 (m, 1H, H-2), 7.05 (d, *J*=8.0 Hz, 1H, N*H*), 12.42 (s, 1H, CO_2H); HRESIMS calcd for $C_9H_{17}NO_4Na$ $[M+Na]^+$ 226.1055, found 226.1049.

4.1.2.2. (*R*)-Methyl 3-(*tert*-butoxycarbonylamino)-pentanoate. Obtained from the (*R*)-2-*tert*-butoxycarbonylaminobutyric acid according to the preparation of **7**. A colorless solid: R_f=0.38 (EtOAc–petroleum ether=1:3); $[\alpha]_D^{29}$ +17.4 (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, *J*=7.3 Hz, 3H, H-5), 1.44 (s, 9H, ^{t}*Bu*), 1.52–1.56 (m, 2H, H-4), 2.48–2.55 (m, 2H, H-2), 3.68 (s, 3H, CO_2CH_3), 3.81–3.86 (m, 1H, H-3), 4.91 (br, 1H, N*H*); ^{13}C NMR ($CDCl_3$) δ 10.6 (C-5), 27.5 (C-4), 28.3 ((CH_3)$_3$COC=O), 38.7 (C-2), 49.0 (C-3), 51.6 (CO_2CH_3), 79.2 ((CH_3)$_3$COC=O), 155.4 ((CH_3)$_3$COC=O), 172.2 (C-1); HRESIMS calcd for $C_{11}H_{21}NO_4Na$ $[M+Na]^+$ 254.1368, found 254.1357.

4.1.2.3. Boc-Ala(Thz)-(*R*)-Apa-OMe. Obtained from the Boc-(*R*)-Apa-OMe and Boc-Ala(Thz)-OEt **4** according to the preparation of **2**. A colorless oil: R_f=0.30 (EtOAc–petroleum ether=1:1); $[\alpha]_D^{29}$ −24.5 (*c* 0.28, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.98 (t, *J*=7.3 Hz, 3H, H-5), 1.47 (s, 9H, ^{t}*Bu*), 1.62 (d, *J*=6.6 Hz, 3H, H-11), 1.67–1.73 (m, 2H, H-4), 2.64 (dd, *J*=16.1, 5.9 Hz, 1H, H-2a), 2.67 (dd, *J*=15.8, 5.5 Hz, 1H, H-2b), 3.70 (s, 3H, CO_2CH_3), 4.32–4.38 (m, 1H, H-3), 5.08 (br, 1H, H-10), 5.14 (br, 1H, N*H*), 7.64 (d, *J*=8.8 Hz, 1H, N*H*), 8.00 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ 10.7 (C-5), 21.6 (C-11), 27.3 (C-4), 28.3 ((CH_3)$_3$COC=O), 38.4 (C-2), 47.5 (C-3), 48.7 (C-10), 51.7 (CO_2CH_3), 80.3 ((CH_3)$_3$COC=O), 123.0 (C-8), 149.9 (C-7), 155.0, 160.6, 171.9 and 174.0 (4 quat. C); HRESIMS calcd for $C_{17}H_{27}N_3O_5SNa$ $[M+Na]^+$ 408.1569, found 408.1586.

4.1.2.4. Linear pentapeptide Boc-Ala(Thz)-(*R*)-Apa-Lac-MeVal-MePhe-O^{t}Bu. Obtained from Boc-Ala(Thz)-(*R*)-Apa-OMe and compound **3** according to the preparation of **11**. A white foam: R_f=0.11 (EtOAc–petroleum ether=3:2); $[\alpha]_D^{27}$ −96.6 (*c* 0.15, MeOH); ^{1}H NMR ($CDCl_3$, one main rotamer) δ 0.77 (d, *J*=6.5 Hz, 3H, H-14), 0.91 (d, *J*=6.2 Hz, 3H, H-15), 0.95 (t, *J*=7.4 Hz, 3H, H-24), 1.15 (d, *J*=6.6 Hz, 3H, H-19), 1.46 (s, 9H, ^{t}*Bu*), 1.47 (s, 9H, *Boc*), 1.49 (d like, overlapped, 3H, H-30), 1.56–1.65 (m, 2H, H-23), 2.23–2.28 (m, 1H, H-13), 2.44 (s, 3H, N-CH_3), 2.64–2.76 (dd and dd like, overlapped, 2H, H-21a and H-21b), 2.79 (s, 3H, N-CH_3), 2.90–2.95 (m, 1H, H-3a), 3.38 (dd, *J*=15.0, 4.4 Hz, 1H, H-3b), 4.33–4.38 (m, 1H, H-22), 4.95 (d, *J*=10.6 Hz, 1H, H-12), 5.05–5.11 (m, overlapped, 2H, H-29 and H-18), 5.53 (dd, *J*=12.0, 4.0 Hz, 1H, H-2), 7.17–7.28 (m, 5H, Ar *H*), 7.82 (d, *J*=8.8 Hz, 1H, 22-N*H*), 8.00 (s, 1H, H-27); ^{13}C NMR ($CDCl_3$, one main rotamer) δ 10.7 (C-24), 16.1 (C-19), 17.8 (C-15), 19.7 (C-14), 21.5 (C-30), 26.8 (C-13), 27.2 (C-23), 28.0 ((CH_3)$_3$C), 28.3 ((CH_3)$_3$COC=O), 29.2 (N-CH_3), 31.4 (N-CH_3), 34.3 (C-3), 38.3 (C-21), 47.6 (C-22), 48.7 (C-29), 57.6 (C-12), 58.3 (C-2), 67.0 (C-18), 80.3 ((CH_3)$_3$COC=O), 81.9 ((CH_3)$_3$COC=O), 122.9 (C-27), 126.6 (C-7), 128.4 and 128.8 (C-5, C-6, C-8, and C-9), 137.2 (C-4), 150.1 (C-26), 154.9, 160.6, 169.6, 170.0, 170.8 (quat. C); HRESIMS calcd for $C_{39}H_{59}N_5O_9SNa$ $[M+Na]^+$ 796.3931, found 796.3962.

4.1.2.5. Cyclic depsipeptide (1a). The same preparation as of compound **1**. A colorless solid: R_f=0.45 ($CHCl_3$–MeOH=20:1); $[\alpha]_D^{27}$ +22.2 (*c* 0.07, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.50 (d, *J*=6.4 Hz, 3H, H-25), 0.86 (d, *J*=6.9 Hz, 3H, H-26), 1.07 (t, *J*=7.3 Hz, 3H, H-5), 1.24 (d, *J*=6.8 Hz, 3H, H-30), 1.43 (d, *J*=6.9 Hz, 3H, H-11), 1.61–1.69 (m, 2H, H-4), 2.28–2.34 (m, 1H, H-24), 2.41 (dd, *J*=11.9, 2.8 Hz, 1H, H-2a), 2.79 (dd, *J*=11.9, 5.0 Hz, 1H, H-2b), 2.90 (dd, *J*=13.7, 6.4 Hz, 1H, H-14a), 3.13 (s, 3H, H-27), 3.14 (s, 3H, H-21), 3.28 (dd, *J*=13.7, 8.2 Hz, 1H, H-14b), 4.40–4.46 (m, 1H, H-3), 5.04–5.07 (m, overlapped, 1H, H-10), 5.07 (d, *J*=10.1 Hz, 1H, H-23), 5.20 (q, *J*=6.8 Hz, 1H, H-29), 5.45 (t like, *J*=8.2, 6.9 Hz, 1H, H-13), 7.08 (t, *J*=7.3 Hz, 1H, H-18), 7.17 (d, *J*=7.8 Hz, 1H, H-16 and H-20), 7.19–7.22 (dd like, 2H, H-17 and H-19), 7.94 (d, *J*=6.0 Hz, 1H, 10-N*H*), 8.02 (s, 1H, H-8), 9.14 (d, *J*=10.1 Hz, 1H, 3-N*H*); ^{13}C NMR ($CDCl_3$) δ 11.2 (C-5), 15.8 (C-30), 18.5 (C-25 and C-26), 24.1 (C-11), 25.9 (C-4), 27.4 (C-24), 29.0 (C-21), 29.8 (C-27), 37.0 (C-14), 38.8 (C-2), 47.3 (C-3), 48.0 (C-10), 57.8 (C-23), 60.7 (C-13), 67.5 (C-29), 123.1 (C-8), 127.0 (C-18), 128.7 (C-16 and C-20), 129.1 (C-17 and C-19), 136.3 (C-15), 148.6 (C-7), 160.4 (C-6), 168.0 (C-12), 169.5 (C-9), 169.9 (C-22), 170.3 (C-1), 172.9 (C-28); HRESIMS calcd for $C_{30}H_{42}N_5O_6S$ $[M+H]^+$ 600.2856, found 600.2875.

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References and notes

1. Burjar, A. M.; Banaigs, B.; Abou-Mansour, E.; Burgess, J. G.; Wright, P. C. *Tetrahedron* **2001**, *57*, 9347–9377.
2. Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 29–31.
3. Tan, T.; Sitachitta, N.; Gerwick, W. H. *J. Nat. Prod.* **2003**, *66*, 764–771.
4. Luesch, H.; Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 996–1000.
5. Zhang, W.; Song, N.; Li, Z.-Z.; Li, Y.-X. *Chin. Chem. Lett.* **2006**, *17*, 285–288.
6. (a) Plucinska, K.; Liberek, B. *Tetrahedron* **1987**, *43*, 3509–3517; (b) Guichard, G.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 187–206; (c) Wiberg, K. B.; Hutton, T. W. *J. Am. Chem. Soc.* **1956**, *78*, 1640–1645.
7. Kigoshi, H.; Yamada, S. *Tetrahedron* **1999**, *55*, 12301–12308.
8. McDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 1915–1918.
9. Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962–1965.
10. Li, P.; Xu, J.-C. *Tetrahedron* **2000**, *56*, 8119–8131.
11. Hruby, V. J.; Ehler, K. W. *J. Org. Chem.* **1970**, *35*, 1690.
12. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.
13. The natural product exhibited cytotoxicity against KB and LoVo cells with IC_{50} values of 1 and 5 μM, respectively.
14. Bischofberger, N.; Waldmann, H.; Saito, T.; Simon, S. E.; Lees, W.; Bednarski, M. D.; Whitesides, G. M. *J. Org. Chem.* **1988**, *53*, 3457–3465; this compound was reported as an oil whereas in our hands it was a white crystalline solid.