Research Article

Journal of PeptideScience

Received: 14 December 2010 Revised: 17 January 2011 Accepted: 18 January 2011 Published online in Wiley Online Library: 14 April 2011

(wileyonlinelibrary.com) DOI 10.1002/psc.1361

Synthesis and biological evaluation of analogues of the marine cyclic depsipeptide obyanamide

Wei Zhang, Ning Ding and Yingxia Li*

On the basis of the total synthesis of obyanamide, 20 analogues of this marine cyclic depsipeptide have been synthesized by (i) preparation of the tripeptide fragments in the western hemisphere using Z/OtBu protocol; (ii) preparation of the dipeptide fragments in the eastern hemisphere using Boc/OMe protocol; and (iii) fragments coupling, removal of protecting groups (Boc and OtBu, in one pot), and macrocyclizaion in the last step. The cytotoxic test showed that three synthetic compounds exhibited moderate activities against HL-60, KB, LOVO, and A549 cell lines. According to the results, the β-amino acid residue was found to play a critical role in the biological activities. Additionally, the ester bond along with the Ala(Thz) moiety was also essential for biological activities. However, it seems too early to draw a conclusion that the *N*-methylation of Val/Phe can lead to higher or lower cytotoxic activities. Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: obyanamide; synthesis; cyclic depsipeptides; analogues; cytotoxicity

Introduction

Marine cyclic (depsi)peptides are a growing research area in the past two decades due to their novel structures and remarkable biological activities [1–3]. Many of them have become the focus of recent synthetic endeavors [4–6]. Obyanamide is a cytotoxic cyclic depsipeptide that was isolated by Moore and co-workers in 2002 [7]. One year later, several structural analogues have also been reported by the same [8] and other [9] groups. Interestingly, most of them were found to have moderate activities against several cancer cell lines. Structure–activity relationship studies on these compounds might help to find more efficient anti-cancer agents. In ongoing efforts on this target, we have reported the total synthesis and revised structure of obyanamide [10,11] (Figure 1). Here we report the results of the synthesis of its analogues along with their biological activities.

Results and Discussions

On the basis of the total synthesis of the natural product, we have established a rapid and efficient route to prepare these cyclic depsipeptides. More specifically, this route includes (i) preparation of the tripeptide fragments in the western hemisphere using Z/OtBu protocol; (ii) preparation of the dipeptide fragments in the eastern hemisphere using Boc/OMe protocol; and (iii) fragments coupling, removal of protecting groups (Boc and OtBu, in one pot), and macrocyclizaion in the last step.

As illustrated in Scheme 1, the synthesis of tripeptide fragments started from alanine or phenylalanine derivatives (**1a–c**). The Z-protected amino acids were hydrogenated to release the free amine compounds which were coupled with Z-Val-OH or Z-MeVal-OH in the following step, to produce the corresponding dipeptides **2a–e** in 70–97% yields. These five dipeptides were used for the next cycle, introducing a new amino/hydroxy acid to give six tripeptide fragments. Notably, (*S*)-lactic acid was used directly to generate the corresponding tripeptides **3e** and **3f** in high yields during the second cycle of coupling. However, when the *N*-terminus of the dipeptides was methylated (**2a, 2d**, and **2e**), it was necessary to mask the hydroxy group of (*S*)-lactic acid before the coupling steps to avoid nucleophilic competition between the amino group of the dipeptides and the free α-hydroxy group of the acid. Thus, after two cycles of coupling and removals of the *N*/*O*-protecting groups in the final step (omitted for compound **3e** and **3f**, see Table 1), six tripeptide fragments were obtained smoothly.

In order to construct the eastern hemispheric dipeptides **7a–j**, in first instance four thiazole/oxazole containing amino acids (**5a–d**) and four β-amino acids (**6b–e**) were prepared from commercially available reagents [12–15]. After Boc group removals and EDC/HOAt intermediate couplings, ten dipeptides building blocks were obtained smoothly in satisfactory yields (Scheme 2).

With six tripeptides (**4a–d, 3e–f**) and ten dipeptides fragments (**7a–j**) in hand, we could now proceed toward the cyclic (depsi)peptides (Scheme 3 and Table 2). Previous studies showed that Yamaguchi's procedure [16] was superior to the standard esterification method (EDC/DMAP). Thus 19 linear pentapeptides were obtained smoothly by this strategy, while for compounds **8l** and **8m** standard peptide coupling conditions (EDC/HOAt) gave satisfactory results. Finally, all these linear pentapeptides were treated with TFA in DCM to remove the OtBu and *N*-Boc groups in one pot and cyclized to afford the desired cyclic (depsi)peptides (**9a–u**).

* *Correspondence to: Yingxia Li, Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Zhangheng Rd. 826, Shanghai 201203, China. E-mail: liyx417@fudan.edu.cn*

Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai 201203, China

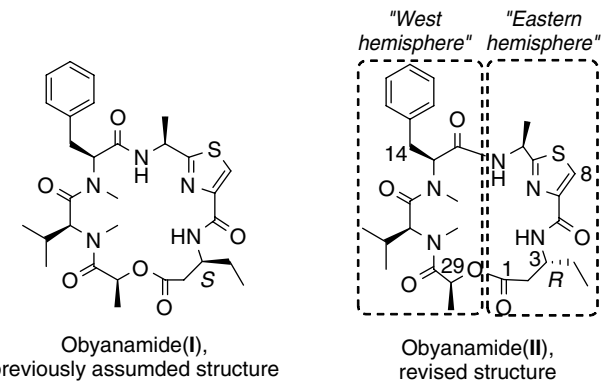


Figure 1. The structure of obyanamide.

Table 1. Yields of tripeptides **4a**–**d** and **3e**–**f**

	R_1	R_2	R_3	W_1	W_2	Yields (%) of first cycle	Yields (%) of second cycle	Yields (%) of *N*/*O*-PG removal
4a	Ph	Me	Me	OBn	OH	78	87	98
4b	Ph	H	Me	OBn	OH	89	100	100
4c	Ph	Me	Me	NHZ	NH_2	78	82	100
4d	H	Me	Me	OBn	OH	70	78	100
3e	Ph	Me	H	OH	OH	97	78	–
3f	Ph	H	H	OH	OH	92	95	–

The cytotoxic activities of the final compounds were determined by the MTT or sulforhodamine B (SRB) assays, using the HL-60, P388, BEL-7402, KB, LOVO, and A549 cell lines. Among the 21 cyclic (depsi)peptides, 3 compounds (**9b, 9d** and **9p**) exhibited moderate cytotoxic activities (Table 3). According to the above results, the β-amino acid residue especially its configuration and side chain may play a critical role in the biological effect. Converting the *R*-configuration (**9b**) to *S*-(**9a**) led to inactive derivative. While when the side chain of this residue was shortened at the same time (**9b** vs. **9d**), the activities decreased dramatically. The ester bond along with the Ala(Thz) moiety was also essential for biological activities. Any changes at these positions led to inactive derivatives (**9b** vs. **9m;9b** vs. **9i, 9j**, and **9k**). However, it seems too early to draw a conclusion that the *N*-methylation of Val/Phe can lead to higher or lower cytotoxic activities (**9p** vs. **9n** and **9o**).

Experimental Section

General Information

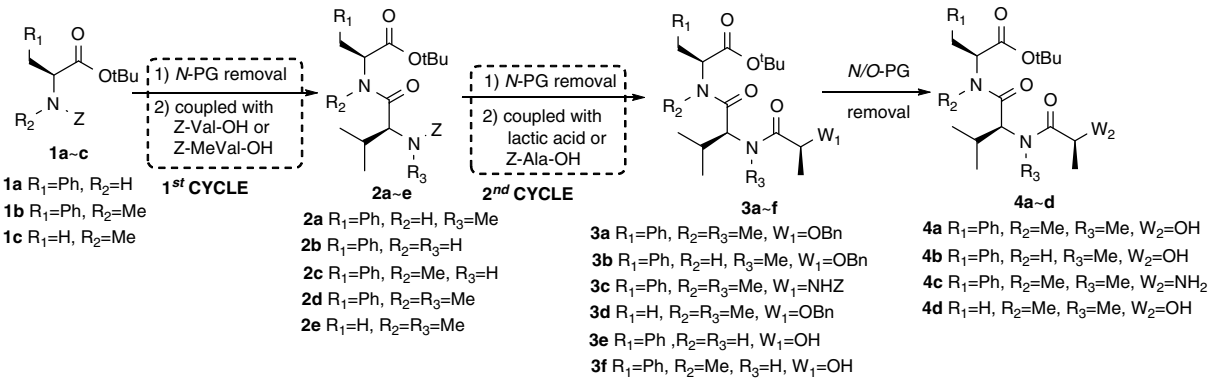
Solvents were purified by standard methods. Amino acids and coupling reagents were purchased from GL Biochem (Shanghai) Ltd. TLC was carried out on Merck 60 F_{254} silica gel plates and visualized by UV irradiation or by staining with iodine absorbed on silica gel, ninhydrin solution, or with aqueous 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. Mass spectra were obtained on a Q-Tof Ultima Global mass spectrometer. The cytotoxic effects were examined using SRB assay against A549, LOVO, KB, BEL-7402, and MTT tetrazolium dye assay against P388 and HL-60 cell lines.

*Synthesis of dipeptide **2a**–**e**: general procedure (first cycle)*

The amino acid derivatives **1a–c** were hydrogenated over 10% Pd/C in EtOAc until all the starting material disappeared (2–4 h). The suspensions were filtered through a pad of celite, washed with EtOAc, and concentrated *in vacuo*. The amine compounds were dried under high vacuum for 2 h and dissolved in dry DCM. After 1 equiv. of Z-Val-OH or Z-MeVal-OH was added, the mixtures were cooled to 0 °C. Then 15 min later, EDC (1.2 equiv.), HOAt (1.2 equiv.), and DIPEA (2 equiv.) were added, respectively. The mixtures were stirred at 0 °C for 2 h and room temperature overnight. After dilution with EtOAc, the mixtures were washed with 10% citric acid, 5% $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residues were purified by column chromatography (EtOAc–petroleum ether) to give the dipeptides **2a–e**.

*N^{α}-Z-N^{α}-Me-L-Val-L-Phe-OtBu (**2a**)*

Yield 89%. Colorless oil. $R_f = 0.48$ (EtOAc : Petroleum ether = 1 : 3); ^{1}H NMR ($CDCl_3$, * as rotamer) δ: 0.81 and 0.83* (d, $J = 6.6$ Hz, 3H), 0.88 and 0.92* (d, $J = 6.6$ Hz, 3H), 1.42 and 1.44* (s, 9H), 2.20–2.24 (m, 1H), 2.71 and 2.80* (s, 3H), 2.91 (dd, $J = 7.7$, 13.9 Hz, 1H), 3.10 (dd, $J = 5.8$, 14.3 Hz, 1H), 3.96 and 4.09* (d, $J = 10.6$ Hz, 1H), 4.69–4.75 (m, 1 H), 5.03–5.17 (m, 2 H), 6.02 and 6.46* (each d, $J = 7.3$ Hz, 1H), 7.00–7.39 (m, 10H); ^{13}C NMR ($CDCl_3$) δ: 18.5, 19.6, 25.9, 27.9, 29.5, 38.1, 53.2, 64.9, 67.5, 82.1, 126.8, 126.9, 128.1, 128.3, 128.6, 129.3, 136.2, 136.5, 157.3, 169.4, 170.3; ESI-MS (*m/z*) calcd. $[M+Na]^+ = 491.3$, $[M+K]^+ = 507.2$, found 491.2, 507.2.



Scheme 1. (a) Conditions of PG removals: Pd/C, H_2, EtOAc, rt, 2–4 h; (b) conditions of couplings: EDC/HOAt/DCM, 0 °C to rt, 6–14 h.

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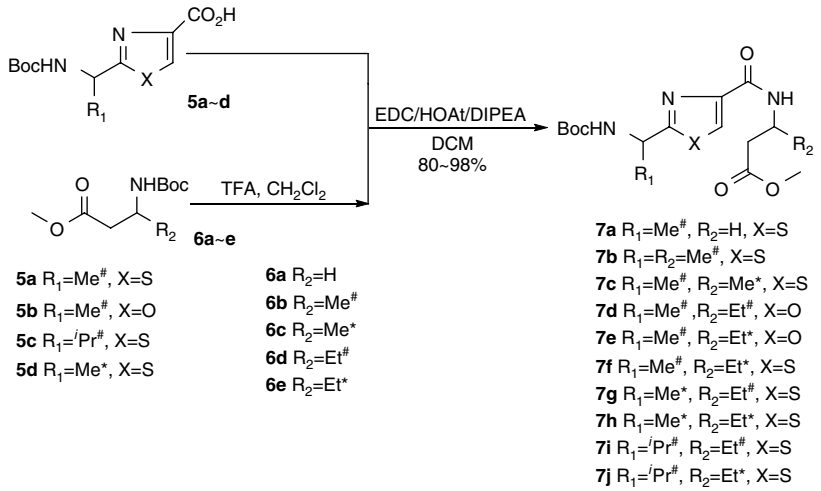
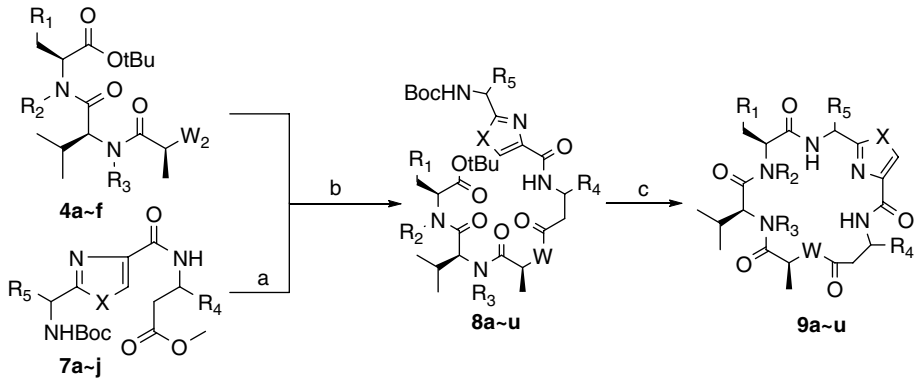


Scheme 2. Synthesis of dipeptide fragments **7a**–**j**. *: *R* configuration; #: *S* configuration.



Scheme 3. Reagents and conditions: (a) LiOH, THF/MeOH/H_2O; (b) Yamaguchi esterification or EDC/HOAt/DIPEA (for **8l** and **8m**); (c) (i) TFA, DCM; (ii) HATU/DIPEA, THF.

*N^{α}-Z-L-Val-L-Phe-OtBu (**2b**)*

Yield 92%. Pale yellow solid. R_f = 0.47 (EtOAc : Petroleum ether = 1 : 2); $[\alpha]^{20}_D$ = −29.5 (*c* = 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ: 0.88 (d, *J* = 7.0 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 1.39 (s, 9H), 2.06–2.12 (m, 1H), 3.07 (d, *J* = 6.4 Hz, 2H), 4.00–4.02 (m, 1H), 4.73–4.77 (m, 1H), 5.09 (d, *J* = 12.4 Hz, 1H), 5.12 (d, *J* = 12.1 Hz, 1H), 5.38 (d, *J* = 8.0 Hz, 1H), 6.36 (br, 1H), 7.14–7.37 (m, 10H); ^{13}C NMR ($CDCl_3$) δ: 17.6, 19.1, 27.9, 31.1, 38.0, 53.5, 60.2, 67.0, 82.4, 127.0, 128.0, 128.1, 128.4, 128.5, 129.4, 135.9, 136.2, 156.3, 170.2, 170.6. ESI-MS (*m/z*) calcd. $[M+H]^+$ = 455.3, $[M+Na]^+$ = 477.2, $[M+K]^+$ = 493.2, found 455.2, 477.2, 493.2.

*N^{α}-Z-L-Val-N^{α}-Me-L-Phe-OtBu (**2c**)*

Yield 97%. Colorless oil. R_f = 0.38 (EtOAc : Petroleum ether = 1 : 3); ^{1}H NMR ($CDCl_3$, one main rotamer of four) δ: 0.90 (d, *J* = 6.6 Hz, 3H), 0.97 (d, *J* = 7.0 Hz, 3H), 1.42 (s, 9H), 1.91–1.97 (m, 1H), 2.96 (s, 3H), 2.91–2.93 (m, 1H), 3.32 (dd, *J* = 5.8, 14.6 Hz, 1H), 4.38 (dd, *J* = 6.2, 9.1 Hz, 1H), 5.01–5.12 (m, 2H), 5.27 (dd, *J* = 5.8, 10.3 Hz, 1H), 5.31 (d, *J* = 9.2 Hz, 1H), 7.12–7.38 (m, 10H); ^{13}C NMR ($CDCl_3$, one main rotamer of four) δ: 17.3, 19.4, 27.9, 31.2, 32.6, 34.5, 55.6, 58.9, 66.7, 81.9, 126.6, 127.9, 128.1, 128.5, 128.9, 129.2, 136.4, 137.0, 156.2, 169.6, 172.3. ESI-MS (*m/z*) calcd. $[M+Na]^+$ = 491.3, $[M+K]^+$ = 507.2, found 491.2, 507.2.

*N^{α}-Z-N^{α}-Me-L-Val-N^{α}-Me-L-Phe-OtBu (**2d**)*

See Ref. 11.

*N^{α}-Z-N^{α}-Me-L-Val-N^{α}-Me-L-Ala-OtBu (**2e**)*

Yield 70%. Colorless oil. R_f = 0.31 (EtOAc : Petroleum ether = 1 : 3); ^{1}H NMR ($CDCl_3$, one main rotamer of four) δ: 0.89 (d, *J* = 6.4 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H), 1.32 (d, *J* = 7.3 Hz, 3H), 1.43 (s, 9H), 2.31–2.37 (m, 1H), 2.92 (s, 3H), 3.03 (s, 3H), 4.74 (d, *J* = 10.6 Hz, 1H), 4.98–5.27 (m, overlapped, 3H), 7.30–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, one main rotamer of four) δ: 14.3, 18.4, 19.4, 27.7, 28.0, 29.1, 31.6, 53.2, 60.0, 67.4, 81.5, 127.6, 128.0, 128.5, 136.7, 156.0, 156.5, 157.0.

*Synthesis of tripeptide **3**: general procedure (second cycle)*

The second cycle was performed in similar mode as the first cycle. The dipeptides **2a**–**e** were hydrogenated and coupled with Z-Ala-OH or L-lactic acid (Lac) to give the corresponding tripeptides **3a**–**f**.

*BnO-L-Lac-N^{α}-Me-L-Val-N^{α}-Me-L-Phe-OtBu (**3a**)*

See Ref. 11.

Table 2. Yields of linear pentapeptides **8a–u** and cyclic (depsi)peptides **9a–u**

	R_1	R_2	R_3	W	R_4	X	R_5	Yields of **8** (%)	Yields of **9** (%)
a	Ph	Me	Me	O	Et#	S	Me#	94	59
b	Ph	Me	Me	O	Et*	S	Me#	98	53
c	Ph	Me	Me	O	H	S	Me#	91	36
d	Ph	Me	Me	O	Me#	S	Me#	54	45
e	Ph	Me	Me	O	Et#	O	Me#	82	35
f	Ph	Me	Me	O	Et#	S	Me*	95	41
g	Ph	Me	Me	O	Et#	S	^{i}Pr#	87	43
h	Ph	Me	Me	O	Me*	S	Me#	96	67
i	Ph	Me	Me	O	Et*	O	Me#	83	67
j	Ph	Me	Me	O	Et*	S	^{i}Pr#	80	50
k	Ph	Me	Me	O	Et*	S	Me*	98	52
l	Ph	Me	Me	NH	Et#	S	Me#	100	40
m	Ph	Me	Me	NH	Et*	S	Me#	77	71
n	Ph	H	Me	O	Et*	S	Me#	87	74
o	Ph	Me	H	O	Et*	S	Me#	92	88
p	Ph	H	H	O	Et*	S	Me#	100	94
q	H	Me	Me	O	Et*	S	Me#	100	24
r	Ph	H	Me	O	Et#	S	Me#	77	64
s	Ph	Me	H	O	Et#	S	Me#	84	75
t	Ph	H	H	O	Et#	S	Me#	72	66
u	H	Me	Me	O	Et#	S	Me#	92	47

* *R* configuration,
# *S* configuration

Table 3. IC_{50} (μM) values of **9b**, **9d**, and **9p**[a]

	HL-60	KB	LOVO	A-549
9b	6.8	6.7	19	0.78
9d	42	16	43	Nd
9p	Nd	Nd	Nd	0.64

[a] All the target compounds (**9a–9u**) were screened for cytotoxicities. Other compounds not listed in the table were inactive in the test (IC_{50} > 100 μM).
Nd, not detected.

*BnO-L-Lac-N$^{\alpha}$-Me-L-Val-L-Phe-OtBu (**3b**)*

Yield 99%. Colorless oil. R_f = 0.24 (EtOAc : Petroleum ether = 1 : 3); ^{1}H NMR ($CDCl_3$, one rotamer of two) δ: 0.83 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H), 1.33 (d, J = 6.6 Hz, 3H), 1.40 (s, 9H), 2.25–2.31 (m, 1H), 2.79 (s, 3H), 2.91–2.94 (dd like, overlapped, 1H), 3.09 (dd, J = 5.5, 13.9 Hz, 1H), 4.24 (q, J = 7.0 Hz, 1H), 4.32 (d, J = 11.7 Hz, 1H), 4.53 (d, J = 11.7 Hz, 1H), 4.58 (d, J = 11.3 Hz, 1H), 5.71–5.75 (m, 1H), 6.51 (br, 1H), 7.13–7.36 (m, 10H); ^{13}C NMR ($CDCl_3$, one rotamer of two) δ: 16.9, 18.5, 19.6, 25.5, 27.9, 30.2, 38.1, 53.2, 63.0, 70.3, 72.6, 82.1, 126.9, 127.8, 128.4, 128.5, 129.1, 129.8, 136.3, 137.5, 169.4, 170.2, 172.9.

*N$^{\alpha}$-Z-L-Ala-N$^{\alpha}$-Me-L-Val-N$^{\alpha}$-Me-L-Phe-OtBu (**3c**)*

Yield 82%. Colorless oil. R_f = 0.21 (EtOAc : Petroleum ether = 1 : 2); $[\alpha]^{20}_D$ = −99.2 (c = 0.4, MeOH); ^{1}H NMR ($CDCl_3$) δ: 0.50 (d, J = 6.4 Hz, 3H), 0.72 (d, J = 6.4 Hz, 3H), 1.30 (d, J = 6.8 Hz, 3H), 1.44 (s, 9H), 2.17–2.21 (m, 1H), 2.82 (s, 3H), 2.94 (s, 3H), 3.33 (dd, J = 5.0,13.9 Hz, 1H), 3.38 (dd, J = 4.6, 14.0 Hz, 1H), 4.64–4.68 (m, 1H), 4.85 (d, J = 11.0 Hz, 1H), 5.07–5.09 (dd like, overlapped, 2H), 5.29 (dd, J = 4.6, 11.5 Hz, 1H), 5.68 (d, J = 8.2 Hz, 1H), 7.16–7.35 (m, 10H); ^{13}C NMR ($CDCl_3$) δ: 18.0, 18.9, 19.0, 26.7, 28.0, 30.1, 32.1, 34.4, 47.2, 58.4, 58.8, 66.8, 81.8, 126.7, 127.9, 128.1, 128.5, 128.7, 128.8, 136.4, 137.1, 155.5, 169.6, 172.9; ESI-MS (m/z) calcd. $[M+Na]^+$ = 576.3, $[M+K]^+$ = 592.3, found 576.2, 592.2.

*BnO-L-Lac-N$^{\alpha}$-Me-L-Val-N$^{\alpha}$-Me-L-Ala-OtBu (**3d**)*

Yield 78%. Colorless oil. R_f = 0.60 (EtOAc : Petroleum ether = 1 : 1); ^{1}H NMR ($CDCl_3$, one main rotamer of four) δ: 0.90 (d, J = 6.6 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 1.30 (d, J = 7.3 Hz, 3H), 1.41 (d like, overlapped, 3H), 1.44 (s, 9H), 2.34–2.39 (m, 1H), 3.00 (s, 3H), 3.04 (s, 3H), 4.33–4.37 (m, 1H), 4.38 (d, J = 11.3 Hz, 1H), 4.59 (d, J = 11.3 Hz, 1H), 5.02 (q, J = 7.3 Hz, 1H), 5.23 (d, J = 11.0 Hz, 1H), 7.27–7.34 (m, 5H); ^{13}C NMR ($CDCl_3$, one main rotamer of four) δ: 14.3, 17.5, 18.4, 19.4, 27.1, 28.0, 29.7, 31.8, 35.4, 58.1, 70.9, 73.3, 81.5, 127.8, 128.0, 128.5, 137.5, 170.6, 171.8, 172.5

*HO-L-Lac-L-Val-L-Phe-OtBu (**3e**)*

Yield 95%. White foam. R_f = 0.24 (EtOAc : Petroleum ether = 1 : 1); $[\alpha]^{20}_D$ = −43.4 (c = 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ: 0.78 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 7.0 Hz, 3H), 1.22 (d, J = 6.6 Hz, 3H), 1.30 (s, 9H), 1.92–1.98 (m, 1H), 2.90 (dd, J = 8.4, 13.4 Hz, 1H), 2.96 (dd, J = 7.0, 13.9 Hz, 1H), 3.94–3.97 (m, 1H), 4.25 (dd, J = 6.2, 9.5 Hz, 1H), 4.35 (m, 1H), 5.69 (d, J = 5.1 Hz, 1H), 7.19–7.28 (m, 5H), 7.39 (d, J = 9.5 Hz, 1H), 8.52 (d, J = 7.7 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ: 17.6, 19.1, 21.3, 27.5, 31.5, 36.8, 54.2, 55.9, 67.3, 80.6, 126.5, 128.2, 129.1, 137.1, 170.4, 170.6, 173.9; ESI-MS (m/z) calcd. $[M+Na]^+$ = 415.2, $[M+K]^+$ = 431.2, found 415.2, 431.2.

*HO-L-Lac-L-Val-N$^{\alpha}$-Me-L-Phe-OtBu (**3f**)*

Yield 78%. Colorless oil. R_f = 0.28 (EtOAc : Petroleum ether = 1 : 1); ^{1}H NMR ($CDCl_3$) δ: 0.90 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.9 Hz, 3H), 1.37 (d, J = 6.9 Hz, 3H), 1.44 (s, 9H), 1.98–2.04 (m, 1H), 2.93 (dd, J = 5.5, 14.2 Hz, 1H), 3.32 (dd, J = 5.5, 14.7 Hz, 1H), 3.57 (br, 1H), 4.10–4.14 (m, 1H), 4.64 (dd, J = 6.9, 9.2 Hz, 1H), 5.33 (dd, J = 5.5, 10.1 Hz, 1H), 6.92 (d, J = 9.2 Hz, 1H), 7.15–7.31 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 17.6, 19.4, 21.2, 28.0, 31.1, 32.6, 34.5, 53.3, 58.8, 68.3, 82.1, 126.4, 128.4, 129.1, 137.1, 169.5, 172.5, 174.3.

*Synthesis of tripeptide fragments **4a–d**: general procedure*

Ten percent Pd/C was added to the solutions of tripeptides **3a–d** in EtOAc. The reaction mixtures were purged with hydrogen three times and stirred for 2 h at room temperature. The suspensions were filtered through a pad of celite, washed with EtOAc, and concentrated *in vacuo* to give **4a–d** in nearly quantitative yields.

*HO-L-Lac-N$^{\alpha}$-Me-L-Val-N$^{\alpha}$-Me-L-Phe-OtBu (**4a**)*

See Ref. 11.

*HO-L-Lac-N$^{\alpha}$-Me-L-Val-L-Phe-OtBu (**4b**)*

Yield 100%. Colorless oil. R_f = 0.32 (EtOAc : Petroleum ether = 1 : 1); ^{1}H NMR ($CDCl_3$) δ: 0.82 (d, J = 7.0 Hz, 3H), 0.96 (d, J = 6.2 Hz, 3H), 1.18 (d, J = 6.6 Hz, 3H), 1.39 (s, 9H), 2.25–2.34 (m, 1H), 2.87 (s, 3H), 2.96 (dd, J = 7.7, 13.9 Hz, 1H), 3.06 (dd, J = 6.2, 14.3 Hz, 1H), 3.82 (d, J = 8.1 Hz, 1H), 4.41–4.17 (m, 1H), 4.65 (d, J = 11.0 Hz, 1H), 4.67–4.76 (m, 1H), 6.85 (d, J = 7.3 Hz, 1H), 7.15–7.28 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 18.4, 19.3, 20.5, 25.7, 27.7, 29.9, 37.9, 53.4, 63.1, 64.4, 82.1, 126.8, 128.3, 129.1, 136.1, 169.2, 170.3, 175.9.

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*H-L-Ala-N$^{\alpha}$-Me-L-Val-N$^{\alpha}$-Me-L-Phe-OtBu (**4c**)*

This intermediate was directly subjected to next step without structural characterization.

*HO-L-Lac-N$^{\alpha}$-Me-L-Val-N$^{\alpha}$-Me-L-Ala-OtBu (**4d**)*

Yield 99%. Colorless oil. R_f = 0.24 (EtOAc : Petroleum ether = 1 : 1); ^{1}H NMR ($CDCl_3$, one main rotamer of four) δ: 0.88 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 1.32 (d, J = 7.0 Hz, 6H), 1.44 (s, 9H), 2.36–2.41 (m, 1H), 2.95 (s, 3H), 3.00 (s, 3H), 3.70 (d, J = 7.7 Hz, 1H), 4.48–4.54 (m, 1H), 5.00 (q, J = 7.0 Hz, 1H), 5.17 (d, J = 11.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, one main rotamer of four) δ: 14.3, 18.3, 19.3, 21.0, 27.0, 27.9, 29.6, 31.7, 53.6, 58.6, 64.5, 81.6, 169.9, 170.4, 175.9.

*Synthesis of dipeptide fragments **7a–j**: general procedure*

TFA was added to the solutions of **6a–e** in CH_2Cl_2 at 0 °C. The reaction mixtures were warmed to room temperature and stirred for 2 h. The solvent was evaporated and the oily residues were dissolved twice in CH_2Cl_2 with evaporation each time to give TFA salts, which were dissolved in dry DCM. After 1 equiv. of **5a–d** was added, the mixtures were cooled to 0 °C. Then 15 min later, EDC (1.2 equiv.), HOAt (1.2 equiv.), and DIPEA (2 equiv.) were added respectively. The mixtures were stirred at 0 °C for 2 h and room temperature overnight. After dilution with EtOAc, the mixtures were washed with 10% citric acid, 5% $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residues were purified by column chromatography (EtOAc–petroleum ether) to give dipeptides **7a–j** in 80-98% yield.

7a

Yield 84%. Colorless oil. R_f = 0.24 (EtOAc : Petroleum ether = 1 : 1); $[\alpha]^{20}_D$ = −24.9 (c = 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ: 1.47 (s, 9H), 1.60 (d, J = 6.9 Hz, 3H), 2.67 (t, J = 6.4 Hz, 3H), 3.69–3.73 (m, 2H), 3.74 (s, 3H), 5.02–5.07 (m, 1H), 5.16 (br, 1H), 7.73 (t, J = 5.5 Hz, 1H), 8.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 21.6, 28.3, 34.0, 34.7, 48.7, 51.8, 80.3, 123.0, 149.7, 154.9, 161.1, 172.7, 174.5; ESI-MS (m/z) calcd. $[M+H]^+$ = 358.2, $[M+Na]^+$ = 380.1, $[M+K]^+$ = 396.1, found 358.7, 380.6, 396.6.

7b

Yield 82%. Colorless oil. R_f = 0.26 (EtOAc : Petroleum ether = 1 : 1); $[\alpha]^{20}_D$ = −7.3 (c = 1.1, MeOH); ^{1}H NMR ($CDCl_3$) δ: 1.35 (d, J = 7.0 Hz, 3H), 1.47 (s, 9H), 1.61 (d, J = 7.0 Hz, 3H), 2.61 (dd, J = 5.9, 15.4 Hz, 1H), 2.68 (dd, J = 5.5, 15.7 Hz, 1H), 3.71 (s, 3H), 4.51–4.56 (m, 1H), 5.07–5.09 (m, 1H), 5.15 (br, 1H), 7.65 (d, J = 8.5 Hz, 1H), 8.00 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 20.2, 21.6, 28.3, 40.2, 42.1, 48.7, 51.7, 80.3, 123.1, 149.8, 154.9, 160.3, 171.8, 174.1; ESI-MS (m/z) calcd. $[M+H]^+$ = 372.2, $[M+Na]^+$ = 394.1, $[M+K]^+$ = 410.1, found 372.7, 394.6, 410.6.

7c

Yield 85%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.35 (d, J = 6.8 Hz, 3H), 1.47 (s, 9H), 1.61 (d, J = 6.4 Hz, 3H), 2.61 (dd, J = 15.6, 6.4 Hz, 1H), 2.69 (dd, J = 15.6, 5.0 Hz, 1H), 3.71 (s, 3H), 4.52–4.56 (m, 1H), 5.08 (br, 1H), 5.14 (br, 1H), 7.67 (d, J = 7.8 Hz, 1H), 8.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 20.2, 21.6, 28.3, 40.1, 42.1, 48.7, 51.7, 80.4, 123.1, 149.8, 154.9, 160.3, 171.8, 174.1.

7d

Yield 92%. Colorless oil. R_f = 0.24 (EtOAc : Petroleum ether = 1 : 1); ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, J = 7.4 Hz, 3H), 1.46 (s, 9H), 1.54 (d, J = 6.8 Hz, 3H), 1.65–1.70 (m, 2H), 2.63 (d, J = 5.5 Hz, 2H), 3.70 (s, 3H), 4.31–4.37 (m, 1H), 4.97–4.99 (m, 1H), 5.11 (br, 1H), 7.23 (d, J = 9.2 Hz, 1H), 8.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 10.6, 20.0, 27.2, 28.3, 38.4, 44.7, 47.2, 51.7, 80.3, 136.0, 141.1, 155.0, 160.0, 164.5, 171.9.

7e

Yield 82%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, J = 7.3 Hz, 3H), 1.47 (s, 9H), 1.54 (d, J = 6.8 Hz, 3H), 1.65–1.70 (m, 2H), 2.62 (d, J = 5.5 Hz, 2H), 3.70 (s, 3H), 4.31–4.36 (m, 1H), 4.97 (brm, 1H), 5.10 (brs, 1H), 7.23 (d, J = 9.1 Hz, 1H), 8.12 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 10.6, 19.9, 27.2, 28.3, 38.4, 44.8, 47.3, 51.7, 80.2, 136.0, 141.1, 154.9, 160.0, 164.6, 171.9.

7f

See Ref. 11.

7g

Yield 85%. Colorless oil. R_f = 0.28 (EtOAc : Petroleum ether = 1 : 1); ^{1}H NMR ($CDCl_3$) δ: 0.98 (t, J = 7.3 Hz, 3H), 1.47 (s, 9H), 1.62 (d, J = 6.2 Hz, 3H), 1.65–1.72 (m, 2H), 2.64 (dd, J = 5.5, 15.7 Hz, 1H), 2.67 (dd, J = 5.5, 15.8 Hz, 1H), 3.70 (s, 3H), 4.32–4.38 (m, 1H), 5.09 (br, 1H), 5.11 (br, 1H), 7.64 (d, J = 8.8 Hz, 1H), 8.00 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 10.7, 21.6, 27.3, 28.3, 38.4, 47.6, 48.7, 51.8, 80.3, 123.1, 149.9, 155.0, 160.6, 172.0, 174.1.

7h

Yield 90%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.98 (t, J = 7.2 Hz, 3H), 1.47 (s, 9H), 1.62 (d, J = 6.9 Hz, 3H), 1.67–1.71 (m, 2H), 2.64–2.66 (m, 2H), 3.70 (s, 3H), 4.33–4.37 (m, 1H), 5.08–5.14 (brm, 2H), 7.63 (d, J = 9.6 Hz, 1H), 8.00 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 10.7, 21.6, 27.3, 28.3, 38.5, 47.6, 48.7, 51.7, 80.3, 123.0, 149.9, 154.9, 160.6, 171.9, 174.1.

7i

Yield 80%. Colorless oil. R_f = 0.31 (EtOAc : Petroleum ether = 1 : 1); ^{1}H NMR ($CDCl_3$) δ: 0.93 (d, J = 6.6 Hz, 3H), 0.97 (d, J = 7.3 Hz, 3H), 0.99 (t, J = 7.0 Hz, 3H), 1.47 (s, 9H), 1.67–1.72 (m, 2H), 2.33–2.39 (m, 1H), 2.64 (dd, J = 5.8, 15.7 Hz, 1H), 2.67 (dd, J = 5.9, 16.0 Hz, 1H), 3.70 (s, 3H), 4.32–4.38 (m, 1H), 4.88 (dd, J = 5.5, 8.8 Hz, 1H), 5.19 (d, J = 8.8 Hz, 1H), 7.62 (d, J = 9.1 Hz, 1H), 8.00 (s, 1H); ^{13}C NMR ($CDCl_3$) δ:10.7, 17.4, 19.3, 27.3, 28.3, 33.2, 38.5, 47.6, 51.7, 57.9, 80.2, 122.6, 150.1, 155.4, 160.7, 171.9, 172.5.

7j

Yield 87%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.93 (d, J = 6.8 Hz, 3H), 0.97–1.01 (overlapped, 6H), 1.47, (s, 9H), 1.68–1.72 (m, 2H), 2.36–2.39 (m, 1H), 2.62–2.68 (m, 2H), 3.70 (s, 3H), 4.33–4.37 (m, 1H), 5.15 (d, J = 8.7 Hz, 1H), 7.61 (d, J = 8.7 Hz, 1H), 7.99 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 10.7, 17.4, 19.3, 27.3, 28.3, 29.7, 33.2, 38.5, 47.6, 51.7, 57.9, 80.2, 122.7, 150.1, 155.4, 160.7, 171.9, 172.5.

*Synthesis of linear pentapeptides **8a–k, 8n–u**: general procedure*

LiOH (2 equiv.) was added to the solutions of dipeptides **7a–j** in THF/MeOH/H_2O (cat. 0.5 M) at 0 °C. The reaction mixtures were warmed to room temperature and stirred until all the starting material disappeared. After most of the solvent was evaporated, the solutions were acidified to pH 2 with 1 M HCl and extracted with EtOAc. The organic extracts were combined and washed with brine. Evaporation of the solvent gave the corresponding carboxylic acids, which were dried under high vacuum for 2 h and then dissolved in dry THF followed by addition of DIPEA (4 equiv.) and 2,4,6-trichlorobenzoyl chloride (3 equiv.). The reaction mixtures were stirred at room temperature for 3 h before concentration to dryness under argon. The residues were dissolved in dry toluene and 0.9 equiv. of the alcohols (**3e–f, 4a–b, 4c–d**) and DMAP (4 equiv.) were added. The mixtures were stirred for 3 h at room temperature, diluted with EtOAc, and washed with 10% citric acid, 5% $NaHCO_3$, and brine. The organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The residues were purified by column chromatography (EtOAc–petroleum) to give the linear pentapeptides.

For compounds **8l** and **8m**, general coupling methods (EDC/HOAt/DIPEA) were adopted.

All the linear pentapeptides gave satisfactory analytical and spectroscopic data in full agreement with the assigned structures.

Selected data of these compounds:

8d

Yield 54%. White foam. R_f = 0.14 (EtOAc : Petroleum ether = 1 : 1); $[\alpha]_D^{27}$ = −109.5 (*c* = 0.11, MeOH); ^{1}H NMR ($CDCl_3$) δ: 0.76 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.2 Hz, 3H), 1.20 (d, *J* = 6.6 Hz, 3H), 1.33 (d, *J* = 7.0 Hz, 3H), 1.45 (s, 9H), 1.47 (s, 9H), 1.60 (d, *J* = 7.0 Hz, 3H), 2.21–2.27 (m, 1H), 2.44 (s, 3H), 2.62 (dd, *J* = 6.2, 15.4 Hz, 1H), 2.69 (dd, *J* = 5.5, 15.4 Hz, 1H), 2.79 (s, 3H), 2.90–2.95 (dd like, overlapped, 1H), 3.38 (dd, *J* = 4.8, 15.4 Hz, 1H), 4.54–4.60 (m, 1H), 4.96 (d, *J* = 10.7 Hz, 1H), 5.09 (q, *J* = 7.0 Hz, 1H), 5.05–5.13 (m, overlapped, 1H), 5.53 (dd, *J* = 4.4, 11.7 Hz, 1H), 7.15–7.29 (m, 5H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.98 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 16.2, 17.7, 19.7, 20.1, 21.5, 26.9, 28.0, 28.3, 29.2, 31.5, 34.3, 40.2, 42.2, 48.7, 57.6, 58.3, 67.1, 80.4, 82.0, 123.0, 126.6, 128.4, 128.8, 137.2, 149.9, 154.9, 160.2, 169.7, 170.1, 170.5, 170.7; ESI-MS (m/z) calcd. $[M + Na]^+$ = 782.4, found 782.3.

8t

Yield 72%. White foam. R_f = 0.31 (EtOAc : Petroleum ether = 1 : 1); $[\alpha]_D^{20}$ = −37.7 (*c* = 0.12, MeOH); ^{1}H NMR ($CDCl_3$) δ: 0.92 (br, overlapped, 6H), 0.98 (t, *J* = 7.4 Hz, 3H), 1.39 (s, 9H), 1.42 (d, *J* = 6.4 Hz, 3H), 1.47 (s, 9H), 1.60 (d, *J* = 6.9 Hz, 3H), 1.66–1.73 (m, 2H), 2.15–2.16 (m, 1H), 2.68 (dd, *J* = 6.9, 16.0 Hz, 1H), 2.77 (dd, *J* = 4.6, 16.0 Hz, 1H), 3.05–3.06 (dd like, overlapped, 2H), 4.21–4.24 (m, 1H), 4.43–4.49 (m, 1H), 4.71–4.74 (m, 1H), 5.07–5.09 (m, 1H), 5.15–5.16 (m, 1H), 6.43 (d, *J* = 6.4 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 7.14–7.26 (m, 5H), 7.47 (d, *J* = 9.2 Hz, 1H), 7.98 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 10.6, 18.1, 19.1, 19.2, 21.5, 27.8, 27.9, 28.3, 31.0, 38.0, 39.4, 47.4, 48.7, 53.5, 58.3, 71.2, 123.2, 127.0, 128.4, 129.4, 141.5, 149.6, 149.7, 151.6, 160.8, 170.0, 170.2.

*Synthesis of target compound, cyclic (depsi)peptide **9a–u**: general procedure*

To the solutions of the linear pentapeptides **8a–u** in dry CH_2Cl_2 was added TFA at 0 °C. The reaction mixtures were warmed to room temperature and stirred for another 4 h. Residual TFA was removed by successive addition and evaporation of CH_2Cl_2. The residues were dissolved in dry THF (*ca.* 1 × 10^{-3} mol/l) and then HATU (5 equiv.) and DIPEA (8 equiv.) were added successively. The resultant mixtures were stirred for 3 days at room temperature and concentrated *in vacuo*. The residues were dissolved in EtOAc and washed with 10% citric acid, 5% $NaHCO_3$, and brine. The organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residues were purified by silica gel column ($CHCl_3$–MeOH) followed by Pharmadex LH-20 ($CHCl_3$–MeOH = 1 : 1) to give compounds **9a–u** in 24–94% yields.

All the cyclic (depsi)peptides gave satisfactory analytical and spectroscopic data in full accord with their assigned structures.

Selected data of these compounds:

9d

Yield 45%. White foam. R_f = 0.55 ($CHCl_3$:MeOH = 20 : 1); $[\alpha]_D^{19}$ = −83.7 (*c* = 0.3, MeOH); ^{1}H NMR ($CDCl_3$) δ: 0.59 (d, *J* = 6.6 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H), 1.44 (d, *J* = 6.6 Hz, 3H), 1.47 (d, *J* = 7.0 Hz, 3H), 1.54 (d, *J* = 6.6 Hz, 3H), 2.26–2.33 (m, 1H), 2.72 (dd, *J* = 4.4, 16.9 Hz, 1H), 2.80–2.84 (m, 2H), 3.08 (s, 3H), 3.22 (s, 3H), 3.44 (dd, *J* = 8.8, 13.6 Hz, 1H), 4.41–4.44 (m, 1H), 5.02 (d, *J* = 10.6 Hz, 1H), 5.16 (dd, *J* = 5.9, 8.8 Hz, 1H), 5.32–5.37 (m, 1H), 5.44 (q, *J* = 6.6 Hz, 1H), 7.15–7.24 (m, 5H), 7.96 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 8.91 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 16.2, 18.7, 18.9, 20.4, 23.6, 27.6, 29.0, 30.1, 36.4, 39.6, 41.5, 46.6, 57.2, 60.9, 67.5, 122.2, 127.0, 128.7, 129.4, 136.3, 150.6, 160.1, 167.2, 168.9, 169.8, 170.4, 171.9; HRESIMS calcd. for $C_{29}H_{39}N_5O_6NaS$ $[M + Na]^+$ 608.2519, found 608.2517.

9p

Yield 94%. white foam. ^{1}H NMR ($CDCl_3$) δ: 0.93 (d, *J* = 6.9 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.96 (t, *J* = 7.3, 7.8 Hz, 3H), 1.17 (d, *J* = 6.8 Hz, 3H), 1.47 (d, *J* = 6.8 Hz, 3H), 1.51–1.59 (m, 2H), 2.11–2.17 (m, 1H), 2.56 (dd, *J* = 3.9, 12.2 Hz, 1H), 2.71 (dd, *J* = 4.1, 12.4 Hz, 1H), 3.06 (dd, *J* = 5.0, 14.2 Hz, 1H), 3.61 (dd, *J* = 6.2, 14.0 Hz, 1H), 4.00 (t like, *J* = 5.0, 4.6 Hz, 1H), 4.41–4.46 (m, 1H), 4.79–4.81 (m, 1H), 5.09 (q, *J* = 6.9 Hz, 1H), 5.43 (m, 1H), 6.12 (d, *J* = 8.7 Hz, 1H), 6.52 (d, *J* = 4.6 Hz, 1H), 7.20 (t, *J* = 7.3 Hz, 1H), 7.24–7.26 (m, 2H), 7.29–7.31 (m, 2H), 7.42 (d, *J* = 6.8 Hz, 1H), 8.03 (s, 1H), 9.08 (d, *J* = 10.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 11.3 (C-5), 17.3 (C-28), 18.0 (C-24), 19.2 (C-25), 24.3 (C-11), 26.3 (C-4), 29.6 (C-23), 36.9 (C-14), 38.2 (C-2), 47.7 (C-3), 47.9 (C-10), 53.4 (C-13), 60.3 (C-22), 69.5 (C-27), 123.2 (C-8), 127.3 (C-18), 128.8 (C-16, C-20), 129.5 (C-17, C-19), 136.5 (C-15), 150.3 (C-7), 160.7 (C-6), 169.3 (C-9), 170.0 (C-12), 170.2 (C-21), 170.7 (C-1), 173.2 (C-26); HRESIMS calcd. for $C_{28}H_{37}N_5O_6NaS$ $[M + Na]^+$ 594.2362, found 594.2354.

Conclusion

In conclusion, 20 analogues of marine cyclic depsipeptide obyanamide have been synthesized by [3+2] strategy. Preliminary SAR studies show that the β-amino acid residue, the ester bond, and the Ala(Thz) moiety were essential for biological activities. However, the role of *N*-methylation of Val/Phe needs further research.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (81001392) and School of Pharmacy, Fudan University.

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