HETEROCYCLES, Vol. 98, No. 6, 2019, pp. 789 - 797. © 2019 The Japan Institute of Heterocyclic Chemistry
Received, 25th March, 2019, Accepted, 16th May, 2019, Published online, 29th May, 2019
DOI: 10.3987/COM-19-14076

TOTAL SYNTHESIS OF THERMOACTINOAMIDE A AND ITS ANALOGUE

Yueguang Sun, Hang-bin Li, Rong Zhou, Hailin Wang, Pengju Feng,* and Shihai Xu*

Department of Chemistry, Jinan University, Guangzhou, China. E-mail: pfeng@jnu.edu.cn; txush@jnu.edu.

Abstract – Thermoactinoamide A, showing remarkable antibacterial properties, is a novel cyclic hexapeptide which was originally isolated from thermophilic bacterium. We disclosed here the first total synthesis of thermoactinoamide A and its analogue, anti-thermoactinoamide A, by the Fmoc solid-phase peptide synthesis and subsequent efficient macrolactamization. Thermoactinoamide A and its analogue can be obtained in 74% and 46% overall yield respectively without any isomerization under the developed methodology.

INTRODUCTION

Cyclic peptides are a particular scaffold of high pharmaceutical and biological interest.[1-3] Much attention has been constantly paid to isolate or synthesize new targets of cyclic peptide skeleton for drug discovery.[4-8] Marine organisms provide a rich resource for the discovery of structurally diverse peptides.[9-12] Recently, thermoactinoamide A (**1a**), with a significant and selective growth inhibition against *Staphylococcus aureus* ATCC 6588 (MIC value of 35 μM), was isolated by Mangoni and co-workers from the thermophilic bacterium *Thermoactinomyces vulgaris* strain ISCAR 2354.[13] Thermoactinoamide A (**1a**) represents a new cyclic hexapeptide, which contains both D and L-amino acids redidues. Interestingly, structurally similar cyclic hexapeptidesdesotamides A and B were also reported for their comparably narrow antibiotic activity against *Staphylococcus aureus* ATCC 29213 with an MIC value of 23 μM.[14] As the viewpoint of Mangoni, the cyclic hexapeptides with mixed D/L configurations, one aromatic amino acid residue and prevalence of lipophilic redidues may be considered as a starting point for searching antiviotic drugs. The unique ring structure of thermoactinoamide A (**1a**) along with its potential biological activities make it an attractive target for synthesis. Herein we wish to report our efficiently synthetic methodology to thermoactinoamide A (**1a**) and its analogue, anti-thermoactinoamide A (**1b**) (Figure 1).

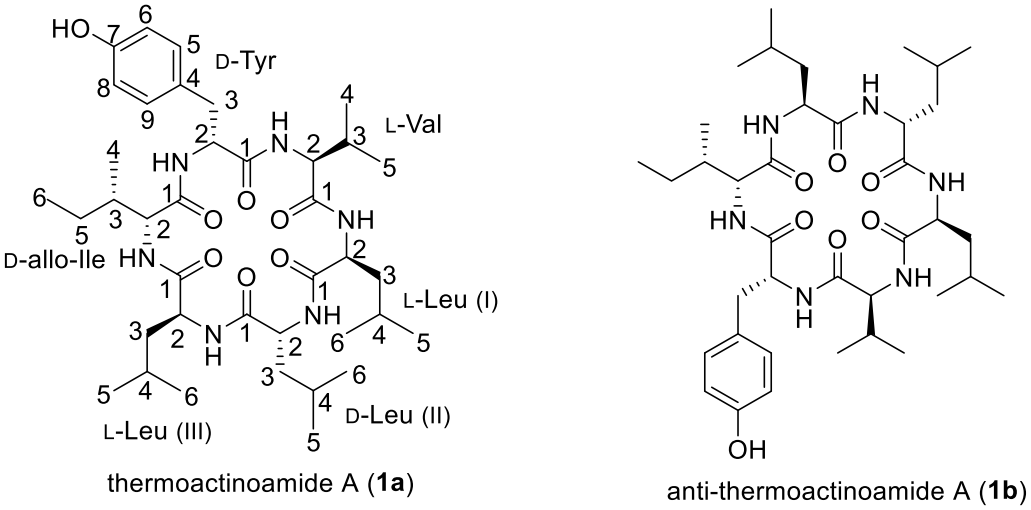
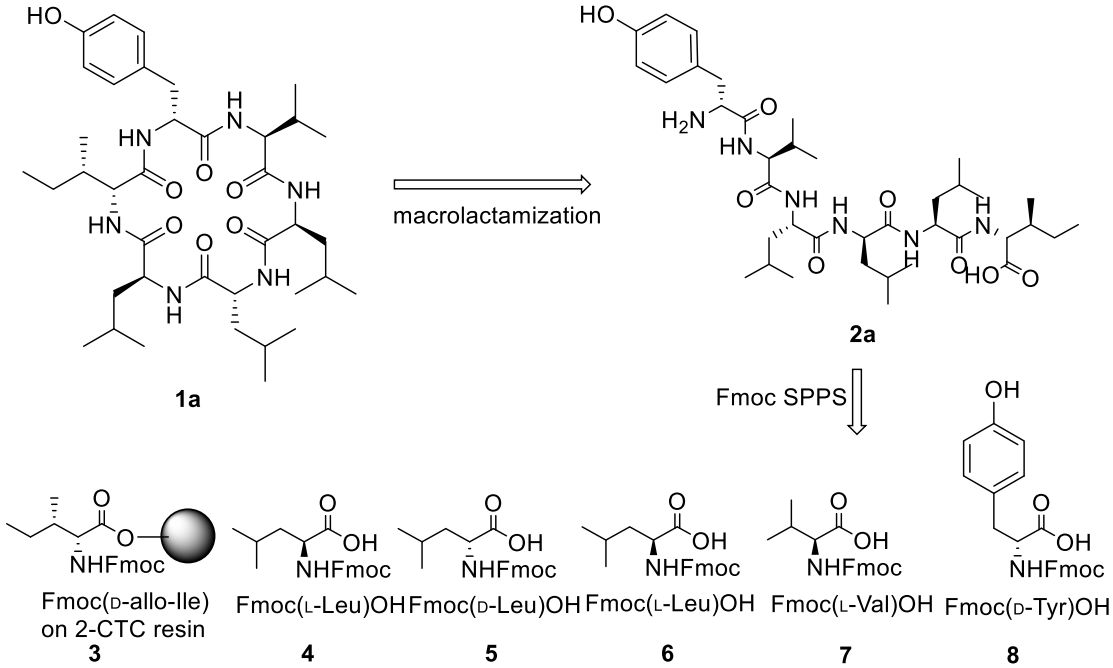


Figure 1. Structures of thermoactinoamideA (**1a**) and anti-thermoactinoamide A (**1b**)

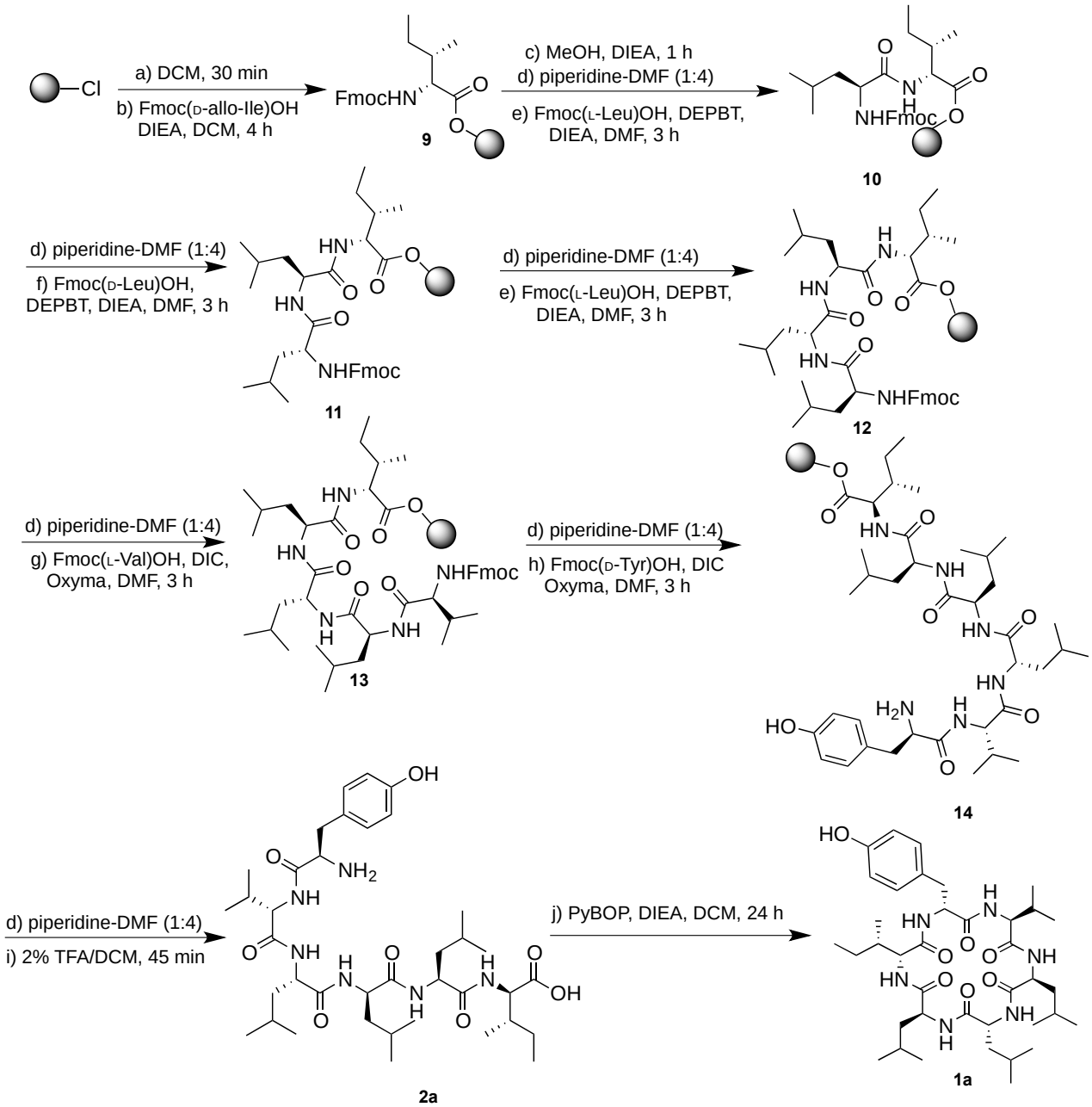
RESULTS AND DISCUSSION

Our retrosynthetic analysis of thermoactinoamide A (**1a**) is outlined in Scheme 1. Thermoactinoamide A (**1a**) could originate from compound **2a** via macrolactamization at *C*-terminus of D-allo-Ile residue and *N*-terminus of D-Tyr residue, so that D-Tyr-OH with a free phenol group which may cause side reaction during the amidation sequence could be introduced to the linear peptide at last. The linear peptide precursor could be formed through Fmoc-based solid-phase peptide synthesis (SPPS) by introduction of D-allo-Ile, L-Leu, D-Leu, L-Val and D-Tyr. For the synthesis of thermoactinoamide, as no Gly or Pro at the *C*-terminus that would avoid epimerization, the most expensive D-allo-Ile was selective firstly reacted with 2-chlorotrityl chloride resin for improving its utilization efficiency (D-allo-Ile could be recycled).



Scheme 1. Retrosynthetic analysis of thermoactinoamide A (**1a**)

The synthesis was commenced to gain the linear hexapeptide via Fmoc SPPS. 2-Chlorotrityl chloride resin[15-23] was selected as solid support due to its synthetic advantages, such as the minimization of diketopiperazine (DKP) formation and mild reaction condition at the support-cleavage step.[24] Fmoc(D-allo-Ile)OH was firstly anchored onto 2-chlorotrityl chloride resin in the presence of diisopropylethylamine (DIEA) in DCM for 4 h at room temperature, followed by addition of MeOH/DIEA (9:1) for capping the unreacted resin sites.[25] Fmoc-protected peptidyl resin **3** was treated



Scheme 2. Synthesis of thermoactinoamide A (**1a**)

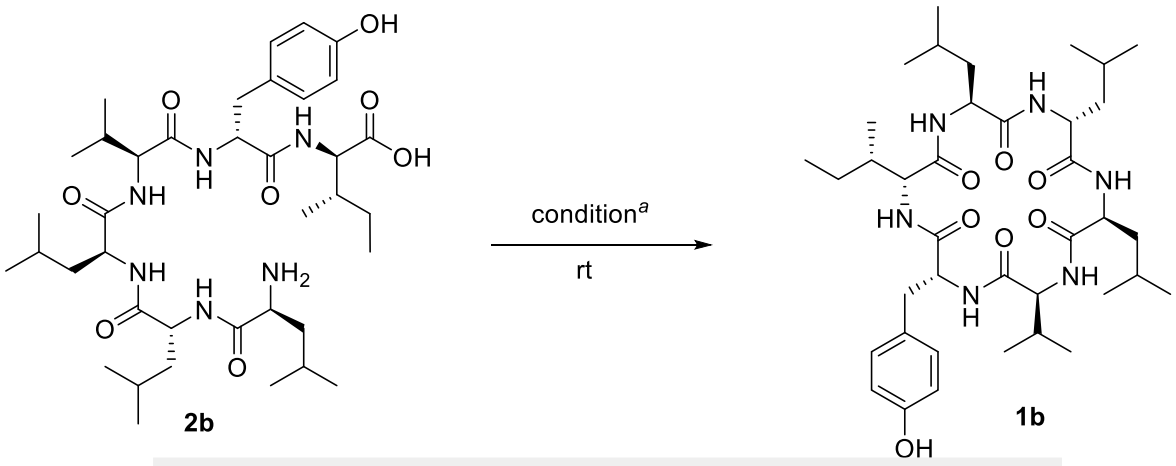
with piperidine/DMF (2:8) to liberate the amine, which was subsequently coupled with Fmoc(D-Leu)OH by mixing with condensation reagents 3-(diethoxyphosphoryloxy)-1,2,3-benzotrizin-4(3*H*)-one (DEPBT) and DIEA in DMF to give linear peptide **10**. Elongation of the peptide sequence was performed by

sequential Fmoc removal and coupling steps.[26] The amide formation efficiency between different amino acids was highly depended on coupling reagents. Compounds **12** and **13** could be totally converted to products in a relevant shorter time when diisopropylcarbodiimide (DIC) and ethyl cyanoglyoxylate-2-oxime (Oxyma) was used,[27-31] comparing to other coupling reagents, such as DEPBT and DIEA.[32,33] Upon incorporation of all amino acid residues, the resin at the *C*-terminus of **14** was detached upon treatment with TFA/DCM (2:98), leading to 6-membered cyclolactam precursor **2a**.[34] Macrocyclization was found to be the most challenging step, the reaction condition of which was carefully examined, including coupling reagents, base, etc. Finally, thermoactinoamide A (**1a**) was smoothly cyclized from crude **2a** by using benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) as coupling reagent, maintaining pH of reaction mixture at 8 with tuning the addition of DIEA.[35,36] Notably, the epimerization of final product does not occur under our developed methodology.

After reversed-phase HPLC purification, thermoactinoamide A (**1a**) was obtained in 74% yield. The pure product was fully characterized. The spectra (^{1}H NMR, HRMS) of our synthetic **1a** are in good agreement with the literature data.[6] In addition, the ^{13}C and DEPT NMR spectrum for **1a** was well documented in supporting information (**Figures S2−S8**), while these spectrum was not shown in previous literature, indicating that it may be very difficult to obtain a sufficient amount by separation.

To demonstrate the utility of synthetic strategy for synthesis similar 6-membered cyclopeptides and provide more information about bioactivity of thermoactinoamide A analog, we succeeded to apply the same method to achieve an analogue of **1a**, anti-thermoactinoamide A (**1b**) which has the same amino acid residues of **1a**, but the amino acid residues connected with each other in the reverse order. The synthetic route of **1b** was outlined in Scheme S1. During the synthesis, we found that the yield limit step was always macrolactamization of the linear peptide. The cyclization condition was further optimized and disclosed in Table 1. The combination of PyBOP with DIEA in DCM gave the highest cyclization yield among other coupling reagents tested thus far, such as PyBOP with collidine, PyBOP/HOAt with DIEA, and PyBOP/HOAt with collidine (Table 1, Entries 1–4). Mixed solvent of DCM-DMF was greatly improved the efficiency of the reaction (Table 1, Entries 5–6). With the best condition, anti-thermoactinoamide A (**1b**) was obtained in a 46% as a sole isomer after reversed-phase HPLC purification. Compound **1b** was fully characterized.

Table 1. Optimization of the cyclization



Entry	Coupling reagent	Base	Solvent	Yield (%)[b]
1	PyBOP	DIEA	DCM	19
2	PyBOP	collidine	DCM	10
3	PyBOP/HOAt	DIEA	DCM	17
4	PyBOP/HOAt	collidine	DCM	13
5	PyBOP	DIEA	DCM-DMF	50
6	PyBOP	collidine	DCM-DMF	32

[a] All reactions were run on a 50 mg scale of **1b** with the pH of reaction mixture at 8. [b] Isolated yields.

To pursue new bioactivity of thermoactinoamides, antitumor activity, antiviral activity, anti-tuberculous activity and anti-inflammatory activity were also selectively evaluated by using compounds **1a**, **2a**, **1b**, **2b** (see ESI for details). Unfortunately, no positive results were obtained.

EXPERIMENTAL

Characterization methods: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker (300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR) spectrometer. Chemical shifts are denoted in δ (ppm) relative to residual solvent peaks as internal standard (CD_3OD, ^{1}H δ 3.31, ^{13}C δ 49.0). HRMS spectra were recorded on a Thermo Scientific Exactive mass spectrometer. High performance liquid chromatography (HPLC) experiments were performed with an Agilent Technologies HPLC system.

Materials: DMF and DCM were purchased from Guangdong GuanghuaSci-Tech Co., Ltd. The HPLC grade solvents (MeOH) were purchased from J&K Scientific. DIC, DIEA, Oxyma were purchased from Aladdin. 2-CTC resin, PyBOP and amino acids such as Fmoc(D-allo-Ile)OH, Fmoc(L-Leu)OH, Fmoc(D-Leu)OH, Fmoc(L-Val)OH, Fmoc(D-Tyr)OH et al, were purchased from GL Biochem.

Procedure for solid-phase peptide synthesis (SPPS)

Step 1: Fmoc group of the solid supported peptide was removed by using 20% piperidine/DMF solution (10 min, room temperature).

Step 2: The resin in the reaction vessel was washed with DMF (10 mL × 3) and CH_2Cl_2 (10 mL × 3).

Step 3: To the solution of amino acid (2.0 eq.) were added DEPBT (4.0 eq.) and DIEA (4 eq.). The mixture was injected to the reaction vessel. The resulting mixture was stirred for 180 min at room temperature.

Step 4: The resin in the reaction vessel was washed with DMF (10 mL × 3) and CH_2Cl_2 (10 mL × 3). Amino acids were condensed onto the solid support by repeating Steps 1–4.

Synthesis of thermoactinoamide A (1a). Fmoc(D-allo-Ile)-2-chlorotrityl resin (**9**): 2-Chlorotrityl resin (loading rate 0.94 mmol/g, 1.1 g, 1.0 mmol) in Libra tube was swollen with CH_2Cl_2, and then excess solvent was removed by filtration. To the resin was added a solution of Fmoc(D-allo-Ile)OH, (500 mg, 1.4 mmol) and *i*-Pr_2NEt (463 μL, 2.8 mmol) in CH_2Cl_2 (10 mL), and stirred for 240 min. The reaction mixture was filtered, washed with DMF (10 mL × 3), CH_2Cl_2 (10 mL × 3), then dried under vacuum to give Fmoc(D-allo-Ile)-resin. Dried Fmoc(D-allo-Ile)-resin was added with 20% piperidine in DMF, and stirred for 10 min.

Peptide (2a). Fmoc(D-allo-Ile)-resin was subjected to 3 cycles [Fmoc(L-Leu)OH, Fmoc(D-Leu)OH, Fmoc(L-Leu)OH] of the SPPS protocol (Steps 1–4) to afford resin-bound peptide (**12**). To peptide (**12**) were added Fmoc(L-Val)OH (950 mg, 2.8 mmol), DIC (353 μL, 2.8 mmol), and Oxyma (398 mg, 2.8 mmol) in DMF (10 mL). After being stirred for 180 min at room temperature, there action mixture was filtered and washed with DMF (10 mL × 3) and CH_2Cl_2 (10 mL × 3), and then 20% piperidine in DMF (10 mL) was added to the resin. After being stirred for 10 min at room temperature, the reaction mixture was filtered and washed with DMF (10 mL × 3) and CH_2Cl_2 (10 mL × 3) to afford resin-bound peptide (**13**). To peptide (**13**) were added Fmoc(D-Tyr)OH (1.1g, 2.8mmol), DIC (353 μL, 2.8 mmol), and Oxyma (398 mg, 2.8 mmol) in DMF (10 mL). After being stirred for 180 min at room temperature, the action mixture was filtered and washed with DMF (10 mL × 3) and CH_2Cl_2 (10 mL × 3), and then 20% piperidine in DMF (10 mL) was added to the resin. After being stirred for 10 min at room temperature, the reaction mixture was filtered and washed with DMF (10 mL × 3) and CH_2Cl_2 (10 mL × 3) to afford resin-bound peptide (**14**). To peptide (**14**) was added CH_2Cl_2/TFA = 98/2 (20 mL). After being stirred for 45 min at room temperature, the reaction mixture was filtered and washed with DCM (10 mL × 3) and MeOH (10 mL × 3). The filtrate was concentrated to afford crude peptide (**2a**), whichwas used in the next step without further purification. The crude peptide (**2a**) HO-D-Ile-Leu-D-Leu-Leu-Val-D-Tyr-NH_2: HRMS calcd for $C_{38}H_{64}N_6O_8$ $[M+H]^+$ *m/z* = 733.4858; found 733.4806. $[\alpha]^{25}_D$ +163 (c 1, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 8.00 (dd, *J* = 8.5, 5.9 Hz, 1H), 7.16–7.09 (m, 2H), 6.83–6.75 (m, 2H), 4.51 (dd, *J* = 8.7, 4.9 Hz, 2H), 4.44 (dd, *J* = 9.3, 5.7 Hz, 1H), 4.28 (t, *J* = 7.2 Hz, 1H), 4.19 (t, *J* = 7.5 Hz, 1H),

3.14 (dd, *J* = 13.9, 7.5 Hz, 1H), 3.01 (dd, *J* = 13.9, 7.8 Hz, 1H), 2.01 (dt, *J* = 12.7, 6.8 Hz, 2H), 1.68 (dp, *J* = 20.6, 7.4, 7.0 Hz, 10H), 1.33 (ddq, *J* = 47.1, 13.9, 7.0 Hz, 3H), 1.02–0.83 (m, 32H); ^{13}C NMR (75 MHz, CD_3OD) δ 10.7, 13.9, 17.4, 18.3, 20.2, 20.5, 20.6, 21.9, 22.1, 22.3, 24.5, 24.6, 24.7, 26.0, 30.2, 36.6, 36.9, 39.6, 40.1, 40.5, 51.9, 52.4, 54.5, 55.6, 55.7, 59.8, 115.5, 124.6, 130.1, 157.0, 169.2, 172.3, 172.9, 173.4, 173.5, 173.6.

To a solution of peptide (**2a**) (50.0 mg, 0.02 mmol) in CH_2Cl_2 (100 mL) were added PyBOP (52.0 mg, 0.10 mmol) and maintained the pH at 8 with the addition of DIEA. After being stirred overnight, the reaction mixture was concentrated. The residue was purified by reversed-phase HPLC (**See Figure S9**) to afford (**1a**) (41.2 mg, 74% for 7 steps) as a white solid: HRMS calcd for $C_{38}H_{63}N_6O_7$ $[M+H]^+$ *m/z* = 715.4746. $[\alpha]^{25}_D$ +64 (c 0.5, MeOH); mp: 310.8-312.2 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.11–7.03 (m, 2H), 6.74–6.68 (m, 2H), 4.54 (dd, *J* = 8.8, 5.9 Hz, 1H), 4.44 (dd, *J* = 9.4, 7.0 Hz, 1H), 4.36 (dd, *J* = 7.7, 6.1 Hz, 2H), 4.30–4.24 (m, 1H), 2.96–2.87 (m, 2H), 2.28–2.18 (m, 2H), 2.05 (d, *J* = 5.3 Hz, 2H), 1.71 (t, *J* = 6.9 Hz, 4H), 1.60 (d, *J* = 5.7 Hz, 6H), 1.02–0.96 (m, 8H), 0.96–0.90 (m, 16H), 0.88 (s, 2H), 0.74 (d, *J* = 7.0 Hz, 2H), 0.52 (d, *J* = 6.9 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 10.0, 14.0, 15.4, 18.0, 19.8, 20.7, 21.2, 21.5, 21.9, 22.2, 24.5, 24.8, 26.7, 28.9, 29.4, 31.7, 37.5, 39.0, 40.1, 40.7, 51.2, 51.9, 52.7, 56.1, 56.5, 58.7, 115.0, 129.5, 130.0, 156.1, 171.1, 172.1, 172.5, 172.9, 173.1, 173.4.

Synthesis of anti-thermoactinoamide A (1b)

According to the procedure of synthesis of thermoactinoamide A (**1a**): the connection order of amino residues is HO-D-Ile-D-Tyr-Val-Leu-D-Leu-Leu-NH_2.

Anti-thermoactinoamide A (**1b**): ^{1}H NMR (300 MHz, CD_3OD) this compound gave broad signals in ^{1}H NMR spectrum; ^{13}C NMR (75 MHz, CD_3OD) this compound gave signals in ^{13}C NMR spectrum, see **Figure S12**. HRMS calcd for $C_{38}H_{63}N_6O_7$ $[M+H]^+$ *m/z* = 715.4756 found: 715.4753; mp: 319.4-321.8 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.13 (d, *J* = 8.5 Hz, 2H), 6.74 (d, *J* = 8.5 Hz, 2H), 4.63 (s, 1H), 4.51 (t, *J* = 7.0 Hz, 1H), 4.43 (d, *J* = 4.5 Hz, 1H), 4.37–4.28 (m, 3H), 3.17 (dd, *J* = 14.3, 4.4 Hz, 1H), 2.81 (dd, *J* = 14.3, 10.5 Hz, 1H), 1.61 (s, 7H), 1.31 (s, 2H), 1.01–0.89 (m, 25H), 0.85 (d, *J* = 6.9 Hz, 3H), 0.78 (d, *J* = 6.8 Hz, 3H), 0.70 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 10.7, 13.7, 17.4, 17.8, 19.8, 21.2, 21.5, 21.7, 22.21, 22.23, 24.5, 24.6, 24.7, 25.9, 31.1, 36.0, 36.1, 39.4, 40.6, 40.1, 51.2, 52.5, 52.6, 56.1, 57.5, 58.4, 115.1, 127.2, 129.7, 156.2, 171.2, 172.4, 172.5, 172.6, 173.3, 173.4.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China [Nos. 21602078, 21672084, and 81874292]; the Science and Technology Planning Project of Guangzhou [Nos. 201704030042 and 201710010088]; the National Key Research and Development Plan of China (No. 2018YFC0310900); the Scientific Research Cultivation and Innovation Fund Research Project of Jinan

University [Nos. 21616113 and 21617472], and the fundamental research funds for the central university.

REFERENCES

1. Y. Lee, C. Phat, and S. Hong, *Peptides*, 2017, **95**, 94.
2. A. Zorzi, K. Deyle, and C. Heinis, *Curr. Opin. Chem. Biol*., 2017, **38**, 24.
3. H. K. Kang, M. C. Choi, C. H. Seo, and Y. Park, *Int. J. Mol. Sci*., 2018, **19**, 2.
4. J. Hur, J. Jang, J. Sim, W. S. Son, H. C. Ahn, T. S. Kim, Y. H. Shin, C. Lim, S. Lee, H. An, S. Kim, and Y. Suh, *Angew. Chem. Int. Ed*., 2018, **57**, 3069.
5. R. A. Medina, D. E. Goeger, P. Hills, S. L. Mooberry, N. Huang, L. I. Romero, O. B. Eduardo, W. H. Gerwick, and K. L. McPhail, *J. Am. Chem. Soc*., 2008, **130**, 6324.
6. O. S. Kwon, C. K. Kim, W. S. Byun, J. Oh, Y. J. Lee, H. S. Lee, C. J. Sim, D. C. Oh, S. K. Lee, K. B. Oh, and J. Shin, *J. Nat. Prod*., 2018, **81**, 1426.
7. T. L. Simmons, L. M. Nogle, O. Media, F. A.Valeriote, S. L. Mooberry, and W. H. Gerwick, *J. Nat. Prod*., 2009, **72**, 1011.
8. A. Tripathi, J. Puddick, M. R. Prinsep, M. Rottmann, and L. T. Tan, *J. Nat. Prod*., 2010, **73**, 1810.
9. S. K. Kim and I. Wijesekara, *J. Funct. Foods*, 2010, **2**, 1.
10. S. H. Md, S. Fareed, S. Ansari, and S. S. khan, *Indian J. Mar. Sci*., 2012, **41**, 27.
11. N. Natalie and O. Till, *Mar. Drugs*, 2015, **13**, 4814.
12. A. Tapeinou, M. T. Matsoukas, C. Simal, and T. Tselios, *Biopolymers*, 2015, **104**, 453.
13. R. Teta, V. T. Marteinsson, and A. Mangoni, *J. Nat. Prod*., 2017, **80**, 2530.
14. Y. Song, Q. X. Li, Y. Liu, Y. Chen, A. Zhang, W. Sun, J. Zhang, and J. Zhang, *J. Nat. Prod*., 2014, **77**, 1937.
15. M. Kikuchi and H. Konno, *Org. Lett*., 2014, **16**, 4324.
16. D. S. Nielsen, H. N. Hoang, R. J. Lohman, F. Diness, and D. P. Fairlie, *Org. Lett*., 2012, **14**, 5720.
17. Z. Pan, C. Wu, W. Wang, Z. Cheng, G. Yao, K. Liu, H. Li, L. Fang, and W. Su, *Org. Lett*., 2017, **19**, 4420.
18. M. M. Sleebs, D. Scanlon, J. Karas, R. Maharani, and A. B. Hughes, *J. Org. Chem*., 2011, **76**, 6686.
19. M. N. Rahimi, L. K. Buckton, S. S. Zaiter, J. Kho, V. Chan, A. Guo, J. Konesan, S. H. Kwon, L. K. O. Lam, M. F. Lawler, M. Leong, G. D. Moldovan, D. A. Neale, G. Thornton, and S. McAlpine, *Med. Chem. Lett*., 2018, **9**, 73.
20. H. Kaur, P. W. R. Harris, P. J. Little, and M. A. Brimble, *Org. Lett*., 2015, **17**, 492.
21. Y. E. Jad, G. A. Acosta, T. Naicker, M. Ramtahal, A. E. Faham, T. Govender, H. G. Kruger, B. Torre, and F. Albericio, *Org. Lett*., 2015, **17**, 6182.
22. J. Spiegel, C. M. Moruno, H. Kessler, and W. D. Lubell, *J. Org. Chem*., 2012, **77**, 5271.

23. J. Chatterjee, B. Laufer, and H. Kessler, *Nat. Protoc.*, 2012, **7**, 432.
24. M. Pelaygimeno, J. Tullapuche, and O. Albericio, *Nat. Protoc.*, 2016, **11**, 1924.
25. H. Kaur, P. W. R. Harris, P. J. Little, and M. A. Brimble, *Org. Lett.*, 2015, **17**, 492.
26. C. Roses, C. Camo, K. Vogels, M. Planas, and L. Feliu, *Org. Lett.*, 2016, **18**, 4140.
27. S. F. Ramon, P. Rafel, B. Rafael, E. F. Ayman, and A. Fernando, *Chem. Eur. J.*, 2009, **15**, 9394.
28. T. Kuranaga, A. Enomoto, H. Tan, K. Fujita, and T. Wakimoto, *Org. Lett.*, 2017, **19**, 1366.
29. L. Corcilius, N. T. Elias, J. L. Ochoa, R. G. Linington, and R. J. Payne, *J. Org. Chem.*, 2017, **82**, 12778.
30. K. Pels and K. Thomas, *ACS Comb. Sci.*, 2015, **17**, 152.
31. M. Stach, A. Weidkamp, S. H. Yang, K. Hung, D. Furkert, P. W. R. Harris, J. B. Smaill, and M. A. Brimble, *Eur. J. Org. Chem.*, 2015, 6341.
32. H. Li, X. Jiang, Y. Ye, C. Fan, T. Romoff, and M. Goodman, *Org. Lett.*, 1999, **1**, 91.
33. W. Xu, X. Liao, S. Xu, J. Diao, B. Du, X. Zhou, and S. Pan, *Org. Lett.*, 2008, **10**, 4569.
34. D. S. Nielsen, H. N. Hoang, R. J. Lohman, F. Diness, and D. P. Fairlie, *Org. Lett.*, 2012, **14**, 5720.
35. E. Marcucci, J. Tulla-Puche, and F. Albericio, *Org. Lett.*, 2012, **14**, 612.
36. R. Zhou, Y. G. Sun, H. B. Li, W. L. Long, X. J. Liao, P. J. Feng, and S. H. Xu, *Chem. Med. Chem.*, 2018, **13**, 2022.