Tetrahedron Letters 55 (2014) 6109–6112

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Total synthesis of proposed structure of coibamide A, a highly N- and O-methylated cytotoxic marine cyclodepsipeptide

Wei He [a], Hai-Bo Qiu [a], Yi-Jie Chen [b], Jie Xi [b], Zhu-Jun Yao [a,b,*]

[a] State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China
[b] State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing, Jiangsu 210093, China

ARTICLE INFO

Article history:
Received 12 August 2014
Accepted 9 September 2014
Available online 16 September 2014

Keywords:
Coibamide A
Cyclodepsipeptide
Anticancer
N-Methylamino acid
Total synthesis

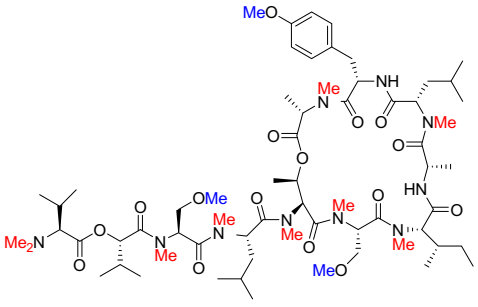
ABSTRACT

Total synthesis of the originally proposed structure of coibamide A, a highly N- and O-methylated cytotoxic marine cyclodepsipeptide, has been accomplished by using a [(4+1)+3+3]-peptide fragment-coupling strategy and careful examination and optimization of the multiple dense N-methylated amide-bond formations. The synthetic sample of the proposed coibamide A could not match the natural product in both ^{1}H and ^{13}C NMR spectra, but was found to exhibit low micromolar cytotoxicity against the proliferation of three tested cancer cells.

© 2014 Elsevier Ltd. All rights reserved.

Marine cyanobacteria are a rich source of complex secondary metabolites, and some of them have shown striking biological activity[1–4] and have consequently become attractive synthetic targets in the quest for new leads for pharmaceutical development.[5] Coibamide A (**1**, Fig. 1) is a cyclodepsipeptide recently isolated by McPhail and co-workers from the marine cyanobacterium *Leptolyngbya* sp. collected from the Coiba National Park, Panama.[6] It was found to exhibit attractive low-nanomolar inhibitory activities against the proliferation of various cancer cells, including MDA-MB-231 (IC_{50} 2.8 nM), LOX IMVI (IC_{50} 7.4 nM), HL-60 (IC_{50} 7.4 nM), and SNB-75 (IC_{50} 7.6 nM). The significant inhibitory potencies and possible new mechanism of action make coibamide A to be a promising lead in anticancer drug discovery.[6–8] For the limited sources of marine natural products, establishment of a practical chemical synthesis of coibamide A is of extreme value to acquire sufficient sample for further pharmaceutical development and exploration of its structure–activity relationship.

Full assignment of natural coibamide A was carried out when its isolation by aid of NMRs and MS studies, as well as chiral HPLC analysis of the degraded amino acids (Fig. 1).[6] Among the amino-acid components, the absolute configuration of threonine was predicted by the MM2 method and was further confirmed with ROESY experiments. Coibamide A comprised a 22-membered macrocycle with a high content of *N*- and *O*-methylamino acids (eight of its eleven amino acids are N-methylated). Such a structural feature may help to improve the metabolic stability and enhance the hydrophobicity,[9] however, synthesis of a macrocyclic peptide containing multiple dense N-methylated amides is often full of challenges. Besides lower efficiency of the amide couplings with *N*-methylamino acids, common problems encountered in the synthesis also include the side reactions of forming diketopiperazine, epimerization, and the lability of N-alkylated peptides to acids.[10,11] Despite many reports have been addressed the development of proper reagents for the coupling of sterically hindered *N*-methylamino acids,[12] a satisfactory solution to resolve all the above problems has not yet been found.



Coibamide A (**1**)

Figure 1. Proposed structure of coibamide A.

* Corresponding author. Tel./fax: +86 21 54925123.
E-mail address: yaoz@sioc.ac.cn (Z.-J. Yao).

http://dx.doi.org/10.1016/j.tetlet.2014.09.047
0040-4039/© 2014 Elsevier Ltd. All rights reserved.

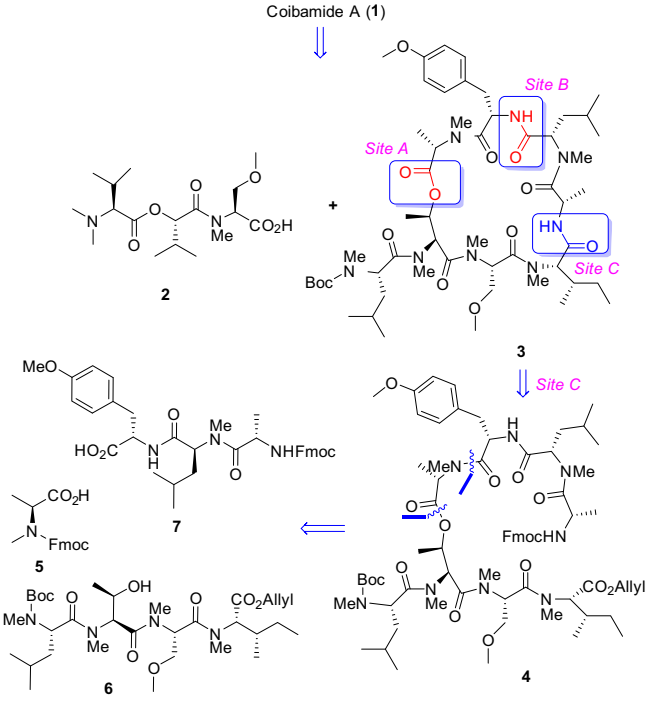
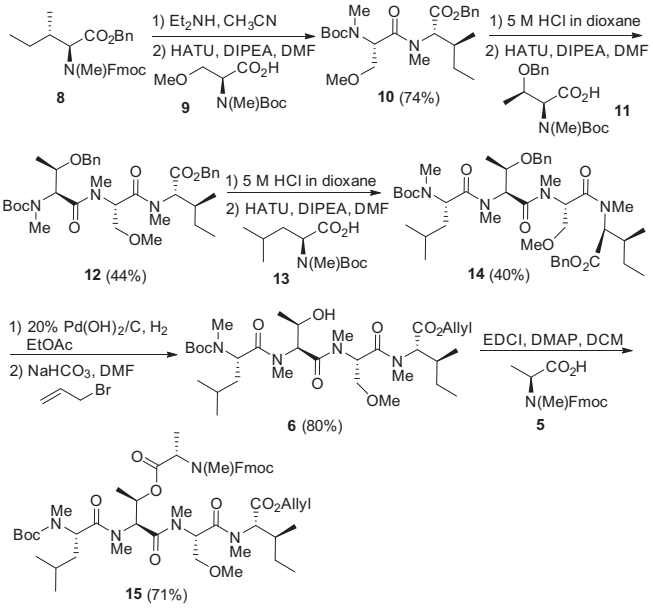
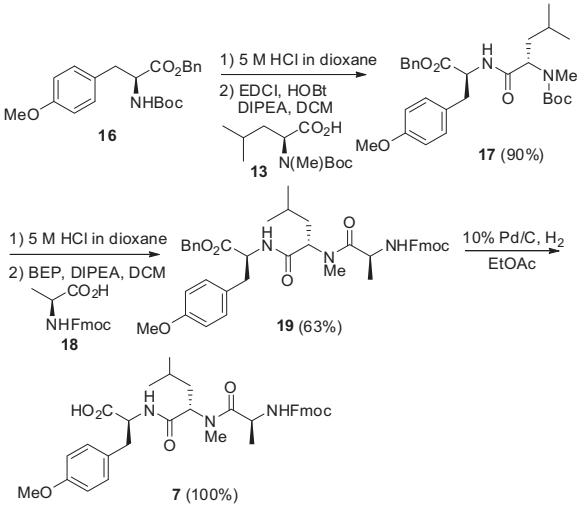


Figure 2. Retrosynthetic analysis of coibamide A.



Scheme 1. Synthesis of tetrapeptide **6**.



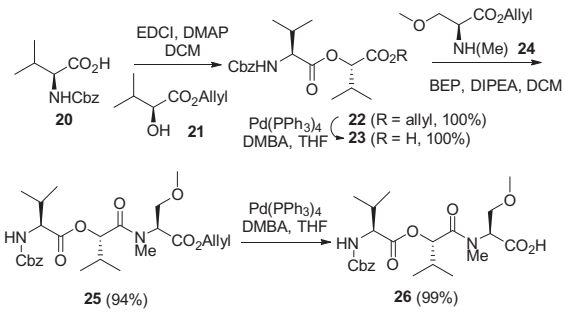
Scheme 2. Synthesis of tripeptide acid **7**.

Our retrosynthetic analysis of coibamide A is shown in Figure 2. At first, the side chain is disconnected from the macrocycle at the Ser(OMe)-*N*-MeLeu junction, affording fragment **2** and macrocycle **3** having a residue of *N*-MeLeu, which would reduce the difficulty of future segment-coupling with **2**. Further disconnection of the macrocycle **3** renders significant strategic importance and may dictate the success of the synthesis. According to the common guidelines of peptide synthesis, a proper cyclization position should avoid those sterically encumbered by the N-methylated amide bond.[10] Esterification is firstly excluded from the potential cyclization protocols, because unfavorable kinetics of lactone-formation based cyclization (at site A) may imply a major risk of racemization. For larger distribution of Z-amide conformer in the N-methylated peptides (compared to the non-N-methylated peptides), cyclization at *N*-MeLeu-Tyr(OMe) junction (site B) would be also unfavorable because of a high risk of 2,5-diketopiperizine formation resulting in the degradation of the linear peptide. Therefore, a potentially suitable macrocyclization position is determined at the *N*-MeIle-Ala amide bond (site C), affording the linear precursor **4**. Precursor **4** can be further broken into three fragments: Fmoc-*N*-Me-Ala-OH (**5**), tetrapeptide **6**, and tripeptide **7**.

The total synthesis began with the preparation of tetrapeptide **6** (Scheme 1). In order to overcome the possible epimerization of the active ester of N-methylated amino acid component (via oxazolone formation) during the couplings, stepwise coupling protocol was applied in the synthesis.[13] Coupling of *N*-Boc-*N*-Me-Ser(OMe) (**9**) with the *N*-Fomc deblocked product derived from **8** yielded dipeptide **10**. Removal of the *N*-Boc group of **10** with 5 M HCl in dioxane followed by condensation with the active ester of *N*-Boc-*N*-Me-Thr(OBzl) (**11**) afforded tripeptide**12**. Using a similar operation, tetrapeptide **14** was obtained after coupling with acid **13**. Considering the orthogonality of the protecting groups, the benzyl ester of **14** was altered with an allyl ester by hydrogenolysis of **14** and subsequent selective O-allylation with allyl bromide in the presence of $NaHCO_3$ in DMF. In order to reduce the steric hindrance of segments coupling, the resulting segment **6** was firstly coupled with the single *N*-methylamino acid **5**, providing the corresponding ester **15**. Coupling with a single *N*-methylamino acid is also advantageous in reducing the extent of epimerization during this ester-bond formation.

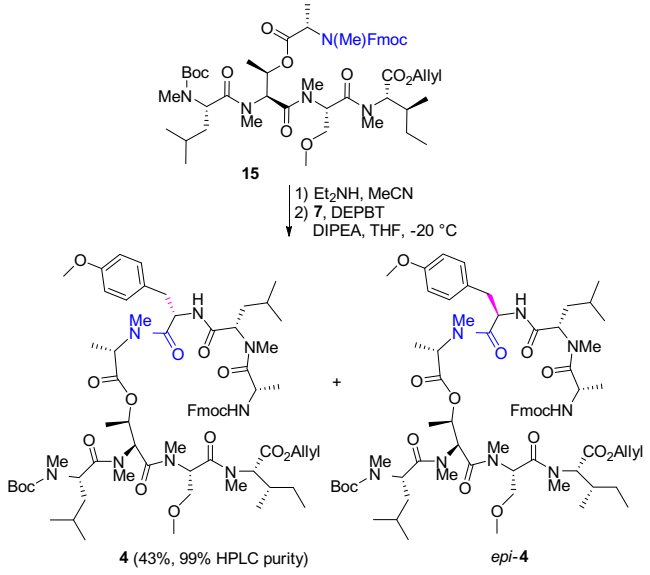
Tripeptide fragment **7** was synthesized in a fashion from the C-terminal to the N-terminal, beginning with the tyrosine derivative **16** (Scheme 2). Use of an *N*-Boc derivative **13** resulted in higher yield and minimized DKP formation in the second step of coupling. The benzyl ester protecting group of tripeptide **19** was then removed by hydrogenolysis, affording tripeptide acid **7** in quantitative yield.

To improve the coupling efficiency, we decided to synthesize the side-chain fragment **26** (a derivative of **2**, Fig. 2) from its N-terminal to C-terminal (Scheme 3). We reasoned that the presence of ester bond could be exploited to decrease the extent of oxazolone formation, which might otherwise lead to epimerization. The chiral α-hydroxy acid **21** was prepared readily from L-Val.[14] Esterification of alcohol **21** with acid **20** was carried out in the presence of EDCI and a catalytic amount of DMAP, affording ester **22** in quantitative yield. Removal of the *O*-allyl group of **22** with



Scheme 3. Synthesis of side-chain acid **26**.

Table 1
Screening conditions for the coupling of **7** and **15**



Entry	Conditions	4/*epi*-4	
		Combined yield[a] (%)	HPLC ratio[b]
1	PyBOP, NMM, DCM, rt	59	61:39
2	BOP, NMM, DCM, rt	67	61:39
3	BEP, DIPEA, DCM, −15 °C to rt	28	95:5
4	HATU, DIPEA, DCM, 0 °C to rt	62	66:34
5	DEPBT, DIPEA, THF, −20 °C	43	99:1
6	DEPBT, DIPEA, THF, 0 °C	75	93:7
7	DEPBT, DIPEA, THF, rt	89	87:13
8	Bop-Cl, DIPEA, DCM, rt	13	81:19
9	EDCI, HOAt, DIPEA, DCM, rt	75	63:37
10	EDCI, HOAt, DIPEA, DCM, −20 °C	53	75:25

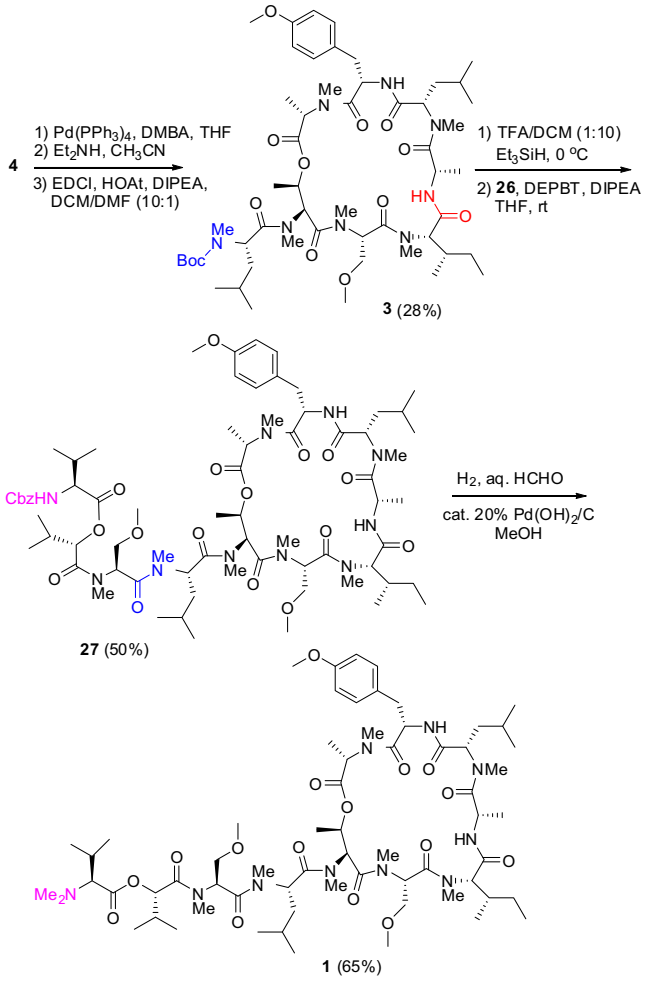
[a] Isolated yields over 2 steps.
[b] HPLC conditions: C18 column, $\lambda = 220$ nm, isocratic 90% CH_3CN/H_2O at a flow rate of 1.0 mL/min.

1 mol % $Pd(PPh_3)_4$ provided quantitative yield of acid **23**, which was further coupled with the freshly prepared amine **24** with BEP (2-bromo-1-ethylpyridinium tetrafluoroborate)[15] and DIPEA in DCM, affording **25** in 94% yield. Deprotection of the *O*-allyl group of **25** gave the required acid **26**.

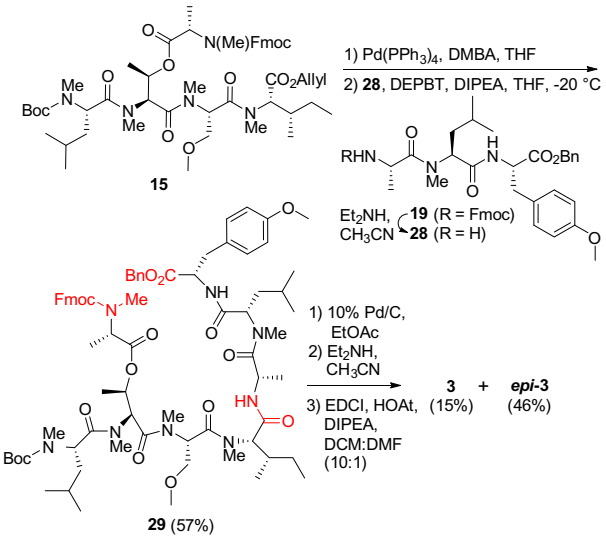
With segments **7** and **15** in hand, we continued to synthesize the key linear precursor **4** for the macrocyclization. Surprisingly, low efficiency was observed in the coupling between acid **7** and the amine derived from **15**, and the degree of epimerization was also increased. After screening of various coupling conditions (Table 1),[16] the two segments was finally jointed upon treatment with DEPBT and DIPEA in THF at −20 °C (entry 5).[17] Though increasing the reaction temperature (entries 5–7) could improve the yield, the extent of epimerization was also increased. Using the optimized conditions, we were able to synthesize the linear precursor **4** in 43% isolated yield over two steps with 99% HPLC purity.

After removing *O*-allyl and *N*-Fmoc groups from the linear precursor **4**, final cyclization was carried out in the presence of EDCI, HOAt, and DIPEA in DCM/DMF (10:1), affording 28% yield of product **3** and a dimerized by-product (~29% yield) (Scheme 4). Use of other condensation reagents[18] and reducing the reaction concentration could not reduce the ratio of dimer and improve the results. The *N*-Boc protecting group was then removed from the macrocycle **3**, and the resulting free amine was immediately coupled to acid **26** using DEPBT as the coupling reagent, affording the whole skeleton **27**.[19] Finally, hydrogenolysis of the *N*-Cbz group of **27** followed by in situ reductive amination with formaldehyde afforded the proposed structure of coibamide A (**1**).

In order to confirm the stereochemical configuration of cyclic peptide **3**, we decided to re-synthesize the same macrocycle using different synthetic sequences. The Tyr(OMe)-*N*-MeAla amide bond was chosen as the alternative cyclization site, and the *N*-MeIle-Ala junction was applied for the coupling of segments. To our delight, the same cyclic peptide **3** was finally afforded in a low yield (15%) along with *epi*-**3** (46%, epimerized at the α-position of Tyr) through the new route (Scheme 5). Generation of a



Scheme 4. Total synthesis of the proposed structure of coibamide A (**1**).



Scheme 5. Alternative route to the cyclized product **3**.

common product **3** through two different routes (Schemes 4 and 5) logically excluded the possibility of epimerization during the previous macrocyclization (Scheme 4), and also verified the stereochemical structure of **3**.

Unfortunately, analytical data of synthetic sample of **1** were inconsistent with those reported for natural coibamide A.[6] Significant differences were observed in both ^{1}H and ^{13}C NMR spectra of our synthetic sample from those of natural product. Based on the results from our study, further determination and reassignment of the full structure of coibamide A would be needed.[20] Interestingly, some of the synthetic samples were found to exhibit low micromolar cytotoxicities against three cancer cell lines in our biological screenings, including the final product **1** (IC_{50} 17.98 μM (MDA-MB-231); 11.77 μM (MCF-7) and 22.80 μM (A549)) and the cyclic intermediate **3** (IC_{50} 8.30 μM (MDA-MB-231); 8.35 μM (MCF-7); and 16.79 μM (A549)).

In summary, a total synthesis of the literature structure of coibamide A has been accomplished, for the first time, using a convergent [(4+1)+3+3]-peptide fragment-coupling strategy. Formations of multiple dense N-methylated amide-bonds of this unique marine natural cyclodepsipeptide were carefully examined and optimized to avoid possible epimerization during the fragment couplings. Reverse-sequence synthesis of the linear peptides and application of an alternative macro-cyclization site have been proven to be two useful tools for examination of the alpha-carbon racemization of the active esters involved in the synthesis. Though the final product **1** could not match the natural product in both ^{1}H and ^{13}C NMR spectra, the synthetic sample of the proposed coibamide A and a late-stage cyclic intermediate was identified to exhibit low-micromolar inhibitory activity against the proliferation of three tested cancer cells.

Acknowledgments

This work was financially supported by the Ministry of Science and Technology of the People's Republic of China (2010CB833200, 2013AA092903) and the National Natural Science Foundation of China (21032002).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.09.047.

References and notes

1. Simmons, T. L.; Coates, R. C.; Clark, B. R.; Engene, N.; Gonzalez, D.; Esquenazi, E.; Dorrestein, P. C.; Gerwick, W. H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4587–4589.
2. Bull, A. T.; Stach, J. E. M. *Trends Microbiol.* **2007**, *15*, 481–499.
3. Luesch, H.; Harrigan, G. G.; Goetz, G.; Horgen, F. D. *Curr. Med. Chem.* **2002**, *9*, 1791–1806.
4. Nunnery, J. K.; Mevers, E.; Gerwick, W. H. *Curr. Opin. Biotechnol.* **2010**, *21*, 787–793.
5. Rawat, D. S.; Joshi, M. C.; Joshi, P.; Atheaya, H. *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 33–40.
6. Medina, R. A.; Goeger, D. E.; Hills, P.; Mooberry, S. L.; Huang, N.; Romero, L. I.; Ortega-Barría, E.; Gerwick, W. H.; McPhail, K. L. *J. Am. Chem. Soc.* **2008**, *130*, 6324–6325.
7. McPhail, K. L.; Medina, R. A.; Gerwick, W. H.; Goeger, D. E.; Capeon, T. L. US 08034780, 2011.
8. Hau, A. M.; Greenwood, J. A.; Löhr, C. V.; Serrill, J. D.; Proteau, P. J.; Ganley, I. G.; McPhail, K. L.; Ishmael, J. E. *PLOS ONE* **2013**, *8*, 1–16.
9. Chatterjee, J.; Gilon, C.; Hoffman, A.; Kessler, H. *Acc. Chem. Res.* **2008**, *41*, 1331–1342.
10. Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243–2266.
11. Teixidó, M.; Albericio, F.; Giralt, E. *J. Peptide Res.* **2005**, *65*, 153–166.
12. For reviews on coupling reagents, see: (a) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557–6602; (b) Valeur, E.; Bradley, M. *Chem. Soc. Rev.* **2009**, *38*, 606–631; (c) Prasad, K. V. S. R. G.; Bharathi, K.; Haseena, B. B. *Int. J. Pharm. Sci. Rev. Res.* **2011**, *8*, 108–119.
13. Synthesis of **6** by sequential N→C extension afforded the racemic product, and it was less efficient than the route by an C→N sequence.
14. Liang, B.; Portonovo, P.; Vera, M. D.; Xiao, D.; Joullié, M. M. *Org. Lett.* **1999**, *1*, 1319–1322.
15. Li, P.; Xu, J. C. *Tetrahedron* **2000**, *56*, 8119–8131.
16. Colucci, W. J.; Tung, R. D.; Petri, J. A.; Rich, D. H. *J. Org. Chem.* **1990**, *55*, 2895–2903.
17. (a) Nagam, A. C.; Radford, S. E.; Warriner, S. L. *Synlett* **2007**, 2517–2520; (b) Wu, X. Y.; Stockdill, J. L.; Wang, P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2010**, *132*, 4098–4100; (c) Wu, X. Y.; Stockdill, J. L.; Park, P. K.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 2378–2384.
18. Representative cyclization conditions in the syntheses of cyclic peptides, see: (a) Sleebs, M. M.; Scanlon, D.; Karas, J.; Maharani, R.; Hughes, A. B. *J. Org. Chem.* **2011**, *76*, 6686–6693; (b) Wen, S.-J.; Yao, Z.-J. *Org. Lett.* **2004**, *6*, 2721–2724; (c) Marcucci, E.; Tulla-Puche, J.; Albericio, F. *Org. Lett.* **2012**, *14*, 612–615; (d) Suenaga, K.; Mutou, T.; Shibata, T.; Itoh, T.; Fujita, T.; Takada, N.; Hayamizu, K.; Takagi, M.; Irifune, T.; Kigoshi, H.; Yamada, K. *Tetrahedron* **2004**, *60*, 8509–8527.
19. An alternative stepwise synthesis of **27** has also been completed from the linear precursor **4** using same condensation conditions to couple the side chain and elongate the side chain. Because the two synthesis routes afforded the same product **27**, we concluded that epimerization did not happen during the side-chain couplings with DEPBT and DIPEA in THF.
20. For inadequate evidences, we are unable to conclude any structural revision at this moment referring to the NMR differences between the proposed structure (the synthesized **1** from this work) and the natural product.