Total Synthesis of the Proposed Structure for Itralamide B

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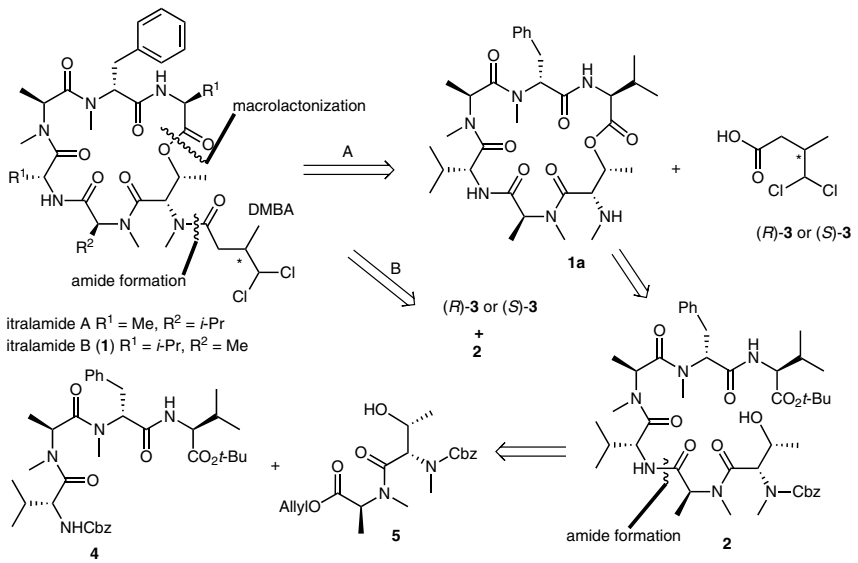
Received: 28.12.2013; Accepted after revision: 05.02.2014

Abstract: A stereocontrolled total synthesis of the cyclodepsipeptide, itralamide B, has been achieved. Both *R*- and *S*-stereoisomers of the side chain were attached to the macrocyclic ring, however, the synthesized structure appears to be different from that of the marine natural product.

Key words: natural products, total synthesis, macrolactonization, configuration determination, stereoselectivity

Naturally occurring cyclodepsipeptides often exhibit unique biological properties and are thus attractive candidates for drug development.[1] The bioactivities of natural cyclodepsipeptides range from insecticidal, antiviral and antibacterial to cytotoxic and antiproliferative. The conformation of the cyclodepsipeptides is largely determined by the skeleton of the macrocycle and the side-chain moieties, some of which may contain different conformers at ambient temperature in solution. This conformational flexibility can complicate structural elucidation and absolute stereochemistry assignment based on NMR techniques and computation-assisted conformational analyses.[2] Total synthesis plays a critical role in marine natural product structure elucidation, and this approach has led to the overwhelming majority of natural product structural revisions. In addition, total synthesis provides materials for biological testing towards pharmaceutical development. We have undertaken the total synthesis of natural marine cyclodepsipeptides,[3] and have successfully revised the structure of several natural products.[4]

Itralamides A and B (**1**; Scheme 1) were isolated from the lipophilic extract of *Lyngbya majuscula* collected from the eastern Caribbean.[5] The structures of these molecules were determined mainly on the basis of NMR, MS spectral data, and chemical manipulation involving the use of Marfey's method.[6] Itralamides A and B featured high content of N-methylation of their amino acid fragments, with four of the six amino acids being modified. The *N*-methylthreonine was identified as an unusual occurrence in nat-



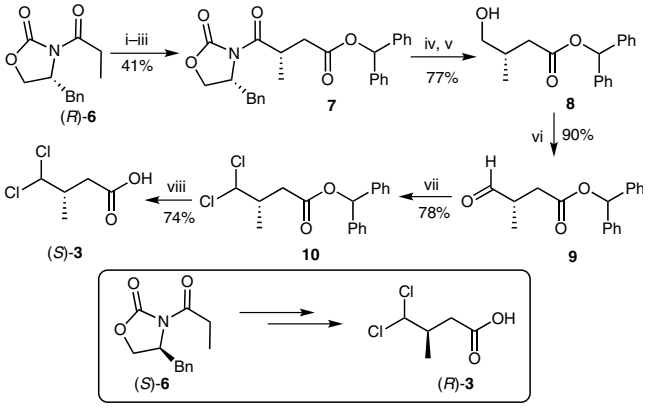
Scheme 1 Retrosynthetic analysis of itralamide B (**1**)

SYNLETT 2014, 25, 1014–1018
Advanced online publication: 14.03.2014
DOI: 10.1055/s-0033-1340872; Art ID: ST-2013-W1173-L

ural product structures; it has been found in only a few bioactive natural cyclopeptides, including coibamide A[7] and papuamides A–D.[8] Itralamides were found to contain an interesting branched chlorinated moiety, 4,4-dichloro-3-methylbutanoic acid (DMBA), linked through an amide bond to *N*-methylthreonine. This chlorinated unit could potentially be derived from valine presumably involves the novel chlorination of unreactive carbon. The presence of this chlorinated moiety also suggested that itralamides may originate from cyanobacteria.[9] Itralamide B (**1**) was found to be cytotoxic toward human embryonic kidney (HEK293) with an IC_{50} value of around 6 μM. Previously, we reported the total synthesis of dichlorinated cyclodepsipeptides lyngbyabellin A[10] and sintokamide C.[11] Here we report on our efforts in the total synthesis of itralamide B with the aim of assigning the absolute stereochemistry of the methyl group of DMBA.

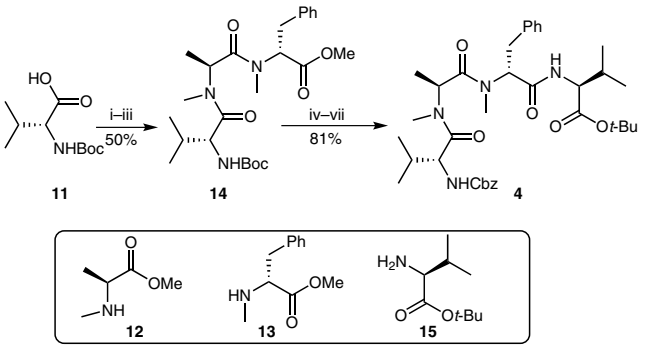
The retrosynthetic analysis is illustrated in Scheme 1.[12] Because the absolute stereochemistry of the side chain was not established, we planned a convergent synthetic approach involving the attachment of two possible diastereomers of the side chain [(*R*)-**3** and (*S*)-**3**] to the cyclodepsipeptide core **1a** in the late stage (Scheme 1, route A). Accordingly, **1a** can be constructed from acyclic precursor **2** through a macrolactonization reaction. Hexapeptide **2** was envisaged to be synthesized from tetrapeptide **4** and dipeptide **5**. However, although this strategy is appealing, macrocyclic intermediate **1a** might undergo *O*,*N*-acyl migration.[13] In this respect, we followed an unambiguous approach that included the installation of the two possible stereoisomers of side chain prior to the formation of the macrocycle (Scheme 1, route B).

The synthesis of 4,4-dichloro-3-methylbutanoic acid (**3**) commenced with an Evans alkylation reaction[14,4d] (Scheme 2). Treatment of (*R*)-4-benzyl-3-propionyloxazolidin-2-one (**6**) with NaHMDS at –78 °C, followed by addition of *tert*-butyl bromoacetate, produced the corresponding alkylation adduct, which was transformed into benzhydryl ester **7** through a two-step sequence including cleavage of the *tert*-butyl ester group through the action of trifluoroacetic acid followed by treatment of the resultant acid with benzophenone hydrazine, in the presence of (diacetoxyiodo)benzene and iodine.[15,4g] Subsequent hydrolysis of the chiral auxiliary of **7**, with sodium carbonate and hydrogen peroxide, afforded the corresponding acid, which was then reduced to alcohol **8** by borane dimethylsulfide in 77% yield over the two steps.[16] Oxidation of alcohol **8** with TCCA and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), buffered with sodium bicarbonate in dichloromethane,[17] gave aldehyde **9** in 90% yield, which was subjected to chlorination with triphenyl phosphite and chlorine in the presence of triethylamine in dichloromethane to afford the dichlorinated product **10** in 78% yield.[18,11] Acidic cleavage of benzhydryl ester **10** afforded the corresponding carboxylic acid (*S*)-**3** in 74% yield, which was ready to be incorporated into the macrocyclic core of itralamide B. The enantiomer (*R*)-**3** was prepared according to the same reaction sequence by the use of (*S*)-**6** as starting material.



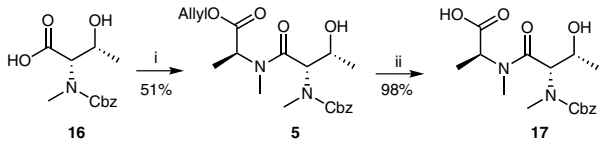
Scheme 2 Synthesis of DMBA fragments (*R*)-**3** and (*S*)-**3**. *Reagents and conditions*: (i) NaHMDS, THF, –78 °C, $BrCH_2CO_2{}^tBu$ (dr > 96:4); (ii) TFA, CH_2Cl_2; (iii) $Ph_2C{=}NNH_2$, DIB, I_2; (iv) Na_2CO_3, H_2O_2, THF–H_2O; (v) $BH_3{\cdot}Me_2S$, THF; (vi) TCCA, TEMPO, $NaHCO_3$, CH_2Cl_2; (vii) $P(OPh)_3$, Cl_2, Et_3N, CH_2Cl_2; (viii) TFA, CH_2Cl_2.

With the requisite subunit **3** in hand, we next turned our attention to the construction of peptide fragments **4** and **5**. The synthesis began with the preparation of different *N*-methylated amino acids, including L-Ala, D-Phe, and L-Thr, by employing a facile, two-pot reductive amination approach.[19] Coupling of D-*N*-Boc-Val-OH (**11**) with *N*-Me-Ala-OMe (**12**) in the presence of HATU[20] and HOAt produced the dipeptide in 72% yield. Hydrolysis of the methyl ester, followed by peptide bond formation with *N*-Me-Phe-OMe (**13**) by using HATU and HOAt as coupling reagents, furnished tripeptide **14** in 69% yield over two steps. The *N*-terminal Boc group of tripeptide **14** was exchanged for a Cbz group through a two-step manipulation, which included trifluoroacetic acid promoted cleavage of Boc group and reprotection of the resulting free amine as its Cbz carbamate through the action of CbzOSu and sodium bicarbonate. Removal of the methyl ester produced the corresponding carboxylic acid, which was then coupled with amino ester **15** by the action of PyAOP[21] to furnish tetrapeptide **4** in 81% yield (Scheme 3).



Scheme 3 Preparation of tetrapeptide **4**. *Reagents and conditions*: (i) **12**, HATU, HOAt, DIPEA, CH_2Cl_2; (ii) LiOH, THF–H_2O; (iii) **13**, HATU, HOAt, DIPEA, CH_2Cl_2; (iv) TFA, CH_2Cl_2; (v) CbzOSu, $NaHCO_3$, MeCN; (vi) LiOH, THF–H_2O; (vii) **15**, PyAOP, HOAt, DIPEA, CH_2Cl_2.

The preparation of dipeptide **5** is illustrated in Scheme 4. Initially, condensation of *N*-Me-*N*-Cbz-Thr-OH (**16**) with the *tert*-butyl *N*-methyl alanine ester by the action of BOPCl[22] and DIPEA produced the corresponding dipeptide, however, selective removal of the *tert*-butyl ester under acidic conditions proved difficult because various reaction conditions led to extensive dehydration of the threonine residue. To circumvent this problem, allyl ester was selected as the appropriate protecting group for this substrate; under similar condensation conditions, dipeptide **5** was obtained in 51% yield. The allyl ester could be easily removed to afford **17** in 98% yield by the action of $Pd_2(dba)_3$ and Ph_3P in the presence of a secondary amine in THF.

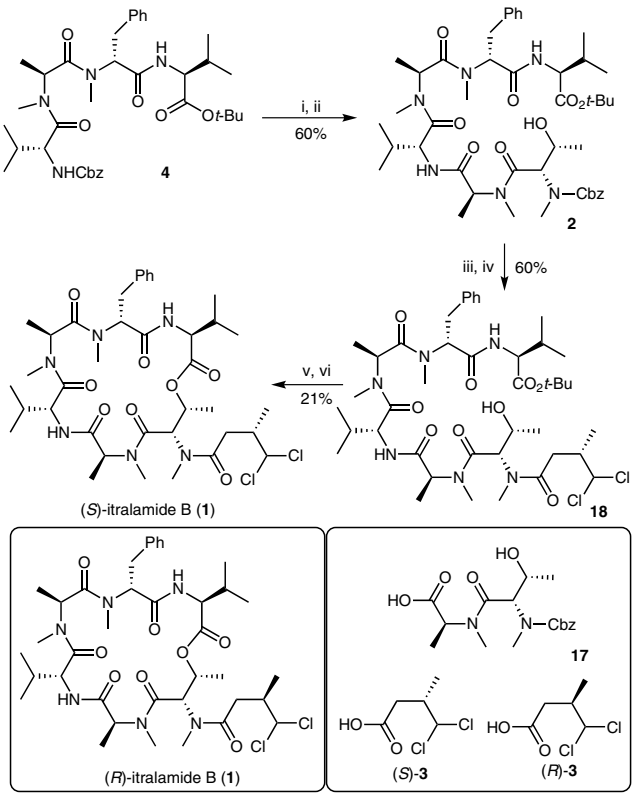


Scheme 4 Preparation of dipeptide **5**. *Reagents and conditions*: (i) *N*-Me-Ala-OAllyl, BOPCl, DIPEA, CH_2Cl_2; (ii) $Pd_2(dba)_3$, Ph_3P, Et_2NH, THF, 0 °C.

Hydrogenolysis of the Cbz group of **4** and condensation of the crude free amine with acid **17** through the action of HATU/HOAt, afforded **2** in 60% yield. Cleavage of the *N*-Cbz protecting group of **2** was facilitated by the use of Pd/C catalyst in methanol, and the resulting amine was then condensed with (*S*)-4,4-dichloro-3-methylbutanoic acid [(*S*)-**3**], employing HATU and HOAt as the coupling reagents, to give **18** in 60% yield. After removal of the *tert*-butyl ester of **18** by the action of $BF_3{\cdot}OEt_2$ in dichloromethane,[23] the resulting hydroxy acid was subjected to macrolactonization by carboxylic acid activation under Shiina's conditions,[24] to afford the (*S*)-DMBA-containing stereoisomer, (*S*)-itralamide B (**1**), in 21% yield.[25] The (*R*)-itralamide B (**1**) was also prepared from (*R*)-4,4-dichloro-3-methylbutanoic acid [(*R*)-**3**] by the same route used for the synthesis of the *S*-stereoisomer (Scheme 5).

To our disappointment, the ^1H and ^{13}C NMR spectra of both synthetic stereoisomers [(*S*)-itralamide B and (*R*)-itralamide B] were different from those of natural itralamide B. Significant discrepancies in the ^{13}C NMR spectra were most apparent for the two isopropyl groups of valine, the two *N*-methyl groups of alanine, and the ester carbonyl and methyl group of threonine.[26] It would appear that the error in the original assignment of the stereochemistry of itralamide B lies somewhere in the macrocycle.

In summary, we have developed a stereocontrolled route for the total synthesis of the proposed structure for itralamide B. Efforts toward the total synthesis of additional diastereoisomers are underway in our laboratory. A more detailed account of this work and further studies toward the establishment of the stereochemistry of itralamide B and biological evaluation of diastereoisomers will be disclosed in due course.



Scheme 5 Completion of the total synthesis of itralamide B (**1**). *Reagents and conditions*: (i) H_2, Pd/C, MeOH; (ii) **17**, HATU, HOAt, DIPEA, CH_2Cl_2; (iii) Pd/C, H_2, MeOH; (iv) (*S*)-**3**, HATU, HOAt, DIPEA, CH_2Cl_2; (v) $BF_3{\cdot}OEt_2$, CH_2Cl_2; (vi) MNBA, DMAP, toluene–THF.

Acknowledgment

We acknowledge financial support from the NSFC (21062006, 21072007, 21272011), the Hong Kong Research Grants Council (Projects: PolyU 5040/10P; PolyU 5037/11P, PolyU 5020/12P; PolyU5030/13P), the Fong Shu Fook Tong Foundation, the Joyce M. Kuok Foundation, and The Shenzhen Bureau of Science, Technology and Information (JC200903160367A, JC201005260220A, JC201005260102A, ZYC201105170351A). X.J.W. would also like to thank the Jiangxi Province Departments of Education (KJLD12036) and Science & Technology ([2013]138).

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(25) **Synthesis of Itralamide B (1)**: Compound **18** (23.0 mg, 0.03 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (1.0 mL) at 0 °C, and $BF_3{\cdot}OEt_2$ (34 μL, 0.3 mmol, 10.0 equiv) was added dropwise to the solution at 0 °C. The reaction solution was allowed to warm to r.t. and stirred for 0.5–1.0 h (reaction monitored by TLC). The reaction was quenched by the addition of saturated NH_4Cl (2 mL) and diluted with CH_2Cl_2 (60 mL). The organic phase was washed with saturated NH_4Cl (3 × 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford the crude hydroxy acid. A solution of this hydroxy acid in THF–PhMe (10 mL, 1:1) was slowly added to a solution of DMAP (33 mg, 0.3 mmol, 10.0 equiv) and MNBA (47 mg, 0.14 mmol, 5.0 equiv) in PhMe (10 mL) at 0 °C. The reaction mixture was warmed to r.t., then stirred and heated to 60 °C for 2 d. The reaction mixture was diluted with EtOAc (100 mL) and washed successively with saturated NH_4Cl (3 × 20 mL) and brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc) to afford (*S*)-itralamide B (**1**; 5.0 mg, 21%).
(*S*)-Itralamide B (1): $[\alpha]_D^{25}$ –44.63 (*c* 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45–7.12 (m, 5 H), 6.90 (d, *J* = 9.6 Hz, 1 H), 6.47 (d, *J* = 8.0 Hz, 1 H), 5.99 (d, *J* = 2.9 Hz, 1 H), 5.75–5.65 (m, 2 H), 5.53–5.43 (m, 1 H), 5.08 (q, *J* = 6.8 Hz, 1 H), 4.98 (dd, *J* = 7.9, 4.2 Hz, 1 H), 4.71 (dd, *J* = 9.4, 4.1 Hz, 1 H), 4.62 (q, *J* = 7.6 Hz, 1 H), 3.66 (dd, *J* = 15.5, 4.9 Hz, 1 H), 3.33 (d, *J* = 23.6 Hz, 3 H), 3.19 (d, *J* = 7.1 Hz, 3 H), 3.17–3.07 (m, 3 H), 3.07–2.94 (m, 3 H), 2.87 (dd, *J* = 15.6, 12.1 Hz, 1 H), 2.80 (d, *J* = 4.0 Hz, 1 H), 2.76–2.65 (m, 1 H), 2.46 (dd, *J* = 16.5, 7.5 Hz, 1 H), 2.28–2.21 (m, 1 H), 2.06–1.99 (m, 1 H), 1.29 (d, *J* = 2.0 Hz, 3 H), 1.26 (d, *J* = 2.8 Hz, 3 H), 1.20 (dd, *J* = 6.6, 3.5 Hz, 3 H), 1.08–1.01 (m, 6 H), 0.92 (dd, *J* = 9.4, 7.2 Hz, 6 H), 0.80 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.89, 172.84, 172.53, 170.65, 170.17, 169.90, 169.47, 137.35, 128.54, 128.34, 126.48, 78.18, 69.62, 56.92, 56.75, 54.69, 54.68, 53.98, 51.43, 40.65, 35.75, 33.92, 33.77,

32.15, 31.83, 31.12, 31.00, 30.54, 19.90, 19.63, 17.82, 17.10, 15.61, 15.35, 14.10, 13.82. HRMS (ESI): *m*/*z* $[M+Na]^+$ calcd for $C_{38}H_{58}Cl_2N_6NaO_8^+$: 819.3585; found: 819.3587.

(*R*)-Itralamide B (1): $[\alpha]_D^{25}$ –42.33 (*c* 0.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.24–7.16 (m, 5 H), 6.89 (d, *J* = 9.1 Hz, 1 H), 6.48 (d, *J* = 7.8 Hz, 1 H), 6.04 (d, *J* = 2.9 Hz, 1 H), 5.78–5.63 (m, 1 H), 5.49 (dd, *J* = 6.6, 3.2 Hz, 1 H), 5.45–5.35 (m, 1 H), 5.12–5.02 (m, 1 H), 4.98 (dd, *J* = 7.8, 4.2 Hz, 1 H), 4.71 (dd, *J* = 9.3, 4.0 Hz, 1 H), 4.67–4.55 (m, 1 H), 3.66 (dd, *J* = 15.3, 5.1 Hz, 1 H), 3.28 (d, *J* = 63.9 Hz, 3 H), 3.18 (s, 3 H), 3.13 (d, *J* = 23.1 Hz, 3 H), 3.01 (d, *J* = 11.3 Hz, 3 H), 2.93 (d, *J* = 15.7 Hz, 1 H), 2.90–2.78 (m, 1 H), 2.78–2.67 (m, 1 H), 2.43 (dd, *J* = 16.7, 6.0 Hz, 1 H), 2.25 (dd, *J* = 11.9, 5.9 Hz, 1 H), 2.04 (d, *J* = 9.5 Hz, 1 H), 1.38 (dd, *J* = 18.4, 7.2 Hz, 3 H), 1.30 (d, *J* = 6.7 Hz, 3 H), 1.20 (d, *J* = 6.6 Hz, 3 H), 1.06 (d, *J* = 6.8 Hz, 6 H), 0.92 (dd, *J* = 11.7, 7.0 Hz, 6 H), 0.80 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.88, 172.83, 172.56, 170.68, 170.17, 169.85, 169.51, 137.37, 128.55, 128.34, 126.48, 78.02, 69.64, 56.88, 56.75, 54.75, 54.22, 53.99, 51.47, 40.45, 35.98, 33.97, 33.78, 32.18, 31.85, 31.12, 31.02, 30.55, 19.89, 19.63, 17.82, 17.09, 17.07, 15.60, 15.05, 13.83 ppm. HRMS (ESI): *m*/*z* $[M+Na]^+$ for $C_{38}H_{58}Cl_2N_6NaO_8^+$: 819.3585; found: 819.3589.

(26) See the Supporting Information for detailed analyses.

