Synthesis of the All-L Cyclopentapeptides Versicoloritides A, B, and C

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Received: 08.06.2012; Accepted: 01.07.2012

Abstract: Herein we describe the first synthesis of the all-L naturally occurring cyclopentapeptides versicoloritides A, B, and C. We found that the extent of oligomerization and epimerization upon head-to-tail cyclization of the linear pentapeptide precursors could be reduced to acceptable levels by modifying the reaction conditions.

Key words: cyclic peptides, versicoloritides, natural products, cyclization, total synthesis

In an on-going search for bioactive natural products from surface-associated marine microorganisms, three new cyclopentapeptides, versicoloritides A (**1**), B (**2**), and C (**3**) were recently isolated from the coral-associated fungus *Aspergillus versicolor* (Figure 1).[1] The versicoloritides differ from each other by a single residue and were found to consist of only L-amino acids with the general sequence cyclo-(FPFPX), where X is L-alanine, glycine, or L-serine in versicoloritides A (**1**), B (**2**), and C (**3**), respectively. Cyclopentapeptides often contain D-amino acids, N-methylated amino acids, and/or unnatural amino acids in addition to L-amino acids.[2] Natural product cyclopentapeptides consisting of only L-amino acids are rarely isolated, and only a few other naturally occurring all-L cyclopentapeptides have been found to date.[3] Our on-going interest in the synthesis of natural product cyclic peptides, the rare occurrence of all-L cyclopentapeptides and the reported challenges associated with the cyclisation of all-L linear pentapeptides prompted our interest in the synthesis of the versicoloritides.[3a,4] We herein report the first synthesis of the three known members of the versicoloritide family, versicoloritides A (**1**), B (**2**), and C (**3**).

There are many methods available for the synthesis of head-to-tail cyclic peptides, of which the final macrolactamization reaction is often carried out in solution phase under high dilution conditions or by using on-resin cyclization techniques such as side-chain anchoring of a trifunctional amino acid or the use of 'safety-catch' linkers.[4a,5] Irrespective of the cyclization technique employed, the synthesis of head-to-tail cyclic peptides containing less than seven residues is known to be challenging due to the small-to-medium ring sizes involved.[4a,b,5,6] In particular, the intramolecular cyclization of linear pentapeptides is associated with oligomerization and C-terminal epimerization.[3a,c,4a] The extent of oligomerization and epimerization during the cyclization of pentapeptides is primarily governed by the sequence of the linear precursor and is further influenced by the choice of coupling reagents, base, solvent, the presence of metal ions, racemization suppressants, and reaction temperature.[3a,c,7] Retrosynthetic disconnection of the versicoloritides, however, affords linear pentapeptides containing all-L amino acids. Linear peptides containing all-L or all-D amino acids prefer to adopt extended conformations in order to minimize allylic strain.[4a] This preferred extended conformation, however, is counteractive for head-to-tail cyclization, as the success of macrolactamization is promoted by the ability of a linear precursor to adopt an intermediate conformation similar to that of the corresponding cyclic peptide.[4a,8]

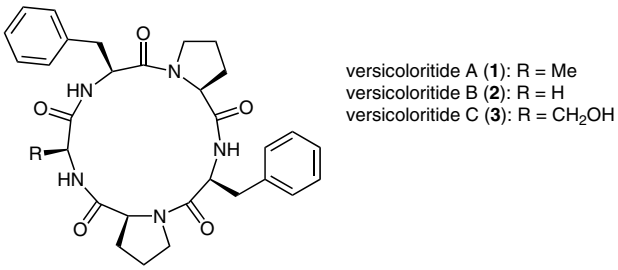


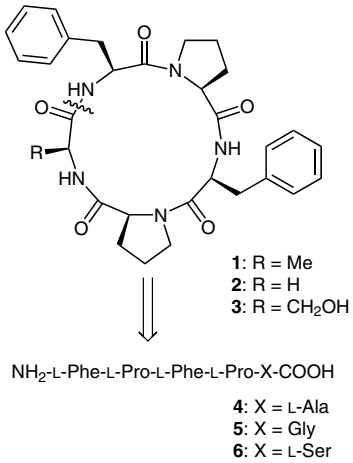
Figure 1 The versicoloritide family of cyclic peptides, versicoloritides A (**1**), B (**2**), and C (**3**)

Fortunately, the pentapeptide precursors to versicoloritides A (**1**), B (**2**), and C (**3**) contain proline residues and a glycine residue in the case of versicoloritide B. These residues are known to induce and stabilize turn structures thereby aligning the N- and C-termini in closer spatial proximity.[4a,9] The linear precursors **4**, **5**, and **6** to versicoloritides A (**1**), B (**2**), and C (**3**), respectively, were therefore chosen with the turn-inducing proline residues located in the middle of the linear sequence rather than positioned at the N- and C-termini (Scheme 1).[4a] To minimize steric hindrance during cyclization the smaller variable residues (alanine, glycine, and serine) were chosen as the C-terminal residues of the pentapeptide precursors **4**, **5**, and **6**. Moreover, the presence of a glycine residue at the C-terminus of **5** also prevents any opportu-

SYNLETT 2012, 23, 2284–2288
Advanced online publication: 14.08.2012
DOI: 10.1055/s-0032-1316994; Art ID: ST-2012-D0486-L

nity for racemization during the preparation of versicoloritide B (**2**).[4a]

The linear precursors **4**, **5**, and **6** were assembled manually by Fmoc-SPPS (see Supporting Information). Briefly, the variable C-terminal residues were attached via an acid-labile Wang-like linker to in-house prepared aminomethyl polystyrene resin.[10]



Scheme 1 Disconnection sites for the synthesis of versicoloritides A (**1**), B (**2**), and C (**3**)

Successful couplings were followed by deprotection of the Fmoc group with 20% piperidine in DMF and each subsequent amino acid (4 equiv) was then attached to the growing resin-bound peptide using a mixture of HBTU (3.9 equiv) and DIPEA (8 equiv) in DMF for 45 minutes. The fully assembled linear resin-bound peptides were then cleaved from the solid support using a mixture of TFA, TIS, H_2O, and 2,2′-(ethylenedioxy)diethanethiol. The hydrophobic nature of the linear peptides, however, rendered the crude peptides miscible in cold diethyl ether that is normally used during workup to precipitate crude peptides. The TFA cocktail–peptide mixture was therefore diluted with a mixture of H_2O and MeCN, lyophilized, and the resultant oil purified by semipreparative RP-HPLC to afford the pure linear peptides **4**, **5**, and **6** as colorless solids.

With linear peptides **4**, **5**, and **6** in hand, our attention turned to the critical macrolactamization step. In order to initially evaluate the conditions that affect oligomerization, we chose to cyclize linear precursor **5** as the achiral C-terminal glycine residue offers no opportunity for racemization. The cyclization was performed by slowly adding a mixture of linear precursor **5** and HATU (3 equiv) in DMF dropwise at the rate of 0.5 mL/h to a stirring solution of DIPEA (5 equiv) in DMF with a final concentration of 0.9 mg/mL (Table 1, entry 1). Analysis of the reaction mixture by LC–MS after complete addition showed that the cyclization had proceeded quickly. The linear precursor **5** was undetected, and the presence of both the desired cyclopentapeptide versicoloritide B (**2**) and the undesired corresponding cyclodecapeptide were detected in equal amounts in a 1:1 ratio as determined by RP-HPLC. In an attempt to reduce the amount of cyclodimerization, the above reaction conditions were modified to use either CH_2Cl_2 as the reaction solvent at the same concentration of 0.9 mg/mL or DMF at a higher dilution with a final concentration of 0.33 mg/mL, neither of which significantly altered the cyclic monomer/dimer ratio (Table 1, entries 2 and 3).[11] Reversing the order of addition of reagents (i.e., adding DIPEA in DMF dropwise to a stirred mixture of **5** and HATU in DMF) resulted in an increase in the amount of the undesired cyclic dimer.[12] We next evaluated the effect of other activating reagents on the cyclic monomer/dimer ratio. In contrast to HATU, the activating reagents BOP and HBTU were both found to improve the 1:1 cyclic monomer/dimer ratio to an acceptable 9:1 ratio (Table 1, entries 5–8).

Table 1 Cyclization of Linear Precursor **5** to **2**

Entry	Reagents and conditions[a]	(**2**):Dimer[b]
1	HATU, DIPEA, DMF, 0.9 mg/mL	1:1
2	HATU, DIPEA, CH_2Cl_2, 0.9 mg/mL	1:1
3	HATU, DIPEA, DMF, 0.33 mg/mL	1:1
4	HATU, DIPEA, DMF, 0.45 mg/mL	1:3[c]
5	PyBOP, DIPEA, DMF, 0.33 mg/mL	major:minor[d]
6	BOP, DIPEA, DMF, 0.33 mg/mL	9:1
7	HBTU, DIPEA, DMF, 0.33 mg/mL	9:1
8	HBTU, DIPEA, CH_2Cl_2–DMF (4:1), 0.33 mg/mL	9:1

[a] Conditions: 3 equiv of coupling reagent and 5 equiv of base were used.
[b] The yield ratios were estimated by analytical RP-HPLC peak area.
[c] Reversed reagent addition order.
[d] Ratio cannot be determined as phosphoramide generated by PyBOP co-eluted with cyclic monomer.

Table 2 Cyclization of Linear Precursor **4** to **1**

Entry	Reagents and conditions[a,b]	Epimer:(**1**):Dimer[c]
1	PyBOP, DIPEA, DMF	major:minor[d]
2	BOP, DIPEA, DMF	1:8:1
3	BOP, collidine, DMF	3:6:1
4	BOP, NMM, DMF	2:6:2
5	HBTU, DIPEA, DMF	1:6:3
6	HBTU, HOBt, DIPEA, DMF	2:7:1
7	HBTU, HOBt, DIPEA, CH_2Cl_2–DMF (19:1)	0:7:3

[a] Conditions: 3 equiv of coupling reagent and 5 equiv of base were used.
[b] The final concentration of linear peptide was 0.33 mg/mL.
[c] The yield ratios were estimated by analytical RP-HPLC peak area.
[d] Ratio cannot be determined as phosphoramide generated by PyBOP co-eluted with cyclic monomer.

Satisfied with the use of the above conditions for the synthesis of versicoloritide B (**2**),[13] the predominant cyclic monomer peak was isolated by concentration of the reaction mixture under reduced pressure, dilution of the remaining residue with 0.1% TFA–H_2O (v/v), and purification by semipreparative RP-HPLC. The optical rotation value and spectroscopic data of synthetic versicoloritide B (**2**) were in agreement with that of naturally occurring versicoloritide B (**2**, Table 2 in Supporting Information). In addition, the *cis*-conformation of both proline residues of the natural product was retained in synthetic versicoloritide B (**2**).[1,14]

With versicoloritide B in hand we next attempted the synthesis of versicoloritide A (**1**) by applying the above reaction conditions to the cyclization of linear precursor **4**. In a manner consistent with the cyclization of **5**, the cyclization of precursor **4** using the activating reagents BOP and HBTU also resulted in a larger proportion of the desired cyclopentapeptide relative to the undesired cyclodecapeptide as confirmed by LC–MS and RP-HPLC (Table 2).[15] In addition to the formation of the desired cyclic monomer **1** and undesired cyclic dimer, analytical LC–MS and RP-HPLC spectra revealed the formation of an additional compound adjacent to versicoloritide A (**1**) which exhibited an identical mass to the cyclopentapeptide (Figure 2, trace A).

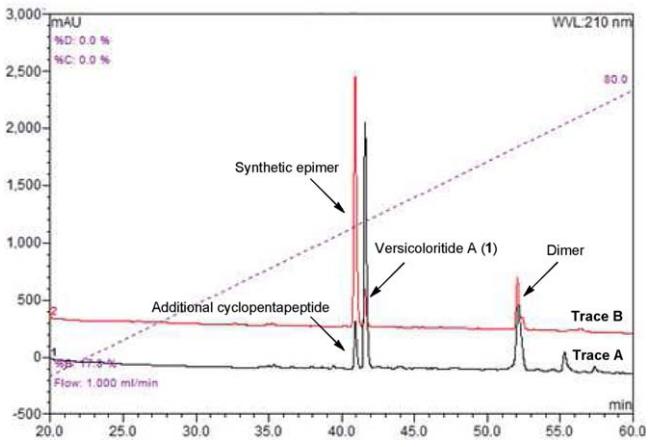


Figure 2 RP-HPLC analysis of the crude reaction mixture after complete addition of reagents of the cyclization of the linear epimer (trace B) and the linear precursor **4** (trace A)

Isolation of both cyclic monomer peaks by RP-HPLC followed by comparison of the ^{1}H NMR data for the synthetic cyclopentapeptide mixture with the reported values for versicoloritide A confirmed that the predominant cyclopentapeptide peak was indeed versicoloritide A (**1**, Figure 3).[1]

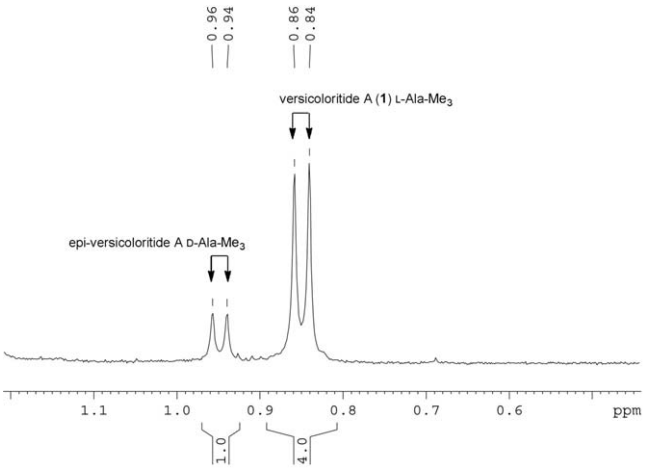


Figure 3 Methyl region of the ^{1}H NMR spectrum of a mixture of versicoloritide A (**1**) and *epi*-versicoloritide A

To determine whether the additional cyclic monomer peak was due to either of the proline residues of the cyclopentapeptide adopting a *trans*-configuration[16] or whether it was an epimer of versicoloritide A resulting from C-terminal epimerization during macrolactamization,[17] we decided to synthesize the possible epimer, namely cyclo-(-L-Phe-L-Pro-L-Phe-L-Pro-D-Ala). The linear epimer precursor NH_2-L-Phe-L-Pro-L-Phe-L-Pro-D-Ala-COOH was

synthesized in a similar manner to the other precursors by manual Fmoc-SPPS and purified by RP-HPLC. The epimer precursor was then subjected to identical cyclization conditions as precursor **4**, by treatment with a mixture HBTU (3 equiv) and DIPEA (5 equiv) in DMF. RP-HPLC analysis of the reaction mixture after addition of the reagents, as shown in Figure 2, established that the retention time of the synthetic cyclic epimer (Figure 2, trace B) matched exactly with the additional peak observed upon cyclization of the all-L linear precursor **4** (Figure 2, trace A), thereby confirming that epimerization of L-Ala to D-Ala took place upon macrolactamization.

Having established that the additional peak was in fact due to C-terminal racemization that took place during the macrocyclization step, we attempted to reduce the undesired base-catalyzed epimerization by substituting DIPEA with less basic tertiary amines such as 2,4,6-collidine and *N*-methylmorpholine.[7a,18] Substitution of DIPEA with both 2,4,6-collidine and *N*-methylmorpholine, however, increased the extent of epimerization (Table 2, entries 3 and 4). Interestingly, similar observations have been reported by others whereby substitution of DIPEA with the sterically hindered base collidine was associated with higher degrees of epimerization, as observed during the synthesis of a highly N-methylated cyclic peptide NMe-IB-01212[12] and the cyclization of other all-L pentapeptides.[4b,12] A cyclization attempt of precursor **4** using the racemization suppressant HOBt also failed to improve the *epi*-versicoloritide A/versicoloritide A ratio (Table 2, entry 6). In a further attempt to reduce the extent of epimerization, we chose to modify the reaction solvent based on studies that showed that the extent of racemization could be altered by the choice of solvent.[7a,19] Using 95% CH_2Cl_2 as the reaction solvent instead of DMF was found to have a significant effect on C-terminal epimerization, in that the *epi*-versicoloritide A/versicoloritide A ratio reflected negligible formation of the undesired epimer (Table 2, entry 7).

Satisfied with the optimized reaction conditions developed herein to reduce undesired epimerization, we isolated synthetic versicoloritide A (**1**) by concentrating the reaction mixture under reduced pressure, diluting the remaining residue with 0.1% TFA–H_2O (v/v) and purifying by semipreparative RP-HPLC. The optical rotation value and spectroscopic data of synthetic versicoloritide A (**1**) were in agreement with that of the naturally occurring versicoloritide A (**1**, Table 1 in Supporting Information).[1]

Having successfully synthesized versicoloritide A (**1**) and B (**2**), we next attempted the synthesis of versicoloritide C (**3**). Interestingly, in contrast to the cyclization of precursors **4** and **5**, the cyclization of precursor **6** proceeded smoothly to form versicoloritide C (**3**) as the predominant product with negligible dimerization and undetectable epimerization in all the conditions that were evaluated, using either DMF or 90% CH_2Cl_2 as the solvent and using either HBTU or BOP as the coupling reagent.[20] Like versicoloritide A (**1**) and B (**2**), the optical rotation value and spectroscopic data of synthetic versicoloritide C (**3**) were in agreement with naturally occurring versicoloritide C (**3**) (Table 3 in Supporting Information).[1]

In conclusion, we herein report the first synthesis of the naturally occurring all-L cyclopentapeptides versicoloritides A (**1**), B (**2**), and C (**3**). We observed for the three similar linear precursor sequences FPFPX significant differences in the degree of dimerization and epimerization observed despite using the same reaction conditions. Importantly, the spectroscopic data of the synthetic versicoloritides (**1**), (**2**), and (**3**) confirmed the structure of the natural products elucidated by Zhuang et al.[1] The peptides synthesized herein are currently undergoing further biological evaluation.

Acknowledgment

We thank the Maurice Wilkins Centre for Molecular Discovery for financial support.

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

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(13) **Versicoloritide B (2)**
To a solution of DIPEA (28 μL, 161 μmol) in CH_2Cl_2 (36 mL) was added **5** (18 mg, 32 μmol) and HBTU (36 mg, 95 μmol) in CH_2Cl_2–DMF (4:1, 15 mL) at 0.5 mL/h. The reaction mixture was concentrated under reduced pressure,

diluted with 0.1% TFA–H_2O (v/v, 18 mL), and purified by RP-HPLC to afford **2** as a colorless solid (10 mg, 57%). HRMS (EI): *m/z* $[M + Na]^+$ calcd for $C_{30}H_{35}N_5NaO_5$: 568.2536; found: 568.2526. IR: 3277, 2956, 1626, 1542, 1447, 1344, 1162, 746, 702 cm^{-1}. $[\alpha]_D^{20}$ –46 (*c* 0.1, MeOH) [lit. –43.6 (*c* 0.1, MeOH)].[1] See the Supporting Information for complete procedures and analytical data.

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(15) **Versicoloritide A (1)**
To a solution of DIPEA (31 μL, 178 μmol) in CH_2Cl_2 (44 mL) was added **4** (22 mg, 38 μmol), HBTU (44 mg, 116 μmol), and HOBt (15 mg, 111 μmol) in CH_2Cl_2–DMF (4.7:1, 17 mL) at 0.5 mL/h. The reaction mixture was concentrated under reduced pressure, diluted with 0.1% TFA–H_2O (v/v, 10 mL) and purified by RP-HPLC to **1** as a colorless solid (5.5 mg, 26%). HRMS (EI): *m/z* $[M + Na]^+$ calcd for $C_{31}H_{37}N_5NaO_5$: 582.2692; found: 582.2675. IR: 3280, 2937, 1633, 1519, 1448, 1346, 1319, 1161, 745, 701 cm^{-1}. $[\alpha]_D^{20}$ –122 (*c* 0.1, MeOH) [lit. –90.7 (*c* 1.7, MeOH)].[1] See the Supporting Information for complete procedures and analytical data.

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(20) **Versicoloritide C (3)**
To a solution of DIPEA (60 μL, 344 μmol) in CH_2Cl_2 (80 mL) was added **6** (40 mg, 67 μmol) and HBTU (77 mg, 200 μmol) in CH_2Cl_2–DMF (5:1, 22 mL) at 0.5 mL/h. After complete addition of the reagents, the reaction mixture was concentrated under reduced pressure, diluted with 0.1% TFA–H_2O (v/v, 18 mL), and purified by RP-HPLC to afford **3** as a colorless solid (17 mg, 44%). HRMS (EI): *m/z* $[M + Na]^+$ calcd for $C_{31}H_{37}N_5NaO_6$: 598.2642; found: 598.2645. IR: 3307, 2956, 1632, 1523, 1443, 1345, 1165, 746, 700 cm^{-1}. $[\alpha]_D^{20}$ –135.5 (*c* 0.2, MeOH) [lit. –118 (*c* 0.2, MeOH)].[1] See Supporting Information for complete procedures and analytical data.

