Concise and Efficient Total Syntheses of Virenamides A and D

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ABSTRACT
Concise total syntheses of linear thiazole-containing peptides virenamides A (1) and D (4), isolated from Australian ascidian Diplosoma virens have been accomplished from Boc-L-valine (6) in 7 steps. A cyclization between thioamide and bromoacetaldehyde was applied to form thiazole ring as a key step.

Indexing terms/Keywords
Total syntheses; Virenamide; Ascidian Diplosoma virens; Thiazole

Academic Discipline And Sub-Disciplines
Organic chemistry

SUBJECT CLASSIFICATION
Heterocyclic compounds

TYPE (METHOD/APPROACH)
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INTRODUCTION

Marine natural products are a rich source of novel peptides, many of which show high levels of biological activity [1-3]. For example, the thiazole-containing cyclic peptide largazole exhibits extremely potent antiproliferative activity against a number of cancer cell-lines including MDA-MB-231 mammary cells (GI₅₀ 7.7 nM), U2OS fibroblastic osteosarcoma cells (GI₅₀ 55 nM), HT29 colon cells (GI₅₀ 12 nM), and IMR-32 neuroblastoma cells (GI₅₀ 16 nM) and linear thiazole-containing peptide dolastatin 10 is one of the most potent antineoplastic agents [4-5]. Over a decade ago, Bowden et. al isolated five cytotoxic linear peptides virenamides A-E (1-5) from Australian ascidian Diplosoma virens and assigned their structures by extensive NMR experiments [6-7]. In 1999, Moody et. al had reported a stereoselective synthesis of virenamide B (2) in which an elegant diastereoselective addition of 2-lithiothiazole to oxime ether was applied to construct the thiazole ring in excellent yield and diastereomeric excess [8]. For a long term concern, we initiated the total synthesis of virenamides in order to investigate the potential bioactivity of the derivatives. Herein, we report the concise efficient total syntheses of virenamides A (1) and D (4) from accessible starting material Boc-L-valine (6), which would provide enough product for further biological studies. 

![Chemical structures of virenamides A-E (1-5)](image)

Our initial retrosynthetic analysis of 1 and 4 is outlined in Scheme 1. We envisaged that the thiazole ring of 1 and 4 could be constructed through a cyclization between thioamide 9 and bromoacetaldehyde 10. Thioamide 9 in turn could be obtained from a cheap starting material Boc-L-valine (6).

![Scheme 1. Retrosynthetic analysis of virenamide A (1) and D (4)](image)

EXPERIMENTAL PROCEDURE

Melting points (mp) are uncorrected and were measured on a microscopic melting point apparatus. The IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer with a KBr disk. The ¹H NMR and ¹³C NMR spectra were taken on a Bruker AV 300 or AV 500 MHz and 75 or 125 MHz spectrometer in CDCl₃, chemical shift are given in part per million (ppm) relative to TMS as an internal standard. Mass spectra and High Resolution Mass spectra were performed on Agilent Q TOF 6520 mass spectrometer with electron spray ionization (ESI) as the ion source. Optical rotations were recorded using a sodium lamp with a Rudolph Autopol I Automatic Polarimeter with 1 dm tube. (S)-tert-butyl 1-amino-3-methyl-1-oxobutan-2-ylcarbamate (7) [9], (S)-tert-butyl 1-amino-3-methyl-1-thioxobutan-2-ylcarbamate (9) [10-12], (S)-2-methyl-1-(thiazol-2-yl)propan-1-amine hydrochloride (12) [8], and (S)-2-[(S)-2-((tert-butoxycarbonylamino)-3-phenylpropanamido)-3-methylbutanoic acid (13) [15], were prepared following the literature procedures.
Dipea (2.09 g, 16 mmol) was added to a solution of compound 9 (0.928 g, 4 mmol) in anhydrous DME (16 mL). Bromoacetaldheyde 10 (1.48 g, 12 mmol) was added and the solution was stirred at rt for 14 h. The mixture was evaporated in vacuo and the residue was partitioned between H₂O (96 mL) and EtO (48 mL). The aqueous layer was further extracted with EtO (48 mL) and the combined organic extracts were washed with brine (24 mL), dried (Na₂SO₄) and evaporated in vacuo. The residue was re-dissolved in anhydrous DME (15 mL) and cooled to 0 °C. A solution of TFA (1.34 g, 5.6 mmol) and dry Pyridine (1.02 g, 12.8 mmol) in anhydrous DME (4.5 mL) was added and the solution was stirred at 0 °C for 0.5 h. The reaction mixture was evaporated in vacuo. The residue was dissolved in CHCl₃ (75 mL), washed with H₂O (40 mL) and brine (40 mL). Evaporation of the solvent followed by purification on silica gel afforded colorless oil 11 (0.839 g, 82%, ee 94.5%).  

[α]D²⁻⁰⁻³.⁹ g (1.10, CHCl₃): IR (CHCl₃) νmax/cm⁻¹: 3409 (NH), 3310 (NH), 3026 (CH₃-), 2964 (CH₂-), 1674 (CH-); 'H NMR spectrum (300 MHz, CDCl₃) δ 7.85 (1H, d, δ = 8.4 Hz, NH); 7.78 (1H, d, J = 3.3 Hz, thiazole H-4), 7.66-7.27 (5H, m, Ar-H); 7.23 (1H, d, J = 3.3 Hz, thiazole H-5); 7.08 (1H, br d, J = 8.4 Hz, NH), 5.82 (1H, br d, J = 7.2 Hz, N=O); 5.84 (1H, d, J = 6.9 Hz, CH), 4.52 (2H, br t, J = 8.1 Hz, 2CH₂), 2.96-3.11 (2H, m, CH₂-Ph), 2.36-2.41 (1H, m, CHMe₂).  

Spectral data of 11: 7.34 (m, 5H, CH₃), 7.25 (m, 5H, CH₃), 7.16 (1H, d, J = 6.6 Hz, 2CH₂-20), 2.88 (dd, 1H, J = 6.0, 14.1 Hz, CH-15), 2.33 (dqq, 1H, J = 5.7, 6.2, 6.3 Hz, CHMe₂-5).  

Compound 12 (0.115 g, 0.50 mmol) in dry DMF (2 mL) was added in one portion, and the mixture was stirred for 1 h. Water, brine, and EtOAc were added, and the layers were separated. The aqueous phase was further extracted with EtOAc. The combined EtOAc extracts were washed with water, dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography on silica gel to give colorless syrup 14 (0.163 g, 54% yield).  

[α]D²⁻⁰⁻⁰.⁹ g (1.06, CHCl₃): IR (CHCl₃) νmax/cm⁻¹: 3442 (C=O); 'H NMR spectrum (300 MHz, CDCl₃) δ 7.88 (1H, d, J = 8.8 Hz, NH); 7.67-7.25 (5H, m, Ar-H); 7.21 (1H, d, J = 3.3 Hz, thiazole H-4), 7.06 (1H, d, J = 6.8 Hz, NH); 5.92 (1H, d, J = 5.8 Hz, CH-9); 3.75 (dd, 1H, J = 5.8, 6.6 Hz, CH-14); 3.31 (dd, 1H, J = 5.8, 6.6 Hz, CH-15); 3.08 (d, 1H, J = 6.6 Hz, 2CH₂-20), 2.88 (dd, 1H, J = 6.0, 14.1 Hz, CH-15).  

To a solution of compound 15 (47 mg, 0.106 mmol) in 2.7 mL dry DMF, TBAI (8 mg, 0.0212 mmol), prenyl bromide (66 mg, 0.424 mmol) and NaHCO₃ (54 mg, 0.636 mmol) were added sequentially at rt and the mixture was stirred at 70 °C for 2 h. The solution was cooled to rt. Water (5 mL), brine (5 mL), and EtOAc (10 mL) were added, and the EtOAc extracts were separated. The aqueous phase was further extracted with EtOAc (10 mL). The combined EtOAc extracts were washed with brine (4 mL), dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography on silica gel to give virenamide A 1 (52 mg, 91% yield). [α]D²⁻⁰⁻⁰.⁹ g (1.06, CHCl₃): IR (CHCl₃) νmax/cm⁻¹: 3405 (NH), 3318 (NH), 3026 (CH₃-), 2964 (CH₂-), 1674 (CH-); 'H NMR spectrum (300 MHz, CDCl₃) δ 7.82 (1H, d, J = 8.8 Hz, NH); 7.67-7.25 (5H, m, Ar-H); 7.21 (1H, d, J = 3.3 Hz, thiazole H-4), 7.06 (1H, d, J = 6.8 Hz, NH); 5.92 (1H, d, J = 5.8 Hz, CH-9); 3.75 (dd, 1H, J = 5.8, 6.6 Hz, CH-14); 3.31 (dd, 1H, J = 5.8, 6.6 Hz, CH-15); 3.08 (d, 1H, J = 6.6 Hz, 2CH₂-20).  

Chemical shifts were referenced to TMS (δ = 0.00) as internal standard. Spectra were recorded on a Bruker (400 MHz) spectrometer. The chemical shifts were reported relative to the solvent peak (CDCl₃, δ = 7.26). Cross-peaks were assigned according to the literature [8].
RESULTS AND DISCUSSION

In the present work we report the first total synthesis of virenamides A and D based on N-(tert-butoxycarbonyl)-L-phenylalanyl-N-[(S)-1-(thiazole-2-yl)-2-methyl-propyl]-L-valinamide (14), which was prepared from (S)-N-(tert-butoxycarbonyl)-1-(2-thiazolyl)-2-methylpropylamine (11) [13], as key intermediate. As illustrated in Scheme 1, the cyclization of thiamide 9 [9-12] with bromoacetaldehyde 10 to form thiazole is the key step for synthesis of Virenamides A and D and is crucial as it is prone to epimerization at the α-stereogenic center. We optimized the reaction conditions for this conversion. As can be seen from Table 1, when bromoacetaldehyde 10 effected the reaction (Entries 1, 2, no base), the deprotection of Boc group of 11 was observed because of the acidic condition where the simultaneous release of HBr during the cyclization. Thus, we treated the cyclization reaction mixture with Boc₂O/TEA to get 11 in moderate yield, but HBr in the reaction mixture resulted in almost entirely racemization of the product [13]. Based on what the literature described [13], we tried several inorganic bases and organic bases to form thiazoline intermediate, which was used for next step without purification. Dehydration of thiazoline intermediate afforded 11 in different yields and ee values. Although inorganic base could give high yield in general (Entries 4, 5), it was found that organic base yield much better ee value (Entries 7-9). DIPEA was proved to be the best acid trapper, which gave a yield of 82% with 94.5% ee (Entry 7) according to chiral-HPLC analysis [14].

Having synthesized the key intermediate 11, the next step was to prepare intermediate 14. We should prepare dipeptide 13 firstly, which was through a two-step sequence. Boc-(L)-Phenylalanine was reacted with L-valine methyl ester to give dipeptide ester in 95% yield, which was saponified with 1M NaOH/THF to give dipeptide 13 [15] in 94% yield. Then the synthesis of 14 was achieved in two steps including removal of Boc group from 11 to provide amine hydrochloride 12 [8], Coupling of 12 with dipeptide 13 with CICOOBu/iNMM to give tripeptide 14 in 54% yield [16]. Likewise, removal of Boc group from 14 with AcCl in MeOH smoothly provided amine hydrochloride 15 in almost quantitative yield, which was used for next step without further purification.

Finally, double alkylation of amine 15 with 4 equiv prenyl bromide in DMF at 70 °C for 2 h afforded virenamide A (1) in 91% yield as a colorless oil ([α]D21 -35.5, c 0.16 in CHCl₃), while mono-alkylation of 15 with 2 equiv prenyl bromide in DMF at room temperature for 5 h smoothly furnished virenamide D (4) in 79% yield as a colorless oil ([α]D21 -65.6, c 0.25 in CHCl₃).
The structures of compound 1 and 4 were determined from spectroscopic as well as optical rotation analytical data, which were consistent with those described for the natural products [6-7]. The $^1$H NMR spectrum of compound 1 revealed a double signal at $\delta$ 3.08 ppm ($J$ 6.6 Hz) due to $\equiv$CH protons, a triple signal at $\delta$ 5.13 ppm ($J$ 6.7 Hz) due to CH$_2$ protons, two single signals at $\delta$ 1.55 and $\delta$ 1.69 due to C22. The high resolution mass spectrum of compound 1 showed [M+H]$^+$ at 539.3409 which is coincident with calculated [M+H]$^+$ (539.3414) as the molecular formula C$_9$H$_8$N$_2$O$_2$S. While, the $^1$H NMR spectrum of compound 4 showed a double signal at $\delta$ 3.10 and 2.98 ppm ($J$ 13.3, 7.6 Hz) due to $\equiv$CH molecular formula, a broad triple signal at $\delta$ 4.98 ppm ($J$ 7.6 Hz) due to CH$_2$ protons, two single signals at $\delta$ 1.46 and $\delta$ 1.63 due to C22. The high resolution mass spectrum of compound 4 showed [M+H]$^+$ at 471.2784 which is coincident with calculated [M+H]$^+$ (471.2788) as the molecular formula C$_{20}$H$_{30}$N$_2$O$_2$S. Analytical data for new compound and copies of $^1$H NMR and $^{13}$C NMR spectra can be found in supporting information.

Table 1. Optimization of reaction conditions$^a$

| Entry | Mol. ratio (9:10:Base) | Step 1 | Step 2 | Reagents | Yield (%)$^c$ | ee (%)$^b$ |
|-------|------------------------|--------|--------|----------|--------------|------------|
| 1     | 1:1:1                  | Dioxane r.t. | r.t. | (Boc)$_2$O (1 eq.), TEA (1.1 eq) | 88           | 8          |
| 2     | 1:1:1                  | DME r.t. | r.t. | (Boc)$_2$O (1 eq), TEA (1.1 eq) | 67           | 6.5        |
| 3     | 1:3:4                  | DME r.t. | KHCO$_3$ 0°C | TFAA (1.4 eq), Pyr (3.2 eq) | 76           | 66         |
| 4     | 1:3:4                  | DME 0°C | KHCO$_3$ 0°C | TFAA (1.4 eq), Pyr (3.2 eq) | 80$^a$       | 61         |
| 5     | 1:3:4                  | DME r.t. | NaHCO$_3$ 0°C | TFAA (1.8 eq), Pyr (4.1 eq) | 82           | 70         |
| 6     | 1:3:4                  | DME r.t. | K$_2$CO$_3$ 0°C | TFAA (1.4 eq), Pyr (3.2 eq) | 69           | 86         |
| 7     | 1:3:4                  | DME r.t. | DIPEA 0°C | TFAA (1.4 eq), Pyr (3.2 eq) | 82           | 94.5       |
| 8     | 1:3:4                  | DME r.t. | TEA 0°C | TFAA (1.4 eq), Pyr (3.2 eq) | 59           | 93         |
| 9     | 1:7:8                  | DME r.t. | NMM 0°C | TFAA (1.1 eq), Pyr (2.6 eq) | 62$^a$       | 76         |

$^a$ Reactions were performed on a 0.5 mmol scale.

$^b$ The ee values were calculated according to reference 8 (entries 3-6, 8, 9) or determined by chiral-HPLC (entries 2, 7).

$^c$ Isolated yields after flash column chromatography.

$^d$ The starting material was completely consumed after 36 h.

$^e$ The starting material was not completely consumed after 36 h.

CONCLUSIONS

In summary, we have developed a very concise route for the first total syntheses of virenamide A and D starting from Boc-L-valine in 7-steps (overall yields: 26% for virenamide A; 22% for virenamide D). Syntheses of these natural products and their derivatives in large scale could be realized by this route, which facilitates further biological experiments. Studies towards the structure modifications of these natural products for further pharmacological investigation are ongoing.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://

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