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Total synthesis of the proposed structure of cyclic hexadepsipeptide veraguamide A†

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We have developed a practical method to assemble the proposed structure of natural product veraguamide A (1) by first preparing the three key fragments followed by optimization of the macrocyclization site. Although the synthetic product gave similar optical rotation to that reported for natural product, significant differences in the ^{1}H and ^{13}C NMR spectra were observed, especially the proton and carbon signals in the two *N*-MeVal moieties.

Veraguamide A (**1**) is a natural cyclic hexadepsipeptide isolated from a *Symploca cf. hydnoides* sample of Cetti Bay, Guam through a cytotoxicity-directed purification process by Luesch and co-workers[1] very recently. Almost at the same time, Gerwick's research group[2] also reported isolation of the same compound from *cf. Oscillatoria margaritifera* obtained from the Coiba National Park, Panama. Veraguamide A contains six amino/hydroxy acid residues, including one proline (Pro), one valine (Val), two *N*-methyl valines (*N*-MeVal), one 2-hydroxy-3-methyl-pentanoic acid (Hmpa), and one (2*S*,3*R*)-8-bromo-3-hydroxy-2-methyloct-7-ynoic acid (Br-Hmoya) (Fig. 1). The terminal alkynyl bromide functionality is very unusual in the cyanobacterial metabolites and has been only reported in one other marine natural product.[3] In addition, in spite of its different origins, this compound was reported possessing cytotoxic activity against several cancer lines including HT29 colorectal adenocarcinoma, HeLa cervical carcinoma and H-460 human lung cell lines.[1,2] The planar structure of veraguamide A was elucidated through NMR and MS experiments, and the absolute configurations of the stereocenters were determined by enantioselective HPLC and Mosher's analysis of acid and base hydrolysates. The interesting structure as well as its inhibitory potency against cancer cells made veraguamide A (**1**) an attractive target for total synthesis and broad biological activity screening.

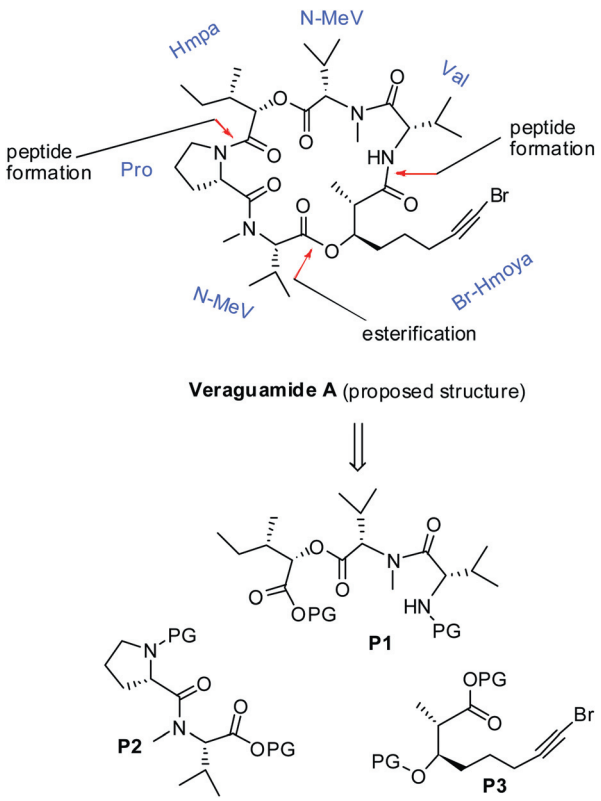

Fig. 1 Structure of veraguamide A and our retrosynthetic analysis.

Among the six key amide–ester linkages in veraguamide A (**1**), three positions can be selected as the preferred sites for closure of the macrocycle (Fig. 1). Therefore, we decided to disconnect this molecule into three fragments **P1**, **P2** and **P3** based on our retrosynthetic analysis and then determine the optimal macrocyclization site.

The fragment **P1** is a tripeptide containing Hmpa–*N*-MeV–Val amino acid sequence. Its synthesis was started from the conversion of L-isoleucine to 2-hydroxy-3-methyl-pentanoic acid (Hmpa). As shown in Scheme 1, diazotization and hydrolysis of L-isoleucine followed by protection with allyl bromide provided allyl ester **2**[4] in 60% overall yield. Condensation of ester **2** with *N*-methyl-*N*-Boc protected valine under DCC–4-PPY at 0 °C produced ester **3** in 91% yield. *N*-Boc protection in **3** was

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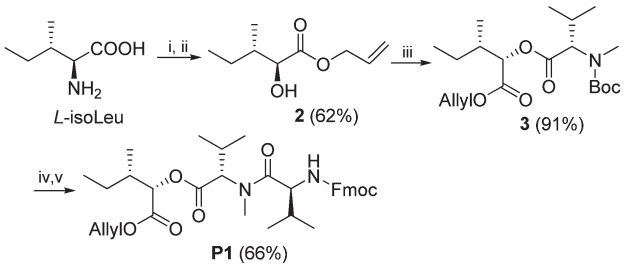
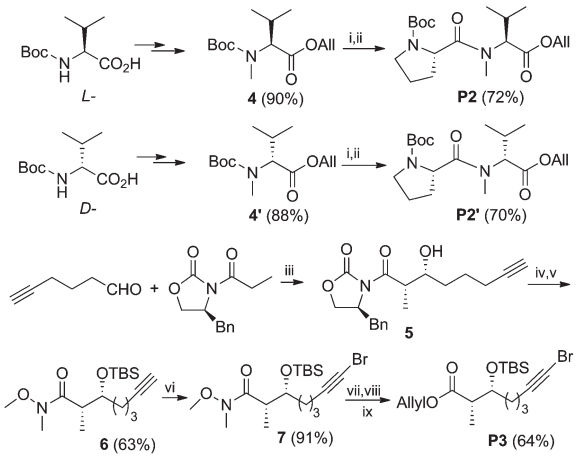


Scheme 1 Synthesis of fragment **P1**. *Reaction condition and reagents*. (i) $NaNO_2$, H_2SO_4; (ii) allyl bromide, K_2CO_3, TBAB; (iii) *N*-Me-*N*-Boc-Val, DCC, 4-PPY, 0 °C; (iv) TFA, CH_2CI_2; (v) *N*-Fmoc-Val, HATU, HOAt, DIPEA.

Table 1 Condensation conditions for preparation of **P1**

Entry	Conditions[a]	Yield (for two steps)
1	TBTU, HOBt, DIPEA, CH_2Cl_2	6%
2	EDCI, HOBt, Et_3N, CH_2Cl_2	7%
3	PyBOP, DIPEA, CH_2Cl_2	33%
4	PyBroP, DIPEA, CH_2Cl_2	52%
5	HATU, HOAt, DIPEA, CH_2Cl_2	66%

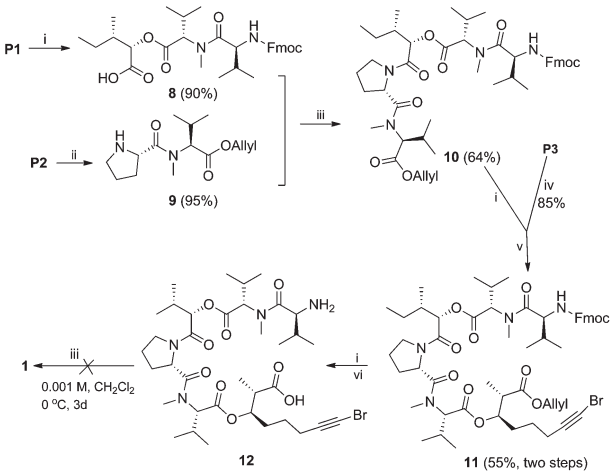
[a] The coupling reaction was performed in the presence of condensation reagent (1.5–2.0 equiv) and base (3.0 equiv) at 0 °C–rt for 12 h.



Scheme 2 Synthesis of fragments **P2** and **P3**. *Reaction condition and reagents*. (i) TFA, CH_2Cl_2; (ii) *N*-Boc-proline; HATU, HOAt, DIPEA; (iii) Bu_2BOTf, Et_3N, −78 °C, 62%; (iv) MeONHMe·HCl, $AlCl_3$, CH_2Cl_2; (v) TBSCl, imidazole, 0 °C-rt; (vi) NBS, $AgNO_3$, acetone, rt; (vii) DIBALH, −78 °C; (viii) $NaClO_2$, NaH_2PO_4; (ix) allyl bromide, K_2CO_3, TBAB.

removed by TFA and the resulting amine was then subjected to condensation with *N*-Fmoc protected valine. After attempts on various conditions (Table 1), the best result was obtained by using HATU (1.5 equiv), HOAt (1.5 equiv) and iPr_2NEt (3.0 equiv) leading to the fragment **P1** in 66% yield.

As outlined in Scheme 2, preparation of the dipeptide fragment **P2** was quite straightforward. First, *N*-methyl-valine (**4**) was prepared[5] in 90% yield by *N*-methylation of *N*-Boc valine followed by protection with allyl bromide. After removal of the



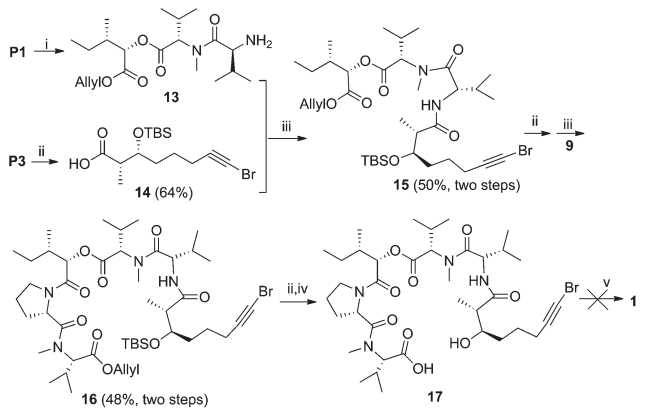
Scheme 3 Synthetic effort through **P1→P2→P3→P1** strategy. *Reaction condition and reagents*. (i) $Pd(PPh_3)_4$, NMA, 0 °C; (ii) TFA, CH_2Cl_2; (iii) HATU, HOAt, DIPEA, CH_2Cl_2; (iv) TBAF, THF; (v) EDCI, CH_2Cl_2, 0 °C-rt; (vi) DEA, CH_3CN, rt.

Boc group, condensation with *N*-Boc protected proline led to the dipeptide **P2** in 72% yield. It has to be mentioned that *N*-methylation of *N*-Boc-valine made both compounds **4** and **P2** existing as mixture of rotamers due to the steric effect. To rule out the racemerization of **P2** during the amide formation, D-configurated *N*-Boc-valine was used, and the corresponding **P2′** was prepared following the same procedure as that for **P2**. Both **P2** and **P2′** gave almost identical ^1H- and ^{13}C-NMR spectra, but showed different polarity in TLC and chiral HPLC, as well as opposite cotton effects in their CD spectra that confirming the optical purity of **P2**.

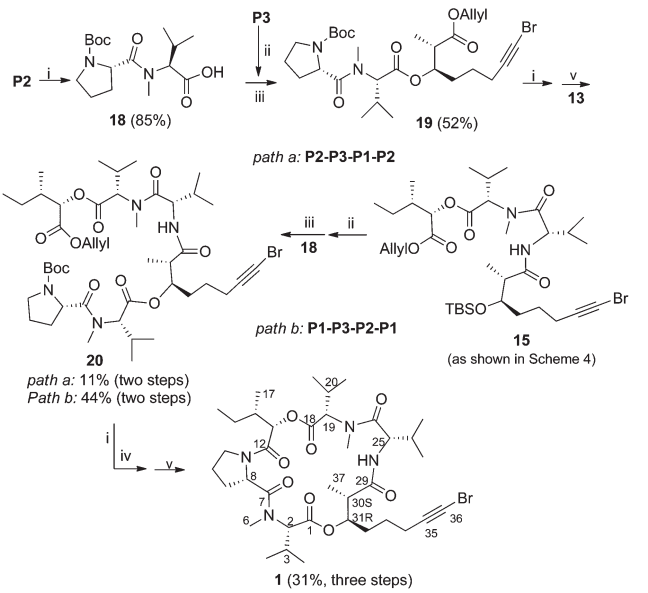
Synthesis of fragment **P3** with configuration (2*S*,3*R*) was achieved by using an asymmetric aldol synthetic strategy induced by Evans' auxiliary.[6] As demonstrated in Scheme 2, treatment of (4*S*)-benzyl-*N*-propionyl oxazolidinone with hex-5-ynal[7] in the presence of Bu_2BOTf and Et_3N at −78 °C afforded the known compound **5**[7] with (2*S*,3*R*)-configuration in 62% yield. Removal of the chiral auxiliary in **5** with trimethylaluminum and *N*,*O*-dimethylhydroxylamine followed by TBS-protection provided amide **6**[8] in 63% overall yield. Bromination of terminal alkyne **6** was succeeded using NBS–$AgNO_3$ in DMF following a similar literature procedure[9] and bromide **7** was obtained in 91% yield. Reduction[10] of oxime ester **7** followed by oxidation and *O*-allyl protection furnished the fragment **P3** in 64% overall yield.

With the three key fragments (**P1**, **P2**, **P3**) in hand, we decided to assemble the natural product **1** by choosing Val–Br-Hmoya (**P1–P3**) amide bond as the macrocyclization site due to it being sterically less hindered. Therefore, a connecting strategy **P1 → P2 → P3 → P1** was initiated.

As outlined in Scheme 3, removal of *O*-allyl group of the fragment **P1** with $Pd(PPh_3)_4$ at 0 °C afforded acid **8** in 90% yield. Meanwhile, removal of *N*-Boc protection in **P2** by TFA provided dipeptide **9** in 95% yield. Peptide formation of acid **8** with amine **9** using HATU–HOAt–DIPEA[5] at 0 °C led to product **10** in 64% yield. After taking off the *O*-allyl group in **10**, the resulting acid was directly reacted with the de-silylated alcohol



Scheme 4 Synthetic effort through **P3→P1→P2→P3** strategy. *Reaction conditions and reagents*. (i) DEA, rt, CH_3CN; (ii) $Pd(PPh_3)_4$, NMA, 0 °C; (iii) HATU, HOAt, DIPEA, 0 °C; (iv) TBAF, 85%; (v) EDCI or MNBA.



Scheme 5 Synthetic efforts of **1** through path a and path b. *Reaction conditions and reagents*. (i) $Pd(PPh_3)_4$, NMA, 0 °C; (ii) TBAF; (iii) EDCI; (iv) TFA, 0 °C-rt; (v) HATU, HOAt, DIPEA, 0 °C.

intermediate prepared by treating the fragment **P3** with TBAF in quantitative yield. The condensation reaction went through smoothly and the diester **11** was obtained in 55% overall yield using EDCI[11] as the condensation agent. Other esterification conditions, including DCC–4-PPY, 2,4,6-trichlorobenzoyl chloride–Et_3N, or oxalyl dichloride–DMAP did not promote this reaction at all.

After cleaving the *O*-allyl protection group in **11** using $Pd(PPh_3)_4$ at 0 °C to free the acid moiety, and removing the Fmoc group using DEA at rt to release the primary amino function, the resulting intermediate **12** was then directly subjected to macrocyclization with HATU–HOAt–DIPEA in relatively dilute solution at 0 °C. Unfortunately, the condensation reaction did not occur, even after three days or under elevated temperature.

The failure of macrocyclization at the Val–Br-Hmoya amide linkage is primarily due to the instability of the primary amine precursor **12**, which readily underwent intramolecular cyclization in addition to its lower activity in the peptide formation. In this regard, an alternative synthetic approach (**P3 → P1 → P2 → P3**) was proposed with the Val–Br-Hmoya primary amide bond formed slightly early and the *N*-MeV–Br-Hmoya (**P2–P3**) ester bond was elected as the macrocyclization site. As shown in Scheme 4, cleavage of the *O*-allyl group of the fragment **P3** with $Pd(PPh_3)_4$ at 0 °C delivered acid **14** in 64% yield. Meanwhile, treatment of the fragment **P1** with DEA yielded amine **13**, which was then directly condensed with acid **14** using HATU–HOAt–DIPEA at 0 °C to provide amide **15** in 50% overall yield.[10] Subsequent de-allylation of **15** followed by condensation with dipeptide **9** under the same peptide formation conditions led to the key intermediate **16** in 48% overall yield. Further removal of the *O*-allyl and *O*-TBS group in **16** afforded the precursor **17**, which was directly subjected to macrocyclization by treating with EDCI or MNBA. Unfortunately, the esterification was found very sluggish and no product was detected.

From the result above, it was apparent that the *N*-MeV–Br-Hmoya (**P2–P3**) ester bond was insufficiently active for the final macrocycle closure step, therefore, the amide bond in the Pro–Hmpa site (**P1–P2**) turned out as the optimal macrocyclization site. Accordingly, a new synthetic path **P2 → P3 → P1 → P2** as shown in Scheme 5 (path a) was proposed. First, de-allylation of the fragment **P2** afforded acid **18** in 85% yield. EDCI-promoted condensation of **18** with the de-silylated intermediate of the fragment **P3** produced ester **19** successfully in 52% yield. Subsequent *O*-de-allylation of **19** using $Pd(PPh_3)_4$, and then condensation with amine **13** using HATU–HOAt–DIPEA delivered the expected precursor **20**. However, the yield of **20** was very low (11% in two steps), again due to the poor efficiency in the formation of the Val–Br-Hmoya primary amide bond. In order to collect a sufficient amount of **20** for further synthetic exploration, a more efficient synthetic strategy was needed.

Upon closely comparing the results obtained in Schemes 4 and 5 (path a), the successful formation of compound **15** bearing the Val–Br-Hmoya amide linkage and the smooth formation of compound **19** bearing the *N*-MeV–Br-Hmoya (**P2–P3**) ester bond led us to adjust the bond connection sequence. As proposed in Scheme 5 (path b), the Val–Br-Hmoya amide bond was formed prior to the formation of the *N*-MeV–Br-Hmoya ester bond, and the Pro–Hmpa amide bond was retained as the macrocyclization site (**P1 → P3 → P2 → P1**). Therefore, as mentioned earlier, intermediate **15** containing the Val–Br-Hmoya amide linkage was established in 50% yield by condensation of amine **13** and acid **14** (as shown in Scheme 4). After removal of the *O*-TBS group in **15** with TBAF, the subsequent condensation with acid **18** proceeded smoothly and the precursor **20** was obtained in 44% overall yield, much better than that from path a. Cleaving both the *O*-allyl group by $Pd(PPh_3)_4$ and the *N*-BOC group by TFA went through readily, and the resulting intermediate was directly subjected to the standard amide formation condition using HATU–HOAt–DIPEA. To our delight, the final product **1** was obtained as the major product in 31% overall yield (three steps), together with some inseparable side products.

All spectroscopic data[12] supported the structure of our product **1**. It showed an optical rotation $[\alpha]_D^{20}$ of −33.0 (*c* MeOH, 0.27),

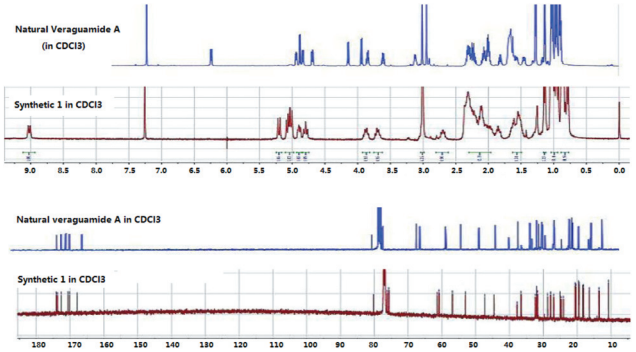


Fig. 2 ^{1}H and ^{13}C NMR comparison between synthetic **1** and natural product.

slightly different from that reported for natural product: −44 (*c* 0.44, MeOH) by Luesch[1] and −14.7 (*c* 0.22, CH_2Cl_2) by Gerwick.[2] However, both the ^{1}H and ^{13}C NMR spectra of our synthetic product were not in agreement with that reported for the natural product. The major difference between our synthetic product **1** and the natural veraguamide A came from both the proton and carbon signals of the tertiary carbon centers (C-2, C-19) in the two *N*-methyl valine fragments. As shown in Fig. 2, in the ^{1}H NMR spectra, the two protons on the C-2 and C-19 of our synthetic **1** showed two doublets with chemical shifts of 5.09 and 5.19 ppm, respectively, whereas two doublets with chemical shifts of 3.94 and 4.15 ppm were reported for the natural product. Therefore, the two protons on the C-2 and C-19 were ∼1.0 ppm downfield-shifting in our synthetic product. Meanwhile, significant difference was also observed in the corresponding ^{13}C NMR spectra. The C-2 and C-19 in our synthetic product **1** displayed chemical shifts of 60.9 and 61.4 ppm, respectively, much upfield-shifting than that for natural product (65.0 and 66.1 ppm, respectively). The signals for the rest protons and carbons were comparable between our synthetic **1** and the natural product.

In conclusion, we have developed a practical method to facilitate the total synthesis of the proposed structure of natural product veraguamide A (**1**) by first preparing the three key fragments followed by optimizing the macrocyclization site. Although the synthetic product gave similar optical rotation to that reported for natural product, significant difference in the ^{1}H and ^{13}C NMR spectra was observed, especially the proton and carbon signals in the two *N*-MeVal amino acid residues. Synthesis of other analogues with different stereo-configurations of the corresponding amino acids, together with biological screening on a panel of cancer cell lines are currently being undertaken.

Acknowledgements

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12 Preparation of natural product **1**. To a solution of peptide **20** (125 mg, 0.135 mmol) in CH_2Cl_2 (7 mL) at 0 °C, was added $[Pd(PPh_3)_4]$ (31.2 mg, 0.027 mmol) and NMA (0.04 mL, 0.405 mmol). The reaction was stirred at rt for 10 h and then quenched with water. The mixture was extracted with CH_2Cl_2, and the combined organic phase was washed with brine and dried over Na_2SO_4. After evaporation of the solvents, the residue was purified by flash chromatography to give the carboxyl acid intermediate as yellow oil, which was then dissolved in CH_2Cl_2 (6 mL). To the solution just obtained, TFA (0.13 mL) was added at 0 °C. The resulting solution was stirred for 6 h, and then concentrated *in vacuo* to give the *N*-deprotected intermediate. To the solution of the *N*-deprotected intermediate in CH_2Cl_2 (100 mL), was added HATU (116 mg, 0.306 mmol), HOAt (42 mg, 0.306 mmol) and DIPEA (0.11 mL, 0.612 mmol) at 0 °C. The reaction mixture was stirred at rt for 3 d, then diluted with H_2O and extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel (petroleum ether–ethyl acetate) to give cyclic peptide **1** (32.1 mg, 31% yield for 3 steps) as a colorless amorphous solid. $[\alpha]_D^{20}$ −33.0 (*c* 0.27, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (d, J = 9.8 Hz, 1H), 5.20 (d, J = 10.1 Hz, 1H), 5.05 (dd, J = 16.9, 8.7 Hz, 3H), 4.89 (d, J = 8.3 Hz, 1H), 4.80 (t, J = 8.6 Hz, 1H), 3.86 (m, 1H), 3.71 (m, 1H), 3.01 (s, 6H), 2.69 (s, 1H), 1.85–2.31 (m, 12H), 1.58 (m, 4H), 1.14 (d, J = 6.3 Hz, 3H), 1.05–0.93 (m, 13H), 0.90–0.76 (m, 11H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.1, 173.8, 172.6, 170.6, 170.2, 167.9, 80.2, 76.2, 75.7, 61.4, 60.8, 56.9, 53.1, 47.3, 44.6, 37.7, 36.6, 32.3, 32.0, 31.9, 31.7, 28.7, 27.9, 26.9, 25.1, 24.7, 24.0, 20.6, 20.4, 19.6, 19.5, 19.5, 18.3, 18.1, 16.4, 13.5, 10.7; ESI-MS *m/z* 789 (M + Na); HRMS Calcd for $C_{37}H_{59}BrN_4NaO_8$ (M + Na): 789.3414, found 789.3411.

