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Total Synthesis of the Marine Cyclic Depsipeptide Viequeamide A

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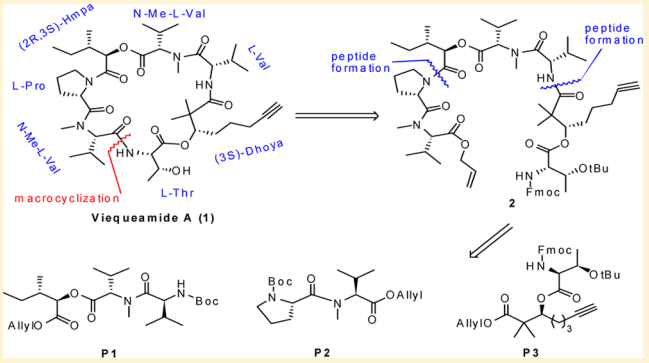
S *Supporting Information*

ABSTRACT: The first total synthesis of viequeamide A, a natural cyclic depsipeptide isolated from a marine button cyanobacterium, was achieved with the N-Me-Val−Thr peptide bond as the final macrocyclization site. The synthetic product gave nearly identical spectroscopic data to that reported for the natural product.



Viequeamide A (**1**) is a natural cyclic depsipeptide recently isolated by Gerwick and co-workers[1] from a marine "button" cyanobacterium (*Rivularia* sp.) near the island of Vieques, Puerto Rico. Structurally, it belongs to the kulolide superfamily featuring a (3*S*)-2,2-dimethyl-3-hydroxy-7-octynoic acid (3*S*-Dhoya) moiety with divergent biological activities.[2] Viequeamide A is a 22-membered macrocycle comprising nine stereogenic centers. In addition to the 3*S*-Dhoya moiety, it contains six other amino/hydroxy acid fragments, including one L-proline (L-Pro), one L-valine (L-Val), one L-threonine (L-Thr), two *N*-methylated-L-valines (L-*N*-MeVal), and one (2*R*,3*S*)-2-hydroxy-3-methylpentanoic acid (2*R*,3*S*-Hmpa) (Figure 1). More attractively, this natural product was found to be highly toxic against H460 human lung cancer cell lines, with an IC_{50} value of 60 nM,[1] strikingly different from other related peptides isolated from the same natural source, which were shown to be inactive to the same cell lines. The structure and significant cytotoxicity made viequeamide A (**1**) an optimal target for total synthesis and further biological activity screening.

Although there are several options to retrosynthetically dissect this molecule, our experience with the synthesis of the related peptide veraguamide A[3] suggested that the esterification between the L-Thr and 3*S*-Dhoya moieties might be troublesome due to the steric hindrance of the highly methylated Dhoya component. Therefore, this coupling needs to be established earlier in the synthesis. Meanwhile, formation of the *N*-MeVal−Thr peptide bond seems optimal for the final macrocyclization in view of the reduced steric hindrance of these two amino acid moieties relative to other residues in the molecule. In this regard, viequeamide A was disconnected into three fragments, the tripeptide **P1**, dipeptide **P2**, and ester **P3**, based on the retrosynthetic analysis as outlined in Figure 1.

The tripeptide fragment **P1** consists of the Hmpa−*N*-Me-Val−Val amino acid sequence. Its synthesis began with the conversion of L-isoleucine to (2*R*,3*S*)-2-hydroxy-3-methylpentanoic acid (2*R*,3*S*-Hmpa). As shown in Scheme 1, diazotization of L-isoleucine with $NaNO_2$, followed by protection of the acid with allyl bromide, provided allyl ester **3**[4] in 62% overall yield. Condensation of ester **3** with *N*-methyl-*N*-Boc-L-valine[7] following a modified Mitsunobu procedure produced the epimerized dipeptide **4**[5] in 91% yield. Removal of the *N*-Boc protecting group in **4** by treating with TFA, followed by condensation with *N*-Boc-protected L-valine under the typical peptide coupling conditions[3,6] (HATU/HOAt/$(iPr)_2NEt$), afforded fragment **P1** in 66% overall yield with no epimerization according to the ^1H and ^{13}C NMR analyses.

Preparation of the dipeptide fragment **P2** has been reported earlier by our group[3] (Scheme 1). It should be mentioned that *N*-methylation of *N*-Boc-L-valine made both compounds **5** and **P2** exist as a mixture of amide rotamers due to steric hindrance. Our group recently prepared the diastereomer of **P2** starting from *N*-Boc-D-valine and ruled out racemization of **P2** during amide bond formation.[3]

The synthesis of ester fragment **P3** is outlined in Scheme 2. First, the commercially available hex-5-yn-1-ol was oxidized[8] to hex-5-ynal with NaOCl/TEMPO in 70% yield. Subsequent aldol condensation[3,9] of hex-5-ynal with chiral amide **9R**

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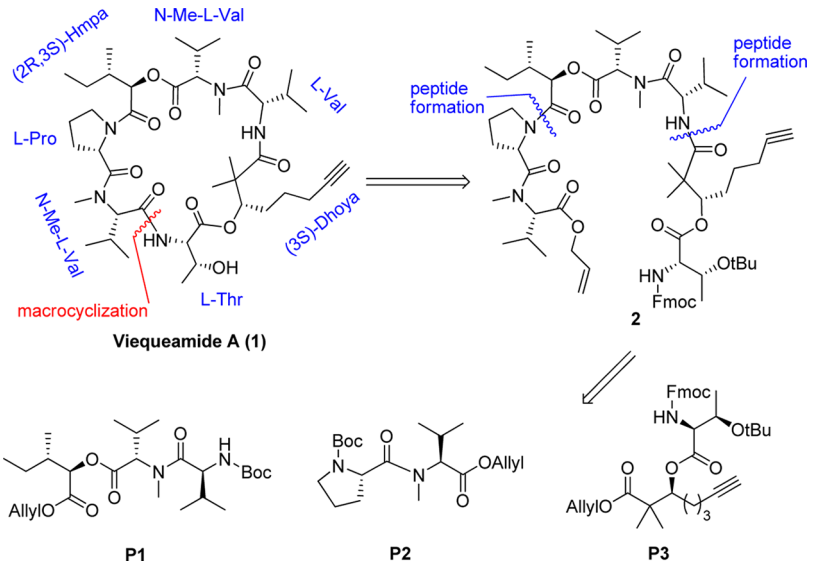
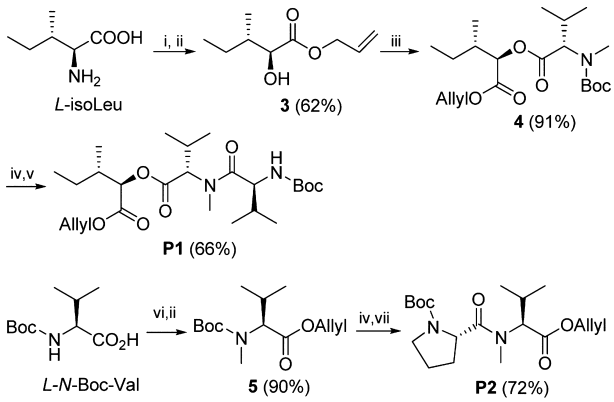


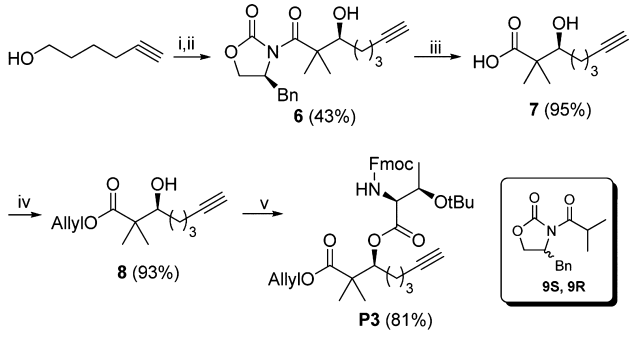
Figure 1. Structure of viequeamide A and our retrosynthetic analysis.

Scheme 1. Synthesis of Fragments P1 and P2[a]



[a]Reaction condition and reagents: (i) $NaNO_2$, H_2SO_4; (ii) allyl bromide, K_2CO_3,TBAB; (iii) *N*-Me-*N*-Boc-L-Val, DEAD, PPh_3, 0 °C to rt; (iv) TFA, CH_2Cl_2; (v) *N*-Boc-L-Val, HATU, HOAt, DIPEA; (vi) MeI, NaH, THF; (vii) *N*-Boc-L-proline, HATU, HOAt, DIPEA.

Scheme 2. Synthesis of Fragment P3[a]



[a]Reaction condition and reagents: (i) NaOCl, TEMPO, NaBr; (ii) **9**, LDA, $Ti(O\text{-}iPr)_3Cl$, THF, −78 °C, then hex-5-ynal, −40 °C; (iii) H_2O_2, LiOH, THF/H_2O; (iv) allyl bromide, $KHCO_3$, DMF; (v) Fmoc-L-Thr(tBu)−OH, EDCI, DMAP.

(prepared by methylation of (*R*)-4-benzyl-3-propionyloxazolidin-2-one[9]) under the standard conditions (Bu_2BOTf/Et_3N at −78 °C) was unsuccessful. Fortunately, by following a modified asymmetric Evans' aldol synthetic strategy[10] reported by Gerwick et al., 5-hexynal reacted with **9S** in the presence of LDA and $Ti(O\text{-}iPr)_3Cl$ to provide the aldol adduct **6** in 61% yield. Removal of the Evans' chiral auxiliary using H_2O_2/LiOH provided 3*S*-Dhoya (**7**) in 95% yield ($[\alpha]^{25}_D$ −24.3 (*c* 0.4, $CHCl_3$), lit.[10] −26.3 (*c* 1.0, $CHCl_3$)).

Esterification of acid **7** with allyl bromide afforded allyl ester **8** in 93% yield (Scheme 2). As expected, the subsequent condensation of ester **8** with L-threonine (L-Thr) proved to be challenging due to steric hindrance. After attempting various conditions with L-Thr substrates (Table 1), we found that the best result could be achieved by using Fmoc-L-Thr(tBu)−OH (5 equiv), EDCI (10 equiv), and DMAP (10 equiv), leading to the **P3** fragment in 81% yield with no epimerization according to the ^1H and ^{13}C NMR analysis.

Table 1. Acylation of Alcohol 8

entry	conditions	yield
1	Fmoc-L-Thr(tBu)-Cl (5.0 equiv), DMAP (0.5 equiv), iPr_2NEt (6.0 equiv), CH_2Cl_2	trace
2	Fmoc-L-Thr(tBu)−OH (2.0 equiv), DCC (3.0 equiv), 4-PPY (3.0 equiv), CH_2Cl_2	33%
3	Fmoc-L-Thr(tBu)−OH (2.0 equiv), 2,4,6-trichlorobenzoyl chloride (2.0 equiv), DMAP (2.0 equiv), iPr_2NEt (2.5 equiv)	48%
4	Fmoc-L-Thr(tBu)−OH (2.0 equiv), EDCI (3.0 equiv), DMAP (3.0 equiv), CH_2Cl_2	52%
5	Fmoc-L-Thr(tBu)−OH (5.0 equiv), EDCI (10.0 equiv), DMAP (10.0 equiv), CH_2Cl_2	81%

With the three key fragments (**P1**, **P2**, **P3**) in hand, we were ready to assemble precursor **2** by choosing the formation of the *N*-MeVal−Thr (**P2**−**P3**) amide bond as the final macrocyclization step. Accordingly, the connecting strategy **P2** → **P1** → **P3** → **P2** was initiated.

As described in Scheme 3, removal of the *O*-allyl group in the **P1** fragment with $Pd(PPh_3)_4$ at 0 °C afforded acid **10** in 85% yield.[3] Meanwhile, removal of *N*-Boc protection in **P2** by treating with TFA followed by condensation with acid **10** under the standard peptide bond-forming conditions[3,6] (HATU/HOAt/DIPEA at 0 °C) yielded product **12** in 60% overall yield (two steps). In order to prepare fragment **14**, we initially

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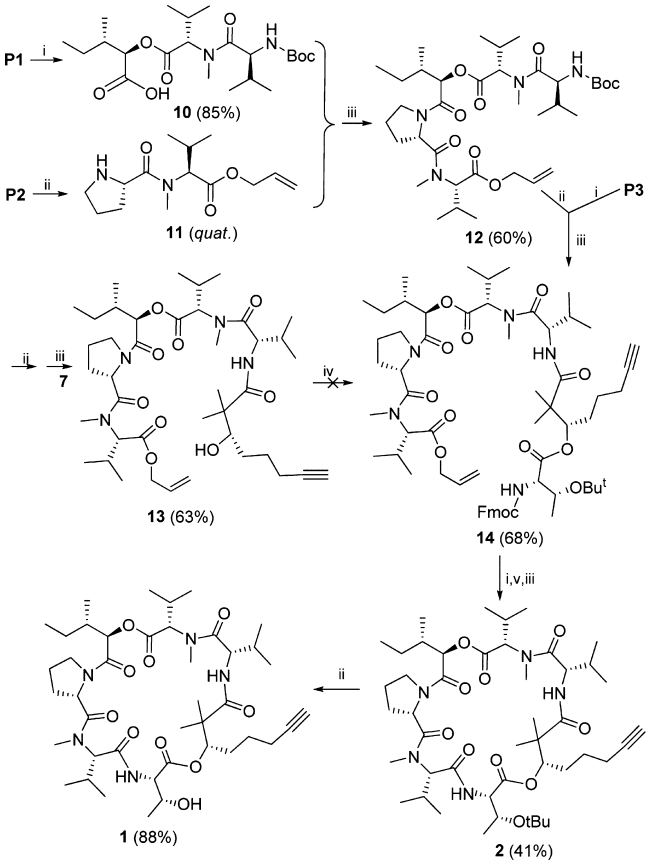
Scheme 3. Final Assembly of Viequeamide A (1)[a]



[a]Reaction condition and reagents: (i) $Pd(PPh_3)_4$, *N*-methylaniline; (ii) TFA, CH_2Cl_2; (iii) HATU, HOAt, DIPEA; (iv) Fmoc-L-Thr(tBu)−OH, EDCI, DMAP; (v) Et_2NH, CH_3CN.

conducted a stepwise procedure by directly using 3*S*-Dhoya (**7**) instead of **P3** to minimize the steric effects. Removal of *N*-Boc protection of **12** provided the primary amine intermediate, which was then condensed with acid **7**. The reaction went smoothly, and product **13** was obtained in 63% overall yield (two steps). However, esterification of **13** with Fmoc-L-Thr(tBu)−OH under the same conditions as used for the preparation of **P3** did not occur.

Alternatively, removal of the allyl protecting group in **P3** with a palladium catalyst ($Pd(PPh_3)_4$/*N*-methylaniline) yielded the corresponding acid intermediate, which was condensed with the liberated primary amine from pentapeptide **12** under the standard coupling conditions.[3,6] Amide **14** was obtained in 68% overall yield. After sequential removal of the allyl group with $Pd(PPh_3)_4$/*N*-methylaniline to liberate the acid function and removal of the Fmoc group by diethylamine to release the amino group in **14**, the product was subjected to macrocyclization under the standard conditions to generate the key cyclic peptide **2** in 41% yield (three steps).[11,12] Finally, treatment of precursor **2** with 20% TFA in CH_2Cl_2 furnished the expected target **1** in 88% yield.

As shown in Figure 2, both the ^1H and ^{13}C NMR spectra of our synthetic product **1** were in good agreement with those reported for the natural product viequeamide A.[1] Meanwhile, our synthetic product **1** showed a specific rotation $[\alpha]^{20}_D$ of −34.7 (*c* 0.15, CH_2Cl_2), also comparable to that for the natural product:[1] −32.6 (CH_2Cl_2). In addition, a direct HPLC analysis with an authentic sample[13] also confirmed the identity of our

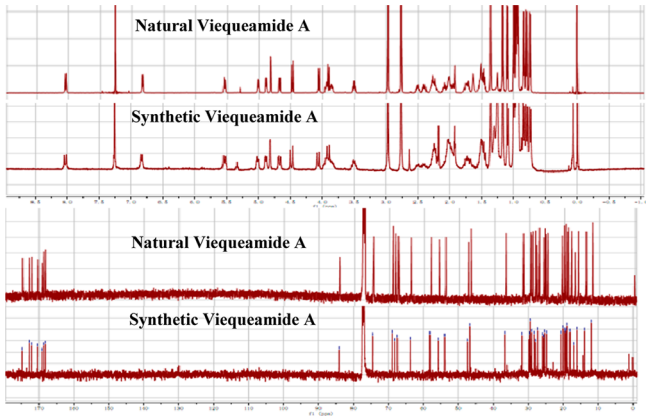


Figure 2. Comparison of ^1H and ^{13}C NMR spectra for synthetic **1** and natural viequeamide A.

synthetic compound. All the comparisons secured the total synthesis of viequeamide A.

The cytotoxicity of synthetic **1**, as well as paclitaxel and etoposide (as positive controls), was evaluated against several cell lines,[14] including human lung cancer A549 cells, squamous cell carcinoma KB cells, vincristine-resistant KB/VCR cells, and human lung cancer H460 cells. Unfortunately, compared to the high potency of paclitaxel (3.2 nM) and etoposide (63.1 nM) in our assays,[14] synthetic compound **1** was found to be inactive in all four cancer cells, with IC_{50} values up to 100 μM. HPLC analysis of the bioassay sample (in DMSO solution) indicated that compound **1**was unstable and decomposed to several side products. Although more investigations are needed, the instability of synthetic **1** may be a partial rationale for its poor cytotoxicity. Further studies on the side products are ongoing.

CONCLUSIONS

In summary, we have achieved the first total synthesis of viequeamide A, a natural cyclic depsipeptide isolated from a marine button cyanobacterium, through 10 linear steps based on the three retrosynthetic fragments with 0.9% overall yield. Our synthesis formed the N-MeVal−Thr peptide bond as the final macrocyclization site, and all of the spectroscopic data of the synthetic product were in good agreement with those reported for the natural product.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were performed in glassware containing a Teflon-coated stir bar. CH_2Cl_2 and THF were purified and dried according to the standard methods prior to use. All reagents were obtained from commercial sources and used without further purifications. NMR spectra were recorded on Varian-MERCURY Plus-300 (300 MHz for ^1H), Variant MR-400 (100 MHz for ^{13}C), and AVANCE III 500 (125 MHz for ^{13}C). ^1H and ^{13}C NMR spectra were recorded with tetramethylsilane as an internal reference. Low- and high-resolution mass spectra were obtained on FinniganLTQ and Micromass Ultra Q-TOF in the ESI mode. Optical rotations were recorded with a Perkin-Elmer 341MC polarimeter. Flash column chromatography on silica gel (200−300 mesh) was used for the routine purification of reaction products, and a mixture of EtOAc and petroleum ether was used as the eluent. The column output was monitored by TLC on silica gel (100−200 mesh) precoated on glass plates (10 cm × 50 cm), and spots were visualized by UV light at 254 nM or by potassium permanganate color agent.

(2*R*,3*S*)-Allyl-2-((*S*)-2-(*tert*-butoxycarbonyl(methyl)amino)-3-methylbutanoyloxy)-3-methylpentanoate (4). A solution of

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diethyl azodicarboxylate (9.96 mmol) in toluene (4.5 mL) was added to a solution of **3** (570 mg, 3.32 mmol), triphenylphosphine (2.9 g,14.94 mmol), and *N*-methyl-*N*-Boc-valine (2.3 g, 9.96 mmol) in THF (30 mL) at 0 °C. The resulting solution was stirred at rt for 14 h. The solvent was removed under reduced pressure and then diluted with H_2O. After extraction with EtOAc, the combined organic phase was washed with brine and dried with Na_2SO_4. Removal of the solvents followed by flash chromatography (petroleum ether/CH_2Cl_2, 3:2) provided **4** as a colorless oil (1.16 g, 91%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.94−5.81 (1H, m), 5.27 (2H, dd, J = 22.7, 13.8 Hz), 5.01 (1H, s), 4.62 (2H, d, J = 5.8 Hz), 4.43 (1H, m), 2.83 (3H, d, J = 11.9 Hz), 2.27−2.12 (1H, m), 2.08−1.92 (1H, m), 1.45 (9H, s), 1.38−1.19 (2H, m), 1.05−0.95 (3H, m), 0.95−0.85 (9H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.6, 169.3, 169.1, 156.2, 155.6, 131.6, 131.5,119.0, 118.8, 80.1, 79.8, 74.9, 65.8, 65.6, 64.6, 63.0, 36.5, 30.5, 29.9, 28.3, 27.5, 27.3, 26.1, 26.0, 19.9, 19.8, 19.1, 18.8, 14.3, 14.2, 11.6; ESIMS m/z 408.2 $[M + Na]^+$; HRMS m/z 408.2352 $[M + Na]^+$ (calcd for $C_{20}H_{35}NNaO_6$, 408.2362).

(2*R*,3*S*)-Allyl-2-((*S*)-2-((*S*)-2-(*tert*-butoxycarbonylamino)-*N*,3-dimethylbutanamido)-3-methylbutanoyloxy)-3-methylpentanoate (P1). To a stirred solution of **4** (100 mg, 0.26 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added TFA (0.6 mL), and the resulting solution was stirred at 0 °C for 6 h. The reaction mixture was concentrated in vacuo to give the crude intermediate. To a solution of *N*-Boc-L-valine (113 mg, 0.52 mmol) in CH_2Cl_2 (5 mL) was added HATU (296 mg, 0.78 mmol) and HOAt (106 mg, 0.78 mmol) followed by addition of the crude intermediate just prepared and DIPEA (0.2 mL, 1.04 mmol). The reaction mixture was allowed to stir for 14 h and then diluted with H_2O. After extraction with CH_2Cl_2, the combined organic phase was washed with brine and dried with Na_2SO_4. Removal of solvent followed by flash chromatography (petroleum ether/EtOAc, 10:1) provided **P1** as a colorless oil (83 mg, 66% in two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93−5.79 (1H, m), 5.35−5.22 (2H, m), 5.19 (1H, d, J = 9.5 Hz), 5.07 (1H, d, J = 10.4 Hz), 5.01 (1H, d, J = 3.4 Hz), 4.60 (2H, ddd, J = 5.7, 2.8, 1.4 Hz), 4.43 (1H dd, J = 9.4, 6.4 Hz), 3.06 (3H, s), 2.29−2.17 (1H, m), 2.05−1.95 (2H, m), 1.82 (1H, d, J = 2.6 Hz), 1.41 (9H, s), 1.33−1.20 (1H, m), 0.99 (8H, dd, J = 11.0, 6.6 Hz), 0.93−0.79 (15H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 172.3, 171.0, 157.9, 133.3, 120.8, 81.3, 77.0, 67.6, 62.8, 57.2, 38.2, 33.3, 32.6, 30.1, 28.7, 27.7, 21.7, 21.4, 20.1, 19.3, 16.1, 13.4; ESIMS m/z 507.2 $[M + Na]^+$; HRMS m/z 485.3223 $[M + H]^+$ (calcd for $C_{25}H_{45}N_2NaO_7$, 485.3227).

(*S*)-*tert*-Butyl-2-(((*S*)-1-(allyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (P2). The fragment **P2** (72% yield in two steps) was obtained from **5** following a similar procedure as that for the preparation of the fragment **P1**. ^{1}H NMR (300 MHz, $CDCl_3$, mixture of rotamers) δ 5.93−5.77 (1H, m), 5.23 (2H, dd, J = 23.8, 13.8 Hz), 4.91 (1H, d, J = 10.4 Hz), 4.68−4.52 (3H, m), 3.64−3.30 (2H, m), 3.00 (3H, d, J = 27.3 Hz), 2.23−1.78 (5H, m), 1.38 (9H, d, J = 7.7 Hz), 0.99 (3H, dd, J = 11.4, 6.6 Hz), 0.89 (3H, dd, J = 6.7, 2.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 173.5, 173.3, 169.5, 169.3, 153.8, 153.7, 131.3, 131.2, 116.9, 116.6, 79.1, 78.8, 64.4, 64.3, 61.7, 56. 7, 56.0, 46.0, 45.7, 29. 9, 29.6, 28.9, 28.2, 26.6, 26.3, 26.0, 23.0, 21.7, 18.2, 17.9; ESIMS m/z 391.1 $[M + Na]^+$; HRMS m/z 391.2192 $[M + Na]^+$ (calcd for $C_{19}H_{32}N_2NaO_5$, 391.2209).

(*S*)-Allyl 3-Hydroxy-2,2-dimethyloct-7-ynoate (8). To a solution of **7** (40 mg, 0.22 mmol) in DMF (3 mL) were added $KHCO_3$ (44 mg, 0.44 mmol) and allyl bromide (0.028 mL, 0.326 mmol). The reaction mixture was stirred for 4 h at rt and then diluted with of H_2O. The mixture was extracted with diethyl ether, washed with brine, dried over Na_2SO_4, and then concentrated under reduced pressure to provide **8** as a colorless oil (46 mg, 93% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.98−5.85 (1H, m), 5.28 (2H, dd, J = 24.3, 13.8 Hz), 4.60 (2H, d, J = 5.6 Hz), 3.64 (1H, d, J = 5.8 Hz), 2.52 (1H, d, J = 5.7 Hz), 2.24 (2H, td, J = 6.7, 2.6 Hz), 1.94 (1H, t, J = 2.6 Hz), 1.89−1.53 (4H, m), 1.21 (6H, d, J = 5.6 Hz).

Allyl 3-((2*S*,3*R*)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-*tert*-butoxybutanoyloxy)-2,2-dimethyloct-7-ynoate (P3). To a solution of ester **8** (110 mg, 0.489 mmol) in CH_2Cl_2 (30 mL) at 0 °C were added Fmoc-L-Thr(tBu)−OH (970 mg, 2.45 mmol), EDCI (937 mg, 4.89 mmol), and DMAP (598 mg, 4.89 mmol). The reaction was stirred at rt overnight, then diluted with H_2O and extracted with CH_2Cl_2. The combined organic phase was washed with brine and dried over Na_2SO_4. Removal of the solvents followed by flash chromatography (petroleum ether/EtOAc, 20:1) afforded **P3** as a colorless oil (239 mg, 81%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (2H, d, J = 7.5 Hz), 7.63 (2H, d, J = 7.4 Hz), 7.41 (2H, t, J = 7.2 Hz), 7.32 (2H, t, J = 7.3 Hz), 5.98−5.83 (1H, m), 5.52 (1H, d, J = 9.3 Hz), 5.36−5.18 (3H, m), 4.57 (2H, d, J = 5.7 Hz), 4.42 (2H, d, J = 6.4 Hz), 4.27 (3H, d, J = 6.3 Hz), 2.19 (2H, s), 1.91 (1H, s), 1.61 (4H, m), 1.20 (15H, s), 1.15 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 170.6, 156.3, 143.8, 143.7, 141.2, 131.9, 127.6, 126.9, 125.1, 125.0, 119.9, 118.3, 83.6, 77.6, 74.2, 68.8, 67.1, 67.0, 65.4, 60.1, 47.1, 46.7, 29.6, 28.7, 24.9, 22.8, 20.0, 19.2, 17.9; ESIMS m/z 626.4 $[M + Na]^+$; HRMS m/z 626.3073 $[M + Na]^+$ (calcd for $C_{36}H_{45}NNaO_7$, 626.3094).

(*S*)-Allyl 2-((*S*)-1-((6*S*,9*S*,12*R*)-12-*sec*-Butyl-6,9-diisopropyl-2,2,8-trimethyl-4,7,10-trioxo-3,11-dioxa-5,8-diazatridecane)-*N*-methylpyrrolidine-2-carboxamido)-3-methylbutanoate (12). To a stirred solution of **P2** (380 mg, 1.03 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added TFA (2 mL), and the resulting solution was stirred at 0 °C for 4 h. The reaction mixture was concentrated in vacuo to give crude intermediate **11**. To the solution of **10** (458 mg, 1.03 mmol) in CH_2Cl_2 (20 mL) were added HATU (783 mg, 2.06 mmol) and HOAt (280 mg, 2.06 mmol) followed by addition of the crude intermediate **11** prepared above and DIPEA (0.72 mL, 4.12 mmol). The reaction was allowed to stir for 14 h and then diluted with H_2O. After extraction with CH_2Cl_2, the combined organic phase was washed with brine and dried with Na_2SO_4. Removal of solvent followed by flash chromatography (petroleum ether/EtOAc, 3:1) provided **12** as a colorless oil (429 mg, 60% in two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.92−5.76 (1H, m), 5.32−5.16 (3H, m), 5.08−4.99 (2H, m), 4.94 (1H, d, J = 10.4 Hz), 4.75 (1H, d, J = 8.7 Hz), 4.57 (2H, d, J = 5.7 Hz), 4.41 (1H, s), 4.04 (1H, s), 3.51 (1H, d, J = 8.4 Hz), 3.03 (4H, s), 2.93 (3H, s), 2.04 (11H, t, J = 56.6 Hz), 1.41 (9H, s), 0.91 (32H, tdd, J = 20.0, 13.8, 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 171.4, 170.7, 169.7, 167.2, 155.7, 131.4, 118.1, 79.0, 75.0, 64.8, 60.8, 60.6, 56.5, 54.8, 46.6, 35.7, 31.2, 30.6, 30.1, 27.1, 26.5, 24.9, 24.1, 19.8, 19.4, 19.3, 18.7, 18.1, 16.9, 14.2, 11.1; ESIMS m/z 717.4 $[M + Na]^+$; HRMS m/z 717.4434 $[M + Na]^+$ (calcd for $C_{36}H_{62}N_4NaO_9$, 717.4414).

(*S*)-Allyl 2-((*S*)-1-((2*R*,3*S*)-2-((*S*)-2-((*S*)-2-((*S*)-3-Hydroxy-2,2-dimethyloct-7-ynamido)-*N*,3-dimethylbutanamido)-3-methylbutanoyloxy)-3-methylpentanoyl)-*N*-methylpyrrolidine-2-carboxamido)-3-methylbutanoate (13). The peptide **13** was obtained from **12** in 63% yield over two steps following a similar procedure to that for the preparation of the fragment **P1**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.63 (1H, d, J = 8.3 Hz), 5.85 (1H, ddd, J = 16.1, 10.6, 5.6 Hz), 5.24 (2H, dd, J = 21.9, 13.8 Hz), 5.07−4.90 (3H, m), 4.80−4.63 (2H, m), 4.57 (2H, d, J = 5.6 Hz), 4.05 (1H, s), 3.55−3.39 (2H, m), 3.03 (3H, s), 2.94 (3H, s), 2.32−2.09 (9H, m), 1.87 (5H, dt, J = 20.0, 8.5 Hz), 1.59 (2H, d, J = 5.7 Hz), 1.34 (2H, dd, J = 19.9, 8.8 Hz), 1.22 (3H, s), 1.17 (3H, s), 1.03−0.76 (24H, m).

(*S*)-Allyl 2-((*S*)-1-((5*S*,8*S*,12*S*,15*S*,18*R*)-5-((*R*)-1-*tert*-Butoxyethyl)-18-*sec*-butyl-1-(9*H*-fluoren-9-yl)-12,15-diisopropyl-9,9,14-trimethyl-3,6,10,13,16-pentaoxo-8-(pent-4-ynyl)-2,7,17-trioxa-4,11,14-triazanonadecane)-*N*-methylpyrrolidine-2-carboxamido)-3-methylbutanoate (14). To a solution of peptide **P3** (85 mg, 0.14 mmol) in CH_2Cl_2 (20 mL) at 0 °C were added $Pd(PPh_3)_4$ (16.5 mg, 0.014 mmol) and *N*-methylaniline (0.045 mL, 0.42 mmol). The reaction was stirred at rt for 4 h. After evaporation in vacuo, the residue was purified by silica gel chromatography (petroleum ether/EtOAc, then CH_2Cl_2/MeOH) to give the carboxylic acid intermediate as a yellow oil (328 mg, 85%). To the solution of acid intermediate just prepared (40 mg, 0.071 mmol) in CH_2Cl_2 (5 mL) were added HATU (54 mg, 0.142 mmol), HOAt (20 mg, 0.142 mmol), DIPEA (0.05 mL, 0.284 mmol), and the de-Boc intermediate of **12** (prepared by treating **12** (100 mg, 0.144 mmol) with TFA (0.5 mL)). The reaction mixture was allowed to stir for 14 h and then diluted with H_2O. After extraction with CH_2Cl_2, the combined organic phase was washed with brine and dried with

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Na_2SO_4. Removal of solvent followed by flash chromatography (petroleum ether/EtOAc, 2:1) provided amide **14** as a colorless oil (429 mg, 68% in two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (2H, d, J = 7.3 Hz), 7.61 (2H, d, J = 7.3 Hz), 7.39 (2H, t, J = 7.2 Hz), 7.30 (2H, t, J = 7.2 Hz), 6.65 (1H, d, J = 8.5 Hz), 5.85 (1H, ddd, J = 16.1, 10.7, 5.4 Hz), 5.71 (1H, d, J = 8.9 Hz), 5.30 (1H, d, J = 6.8 Hz), 5.22 (2H, t, J = 9.3 Hz), 5.07−4.90 (3H, m), 4.76 (2H, d, J = 7.6 Hz), 4.57 (2H, d, J = 4.8 Hz), 4.39 (3H, d, J = 7.1 Hz), 4.27 (2H, d, J = 7.5 Hz), 4.07 (1H, s), 3.51 (1H, d, J = 9.0 Hz), 3.03 (3H, s), 2.92 (3H, s), 2.27−1.52 (17H, m), 1.18 (18H, t, J = 8.9 Hz), 1.01−0.71 (38H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 174.3, 173.6, 172.9, 172.4, 171.7, 169.4, 158.3, 145.8, 145.6, 143.1, 133.6, 129.6, 128.9, 127.0, 121.9, 120.4, 85.6, 80.6, 77.2, 76.2, 70.7, 69.0, 68.9, 67.1, 63.1, 62.9, 62.1, 58.7, 55.7, 49.0, 48.9, 48.5, 38.0, 33.4, 32.8, 32.5, 32.0, 30.6, 30.1, 29.3, 28.6, 27.1, 27.0, 26.3, 25.9, 22.2, 22.0, 21.9, 21.6, 20.9, 20.3, 20.0, 19.3, 16.4, 13.4; ESIMS m/z 1162.5 $[M + Na]^+$.

(3*S*,6*S*,9*S*,13*S*,16*S*,19*R*,24*aS*)-19-*sec*-Butyl-6-((*R*)-1-hydroxyethyl)-3,13,16-triisopropyl-2,10,10,15-tetramethyl-9-(pent-4-ynyl)tetradecahydropyrrolo[2,1-*i*][1,13,4,7,10,16,19]-dioxapentaazacyclodocosine-1,4,7,11,14,17,20(19*H*)heptaone (viequeamide A, 1). To a solution of peptide **14** (60 mg, 0.053 mmol) in CH_2Cl_2 (7 mL) at 0 °C were added $Pd(PPh_3)_4$ (9 mg, 0.008 mmol) and *N*-methylaniline (0.017 mL, 0.157 mmol). The reaction was stirred at rt for 4 h. After evaporation of the solvents in vacuo, the residue was purified by silica gel chromatography (petroleum ether/EtOAc, then CH_2Cl_2/MeOH) to give the carboxylic acid intermediate as a yellow oil, which was then dissolved in CH_3CN (3 mL). To the solution just prepared, diethylamine (0.6 mL) was added at 0 °C. The resulting solution was stirred for 1 h and then concentrated in vacuo to give the *N*-deprotected intermediate, which was then dissolved in CH_2Cl_2 (50 mL). HATU (40 mg, 0.105 mmol), HOAt (14 mg, 0.105 mmol), and DIPEA (0.028 mL, 0.215 mmol) were added sequentially at 0 °C. The reaction mixture was allowed to stir at rt for 26 h, 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/EtOAc, 2:1) to give cyclic peptide **2** (18.7 mg, 41% yield for 3 steps) as a colorless oil: ESIMS m/z 882.8 $[M + Na]^+$; HRMS m/z 882.5566 $[M + Na]^+$ (calcd for $C_{46}H_{77}N_5NaO_{10}$, 882.5568).

To a solution of peptide **2** (18.7 mg, 0.022 mmol) in CH_2Cl_2 (2 mL) was added TFA (0.5 mL) at 0 °C. The resulting solution was stirred for 1 h and then concentrated in vacuo. The residue was purified by thin-layer chromatography (CH_2Cl_2/EtOAc, 1:1) to give product **1** (15.6 mg, 88% yield) as an amorphous solid: $[\alpha]^{20}_D$ −34.7 (c 0.15, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (1H, d, J = 10.4 Hz), 6.83 (1H, d, J = 7.2 Hz), 5.53 (1H, d, J = 8.7 Hz), 5.01 (1H, d, J = 7.8 Hz), 4.88 (1H, d, J = 5.3 Hz), 4.82 (1H, d, J = 1.7 Hz), 4.67 (1H, dd, J = 10.3, 3.1 Hz), 4.50 (1H, d, J = 12.4 Hz), 4.07 (1H, d, J = 10.7 Hz), 3.88 (3H, dt, J = 12.6, 7.2 Hz), 3.57−3.44 (1H, m), 2.97 (3H, s), 2.77 (3H, s), 2.57−2.35 (2H, m), 2.23 (3H, dd, J = 13.6, 6.5 Hz), 2.03 (4H, t, J = 17.2 Hz), 1.93 (1H, t, J = 2.5 Hz), 1.74 (1H, s), 1.68−1.65 (1H, m), 1.56−1.42 (5H, m), 1.36 (3H, s), 1.17 (3H, s), 1.09 (3H, d, J = 6.7 Hz), 1.01−0.91 (15H, m), 0.86−0.72 (9H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.7, 172.7, 172.1, 170.3, 169.2, 168.7, 168.2, 84.1, 77.4, 74.5, 68.9, 68.1, 67.4, 63.6, 58.1, 55.7, 53.9, 47.3, 46.6, 36.6, 31.7, 29.6, 29.4, 29.0, 28.2, 27.9, 27.2, 25.9, 25.6, 25.3, 24.7, 20.6, 20.0, 19.5, 19.3, 19.1, 18.8, 18.1, 17.0, 16.1, 13.8, 11.9; ESIMS m/z 804.5 $[M + H]^+$, 826.6 $[M + Na]^+$; HRMS m/z 804.5103 $[M + H]^+$ (calcd for $C_{42}H_{70}N_5O_{10}$, 804.5123).

Cytotoxicity Assay. Cytotoxic effects on the cells were assessed by sulforhodamine B assays as reported previously.[14] The concentration required for 50% inhibition (IC_{50}) of the tested cells was calculated using the Logit method.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the preparation of compounds **3**, **5**, **6**, **7**, **9R**, **10** and ^{1}H and ^{13}C NMR spectra for compounds **3**, **4**, **P1**, **5**, **P2**, **6**, **7**, **8**, **9**, **P3**, **12**, **13**, **14**, **1**. ^{1}H NMR and ^{13}C NMR data, comparison of synthetic with natural viequeamide A (**1**), and comparison of HPLC for synthetic and natural viequeamide A (**1**). These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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