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# Application of cyano ylide methodology to the synthesis of cyclotheonamides $E_2$ and $E_3$

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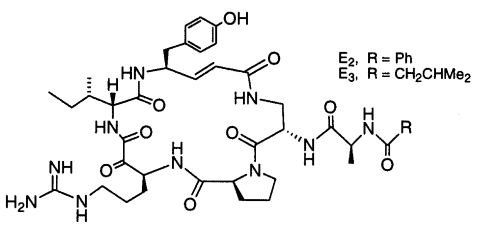
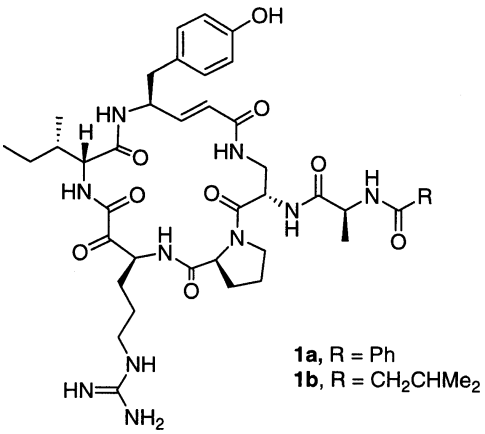
**Abstract**—A total synthesis of cyclotheonamides $E_2$ and $E_3$ is reported. A key step in the synthesis involves the formation of the α-keto amide linkage by application of the cyano ylide activation of a carboxyl group as developed in our earlier syntheses of cyclic peptide protease inhibitors. 

The cyclotheonamides (A–E), recently isolated from marine sources, are cyclic pentapeptides which show notable activity as inhibitors of serine proteases.[1,2] Because of their biological properties and unusual structural features, considerable attention has been given to the study of the mode of enzyme inhibition and synthesis of these macrocyclic α-keto lactams. Total syntheses of cyclotheonamides A and B have been reported[2] by Schreiber, Maryanoff, Wipf, Ottenheijm and Shioiri.

Adding to the interest in this area has been the recent isolation of two new cyclotheonamides, $E_2$ (**1a**) and $E_3$ (**1b**),[1b] which contain most of the elements of cyclotheonamides A and B, except for the presence of a D-alloisoleucine residue in the place of D-phenylalanine, and side-chains containing benzoylalanine and isovalerylalanine residues. A unique functional unit in all cyclotheonamides is associated with the extra carbonyl α- to the amide linkage in the arginine residue. It has been suggested that this active carbonyl group is involved in the deactivation of a protease by imparting the characteristics of an enzyme transition state analog.[2b] It is also noteworthy that the cyclotheonamides have structural features similar to those of the immunosuppressants bearing α-keto amide functions such as FK-506 and rapamycin.[2f]




In a preliminary communication,[3] we have outlined a total synthesis of cyclotheonamides $E_2$ and $E_3$. We now provide details of this synthesis involving cyano ylide activation[4] of an arginine carboxyl in the formation of the key α-keto lactam unit. A number of the steps in our synthesis make use of protecting groups in common with the procedures published in the early synthetic work. However, our strategy has unique features which should be of considerable value in future syntheses of compounds in this family. In particular, the formation of the relatively robust α-keto amide at an early stage of the synthesis avoids problems in earlier syntheses associated with α-hydroxy precursors generated en route to the α-keto amide residue. It precludes the necessity for carrying a mixture of diasteromers through subsequent steps in the synthesis, as well as the protection of the hydroxyl group needed to avoid the possibility of competing intramolecular lactone formation. Overall, the simplicity of forming the α-keto amide residue using the cyano ylide activation procedure avoids the extra steps needed to generate, protect, deprotect and oxidize an alcohol intermediate to the α-keto function.[5,6]

*Keywords*: cyano ylide methodology; cyclotheonamides; synthesis.

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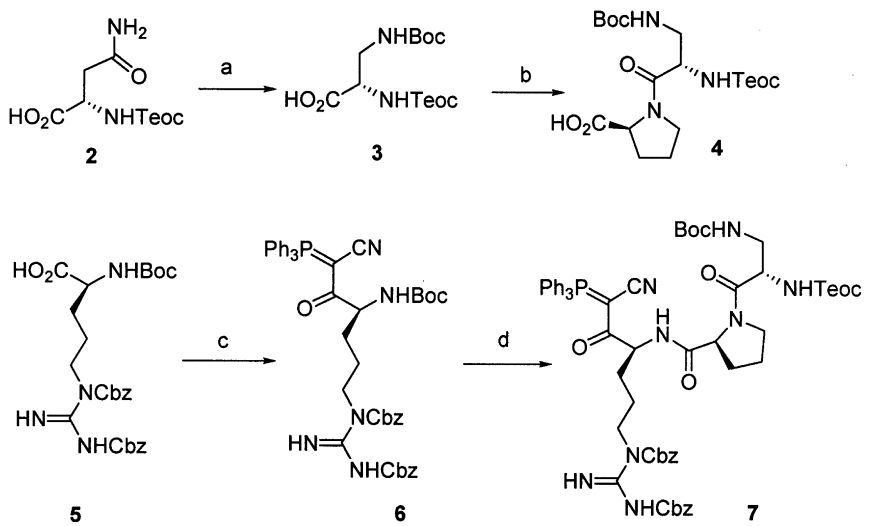


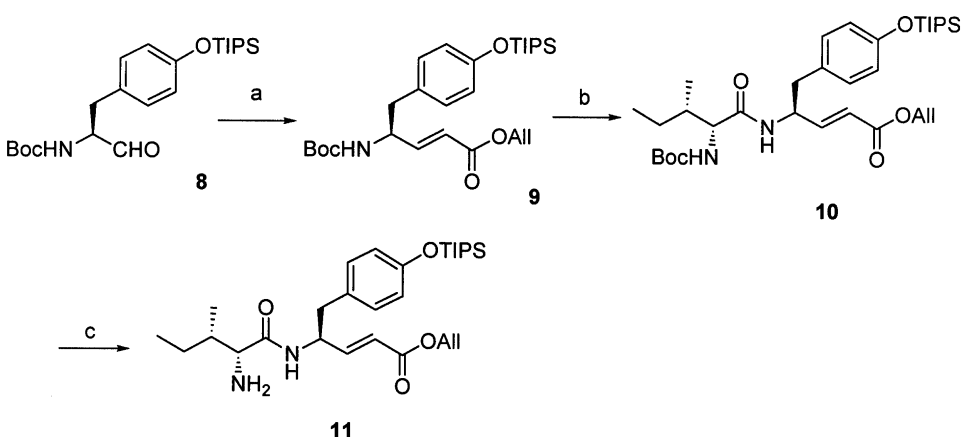

**Scheme 1.** *Regents and conditions*: (a) TIB, Py; then $Boc_2O$, NaOH, 77%; (b) EDCI, HOBt, Pro-OBn, 88%; then Pd–C, $H_2$, 100%; (c) EDCI, DMAP, $Ph_3P{=}CHCN$, 86%; (d) TFA; then EDCI, HOBt; **4**, 78%.

Our synthesis began with the formation of the tripeptide from arginine, proline and diaminopropionic acid (Scheme 1). The latter acid, formed by a Curtius-type degradation of Teoc-aspargine (**2**)[7] using bis(trifluoroacetyl) iodobenzene (TIB), was then converted to the *N*-Boc derivative **3**. The acid **3** was then coupled (EDCI) with proline benzyl ester to form the protected dipeptide which, on hydrogenation (Pd–C), yielded **4**. In a separate pathway, the ylide **6** was prepared from arginine having a Boc-protected amino group, and a doubly Cbz-protected guanidino residue (**5**).[8] Reaction of **5** with (triphenylphosphoranylidine)acetonitrile yielded the acyl cyano ylide **6** (86%).[4a] TFA removal of the Boc group from **6** then yielded the free amine for coupling with **4** (EDCI, HOBt) to form the tripeptide **7** (78%).

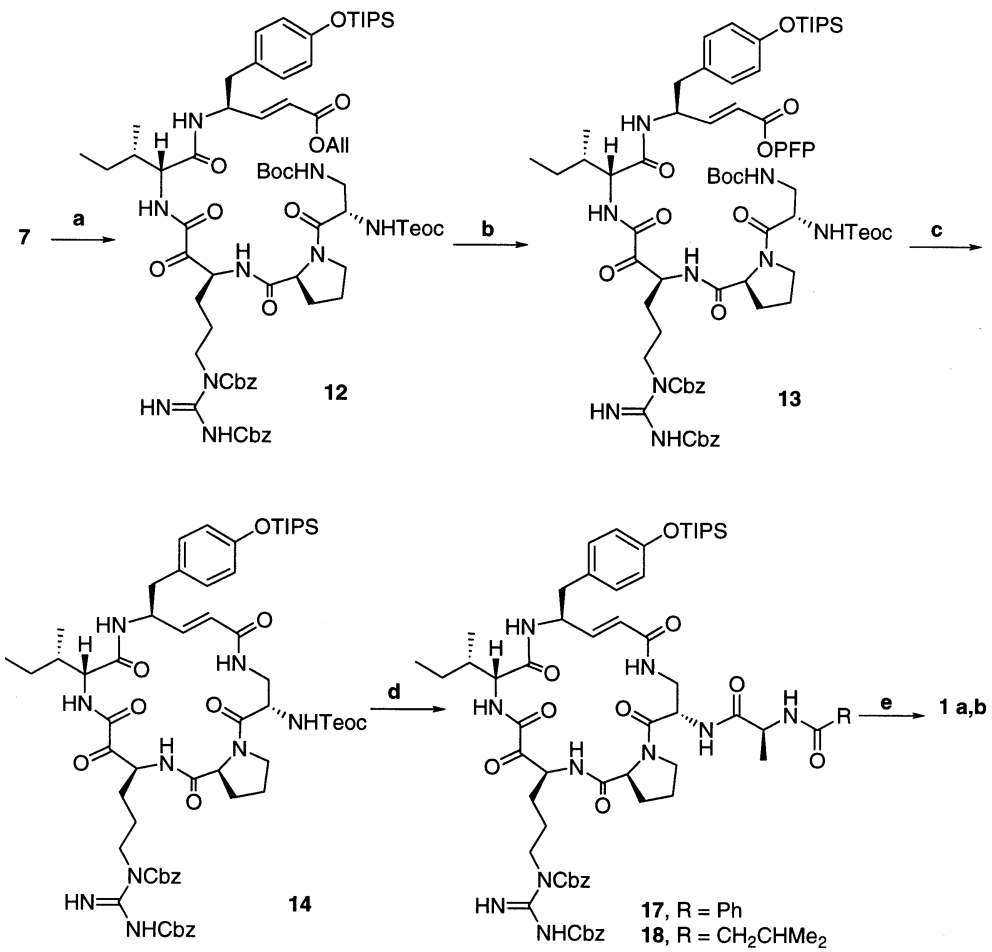
To continue the synthesis, L-Boc-tyrosine methyl ester was converted to the TIPS derivative,[9] and then reduced with DIBAL-H to the aldehyde **8**. A Wittig reaction of **8** with allyl (triphenylphosphoranylidine)acetate (Scheme 2) yielded the allyl protected α,β-unsaturated ester **9** which, on treatment with TFA and coupling (EDCI, HOBt, $Et_3N$) with D-Boc-alloisoleucine gave the dipeptide **10** containing a vinylogous tyrosine. Removal of the Boc group yielded **11** for reaction in the next stage with **7**.

Tripeptide (**7**) was now ozonized to generate the strongly electrophilic α,β-diketonitrile intermediate, not isolable, which formed an amide bond with **11** to yield the pentapeptide **12** (75%) (Scheme 3). At this point in the synthesis, we found it expedient to change the allyl-protecting group to a pentafluorophenoxy group for enhanced activation of the ester. This was readily accomplished, first by the use of $Pd^0$ to regenerate the carboxyl group (86%)[10] and then DCC-promoted coupling with pentafluorophenol (PFPOH) yielding **13** (88%).[11] For the ring closure of **13** to **14**, we first selectively cleaved the Boc group with HCl in $Et_2O–CH_2Cl_2$ in the presence of the acid-labile Teoc group[12] and then carried out lactam formation with DMAP, $NaHCO_3$ (61%). The removal of the Teoc protecting group to yield **17** and **18** took place with TFA, permitting the installation of side-chains (EDCI, HOBt) with *N*-benzoylalanine (**15**) to yield **17** and isovaleryalanine (**16**), forming **18**.

The Cbz and TIPS protecting groups in **17** and **18** were then removed smoothly with HF·Py, yielding synthetic cyclotheonamides $E_2$ (**1a**) and $E_3$ (**1b**) which were fully characterized by HRMS, $^1H$ NMR and $^{13}C$ NMR. The NMR spectra of **1a** and **1b** were identical in all significant respects




**Scheme 2.** *Reagents and conditions*: (a) $Ph_3P{=}CHCO_2All$, 90%; (b) TFA; then EDCI; HOBt, $Et_3N$, D-Boc-alle, 85%; (c) TFA; then aq. $NaHCO_3$, 100%.




**Scheme 3.** *Regents and conditions*: (a) $O_3$; then **11**, 75%; (b) $Pd(PPH_3)_4$, $PhSiH_3$, 86%; then DCC, PFP-OH, 88%; (c) HCl in $Et_2O-CH_2Cl_2$; then DMAP, $NaHCO_3$, 61%; (d) TFA; then aq. $NaHCO_3$; then EDCI, HOBt, **15** or **16**, 83–85%; (e) HF·Py, 70–72%.

with the corresponding spectra of the natural materials, kindly sent to us by Professors Fusetani and Nakao.

## 1. Experimental

### 1.1. General

**1.1.1. Curtius degradation of Teoc-asparagine to *N*-Boc derivative 3.** Teoc-Asn **2** (3.77 g, 13.6 mmol) was dissolved in a mixture of DMF (3 mL), dioxane (50 mL) and distilled water (53 mL). [Bis(trifluoroacetoxy)iodo]benzene (9.07 g, 21.1 mmol) was added in one pot. After 15 min, pyridine (2.2 mL, 27.2 mmol) was added, and the reaction mixture was stirred at room temperature for 3 h. The solvents were evaporated under reduced pressure and the residue was then dissolved in water (50 mL) and extracted with ether (3×50 mL). The aqueous layer was concentrated under reduced pressure to afford a residue, which was used without purification.

The residue was basified with 1N NaOH (35 mL) at 0°C. Di-*tert*-butyl dicarbonate (4.46 g, 20.4 mmol) in dioxane (10 mL) was added dropwise. Then the reaction mixture was warmed to room temperature, and stirred for 2 h. After it was acidified with 1N HCl, the reaction mixture was extracted with EtOAc (3×50 mL), and the combined organic extracts were dried and concentrated. The residue was purified by flash chromatography (hexanes–EtOAc, 5:4) to give **3** (3.65 g, yield 77%) as a white foam. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 11.14 (br s, 1H), 6.39 (br s, 0.5H), 6.04 (br s, 0.5H), 5.70 (br s, 0.5H), 5.28 (br s, 0.5H), 4.35 (m, 1H), 4.14 (m, 2H), 3.57 (m, 1H), 3.50 (m, 1H), 1.43 (s, 3.6H), 1.39 (s, 5.4H), 0.96 (t, $J$=8.0 Hz, 2H), 0.0 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 173.5, 173.0, 158.2, 157.1, 157.0, 156.1, 81.9, 80.2, 63.8, 63.5, 54.9, 54.5, 54.1, 43.1, 42.1, 28.2, 28.1, 17.6, −1.6; HRMS (ES) calcd for $C_{14}H_{28}N_2O_6SiNa$ $(M+Na)^+$: 371.1614 $(M+Na)^+$, found 371.1609.

**1.1.2. Coupling of 3 with proline benzyl ester.** Compound **3** (2.82 g, 8.09 mmol) was dissolved in $CH_2Cl_2$ (10 mL), and EDCI (1.86 g, 9.71 mmol) and HOBt (1.31 g, 9.71 mmol) were added, followed by the addition of a mixture of Pro-OBn hydrochloride (2.35 g, 9.71 mmol) and triethylamine (1.36 mL, 9.71 mmol). The resulting mixture was stirred overnight. 1N HCl (50 mL) was added, and after extraction with $CH_2Cl_2$ (50 mL), the organic layer was washed with sat. $NaHCO_3$, brine, and then dried over $Na_2SO_4$ and concentrated. Flash chromatography of the residue afforded the benzyl ester of protected dipeptide **4** (3.81 g, yield 88%) as a glassy solid. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.31 (m, 5H), 5.90 (m, 1H), 5.34 (m, 1H), 5.18 (d, $J$=12.3 Hz, 1H), 5.06 (d, $J$=12.3 Hz, 1H), 4.65 (m, 1H),

4.58 (m, 1H), 4.11 (m, 2H), 3.75 (m, 2H), 3.42 (m, 1H), 3.23 (m, 1H), 2.18 (m, 1H), 1.96 (m, 3H), 1.41 (s, 9H), 0.95 (t, $J$=8.5 Hz, 2H), 0.04 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 171.6, 169.9, 156.7, 156.1, 135.5, 128.6, 128.3, 128.1, 128.0, 79.3, 77.4, 66.9, 63.3, 59.0, 52.8, 52.2, 47.1, 46.5, 42.3, 31.1, 28.9, 28.4, 24.9, 22.0, 17.7, −1.5; HRMS (ES) calcd for $C_{26}H_{41}N_3O_7SiNa$: 558.2612 $(M+Na)^+$, found 558.2606.

**1.1.3. Debenzylation of the benzyl ester of 4.** The benzyl ester of **4** (3.24 g, 6.05 mmol) in EtOAc (50 mL) was treated with 10% Pd–C (322 mg, 5 mol%), and the reaction mixture was stirred under a balloon of $H_2$ overnight, and then filtered through Celite followed by washing of the Celite with EtOAc–MeOH. The filtrate was concentrated to give the free acid **4** (2.70 g, 100%) as a white foam. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 5.65 (d, $J$=7.0 Hz, 1H), 5.37 (br s, 1H), 4.66 (m, 1H), 4.56 (t, $J$=6.2 Hz, 1H), 4.11 (m, 2H), 3.76 (m, 2H), 3.37 (m, 2H), 2.16 (m, 2H), 2.05 (m, 2H), 1.46 (s, 1.8H), 1.40 (s, 7.2H), 0.95 (t, $J$=8.5 Hz, 2H), 0.0 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 174.3, 171.1, 156.8, 156.5, 79.6, 63.6, 59.3, 52.4, 47.6, 42.2, 28.9, 28.5, 24.9, 17.8, −1.4; HRMS (ES) calcd for $C_{19}H_{35}N_3O_7$ SiNa: 468.2142 $(M+Na)^+$, found 468.2141.

**1.1.4. Conversion of protected arginine to the cyano ylide 6.** A solution of Boc-Arg(Cbz)$_2$-OH **5** (56.46, 11.9 mmol) in $CH_2Cl_2$ (20 mL) was treated with DMAP (1.45.4 mg, 10 mol%) and EDCI (2.51 g, 13.1 mmol), followed by (cyanomethylene)triphenylphosphorane (7.17 g, 23.8 mmol). The reaction mixture was stirred for 4 h, and then concentrated. Flash chromatography of the residue with 1:1 hexane–EtOAc gave **6** (8.45 g, yield 86%) as a white foam: IR (neat); 3388 (br), 3275 (shoulder), 2177, 1715, 1607, 1507, 1497, 1439, 1378, 1367, 1253, 1176, 1108 cm$^{-1}$. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.53 (br s, 1H), 9.29 (br s, 1H), 7.60, 7.56 (m, 9H), 7.50–7.46 (m, 6H), 7.42–7.30 (m, 6H), 7.32–7.20 (m, 4H), 5.33 (d, $J$=7.7 Hz, 1H), 5.23 (s, 2H), 5.13 (s, 2H), 4.92 (m, 1H), 4.13 (m, 1H), 3.97 (m, 1H), 2.02 (m, 1H), 1.78 (m, 3H), 1.44 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 194.3, 163.8, 160.5, 160.4, 155.8, 155.5, 136.9, 134.7, 133.5, 133.4, 133.2, 129.0, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 127.6, 127.5, 123.0, 122.2, 120.8, 120.6, 78.8, 77.4, 68.7, 67.0, 55.9, 55.8, 47.8, 46.8, 44.5, 30.3, 28.4, 28.3, 24.8; HRMS (ES) calcd for $C_{47}H_{48}N_5O_7PNa$: 848.3189 $(M+Na)^+$, found 848.3184.

**1.1.5. Coupling of cyano ylide 6 with acid 4 to form 7.** Cyano ylide **6** (2.58 g, 3.12 mmol) was treated with $CH_2Cl_2$–TFA (1:1, 50 mL). After 1 h, the reaction mixture was concentrated under vacuum, and the residue was treated with 10% $Na_2CO_3$ (50 mL) and extracted with $CH_2Cl_2$ (3×60 mL). The combined organic extracts were dried over $Na_2SO_4$, and concentrated to yield a foam. Without purification, it was added to a reaction mixture of **4** (1.32 g, 2.97 mmol), EDCI (626 mg, 3.27 mmol), and HOBt (441.8 mg, 3.27 mmol) in $CH_2Cl_2$ (25 mL). The reaction mixture was stirred overnight, and then concentrated. Flash chromatography of the residue with 2.5:1 EtOAc–hexane afforded **7** (2.67 g, 78% yield) as a white foam: IR (neat) 3391 (br), 3307 (shoulder), 2181, 1717, 1652, 1646, 1608, 1507, 1437, 1250 cm$^{-1}$. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.46 (br s, 1H), 9.27 (br s, 1H), 7.62–7.60 (m, 3H), 7.55–7.45 (m, 12H), 7.29 (m, 1H), 6.77 (d, $J$=7.5 Hz, 1H), 6.13 (m, 1H), 5.33 (d, $J$=7.9 Hz, 1H), 5.23 (s, 2H), 5.15 (m, 1H), 5.06 (d, $J$=3.0 Hz, 2H), 4.63 (m, 1H), 4.38 (m, 1H), 4.10 (m, 2H), 4.00 (m, 2H), 3.57 (m, 2H), 3.20 (m, 1H), 2.79 (m, 1H), 1.98 (m, 2H), 1.93 (m, 1H), 1.83 (m, 1H), 1.75 (m, 2H), 1.64 (m, 1H), 1.33 (s, 9H), 0.93 (t, $J$=8.3 Hz, 2H), 0.0 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 193.4, 170.7, 170.4, 163.9, 160.5, 156.3, 156.0, 155.8, 136.7, 134.7, 133.4, 133.3, 133.2, 129.2, 129.1, 128.6, 128.4, 128.2, 128.0, 127.8, 127.6, 122.6, 121.9, 120.1, 120.0, 78.8, 77.4, 68.7, 66.9, 63.1, 60.7, 54.3, 54.2, 51.3, 48.8, 47.8, 47.3, 44.5, 43.0, 30.3, 29.1, 28.2, 24.6, 24.4, 17.6, −1.6; HRMS (ES) calcd for $C_{61}H_{73}N_8O_{11}$PSiNa: 1175.4803 $(M+Na)^+$, found 1175.4802.

**1.1.6. Wittig reaction of aldehyde 8 to form unsaturated ester 9.** The TIPS derivative of L-Boc-tyrosine methyl ester[9] was reduced with DIBAL-H to the aldehyde **8** (90%). A solution of aldehyde **8** (2.60 g, 8.54 mmol) in DMF (20 mL) was treated with [(allyloxycarbonyl)methylene]-triphenylphosphorane (3.38 g, 9.39 mmol) and stirred at room temperature for 5 h. The reaction mixture was diluted with water (80 mL), and extracted with ether (3×100 mL). The combined organic extracts were dried over $Na_2SO_4$, concentrated, and purified by flash chromatography (5:1 hexanes–EtOAc) to give the allyl ester **9** (4.0 g, 95%) as a colorless oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.01 (d, $J$=8.2 Hz, 2H), 6.92 (dd, $J$=5.1, 15.6 Hz, 1H), 6.81 (d, $J$=8.2 Hz, 2H), 5.92 (m, 1H), 5.86 (dd, $J$=1.5, 15.6 Hz, 1H), 5.31 (dd, $J$=1.5, 17.2 Hz, 1H), 5.22 (dd, $J$=1.1, 10.4 Hz, 1H), 4.67 (d, $J$=8.6 Hz, 1H), 4.62 (d, $J$=5.4 Hz, 2H), 4.56 (br s, 1H), 2.81 (d, $J$=6.3 Hz, 2H), 1.41 (s, 9H), 1.23 (m, 3H), 1.10 (s, 9H), 1.09 (s, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 165.9, 155.1, 155.0, 148.5, 132.3, 130.4, 128.9, 120.8, 120.1, 118.4, 118.2, 79.8, 77.4, 65.2, 52.7, 40.1, 28.4, 18.0, 12.7; HRMS (ES) calcd for $C_{28}H_{45}NO_5SiNa$: 526.2965 $(M+Na)^+$, found 526.2966.

**1.1.7. Coupling of 9 with D-Boc-alloisoleucine.** The unsaturated ester **9** (4.18 g, 8.25 mmol) was treated with $CH_2Cl_2$–TFA (1:1, 50 mL) for 1 h, then concentrated under vacuum. The residue was dissolved in $CH_2Cl_2$ (10 mL), and triethylamine (1.26 mL, 9.08 mmol) was added. The reaction mixture was added to a solution of Boc-D-AIle (1.73 g, 7.50 mmol), EDCI (1.73 g, 9.00 mmol), HOBt (1.22 g, 9.00 mmol) in $CH_2Cl_2$ (50 mL). The resulting mixture was stirred at room temperature overnight and then diluted with $CH_2Cl_2$ (50 mL), and washed with 1N HCl (100 mL), sat. $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over $Na_2SO_4$ and concentrated. Flash chromatography of the residue with 7:1 hexanes–EtOAc afforded coupling product **10** (3.93 g, 85% yield) as a white foam. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.99 (d, $J$=8.4 Hz, 2H), 6.94 (dd, $J$=4.9, 15.7 Hz, 1H), 6.79 (d, $J$=8.4 Hz, 2H), 6.30 (m, 1H), 5.89 (m, 2H), 5.29 (dd, $J$=1.5, 17.2 Hz, 1H), 5.22 (dd, $J$=1.1, 10.5 Hz, 1H), 5.08 (m, 1H), 4.91 (m, 1H), 4.60 (d, $J$=5.7 Hz, 2H), 3.96 (m, 1H), 2.88 (m, 1H), 2.79 (m, 1H), 1.78 (m, 1H), 1.44 (s, 9H), 1.23 (m, 4H), 1.09 (s, 9H), 1.08 (s, 9H), 1.05 (m, 1H), 0.84 (t, $J$=6.5 Hz, 3H), 0.77 (d, $J$=6.8 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 171.6, 165.8, 156.1, 155.2, 147.6, 132.3, 130.3, 128.7, 121.1, 120.1, 118.3, 80.1, 65.2, 58.8, 51.2, 39.7, 36.9, 28.5, 26.2, 18.0, 14.5, 12.8, 11.6; HRMS (ES) calcd for $C_{34}H_{56}N_2O_6$SiNa: 639.3805 $(M+Na)^+$, found 639.3804.

**1.1.8. Formation of the free amine 11 from Boc derivative 10.** Product **10** containing a vinylogous tyrosine residue (980.0 mg, 1.59 mmol) was treated with $CH_2Cl_2$–TFA (1:1, 20 mL) at room temperature for 1 h, then the solvents were evaporated under vacuum. The residue was basified with 10% $Na_2CO_3$ (15 mL), and extracted with $CH_2Cl_2$ (3×15 mL). The combined extracts were dried over $Na_2SO_4$, and concentrated to give the free amine **11** (822 mg, 100% yield) as a pale yellow oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.57 (d, $J$=8.6 Hz), 6.99 (d, $J$=8.2 Hz, 2H), 6.93 (dd, $J$=5.2, 15.7 Hz, 1H), 6.78 (d, $J$=8.2 Hz, 2H), 5.89 (m, 1H), 5.79 (d, $J$=15.7 Hz, 1H), 5.28 (d, $J$=18.2, 1H), 5.20 (d, $J$=10.4 Hz, 1H), 4.87 (m, 1H), 4.60 (d, $J$=5.6 Hz, 2H), 3.25 (m, 1H), 2.88 (dd, $J$=6.7, 13.8 Hz, 1H), 2.80 (dd, $J$=7.2, 13.8 Hz, 1H), 2.03 (m, 1H), 1.30–1.19 (m, 7H), 1.07 (s, 9H), 1.06 (s, 9H), 0.89 (t, $J$=7.4 Hz, 3H), 0.72 (d, $J$=6.9 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 174.3, 165.8, 155.1, 148.2, 132.3, 130.4, 130.3, 129.0, 120.8, 120.6, 120.1, 118.2, 77.4, 65.2, 57.9, 50.9, 39.9, 37.1, 26.9, 18.0, 17.6, 13.3, 13.0, 12.0; HRMS calcd for $C_{29}H_{49}N_2O_4Si$: 517.3461 $(M+H)^+$, found 517.3467.

**1.1.9. Formation of the pentapeptide 12.** A solution of the cyano ylide **7** (540 mg, 0.47 mmol) in $CH_2Cl_2$ (15 mL) was cooled to −78°C, then ozone was bubbled through until the solution became bluish. Nitrogen was then bubbled through for 10 min to remove extra ozone. A solution of amine **11** (255 mg, 0.49 mmol) in $CH_2Cl_2$ (2 mL) was added, and the resulting reaction was kept at −78°C for 15 min, then warmed to room temperature, and the solvent was evaporated. $AgNO_3$ (5 mL, 1 M in 4:1 of THF–$H_2O$) was added, and the reaction stirred for 4 h. The reaction mixture was extracted with EtOAc (3×10 mL). The combined organic layers were dried over $Na_2SO_4$ and concentrated. Flash chromatography of the residue with 1.5:1 EtOAc–hexanes gave pentapeptide **12** (492.3 mg, 75% yield) as a white foam. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.41–7.27 (m, 10H), 7.09 (m, 2H), 6.94 (m, 1H), 6.79 (m, 2H), 5.91 (m, 2H), 5.27 (m, 3H), 5.18 (m, 1H), 5.12 (s, 2H), 4.79 (m, 1H), 4.57 (m, 3H), 4.33 (m, 2H), 4.14 (m, 2H), 3.90 (m, 2H), 3.70 (m, 2H), 3.41 (m, 1H), 3.21 (m, 1H), 2.94 (m, 1H), 2.69 (m, 1H), 2.18–1.69 (m, 5H), 1.62 (m, 2H), 1.52 (m, 2H), 1.42 (s, 9H), 1.24 (m, 4H), 1.14 (m, 1H), 1.11 (s, 9H), 1.09 (s, 9H), 0.95 (m, 3H), 0.80 (m, 3H), 0.65 (m, 3H), 0.02 (s, 9H); $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta$ 196.5, 175.4, 174.3, 172.9, 172.5, 171.9, 171.6, 167.5, 167.3, 165.2, 165.1, 162.1, 158.7, 158.5, 157.2, 157.1, 157.0, 156.2, 149.5, 149.3, 149.2, 138.6, 136.9, 136.7, 136.6, 133.8, 133.7, 131.4, 131.3, 129.9, 129.7, 129.6, 129.5, 129.4, 129.2, 129.1, 129.0, 121.9, 121.8, 120.9, 118.6, 118.5, 100.2, 99.4, 80.5, 80.4, 70.2, 70.1, 70.0, 68.4, 66.3, 66.2, 64.4, 62.1, 62.0, 61.4, 58.7, 58.4, 57.2, 56.2, 55.8, 54.4, 54.3, 54.2, 53.5, 53.4, 46.2, 45.7, 42.8, 40.2, 40.1, 38.3, 38.4, 30.9, 30.4, 29.0, 27.5, 26.0, 18.8, 18.6, 15.3, 15.2, 14.0, 12.2, 12.1, −1.3; HRMS (ES) calcd for $C_{72}H_{109}N_9O_{17}SiNa$: 1450.7378 $(M+MeOH+Na)^+$, found 1450.7375.

**1.1.10. Formation of the pentafluorophenoxy derivative 13.** Pentapeptide **12** (435 mg, 0.31 mmol) was dissolved in $CH_2Cl_2$ (8 mL), the flask was wrapped with aluminum foil, and phenylsilane (39 mL, 0.62 mmol) was added, followed by the addition of tetrakis (triphenylphosphine)palladium (30.0 mg, 0.03 mmol). The resulting mixture was stirred at room temperature for 15 min, then 1N HCl (10 mL) was added, and stirred continuously for 1 h at room temperature. The aqueous layer was extracted with $CH_2Cl_2$ (3×10 mL), and the combined extracts were dried over $Na_2SO_4$, filtered and concentrated to give the acid which was dissolved in $CH_2Cl_2$ (2 mL). DCC (96.0 mg, 0.47 mmol) and pentafluorophenol (114.1 mg, 0.62 mmol) were then added, and the resulting mixture was stirred overnight. The solvent was evaporated under reduced pressure and the residue was separated on silica gel with 1.5:1 hexane–EtOAc to give the pentafluorophenoxy derivative **13** (358.8 mg, 76% yield for the two steps) as a white foam. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.40–7.27 (m, 11H), 7.12 (d, $J$=8.4 Hz, 2H), 6.81 (d, $J$=8.4 Hz, 2H), 6.20 (m, 1H), 5.27 (br s, 2H), 5.11 (br s, 2H), 4.56 (m, 1.4H), 4.40–4.27 (m, 2.6H), 4.12 (m, 2H), 3.97 (m, 2H), 3.73 (m, 2H), 3.40 (m, 1H), 3.14 (m, 1H), 3.01 (m, 1H), 2.98 (m, 1H), 2.30–1.60 (m, 9H), 1.41 (s, 9H), 1.25 (m, 4H), 1.14 (m, 1H), 1.11 (s, 9H), 1.09 (s, 9H), 0.96 (m, 3H), 0.85–0.62 (m, 6H), 0.03 (s, 8H), −0.04 (s, 1H); $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta$ 196.5, 174.4, 173.2, 173.1, 172.8, 172.1, 171.6, 165.2, 163.3, 163.2, 162.1, 162.0, 158.7, 158.5, 157.3, 157.2, 156.4, 154.9, 154.7, 143.7, 141.8, 140.3, 138.6, 136.9, 136.7, 131.5, 131.3, 131.2, 129.8, 129.7, 129.6, 129.5, 129.2, 129.1, 129.0, 121.0, 118.6, 100.2, 99.5, 80.5, 70.1, 68.4, 64.4, 62.1, 62.0, 61.5, 58.9, 58.7, 58.5, 57.2, 55.9, 54.4, 53.9, 53.7, 46.1, 45.7, 42.8, 39.8, 38.8, 38.6, 38.3, 34.9, 30.9, 30.7, 28.9, 27.5, 27.3, 26.8, 26.7, 26.2, 26.0, 18.8, 18.6, 15.4, 15.2, 15.0, 14.0, 12.2, 12.1, 12.0, −1.2; MS (ES) calcd for $C_{75}H_{104}N_9O_{17}F_5Si_2Na$: 1576.6907 $(M+MeOH+Na)^+$, found 1576.76.

**1.1.11. Ring-closure of 13 to the protected macrocyclic pentapeptide 14.** The open-chain pentapeptide **13** (175 mg, 0.115 mmol) was dissolved in $CH_2Cl_2$ (10 mL), sat. HCl in $Et_2O$ (6 mL) was added, and the mixture was stirred for 15 min, then concentrated under vacuum. The residue in DMF (50 mL) was added slowly through a syringe pump over 1 h to a mixture of $NaHCO_3$ (145 mg, 1.72 mmol) and DMAP (2.81 mg, 0.02 mmol) in DMF (50 mL) which was cooled to −5°C, and the reaction mixture was stirred at −5°C for 20 h, then quenched with 1N HCl and extracted with $Et_2O$ (3×150 mL). The organic extracts were combined, dried over $Na_2SO_4$ and concentrated. The residue was purified on silica gel with EtOAc–hexane (7:3) to give the macrocyclic product **14** (86.8 mg, yield 61%) as a white foam. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.37–7.22 (m, 10H), 7.09 (d, $J$=8.4 Hz, 2H), 6.77 (m, 3H), 6.16 (d, $J$=15.3 Hz, 1H), 5.21 (s, 2H), 5.09 (m, 2H), 4.75 (m, 1H), 4.42 (m, 2H), 4.28 (m, 1H), 4.16–3.90 (m, 5H), 3.88 (m, 1H), 3.72 (m, 1H), 3.43 (m, 1H), 3.06 (m, 1H), 2.75 (m, 1H), 2.58 (m, 1H), 2.13 (m, 1H), 2.05 (m, 1H), 1.95–1.72 (m, 4H), 1.67 (m, 1H), 1.55 (m, 1H), 1.49–1.31 (m, 2H), 1.21 (m, 4H), 1.08 (s, 9H), 1.06 (s, 9H), 0.90 (m, 2H), 0.77 (m, 3H), 0.60 (d, $J$=6.6 Hz, 0.9H), 0.54 (d, $J$=6.6 Hz, 2.1H), −0.03 (s, 9H); $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta$ 174.1, 173.9, 172.9, 172.2, 171.9, 171.8, 170.5, 168.0, 165.1, 162.3, 158.5, 157.2, 156.2, 144.4, 144.0, 138.6, 138.5, 136.7, 131.9, 131.3, 131.2, 129.9, 129.8, 129.7, 129.6, 129.5, 129.2, 129.1, 129.0, 125.3, 124.7, 120.8, 99.8, 99.6, 70.1, 68.4, 68.3, 64.5, 61.8, 58.4, 57.8, 56.8, 56.1, 53.1, 52.9, 52.6, 52.5, 46.3, 46.2, 41.5, 41.2, 40.8, 40.0, 39.6, 31.5, 30.9, 27.3, 26.7,

26.4, 26.2, 24.9, 24.7, 18.8, 18.7, 18.6, 15.1, 14.8, 14.0, 12.7, 12.4, −1.3; HRMS (ES) calcd for $C_{63}H_{95}N_9O_{14}Si_2Na$: 1292.6435 $(M+MeOH+Na)^+$, found 1292.6444.

**1.1.12. Removal of Teoc protecting group to form the protected cyclotheonamides 17 and 18.** The macrocyclic pentapeptide with TIPS, Teoc, and Cbz protection (40 mg, 0.032 mmol) was treated with TFA–$CH_2Cl_2$ (1:1, 6 mL), and the reaction mixture was stirred for 90 min, then concentrated. The residue was dissolved in $CH_2Cl_2$ (15 mL), and washed with aqueous $NaHCO_3$ (2×10 mL). The organic layer was dried over $Na_2SO_4$ and concentrated. The solution of the residue in $CH_2Cl_2$ (1 mL) was added to a mixture of EDCI (12.3 mg, 0.064 mmol), HOBt (8.6 mg, 0.064 mmol) and benzoylalanine **15** (6.8 mg, 0.0352 mmol) or isovalerylalanine **16** (8.3 mg, 0.0352 mmol) in $CH_2Cl_2$ (1 mL), and the resulting mixture was stirred overnight. It was then diluted with $CH_2Cl_2$ (15 mL) and washed with sat. $NaHCO_3$, and then brine. The organic layer was dried over $Na_2SO_4$ and concentrated. Flash chromatography of the residue in each case with 5:1 EtOAc–hexanes gave **17** (34.5 mg, yield 85%) or **18** (33.2 mg, yield 83%) as white foams.

*For **17***. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.84 (m, 2H), 7.51 (m, 1H), 7.44–7.26 (m, 12H), 7.18 (d, $J$=8.5 Hz, 2H), 6.8 (m, 1H), 6.79 (d, $J$=8.5 Hz, 2H), 6.19 (dd, $J$=1.5, 15.4 Hz, 1H), 5.24 (s, 2H), 5.11 (m, 2H), 4.81 (m, 1H), 4.66 (m, 1H), 4.57 (m, 1H), 4.47 (m, 1H), 4.31 (m, 1H), 4.25 (m, 1H), 4.09–3.92 (m, 2H), 3.91 (m, 1H), 3.78 (m, 1H), 3.50 (m, 1H), 3.09 (m, 1H), 2.86 (m, 1H), 2.62 (m, 1H), 2.20–1.73 (m, 6H), 1.67 (m, 1H), 1.58 (m, 1H), 1.40 (m, 5H), 1.25 (m, 3H), 1.11 (s, 9H), 1.10 (s, 9H), 0.81 (m, 4H), 0.64 (d, $J$=6.8 Hz, 1H), 0.58 (d, $J$=6.6 Hz, 2H); $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta$ 175.5, 174.4, 174.2, 173.2, 172.3, 172.1, 171.8, 170.8, 170.4, 168.3, 165.4, 162.6, 157.5, 156.5, 144.5, 144.1, 138.7, 138.6, 136.7, 135.2, 133.0, 131.9, 131.3, 131.2, 129.9, 129.8, 129.7, 129.7, 129.6, 129.2, 129.1, 129.0, 128.7, 125.3, 124.6, 120.9, 99.8, 99.6, 70.1, 68.5, 68.4, 61.8, 58.5, 57.8, 56.8, 56.1, 53.2, 53.0, 51.5, 51.4, 50.9, 50.8, 46.3, 46.2, 41.6, 41.2, 41.1, 40.8, 40.0, 39.6, 31.5, 30.9, 27.3, 26.7, 26.4, 26.2, 24.9, 24.7, 18.6, 18.2, 15.1, 14.8, 14.0, 12.6, 12.6, 12.4; HRMS (ES) calcd for $C_{68}H_{92}N_{10}O_{14}SiNa$: 1323.6461 $(M+MeOH+Na)^+$, found 1323.6459.

*For **18***. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.42–7.27 (m, 10H), 7.13 (d, $J$=8.4 Hz, 2H), 6.83 (m, 1H), 6.80 (d, $J$=8.4 Hz, 2H), 6.18 (d, $J$=15.1 Hz, 1H), 5.26 (s, 2H), 5.14 (m, 2H), 4.80 (m, 1H), 4.61 (m, 1H), 4.47 (m, 1H), 4.38–4.25 (m, 2H), 4.22 (m, 1H), 4.10–3.85 (m, 3H), 3.77 (m, 1H), 3.51 (m, 1H), 3.09 (m, 1H), 2.83 (m, 1H), 2.62 (m, 1H), 2.14–2.00 (m, 5H), 1.95 (m, 1H), 1.86 (m, 2H), 1.68 (m, 1H), 1.60 (m, 1H), 1.48–133 (m, 2H), 1.32–1.21 (m, 7H), 1.11 (s, 9H), 1.10 (s, 9H), 0.99–0.90 (m, 6H), 0.81 (m, 4H), 0.64 (d, $J$=6.8 Hz, 1H), 0.58 (d, $J$=6.7 Hz, 2H); $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta$ 175.5, 175.1, 174.1, 173.9, 172.0, 171.8, 171.4, 170.5, 165.1, 162.3, 157.2, 156.2, 144.4, 144.1, 138.7, 138.6, 136.8, 131.9, 131.3, 131.2, 129.9, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 125.3, 124.7, 120.9, 99.8, 99.6, 70.1, 68.5, 68.4, 61.8, 58.4, 57.8, 56.8, 56.1, 53.2, 53.0, 51.4, 51.3, 46.3, 46.0, 41.6, 41.2, 41.1, 40.8, 40.0, 39.6, 33.2, 31.5, 30.9, 30.8, 27.5, 27.3, 26.7, 26.4, 26.1, 24.9, 24.7, 23.8, 22.9, 22.8, 18.5, 18.1, 15.1, 14.8, 14.0, 12.6, 12.4; HRMS (ES) calcd for $C_{66}H_{96}N_{10}O_{14}SiNa$: 1303.6774 $(M+MeOH+Na)^+$, found 1303.6775.

**1.1.13. Cyclotheonamides $E_2$ and $E_3$.** Product **17** (20 mg, 0.0158) or product **18** (20 mg, 0.016 mmol) was treated with HF·Py (1 mL) and anisole (0.15 mL) and the resulting mixture was stirred at room temperature for 3 h. Nitrogen was then bubbled through the solution for 1 h to remove excess HF, $H_2O$ (8 mL) was added, and the resulting solution was lyophilized to give crude products. HPLC purification (C18, gradient eluting with $CH_3CN$–$H_2O$ 1:99 to 40:60 over 25 min, 1% TFA) furnished cyclotheonamide $E_2$ (9.4 mg, 70%) or cyclotheonamide $E_3$ (9.5 mg, 72%) as white foams.

*For $E_2$ (**1a**)*. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.87 (d, $J$=7.2 Hz, 2H), 7.56 (t, $J$=7.3 Hz, 1H), 7.47 (m, 2H), 7.08 (d, $J$=8.4 Hz, 2H), 6.82 (dd, $J$=2.5, 15.5 Hz, 1H), 6.72 (d, $J$=8.5 Hz, 2H), 6.17 (dd, $J$=2.2, 15.5 Hz, 1H), 4.75 (m, 1H), 4.67 (m, 1H), 4.58 (m, 1H), 4.52 (m, 1H), 4.28 (m, 2H), 4.14 (m, 0.7H), 4.06 (m, 0.3H), 3.86 (m, 1H), 3.55 (m, 1H), 3.15 (m, 2H), 3.08 (m, 1H), 2.87 (m, 1H), 2.59 (m, 1H), 2.25 (m, 1H), 1.97 (m, 4H), 1.70 (m, 1H), 1.58 (m, 2H), 1.45 (d, $J$=7.2 Hz, 3H), 1.41 (m, 1H), 1.31 (m, 1H), 0.83 (m, 4H), 0.63 (d, $J$=6.7 Hz, 1H), 0.57 (d, $J$=6.7 Hz, 2H); $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta$ 175.3, 175.2, 174.4, 174.3, 174.1, 173.2, 173.1, 172.4, 172.3, 171.8, 171.6, 170.5, 170.3, 168.1, 158.7, 157.5, 144.4, 144.0, 135.2, 133.0, 131.2, 131.1, 130.0, 129.7, 128.7, 125.2, 124.6, 116.4, 99.8, 99.7, 71.5, 62.0, 58.6, 58.1, 55.9, 55.3, 55.2, 53.6, 53.5, 53.4, 53.3, 51.8, 51.7, 50.9, 50.8, 42.2, 42.1, 41.6, 41.2, 41.0, 40.9, 40.0, 39.6, 31.6, 31.0, 27.4, 27.3, 26.6, 26.3, 26.2, 25.3, 24.8, 17.9, 14.8, 14.6, 12.5, 12.3; HRMS (ES) calcd for $C_{43}H_{61}N_{10}O_{10}$: 877.4572 $(M+MeOH+H)^+$, found 877.4575.

*For $E_3$ (**1b**)*. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.08 (d, $J$=8.5 Hz, 2H), 6.82 (m, 1H), 6.71 (d, $J$=8.4 Hz, 2H), 6.16 (d, $J$=15.4 Hz, 1H), 4.75 (m, 1H), 4.62 (m, 1H), 4.51 (m, 1H), 4.34 (m, 1H), 4.26 (m, 2H), 4.12 (m, 0.7H), 4.04 (m, 0.3H), 3.84 (m, 1H), 3.53 (m, 1H), 3.14 (m, 2H), 3.07 (m, 1H), 2.82 (m, 1H), 2.58 (m, 1H), 2.24 (m, 1H), 2.12–1.92 (m, 6H), 1.70 (m, 1H), 1.59 (m, 2H), 1.38 (m, 1H), 1.35 (m, 2H), 1.30 (d, $J$=7.1 Hz, 3H), 0.95 (d, $J$=5.6 Hz, 3H), 0.94 (d, $J$=5.9 Hz, 3H), 0.82 (m, 4H), 0.62 (d, $J$=6.7 Hz, 1H), 0.56 (d, $J$=6.8 Hz, 2H); $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta$ 175.7, 175.1, 175.0, 174.3, 174.1, 173.2, 173.1, 172.4, 172.3, 171.8, 170.5, 168.1, 158.7, 157.5, 144.4, 144.0, 131.2, 131.1, 130.0, 125.2, 124.6, 116.4, 99.8, 99.7, 71.4, 62.0, 58.5, 58.1, 55.9, 55.3, 55.2, 53.7, 53.6, 51.6, 50.9, 46.0, 42.2, 42.1, 41.6, 41.1, 40.9, 39.7, 39.6, 31.5, 31.0, 27.6, 27.4, 27.3, 26.6, 26.4, 26.2, 25.3, 22.9, 22.8, 17.8, 14.8, 14.6, 12.5, 12.3; HRMS (ES) calcd for $C_{41}H_{65}N_{10}O_{10}$: 857.4885 $(M+MeOH+H)^+$, found 857.4888.

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5. An additional difference between our synthesis and earlier work lies in the final macrolactamization site. In contrast to the other synthetic routes, the ring closure in our sequence took place between the NH terminus of the diaminopropionic acid residue and the carboxyl group of the α,β-vinylogous tyrosine. Di-Cbz protection of the guanidine residue provided excellent protection throughout the sequence and permitted easy removal at the last stage with HF·Py and anisole (70–72%).[6] As outlined, all steps were accomplished in good to excellent yields.
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