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Asymmetric Synthesis of Apratoxin E

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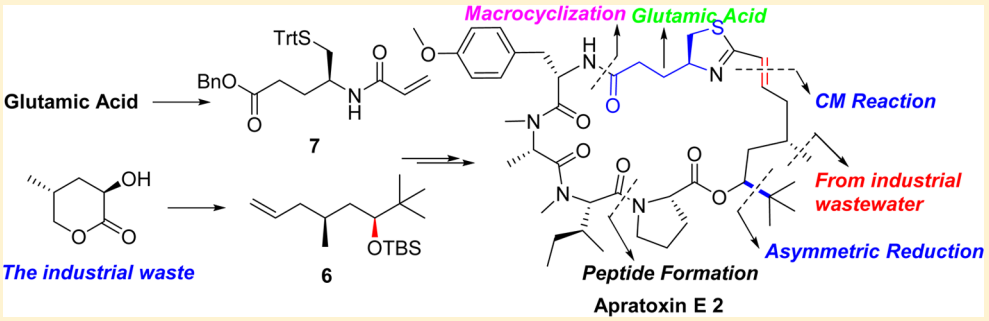
Supporting Information



ABSTRACT: An efficient method for asymmetric synthesis of apratoxin E **2** is described in this report. The chiral lactone **8**, recycled from the degradation of saponin glycosides, was utilized to prepare the non-peptide fragment **6**. In addition to this "*from nature to nature*" strategy, olefin cross-metathesis (CM) was applied as an alternative approach for the formation of the double bond. Moreover, pentafluorophenyl diphenylphosphinate was found to be an efficient condensation reagent for the macrocyclization.

INTRODUCTION

As a class of promising compounds in drug discovery, marine cyanobacteria produce suites of biological secondary metabolites, which possess a variety of physiological activities including antimicrobial, antimalarial, cytotoxic, and neorotoxic properties.[1] Cyanobacterial cyclodepsipeptides, predominantly with structural skeletons as depsipeptides and peptide−polyketide hybrids, have attracted considerable attention as novel pharmaceuticals. A number of them have been advanced to therapeutic lead compounds in phase II clinical trials for cancer treatment.[2] As a prime instance, a family of apratoxins, which are cyclodepsipeptides, exhibit potent cancer growth inhibitory activity by inducing G1 phase specific cell cycle arrest and apoptosis (Figure 1).[3]

Apratoxins A (**1**) and E (**2**) were isolated from the remarkably prolific *Lyngbya majuscula* collected in Guam and Palau, while apratoxin D (**3**) was obtained from the same species collected in Papua New Guinea (Figure 1). All of these isolated marine secondary metabolites show potent in vitro cytotoxicity against LoVo cell lines (IC_{50}: 0.36−10.8 nM) and the KB (IC_{50}: 0.52−21.3 nM).[4] Because of their unique scaffolds and striking biological activities, tremendous efforts have been devoted to the asymmetric synthesis of apratoxins A, C, D, F[5] and corresponding analogues,[6] as well as the studies on their mechanism of action[7] and biosynthetic pathways.[8] We demonstrated an asymmetric method for synthesis of dehydroapratoxin A in 2011.[9] Interestingly, apratoxin E (**2**), a new peptide−polyketide hybrid of the apratoxin class, seemed to be ignored by synthetic chemistry. As part of our continuous efforts in searching for divergent synthesis of biological secondary metabolites[10] to expand our knowledge about their structure−activity relationships, herein we present the first asymmetric synthesis of apratoxin E **2**.

RESULTS AND DISCUSSION

Our strategy for asymmetric synthesis of apratoxin E **2** is illustrated in Figure 2, with stereoselective synthesis of non-peptide fragment **5** and effective macrocyclization as our main focus in constructing this target molecule. As we aim to achieve an effective method for synthesis of apratoxin E **2**, we proposed a cross-metathesis (CM)[11] reaction to form the double bond between C34 and C35 and explored macrolactamization at two different connection points for the final ring closure. Moreover, the concept of "*from nature to nature*" was applied in our asymmetric synthesis of apratoxin E. In other words, the key fragment **6** could be prepared from industrial waste, and another non-peptide fragment **7** could be derived from glutamic acid.

The preparation of non-peptide fragment **6** is shown in Scheme 1. Chiral lactone **8** was isolated from the industrial wastewater during the degradation of saponin glycosides[12] in 22% yield. Treatment of **8** with benzyl chloride (BnCl) and NaOH in toluene under refluxing conditions resulted in the

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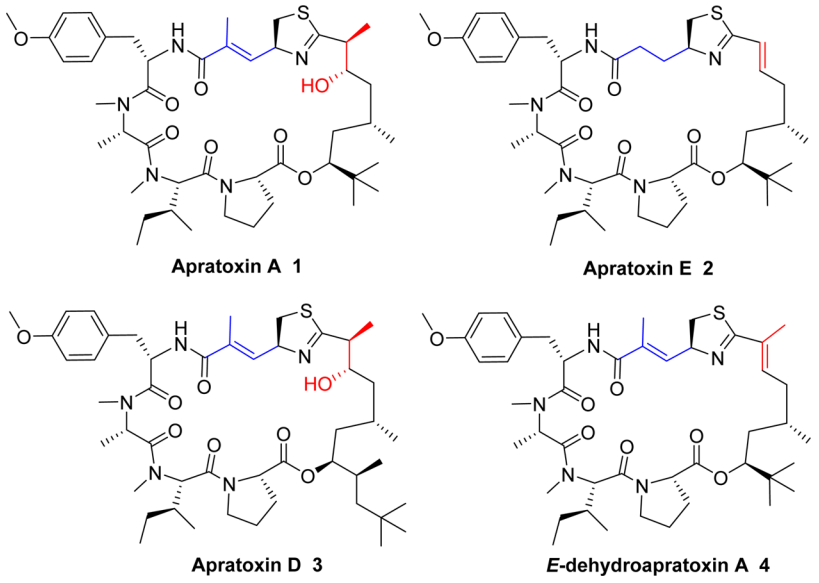


Figure 1. Structures for the representative apratoxins.

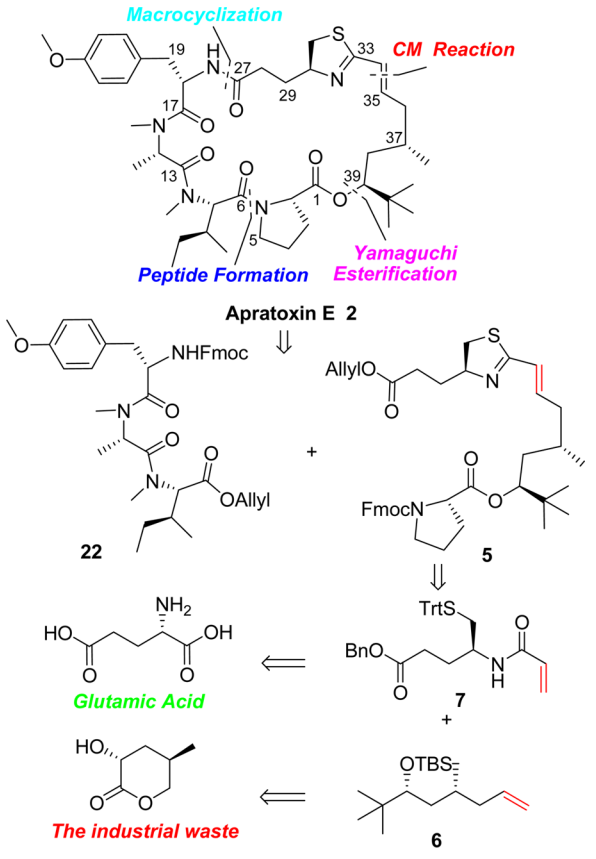
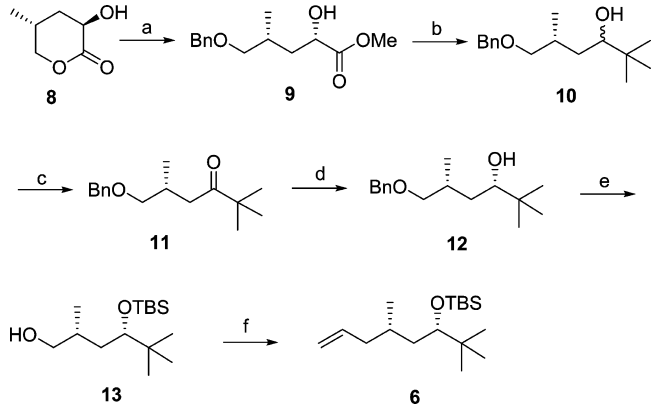


Figure 2. Our strategy to access apratoxin E.

ring opening and simultaneous selective benzylation of the primary alcohol. Upon the protection of carboxylic acid, the crude ester **9** was reduced with $LiAlH_4$, and the resulting diol was subjected to oxidative cleavage ($NaIO_4$) to give the aldehyde. The introduction of a *t*-butyl group was achieved by nucleophilic addition with a solution of *tert*-butylmagnesium chloride, which obviously gave a mixture of two diastereomers (*dr* = 1:1). This new stereocenter could be enriched through an oxidation and stereoselective reduction process. Thus, compound **10** was oxidized with DMP to give desired ketone **11**, which was treated with BH_3·DMS and a catalytic amount of Corey's chiral borane, *R*-CBS catalyst.[13] This sequence led to the enhancement of desired isomer with *syn*/*anti* = 88:12, and each isomer could be separated by silica gel chromatography. The subsequent transformations were straightforward. Protection (TBSOTf, 2,6-lutidine) of compound **12** and subsequent hydrogenation (Pd/C, H_2) generated the desired primary alcohol **13** in 76% overall yield. Iodination of the alcohol and then covered to olefin **6** via a copper-catalyzed Grignard coupling reaction[14] with vinylmagnesium bromide in 84% overall yield.

Scheme 1. Preparation of Olefin 6



[a]Reagents and conditions: a. (1) BnCl, NaOH, toluene, reflux, 12 h; (2) MeOH, H_2SO_4, reflux, 4 h; b. (1) $LiAlH_4$, THF, 10 h; (2) $NaIO_4$, H_2O, 1.5 h; (3) *t*-BuMgCl, THF, overnight, 47% (5 steps); c. DMP, $NaHCO_3$, DCM, 3 h, 87%; d. (*R*)-CBS, BH_3·DMS, toluene, −20 to 35 °C, 72%; e. (1) TBSOTf, 2,6-lutidine, DCM, 0 °C, 3 h; (2) Pd/C, $Pd(OH)_2$, H_2, MeOH, 3 h, 76% (2 steps); f. (1) I_2, imid., PPh_3, DCM, 15 min; (2) CH_3═$CHMgBr$, Li_2CuCl_4, THF, −78 °C to rt, overnight, 84% (2 steps).

Next, we turned our attention to prepare the peptide unit **7** of apratoxin E (Scheme 2). Protection (Boc_2O) and subsequent reduction[15] ($NaBH_4$) of the glutamic acid derivative **14** gave the desired alcohol **15** in 69% overall yield, which was

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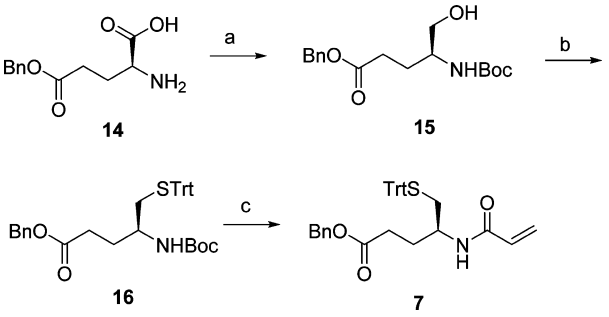
Scheme 2. Preparation of the Peptide Unit 7

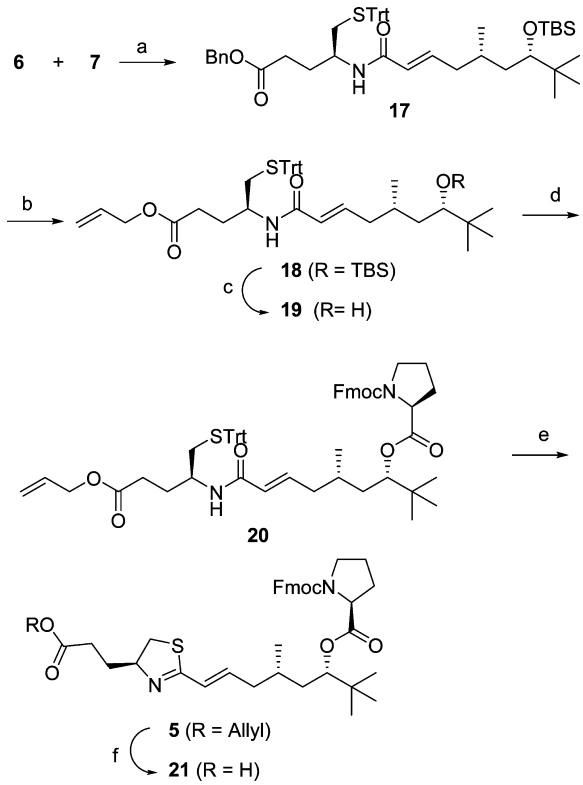


[a]Reagents and conditions: a. (1) Boc_2O, $NaHCO_3$, H_2O/dioxane, 0 °C to rt, overnight; (2) 4-methylmorpholine, ethyl chloroformate, −10 °C, 1 h then $NaBH_4$, THF, rt, 1 h, 69% (2 steps); b. (1) MsCl, TEA, DCM, 0 °C, 40 min; (2) NaH, TrtSH, DMF, 0 °C to rt, overnight, 79% (2 steps); c. (1) TFA, DCM, 0 °C, 30 min; (2) acrylic acid, HATU, DIPEA, DCM, 5 h, 71% (2 steps).

conveniently converted to its sulfide **16** by the known method[16] in 79% yield. Deprotection (TFA) of **16** and subsequent coupling with acrylic acid using HATU[17] generated amide **7** in 71% yield.

With the intermediates **6** and **7** in hand, the fragment **17** was prepared by CM reaction in 60% yield (*E*:*Z* > 99:1) (Scheme 3). After the benzyl ester was switched to allylic protection through hydrolysis (LiOH) and subsequent alkylation (AllylBr/K_2CO_3), the resulting compound **18** was subjected to a solution of HCl(6N)/EtOAc to produce the secondary alcohol **19** in 82% isolated yield, which was coupled with *N*-Fmoc-proline under Yamaguchi conditions[18] to give **20** in 89% yield. Then, compound **20** was treated with $POPh_3/Tf_2O$[19] in CH_2Cl_2 at 0 °C to form the desired thiazoline **5** in 86% yield. Finally, the deprotection of the allyl protected carboxylic acid in thiazoline **5** was achieved by treatment with catalytic amount of $Pd(PPh_3)_4$ in *N*-methylaniline to give the acid **21** in 88% yield.[20]

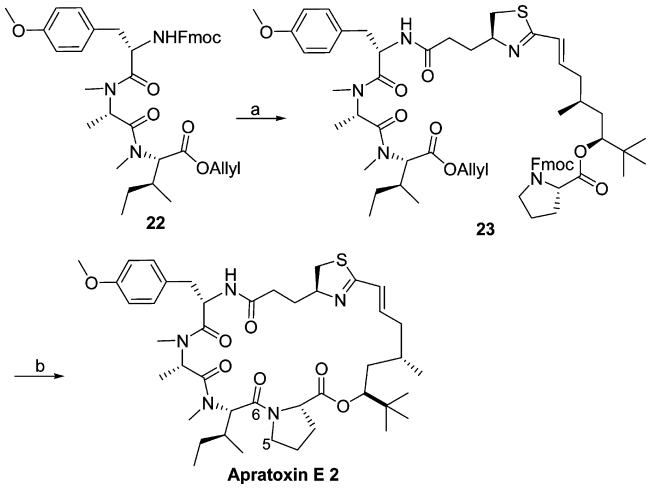
Scheme 3. Preparation of the Non-peptide Fragment 21



[a]Reagents and conditions: a. Grubbs 2nd, DCM, reflux, 24 h, 60%; b. (1) LiOH, THF/MeOH/H_2O, 0 °C to rt, 7 h; (2) AllylBr, K_2CO_3, DMSO, rt, 4 h, 84% (2 steps); c. HCl, EtOAc, 0 °C to rt, 3 h, 82%; d. *N*-Fmoc-proline, 2,4,6-trichlorobenzoylchloride, DIPEA, DMAP, toluene, 89%; e. $Ph_3P{=}O$, Tf_2O, DCM, 0 °C, 20 mim, 86%; f. $Pd(PPh_3)_4$, *N*-methylaniline, THF, 1 h, 88%.

Tripeptide **22** was prepared by sequential condensation of *N*-methylisoleucine allyl ester with *N*-Boc-*N*-methylalanine and *N*-Fmoc-*O*-methyltyrosine by the known method.[5d] Upon the treatment with Et_2NH, the resulting free amine was coupled with carboxylic acid **21** using pentafluorophenyl diphenylphosphinate (FDPP)[21] in acetonitrile to afford the amide **23** in 75% yield. The sequential cleavage of allyl ester by $Pd(PPh_3)_4$ in *N*-methylaniline and removal of the Fmoc protecting group with Et_2NH/CH_3CN afforded the crude cyclization precursor, which was subjected to macrolactamization (HATU/DIPEA) to give apratoxin E **2** in 19% overall isolated yield (Scheme 4).

Scheme 4. Synthesis of Apratoxin E (2) by Macrolactamization at C5 and C6



[a]Reagents and conditions: a. (1) Et_2NH, MeCN, 30 min; (2) **21**, FDPP, DIPEA, MeCN, overnight, 75%; b. (1) $Pd(PPh_3)_4$, *N*-methylaniline, THF, 30 min; (2) Et_2NH, MeCN, 30 min; (3) HATU, DIPEA, DCM, 40 h, 19%.

To improve the yield of macrolactamization, we turned our attention to the final amidation between the less hindered carboxylic acid and primary amino group, i.e., C27 and C18 (Scheme 5). After the deprotection of the allyl protected carboxylic acid in tripeptide fragment **22** and *N*-Fmoc protecting group in non-peptide fragment **5**, the resulting free carboxylic acid **24** and free amine **25** were coupled in the presence of HATU/DIPEA to generate **26** in 73% overall yield. Similar sequential cleavage of allyl ester by $Pd(PPh_3)_4$ in *N*-methylaniline and removal of the Fmoc protecting group with Et_2NH/CH_3CN gave the cyclization precursor, which, upon the subsequent macrolactamization use of FDPP as condensation reagent, afforded apratoxin E **2** in 43% overall isolated yield. The product could be further purified by RP C_{18} HPLC with 80% aqueous CH_3CN (Sepax-tech Amethyst C18 semipreparative column, 250 mm × 150 mm, 10 mL/min,

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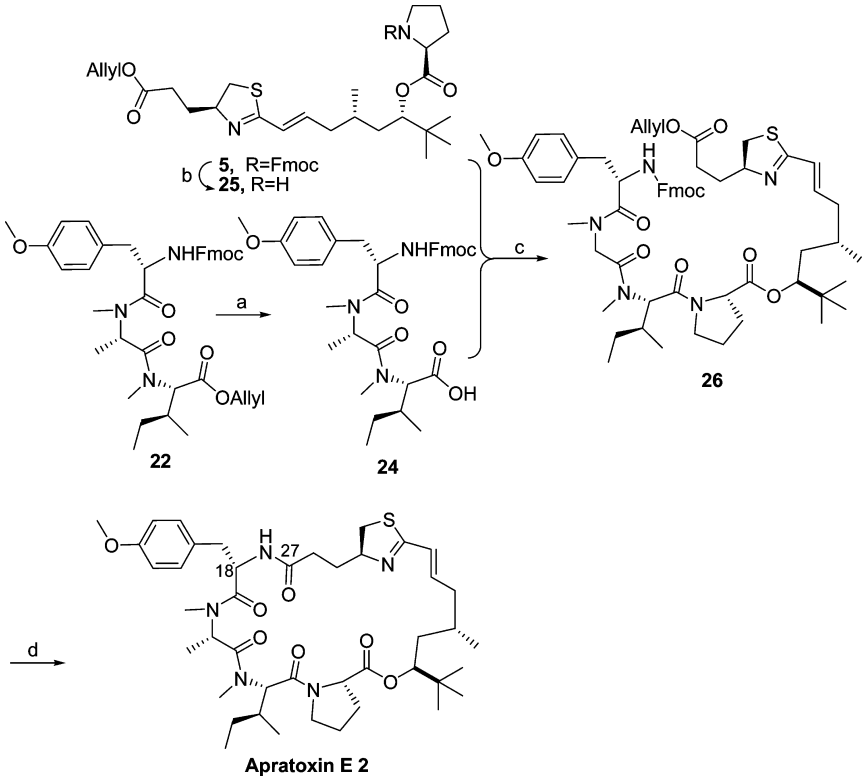
Scheme 5. Synthesis of Apratoxin E 2 by Macrolactamization at C18 and C27



[a]Reagents and conditions: a. $Pd(PPh_3)_4$, *N*-methylaniline, THF, 1 h; b. Et_2NH, MeCN, 30 min; c. HATU, DIPEA, DCM, overnight, 73%; d. (1) $Pd(PPh_3)_4$, *N*-methylaniline, THF, 30 min; (2) Et_2NH, MeCN, 30 min; (3) FDPP, DIPEA, MeCN, 40 h, 43%.

refractive index detection){$[\alpha]_D^{23}$ −150.4 (*c* 0.24, MeOH), lit.[4d] $[\alpha]_D^{20}$ −69 (*c* 0.12, MeOH)}, to afford >99% purity. Both ^1H and ^{13}C NMR spectroscopic data are consistent with those in the literature.[4d] Notably, apratoxin E **2** exists as a mixture of two conformers (2:3) in $CDCl_3$ due to the restricted rotation around the *O*-Me-Tyr-*N*-Me-Ala amide bond.[4b,d]

CONCLUSION

In summary, we first completed the enantioselective total synthesis of natural apratoxin E **2**. The main feature of our strategy is "*from nature to nature*", namely, the non-peptide fragment **6** was effectively prepared from industrial waste generated during the degradation of saponin glycosides. The cross-metathesis (CM) was applied as an alternative approach for the formation of the double bond. Moreover, FDPP was found to be an efficient condensation reagent for the macrocyclization. Further efforts on the extension of this strategy are ongoing in our laboratory to synthesize other analogues of apratoxin E. The chemistry and related biological data will be published in due course.

EXPERIMENTAL SECTION

General. THF was distilled from sodium/benzophenone. Reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel with a fluorescent indicator. Flash chromatography was performed on silica gel (300−400) with petroleum/EtOAc as eluent. Optical rotations were measured on a polarimeter with a sodium lamp. HRMS were measured on a LCMS-IT-TOF apparatus. IR spectra were recorded using film on a Fourier transform infrared spectrometer. NMR spectra were recorded at 400 or 600 MHz, and chemical shifts are reported in δ (ppm) referenced to an internal TMS standard for ^1H NMR and $CDCl_3$ (77.0 ppm) for ^{13}C NMR.

Methyl (2*S*,4*R*)-5-(Benzyloxy)-2-hydroxy-4-methylpentanoate 9. The lactone **8** (5.00 g, 38.43 mmol) was dissolved in toluene (60 mL); then NaOH (6.15 g, 153.72 mmol) and BnCl (8.80 mL, 76.86 mmol) were added. After being refluxed for 12 h, water was added. Extraction was performed with Et_2O three times, and the aqueous phase was acidified with hydrochloric acid. The resulting mixture was extracted with EtOAc (60 mL × 3), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give the crud acid without further purification. The above crude acid was dissolved in MeOH (60 mL), and then H_2SO_4 (1.00 mL, 98%) was carefully dropped. After being refluxed for 4 h, the reaction was quenched with water. The mixture was extracted with EtOAc (50 mL × 3), and the combined organic layers were washed with brine, dried, filtrated, and concentrated to give the crude ester without further purification. $[\alpha]_D^{23}$ = +6.2 (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3450, 2958, 2851, 2359, 1737, 1458, 1211, 1092, 740, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.17−7.05 (m, 5H), 4.35−4.27 (m, 2H), 4.14−4.03 (m, 1H), 3.57 (s, 3H), 3.17−3.15 (m, 1H), 3.15−3.12 (m, 1H), 1.97−1.85 (m, 1H), 1.60−1.42 (m, 2H), 0.82−0.78 (m, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.8, 138.2, 133.6, 130.2, 128.4, 127.6, 75.9, 73.1, 69.3, 52.4, 39.3, 30.6, 16.8 ppm; HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{14}H_{20}O_4H$: 253.1440, found: 253.1434.

(*R*)-6-(Benzyloxy)-2,2,5-trimethylhexan-3-one 11. A solution of the above ester **9** in THF (30 mL) was carefully dropped to a suspension of LAH (1.75 g, 46.15 mmol) in THF (30 mL). After being stirred for 10 h, the resulting mixture was carefully quenched with water (0.5 mL) and filtrated. The filtrate was concentrated to give the crude diol without further purification. The above diol was dissolved in water (50 mL), and then the mixture was acidified with hydrochloric acid to pH = 6 at 0 °C. $NaIO_4$ (9.87 g, 46.15 mmol) was added in one portion and stirred for 1.5 h. The mixture was extracted with EtOAc (30 mL × 3), and the combined organic layers were washed with brine, dried, filtrated, and concentrated to give the

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aldehyde without further purification. The above crude aldehyde was dissolved in dry THF (60 mL), and the mixture was cooled to 0 °C. Then, a solution of *tert*-butylmagnesium chloride (1 M in THF, 58.0 mL, 58.00 mmol) was slowly dropped. After being stirred for overnight, the reaction was quenched with an aqueous solution of saturated NH_4Cl and the resulting mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 10:1) to give **10** (4.52 g, 47%, 5 steps) as a colorless oil. The above alcohol (4.17 g, 16.65 mmol) was dissolved in cooled DCM (60 mL, 0 °C); then DMP (10.60 g, 24.98 mmol) was added in several portions. After being stirred for 3 h, the mixture was carefully quenched with a solution of saturated aqueous $NaHCO_3$ and solid $Na_2S_2O_3$. The resulting mixture was extracted with DCM (30 mL × 3), and the combined organic layers were washed with brine, dried, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 20:1) to give **11** (3.60 g, 87%) as a colorless oil. $[\alpha]_D^{23}$ = +6.7 (*c* 4.00, $CHCl_3$); IR (film): ν_{max} 2966, 1705, 1477, 1454, 1365, 1097, 738, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38−7.24 (m, 5H), 4.50−4.48 (m, 2H), 3.37−3.26 (m, 2H), 2.65 (dd, *J* = 16.8, 4.8 Hz, 1H), 2.46−2.30 (m, 2H), 1.12 (s, 9H), 0.94−0.92 (m, 3H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 214.6, 138.0, 127.7, 126.9, 126.8, 74.3, 72.2, 43.6, 39.8, 28.6, 25.7, 16.6 ppm; HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{16}H_{24}O_2H$: 249.1855, found: 249.1849.

(3*S*,5*R*)-6-(Benzyloxy)-2,2,5-trimethylhexan-3-ol 12. A solution of (*R*)-CBS (1 M in toluene, 1.23 mL, 1.23 mmol) was dissolved in dry toluene (15 mL) and cooled to −20 °C, and then BH_3·DMS (0.23 mL, 2.43 mmol) was slowly dropped. After being stirred for 30 min at the same temperature, a solution of **11** (0.61 g, 2.44 mmol) in toluene (4 mL) was slowly dropped. After being stirred for 10 h at −20 to 35 °C, the mixture was concentrated and diluted with MeOH. The resulting mixture was concentrated, and the residue was purified by flash chromatography on silica gel (PE/EA = 10:1) to give **12** (0.44 g, 72%) as a colorless oil. $[\alpha]_D^{23}$ = −26.2 (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3447, 2959, 2093, 1636, 1085, 697, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37−7.24 (m, 5H), 4.54 (brs, 2H), 3.41−3.35 (m, 1H), 3.30−3.25 (m, 2H), 2.52 (brs, 1H), 1.97 (ddd, *J* = 13.6, 12.8, 6.4 Hz, 1H), 1.42−1.36 (m, 2H), 0.95−0.91 (m, 3H), 0.90 (s, 9H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.6, 127.8, 127.1, 127.0, 77.4, 75.9, 72.4, 36.2, 34.2, 31.0, 25.2, 17.0 ppm; HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{16}H_{26}O_2H$: 251.2006, found: 251.2005.

(2*R*,4*S*)-4-((*tert*-Butyldimethylsilyl)oxy)-2,5,5-trimethylhexan-1-ol 13. A cooled (0 °C) DCM (30 mL) solution of **12** (1.62 g, 6.47 mmol) and 2,6-lutidine (1.13 mL, 9.72 mmol) was carefully treated with TBSOTf (2.28 mL, 9.72 mmol) for 3 h. Then, the mixture was quenched with a saturated aqueous of NH_4Cl and extracted with DCM (15 mL × 3). The combined organic layers were washed with brine, dried, filtrated, and concentrated to give the crude product without further purification. The above product, 10% Pd/C (150 mg), and $Pd(OH)_2$ (150 mg) were stirred in MeOH (70 mL) for 3 h under a H_2 atmosphere. Then, the mixture was filtered and the filtrate was concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 30:1) to give **13** (1.35 g, 76%, 2 steps) as a colorless oil. $[\alpha]_D^{23}$ = +25.2 (*c* 2.00, $CHCl_3$); IR (film): ν_{max} 2345, 2082, 1635, 1249, 1084, 1031, 772 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.50−3.37 (m, 2H), 3.32 (dd, *J* = 8.4, 1.2 Hz, 1H), 1.84−1.72 (m, 1H), 1.46−1.35 (m, 2H), 1.25−1.17 (m, 1H), 0.93−0.87 (m, 12H), 0.85 (s, 9H), 0.05 (s, 6H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 77.6, 68.7, 36.2, 35.1, 32.0, 25.8, 25.6, 17.9, 15.6, −3.9, −4.4 ppm; HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{15}H_{34}O_2SiH$: 275.2401, found: 275.2402.

***tert*-Butyldimethyl((3*S*,5*S*)-2,2,5-trimethyloct-7-en-3-yloxy)-silane 6.** To a stirred solution of **13** (1.08 g, 3.93 mmol) in DCM (20 mL) was added PPh_3 (1.34 g, 5.11 mmol) and imidazole (0.54 g, 7.86 mmol), and then I_2 (1.30 g, 5.11 mmol) was added in several portions. After stirring for 15 min, the reaction was quenched with a saturated aqueous solution of Na_2SO_3 and extracted with DCM (30 mL × 3). The combined organic layers were washed with brine, dried, filtrated, and concentrated to give the crude product without further purification. The above crude compound was dissolved in dry THF (20 mL) and cooled to −78 °C. Once a solution of vinylmagnesium bromide (0.7 M in THF, 28.07 mL, 19.65 mmol) was dropped, a solution of Li_2CuCl_4 (0.1 M in THF, 3.90 mL, 0.39 mmol) was slowly dropped. After being stirred for overnight at −78 °C to room temperature, the mixture was quenched with a saturated aqueous solution of NH_4Cl and extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (PE) to give **6** (0.94 g, 84%, 2 steps) as a colorless oil. $[\alpha]_D^{25}$ = −14.8 (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3493, 2946, 1742, 1581, 1519, 1453, 1411, 1312, 1216, 1172, 1001, 767 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.88−5.74 (m, 1H), 5.08−5.01 (m, 2H), 3.36 (dd, *J* = 7.6, 2.8 Hz, 1H), 2.26−2.17 (m, 1H), 1.84−1.65 (m, 2H), 1.49 (ddd, *J* = 14.4, 9.2, 2.8 Hz, 1H), 1.23 (ddd, *J* = 14.4, 7.6, 4.4 Hz, 1H) 0.96−0.84 (m, 21H), 0.09 (s, 6H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.1, 116.0, 78.5, 40.9, 40.4, 35.8, 29.7, 26.4, 26.2, 20.7, 18.5, −3.4, −3.8 ppm; HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ Calcd for $C_{17}H_{36}OSiH$: 285.2608, found: 285.2609.

Benzyl (*S*)-4-((*tert*-Butoxycarbonyl)amino)-5-hydroxypentanoate 15. To a stirred solution of (*S*)-2-amino-5-(benzyloxy)-5-oxopentanoic acid **14** (5.00 g, 21.08 mmol) in H_2O/dioxane (30 mL/30 mL) was added $NaHCO_3$ (1.99 g, 23.64 mmol) and Boc_2O (5.52 g, 25.30 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for overnight. After concentrated, the aqueous phase was acidified with hydrochloric acid, and the mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were dried, filtrated, and concentrated to give the crude acid as a colorless oil without further purification. The above crude acid was dissolved in THF (100 mL) at room temperature, and then 4-methylmorpholine (2.32 mL, 21.08 mmol) was dropped. After stirring for 10 min, the mixture was cooled to −10 °C and ethyl chloroformate (2.42 mL, 25.30 mmol) was dropped. The reaction mixture was stirred for another 1 h at the same temperature and filtrated. Then, a solution of $NaBH_4$ (2.39 g, 63.24 mmol) in H_2O (20 mL) was dropped to the filtrate and stirred for 1 h at room temperature. The mixture was quenched with a saturated aqueous solution of NH_4Cl and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 2:1) to give **15** (4.71 g, 69%, 2 steps) as a white solid. mp 76−77 °C {lit.[15] mp 75−76 °C; $[\alpha]_D^{25}$ = −10.3 (*c* 1.00, $CHCl_3$) {lit.[15] $[\alpha]_D^{25}$ = −10.0 (*c* 1.00, MeOH)}; IR (film): ν_{max} 3346, 2985, 2947, 1727, 1682, 1523, 1451, 1368, 1298, 1167, 1070, 1021, 748, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.42−7.33 (m, 5H), 5.15 (s, 2H), 4.86 (d, *J* = 6.8 Hz, 1H), 3.71−3.62 (m, 2H), 3.61−3,54 (m, 1H), 2.60 (brs, 1H), 2.52−2.45 (m, 2H), 1.99−1.88 (m, 1H), 1.86−1.75 (m, 1H), 1.45 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 156.1, 135.6, 128.4, 128.2, 128.1, 79.5, 66.4, 65.0, 52.1, 30.7, 28.2, 26.1 ppm; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{17}H_{25}NO_5Na$: 346.1625, found: 346.1613.

Benzyl (*S*)-4-((*tert*-Butoxycarbonyl)amino)-5-(tritylthio)pentanoate 16. Compound **15** (2.50 g, 7.73 mmol) and TEA (2.20 mL, 15.46 mmol) were stirred in dry DCM (30 mL) for 10 min at 0 °C. MsCl (0.90 mL, 11.60 mmol) was dropped, and the resulting mixture was stirred for another 30 min. Then, the reaction was quenched by adding a saturated aqueous solution of NH_4Cl and then extracted with DCM (30 mL × 3). The organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated to give the intermediate without further purification. A suspension of NaH (60%) (0.35 g, 9.26 mmol) in DMF (10 mL) was treated with a solution of TrtSH (2.35 g, 8.50 mmol) in DMF (5 mL) at °C. After being stirred for 45 min, a solution of the above intermediate in DMF (15 mL) was slowly dropped, and the resulting mixture was stirred for overnight 0 °C to room temperature. The resulting mixture was quenched with water and diluted with EtOAc. The mixture was separated, the aqueous layer was extracted with EtOAc (30 mL × 3), and the combined organic layers were washed with water and brine, respectively, dried, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 10:1) to give **16** (3.55 g, 79%) as a white amorphous solid. $[\alpha]_D^{25}$ = −10.5 (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3364,

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3058, 3031, 2924, 2851, 1713, 1494, 1366, 1247, 1169, 1028 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.45−7.20 (m, 20H), 5.15−5.05 (m, 2H), 4.49 (d, J = 8.8 Hz, 1H), 3.75−3.60 (m, 1H), 2.38−2.33 (m, 1H), 2.32−2.27 (m, 2H), 1.85−1.65 (m, 2H), 1.45 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 155.0, 144.4, 135.7, 129.4, 128.4, 128.1, 127.8, 126.6, 79.2, 66.5, 66.2, 49.2, 37.0, 30.8, 29.4, 28.2 ppm; HRMS (ESI-TOF) m/z: [M + Na^+] Calcd for $C_{36}H_{39}NO_4SNa$: 604.2492, found: 604.2470.

Benzyl (*S*)-4-Acrylamido-5-(tritylthio)pentanoate 7. A cooled (0 °C) solution of **16** (2.36 g, 4.05 mmol) in DCM (7 mL) was treated with TFA (7 mL) for 30 min; then the mixture was concentrated under reduced pressure. The residue was dissolved in dry DCM (15 mL), and HATU (2.31 g, 6.08 mmol), DIPEA (2.10 mL, 12.15 mmol), and acrylic acid (0.33 mL, 4.86 mmol) were added under a N_2 atmosphere. After being stirred for 5 h at room temperature, the mixture was quenched with a saturated aqueous solution of NH_4Cl and extracted with DCM (30 mL × 3). The combined organic layers were washed with brine, dried, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 3:1) to give the **7** (1.53 g, 71%) as a white amorphous solid. $[\alpha]_D^{25}$ = −23.5 (c 1.00, $CHCl_3$); IR (film): ν_{max} 3272, 3059, 3031, 2953, 1735, 1656, 1627, 1540, 1490, 1243, 1181, 983 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.47−7.21 (m, 20H), 6.27 (dd, J = 17.2, 1.6 Hz, 1H), 5.99 (dd, J = 17.2, 10.4 Hz, 1H), 5.79 (d, J = 8.8 Hz, 1H), 5.64 (dd, J = 10.4, 1.2 Hz, 1H), 5.10 (s, 2H), 4.16−4.05 (m, 1H), 2.50 (dd, J = 12.4, 5.6 Hz, 1H), 3.67 (dd, J = 12.4, 5.2 Hz, 1H), 2.39−2.25 (m, 2H), 1.90−1.77 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 165.0, 144.6, 135.8, 130.8, 130.2, 129.6, 128.6, 128.3, 128.0, 127.1, 126.9, 126.7, 66.8, 66.5, 48.3, 36.6, 31.0, 29.0 ppm; HRMS (ESI-TOF): m/z: [M + $Na]^+$ Calcd for $C_{34}H_{33}NO_3SNa$: 558.2073, found: 558.2053.

Benzyl (*S*)-4-((5*S*,7*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-5,8,8-trimethylnon-2-enamido)-5-(tritylthio)pentanoate 17. Compound **7** (1.20 g, 2.24 mmol), compound **6** (637 mg, 2.24 mmol), and Grubbs 2nd (cat.) were refluxed in DCM (80 mL) under an Ar atmosphere for 24 h to give a crude product, which was purified by flash chromatography on silica gel (PE/EA = 8:1) to give the **17** (1.06 g, 60%) as a white amorphous solid. $[\alpha]_D^{25}$ = −19.0 (c 1.00, $CHCl_3$); IR (film): ν_{max} 3272, 3031, 3060, 2926, 2855, 1738, 1667, 1630, 1539, 1445, 1361, 1257, 1081, 1031 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46−7.20 (m, 20H), 6.87−6.78 (m, 1H), 5.69 (d, J = 15.2 Hz, 1H), 5.51 (d, J = 8.8 Hz, 1H), 5.17−5.08 (m, 2H), 4.18−4.06 (m, 1H), 3.36 (dd, J = 7.2, 2.4 Hz, 1H), 2.52−2.41 (m, 2H), 2.40−2.28 (m, 3H), 1.95−1.77 (m, 4H), 1.55−1.45 (m, 1H), 1.35−1.30 (m, 1H), 0.98 (s, 3H), 0.95 (s, 9H), 0.90 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 165.2, 144.6, 143.6, 135.9, 129.6, 128.6, 128.3, 128.2, 128.0, 126.8, 125.0, 78.5, 66.8, 66.4, 48.1, 41.2, 38.7, 36.7, 35.9, 31.1, 29.7, 29.1, 26.4, 26.2, 21.0, 18.5, −3.2, −3.7 ppm; HRMS (ESI-TOF): m/z: [M + $Na]^+$ Calcd for $C_{49}H_{65}NO_4SSiNa$: 814.4296, found: 814.4290.

Allyl (*S*)-4-((5*S*,7*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-5,8,8-trimethylnon-2-enamido)-5-(tritylthio)pentanoate 18. Compound **17** (0.95 g, 1.20 mmol) was dissolved in a mixture of THF, MeOH, and H_2O (12 mL *V*:*V*:*V* = 1:1:1); then $LiOH·H_2O$ (75 mg, 1.80 mmol) was added in one portion. After being stirred for 7 h, the mixture was quenched with a saturated aqueous solution of NH_4Cl and extracted with EtOAc (30 mL × 3). The combined organic layers were dried over $MgSO_4$, filtrated, and concentrated. The residue was dissolved in DMSO (4 mL), and K_2CO_3 (332 mg, 2.40 mmol) and AllylBr (0.13 mL, 1.80 mmol) were added. After being stirred for 4 h, the reaction was diluted with water and extracted with EtOAc (25 mL × 3). The combined organic layers were washed with water and brine respectively, dried over $MgSO_4$, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 6:1) to give **18** (747 mg, 84%, 2 steps) as a white amorphous solid. $[\alpha]_D^{25}$ = −22.4 (c 1.00, $CHCl_3$); IR (film): ν_{max} 3059, 2956, 2857, 1712, 1662, 1493, 1362, 1256, 1084, 1032, 832, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46−7.41 (m, 6H), 7.35−7.20 (m, 9H), 6.81 (ddd, J = 15.2, 8.0, 6.8 Hz, 1H), 5.96−5.85 (m, 1H), 5.70 (d, J = 15.2 Hz, 1H), 5.49 (d, J = 8.8 Hz, 1H), 5.35−5.28 (m, 1H), 5.27−5.22 (m, 1H), 4.58−4.52 (m, 2H), 4.15−4.05 (m, 1H), 3.35 (dd, J = 7.6, 2.8 Hz, 1H), 2.52−2.40 (m, 2H), 2.34−2.23 (m, 2H), 1.95−1.75 (m, 4H), 1.54−1.45 (m, 1H), 1.34−1.25 (m, 2H), 0.97−0.93 (m, 12H), 0.89 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 165.2, 144.6, 143.6, 132.1, 129.6, 128.0, 126.8, 125.0, 118.3, 78.4, 66.8, 65.2, 48.1, 41.2, 38.7, 36.7, 31.0, 29.7, 29.1, 26.4, 26.2, 21.0, 18.5, −3.3, −3.8 ppm; HRMS (ESI-TOF) m/z: [M + $Na]^+$ Calcd for $C_{45}H_{63}NO_4SSiNa$: 764.4139, found: 764.4127.

Allyl (*S*)-4-((5*S*,7*S*,*E*)-7-Hydroxy-5,8,8-trimethylnon-2-enamido)-5-(tritylthio)pentanoate 19. Compound **18** (747 mg, 1.01 mmol) was stirred in EtOAc (6 mL) at 0 °C; then hydrochloric acid (6 N, 4 mL) was dropped and the reaction was stirred for 3 h at 0 °C to room temperature. The resulting mixture was extracted with EtOAc (10 mL × 3), and the combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ and brine, respectively, dried over $MgSO_4$, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 2:1) to give **19** (521 mg, 82%) as a white amorphous solid. $[\alpha]_D^{25}$ = −27.7 (c 1.00, $CHCl_3$); IR (film): ν_{max} 3391, 3059, 2955, 2869, 1708, 1660, 1626, 1493, 1448, 1256, 1083, 989, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.45−7.39 (m, 6H), 7.33−7.27 (m, 6H), 7.26−7.20 (m, 3H), 6.86−6.77 (m, 1H), 5.95−5.84 (m, 1H), 5.70 (d, J = 15.2 Hz, 1H), 5.49 (d, J = 8.8 Hz, 1H), 5.33−5.27 (m, 1H), 5.26−5.21 (m, 1H), 4.56−4.52 (m, 2H), 4.14−4.03 (m, 1H), 3.35−3.28 (m, 1H), 2.50−2.35 (m, 3H), 2.32−2.20 (m, 2H), 2.05−1.95 (m, 1H), 1.83−1.73 (m, 2H), 1.48−1.39 (m, 2H), 1.32−1.22 (m, 2H), 1.02−0.98 (m, 3H), 0.91 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 165.1, 144.4, 143.5, 131.9, 129.4, 127.8, 126.7, 124.7, 118.2, 66.6, 65.1, 47.9, 38.4, 37.9, 36.6, 34.9, 30.8, 29.6, 28.9, 25.5, 20.9, 20.6 ppm; HRMS (ESI-TOF) m/z: [M + $Na]^+$ Calcd for $C_{39}H_{49}NO_4SNa$: 650.3275, found: 650.3257.

1-((9*H*-Fluoren-9-yl)methyl) 2-((3*S*,5*S*,*E*)-9-(((*S*)-5-(Allyloxy)-5-oxo-1-(tritylthio)pentan-2-yl)amino)-2,2,5-trimethyl-9-oxonon-7-en-3-yl) (*S*)-Pyrrolidine-1,2-dicarboxylate 20. A cooled (0 °C) solution of *N*-Fmoc-proline (536 mg, 1.59 mmol) and DIPEA (0.40 mL, 2.38 mmol) in toluene (5 mL) was carefully treated with 2,4,6-trichlorobenzoyl chloride (0.37 mL, 2.38 mmol) for 30 min; then a solution of compound **19** (498 mg, 0.79 mmol) in toluene and DMAP (194 mg, 1.59 mmol) was added separately. After being stirred for 3 h, the mixture was quenched with a saturated aqueous solution of NH_4Cl and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 3:1) to give **20** (669 mg, 89%) as a white amorphous solid. $[\alpha]_D^{25}$ = −47.1 (c 1.00, $CHCl_3$); IR (film): ν_{max} 3066, 3015, 2959, 2928, 1708, 1662, 1633, 1450, 1419, 1366, 1253, 1180, 1122, 989 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.82−7.74 (m, 2H), 7.68−7.65 (m, 1.6H), 7.60−7.57 (m, 0.4H), 7.45−7.37 (m, 7H), 7.34−7.18 (m, 12H), 6.85−6.70 (m, 1H), 5.95−5.77 (m, 2H), 5.67−5.47 (m, 1H), 5.32−5.25 (m, 1H), 5.25−5.18 (m, 1H), 4.95−4.80 (m, 1H), 4.56−4.42 (m, 4H), 4.35−4.15 (m, 2H), 4.10−4.00 (m, 1H), 3.70−3.64 (m, 1H), 3.61−3.47 (m, 1H), 2.49−2.29 (m, 3H), 2.28−2.06 (m, 4H), 2.04−1.90 (m, 3H), 1.69−1.62 (m, 2H), 1.60−1.51 (m, 0.8H), 1.48−1.40 (m, 1.2H), 1.28 (s, 1H), 1.02−0.96 (m, 2H), 0.90 (s, 9H), 0.80−0.75 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 172.9, 172.8, 172.7, 172.2, 165.5, 165.2, 154.8, 154.4, 144.6, 144.2, 144.1, 143.9, 143.7, 143.0, 142.7, 141.3, 132.1, 129.6, 128.0, 127.9, 127.7, 127.6, 127.1, 127.0, 126.8, 126.7, 125.5, 125.3, 125.3, 125.2, 119.9, 118.2, 79.5, 79.0, 67.7, 67.5, 66.8, 66.7, 65.2, 59.6, 59.4, 48.1, 47.9, 47.3, 47.2, 47.0, 46.4, 37.1, 36.8, 36.6, 36.4, 34.9, 34.7, 31.3, 30.9, 30.0, 29.8, 29.7, 29.4, 29.3, 29.2, 25.9, 24.3, 23.3, 20.9, 20.7 ppm; HRMS (ESI-TOF) m/z: [M + $Na]^+$ Calcd for $C_{59}H_{66}N_2O_7SNa$: 969.4483, found: 969.4459.

1-((9*H*-Fluoren-9-yl)methyl) 2-((3*S*,5*S*,*E*)-8-((*S*)-4-(3-(Allyloxy)-3-oxopropyl)-4,5-dihydrothiazol-2-yl)-2,2,5-trimethyloct-7-en-3-yl) (*S*)-Pyrrolidine-1,2-dicarboxylate 5. Triphenylphosphine oxide (746 mg, 2.70 mmol) was dissolved in DCM (3 mL) and cooled to 0 °C; then trifluoromethanesulfonic anhydride (0.23 mL, 1.35 mmol) was added at 0 °C. After being stirred for 10 min, a solution of compound **20** (423 mg, 0.45 mmol) in DCM (2 mL) was dropped, and the mixture was stirred for 10 min. Then, the mixture

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was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with DCM (20 mL × 3). The combined organic layers were washed with brine, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/EA = 4:1) to give **5** (265 mg, 86%) as a white amorphous solid. $[\alpha]_D^{25}$ = −102.7 (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3018, 2961, 1706, 1580, 1419, 1357, 1245, 1217, 1177, 1122, 990 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.81−7.76 (m, 2H), 7.68−7.64 (m, 1.6H), 7.60−7.57 (m, 0.4H), 7.45−7.38 (m, 2H), 7.37−7.30 (m, 2H), 6.37−6.25 (m, 1.6H), 6.21−6.13 (m, 0.4H), 5.99−5.87 (m, 1H), 5.37−5.30 (m, 1H), 5.27−5.22 (m, 1H), 4.94−4.86 (m, 1H), 4.63−4.58 (m, 2H), 4.56−4.37 (m, 3.6H), 4.32−4.27 (m, 0.6H), 4.24−4.16 (m, 0.8H), 3.77−3.65 (m, 1H), 3.63−3.55 (m, 1H), 3.37−3.30 (m, 0.4H), 3.27−3.21 (m, 0.6H), 2.92−2.85 (m, 0.4H), 2.80−2.74 (m, 0.6H), 2.63−2.46 (m, 2.6H), 2.40−2.25 (m, 1H), 2.12−1.87 (m, 6H), 1.58−1.49 (m, 1H), 1.46−1.40 (m, 1H), 0.95−0.89 (m, 12H), 0.74−0.71 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 173.0, 172.6, 172.5, 166.7, 166.4, 154.7, 154.3, 144.4, 144.2, 144.1, 144.0, 143.8, 143.0, 141.3, 132.2, 127.7, 127.6, 127.1, 127.0, 126.9, 126.4, 125.3, 125.2, 120.0, 118.2, 79.6, 79.1, 76.0, 75.9, 67.7, 67.5, 65.2, 59.5, 59.3, 47.3, 47.0, 46.5, 38.6, 38.3, 37.1, 37.0, 36.7, 34.7, 31.5, 31.3, 30.3, 30.0, 29.7, 29.3, 28.7, 25.9, 24.5, 23.3, 20.9, 20.6 ppm; HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{40}H_{50}N_2O_6SH$: 687.3462, found: 687.3459.

1-((9*H*-Fluoren-9-yl)methyl) 2-((3*S*,5*S*,*E*)-8-((*S*)-4-((5*S*,8*S*,11*S*)-11-((*S*)-*sec*-Butyl)-5-(4-methoxybenzyl)-7,8,10-trimethyl-3,6,9,12-tetraoxo-13-oxa-4,7,10-triazahexadec-15-en-1-yl)-4,5-dihydrothiazol-2-yl)-2,2,5-trimethyloct-7-en-3-yl) (*S*)-Pyrrolidine-1,2-dicarboxylate 23. To a solution of **5** (265 mg, 0.39 mmol) in THF (5 mL) was added $Pd(PPh_3)_4$ (46 mg, 0.04 mmol) and *N*-methylaniline (0.10 mL, 0.96 mmol). After being stirred for 1 h, the mixture was concentrated and purified by flash chromatography on silica gel (DCM/MeOH = 10:1) to give acid **21** (221 mg, 88%) as a white amorphous solid. Tripeptide **22** (228 mg, 0.34 mmol) was dissolved in MeCN (5 mL); then Et_2NH (2.50 mL) was added. After being stirred for 30 min, the reaction mixture was concentrated. The residue and the above acid **21** (221 mg, 0.34 mmol), FDPP (142 mg, 0.37 mmol), and DIPEA (0.08 mL, 0.51 mmol) were stirred in MeCN (2 mL) for overnight at room temperature. The resulting mixture was quenched with a saturated aqueous solution of NH_4Cl and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/Acetone = 2:1) to give **23** (274 mg, 75%) as a white amorphous solid. $[\alpha]_D^{25}$ = −117.3 (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 2111, 2958, 2924, 1738, 1704, 1645, 1514, 1355, 1247, 1179, 1088 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.82−7.75 (m, 2H), 7.71−7.60 (m, 2H), 7.45−7.37 (m, 2H), 7.36−7.30 (m, 2H), 7.21−7.13 (m, 2H), 6.85−6.75 (m, 2H), 6.15−6.09 (m, 0.5H), 5.98−5.77 (m, 1.5H), 5.59−5.45 (m, 1H), 5.35−5.23 (m, 2H), 4.97−4.78 (m, 2H), 4.65−4.57 (m, 2H), 4.55−4.40 (m, 3H), 4.39−4.32 (m, 1H), 4.31−4.19 (m, 1.5H), 4.13−4.07 (m, 0.5H), 3.80−3.74 (m, 3H), 3.70−3.63 (m, 1H), 3.61−3.52 (m, 1H), 3.10−2.98 (m, 2H), 2.95−2.88 (m, 1H), 2.86−2.77 (m, 3H), 2.71 (s, 3H), 2.63−2.60 (m, 0.5H), 2.53−2.50 (m, 1.5H), 2.36−2.17 (m, 3H), 2.10−1.89 (m, 4H), 1.86−1.79 (m, 1H), 1.58−1.51 (m, 1H), 1.46−1.37 (m, 2H), 1.36−1.17 (m, 8H), 0.98−0.81 (m, 18H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ 172.6, 172.0, 171.9, 171.5, 170.7, 158.6, 154.7, 154.3, 144.2, 144.1, 141.3, 131.8, 130.4, 130.3, 128.2, 128.1, 127.7, 127.6, 127.0, 126.9, 126.3, 125.3, 125.2, 119.9, 118.6, 113.9, 79.6, 79.1, 75.9, 75.6, 67.7, 67.6, 66.0, 65.4, 60.5, 59.5, 59.3, 55.2, 50.3, 49.6, 47.3, 47.0, 46.4, 38.6, 38.1, 37.8, 37.1, 37.0, 36.7, 34.7, 33.6, 33.5, 33.3, 31.3, 31.0, 30.5, 30.0, 29.7, 29.5, 29.3, 28.7, 25.9, 25.0, 24.4, 23.3, 20.9, 20.6, 15.8, 14.3, 10.6 ppm; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for: $C_{61}H_{81}N_5O_{10}SNa$: 1098.5596, found: 1098.5602.

(3*S*,5*S*,*E*)-8-((*S*)-4-(3-(Allyloxy)-3-oxopropyl)-4,5-dihydrothiazol-2-yl)-2,2,5-trimethyloct-7-en-3-yl *N*-(*N*-((*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-methoxyphenyl)propanoyl)-*N*-methyl-L-alanyl)-*N*-methyl-L-isoleucyl-L-prolinate 26. A solution of tripetide **22** (120 mg, 0.18 mmol) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol) in THF (3 mL) was treated with *N*-methylaniline (0.05 mL, 0.45 mmol) for 1 h. The mixture was concentrated and purified by flash chromatography on silica gel (DCM/MeOH = 15:1) to give the crude acid without further purification. Compound **5** (124 mg, 0.18 mmol) was dissolved in MeCN (2 mL); then Et_2NH (1 mL) was added. After being stirred for 30 min, the reaction mixture was concentrated. The residue was azeotroped with toluene three times and dissolved in DCM (2 mL); then the above acid, HATU (103 mg, 0.27 mmol), and DIPEA (0.09 mL, 0.54 mmol) were added. After being stirred for overnight at room temperature, the resulting mixture was quenched with a saturated aqueous solution of NH_4Cl and extracted with EtOAc (15 mL × 3). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated, and concentrated. The residue was purified by flash chromatography on silica gel (PE/Acetone = 2:1) to give **26** (141 mg, 73%) as a white amorphous solid. $[\alpha]_D^{23}$ = −108.3 (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3800, 3320, 2958, 2214, 1736, 1640, 1513, 1450, 1247, 1180, 1142, 1075, 1034, 742 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ 7.69−7.65 (m, 2H), 7.48−7.43 (m, 2H), 7.33−7.27 (m, 2H), 7.05−7.01 (m, 2H), 6.74−6.67 (m, 2H), 6.30−6.17 (m, 2H), 5.87−5.79 (m, 1H), 5.52 (d, *J* = 9.0 Hz, 1H), 5.37 (dd, *J* = 13.8, 6.6 Hz, 1H), 5.25−5.21 (m, 1H), 5.16−5.12 (m, 1H), 4.98 (d, *J* = 11.4 Hz, 1H), 4.84−4.78 (m, 2H), 4.51−4.49 (m, 2H), 4.44−4.40 (m, 1H), 4.36 (dd, *J* = 5.6, 3.2 Hz, 1H), 4.28 (dd, *J* = 7.2, 4.8 Hz, 1H), 4.18 (dd, *J* = 7.2, 4.8 Hz, 1H), 4.07 (dd, *J* = 7.2, 6.6 Hz, 1H), 3.84−3.77 (m, 1H), 3.70−3.64 (m, 1H), 3.65 (s, 3H), 3.26 (dd, *J* = 10.8, 8.4 Hz, 1H), 2.98−2.88 (m, 4H), 2.85 (s, 2.5H), 2.80 (dd, *J* = 10.8, 8.4 Hz, 1H), 2.77−2.70 (m, 1.5H), 2.53−2.42 (m, 3H), 2.16−2.05 (m, 2H), 1.95−1.83 (m, 5H), 1.81−1.73 (m, 2H), 1.52−1.46 (m, 1H), 1.32−1.26 (m, 1H), 1.20−1.14 (m, 5H), 0.92−0.88 (m, 3H), 0.82−0.76 (m, 15H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ 171.9, 171.4, 170.8, 170.5, 168.3, 165.6, 157.6, 154.7, 143.8, 142.8, 142.7, 140.2, 131.2, 129.3, 126.9, 126.7, 126.0, 125.3, 124.1, 124.0, 118.9, 117.2, 112.9, 77.8, 77.4, 74.9, 66.0, 64.1, 59.4, 58.1, 56.7, 54.1, 51.3, 48.7, 46.3, 46.1, 37.5, 37.1, 36.0, 35.8, 33.6, 32.3, 30.4, 29.5, 29.4, 29.3, 28.3, 27.2, 24.9, 23.9, 23.7, 19.9, 13.7, 13.5, 9.4 ppm; HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{61}H_{81}N_5O_{10}SH$: 1076.5777, found: 1076.5778.

Apratoxin E 2. *Method 1: The Synthesis of Apratoxin E by Macrolactamization at C5 and C6.* A solution of compound **23** (220 mg, 0.20 mmol) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol) in dry THF (5 mL) was treated with *N*-methylaniline (0.06 mL, 0.50 mmol) for 30 min. The mixture was concentrated, and the residue was purified by flash chromatography (DCM/MeOH = 20:1) to give the acid. The above acid was dissolved in MeCN (2 mL); then Et_2NH (1 mL) was added. After being stirred for 30 min at room temperature, the mixture was concentrated, which was dissolved in DCM (200 mL). HATU (114 mg, 0.30 mmol) and DIPEA (0.66 mL, 4.00 mmol) were added, and the mixture was stirred for 40 h. The mixture was concentrated, and the residue was purified by flash chromatography on silica gel (PE/EA = 1:1) to give apratoxin E **2** (30.2 mg, 19%, 3 steps).

Method 2: The Synthesis of Apratoxin E by Macrolactamization at C18 and C27. A solution of compound **26** (110 mg, 0.10 mmol) and $Pd(PPh_3)_4$ (11.5 mg, 0.01 mmol) in dry THF (3 mL) was treated with *N*-methylaniline (0.03 mL, 0.25 mmol) for 30 min. Then, the reaction was concentrated and purified by flash chromatography on silica gel (DCM/MeOH = 20:1) to give the acid, which was dissolved in MeCN (2 mL), and Et_2NH (1 mL) was added. After being stirred for 30 min at room temperature, the mixture was concentrated and the residue was dissolved in MeCN (100 mL). Then, FDPP (57.6 mg, 0.15 mmol) and DIPEA (0.33 mL, 2.00 mmol) were added, and the reaction was stirred for 40 h. The mixture was concentrated and purified by flash chromatography on silica gel (PE/EA = 1:1) to give apratoxin E **2** (34.2 mg, 43%, 3 steps).

$[\alpha]_D^{24}$ = −150.4 (*c* 0.24, MeOH), {lit.[4d] $[\alpha]_D^{20}$ = −69 (*c* 0.12, MeOH)}; ^1H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ 7.19−7.14 (m, 2H), 6.85−6.80 (m, 2H), 6.62 (d, *J* = 15.6 Hz, 0.67H), 6.46−6.37 (m, 1H), 6.41−6.38 (m, 0.33H), 6.11 (d, *J* = 9.0 Hz, 0.67H), 5.81 (d, *J* = 10.2 Hz, 0.33H), 5.28−5.22 (m, 1H), 5.20−5.15 (m, 0.67H), 5.01−4.97 (m, 0.67H), 4.95−4.90 (m, 1.33H), 4.67 (dd, *J* = 13.2, 6.6 Hz, 0.67H), 4.36−4.32 (m, 1.33H), 4.26−4.23 (m, 0.33H), 4.12−4.06 (m, 1H), 3.80 (s, 1H), 3.79 (s, 2H), 3.73−3.68 (m, 0.67H), 3.68−3.63

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(m, 1H), 3.36−3.31 (m, 1.33H), 3.13−3.08 (m, 1H), 3.00 (s, 2), 2.97−2.91 (m, 2H), 2.86 (s, 1H), 2.84 (s, 1H), 2.64 (s, 2H), 2.53−2.45 (m, 2H), 2.30−2.21 (m, 2H), 2.12−2.03 (m, 2H), 1.97−1.86 (m, 4H), 1.78−1.71 (m, 2H), 1.46−1.44 (m, 0.67H), 1.30−1.24 (m, 4H), 1.08−1.04 (m, 3H), 0.97−0.94 (m, 3H), 0.91−0.88 (m, 9H), 0.87−0.84 (m, 2.33H), 0.60−0.56 (m, 2H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ 172.1, 171.7, 171.4, 171.0, 170.6, 169.8, 169.7, 169.5, 169.3, 169.0, 165.9, 165.6, 158.1, 158.0, 144.3, 144.0, 129.9, 129.8, 128.0, 127.9, 125.5, 125.4, 113.5, 113.2, 77.0, 76.9, 75.8, 75.7, 60.0, 58.8, 58.5, 57.2, 56.5, 54.7, 54.6, 53.4, 50.0, 49.2, 46.8, 46.7, 39.2, 37.7, 37.1, 36.7, 36.6, 36.5, 36.1, 34.5, 34.1, 33.5, 33.0, 32.6, 32.5, 30.3, 29.9, 29.7, 29.1, 28.9, 28.8, 28.7, 28.6, 28.5, 28.1, 25.5, 25.3, 24.9, 24.6, 24.5, 19.9, 18.8, 14.3, 13.5, 13.3, 13.2, 9.5, 9.4 ppm; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{43}H_{65}N_5O_7SH$: 796.4683, found: 796.4679.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02086.

Copies of ^{1}H and ^{13}C NMR spectra of related compounds and comparison of NMR data of natural and synthetic Apratoxin E (PDF)

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Notes

The authors declare no competing financial interest.

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