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Asymmetric synthesis of (*E*)-dehydroapratoxin A

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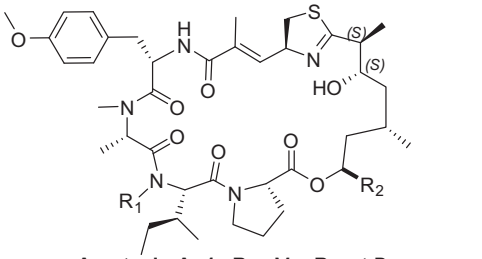
ABSTRACT

An asymmetric approach to key intermediate **17** starting from lactone **7** is described, in which Evan's alkylation and CBS-catalyzed reduction are used for construction of the chiral centers, respectively. Thus, the synthesis of (*E*)-dehydroapratoxin A **6** could be accomplished in a general fashion, therein FDPP has been proven as an efficient condensation reagent for the coupling of amine **25** and carboxylic acid **24**.

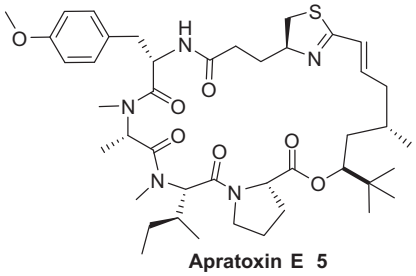
Introduction

Marine cyanobacteria have stimulated enormous efforts for drug discovery or probe due to their complex molecular structures and remarkable biological activities.[1] Most of these secondary metabolites are predominantly modified peptides, depsipeptides, polyketides, and peptide–polyketide hybrids.[2] Although several bioactive compounds have been advanced to phase II clinical trial for treatment cancers,[3,4] many other cyanobacterial metabolites still remain further search either for new pharmaceutically relevant lead compounds or for their modes of action. As a prime instance, apratoxins exhibit potent cancer growth inhibitory activity by inducing G1 phase specific cell cycle arrest and apoptosis.[4]

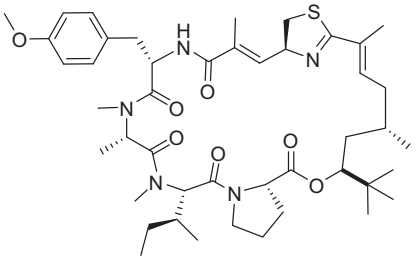


Apratoxin A 1 R_1= Me; R_2 = *t*-Bu
Apratoxin B 2 R_1= H; R_2 = *t*-Bu
Apratoxin C 3 R_1= Me; R_2 = *i*-Pr
Apratoxin D 4 R_1= Me; R_2 =

Apratoxins A–C (**1**–**3**) were isolated from the remarkably prolific *Lyngbya majuscula* collected in Guam and Palau.[5] Apratoxin E **5** was also isolated from *Lyngbya bouillonii* collected in Guam,[6] while apratoxin D **4** from a variety of the same species was collected in Papua New Guinea (Fig. 1).[7] All of those isolated marine



Apratoxin E 5



(*E*)-Dehydroapratoxin A 6

Figure 1. The structures of apratoxin A–E and (*E*)-dehydroapratoxin A.

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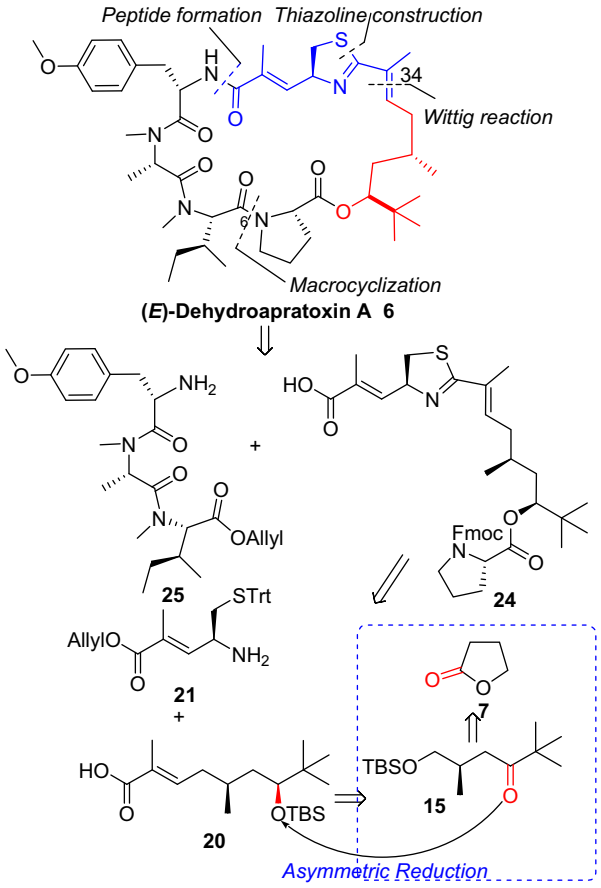
secondary metabolites show potent in vitro cytotoxicity against LoVo cell lines (0.36–10.8 nM) and the KB (0.52–21.3 nM).[5a,6] Due to its high efficacy for cancer cell growth inhibition and intriguing structure, tremendous efforts have been devoted to the asymmetric synthesis of apratoxin A[8] and its analogs,[8f,g,9] as well as its mechanism of action.[4,10] Dehydroapratoxin A[5b] **6**, eliminated from apratoxin A under weakly acidic conditions, suggests that the elimination obviously affects the potency of cytotoxicity against the HT-29 colon adenocarcinoma (IC_{50} 41 nM for **6**) and LeLa cervical carcinoma (IC_{50} 121 nM for **6**).[6] Interestingly, there exists a significant correlation between the cytotoxicity of apratoxin family members and their proportion of the *trans* amide isomer [in NMR ($CDCl_3$), apratoxins A **1**, >90%; apratoxin B **2**, ~75%; apratoxin C **3**, >90%; apratoxin E **5**, ~60% and (*E*)-dehydroapratoxin A **6**, ~40%],[5b] but it is unknown whether those family compounds display different stereochemistry in the aqueous solution.[6] In more recent years, most studies have been focused on apratoxin A and its oxazoline analogs, only little research on other members of apratoxins and their analogs.[8g] It prompts us to develop a general approach for synthesis of a series of apratoxins, and to study their structure–activity relationship, as one part of our continuous interest in pursuing some concise synthesis of piperidine alkaloids, depsipeptides and ceramides.[11] Herein we present a general method for asymmetric synthesis of (*E*)-dehydroapratoxin A **6**.

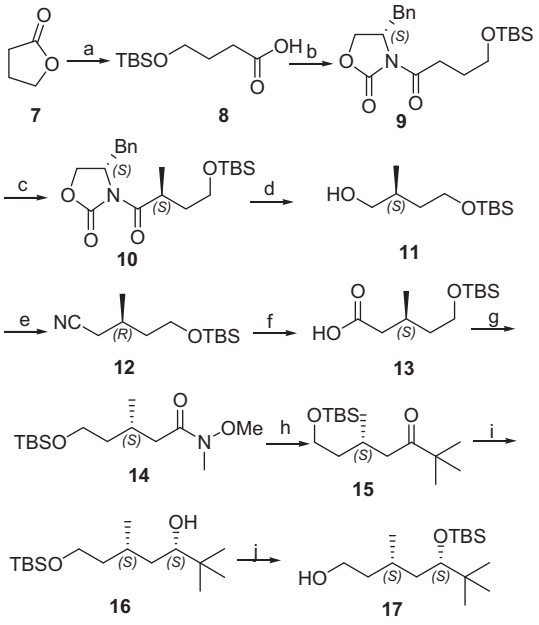
Results and discussion

Our synthetic strategy for asymmetric synthesis of (*E*)-dehydroapratoxin A **6** has been illustrated in Scheme 1. We envisaged to select the macrolactamization as the final step, without trouble for the epimerization at the C34 position in our target, which usually occurred in the synthesis of apratoxin A and its analog.[8,9] The methods for the formation of thiazoline ring and tripeptide fragment **25** are similar to the previously reported method for the synthesis of apratoxin A and its analogs.[8,9] However, we hope to improve the yield by other condensation reagents in the connection of fragment **25** and **24**. Furthermore, in our synthetic route, our aim is to achieve a scalable method for preparation of the free acid part **20** using the asymmetric methylation induced by chiral auxiliary and stereoselective reduction, which could be further used for asymmetric synthesis of a series of apratoxins and their analogs.

The first challenge in our synthetic synthesis was the preparation of the enantiopure (3*S*,5*S*)-7-(*tert*-butyldimethylsilyloxy)-2,2,5-trimethylheptan-3-ol **17**. The synthesis was achieved from commercially starting material lactone **7**, as outlined in Scheme 2. Following treatment of lactone **7** with sodium hydroxide and subsequent protection (TBSCl, Imidazole) of the primary alcohol, free acid **8** was achieved in 81% overall yield. Then, intermediate **8** was activated with ethyl chlorocarbonate and subsequently the resulting mixture was treated with lithium salt of oxazolidinone to afford amide **9** in 74% yield, which was methylated with MeI at −78 °C to afford product **10** with high diasteroselectivity (dr >98:2) in 76% yield. Removal of the auxiliary[12] ($LiBH_4$, H_2O) produced the primary alcohol **11** in 93% yield. Mesylation (MsCl, TEA) and nucleophilic substitution (NaCN, DMSO) afforded cyanide **12** in 95% overall yield. Then reduction of the compound **12** with DIBAL-H[13] at −78 °C gave the corresponding aldehyde without further purification, which was oxidized with $NaClO_2$[14] in the presence of 2-methyl-2-butene to afford acid **13** in 90% overall yield. The compound **13** was easily converted to the Weinreb amide **14** in the presence of HATU[15] in 89% yield. To prepare the ketone **15**, the Weinreb amide **14** was treated with a solution of *tert*-butylmagnesium chloride to give desired ketone **15** in low yield. But when a solution of *tert*-butyllithium (2.0 mmol in Et_2O) was used, the reaction was quickly finished at −10 °C for 5 min and produced **15** in 81% yield. The keto functionality in **15** was stereoselectively reduced to alcohol by using a catalytic



Scheme 1. The retrosynthetic analysis of (*E*)-dehydroapratoxin A.

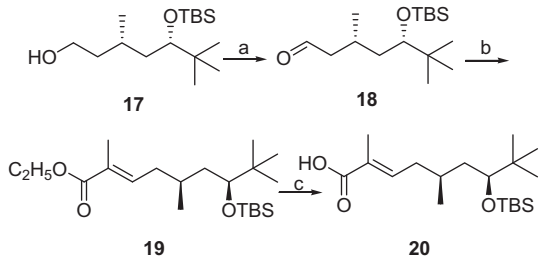


Scheme 2. *Reagents and conditions:* (a) (i) NaOH, H_2O, reflux, 4 h; (ii) TBSCl, Imi., DMAP, DMF, overnight, 81% (two steps); (b) $ClCOOC_2H_5$, THF, 0 to −78 °C, 1 h, then (*S*)-4-benzyl-oxazolidinone, *n*-BuLi, THF, −78 °C to rt, 6 h, 74%; (c) NaHMDS, MeI, THF, −78 °C to rt, overnight, 76%; (d) $LiBH_4$, MeOH, THF, 0 °C, 4 h, 93%; (e) (i) MsCl, TEA, DCM, rt, 30 min; (ii) KCN, DMSO, 60 °C, 4 h, 95% (two steps); (f) (i) DIBAL-H, DCM, −78 °C, 2 h; (ii) $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, 2-methyl-2-butene, *t*-BuOH, H_2O, 0 °C, 2 h, 90%; (g) NHMe(OMe)·HCl, HATU, DIPEA, DMF, 0 °C to rt, 5 h, 89%; (h) *t*-BuLi, Et_2O, −10 °C, 5 min, 81%; (i) (*R*)-CBS, toluene, 40 °C, 6 h, 92%; (j) (i) 2,6-lutidine, TBSOTf, DCM, 0 °C to rt, 3 h; (ii) *p*-TsOH, MeOH, rt, 2 h, overall yield 92%.

amount of Corey's chiral borane, R-CBS catalyst[16] in the presence of BH_3·DMS to yield a partly separable mixture of products **16** (*syn*/*anti* = 88:12) in 92% combined yield. Finally, protection (TBSOTf, 2,6-lutidine) of the compound **16** and subsequent selective deprotection (*p*-TsOH, MeOH) afforded the desired primary alcohol **17** in 92% overall yield.

With key intermediate **17** in hand, the fragment **20** was easily prepared by Wittig reaction (Scheme 3). Swern oxidization[17] of alcohol **17** with DMSO/$(COCl)_2$ at −78 °C provided unpurified aldehyde **18**, which was directly subjected to Wittig reaction with $Ph_3P{=}C(CH_3)COOC_2H_5$ to generate single isomer of olefin **19** in 86% isolated overall yield. Then, hydrolysis of olefin **19** with LiOH·H_2O gave the corresponding carboxylic acid **20** in 94% yield.

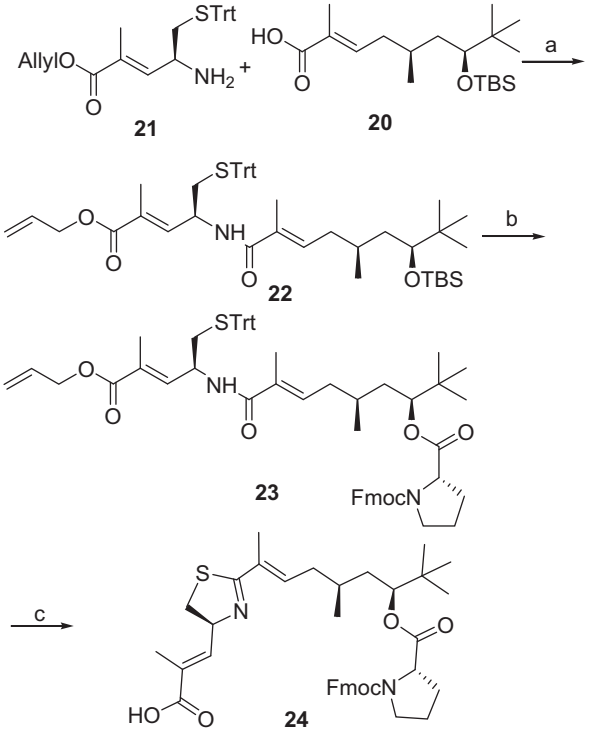
The fragment **21** was easily synthesized by the known method.[8e] Then condensation between amine **21** and carboxylic acid **20** in the presence of HATU afforded amide **22** in 89% yield. Removal of silyl-protecting group with a solution of 6 N HCl/EtOAc



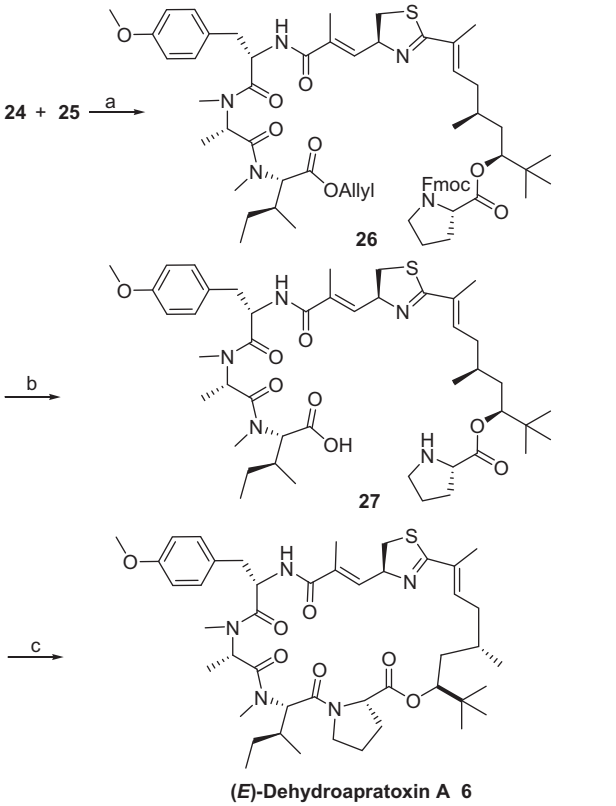
Scheme 3. *Reagents and conditions:* (a) DMSO, $(COCl)_2$, −78 °C, 30 min, 96%; (b) $Ph_3P{=}C(CH_3)COOC_2H_5$, toluene, rt, 4 h, 90%; (c) LiOH·H_2O, THF, MeOH, H_2O, rt, 3 h, 94%.

produced secondary alcohol, which was directly esterified with *N*-Fmoc-Pro-OH under Yamaguchi condition[18] to give **23** in 94% yield. Then compound **23** was treated with $POPh_3/Tf_2O$[19] in CH_2Cl_2 at 0 °C to form the desired thiazoline, which was subsequently treated with *N*-methylaniline in the presence of $Pd(PPh_3)_4$[20] to give free acid **24** in 78% overall yield[21] (Scheme 4).

Tripeptide **25** was prepared by sequential coupling of *N*-methylisoleucine allyl ester with *N*-Boc-*N*-methylalanine and *N*-Fmoc-*O*-methyltyrosine by the known method.[8e] Although HATU or PyAOP was reported to be an effective reagent for the condensation between similar amine **25** and carboxylic acid **24** in robust chemistry,[8b,e] we had tested several condensation reagents and found that the yield of compound **26** was very low (about 39%). We thought that the slightly different structure might affect the yield of condensation. Gratefully, when pentafluorophenyl diphenylphophinate (FDPP)[22] was used, the condensation of amine **25** with carboxylic acid **24** in acetonitrile at 0 °C smoothly produced amide **26** in 91% yield.[23] Sequential cleavage of allyl ester by *N*-methylaniline in the presence of $Pd(PPh_3)_4$ and removal of the Fmoc protecting group with Et_2NH/CH_3CN afforded the cyclization precursor without further purification. Finally, the macrolactamization of the crude cyclization precursor was performed with HATU/DIEA to give crude (*E*)-dehydroapratoxin A **6** in 42% yield[24] over three steps. The crude dehydroapratoxin A **6** was 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, refractive index detection) {$[\alpha]_D^{25}$ −146.8 (*c* 0.4, MeOH), lit.[5b] $[\alpha]_D^{25}$ −133 (*c* 0.30, MeOH)}, and afforded the sample with a satisfied purity (up to 99%), which was determined by HPLC (Kromasil C18 column, 5 μm, 4.6 × 150 mm; 1.0 mL/min; UV 220 nm; retention time is 11.29 min) using 80% aqueous CH_3CN. The structure of



Scheme 4. *Reagents and conditions:* (a) HATU, DIPEA, DMF, 0 °C to rt, overnight, 89%; (b) (i) 6 N HCl, EA, 0 °C, 3 h, 97%; (ii) Fmoc-Pro-OH, 2,4,6-trichlorobenzoyl chloride, DIPEA, DMAP, 0 °C to rt, overnight, 94%; (c) (i) $POPh_3$, Tf_2O, DCM, 0 °C, 30 min, 87%; (ii) $Pd(PPh_3)_4$, *N*-methylaniline, rt, 2 h, 90%.



Scheme 5. *Reagents and conditions:* (a) FDPP, DIPEA, 0 °C to rt, overnight, 91%; (b) (i) $Pd(PPh_3)_4$, *N*-methylaniline, rt, 2 h; (ii) Et_2NH, MeCN, rt, 15 min; (c) HATU, DIPEA, DCM(0.001 M), 0 °C to rt, 2d, 42% (three steps).

dehydroapratoxin A **6** was further confirmed by two-dimensional NMR spectroscopic data, and the spectroscopic data of ^{1}H and ^{13}C NMR agreed with that of the reported data (Scheme 5).[5b]

Conclusion

In summary, the key intermediate **17** was achieved by a gram scalable method, thereby one approach was established by asymmetric synthesis of (*E*)-dehydroapratoxin A **6** from the commercially starting material lactone **7** in a general fashion. Furthermore, FDPP was found to be an efficient condensation reagent for the coupling of amine **25** and free acid **24**. Synthesis of other apratoxins and their analogs through the intermediate **17** is now in progress in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data ((copies of ^{1}H, ^{13}C NMR for compounds **24**, **26** and (*E*)-dehrdroapratoxin A **6**) are available online with this paper in Science Direct) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.05.107.

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21. The date of compound **24**: $[\alpha]_D^{25} = -80.6$ (*c* 3.72, $CHCl_3$); IR (film): ν_{max} 3418, 2958, 2924, 2860, 1705, 1652, 1575, 1451, 1417, 1356, 1196, and 1176 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ: 10.12 (brs, 1H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.71–7.63 (m, 1.5H), 7.60 (d, *J* = 7.6 Hz, 0.5H), 7.42 (dd, *J* = 7.6, 7.2 Hz, 2H), 7.37–7.31 (m, 2H), 6.95–6.88 (m, 1H), 6.25 (dd, *J* = 6.8, 7.2 Hz, 0.6H), 6.12 (dd, *J* = 6.8, 7.2 Hz, 0.4H), 5.35–5.25 (m, 1H), 4.97–4.88 (m, 1H), 4.59–4.46 (m, 2H), 4.39 (dd, *J* = 10.0, 7.6 Hz, 0.6H), 4.31 (dd, *J* = 7.4, 7.2 Hz, 0.6H), 4.27–4.18 (m, 0.8H), 3.78–3.66 (m, 1H), 3.65–3.55 (m, 1H), 3.39 (dd, *J* = 10.8, 8.4 Hz, 0.4H), 3.29 (dd, *J* = 10.4, 8.4 Hz, 0.6H), 2.93 (dd, *J* = 10.8, 9.6 Hz, 0.4H), 2.81 (dd, *J* = 10.8, 8.4 Hz, 0.6H), 2.48–2.23 (m, 2.1H), 2.22–2.04 (m, 3.1H), 2.03–1.88 (m, 6H), 1.85–1.74 (m, 0.8H), 1.62–1.41 (m, 2.8H), 1.35–1.24 (m, 0.4H), 0.96 (d, *J* = 6.8 Hz, 1.6H), 0.93 (s, 9H), 0.75 (d, *J* = 6.8 Hz, 1.4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 172.6, 172.5, 172.1, 154.8, 154.5, 144.2, 144.1, 143.9, 141.9, 141.8, 141.3, 140.5, 139.0, 134.6, 132.5, 132.2, 132.0, 130.8, 129.1, 128.6, 127.7, 127.1, 125.3, 120.0, 79.8, 79.3, 74.4, 74.1, 67.8, 67.6, 59.6, 59.4, 47.3, 47.0, 46.5, 37.4, 37.1, 36.8, 34.7, 34.3, 34.1, 31.3, 30.0, 29.7, 29.3, 25.9, 24.4, 23.4, 21.1, 20.8, 14.6, and 12.8 ppm; MS(ESI) *m/z*: 694.9 ($M+Na^+$); HRMS (MALDI/DHB) calcd for $[C_{39}H_{48}N_2O_6S+H^+]$: 673.3311, found: 673.3292.
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23. The date of compound **26**: $[\alpha]_D^{25} = -115.6$ (*c* 1.01, $CHCl_3$); IR (film): ν_{max} 3428, 2959, 2925, 2855, 1738, 1705, 1634, 1583, 1513, 1451, 1416, 1355, 1248, and 1179 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ: 7.78 (d, *J* = 6.8 Hz, 2H), 7.71–7.57 (m, 2H), 7.41 (dd, *J* = 7.6, 7.2 Hz, 2H), 7.32 (dd, *J* = 7.6, 7.2 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.45 (dd, *J* = 6.8, 6.2 Hz, 0.7H), 6.30 (dd, *J* = 7.2, 6.8 Hz, 1H), 6.21 (dd, *J* = 7.2, 6.8 Hz, 1H), 6.09 (dd, *J* = 6.8, 6.4 Hz, 0.3H), 5.96–5.84 (m, 1H), 5.55 (dd, *J* = 13.6, 6.4 Hz, 0.2H), 5.44 (dd, *J* = 13.2, 6.8 Hz, 0.8H), 5.32 (d, *J* = 17.6 Hz, 1H), 5.28–5.13 (m, 3H), 4.97 (d, *J* = 10.0 Hz, 1H), 4.91 (dd, *J* = 10.2, 10.0 Hz,1H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.57–4.43 (m, 2H), 4.34 (dd, *J* = 10.0, 7.6 Hz, 0.5H), 4.30 (dd, *J* = 7.2, 6.4 Hz, 0.5H), 4.25–4.17 (m, 1H), 3.78 (s, 3H), 3.75–3.65 (m, 1H), 3.64–3.54 (m, 1H), 3.36 (dd, *J* = 10.0, 9.6 Hz, 0.4H) 3.27 (dd, *J* = 10.4, 8.8 Hz, 0.6H), 3.13–3.03 (m, 1H), 3.01 (s, 3H), 2.94–2.81 (m, 2H), 2.79 (s, 3H), 2.45–2.22 (m, 2H), 2.20–2.02 (m, 3H), 1.99 (s, 3H), 1.94 (s, 3H), 1.84–1.72 (m, 1H), 1.60–1.41 (m, 2H), 1.35–1.22 (m, 6H), 1.05–0.94 (m, 7H), 0.94–0.86 (m, 10.5H), 0.75 (d, *J* = 6.4 Hz, 1.5H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 172.6, 171.9, 171.5, 170.6, 168.2, 158.7, 154.7, 144.3, 144.1, 141.3, 132.9, 132.6, 132.2, 131.8, 130.4, 128.5, 128.0, 128.6, 128.4, 127.7, 127.1, 125.2, 120.0, 118.7, 114.0, 79.3, 74.5, 67.5, 65.4, 60.5, 59.6, 59.3, 55.2, 50.5, 49.6, 47.3, 47.0, 46.4, 37.8, 37.5, 36.8, 34.7, 34.3, 34.0, 33.3, 31.3, 31.0, 30.6, 30.0, 29.7, 29.4, 25.9, 25.1, 24.4, 23.3, 21.0, 20.7, 15.8, 14,6, 14.4, 13.4, and 10.6 ppm; MS(ESI) *m/z*: 1124.8 ($M+Na^+$); HRMS (MALDI/DHB) calcd for $[C_{63}H_{83}N_5O_{10}S+H^+]$: 1102.5939, found: 1102.5933.
24. The date of *E*-dehydroapratoxin A **6**: $[\alpha]_D^{25} = -146.8$ (*c* 0.40, MeOH); IR (film): ν_{max} 3306, 2958, 2924, 2853, 1742, 1644, 1512, 1455, 1247, and 1177 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers M/m = 3:2) δ: 7.22–7.12 (m, 2H), 6.88–6.79 (m, 2H), 6.52 (d, *J* = 10.2 Hz, 0.6H), 6.39–6.30 (m, 1.4H), 6.19 (d, *J* = 9.4 Hz, 0.4H), 5.95 (br d, *J* = 9.2 Hz, 0.3H), 5.49–5.45 (m, 0.6H), 5.43–5.38 (m, 0.4H), 5.38–5.33 (m, 0.6H), 5.11 (d, *J* = 10.8 Hz, 0.4H), 5.10–5.06 (m, 0.4H), 5.02 (dd, *J* = 11.6, 1.2 Hz, 0.4H), 4.99 (dd, *J* = 12.4, 1.6 Hz, 0.6H), 4.82 (d, *J* = 11.2 Hz, 0.6H), 4.78–4.73 (m, 0.6H), 4.40–4.36 (m, 0.6H), 4.22–4.18 (m, 0.4H), 4.17–4.12 (m, 0.4H), 4.06–4.01 (m, 0.6H), 3.80–3.77 (m, 3H), 3.67–3.59 (m, 1H), 3.48 (dd, *J* = 10.2, 8.8 Hz, 0.4H), 3.43 (dd, *J* = 10.2, 9.0 Hz, 0.6H), 3.37–3.33 (m, 0.4H), 3.23 (dd, *J* = 12.6, 11.4 Hz, 0.6H), 3.19–3.11 (m, 1H), 3.10–3.06 (m, 0.4H), 2.94–2.89 (m, 2H), 2.88–2.85 (m, 1H), 2.84–2.78 (m, 2H), 2.71–2.59 (m, 3H), 2.31–2.22 (m, 1H), 2.08–1.87 (m, 10H), 1.74–1.53 (m, 3H), 1.35–1.45 (m, 0.6H), 1.36–1.24 (m, 1.8H), 1.13 (d, *J* = 6.0 Hz, 1.8H), 1.02 (d, *J* = 5.6 Hz, 1.8H), 0.98–0.86 (m, 13.6H), 0.86–0.82 (m, 1.8H), 0.66 (d, *J* = 6.0 Hz, 1.8H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, mixture of rotamers) δ: 173.1, 173.0, 172.6, 172.0, 171.1, 170.6, 170.4, 170.2, 170.0, 169.9, 169.5, 167.5, 158.6, 139.4, 139.3, 136.4, 135.6, 132.1, 131.7, 130.7, 130.5, 128.6, 128.4, 128.3, 114.0, 113.8, 77.9, 73.1, 72.9, 60.5, 59.6, 58.8, 58.0, 57.0, 55.3, 53.9, 50.7, 50.3, 47.5, 47.3, 39.3, 38.8, 38.0, 37.1, 37.0, 36.7, 35.1, 34.7, 34.1, 34.0, 33.8, 30.8, 30.1, 29.7, 29.5, 29.4, 29.3, 28.8, 28.2, 26.1, 26.0, 25.9, 25.8, 25.7, 25.4, 25.1, 21.2, 21.1, 15.4, 14.7, 14.3, 14.1, 13.9, 13.8, 13.4, 12.7, 10.2, and 9.8 ppm; MS(ESI) *m/z*: 844.5 ($M+Na^+$); HRMS (MALDI/DHB) calcd for $[C_{45}H_{67}N_5O_7S+Na^+]$: 844.4659, found: 844.4652.