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SYNTHESIS OF A BIPHENYLALANINE ANALOGUE OF APRATOXIN A DISPLAYING SUBSTANTIALLY ENHANCED CYTOTOXICITY

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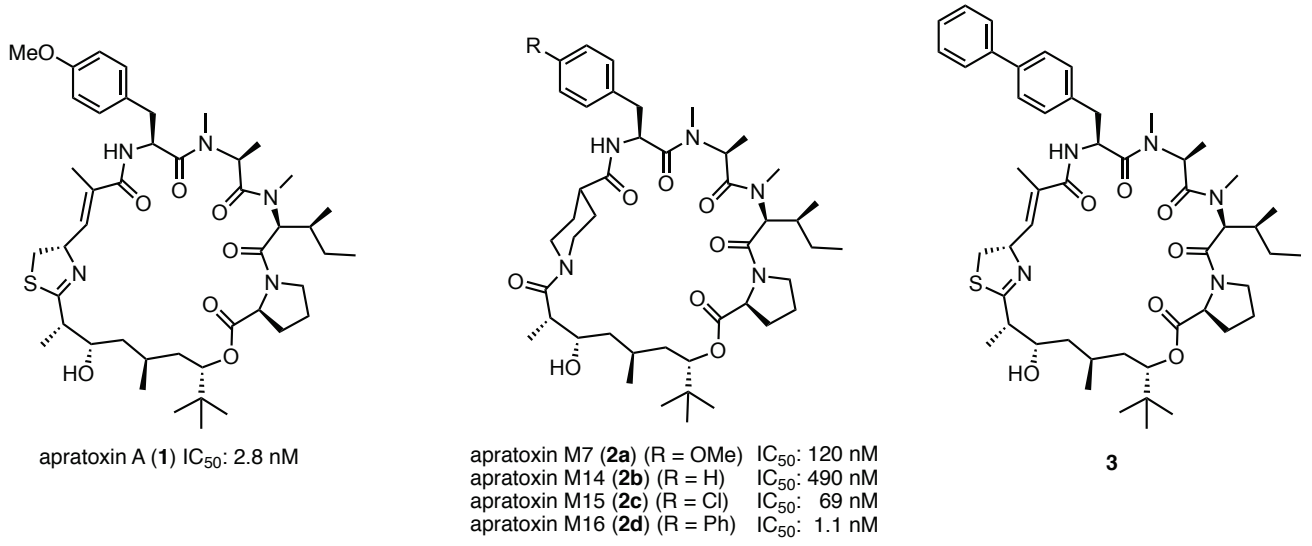
Dedicated to Professor Kaoru Fuji on the occasion of his 80th birthday

Abstract – The concise synthesis of the 3,7-dihydroxy-2,5,8,8-tetramethylnonanoic acid moiety of apratoxin A and the total synthesis of compound **3**, a 4-biphenylalanine (Bph) analogue of apratoxin A, have been demonstrated. The Bph analogue **3** exhibited a 16-fold increase in cytotoxicity against HCT-116 cells with respect to apratoxin A. This evidence indicated that existing the 4-phenyl group of Bph in **3** significantly enhanced its cytotoxicity, a conclusion corroborated by the 100-fold difference in cytotoxicity against HCT-116 cells observed between apratoxin M7 and apratoxin M16, which is characterized by the presence of a 4-phenyl group where apratoxin M7 displays a 4-methoxy group. Results from a conformational study using a distance geometry method suggested that **3** and apratoxin A adopt similar conformations in CD_3CN.

Apratoxin A (**1**) isolated from the marine cyanobacterium *Lyngbya majuscula* exhibits potent cytotoxicity against various cancer cell lines and in vivo anticancer activity (Figure 1).[1–3] Recently, its analogues, apratoxins S4, S8, and S10 that lack a Michael acceptor existing in apratoxin A have been extensively studied by Luesch *et al.* as antitumor agents without causing lethal toxicity.[4]

We have recently reported that apratoxin M7 (**2a**), which can be viewed as a derivative of apratoxin A in which a piperidine-4-carboxylic acid moiety has replaced apratoxin A's modified cysteine moiety, exhibited potent cytotoxicity against HCT-116 cancer cells with an IC_{50} value of 120 nM. Further modification of the compound consisting in the replacement of Tyr(Me) with Phe, Phe(4-Cl), and

4-biphenylalanine (Bph) resulted in the syntheses of apratoxins M14, 15, and 16 (**2b**–**2d**), respectively.[5] Interestingly, evidence indicated that the identity of the substituent R (H, Cl, or Ph) affected the cytotoxicity of **2b**–**2d** against HCT-116 cells. In particular, the Bph derivative of **2a**, apratoxin M16 (**2d**) displayed a 100-fold increase in potency with respect to **2a**, with a measured IC_{50} value of 1.1 nM, which of the same order of magnitude as that of apratoxin A. Results from a conformational analysis performed on apratoxins M7 and M16 using a distance geometry method based on NMR spectroscopy data indicated that both analogues have similar conformations to that of apratoxin A. These results led us to plan the synthesis of a new Tyr(Me)/Bph analogue of apratoxin A **3** to investigate whether the replacement of the methoxy group on Tyr(Me) in the parent compound apratoxin A with a 4-phenyl substituent is an effective approach to causing the IC_{50} value against HCT-116 cells to decrease. Thus, we synthesized compound **3** via the concise asymmetric synthesis of a 3,7-dihydroxy-2,5,8,8-tretramethylnonanoic acid (Dtena) moiety and discovered that **3** exhibited a much more potent activity than **1** against HCT-116 cells.



apratoxin A (**1**) IC_{50}: 2.8 nM

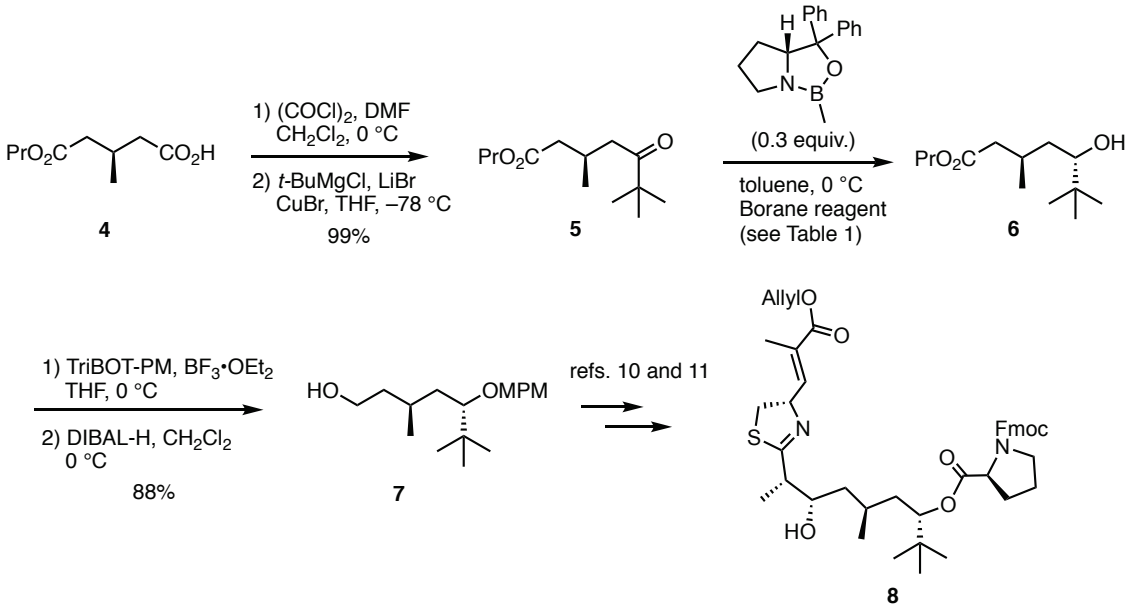
apratoxin M7 (**2a**) (R = OMe) IC_{50}: 120 nM
apratoxin M14 (**2b**) (R = H) IC_{50}: 490 nM
apratoxin M15 (**2c**) (R = Cl) IC_{50}: 69 nM
apratoxin M16 (**2d**) (R = Ph) IC_{50}: 1.1 nM

3

Figure 1. The structures of apratoxin A (**1**), its analogues **2a**–**2d**, and the newly synthesized analogue **3**, and the reported IC_{50} values against HCT-116 cells.

Although the total synthesis of apratoxin A has been reported in several papers,[6] we planned to perform the concise synthesis of **7** by way of a modification of the synthesis of apratoxin C, which we reported previously.[7] The optically active mono-acid **4**, obtained from the enzymatic alcoholysis of 3-methylglutaric anhydride, was quantitatively transformed into *tert*-butyl ketone **5** via acid chloride formation, followed by copper-mediated addition of a *tert*-butyl Grignard reagent (Scheme 1). The (*R*)-2-methyl-CBS-oxazaborolidine-catalyzed stereoselective reduction of ketone **5** was then investigated (Table 1).[8] This reaction was performed at 0 °C using BH_3•THF to provide compound **6** in 57–59% combined yield and up to 90% diastereoselectivity (Entries 1 and 2). On the other hand, use of

catecholborane as stoichiometric reductant resulted in high stereoselection (Entries 3–6)[8] with the best result being obtained when 5 equivalents of catecholborane were utilized. The desired compound **6** was produced in 88% yield and 99% stereoselectivity (Entry 5). 4-Methoxyphenylmethyl (MPM) protection of **6** using 2,4,6-tris(4-methoxybenzyloxy)-1,3,5-triazine (TriBOT-PM)[9] and reduction of the propyl ester moiety afforded alcohol **7**, which was converted to the synthetic intermediate **8** through the published procedure.[10,11]



Scheme 1. Concise synthesis of alcohol **7** for the preparation of compound **8**.

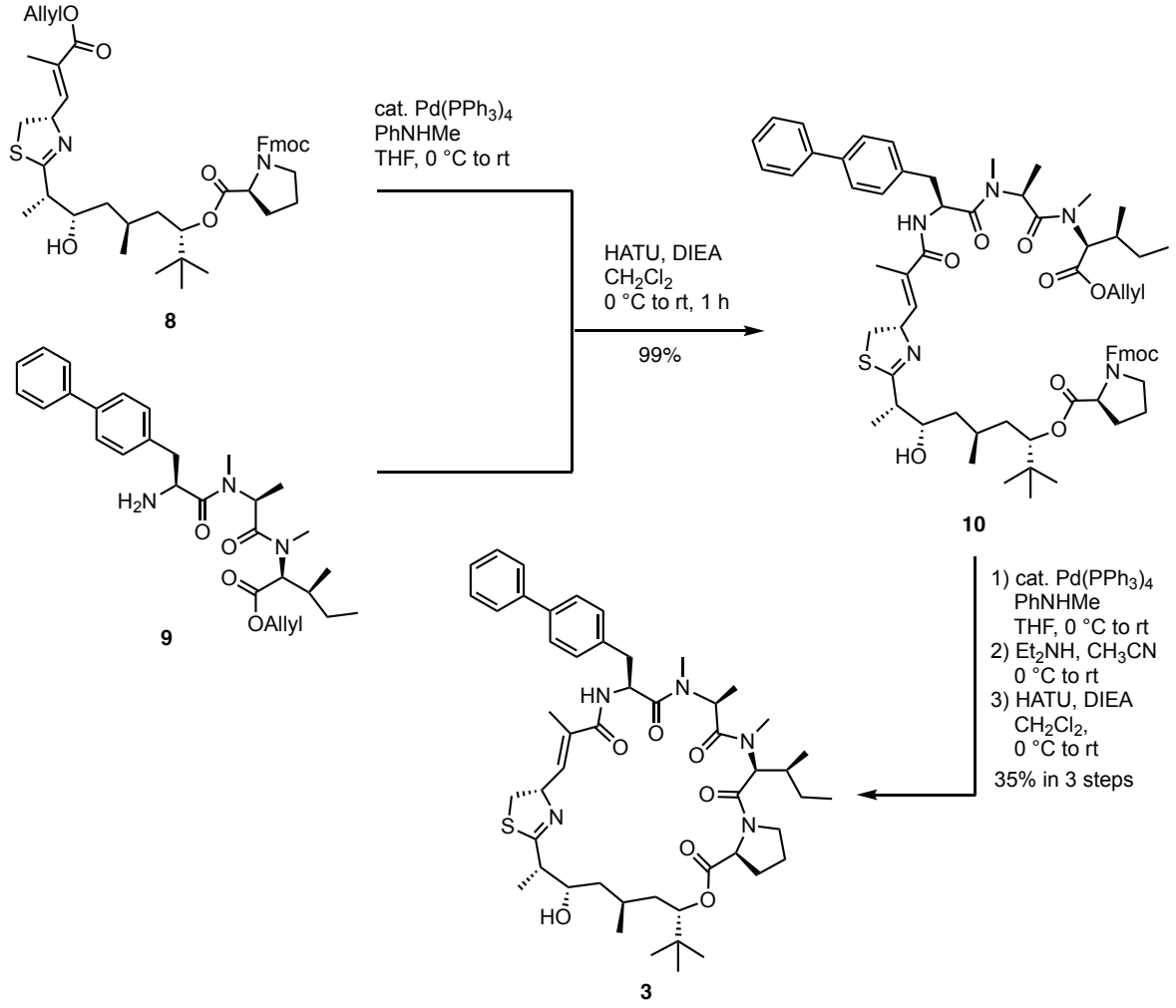
Table 1. Applied conditions and results of the CBS reduction of ketone **5** to produce compound **6**.

Entry	Borane reagent[a]	Equivalents	Concentration of **5** (M)	Time (h)	Yield[b] (%)	dr[c]
1	BH_3•THF	1.0	0.33	0.75	59	85:15
2	BH_3•THF	1.0	0.09	11	57	90:10
3	Catecholborane	1.5	0.15	9.5	9	97:3
4	Catecholborane	3.0	0.13	15	83	98:2
5	Catecholborane	5.0	0.15	9	88	99:1
6	Catecholborane	10.0	0.08	14	79	99:1

[a]THF solution. [b]Combined yield. [c]The diastereomeric ratio of **6** was determined by HPLC.

The synthesis of **3** was carried out in a similar manner to the synthesis of apratoxin A [12] (Scheme 2). Removal of the allyl group in **8** (cat. $Pd(PPh_3)_4$/PhNHMe/THF), followed by coupling of the resulting acid with tripeptide **9**[13] (HATU/DIEA/CH_2Cl_2) provided the desired product **10** in 99% yield over two steps. The sequential removal of the protecting groups at the C and N termini of **10**, followed by macrolactamization under high dilution conditions (HATU/DIEA/CH_2Cl_2/1 mM) furnished the apratoxin

A's Bph analogue **3** in 35% overall yield. The cytotoxicities of **3** and **1** against HCT-116 cells were then evaluated side-by-side. The IC_{50} for **3** was found to have a value of 0.20 nM, corresponding to a 16-fold increase in potency with respect to apratoxin A (IC_{50}: 3.3 nM). Notably, the 4-phenyl substituent of Bph greatly increased the cytotoxicity toward HCT-116 cells of the parent compound apratoxin A similarly to what had been observed for apratoxin M16.[5]

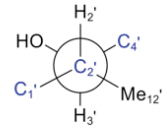
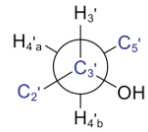
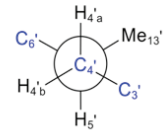
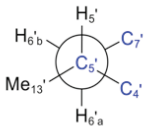
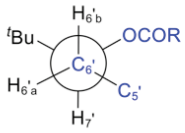


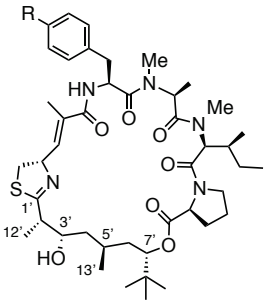
Scheme 2. Synthesis of compound **3**, the 4-biphenylalanine analogue of apratoxin A.

The 3D structure of **3** in CD_3CN was analyzed by a distance geometry method based on NMR spectral data (Table 2). Results from this analysis suggest that the 3D structure of **3** is almost identical to that of apratoxin A (Figure 2), indicating that the substitution of apratoxin A's Tyr(Me) with Bph did not induce any conformational change, although it significantly increased the compound's cytotoxicity against HCT-116 cells. Given that Huang *et al.* have reported that a D-Tyr(Me) analogue of apratoxin A does not exhibit cytotoxicity against A2058 cells,[3] it is conceivable that the 4-methoxyphenyl group of Tyr(Me) of apratoxin A interacts with a target molecule, Sec61 protein translocation channel,[3,13] and the affinity for

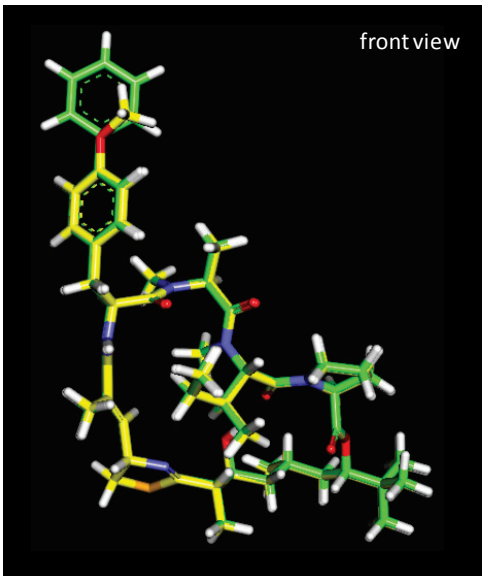
this target molecule could be further enhanced by replacing the 4-methoxyphenyl group of apratoxin A with a 4-biphenyl group.

Table 2. Spectral data of the Dtena region observed in ^{1}H NMR of **3** and **1** (CD_3CN).

	Apratoxin A Bph analogue (**3**)	Apratoxin A (**1**)
	$^{3}J_{H\text{-}2',H\text{-}3'}$ = 10.1 Hz (Large)	$^{3}J_{H\text{-}2,H\text{-}3}$ = 10.5 Hz (Large)
	$^{3}J_{H\text{-}3',H\text{-}4'a}$ = 2.9 Hz (Small) $^{3}J_{H\text{-}3',H\text{-}4'b}$ = 11.3 Hz (Large)	$^{3}J_{H\text{-}3,H\text{-}4a}$ = 3.4 Hz (Small) $^{3}J_{H\text{-}3,H\text{-}4b}$ = 11.2 Hz (Large)
	$^{3}J_{H\text{-}4'a,H\text{-}5'}$ = 10.9 Hz (Large) $^{3}J_{H\text{-}4'a,H\text{-}5'}$ = 3.8 Hz (Small)	$^{3}J_{H\text{-}4a,H\text{-}5}$ = 10.8 Hz (Large) $^{3}J_{H\text{-}4a,H\text{-}5}$ = 4.1 Hz (Small)
	$^{3}J_{H\text{-}5',H\text{-}6'a}$ = 11.9 Hz (Large) $^{3}J_{H\text{-}5',H\text{-}6'b}$ = 3.3 Hz (Small)	$^{3}J_{H\text{-}5,H\text{-}6a}$ = 11.9 Hz (Large) $^{3}J_{H\text{-}5,H\text{-}6b}$ = 3.3 Hz (Small)
	$^{3}J_{H\text{-}6'a,H\text{-}7'}$ = 2.5 Hz (Small) $^{3}J_{H\text{-}6'b,H\text{-}7'}$ = 12.5 Hz (Large)	$^{3}J_{H\text{-}6a,H\text{-}7}$ = 2.5 Hz (Small) $^{3}J_{H\text{-}6b,H\text{-}7}$ = 12.4 Hz (Large)



3 (R = Ph)
1 (R = OMe)



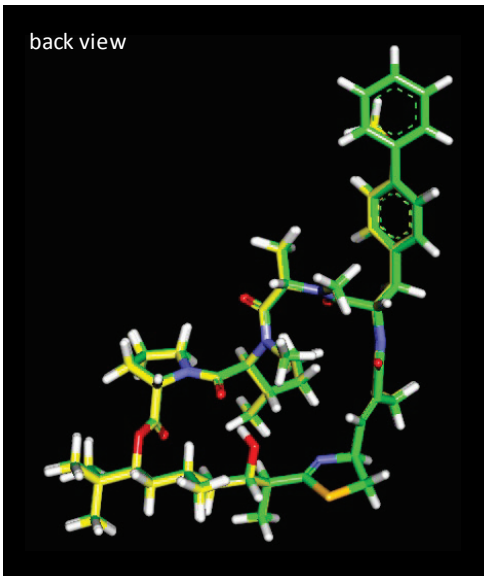


Figure 2. Superposition of the lowest energy conformers of apratoxin A's 4-biphenylalanine analogue **3** (structure in green) and apratoxin A (**1**) (structure in yellow) obtained by a conformational analysis performed using a distance geometry method.

In summary, we have demonstrated a concise synthesis of the Dtena moiety and the total synthesis of the apratoxin A's Bph analogue **3**. Biological evaluation indicated the 4-phenyl group of Bph in **3** caused the compound's cytotoxicity against HCT-116 cells to increase substantially with respect to apratoxin A. A similar trend, in this case with respect to apratoxin M7's cytotoxicity versus HCT-116 cells, was observed in the case of apratoxin M16 (**2d**). Results from a conformational study performed using a distance geometry method suggest that **3** and apratoxin A (**1**) adopt similar conformations in CD_3CN. These results should provide important structure-activity relationship information that could be used in the future design of useful apratoxin A analogues.

EXPERIMENTAL

General. All commercially available reagents were used as received. Dry THF and CH_2Cl_2 (Kanto Chemical Co.) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina column. All reactions in the solution phase were monitored by TLC carried out on Merck silica gel plates (0.2 mm, 60F-254) with UV light, and visualized by *p*-anisaldehyde H_2SO_4−EtOH solution or phosphomolybdic acid−EtOH solution or ninhydrin−AcOH−1-BuOH solution. Silica gel 60N (Kanto Chemical Co. 100~210 mm) was used for column chromatography. SHIMADZU LC-10AT and Shodex RI-101 were used for normal-phase HPLC analysis. Waters 1525 binary pump and Waters 2489 UV/visible detector were used for reversed-phase HPLC purification of the final product. ^{1}H NMR spectra (400 and 600 MHz) and ^{13}C NMR spectra (100 and 150 MHz) were recorded on JEOL JNM-AL400 and JEOL JNM-ECA600 spectrometers in the indicated solvent. Chemical shifts (δ) are reported in units parts per million (ppm) relative to the signal for internal TMS (0.00 ppm for ^{1}H) for solutions in $CDCl_3$. NMR spectral data are reported as follows: chloroform (7.26 ppm for ^{1}H) or chloroform-*d* (77.0 ppm for ^{13}C), dichloromethane (5.32 ppm for ^{1}H) or dichloromethane-d_2 (53.8 ppm for ^{13}C), and acetonitrile (1.94 ppm for ^{1}H) or acetonitrile-d_3 (118.26, 1.32 ppm for ^{13}C) when internal standard is not indicated. Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), brs (broad singlet), brd (broad doublet) and *J* (coupling constants in Hertz). High-resolution mass spectra were measured on Thermo Scientific™ Exactive™ Plus Orbitrap Mass Spectrometer (for ESI). IR spectra were recorded on a JASCO FTIR-4100. Only the strongest and/or structurally important absorption are reported as the IR data afforded in wavenumbers (cm^{-1}). Specific rotations were measured on a JASCO P-1010 polarimeter.

Propyl (*R*)-3,6,6-trimethyl-5-oxoheptanoate (5): To a solution of the carboxylic acid **4**[7] (1.00 g, 5.31 mmol, 1.0 equiv) in dry CH_2Cl_2 (11 mL) were added DMF (20.0 μL, 0.260 mmol, 0.05 equiv) and $(COCl)_2$ (690 μL, 7.96 mmol, 1.5 equiv) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated in vacuo. The resulting crude acid chloride was used for the next reaction without further purification.

To an oven-dried flask were added $LiBr \cdot H_2O$ (2.68 g, 25.5 mmol, 4.8 equiv) and CuBr (1.82 g, 12.7 mmol, 2.4 equiv), and the flask was dried using a heat-gun under vacuum. Once the flask had cooled, dry THF (15 mL) was added, and the suspension was cooled to −78 °C under an argon atmosphere. To the green suspension was added a solution of *t*-BuMgCl (12.7 mL, 12.7 mmol, 1.0 M in THF, 2.4 equiv), followed immediately by addition of a solution of the crude acid chloride in dry THF (10 mL). After being stirred at the same temperature for 15 min, the reaction mixture was quenched with saturated aqueous NH_4Cl. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 9:1) to afford the ketone **5** (1.20 g, 5.25 mmol, 99% over 2 steps) as a colorless oil. $[\alpha]^{19}_D$ +4.3 (*c* 0.97, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 (t, 2H, *J* = 6.7 Hz), 2.41–2.58 (m, 3H), 2.34 (dd, 1H, *J* = 14.9, 5.8 Hz), 2.20 (dd, 1H, *J* = 14.9, 6.8 Hz), 1.60–1.69 (m, 2H), 1.12 (s, 9H), 0.92–0.97 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 214.6, 172.7, 65.8, 44.1, 42.7, 40.9, 26.3, 26.0, 22.0, 19.9, 10.4; IR (neat) 2968, 2878, 1734, 1706, 1464, 1366, 1223, 1196, 1166, 1059 cm^{-1}; HRMS[ESI] calcd for $C_{13}H_{24}O_3Na$ $[M+Na]^+$ 251.1618, found 251.1616.

Propyl (3*R*,5*S*)-5-hydroxy-3,6,6-trimethylheptanoate (6): To a solution of (*R*)-2-methyl-CBS-oxazaborolidine (87.3 μL, 0.0873 mmol, 1 M in toluene, 0.30 equiv), was added a solution of catecholborane (1.5 mL, 1.46 mmol, 1 M in THF, 5.0 equiv) at 0 °C under an argon atmosphere, and the mixture was stirred at the same temperature for 30 min. A solution of the ketone **5** (66.4 mg, 0.291 mmol, 1.0 equiv) in dry toluene (0.6 mL) was then added to above mixture dropwise. After being stirred at 0 °C for 9 h, the reaction mixture was quenched with MeOH and saturated aqueous NH_4Cl. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 15:1) to afford the alcohol **6** (58.9 mg, 0.256 mmol, 88%) as a colorless oil. The diastereomeric ratio of **6** was determined by HPLC analysis (column: Senshu Pak Silica-3301-N (8Φ × 300 mm); flow rate: 1.0 mL/min; elution rate: hexane/EtOAc = 3:1 (isocratic); retention time: 19.7 min for **6**, 21.1 min for C5 diastereomer of **6**). $[\alpha]^{21}_D$ −34 (*c* 0.97, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99–4.09 (m, 2H), 3.22 (brd, 1H, *J* = 10.2 Hz), 2.41 (dd, 1H, *J* = 14.5, 5.4 Hz), 2.17–2.26 (m, 1H), 2.15 (dd, 1H, *J* = 14.5, 7.3 Hz),

1.91 (brs, 1H), 1.61–1.70 (m, 2H), 1.27–1.44 (m, 2H), 1.03 (d, 3H, J = 6.6 Hz), 0.95 (t, 3H, J =7.4 Hz), 0.89 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 66.0, 40.6, 38.7, 34.8, 27.5, 25.6, 22.0, 21.5, 10.4; IR (neat) 3461, 2961, 2872, 1734, 1364, 1315, 1279, 1194, 1167, 1065 cm^{-1}; HRMS[ESI] calcd for $C_{13}H_{27}O_3$ $[M+H]^+$ 231.1955, found 231.1954.

(*S*)-5-(4-methoxybenzyloxy)-3,6,6-trimethylheptan-1-ol (7): To a solution of the alcohol **6** (100 mg, 0.430 mmol, 1.0 equiv) in dry THF (2.2 mL) were added TriBOT-PM (318 mg, 0.650 mmol, 1.5 equiv) and a solution of $BF_3{\cdot}OEt_2$ (220 μL, 0.0430 mmol, 200 mM in THF, 0.10 equiv) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 5 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was passed through a short pad of silica gel (eluted with hexane/EtOAc = 30:1). The resulting PMB ether was used for the next reaction without further purification.

To a solution of the above ester in dry CH_2Cl_2 (2.2 mL) was added a solution of DIBAL-H (1.1 mL, 1.10 mmol, 1.0 M in hexane, 2.6 equiv) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 10 min, the reaction mixture was quenched with MeOH and Rochelle salt at 0 °C, and stirred at room temperature for 1 h. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 20:1) to afford the alcohol **7** (107 mg, 0.380 mmol, 88% over 2 steps) as a colorless oil. The spectroscopic data of **7** were in good agreement with those reported in the literature.[10,11] $[\alpha]^{22}_D$ −31 (*c* 1.4, $CHCl_3$); HRMS[ESI] calcd for $C_{18}H_{30}O_3Na$ $[M+Na]^+$ 317.2087, found 317.2085.

N-Fmoc derivative of H-Bph-MeAla-MeIle-OAllyl (9): To Boc-MeAla-MeIle-OAllyl[6] (479 mg, 1.29 mmol, 1.5 equiv) was added 4 M HCl/dioxane (6.7 mL) at 0 °C. After being stirred at room temperature for 6 h, the reaction mixture was concentrated in vacuo. The residue was azeotroped with toluene and CH_2Cl_2. The crude amine was used for the next reaction without further purification.

To a solution of the crude amine in dry CH_2Cl_2 (4.3 mL) were added Fmoc-Bph-OH (400 mg, 0.862 mmol, 1.0 equiv), DIEA (676 μL, 3.88 mmol, 4.5 equiv) and HATU (490 mg, 1.29 mmol, 1.5 equiv) at room temperature under an argon atmosphere. After being stirred at the same temperature for 1 h, the reaction mixture was quenched with H_2O at 0 °C. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 4:1) to afford the tripeptide Fmoc-Bph-MeAla-MeIle-OAllyl (605 mg, 0.845 mmol, 98%) as a white amorphous solid. $[\alpha]^{23}_D$ −83 (*c* 0.55, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, major rotamer) δ 7.72–7.78 (m, 2H), 7.27–7.56 (m, 15H),

5.85–5.91 (m, 1H), 5.53 (d, 1H, J = 8.9 Hz), 5.43 (q, 1H, J = 6.8 Hz), 5.17–5.32 (m, 2H), 4.97–5.02 (m, 1H), 4.92 (d, 1H, J = 10.3 Hz), 4.52–4.62 (m, 2H), 4.28–4.43 (m, 2H), 4.13–4.20 (m, 1H), 3.13 (dd, 1H, J = 13.7, 7.2 Hz), 3.00 (s, 3H), 2.93 (dd, 1H, J = 13.7, 6.8 Hz), 2.75 (s, 3H), 1.88–1.95 (m, 1H), 1.29 (d, 3H, J = 6.8 Hz), 1.18–1.28 (m, 1H), 0.90–0.96 (m, 1H), 0.89 (d, 3H, J = 6.8 Hz), 0.77 (t, 3H, J = 7.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, mixture of rotamers) δ 171.8, 171.3, 170.6, 155.7, 143.8, 143.7, 141.3, 140.7, 140.6, 139.9, 135.5, 135.1, 131.7, 129.9, 128.8, 127.7, 127.2, 127.1, 126.9, 125.1, 120.0, 118.7, 67.0, 65.4, 60.5, 52.1, 49.7, 47.1, 38.6, 34.4, 33.2, 31.0, 30.6, 25.0, 16.1, 15.7, 14.3, 11.6, 10.5; IR (neat) 3293, 2965, 1734, 1647, 1486, 1450, 1246, 760, 741 cm^{-1}; HRMS[ESI] calcd for $C_{44}H_{49}N_3O_6Na$ $[M+Na]^+$ 738.3514, found 738.3502.

Preparation of H-Bph-MeAla-MeIle-OAllyl (9): To a solution of the above *N*-Fmoc tripeptide (169 mg, 0.236 mmol, 2.0 equiv) in dry MeCN (9.4 mL) was added Et_2NH (4.7 mL) at room temperature under an argon atmosphere. After being stirred at the same temperature for 50 min, the reaction mixture was concentrated in vacuo. The residue was azeotroped twice with CH_2Cl_2. The resulting amine **9** was used for the next reaction without purification.

Hexadepsipeptide 10: To a solution of the allyl ester **8**[10,11] (86.4 mg, 0.118 mmol, 1.0 equiv) in dry THF (2.0 mL) were added *N*-methylaniline (32.0 μL, 0.295 mmol, 2.5 equiv) and a catalytic amount of $Pd(PPh_3)_4$ (13.6 mg, 0.0118 mmol, 0.01 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 1.5 h, the reaction mixture was concentrated in vacuo. The residue was passed through a short pad of silica gel (eluted with hexane/EtOAc = 4:1, then CH_2Cl_2/MeOH = 9:1). The resulting carboxylic acid was used for the next reaction without further purification.

To a solution of the above-mentioned amine **9** in dry CH_2Cl_2 (1.0 mL) were added a solution of the above carboxylic acid in dry CH_2Cl_2 (1.0 mL), DIEA (62.0 μL, 0.354 mmol, 3.0 equiv) and HATU (67.0 mg, 0.177 mmol, 1.5 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel (eluted with hexane/acetone = 9:1) to afford the hexadepsipeptide **10** (137 mg, 0.117 mmol, 99% over 2 steps) as a white amorphous solid. $[\alpha]^{19}_D$ −1.0 X 10^2 (*c* 0.25, CH_2Cl_2); ^{1}H NMR (600 MHz, CD_2Cl_2, major rotamer) δ 7.25–7.79 (m, 17H), 6.49–6.60 (m, 1H), 6.30 (d, 1H, J = 8.6 Hz), 5.86–5.94 (m, 1H), 5.08–5.42 (m, 5H), 4.79–4.92 (m, 2H), 4.58 (m, 2H), 4.12–4.47 (m, 3H), 3.31–3.76 (m, 5H), 2.61–3.20 (m, 10H), 0.76–2.31 (m, 39H); ^{13}C NMR (150 MHz, CD_2Cl_2 mixture of rotamers) δ 172.8, 172.0, 171.4, 170.8, 168.2, 155.2, 144.7, 144.5, 141.7, 141.6, 140.9, 140.0, 136.0, 132.4, 130.4, 129.1, 128.0, 127.9, 127.6, 127.4, 127.3, 127.2, 125.8, 125.7, 125.6, 120.2, 118.6, 78.7, 71.7, 68.0, 67.9, 65.6, 60.9, 60.1, 50.7, 50.1, 47.6, 47.5, 46.9, 46.1, 39.4, 38.4, 38.2, 37.9, 35.0, 34.9, 33.4, 31.5, 31.2, 30.8, 30.3, 26.1, 26.0, 25.4, 25.3, 24.9, 23.6, 20.5, 20.4, 16.5, 15.8, 15.7, 14.5, 13.7, 13.6, 10.5; IR

(neat) 3477, 3327, 2963, 2931, 2875, 1738, 1706, 1644, 1486, 1451, 1416, 1364, 1274, 1181, 1123, 1087, 988, 760, 740 cm^{-1}; HRMS[ESI] calcd for $C_{68}H_{88}N_5O_{10}S$ $[M+H]^+$ 1166.6246, found 1166.6224.

Apratoxin A's Bph analogue (3): To a solution of the allyl ester **10** (130 mg, 0.111 mmol, 1.0 equiv) in dry THF (2.0 mL) were added *N*-methylaniline (32.0 μL, 0.295 mmol, 2.7 equiv) and a catalytic amount of $Pd(PPh_3)_4$ (13.6 mg, 0.0118 mmol, 0.11 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 40 min, the reaction mixture was concentrated in vacuo. The residue was passed through a short pad of silica gel (eluted with hexane/EtOAc = 4:1, then CH_2Cl_2/MeOH = 9:1). The resulting carboxylic acid was used for the next reaction without further purification.

To a solution of the above N-Fmoc amine in dry MeCN (4.7 mL) was added Et_2NH (2.4 mL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 1.5 h, the reaction mixture was concentrated in vacuo. The residue was azeotroped twice with CH_2Cl_2. The crude cyclization precursor was used for the next reaction without further purification.

To a solution of the crude cyclization precursor in dry CH_2Cl_2 (118 mL) were added DIEA (183 μL, 1.06 mmol, 9.5 equiv) and HATU (134 mg, 0.354 mmol, 3.2 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 14 h, the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 4:1, then CH_2Cl_2/MeOH = 9:1) and subsequent reversed-phase HPLC (column: YMC-Pack R&D ODS-A 20 mm × 150 mm; flow rate: 10.0 mL/min; elution method: H_2O/MeOH = 20:80−0:100 linear gradient (0.0−10.0 min), H_2O/MeOH = 0:100 isocratic (10.0−20.0 min); retention time: 12.4 min) to afford apratoxin A's Bph analogue (**3**) (34.6 mg, 0.0390 mmol, 35% over 3 steps) as a white amorphous solid. $[\alpha]^{27}_D$ −2.2 × 10^2 (*c* 0.58, MeOH); ^{1}H NMR (600 MHz, CD_3CN, major rotamer) δ 7.34–7.63 (m, 9H), 6.56–6.67 (m, 1H), 6.11–6.19 (m, 1H), 5.24–5.29 (m, 1H), 5.10 (d, 1H, J = 11.7 Hz), 4.98–5.10 (m, 1H), 4.94 (dd, 1H, J = 13.0, 1.8 Hz), 4.51 (d, 1H, J = 11.0 Hz), 4.04–4.12 (m, 2H), 2.95–3.62 (m, 7H), 2.92 (s, 3H), 2.53–2.63 (m, 4H), 2.22–2.30 (m, 1H), 1.99–2.19 (m, 3H), 1.94 (s, 3H), 1.74–1.88 (m, 3H), 1.48–1.54 (m, 1H), 1.13–1.36 (m, 3H), 1.07 (d, 3H, J = 6.5 Hz), 1.03 (d, 3H, J = 7.2 Hz), 0.94 (d, 3H, J = 6.5 Hz), 0.87 (s, 9H), 0.82–0.86 (m, 7H); ^{13}C NMR (150 MHz, CD_3CN, major rotamer) δ 176.8, 173.3, 171.9, 171.2, 170.7, 170.0, 141.5, 140.2, 137.4, 136.7, 131.00, 130.96, 129.8, 128.2, 127.74, 127.66, 77.9, 73.0, 72.2, 61.4, 60.6, 57.2, 51.2, 50.1, 48.4, 38.9, 38.3, 37.6, 37.4, 35.6, 32.4, 30.5, 30.1, 26.2, 26.1, 25.6, 25.1, 19.8, 16.9, 14.4, 14.3, 13.4, 9.2; IR (neat) 3421, 2965, 2931, 2874, 1740, 1624, 1486, 1455, 1392, 1371, 1319, 1277, 1219, 1178, 1116, 1078, 733, 699 cm^{-1}; HRMS[ESI] calcd for $C_{50}H_{72}N_5O_7S$ $[M+H]^+$ 886.5147, found 886.5128.

Cytotoxicity Assay. Human colorectal carcinoma HCT-116 cells were kindly provided by Prof. Yoshiteru Oshima at the Graduate School of Pharmaceutical Sciences in Tohoku University. They were cultured in an RPMI 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan) containing 10% fetal bovine

serum (Equitech-Bio, Inc., Texas, USA), 100 U/mL of penicillin, and 100 mg/mL of streptomycin (Nacalai Tesque, Inc., Kyoto, Japan) at 37 °C under 5% CO_2. For the cytotoxicity assay, near-confluent cultures of the cells were plated at 5×10^3 cells/100 μL/well fresh culture medium in a 96-well clear bottom plate and incubated at 37 °C under 5% CO_2 for 24 h before the assays.

Each compound was dissolved in DMSO at concentrations ranging from 0.01 to 100 μM. One microliter of the resultant solution was added to the above-mentioned 100 μL cell culture, resulting in various concentrations of the compound (0.1 – 1000 nM) or solvent control (DMSO 1%). After a 48 h incubation at 37 °C under 5% CO_2, 10 μL of WST-8 reagent solution (Cell Count Reagent SF, Nacalai Tesque, Inc.)[14] was added to the cell culture. The cell culture was then incubated at 37 °C under 5% CO_2 for 2 h. Colorimetric determination of WST-8 was conducted at 450 nm with an optional reference wavelength at 595 nm using a microplate reader (Model 680, Bio-Rad, Hercules, CA). The absorbance obtained upon the addition of the vehicle was considered as 100%. Data are expressed from the dose–response curves at three independent experiments. IC_{50} values were calculated by probit analysis using the PriProbit 1.63 software.[15]

Molecular Modeling Based on NMR Data. A NMR measurement for 3D structural analysis was conducted using a NMR spectrometer (600 MHz for ^1H) at 298 K using compound **3**. $^3J_{H,H}$ values were determined by 1D ^1H spectra and ^1H-^1H *J*-resolved 2D NMR spectra. According to a *J*-based configuration analysis (JBCA) method,[16] $^3J_{H,H}$ coupling constants for clearly anti-oriented vicinal protons ($^3J_{H,H} \geq 10$ Hz) were interpreted as torsional angle constraints.

Molecular modeling was performed on the MacroModel (version 9.9) program[17] by the distance geometry method. We used an OPLS-2005 force field and a generalized Born/solvent-accessible surface area (GB/SA) solvent model.[18] The calculations were conducted in a chloroform environment. To find 3D structures that were in agreement with the experimental data and also had low energies in a given force field, we selected a protocol that comprised two steps. First, a conformational search was performed using Monte Carlo-based torsional sampling with the above torsional angle constraints at 20,000 iterations with 500 times of energy minimization. Then, energy minimization was applied to each found structure without constraints.

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