JOC The Journal of Organic Chemistry

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Total Synthesis and Conformational Analysis of Apratoxin C

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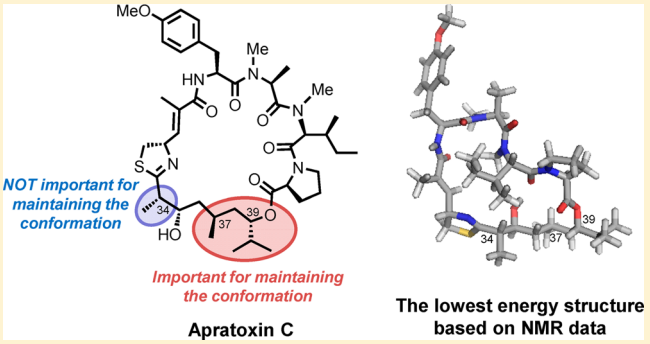
Supporting Information

ABSTRACT: Total synthesis of apratoxin C, a cyanobacterial cyclodepsipeptide with highly potent cytotoxicity against some cancer cell lines, was achieved using the apratoxin A synthetic strategy developed by us. To elucidate the relationship between conformation and activity, the tertiary structure of apratoxin C was analyzed by NMR spectroscopy. We obtained 37 ROEs and five $^{3}J_{H,H}$ values, which were translated into distance and dihedral angle constraints, respectively. Molecular modeling was performed with a restrained conformational search by a distance geometry method. The lowest energy structure indicated that the methyl group at C37 and the isopropyl group at C39 play critical roles in maintaining the conformation, whereas the methyl group at C34 does not. Moreover, we confirmed that apratoxin A and C possess similar conformations, providing a likely explanation for their nearly equivalent cytotoxicities.



INTRODUCTION

Marine cyanobacteria produce a number of secondary metabolites that possess interesting molecular architectures and biological properties.[1−3] Among the metabolites, cyanobacterial cyclodepsipeptides, which have unique scaffolds and nonribosomally synthesized peptide motifs, attract considerable attention as novel pharmaceuticals because of their striking biological activities.[1−3] One example is the apratoxin family (Figure 1), which exhibits highly potent cytotoxicity against some cancer cell lines.[4−9] Apratoxins A−D (**1**−**4**)[4−6] are the cyclodepsipeptides that feature a proline residue, *N*-methylated amino acids, a modified cysteine residue, and a dihydroxylated fatty acid moiety (Figure 1). Because of their unique structure and biological activity, several researchers have demonstrated the total syntheses of apratoxins; Forsyth's group was the first to complete the total synthesis of apratoxin A (**1**).[10,11] Furthermore, two groups including us have achieved total synthesis of **1**.[12−14] We have also demonstrated the solid-phase total synthesis of apratoxin A (**1**) and its analogues.[15] Recently, the total synthesis of apratoxin D (**4**) was reported.[16] Because apratoxins have potential as novel anticancer lead compounds, their mode of action has been studied by genomics and chemical biological approaches.[17−20]

The cytotoxicity of apratoxins is sensitive to certain structural modifications in the fatty acid region. Ma et al. reported that reversal of the configuration at C37 from 37*S* to 37*R* or removal of the methyl group at C37 abolished the cytotoxicity of apratoxins in oxazoline analogues.[14] Our group reported that the protection of the hydroxyl group at C35 with a triethylsilyl (TES) group led to lack of cytotoxicity, whereas the (34*R*)-diastereomer of apratoxin A showed equipotent activity.[13,15] In addition, we reported the synthesis and biological evaluation of apratoxin analogues in which one of the residues was replaced with an azido-derivatized amino acid.[15] Recently, Luesch et al. conducted a structure-activity relationship (SAR) study of apratoxins and developed an apratoxin A/E hybrid analogue with improved *in vivo* antitumor activity.[21,22] According to the above-mentioned studies, the modification of apratoxins could affect not only the local structure but also the main chain conformation because of the flexibility of the molecular frame of cyclic peptides. Luesch et al. analyzed the tertiary structures of apratoxin A (**1**), apratoxin B (**2**), and *E*-dehydroapratoxin A (**5**) by NMR spectroscopy.[4,5] They indicated that the difference in the cytotoxicities could be derived from conformational changes; thus, it is essential to understand the effect of modification on the conformations for SAR and drug design based on the cyclodepsipeptide scaffolds. Since the *tert*-butyl group in apratoxin A (**1**) is thought to be important to regulate its conformation, we focused on apratoxin C (**3**), which possesses an isopropyl group instead of the *tert*-butyl group in **1**. Herein, we report the synthesis of apratoxin C (**3**) and the analysis of the conformation by NMR spectroscopy.

RESULTS AND DISCUSSION

Total Synthesis of Apratoxin C (3). According to our total synthesis of apratoxin A (**1**),[12,13] synthesis of apratoxin C (**3**) can be performed using a similar strategy (Scheme 1). In principle, apratoxin C (**3**) can be synthesized by macro-

Received: May 22, 2014
Published: August 5, 2014

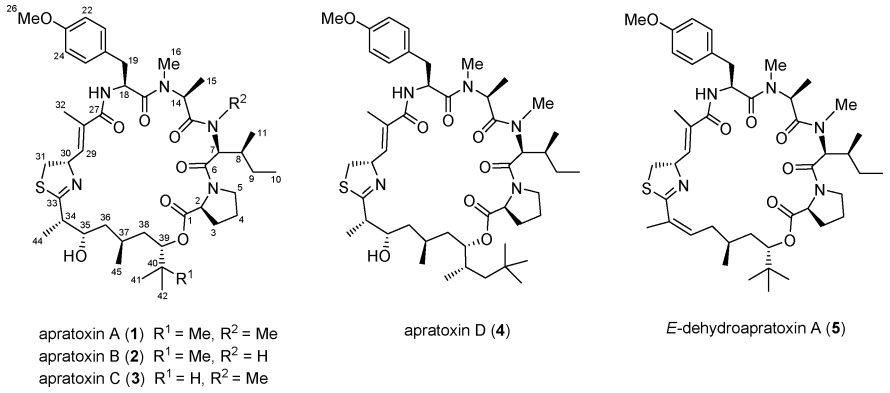
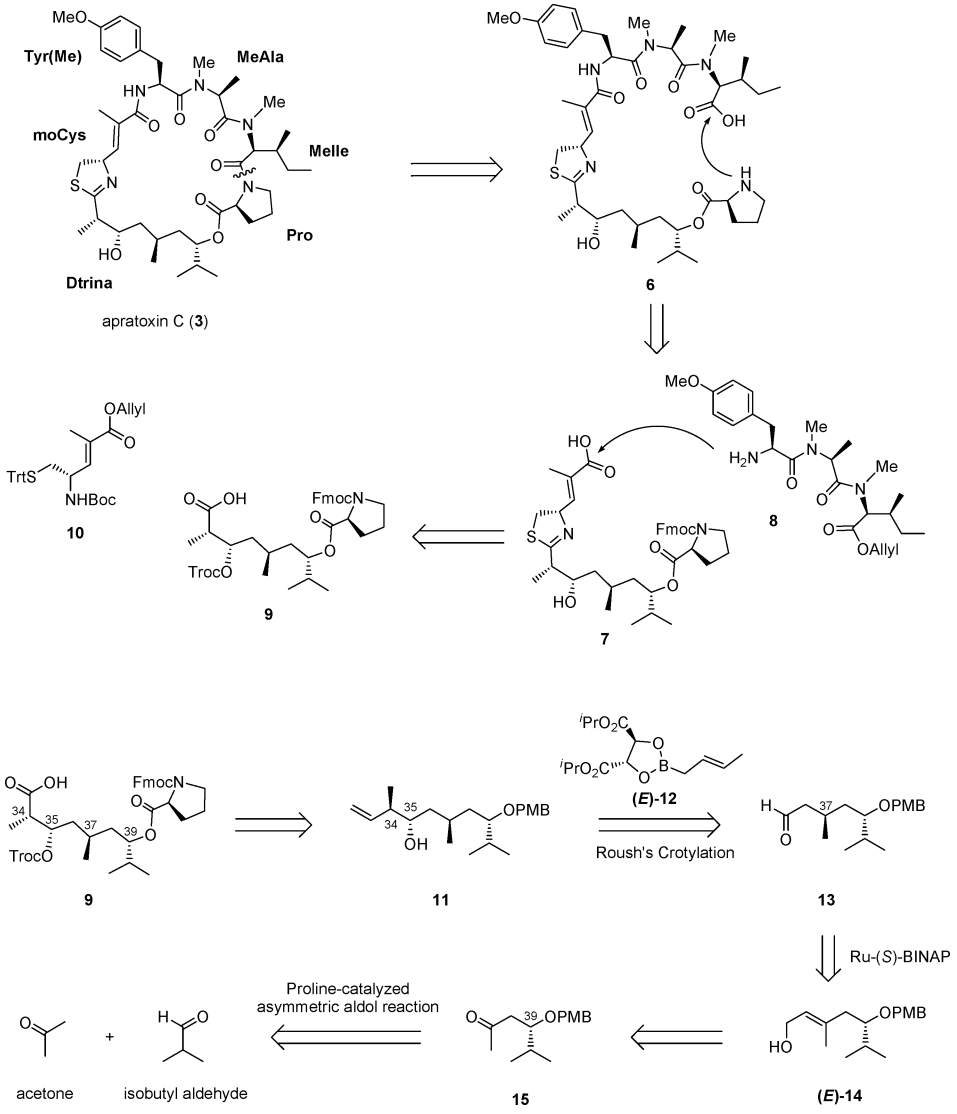


Figure 1. Chemical structures of apratoxins.

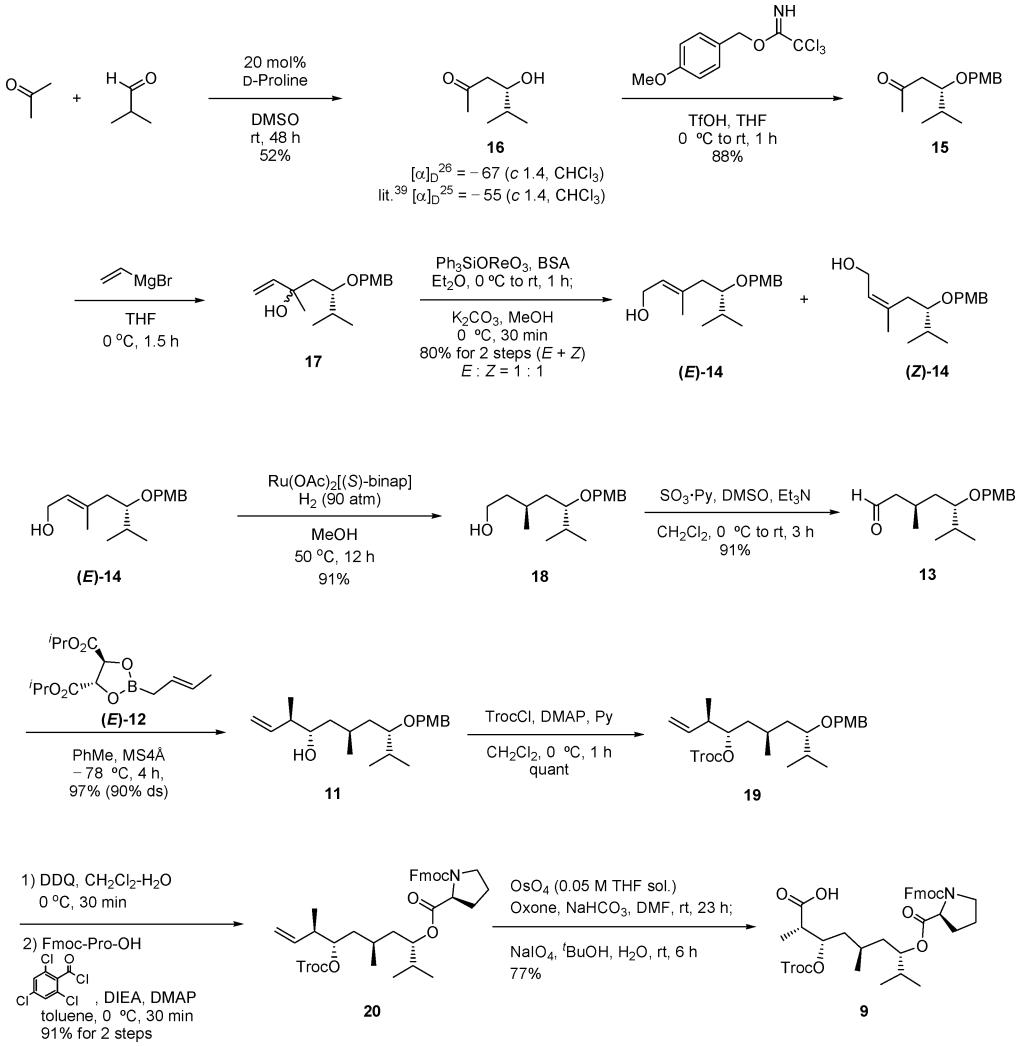
Scheme 1. Retrosynthetic Analysis of Apratoxin C (3)



lactamization of the linear compound **6**, which can be obtained by coupling carboxylic acid **7** with tripeptide **8**. Acid **7** can be prepared from the coupling of Pro-Dtrina (3,7-dihydroxy-2,5,8-trimethylnonanoic acid) moiety **9** with a derivative of compound **10**, followed by construction of a thiazoline ring. Constructions of the stereogenic centers at the C34, C35, C37, and C39 positions are vital for the synthesis of Pro-Dtrina **9**, which can be synthesized from a derivative of compound **11** via diastereoselective crotylation[23,24] of aldehyde **13** with *E*-crotyl borate (*E*)-**12**. The stereogenic center at C37 can be introduced by asymmetric hydrogenation of allylic alcohol (*E*)-**14** in the presence of a Ru-(*S*)-BINAP catalyst.[25] Compound (*E*)-**14** can be transformed from compound **15**,

dx.doi.org/10.1021/jo501130b | J. Org. Chem. 2014, 79, 8000−8009

Scheme 2. Synthesis of Pro-Dtrina 9



which is obtained by proline-catalyzed enantioselective aldol reaction[26] of acetone with isobutylaldehyde.

First, we investigated the synthesis of Pro-Dtrina **9** (Scheme 2). After a proline-catalyzed aldol reaction of acetone with isobutylaldehyde,[26] protection of the resulting hydroxyl group of **16** with a *p*-methoxybenzyl (PMB) ether using PMB trichloroacetimidate in the presence of trifluoromethanesulfonic acid provided compound **15**, which was converted into the alcohol **17** by 1,2-addition of a vinyl Grignard reagent to a ketone moiety. Rhenium-catalyzed isomerization of allylic alcohol with *N*,*O*-bis(trimethylsilyl) acetamide (BSA)[27,28] provided **14** at an *E*:*Z* isomer ratio of 1:1, and these diastereomers were separated by silica gel column chromatography to afford (*E*)-**14** and (*Z*)-**14**[29] in a combined yield of 80%. Ru(OAc)$_2$[(*S*)-binap]-catalyzed asymmetric hydrogenation[25] of (*E*)-**14** under 90 atm of hydrogen afforded compound **18** in a 91% yield as a single diastereomer confirmed by ^{1}H NMR. After oxidation of the primary alcohol in **18** to the aldehyde **13**, diastereoselective crotylation of **13** with (*E*)-crotyl borate (*E*)-**12** afforded **11** and its diastereomer at a ratio of 9:1 (determined by ^{1}H NMR) in a combined yield of 97%. The mixture of the diastereomers was partially purified by preparative RP-HPLC to afford pure **11**, whose structure was determined by instrumental analyses. As its selectivity was good enough to contuinue the synthesis, we utilized **11** as a mixture of the diastereomers for the next reaction. The alcohol **11** and its diastereomer were protected with a 2,2,2-trichloroethoxycarbonyl (Troc) group to provide **19** and its diastereomer. Removal of the PMB group with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), followed by coupling with Fmoc-Pro-OH by the Yamaguchi method,[30] gave compound **20** without epimerization at the α-position of the proline moiety. The diastereomer of **20** was separated by silica gel column chromatography. Finally, oxidative cleavage of the terminal alkene and subsequent oxidation of the resulting aldehyde furnished the desired Pro-Dtrina **9**.

After obtaining the desired **9**, we synthesized the linear peptide **23** leading to apratoxin C (**3**) (Scheme 3). After removal of the *N*-Boc group in **10** (TMSOTf/2,6-lutidine, rt, 7 h),[12,13] coupling of the resulting amine with **9** using *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide (EDCI)/ 1-hydroxy-7-azabenzotriazole (HOAt) provided the amide **21** in a 55% yield. Thiazoline formation was performed by treatment with trifluomethanesulfonic anhydride/triphenylphosphine oxide (Tf_2O/Ph_3PO, 0 °C, 1 h),[31] and subsequent removal of the Troc group (Zn/NH_4OAc, rt, 1.5 h) afforded thiazoline **22** in a 90% yield for two steps. Removal of the allyl group and coupling of the resulting acid with tripeptide **8**[12,13] provided the desired **23** in a 95% yield for two steps. Finally, the removal of the protecting groups at both the N- and C-terminus, followed

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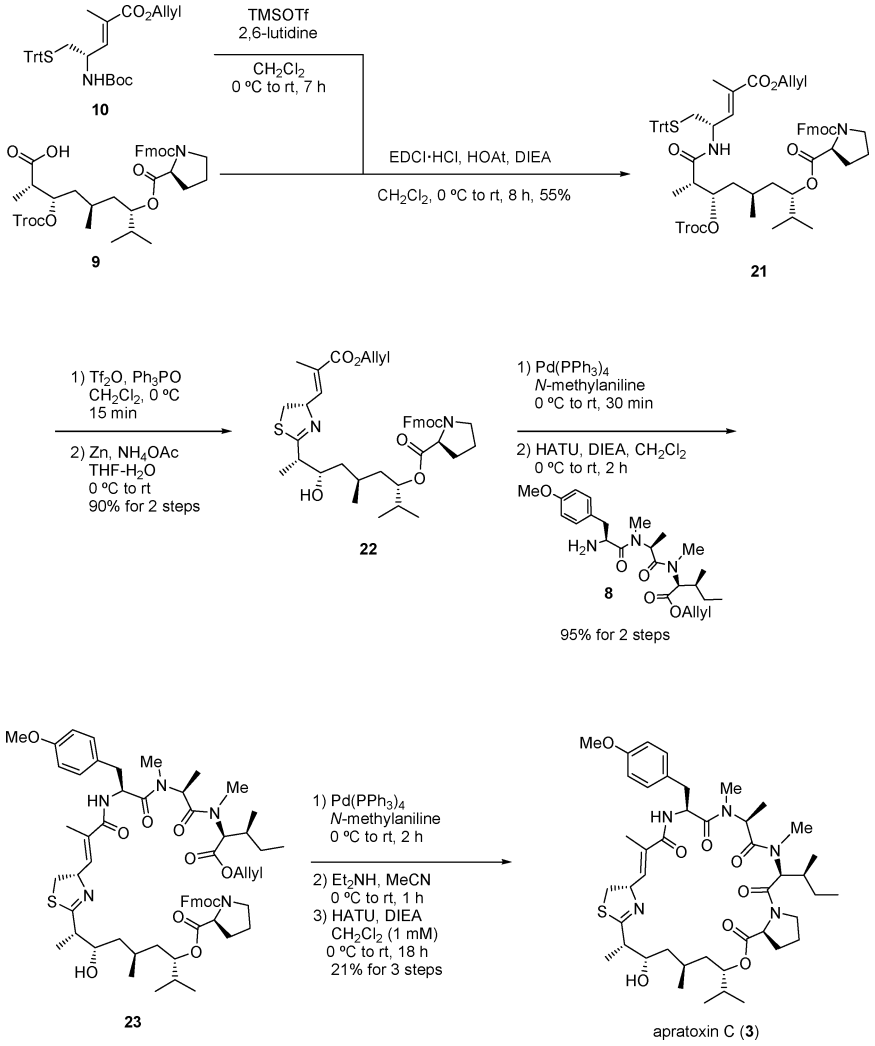
Scheme 3. Total Synthesis of Apratoxin C (3)



by macrolactamization utilizing HATU[32]/diisopropylethylamine (DIEA) under high dilution conditions (1 mM), furnished apratoxin C (**3**) in a 21% yield for three steps. Although trace amount of the diastereomers were also detected in the reaction mixture of the macrolactamization by LC−MS, they were readily removed by preparative HPLC. Spectroscopic data, including optical rotation, of synthetic **3** were identical to those of the natural product, apratoxin C (Figure S1, Table S1 in Supporting Information). Cytotoxic activities of the synthetic apratoxin C (**3**) against HCT-116 cells were evaluated by a WST assay.[33,34] The cytotoxicity of apratoxin C (**3**) (IC_{50} value 4.1 nM) was found to be slightly weaker than that of apratoxin A (**1**) (IC_{50} value 3.2 nM), which agrees with previous reports.[5]

Conformational Analysis of Apratoxin C (3) by NMR. To investigate the conformation of apratoxin C (**3**), we analyzed its tertiary structure by NMR. Because apratoxin C (**3**) might be labile under acidic conditions in $CDCl_3$,[5] NMR experiments were performed using CD_3CN as the solvent. Dihedral angle constraints were determined by *J*-coupling constants between vicinal protons ($^3J_{H,H}$) using a *J*-based configuration analysis (JBCA) method[35] (Table S2 in Supporting Information). For distance constraints, 2D ROESY experiments were performed, and the cross peaks were semiquantitatively translated into three distance constraint categories (strong, ≤2.5 Å; medium, ≤3.5 Å; weak, ≤5.0 Å) according to their signal intensities (Table S2 in Supporting Information).

$^3J_{H,H}$ values and ROEs clearly identified the major conformation of the Dtrina region (Figure 2A), the Newman projections of which are shown in Figure 2B−F. The carbons of the main chain at the C33−C36, C34−C37, and C35−C38 positions were in *anti* orientations to avoid steric hindrance (Figure 2B−D). On the other hand, the main chain atoms at the C36−C39 and C37−OCO positions were in *gauche* orientations (Figure 2E and F). The isopropyl group at C39 (^{i}Pr in Figure 2F) was in *anti* orientation against the main chain (C_{37} in Figure 2F), indicating that this bulky group could be important for maintaining the conformation. Surprisingly, the methyl group at C37 (Me_{45} in Figure 2E) was also in *anti* orientation to the main chain carbon (C_{39} in Figure 2E). This suggests that the methyl group at C37 would play a crucial role in maintaining the conformation. On the other hand, the methyl group at C34 (Me_{44} in Figure 2B) was not in *anti* orientation against the main chain (C_{36} in Figure 2B). The methyl group at C34 seems not to be important for maintaining the conformation.

Molecular modeling was performed on a MacroModel (version 9.9) program[36−38] using distance geometry followed by a conformational search using 10,000-step Monte Carlo-based torsional sampling with 37 distance and 5 dihedral angle

dx.doi.org/10.1021/jo501130b | J. Org. Chem. 2014, 79, 8000−8009

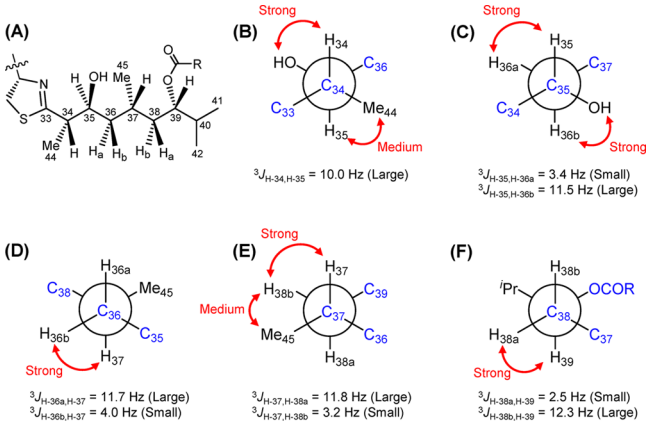


Figure 2. Major conformation of the Dtrina region in apratoxin C (**3**) confirmed by the NMR data measured in CD_3CN. (A) The structure of the Dtrina region. (B–F) Newman projections of the Dtrina region. $^3J_{H,H}$ values are shown below the Newman projections and "Large" and "Small" refer to the magnitude of the $^3J_{H,H}$ values, resulting in the identification of *anti* or *gauche* orientations. Red double-headed arrows indicate the observed ROEs ("Strong" and "Medium" represent relative intensities), which support the proposed conformations. Blue letters indicate the carbons of the main chain.

constraints derived from the NMR data. We applied an OPLS-2005 force field and a generalized Born/solvent-accessible surface area (GB/SA) solvent model.[39] The calculation was conducted in a chloroform environment.[40] The stable structures obtained are shown in Figure 3. The side chain of

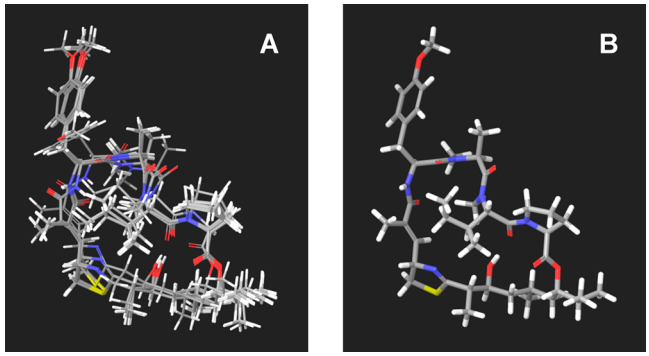


Figure 3. Molecular modeling of apratoxin C (**3**) with a restrained conformational search by the distance geometry method: (A) superposition of top 10 stable conformers consistent with NMR data ($^3J_{H,H}$ and ROEs) and (B) the lowest energy conformer generated by the conformational search.

N-methylisoleucine is close to the Dtrina moiety, and this was indicated by the ROE cross peaks between them (Table S2 in Supporting Information). Despite apratoxin C (**3**) comprising a proline and two *N*-methylated amino acids, all the amide bonds were found to be in *s-trans* forms in this model (Figure 3). Since the hydroxy proton at C35 is proximal to the carbonyl oxygen of Pro in the structural model (ca. 1.8 Å in Figure 3B), we investigated the existence of a hydrogen bond between them by hydrogen–deuterium exchange experiment of apratoxin C in CD_3CN (Figure S2 in Supporting Information). The hydroxy proton at C35 was exchanged to deuterium immediately upon addition of D_2O to the NMR solvent (Figure S2 in Supporting Information), suggesting that the hydroxy group at C35 does not form a strong hydrogen bond with the carbonyl group of Pro.

Because we previously accomplished the total synthesis of apratoxin A (**1**),[12,13] we also analyzed its tertiary structure by NMR measured in CD_3CN (Figures S3 and S4, Table S3 in Supporting Information). The 3D structure of apratoxin A (**1**) obtained was almost identical to that of apratoxin C (**3**) (Figure 4). The *tert*-butyl group is in *anti* orientation against the main

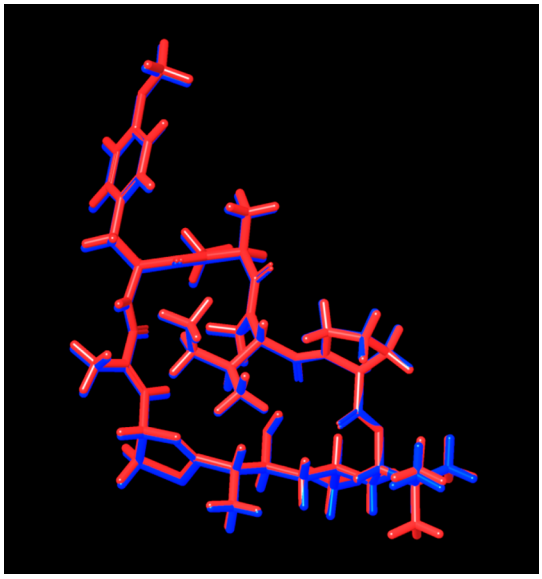


Figure 4. Superposition of the lowest energy conformers of apratoxin A (structure in red) and apratoxin C (structure in blue) obtained with a conformational search by the distance geometry method.

chain (Figure S3F in Supporting Information) like the isopropyl group in apratoxin C (**3**) (Figure 3F). This indicates that both the *tert*-butyl group in apratoxin A (**1**) and the isopropyl group in apratoxin C (**3**) play significant roles in maintaining their conformations as bulky hydrophobic groups. The nearly equivalent cytotoxicity of apratoxin A (**1**) and C (**3**) could arise from the similarity of their conformations. Luesch et al. have proposed a 3D structural model of apratoxin A (**1**) in $CDCl_3$ by NMR.[4] Our structural models of apratoxin A (**1**) and apratoxin C (**3**) in CD_3CN were very similar to that of apratoxin A (**1**) in $CDCl_3$ proposed by Luesch et al.[4] Apratoxin A (**1**) exists in similar conformations in both CD_3CN and $CDCl_3$.

■ CONCLUSION

To the best of our knowledge, we accomplished the total synthesis of apratoxin C (**3**) for the first time. Our synthetic strategy of apratoxin A (**1**)[12,13] was demonstrated to be applicable to the synthesis of apratoxin C (**3**) without any major tactical alteration. On the basis of the molecular modeling with constraints from the NMR data measured in CD_3CN, we presented the structural model of apratoxin C (**3**) in an aprotic solvent. Our structural model indicated that the methyl group at C37 and the isopropyl group at C39 play critical roles in maintaining the conformation, whereas the methyl group at C34 does not. In addition, we confirmed that apratoxin A (**1**) and C (**3**) form similar conformations in CD_3CN, which is in good agreement with their comparable cytotoxicities.

dx.doi.org/10.1021/jo501130b | J. Org. Chem. 2014, 79, 8000–8009

EXPERIMENTAL SECTION

General Techniques. All commercially available reagents were purchased from commercial suppliers and used as received. All solution-phase reactions were monitored by thin layer chromatography (TLC) carried out on silica gel plates (60F-254) with UV light, visualized by *p*-anisaldehyde H_2SO_4/ethanol solution, phosphomolybdic acid/ethanol solution, or ninhydrin/acetic acid/1-butanol solution. Flash column chromatography was performed with silica gel (40−100 μm) with the indicated solvent system. ^{1}H NMR spectra (400 and 600 MHz) and ^{13}C NMR spectra (100 and 150 MHz) were recorded using the indicated solvent. Chemical shifts (δ) for ^{1}H NMR spectra are given from TMS (0.00 ppm) in $CDCl_3$ and from residual nondeuterated solvent peaks in other solvents (CD_2Cl_2, 5.32 ppm; acetonitrile-d_3, 1.94 ppm; methanol-d_4, 3.30 ppm) as internal standards. Chemical shifts (δ) for ^{13}C NMR spectra are given from $^{13}CDCl_3$ (77.0 ppm), $^{13}CD_2Cl_2$ (54.0 ppm), acetonitrile-d_3 (118.26, 1.32 ppm), and methanol-d_4 (49.0 ppm) as internal standards. Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), dt (double triplet), dq (double quartet), ddd (double double doublet), ddt (double double triplet), dddd (double double double doublet), brs (broad singlet), brt (broad triplet), *J* (coupling constants in hertz). High-resolution mass spectra were measured on TOF-MS with EI, FAB, or ESI probe. IR spectra were reported in reciprocal centimeters (cm^{-1}). Optical rotations were measured at 589 nm. Melting points were measured on a melting point apparatus and are not corrected. Preparative RP-HPLC was carried out by using UV detection at 214 and 254 nm.

(*S*)-Hydroxy-5-methylhexan-2-one (16). To a solution of D-proline (4.50 g, 39.1 mmol) in dimethyl sulfoxide (DMSO) (520 mL) and acetone (130 mL) was added isobutylaldehyde (9.39 g, 130 mmol) at room temperature. After being stirred at the same temperature for 2 d, the reaction mixture was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (10% ethyl acetate/hexane) to afford **16** (8.82 g, 67.7 mmol, 52%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (ddd, *J* = 12.4, 6.0, 3.8 Hz, 1 H), 2.91 (d, *J* = 3.8 Hz, 1 H), 2.62 (dd, *J* = 17.4, 2.8 Hz, 1 H), 2.53 (dd, *J* = 17.4, 9.2 Hz, 1 H), 2.20 (s, 3 H), 1.68 (m, 1 H), 0.94 (d, *J* = 6.8 Hz, 3 H), 0.91 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.4, 72.2, 46.9, 32.9, 30.8, 18.3, 17.7. $[\alpha]^{26}_D$ = −66.7 (*c* 1.40, $CHCl_3$) [lit.[41] $[\alpha]^{25}_D$ −55 (*c* 1.4, $CHCl_3$)]; IR (neat) 3438, 2962, 2877, 1713, 1362, 1065 cm^{-1}.

(*S*)-(4-Methoxybenzyloxy)-5-methylhexan-2-one (15). To a solution of **16** (6.37 g, 48.9 mmol) and 4-methoxybenzyl-2,2,2-trichloroacetimidate in tetrahydrofuran (THF) (200 mL) was added trifluoromethanesulfonic acid (0.043 mL, 0.489 mmol) at 0 °C under argon. After being stirred at the same temperature for 1 h, the reaction mixture was quenched with aqueous HCl (1 M) and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (5% ethyl acetate/hexane) to afford **15** (10.8 g, 43.1 mmol, 88%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (ddd, *J* = 12.4, 6.0, 3.8 Hz, 1 H), 2.91 (d, *J* = 3.8 Hz, 1 H), 2.62 (dd, *J* = 17.4, 2.8 Hz, 1 H), 2.53 (dd, *J* = 17.4, 9.2 Hz, 1 H), 2.20 (s, 3 H), 1.68 (m, 1 H), 0.94 (d, *J* = 6.8 Hz, 3 H), 0.91 (d, *J* = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.3, 159.1, 130.8, 129.3, 113.7, 80.0, 71.8, 55.2, 45.0, 31.2, 31.0, 18.3, 17.5; $[\alpha]^{26}_D$ = −32.5 (*c* 1.10, $CHCl_3$); IR (neat) 3438, 2962, 2877, 1713, 1362, 1065 cm^{-1}; HRFABMS calcd for $C_{15}H_{23}O_3$ $[M + H]^+$ 251.1642, found 251.1605.

(*E*)-(*S*)-5-(4-Methoxybenzyloxy)-3,6-dimethylhept-2-en-1-ol ((*E*)-14) and (*Z*)-(*S*)-5-(4-Methoxybenzyloxy)-3,6-dimethylhept-2-en-1-ol ((*Z*)-14). To a solution of **15** (3.60 g, 14.4 mmol) in THF (40 mL) was added vinylmagnesium bromide (29 mL, 2.0 M in THF, 28.8 mmol) at 0 °C under argon. After being stirred at the same temperature for 20 min, the mixture was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was passed through a short pad of silica gel to afford crude **17** and used for the next reaction without further purification.

To a solution of allylic alcohol **17** and $Ph_3SiOReO_3$ (219 mg, 0.431 mmol) in diethyl ether (70 mL) was added *N*,*O*-bis(trimethylsilyl) acetamide (BSA) (4.21 mL, 17.2 mmol) at 0 °C dropwise over 30 min. After being stirred at the same temperature for 1 h, the reaction mixture was quenched with Et_3N (1.0 mL), stirred at 0 °C for 20 min, and concentrated in vacuo. The residue was diluted with MeOH (70 mL) and treated with K_2CO_3 (3.97 g, 28.7 mmol) at 0 °C for 30 min. The reaction mixture was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (5% to 10% ethyl acetate/hexane) to afford **(*E*)-14** (1.62 g, 5.82 mmol, 40%) and **(*Z*)-14** (1.61 g, 5.79 mmol, 40%) as colorless oil. **(*E*)-14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.0 Hz, 2 H), 6.85 (d, *J* = 8.0 Hz, 2 H), 5.49 (td, *J* = 6.8, 0.9 Hz, 1 H), 4.43 (m, 2 H), 4.14 (d, *J* = 6.8 Hz, 2 H), 3.79 (s, 3 H), 3.32 (dt, *J* = 7.6, 4.8 Hz, 1 H), 2.24−2.14 (m, 2 H), 1.92−1.84 (m, 1 H), 0.92 (d *J* = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0, 137.3, 131.1, 129.3, 125.7, 113.6, 81.8, 71.4, 59.3, 55.2, 40.9, 30.8, 18.3, 17.8, 16.7; $[\alpha]^{22}_D$ = −0.60 (*c* 1.04, $CHCl_3$); IR (neat) 3380, 2872, 1612, 1513, 1248, 1067, 1037, 821 cm^{-1}; HRFABMS calcd for $C_{17}H_{27}O_3$ $[M + H]^+$ 279.1960, found 279.1977. **(*Z*)-14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.0 Hz, 2 H), 6.85 (d, *J* = 8.0 Hz, 2 H), 5.71 (brt, *J* = 7.4 Hz, 1 H), 4.49 (d, *J* = 11.0 Hz, 1 H), 4.34 (d, *J* = 11.0 Hz, 1 H), 4.12 (dd, *J* = 11.3, 8.4 Hz, 1 H), 3.83 (m, 1 H), 3.79 (s, 3 H), 3.31 (ddd, *J* = 10.8, 2.8, 2.8 Hz, 1 H), 2.54 (dd, *J* = 13.2, 10.8 Hz, 1 H), 2.06−2.02 (m, 1 H), 1.86 (dd, *J* = 13.2, 2.4 Hz, 1 H), 1.70 (s, 3 H), 0.95 (d, *J* = 6.8 Hz, 6 H), 0.94 (d, *J* = 6.8 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.2, 138.3, 130.0, 129.7, 126.8, 113.7, 80.3, 71.7, 57.9, 55.2, 32.7, 30.4, 23.5, 18.5, 16.8; $[\alpha]^{24}_D$ = −2.55 (*c* 1.15, $CHCl_3$); IR (neat) 3419, 2960, 1613, 1514, 1249, 1062, 1036, 822 cm^{-1}; HRFABMS calcd for $C_{17}H_{27}O_3$ $[M + H]^+$ 279.1960, found 279.1951.

(3*R*,5*S*)-5-(4-Methoxybenzyloxy)-3,6-dimethylheptan-1-ol (18). To a solution of **(*E*)-14** (1.22 g, 4.38 mmol) in MeOH (8.7 mL) was added $Ru(OAc)_2$[(*S*)-binap] (73 mg, 0.087 mmol), and the mixture was placed in an autoclave. The autoclave was filled with hydrogen (90 atm) after repeated filling and purging of hydrogen (3 times). After the reaction was carried out under the appropriate hydrogen pressure (ca. 90 atm) at 50 °C for 12 h, the reaction mixture was diluted with MeOH and additionally stirred with florisil at room temperature for 15 min. Then the reaction mixture was filtered through a pad of silica gel, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (5% ethyl acetate/hexane) to afford **18** (1.86 g, 6.71 mmol, 91%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.0 Hz, 2 H), 6.87 (d, *J* = 8.0 Hz, 2 H), 4.49 (d, *J* = 10.8 Hz, 1 H), 4.39 (d, *J* = 10.8 Hz, 2 H), 3.80 (s, 3 H), 3.71−3.57 (m, 2 H), 3.28 (td, *J* = 8.0, 3.6 Hz, 1 H), 2.00−1.93 (m, 1 H), 1.74−1.68 (m, 1 H), 1.65−1.56 (m, 1 H), 1.45−1.25 (m, 3 H), 0.93−0.89 (m, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 131.1, 129.3, 113.6, 81.1, 70.8, 60.8, 55.1, 39.2, 37.5, 30.0, 26.4, 20.6, 17.9, 17.5; $[\alpha]^{24}_D$ = −37.7 (*c* 1.22, $CHCl_3$); IR (neat) 3383, 2957, 1613, 1514, 1302, 1248, 1038, 821 cm^{-1}; HRFABMS calcd for $C_{17}H_{29}O_3$ $[M + H]^+$ 281.2117, found 281.2109.

(3*R*,5*S*)-5-(4-Methoxybenzyloxy)-3,6-dimethylheptanal (13). To a solution of **18** (0.756 g, 2.69 mmol) in CH_2Cl_2 (27 mL) were added Et_3N (1.87 mL, 13.5 mmol), DMSO (2.86 mL, 40.4 mmol), and sulfur trioxide pyridine complex (1.07 g, 6.74 mmol) at 0 °C under argon. After being stirred at 0 °C to room temperature for 3 h, the mixture was quenched with H_2O, and the aqueous layer was extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (0% to 5% ethyl acetate/hexane) to afford **13** (0.691 g, 2.48 mmol, 91%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (t, *J* = 1.6 Hz, 1 H), 7.25 (d, *J* = 8.0 Hz, 2 H), 6.86 (d, *J* = 8.0 Hz, 2 H), 4.50 (d, *J* = 7.2 Hz, 1 H), 4.34 (d, *J* = 7.2 Hz, 1 H), 3.80 (s, 3 H), 3.22 (dt, *J* = 8.8, 4.0 Hz, 1 H), 2.34 (ddd, *J* = 15.6,

dx.doi.org/10.1021/jo501130b | J. Org. Chem. 2014, 79, 8000−8009

4.8, 1.6 Hz, 1 H), 2.23−1.97 (m, 2 H), 1.47 (ddd, J = 14.0, 8.4, 5.6 Hz, 1 H), 1.32 (ddd, J = 14.0, 8.4, 4.0 Hz, 1 H), 0.97 (d, J = 6.8 Hz, 3 H), 0.91 (d, J = 6.8 Hz, 3 H), 0.89 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.9, 159.1, 131.0, 129.5, 113.8, 80.6, 70.1, 55.3, 50.3, 36.8, 29.8, 25.0, 21.1, 18.2, 17.1; $[\alpha]^{24}_D$ = −30.2 (c 1.05, $CHCl_3$); IR (neat) 2872, 1725, 1612, 1514, 1248, 1067, 1036, 821 cm^{-1}; HRFABMS calcd for $C_{17}H_{27}O_3$ $[M + H]^+$ 279.1960, found 279.1920.

(3*R*,4*S*,6*S*,8*S*)-8-(4-Methoxy-benzyloxy)-3,6,9-trimethyldec-1-en-4-ol (11). To a suspension of aldehyde **13** (691 mg, 2.4 mmol) and molecular sieves 4 Å (691 mg) in toluene (4.8 mL) was added a solution of crotylborane (*E*)-**12** (12.7 mL, ca. 0.39 M solution in toluene, ca. 4.9 mmol) at −78 °C dropwise over 50 min. After being stirred at the same temperature for 4 h, the reaction mixture was quenched with aqueous NaOH (7.0 mL, 2 M) and stirred at 0 °C for 50 min. The aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (5% ethyl acetate/hexane) to give **11** and its diastereomer at a ratio of 9:1 (combined yield 779 mg, 97%) as colorless oil. The mixture of the diastereomers was partially purified by preparative RP-HPLC (column, YMC-Pack R&D ODS-A 20 mm × 150 mm; flow rate, 10.0 mL/min; elution method, H_2O/MeOH = 30:70−5:95 linear gradient (0.0−10.0 min), H_2O/MeOH = 5:95 isocratic (10.0−15.0 min); retention time, 13.1 min) to afford pure **11**, whose structure was determined by instrumental analyses: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 (d, J = 8.9 Hz, 2 H), 6.86 (d, J = 8.2 Hz, 2 H) 5.73 (ddd, J = 17.1, 10.9, 8.2 Hz, 1 H), 5.13−5.06 (m, 2 H), 4.48 (d, J = 10.9 Hz, 1 H), 4.42 (d, J = 10.9 Hz, 1 H), 3.80 (s, 3 H), 3.47 (br. s., 1 H), 3.29 (dt, J = 8.7, 4.2 Hz, 1 H), 2.19−2.07 (m, 1 H), 2.01−1.82 (m, 2 H), 1.53−1.40 (m, 2 H), 1.35−1.28 (m, 1 H), 1.14 (ddd, J = 14.0, 9.9 2.1 Hz, 1 H), 1.01 (d, J = 6.8 Hz, 3 H), 0.95−0.86 (m, 9 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.0, 140.6, 131.2, 129.5, 116.0, 113.7, 81.1, 72.5, 71.0, 55.3, 45.0, 41.1, 38.1, 30.2, 26.5, 20.3, 18.1, 17.5, 16.1; IR ($CHCl_3$) 2958, 2932, 2871, 2362, 1612, 1514, 1464, 1375, 1302, 1248, 1173, 1066, 1051, 1037, 911, 821, 757 cm^{-1}; $[\alpha]^{25}_D$ −31.8 (c 0.055, $CHCl_3$); HRESIMS calcd for $C_{21}H_{34}O_3Na$ $[M + Na]^+$ 357.2400, found 357.2391.

(3*R*,4*S*,6*S*,8*S*)-5-(4-Methoxybenzyloxy)-3,6,9-trimethyl-1-en-4-yl 2,2,2-trichloroethyl Carbonate (19). To a solution of **11** (0.758 g, 2.26 mmol) and pyridine (0.54 mL, 6.78 mmol) in CH_2Cl_2 (11 mL) were added 2,2,2-trichloroethoxycarbonyl chloride (0.37 mL, 2.71 mmol) and 4-dimethylaminopyridine (13 mg, 0.11 mmol) at 0 °C. After being stirred at the same temperature for 2 h, the reaction mixture was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (5% ethyl acetate/hexane) to give carbonate **19** and its diastereomer (1.15 g, 2.26 mmol, quant) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J = 8.3 Hz, 2 H), 6.87 (d, J = 8.3 Hz, 2 H), 5.79−5.66 (m, 1 H), 5.12−5.01 (m, 2 H), 4.89−4.82 (m, 1 H), 4.77 (d, J = 12.7 Hz, 1 H), 4.64 (d, J = 13.2 Hz, 1 H), 4.47 (d, J = 11.2 Hz, 1 H), 4.42 (d, J = 11.7 Hz, 1.0 H), 3.79 (s, 3 H), 3.23 (dt, J = 8.1, 3.8 Hz, 1 H), 2.49−2.37 (m, 1 H), 1.98−1.87 (m, 1 H), 1.80−1.68 (m, 2 H), 1.44 (ddd, J = 13.9, 8.3, 5.6 Hz, 1 H), 1.32−1.22 (m, 1 H), 1.10−1.20 (m, 1 H), 1.04 (d, J = 6.8 Hz, 3 H), 0.95 (d, J = 6.3 Hz, 3 H), 0.91−0.85 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 154.3, 139.0, 131.3, 129.3, 116.2, 113.8, 94.8, 80.9, 80.7, 76.6, 71.1, 55.2, 42.5, 38.3, 37.9, 30.2, 25.9, 20.2, 18.0, 17.4, 15.6; IR ($CHCl_3$) 2959, 2363, 2359, 1756, 1613, 1513, 1466, 1380, 1248, 1065, 1038, 923, 820, 777, 733 cm^{-1}; HRESIMS calcd for $C_{24}H_{35}O_5Cl_3Na$ $[M + Na]^+$ 531.1442, found 531.1433.

Prolyl Ester 20. To a solution of **19** (1.53 g, 2.26 mmol) in CH_2Cl_2 (20 mL) and H_2O (2.0 mL) was added 2,3-dichloro-5,6-dicyanobenzoquinone (1.03 g, 4.52 mmol) at 0 °C. After being stirred at the same temperature for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of *N*-Fmoc-L-proline (1.65 g, 6.78 mmol) in toluene (23 mL) was added DIEA (1.18 mL, 6.78 mmol) and 2,4,6-trichlorobenzoyl chloride (1.06 mL, 6.78 mmol) at 0 °C under argon. The solution was stirred at the same temperature for 10 min. To the resultant mixture were added a solution of the crude alcohol in toluene (23 mL) and 4-(dimethylamino)pyridine (0.96 g, 7.91 mmol) at 0 °C under argon. After being stirred at room temperature for 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (10% to 15% ethyl acetate/hexane) to give **20** (1.47 g, 2.06 mmol, 91% in 2 steps) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.84−7.69 (m, 2 H), 7.66−7.51 (m, 2 H), 7.43−7.35 (m, 2 H), 7.34−7.27 (m, 2 H), 5.80−5.58 (m, 1 H), 5.13−4.95 (m, 2 H), 4.93−4.79 (m, 2 H), 4.79−4.67 (m, 2 H), 4.50−4.14 (m, 4 H), 3.72−3.45 (m, 2 H), 2.52−2.16 (m, 2 H), 2.16−1.87 (m, 3 H), 1.86−1.07 (m, 6 H), 1.06−0.90 (m, 4.5 H), 0.90−0.79 (m, 7.5 H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 172.3, 154.7, 154.4, 154.17, 154.15, 144.25, 144.23, 144.0, 143.8, 141.35, 141.31, 141.26, 138.8, 138.7, 127.71, 127.69, 127.67, 127.13, 127.08, 127.05, 127.02, 125.4, 125.23, 125.16, 119.96, 119.95, 116.3, 94.8, 80.5, 80.3, 77.09, 77.06, 76.58, 76.56, 67.8, 67.3, 59.6, 59.4, 47.2, 47.0, 46.3, 42.35, 42.33, 38.54, 38.43, 37.98, 37.80, 31.2, 30.9, 29.9, 26.2, 26.1, 24.3, 23.3, 19.3, 19.2, 18.5, 18.4, 16.63, 16.57, 15.5, 15.4; IR ($CHCl_3$) 2963, 2362, 2357, 2342, 1754, 1707, 1451, 1417, 1379, 1348, 1249, 197, 1120, 1087, 989, 944, 923, 821, 758, 740 cm^{-1}; $[\alpha]^{25}_D$ −45.6 (c 1.86, $CHCl_3$); HRESIMS calcd for $C_{36}H_{44}NO_7Cl_3Na$ $[M + Na]^+$ 730.2076, found 730.2056.

Pro-Dtrina 9. To a solution of **20** (111 mg, 0.157 mmol) in DMF (1.5 mL) were added OsO_4 (0.031 mL, 0.05 M solution in THF, 1.57 μmol), oxone (0.386 g, 0.628 mmol), and $NaHCO_3$ (52.7 mg, 0.628 mmol) at room temperature. After being stirred at the same temperature for 23 h, the reaction mixture was diluted with H_2O (3.0 mL) and 2-methyl-2-propanol (1.57 mL). To the mixture was added $NaIO_4$ (0.067 mg, 0.314 mmol) at room temperature. The resulting mixture was stirred at the same temperature for 6 h and poured into aqueous HCl (1 M) and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with an aqueous solution of $Na_2S_2O_3$ (10 wt %) and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (40% ethyl acetate/hexane) to give **9** (87.9 mg, 0.121 mmol, 77%) as a white amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.80−7.72 (m, 2 H), 7.66−7.53 (m, 2 H), 7.44−7.35 (m, 2 H), 7.35−7.28 (m, 2 H), 5.23−5.04 (m, 1 H), 4.95−4.84 (m, 1 H), 4.83−4.69 (m, 2 H), 4.56−4.13 (m, 4 H), 3.70−3.44 (m, 2 H), 2.99−2.77 (m, 1 H), 2.39−2.16 (m, 1 H), 2.15−1.64 (m, 6 H), 1.63−1.31 (m, 3 H), 1.29−1.09 (m, 3 H) 1.04−0.93 (m, 1.5 H), 0.86 (d, J = 5.4 Hz, 7.5 H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 172.1, 155.1, 154.5, 153.78, 153.74, 144.20, 144.11, 143.89, 143.82, 141.4, 127.7, 127.1, 125.40, 125.26, 125.21, 125.15, 120.0, 94.65, 94.62, 77.7, 77.2, 76.9, 67.8, 67.6, 59.5, 59.4, 47.18, 47.12, 47.04, 47.0, 46.4, 43.2, 43.0, 38.7, 37.8, 37.7, 37.3, 37.0, 31.3, 31.2, 31.0, 30.0, 25.9, 25.7, 24.2, 23.8, 23.3, 20.2, 19.3, 19.1, 18.4, 18.2, 17.1, 16.7, 11.9, 11.7, 11.5; IR ($CHCl_3$) 2962, 1757, 1741, 1708, 1452, 1420, 1378, 1352, 1248, 1199, 1121, 1090, 942, 819, 758, 740 cm^{-1}; $[\alpha]^{26}_D$ −35.7 (c 1.49, $CHCl_3$); HRESIMS calcd for $C_{35}H_{42}NO_9Cl_3Na$ $[M + Na]^+$ 748.1817, found 748.1796.

Amide 21. To a solution of **10**[12,13] (119 mg, 0.219 mmol) in CH_2Cl_2 (0.8 mL) were added a solution of **9** (123 mg, 0.169 mmol) in CH_2Cl_2 (0.8 mL), DIEA (0.117 mL, 0.67 mmol), HOAt (27.0 mg, 0.202 mmol), and EDCI·HCl (38.7 mg, 0.202 mmol) at 0 °C under argon. After being stirred at the same temperature for 12 h, the reaction mixture was diluted with ethyl acetate and poured into aqueous HCl (1 M) at 0 °C. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (15% to 30% ethyl acetate/hexane) to give **21** (106 mg, 0.092 mmol, 55%) as white amorphous solid: ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ 7.77−7.75 (m, 2 H), 7.70−7.68 (m, 0.4H), 7.58 (m, 1.6 H), 7.41−7.19 (m, 19 H), 6.39 (dd, J = 9.0, 1.2 Hz, 0.6 H), 6.32 (dd, J = 9.0, 1.8 Hz, 0.4 H), 6.25 (brd, J = 4.2 Hz, 0.6 H), 5.98−5.83 (m, 1

dx.doi.org/10.1021/jo501130b | J. Org. Chem. 2014, 79, 8000−8009

H), 5.35−5.17 (m, 2.4 H), 5.01−4.95 (m, 1 H), 4.90−4.83 (m, 1 H), 4.82 (d, J = 12.0 Hz, 0.6 H), 4.73 (d, J = 12.0 Hz, 0.4 H), 4.63−4.61 (m, 2 H), 4.54 (d, J = 5.4 Hz, 1 H), 4.48−4.23 (m, 5 H), 3.65−3.50 (m, 2 H), 2.51−2.29 (m, 2.7 H), 2.20−1.93 (m, 4.3 H), 1.77−1.70 (m, 2 H), 1.73 (s, 1.8 H), 1.69 (s, 1.2 H), 1.68−1.63 (m, 0.6 H), 1.52−1.44 (m, 2.4 H), 1.15- 1.10 (m, 1 H), 1.09 (d, J = 7.0 Hz, 1.8 H), 0.98 (d, J = 7.0 Hz, 1.2 H), 0.92 (d, J = 7.0 Hz, 1.8 H), 0.87−0.82 (m, 6 H), 0.81 (d, J = 7.0 Hz, 1.2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.4, 172.2, 171.7, 167.2, 154.8, 154.6, 153.8, 153.7, 144.5, 144.4, 144.1, 143.9, 143.8, 139.4, 139.2, 132.2, 130.4, 130.2, 129.6, 128.05, 128.04, 127.7, 127.2, 126.9, 126.8, 125.7, 125.4, 125.14, 125.09, 119.97, 118.2, 94.8, 79.0, 78.4, 76.5, 76.4, 67.9, 67.5, 67.2, 67.0, 65.5, 65.4, 59.6, 59.3, 47.2, 47.0, 46.4, 45.1, 44.8, 38.5, 38.3, 37.5, 37.0, 36.0, 35.7, 31.73, 31.67, 31.2, 31.1, 30.0, 25.9, 25.5, 24.3, 23.4, 19.6, 19.3, 18.4, 18.3, 17.0, 13.4, 13.0, 12.9; IR ($CHCl_3$) 2964, 2960, 2362, 2359, 2342, 2332, 1758, 1710, 1679, 1451, 1447, 1419, 1248, 1198, 1182, 1121, 1087, 757, 742, 701, 668 cm^{-1}; $[\alpha]^{26}_D$ −24 (c 1.2, $CHCl_3$); HRFABMS calcd for $C_{63}H_{70}Cl_3N_2O_{10}S$ $[M + H]^+$ 1151.3817, found 1151.3807.

Thiazoline 22. To a solution of Ph_3PO (425 mg, 1.56 mmol) in CH_2Cl_2 (3 mL) was added Tf_2O (131 μL, 0.78 mmol) at 0 °C under argon, and the mixture was stirred at the same temperature for 30 min. The resulting mixture (600 μL) was added slowly to a solution of **21** (60 mg, 52.1 μmol) in CH_2Cl_2 (1.5 mL) at 0 °C under argon. After the mixture was stirred at the same temperature for 15 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0 °C, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of the crude thiazoline in THF (1.6 mL) and aqueous 1 M NH_4Cl (800 μL) was added Zn dust (68.1 mg, 1.04 mmol) at room temperature. After being stirred for 1.5 h at the same temperature, the mixture was partitioned between ethyl acetate and brine. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (30% ethyl acetate/hexane) to afford **22** (33.6 mg, 46.9 μmol, 90%) as a white amorphous solid: ^{1}H NMR (600 MHz, CD_2Cl_2, mixture of rotamers) δ 7.78 (d, J = 7.6 Hz, 2 H), 7.69−7.56 (m, 2 H), 7.43−7.37 (m, 2 H), 7.35−7.27 (m, 2 H), 6.80−6.71 (m, 1 H), 6.01−5.87 (m, 1 H), 5.31−5.15 (m, 3 H), 4.96−4.88 (m, 1 H), 4.66−4.55 (m, 2 H), 4.48−4.19 (m, 4 H), 3.76−3.33 (m, 4 H), 3.00−2.90 (m, 1 H), 2.71−2.50 (m, 1 H), 2.34−2.20 (m, 1 H), 2.15−1.82 (m, 7 H), 1.82−1.47 (m, 4 H), 1.22−1.17 (m, 3 H), 1.08−1.00 (m, 1 H), 0.96−0.77 (m, 9 H); ^{13}C NMR (150 MHz, CD_2Cl_2) δ 173.3, 173.0, 167.6, 155.4, 154.7, 145.1, 144.9, 144.7, 144.5, 141.9, 141.8, 141.7, 133.1, 133.0, 128.2, 128.2, 127.6, 127.6, 126.0, 125.9, 125.8, 125.6, 120.5, 120.4, 118.2, 118.2, 77.6, 76.6, 72.0, 71.9, 68.2, 68.1, 66.0, 65.9, 65.9, 60.2, 60.1, 47.8, 47.7, 47.5, 47.1, 46.3, 45.6, 41.6, 40.3, 39.9, 39.7, 38.1, 32.8, 32.3, 32.1, 31.7, 30.6, 30.5, 30.3, 30.2, 26.3, 25.7, 25.1, 23.9, 20.7, 20.3, 20.1, 19.0, 18.9, 17.8, 17.4, 17.4, 16.8, 16.1, 13.5, 13.5; IR (CH_2Cl_2) 2964, 2359, 2342, 2328, 1739, 1734, 1714, 1684, 1457, 1451, 1437, 1423, 1419, 1245, 1199, 1185, 1120, 740, 669 cm^{-1}; $[\alpha]^{22}_D$ −64 (c 0.32, CH_2Cl_2); HRFABMS calcd for $C_{41}H_{53}N_2O_7S$ $[M + H]^+$ 717.3573, found 717.3563.

Cyclization Precursor 23. To a solution of allyl ester **22** (31 mg, 43 μmol) and *N*-methylaniline (11.6 μL, 107 μmol) in THF (2.0 mL) was added a catalytic amount of $Pd(PPh_3)_4$ (4.9 mg, 4.3 μmol) at 0 °C under argon. After being stirred at 0 °C to room temperature for 30 min, the reaction mixture was concentrated in vacuo. The residue was passed through a pad of silica gel (20% to 100% ethyl acetate/hexane) to afford the crude carboxylic acid, and the resultant acid was used for the next reaction without further purification.

To a solution of tripeptide **8**[12,13] (57 mg, 86 μmol) in MeCN (3.4 mL) was added diethylamine (1.7 mL) at room temperature. After being stirred at the same temperature for 20 min, the reaction mixture was concentrated in vacuo. The residue was azeotroped with CH_2Cl_2 twice, and then dissolved in CH_2Cl_2 (1 mL). This solution was added to the solution of the carboxylic acid, DIEA (15 μL, 86 μmol) and HATU (16 mg, 43 μmol) in CH_2Cl_2 (1 mL) at 0 °C under argon. After being stirred at 0 °C to room temperature for 80 min, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (10% to 30% acetone/hexane) to afford **23** (45 mg, 41 μmol, 95%) as a white amorphous solid. ^{1}H NMR (600 MHz, CD_2Cl_2, mixture of rotamers) δ 7.78 (m, 2 H), 7.69−7.56 (m, 2 H), 7.40 (m, 2 H), 7.32 (m, 2 H), 7.15−7.04 (m, 2 H), 6.82−6.73 (m, 2 H), 6.63−6.43 (m, 1 H), 6.32−6.21 (m, 1 H), 5.98−5.85 (m, 1 H), 5.50−5.00 (m, 5 H), 4.96−4.85 (m, 2 H), 4.62−4.54 (m, 2 H), 4.49−4.19 (m, 3 H), 3.80−3.69 (m, 4 H), 3.67−3.30 (m, 4 H), 3.08−2.76 (m, 6 H), 2.75−2.68 (m, 3 H), 2.34−2.20 (m, 1 H), 2.14−1.44 (m, 15 H), 1.43−1.14 (m, 10 H), 1.12−0.73 (m, 12 H); ^{13}C NMR (150 MHz, CD_2Cl_2, mixture of rotamers) δ 173.0, 172.2, 171.7, 171.0, 168.3, 159.2, 155.4, 154.7, 145.1, 144.9, 144.7, 144.5, 141.8, 132.6, 131.1, 130.1, 128.9, 128.2, 127.6, 126.0, 125.8, 125.8, 120.5, 118.8, 114.3, 114.2, 77.5, 76.7, 72.0, 68.2, 68.1, 65.8, 61.1, 60.3, 60.0, 55.7, 51.2, 50.3, 47.8, 47.8, 47.2, 46.2, 41.6, 40.3, 39.9, 38.2, 33.7, 32.8, 32.1, 31.7, 31.4, 31.4, 31.0, 30.9, 30.5, 26.2, 25.7, 25.5, 25.1, 23.9, 20.2, 20.1, 18.9, 17.8, 17.4, 16.9, 16.1, 14.6, 13.8, 10.8; IR (CH_2Cl_2) 2963, 2932, 2877, 1739, 1706, 1652, 1646, 1634, 1513, 1478, 1464, 1452, 1417, 1249, 1180, 1122, 990, 759, 740 cm^{-1}; $[\alpha]^{25}_D$ −1.2 × 10^2 (c 0.37, CH_2Cl_2); HRESIMS calcd for $C_{62}H_{83}N_5O_{11}SNa$ $[M + Na]^+$ 1128.5702, found 1128.5670.

Apratoxin C (3). To a solution of allyl ester **23** (55 mg, 0.049 mmol) and *N*-methylaniline (0.013 mL, 0.122 mmol) in THF (5 mL) was added a catalytic amount of $Pd(PPh_3)_4$ (5.6 mg, 0.0049 mmol) at 0 °C under argon. After being stirred at 0 °C to room temperature for 2 h, the reaction mixture was concentrated in vacuo. The residue was passed through a pad of silica gel (20% to 50% ethyl acetate/hexane, 5% MeOH/CH_2Cl_2) to afford the crude carboxylic acid, and the resultant acid was used for next reaction without further purification.

To a solution of the carboxylic acid in MeCN (2.0 mL) was added diethylamine (1.0 mL) at 0 °C under argon. After being stirred at 0 °C to room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was azeotroped with CH_2Cl_2 twice and then dissolved in CH_2Cl_2 (49 mL). To this solution were added DIEA (0.076 mL, 0.44 mmol) and HATU (56 mg, 0.15 mmol) at 0 °C under argon. After being stirred at 0 °C to room temperature for 18 h, the reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (50% to 100% hexane/ethyl acetate) 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 (10.0−15.0 min); retention time, 10.5 min) to give apratoxin C (**3**) (8.5 mg, 0.010 mmol, 21%) as a white amorphous solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.15 (d, J = 8.6 Hz, 2 H), 6.80 (d, J = 8.6 Hz, 2 H), 6.35 (d, J = 9.6 Hz, 1 H), 6.06 (d, J = 9.6 Hz), 5.24 (ddd, J = 9.0, 8.6, 4.2 Hz, 1 H), 5.19 (d, J = 11.4 Hz, 1 H), 5.05 (ddd, J = 10.8, 8.4, 4.8 Hz, 1 H), 4.99 (ddd, J = 12.0, 6.6, 2.4 Hz, 1 H), 4.66 (d, J = 10.2 Hz, 1 H), 4.22 (m, 1 H), 4.18 (t, J = 7.8 Hz, 1 H), 3.78 (s, 3 H), 3.67 (m, 1 H), 3.55 (dddd, J = 10.2, 10.2, 10.2, 1.8 Hz, 1 H), 3.46 (dd, J = 10.2, 9.8 Hz, 1 H), 3.30 (brq, J = 7.2 Hz, 1 H), 3.13 (dd, J = 10.2, 4.2 Hz, 1 H), 3.11 (brt, J = 12.0 Hz, 1 H), 2.86 (dd, J = 12.0, 6.0 Hz, 1 H), 2.82 (s, 3 H), 2.71 (s, 3 H), 2.64 (m, 1 H), 2.22 (m, 3 H), 2.06 (m, 1 H), 1.96 (brs, 3 H), 1.89 (m, 2 H), 1.77 (ddd, J = 14.3, 12.0, 3.0 Hz 1 H), 1.71 (m, 1 H), 1.54 (ddd, J = 10.8, 10.8, 4.2 Hz, 1 H), 1.29 (m, 2 H), 1.22 (d, J = 7.2 Hz, 3 H), 1.12 (ddd, J = 13.8, 11.4, 2.4 Hz, 1 H), 1.07 (d, J = 7.2 Hz, 3 H), 0.98 (d, J = 6.6 Hz, 3 H), 0.95 (m, 1 H), 0.92 (m, 6 H), 0.89 (d, J = 7.2 Hz, 3 H), 0.86 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.4, 172.8, 170.53, 170.47, 170.0, 169.6, 158.6, 136.3, 130.6, 130.5, 128.2, 113.9, 75.2, 72.5, 71.6, 60.6, 59.7, 56.8, 55.3, 50.4, 49.1, 47.7, 40.4, 38.2, 37.6, 37.1, 36.7, 33.2, 31.7, 30.4, 29.2, 25.6, 24.6, 24.2, 19.7, 18.8, 18.0, 16.6, 14.0, 13.9, 13.3, 9.1; IR (solid) 3418, 2961, 2931, 2873, 1734, 1623, 1507, 1457, 1248, 1181 cm^{-1}; $[\alpha]^{28}_D$ −1.8 × 10^2 (c 0.43, MeOH) [lit.[5] $[\alpha]^{25}_D$ = −171 (c 0.22, MeOH)]; HRFABMS calcd for $C_{44}H_{68}N_5O_8S$ $[M + H]^+$ 826.4789, found 826.4810.

^{1}H NMR (600 MHz, CD_3CN) δ 7.17 (d, J = 8.3 Hz, 2 H), 6.84 (d, J = 8.3 Hz, 2 H), 6.60 (brs, 1 H), 6.14 (m, 1 H), 5.26, (ddd, J = 10.3, 8.7, 2.8 Hz, 1 H), 5.09 (d, J = 11.7 Hz, 1 H), 4.95 (ddd, J = 12.3, 6.1, 2.5 Hz, 1 H), 4.90 (m, 1 H), 4.45 (d, J = 11.2 Hz), 4.09 (t, J = 7.8 Hz,

dx.doi.org/10.1021/jo501130b | J. Org. Chem. 2014, 79, 8000−8009

1 H), 4.05 (m, 1 H), 3.75 (s, 3 H), 3.59 (m, 1 H), 3.44 (dddd, J = 11.5, 11.2, 10.0, 3.4 Hz, 1 H), 3.41, (dd, J = 11.1, 8.7 Hz, 1 H), 3.40 (brs, 1 H), 3.10 (m, 1 H), 3.03 (ddd, J = 11.1, 2.8, 1.1 Hz, 1 H), 2.89 (m, 1 H), 2.80 (s, 3 H), 2.57 (m, 3 H), 2.55 (dq, J = 10.0, 7.0 Hz, 1 H), 2.25 (m, 1 H), 2.13 (m, 1 H), 2.11 (ddqdd, J = 11.8, 11.7, 6.7, 4.0, 3.2 Hz, 1 H), 2.03 (m, 1 H), 1.92 (s, 3 H), 1.84 (m, 1 H), 1.78 (m, 1 H), 1.74 (ddd, J = 14.3, 12.3, 3.2 Hz, 1 H), 1.67 (m, 1 H), 1.47 (ddd, J = 13.3, 11.5, 4.0 Hz, 1 H), 1.32 (ddd, J = 14.3, 11,8, 2.5 Hz, 1 H), 1.21 (m, 1 H), 1.17 (ddd, J = 13.3, 11.7, 3.4 Hz, 1 H), 1.06 (d, J = 6.8 Hz, 3 H), 1.02 (d, J = 6.9 Hz, 3 H), 0.93 (d, J = 6.7 Hz, 3 H), 0.88 (d, J = 6.8 Hz, 3 H), 0.84 (d, J = 6.8 Hz, 3 H), 0.83 (m, 1 H), 0.83 (m, 3 H), 0.82 (m, 3 H); ^{13}C NMR (150 MHz, CD_3CN) δ 176.8, 173.5, 171.4, 170.7, 170.0, 159.5, 136.7, 131.4, 130.3, 129.9, 114.6, 75.7, 73.0, 72.2, 60.6, 57.4, 55.8, 50.0, 48.4, 40.8, 39.1, 38.3, 37.0, 33.9, 32.3, 30.4, 30.0, 26.1, 25.2, 25.0, 19.7, 19.0, 18.2, 17.0, 14.5, 14.3, 13.3, 9.2. (^{1}H and ^{13}C NMR chemical shift assignments of apratoxin C (**3**) in CD_3CN are summarized in Table S4 in Supporting Information).

Cytotoxicity Assay. Human colon adenocarcinoma HCT116 cells were kindly provided by Prof. Yoshiteru Ohshima at the Graduate School of Pharmaceutical Sciences in Tohoku University. They were cultured in an RPMI 1640 medium (Gibco, Thermo Fisher Scientific, Life Technologies) supplemented with 10% fetal bovine serum (Equitech-Bio, Inc.) 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 in fresh culture medium in a 96-well clear bottom plate and incubated at 37 °C under 5% CO_2 for 24 h before the experiments.

Apratoxin A (**1**) or C (**3**) was dissolved in DMSO at concentrations ranging from 0.01 to 10 μM. One microliter of the resultant solution was added to the above-mentioned 100 μL cell culture, resulting in various concentrations of the compound (0.1−100 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.)[33,34] 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 595 nm using a microplate reader. The absorbance obtained upon the addition of the vehicle was considered as 100%.

Molecular Modeling Based on NMR Data. NMR measurements for tertiary structural analysis were conducted using a NMR spectrometer (600 MHz for ^{1}H) at 298 K using samples of apratoxin C (**3**) (3.8 mg in 0.25 mL of CD_3CN) and apratoxin A (**1**) (3.1 mg in 0.25 mL CD_3CN) in 5 mm Shigemi NMR microtubes. $^3J_{H,H}$ values were determined by 1D ^{1}H spectra and ^{1}H−^{1}H J-resolved 2D NMR spectra. According to a J-based configuration analysis (JBCA) method,[35] $^3J_{H,H}$ coupling constants for clearly antioriented vicinal protons ($^3J_{H,H} \geq 10$ Hz) were interpreted as dihedral angle constraints (Tables S2 and S3 in Supporting Information). To obtain information regarding ^{1}H−^{1}H internuclear distances, transverse ROESY experiments were performed at a mixing time of 250 ms. ROESY cross peak intensities were roughly determined by their peak area in 1D slices of the 2D spectra. ROE cross peak intensities were classified as "Strong" (upper distance constraint ≤2.5 Å), "Medium" (≤3.5 Å), and "Weak" (≤5.0 Å) (Tables S2 and S3 in Supporting Information). The cross peaks between the geminal H36a-H36b and H38a-H38b were used as internal standards for calibration. To address the possibility of conformational averaging, intensities were classified conservatively and only upper distance limits were included in the calculations to allow the largest possible number of conformers to fit the experimental data.

Molecular modeling was performed on the MacroModel (version 9.9) program[36−38] by the distance geometry method. We utilized an OPLS-2005 force field and a generalized Born/solvent-accessible surface area (GB/SA) solvent model.[39] The calculations were conducted in a chloroform environment. To find 3D structures that were in agreement with the experimental data (J-coupling and ROEs, summarized in Tables S2 and S3 in Supporting Information) 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 ^{1}H−^{1}H distance constraints (force constant, 10 kJ mol^{-1} Å^{-2}) and *anti*-oriented dihedral angle constraints (^{1}H−C−C−^{1}H angle, 180 ± 30°) at 10,000 iterations with 500 times of energy minimization. Then, energy minimization was conducted on each found structure without constraints.

H/D exchange experiments were performed on 3.2 mg of apratoxin C (**3**) in 0.5 mL of CD_3CN with the addition of 10 μL of D_2O.

■ ASSOCIATED CONTENT

Supporting Information

Supporting figures and tables, copies of ^{1}H and ^{13}C NMR spectra for synthetic compounds, and 2D NMR spectra of apratoxin C (**3**) and apratoxin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by Grant-in-Aid for Scientific Research (B) (nos. 23310145 and 26282208 for T.D.) from MEXT. The authors are grateful to Prof. Yoichi Nakao at School of Advanced Science and Engineering at Waseda University for kindly providing the ^{1}H NMR spectrum of natural apratoxin C in CD_3CN. The authors are grateful to Prof. Yoshiteru Ohshima and Dr. Teigo Asai at the Graduate School of Pharmaceutical Sciences in Tohoku University for allowing us to use their facility for the cell-based assay.

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