Total synthesis of the marine cyanobacterial cyclodepsipeptide apratoxin A

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A total synthesis of apratoxin A was developed. Apratoxin A, isolated from *Lyngbya* spp. cyanobacteria, is representative of a growing class of marine cyanobacterial cyclodepsipeptides wherein discrete polypeptide and polyketide domains are merged by ester and amide or amide-derived linkages. In the apratoxins, the N terminus of the peptide domain [(Pro)-(*N*-Me-Ile)-(*N*-Me-ala)-(*O*-Me-Tyr)-(moCys)] is a modified vinylogous cysteine that is joined to a novel ketide [3,7-dihydroxy-2,5,8,8-tetramethylnonanoic acid (Dtna)] by an acid-sensitive thiazoline. The C-terminal proline is esterified to a hindered hydroxyl vicinal to the ketide's *tert*-butyl terminus. Major synthetic challenges included assembly and maintenance the thiazoline-containing moiety and macrolide formation involving acylation of the C39 hydroxyl. The Dtna domain was assembled in the biogenetic direction beginning with a Brown allylation of trimethylacetaldehyde to establish the C39 alcohol configuration. Diastereofacial selective addition of a higher-order dimethylcuprate upon a ring-closing metathesis-derived α,β-unsaturated valerolactone installed the C37 methyl-bearing center. A Paterson anti-aldol process was used to incorporate the remaining two ketide stereogenic centers at C34 and C35. Although attempts to incorporate the thiazoline moiety by condensations of thiol esters bearing α-amino carbamate derivatives failed, an intramolecular Staudinger reduction–aza-Wittig process using α-azido thiol esters was uniquely successful. Late-stage macrocycle closure proceeded well by lactam formation between Pro and *N*-Me-Ile residues, but attempted lactonizations of the Pro carboxylate with the C39 hydroxyl failed. Optimization of C35 hydroxyl group protection-deprotection completed the effort, which culminated in the first total synthesis of apratoxin A and will enable analog generation toward improving differential cytotoxicity.

Marine cyanobacteria are known for a wide biosynthetic potential that is manifested in the generation of an array of architecturally novel and biologically potent secondary metabolites (1–3). The recent screening of *Lyngbya* spp. cyanobacteria collected in Guam and Palau by Moore, Paul, and coworkers (4, 5) for antitumor compounds led to the isolation of apratoxins A–C (**1**–**3**, respectively, Fig. 1). The apratoxins are the most cytotoxic entry of several cyclodepsipeptides containing both polypeptide and polyketide fragments that have been isolated from *Lyngbya* spp. cyanobacteria (6–9). In the apratoxins, these domains include highly methylated amino acids joined by proline ester and thiazoline moieties to the novel ketide 3,7-dihydroxy-2,5,8,8-tetramethylnonanoic acid. Similar ketide motifs (e.g., 7-dihydroxy-2,5,8,8-tetramethylnonanoic acid) have been encountered in isolates of the cyanobacterium *Lyngbya bouillonii* (10, 11).

The α,β-unsaturated thiazoline moiety of **1** is likely to have been derived biogenetically from a vinylogous cysteine residue, wherein both primary thiol and secondary amine have condensed with the carboxylate of a 3,7-dihydroxy-2,5,8,8-tetramethylnonanoic acid derivative (12). Although thiazolines are generally well represented in natural products, the novel substitution of the apratoxin thiazoline moiety lends particular chemical instability to these secondary metabolites. The stereogenic centers resident at the thiazoline's 2-position substituent's α-center and at the 4-position may be prone to epimerization, whereas the 2-β-hydroxyl group is sensitive toward acid-induced dehydration leading to (*E*)-34,35-dehydroapratoxin A (**4**) (4, 5). Furthermore, the C-terminal proline residue is

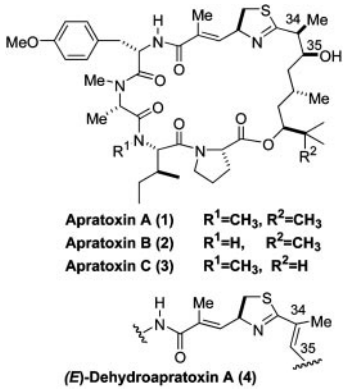

Fig. 1. Structures of the apratoxins.

intramolecularly esterified to the sterically hindered C39 hydroxyl bearing an adjacent *tert*-butyl (**1** and **2**) or *iso*-propyl (**3**) group. Therefore, a successful total synthesis must use both strategy and tactics that allow the incorporation and maintenance of these key structural motifs.

The high levels of cytotoxicity associated with the apratoxins make them interesting lead compounds, but structural modifications will be required to fully develop their biomedical potential. High levels of cytotoxicity against KB (IC_{50} = 0.52 nM) and LoVo cancer cells (IC_{50} = 0.36 nM) have been recorded for **1** (4, 5). However, **1** also demonstrated unfavorably high levels of toxicity toward mice. The mode of action of **1** is unknown, but **1** apparently affects neither microtubule polymerization dynamics nor topoisomerase I. Apratoxin C displayed an *in vitro* cytotoxicity profile similar to that of **1**, but, interestingly, **2** and **4** were one or two orders of magnitude less potent. The *N*-methylation of the *iso*-leucyl residue in **1** and **3**, but absent from **2**, is believed to induce a substantial conformational difference between the macrolides of the primary (**2**) and secondary amides (**1** and **3**) (4, 5). The substantial loss of cytotoxic activity resulting from the dehydration of **1** to give **4** may also reflect the disruption of a biologically active conformation. The small amounts of apratoxins available by isolation from natural sources and the limited number of structural variants have prevented more in-depth biological studies.

As modular products of merged nonribosomal peptide (13) and polyketide (14) biosynthetic pathways (15), the apratoxins may be prime candidates for engineered combinatorial biosynthesis (16–18) to generate analogs with improved chemical stability and differential cytotoxicity profiles. Chemical synthesis may complement this potential by offering an expedient route to structurally

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Abbreviations: THF, tetrahydrofuran; rt, room temperature; TES, triethylsilyl; TFA, trifluoroacetic acid; HRMS, high-resolution MS; ESI, electrospray ionization; PyAOP, (7-azabenzotriazole-1-yloxy) tripyrrolidino-phosphonium hexafluorophosphate; TBAF, tetra-*n*-butylammonium fluoride; TBS, *tert*-butyldimethylsilyl; OTf, trifluoromethanesulfonate; TIPS, triisopropylsilyl; Boc, *tert*-butyloxycarbonyl.

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diversify the apratoxin chemotype. Accordingly, a few studies related to the chemical synthesis of the apratoxins have been published (19, 20), including the synthesis an oxazoline-containing analog (21). A complete total synthesis can also address the immediate apratoxin supply limitation and corroborate the assigned structures of the natural products. To meet these objectives, a modular total synthesis of apratoxin A has been developed (22). The evolution of this versatile and effective total synthesis are detailed herein.

Materials and Methods

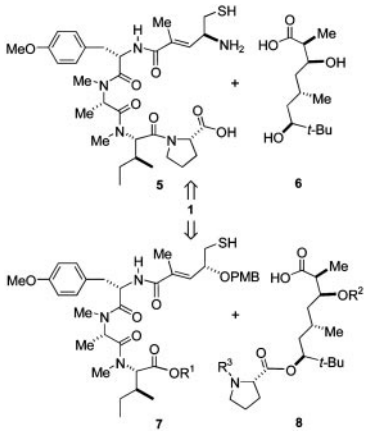
General. General experimental procedures, experimental details, and compound characterization data for additional key synthetic compounds and comparison ^{1}H NMR spectra of synthetic and naturally occurring **1** are provided in the supporting information, which is published on the PNAS web site.

Pyrrolidine-1,2-dicarboxylic Acid 1-*tert*-Butyl Ester 2-[1-*tert*-Butyl-5-triethylsilanyl-oxy-6-[2-(4-{2-[1-({1-[(1-methoxycarbonyl-3-methylbutyl)-methylcarbamoyl]-ethyl}-methylcarbamoyl)-2-(4-methoxyphenyl)-ethylcarbamoyl]-propenyl}-4,5-dihydrothiazol-2-yl)-3-methyl-heptyl] Ester (74). To a stirred rt solution of the azide **72** (13.2 mg, 11.7 μmol) in THF (1 ml) was added triphenylphosphine (6.1 mg, 23 μmol). The mixture was heated to and maintained at 50°C for 12 h. The solvent was removed under reduced pressure, and the residue was purified by preparative TLC to yield **74** (8.0 mg, 63%) as a colorless oil: R_f 0.29 (hexanes-ethyl acetate, 1:2, vol/vol); $[\alpha]_D^{25}$ −94.7 (*c* 0.71, $CHCl_3$); IR (neat, cm^{-1}) 3423, 2962, 1738, 1689, 1640, 1513, 1404, 1252, 759; ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) δ 7.09 (d, *J* = 7.5 Hz, 2H), 6.78 (d, *J* = 7.5 Hz, 2H), 6.44 (d, *J* = 7.5 Hz, 1H), 6.29 (dq, *J* = 1.5, 7 Hz, 1H), 5.42 (q, *J* = 7 Hz, 1H), 5.22 (ddd, *J* = 6.5, 6.5, 7.5 Hz, 1H), 5.11 (ddd, *J* = 8.5, 8.5, 8.5 Hz, 1H), 4.92 (d, *J* = 10 Hz, 1H), 4.78 (m, 1H), 4.37 (dd, *J* = 3, 7 Hz, 0.5H), 4.32 (dd, *J* = 3, 8.5 Hz, 1H), 4.07 (m, 1H), 3.77 (s, 3H), 3.70 (s, 3H), 3.51 (m, 2H), 3.38 (dd, *J* = 8.5, 11 Hz, 1H), 3.06 (dd, *J* = 8, 13.5 Hz, 1H), 2.98 (s, 1H), 2.97 (m, 1H), 2.87 (m, 2H), 2.75 (s, 3H), 2.17 (m, 1H), 2.05 (m, 2H), 1.93 (d, *J* = 1.5 Hz, 3H), 1.93 (m, 2H) 1.54 (m, 1H), 1.45 (s, 4.5H), 1.43 (s, 4.5H), 1.37 (m, 1H), 1.29 (d, *J* = 7 Hz, 3H), 1.28 (m, 1H), 1.15 (m, 3H), 0.99 (t, *J* = 7 Hz, 9H), 0.97 (m, 1H), 0.93 (d, *J* = 7 Hz, 3H), 0.92 (m, 1H), 0.91 (t, *J* = 6.5 Hz, 3H), 0.88 (s, 4.5H), 0.87 (s, 4.5H), 0.63 (q, *J* = 7 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, mixture of rotamers) δ 174.1, 173.9, 172.2, 172.0, 171.6, 171.3, 171.2, 170.8, 170.6, 167.9, 167.8, 158.5, 153.8, 153.7, 134.5, 132.6, 130.3, 127.7, 113.7, 79.8, 79.7, 79.6, 79.3, 73.6, 71.5, 71.3, 64.2, 60.1, 59.5, 59.2, 55.0, 51.7, 50.3, 49.5, 46.3, 46.0, 45.7, 38.7, 37.9, 37.6, 34.9, 33.1, 30.7, 30.4, 29.8, 29.6, 28.4, 28.3, 26.9, 25.8, 25.7, 24.8, 24.2, 23.2, 19.9, 19.8, 15.6, 14.8, 14.2, 13.3, 12.1, 11.9, 10.4, 6.9, 5.0; HRMS (ESI) calcd for $[C_{57}H_{96}N_5O_{11}SSi+Na]^+$ 1109.6489, found 1109.6488.

Pyrrolidine-2-carboxylic Acid 2-[1-*tert*-Butyl-5-triethylsilanyloxy-6-[2-(4-{2-[1-({1-[(1-methoxycarbonyl-3-methylbutyl)-methylcarbamoyl]-ethyl}-methylcarbamoyl)-2-(4-methoxyphenyl)-ethylcarbamoyl]-propenyl}-4,5-dihydrothiazol-2-yl)-3-methyl-heptyl] Ester (76). To a magnetically stirred rt solution of **74** (8.0 mg, 7.4 μmol) in CH_2Cl_2 (1 ml) were sequentially added 2,6-lutidine (1 μl, 0.07 mmol) and *t*-butyldimethylsilyltrifluoromethane sulfonate (3.4 μl, 15 μmol). After 30 min, ethyl acetate (3 ml) and saturated aqueous NH_4Cl (1 ml) were added. The separated aqueous phase was extracted with ethyl acetate (3 × 3 ml). The organic layers were combined, dried over Na_2SO_4, and filtered. The solvent was removed under reduced pressure, the residue was dissolved in THF (1 ml), and the solution was cooled to 0°C. A THF solution of tetra-*n*-butylammonium fluoride (11 μl of a 1.0 M solution, 11 μmol) was added, and after 10 min the reaction mixture was partitioned with H_2O (1 ml) and $CHCl_3$ (3 ml). The aqueous phase was extracted with $CHCl_3$ (3 × 3 ml). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate-methanol, 10:1, vol/vol) to give **76** (6.3 mg, 86% from **74**) as a foam: R_f 0.23 (ethyl acetate-methanol, 9:1, vol/vol); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.09 (d, *J* = 8.5 Hz, 2H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.54 (d, *J* = 8.5 Hz, 1H), 6.29 (dq, *J* = 1.5, 8 Hz, 1H), 5.41 (q, *J* = 6.5 Hz, 1H), 5.24 (ddd, *J* = 6.5, 6.5, 7.5 Hz, 1H), 5.15 (m, 2H), 4.91 (d, *J* = 10 Hz, 1H), 4.85 (dd, *J* = 2, 10 Hz, 1H), 4.05 (m, 1H), 3.77 (s, 3H), 3.70 (s, 3H), 3.41 (m, 1H), 3.23 (m, 2H), 3.09 (m, 1H), 2.98 (s, 1H), 2.96 (m, 2H), 2.87 (m, 2H), 2.73 (s, 3H), 2.40 (m, 1H), 2.17 (m, 1H), 1.93 (d, *J* = 1.5 Hz, 3H), 1.93 (m, 1H), 1.83 (m, 1H), 1.75 (m, 1H), 1.64 (m, 1H), 1.54 (m, 1H), 1.45 (m, 2H), 1.26 (d, *J* = 7 Hz, 3H), 1.24 (m, 1H), 1.22 (d, *J* = 7 Hz, 3H), 0.99 (t, *J* = 7 Hz, 9H), 0.93 (d, *J* = 7 Hz, 3H), 0.92 (m, 1H), 0.91 (t, *J* = 6.5 Hz, 3H), 0.87 (s, 9H), 0.63 (q, *J* = 7 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 171.6, 171.7, 171.5, 171.4, 168.1, 158.6, 134.6, 132.8, 130.4, 127.9, 113.9, 79.4, 73.9, 71.3, 60.3, 60.0, 58.8, 55.2, 51.8, 50.5, 49.7, 46.6, 45.9, 38.4, 38.0, 37.9, 37.8, 35.1, 33.2, 30.9, 30.6, 30.1 26.5, 25.9, 25.0, 24.9, 23.9, 19.7, 15.7, 14.4, 13.6, 13.5, 11.9, 10.6, 7.0, 5.1; HRMS (ESI) calcd for $[C_{52}H_{88}N_5O_9SSi+Na]^+$ 986.6067, found 986.6112.

35-*O*-(Triethylsilyl)-apratoxin A (80). To a stirred rt solution of **76** (4.0 mg, 4.1 μmol) in *t*-butanol, THF, and H_2O (0.1, 0.05, and 0.05 ml, respectively) was added LiOH (1.7 mg, 41 μmol). After 5 h, the reaction mixture was evaporated under a stream of N_2. The residue was dissolved in $CHCl_3$ (2 ml) and neutralized with aqueous phosphate buffer (pH = 5.5, 1 ml). The separated aqueous phase was extracted with $CHCl_3$ (10 × 2 ml). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue (**78**) was azeotropically dried with toluene twice, then dissolved in CH_2Cl_2 (1.5 ml). *N,N*-Di-*iso*-propylethylamine (2 μl) and (7-azabenzotriazole-1-yloxy) tripyrrolidino-phosphonium hexafluorophosphate (PyAOP, 4 mg) were added sequentially. The reaction mixture was allowed to stir at rt for 2 h before it was concentrated under a stream of N_2. The residue was purified by preparative TLC to yield **80** (2.8 mg, 73% from **76**) as a foam: R_f 0.53 (ethyl acetate-hexanes, 2:1, vol/vol); $[\alpha]_D^{25}$ −56.9 (*c* 1.13, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (d, *J* = 7 Hz, 2H), 6.81 (d, *J* = 7 Hz, 2H), 6.65 (dq, *J* = 1.5, 9 Hz, 1H), 6.32 (d, *J* = 9.5 Hz, 1H), 5.42 (ddd, *J* = 5.5, 10, 10 Hz, 1H), 5.10 (ddd, *J* = 9.5, 9.5, 9.5 Hz, 1H), 4.90 (d, *J* = 11.5 Hz, 1H), 4.83 (q, *J* = 6.5 Hz, 1H), 4.78 (dd, *J* = 4, 10.5 Hz, 1H), 4.12 (m, 2H), 3.99 (m, 1H), 3.78 (s, 3H), 3.57 (m, 2H), 3.33 (dd, *J* = 8.5, 11 Hz, 1H), 3.17 (dd, *J* = 8, 13 Hz, 1H), 2.88 (m, 3H), 2.86 (s, 3H), 2.62 (s, 3H), 2.22 (m, 1H), 2.04 (m, 2H), 1.92 (d, *J* = 1.5 Hz, 3H), 1.92 (m, 2H), 1.68 (m, 1H), 1.54 (m, 1H), 1.19–1.43 (m, 3H), 1.23 (d, *J* = 7 Hz, 3H), 1.02 (d, *J* = 7 Hz, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.97 (m, 1H), 0.96 (t, *J* = 7 Hz, 9H), 0.92 (m, 1H), 0.86 (s, 9H), 0.83 (t, *J* = 7 Hz, 3H), 0.65 (d, *J* = 6.5 Hz, 3H), 0.61 (q, *J* = 7 Hz, 6H); HRMS (ESI) calcd for $[C_{51}H_{83}N_5O_8SSi+Na]^+$ 976.5624, found 976.5630.

Apratoxin A (1). To a stirred rt solution of **80** (2.8 mg, 2.9 μmol) in THF was added HF-pyridine complex (*ca.* 0.2 ml). The reaction mixture was allowed to stir at rt for 30 min before it was diluted with ethyl acetate (2 ml) and neutralized with saturated aqueous $NaHCO_3$ (0.5 ml). The separated aqueous phase was extracted with ethyl acetate (5 × 2 ml) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by preparative TLC with ethyl acetate to give **1** as an amorphous white solid (1.6 mg, 65%). Synthetic **1** matched the natural product by ^{1}H NMR spectroscopy, TLC, specific rotation, and MS: R_f 0.33 (ethyl acetate). $[\alpha]_D^{25}$ −121 (*c* 1.13, methanol) [lit. (4). $[\alpha]_D$ −161 (*c* 1.33, methanol)]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 6.35 (dq, *J* = 1.3, 9.7 Hz, 1H), 5.97 (d, *J* = 9 Hz, 1H), 5.25 (ddd, *J* = 4.5, 9, 9.8 Hz, 1H), 5.20 (d, *J* = 11.5 Hz, 1H), 5.05 (ddd, *J* = 4.5, 9.5 Hz, 11, 1H), 4.97 (dd, *J* = 2.5, 13 Hz, 1H), 4.69 (d, *J* = 11 Hz, 1H), 4.23 (m, 1H), 4.19 (t, *J* = 8 Hz, 1H), 3.78 (s, 3H), 3.66 (m, 1H), 3.54 (dddd, *J* = 3, 10, 11, 11.5 Hz, 1H), 3.46 (dd, *J* = 8.5, 11 Hz, 1H), 3.28 (m, 1H),



Scheme 1. Major retrosynthetic dissections of apratoxin A.

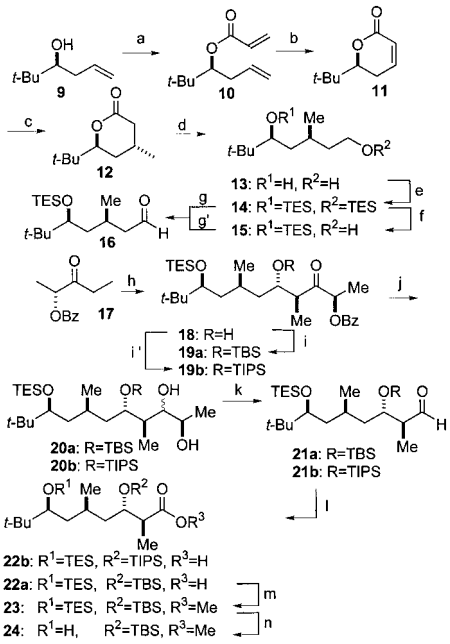
3.14 (dd, $J = 4$, 11 Hz, 1H), 3.11 (dd, $J = 11$, 12.5 Hz, 1H), 2.86 (dd, $J = 5$, 12.5 Hz, 1H), 2.81 (s, 3H), 2.72 (s, 3H), 2.64 (dq, $J = 7$, 10 Hz, 1H), 2.24 (m, 1H), 2.16 (m, 1H), 2.05 (m, 1H), 1.96 (d, $J = 1.5$ Hz, 1H), 1.90 (m, 1H), 1.88 (m, 1H), 1.79 (m, 1H), 1.58 (m, 1H), 1.31 (m, 1H), 1.26 (m, 1H), 1.21 (d, $J = 6.6$ Hz, 3H), 1.11 (m, 1H), 1.07 (d, $J = 7$ Hz, 3H), 0.99 (d, $J = 6.6$ Hz, 3H), 0.96 (m, 1H), 0.95 (d, $J = 7$ Hz, 3H), 0.91 (t, $J = 7$ Hz, 3H), 0.87 (s, 9H). HRMS (ESI) calcd for $[C_{45}H_{69}N_5O_8S+Na]^+$ 862.4759, found 862.4732.

Results and Discussion

Synthetic Plan. A logical disconnection of **1** at the proline ester and thiazoline moieties fully disengages polypeptide and ketide domains to provide tetraamide **5** and carboxylic acid **6** (Scheme 1). This dissection requires potentially challenging late-stage condensations of the vicinal thiol-amine and proline carboxylate of **5** with the carboxylic acid and hindered C39 hydroxyl of **6**, respectively. Whereas **5** can be accessed from component amino acids by established polypeptide synthesis methods, polyketide acid **6** represents a unique synthetic target. Alternatively, the sterically demanding C39 alcohol may be acylated with a single proline unit at an early stage to allow an anticipated higher yielding amide formation between *iso*-leucine carboxylate and proline amine moieties at a late stage to merge ketide and peptide domains of apratoxin A (Scheme 1). Furthermore, the sensitivity of the β-oxythiazoline moiety of **1** may be addressed by step-wise assembly under uniquely mild conditions by a surrogate (**7**) of the vicinal thiol-amine of **5**. Hence, a different dissection that likely violates a modular biosynthetic assembly but which may facilitate laboratory synthesis relies on truncated triamide **7** and proline-ketide ester **8**.

Common to both major retrosynthetic disconnections is the *de novo* synthesis of a functionalized equivalent of the novel polyketide **6**. An anti-aldol reaction between a propionate unit and an aldehyde representing (3*R*,5*S*)-5-hydroxy-3,6,6-trimethylheptanal would establish the assigned stereochemical array of **6**. The 1,5-dioxygenation pattern of the heptanal moiety suggested an α,β-unsaturated valerolactone precursor wherein a single priming C39 stereogenic center could dictate the establishment of the C37 methyl-bearing stereogenic center by 1,3-chirality transfer in a facial selective conjugate addition. The total synthesis of **1** thus began with the establishment of the C39 stereogenic center in the polyketide domain.

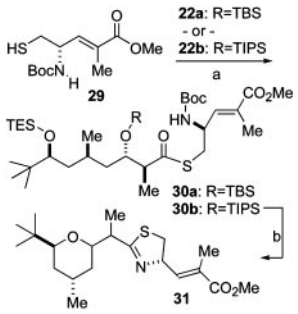
Polyketide Domain Synthesis. The synthesis of the polyketide fragment representing **6** began with Brown allylation of trimethylacetaldehyde mediated with (−)-DIPCl to yield allyl *tert*-butyl carbinol **9** (Scheme 2) (23). Esterfication of **9** with acrylic acid provided diene ester **10** (24) which was cyclized by ring-closing metathesis to lactone **11** by using Grubbs' first-generation catalyst (25). Diastereoselective conjugate addition with a higher-order methyl cuprate then successfully introduced the C37 methyl substituent in **12** (26), as confirmed by nuclear Overhauser effect analysis. Reductive opening of lactone **12** with lithium aluminum hydride provided diol **13**, which was bis-silylated with TESCl to yield **14**. Selective silyl ether cleavage and oxidation of the resultant alcohol **15** delivered aldehyde **16** (27). Alternatively, **16** could be obtained directly from **14** under Swern oxidation conditions (28).



Scheme 2. Synthesis of the 3,7-dihydroxy-2,5,8,8-tetramethylnonanoic acid moiety. (a) acrylic acid, *N*-methyl-2-chloropyridinium iodide, Et_3N, CH_2Cl_2, reflux, 71%; (b) $Ti(O^iPr)_4$ (0.3 eq), Grubbs' catalyst (0.1 eq), CH_2Cl_2 97%; (c) $Me_2CuCNLi_2$, Et_2O, −78°C, 86%; (d) $LiAlH_4$, Et_2O, 0°C, 83%; (e) TESCl, imidazole, *N*,*N*-dimethyl-4-aminopyridine, CH_2Cl_2, 98%;(f) pyridinium *para*-toluene sulfonate, CH_2Cl_2, MeOH, 0°C, 84%; (g) $(COCl)_2$, DMSO, *i*-Pr_2NEt, CH_2Cl_2, 84%; (g′) 2,2,6,6-tetramethylpiperidine-*N*-oxyl, bisacetoxyiodobenzene, CH_2Cl_2, 82%; (h) Me_2NEt, (*c*-hex)$_2BCl$, Et_2O, **16**, −78 to −20°C; (i) TBSOTf, CH_2Cl_2, 2,6-lutidine, −50°C (94% for two steps); (i′) TIPSOTf, CH_2Cl_2, 2,6-lutidine, 0°C (94% for two steps); (j) (*i*) $NaBH_4$, MeOH; (*ii*) K_2CO_3, MeOH, 0°C, 90%; (k) $NaIO_4$, MeOH, H_2O, 73%; (l) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, 86%; (m) CH_2N_2, Et_2O, 100%; n) HF-pyridine, THF, 0°C, 82%.

(*R*)-α-Benzoyloxy-diethylketone **17**, representing the biogenetic C34-C35 propionate unit, was prepared from (*R*)-*iso*-butyl lactate in three steps (Scheme 2). Aldehyde **16** and ketone **17** were then joined by a Paterson *anti*-aldol reaction (29) to give **18**. Alcohol **18** was initially protected as its *tert*-butyldimethylsilyl (TBS) ether **19a**. Thereafter, the more stable triisopropylsilyl (TIPS) ether (**19b**) was also explored. In parallel experiments, reduction of ketones **19a**,**b** followed by hydrolysis of the benzoate moieties efficiently yielded diols **20a**,**b**. Oxidative cleavage and subsequent Lindgren oxidation furnished carboxylic acids **22a**,**b**. Esterification of TBS ether **22a** followed by selective removal of triethylsilyl (TES) group from **23** with HF-pyridine delivered the C39 carbinol **24**. The installed orthogonal carboxylate and diol protection of **23** facilitated the exploration of complementary major fragment coupling strategies.

Thiazoline Assembly. A key structural feature and synthetic challenge of the apratoxins is the β-hydroxy-2,4-disubstituted thiazoline moiety. This fragment could be prone to several side reactions, including acid hydrolysis, epimerization, and elimination of the 2-position β-substituent to give an α,β-unsaturated derivative analogous to **4** (Fig. 1). Among the methods established for the *de novo* construction of thiazolines in general (30) are the direct thermal



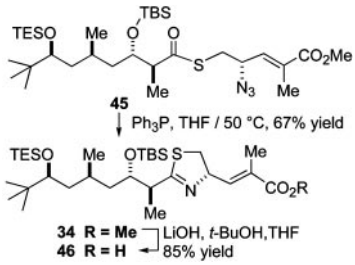
Scheme 3. Modified cysteine and thiazoline formation. (a) diphenyl phosphoryl azide, Et_3N, **22a**, dimethylformamide, 0°C, 85%; (b) (*i*) TFA, CH_2Cl_2; (*ii*) benzene, 80°C, ≈80%.

intramolecular cyclization of thiol esters derived from acidic hydrolysis of vicinal *N-tert*-butyloxycarbonyl (Boc)-aminothiols (31), condensation of nitriles with 2-aminothiols (32), addition of aminothiols to imidate esters (33), sulfurization of oxazolines (34), cyclization of thiol amides (35–37), phosphine-induced annulation of thiol amides and 2-alkynoates (38), and an intramolecular aza-Wittig reaction of a thiol ester (39). The directness of Fukuyama's acid-induced cyclization of an α-aminothiol ester (31) dictated that it be explored first, despite the obvious potential for side reactions.

The relevant α-aminothiol ester **30a** was prepared from the D-serine-derived thiol **29** and carboxylic acid **22a** (Scheme 3). The thiol ester was treated with trifluoroacetic acid (TFA) in CH_2Cl_2 to cleave the carbamate. Removal of solvent and excess TFA gave a residue that was heated at reflux in benzene (31) to provide two major diastereomeric products **31**, both of which contained the anticipated thiazoline moiety. However, both diastereomers had also incorporated a tetrahydropyran at the β-position of the thiazoline's 2-substituent. This finding was attributed to acid-induced β-elimination followed by conjugate addition of the distal hydroxyl group (Scheme 3). This process is clearly related to the acid-induced dehydration of apratoxin A to form (*E*)-34,35-dehydroapratoxin A (**4**, Fig. 1) (4, 5). Various attempts to liberate the amine from the *t*-butyl carbamate of **30a** without β-elimination of the C35 oxy substituent under alternative reaction conditions were unsuccessful. Use of the more robust TIPS group in place of the TBS ether also led to formation of tetrahydropyran **31** from **30b** under the acidic carbamate cleavage–thiazoline formation conditions.

To suppress β-elimination, alternative amine-protecting groups that could be cleaved under basic or neutral reaction conditions were examined. Both fluorenylmethoxycarbonyl- and 2,2,2-trichloroethylcarbamate-protected α-aminothiols were prepared from serine-derived intermediates and esterified with carboxylate **22a** to generate the corresponding thiol esters **32a**,**b**. Removal of the fluorenylmethoxycarbonyl group from **32a** under mildly basic conditions resulted in quantitative acyl migration from thiol to amine to generate the corresponding amide **33**. Amide **33** also resulted on removal of the corresponding 2,2,2-trichloroethylcarbamate group from **32b** by using activated zinc. All attempts to convert thiol amide **33** into thiazoline **34** without C34,35 elimination were unsuccessful.

Staudinger/aza-Wittig Thiazoline Synthesis. The complications above led to the adoption of a uniquely mild process for thiazoline formation: an intramolecular variation of an aza-Wittig reaction triggered by the Staudinger reduction of an α-azido thiol ester (40, 41). In this sequence, a free amine would not be exposed, and neutral reaction conditions could be used. Such an intramolecular *S-aW* process for thiazoline synthesis is precedented in the generation of pyranoside-fused thiazolines (42) and similarly to form an oxazoline (43). However, the acute sensitivity of the apratoxins' 2-(α-methyl-β-hydroxy)-4-(propenoate)-thiazoline moiety presented a unique challenge for *de novo* thiazoline synthesis.

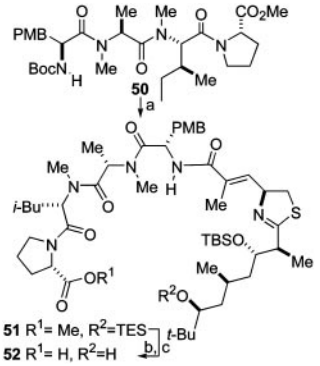


Scheme 4. Synthesis of the MoCys-polyketide domain by *S-aW* reactions.

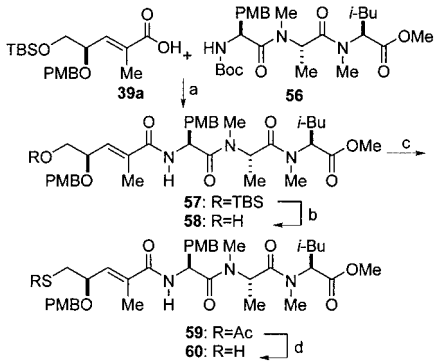
Implementation of the *S-aW* strategy required an advanced intermediate bearing the essential α-azido thiol ester. This was obtained in a straightforward fashion. In the key event, exposure of thiol ester azide **45** to Ph_3P in refluxing anhydrous THF generated thiazoline **34** in high yield without any complications (Scheme 4). The phosphinimine generated *in situ* under the Staudinger reaction of the azide with Ph_3P allowed subsequent intramolecular aza-Wittig reaction to occur with the adjacent thiol ester under the neutral, anhydrous reaction conditions. Saponification of the methyl ester of thiazoline **34** then furnished carboxylic acid **46**.

Synthesis of Apratoxin A Seco-Acid. To match thiazoline **46**, the peptide **50** was targeted next (Scheme 5). The marriage of **46** and **50** would involve macrolide closure involving sequential *p-O*-Me-Tyr–MoCys amide and Pro–C39 OH ester formation. The first task was accomplished uneventfully (Scheme 5). Selective cleavage of the C39 TES ether of **51** with HF-pyridine followed by saponification of the proline methyl ester provided hydroxy carboxylic acid **52**, the C35-*O*-TBS seco-acid of apratoxin A. Attempts to lactonize **52** under modified Yamaguchi macrolactonization conditions, however, led to decomposition. Alternative esterification methods also failed to yield the anticipated lactone. These results can be ascribed to the steric hindrance of the α-*t*-butyl secondary C39 alcohol and the sensitivity of the highly functionalized thiazoline moiety. The inability to lactonize **52** led to an alternative ordering of prolyl acid esterification and thiazoline formation steps.

Alternative Fragment-Coupling Strategy. The alternative disconnection of **1** at the *iso*-leucine-proline amide and thiazoline moieties yielded triamide **7** and polyketide-prolyl ester **8** (Scheme 1). In this approach, the robust proline ester would be installed at the outset, whereas the sensitive thiazoline moiety would be assembled at a late stage. Finally, macrolide closure would involve amide formation



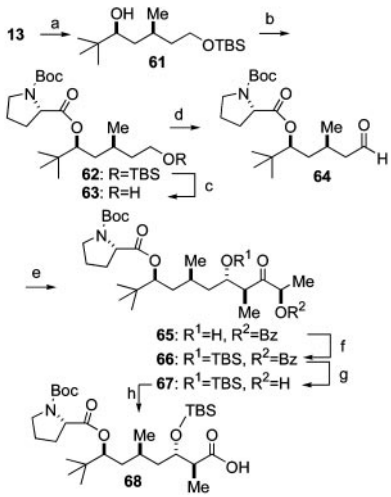
Scheme 5. Synthesis of the apratoxin A seco-acid. (a) (*i*). TFA, CH_2Cl_2; (*ii*) PyAOP, *i*-Pr_2NEt, **46**, dimethylformamide, 67%; (b) HF-pyridine, THF, 70%; (c) LiOH, *t*-BuOH/THF/H_2O = 4:1:1.



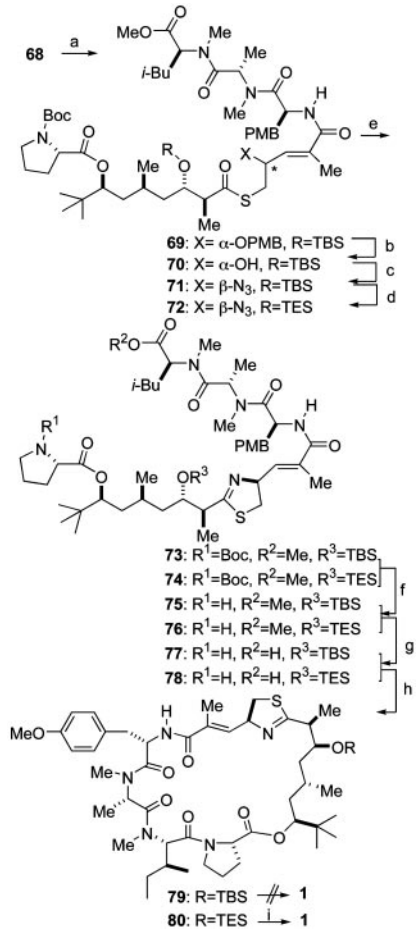
Scheme 6. Assembly of the C6-C32 intermediate. (a) (*i*) TFA, CH_2Cl_2; (*ii*) PyAOP, **39a**, Et_3N, dimethylformamide, 76%; (b) HF-pyridine, THF, 98%; (c) diisopropylazodicarboxylate, Ph_3P, AcSH, THF, 0°C, 85%; (d) K_2CO_3, MeOH, 0°C.

between proline amine and *iso*-leucine carboxylic acid residues instead of macrolactonization of the proline carboxylate.

In pursuit of this macrolactamization plan, tripeptide **56** and carboxylic acid **39a** were joined to form amide **57** (Scheme 6). The primary thiol was then installed to yield thiol acetate **59** (44). Selective saponification of the acetate (45) gave thiol **60** (compare **7**). The synthesis of the proline ester-ketide acid (compare **8**) was initiated with diol **13** (Scheme 2). Selective silylation of the primary hydroxyl group gave secondary alcohol **61** (Scheme 7). In contrast to the failed attempts to lactonize by prolyl acid condensation with the C39 hydroxyl (Scheme 6), esterification of **61** with *N*-Boc-proline proceeded efficiently under Yamaguchi conditions to yield ester **62** (46). Conversion of the primary TBS ether **62** into aldehyde **64** followed by an *anti*-selective aldol reaction with (*R*)-2-benzoyloxy-3-pentanone (**17**) gave β-hydroxyketone **65** (29). Upon silylation of **65** with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) at −50°C to generate silyl ether **66**, it was noted that the *tert*-butyl carbamate could be simultaneously converted into the corresponding TBS carbamate if the reaction temperature was raised to rt (47). Selective hydrolysis of the benzoate of **66** and



Scheme 7. Synthesis of prolyl-polyketide acid **68**. (a) TBSCl, imidazole, CH_2Cl_2, 98%; (b) *N*-Boc-Pro-OH, $Cl_3C_6H_2COCl$, *N*,*N*-diisopropylethylamine, THF; *N*,*N*-dimethyl-4-aminopyridine, benzene, 91%; (c) TBAF, THF, 88%; (d) tetra-*n*-propylammonium perruthenate, *N*-methylmorpholine-*N*-oxide, 4 Å MS, CH_2Cl_2, 89%; (e) **17**, Me_2NEt, c-Hex_2BCl, Et_2O, −78 to −20°C; (f) TBSOTf, CH_2Cl_2, 2,6-lutidine, −50°C, 74% (two steps); (g) K_2CO_3, MeOH; (h) $NaIO_4$, *t*-BuOH, H_2O, 75% (two steps).



Scheme 8. Completion of the total synthesis of apratoxin A. (a) diphenyl phosphoryl azide, Et_3N, **60**, dimethylformamide, 80%; (b) DDQ, CH_2Cl_2, H_2O, 0°C to rt, 93%; (c) diphenyl phosphoryl azide, Ph_3P, diisopropylazodicarboxylate, THF, 97%; (d) (*i*) HF-Pyridine, THF, (*ii*) TESOTf, 2,6-lutidine, CH_2Cl_2, −78°C, 86% (two steps); (e) Ph_3P, THF, 50°C, ≈63%; (f) TBSOTf, CH_2Cl_2, 2,6-lutidine; TBAF, THF, 0°C, 86%; (g) LiOH, *t*-BuOH, THF, H_2O; (h) PyAOP, CH_2Cl_2, *N*,*N*-diisopropylethylamine, ≈73% (two steps); (i) HF-pyridine, THF, 65%.

oxidative cleavage of the resultant α-hydroxyketone **67** completed the synthesis of the prolyl-polyketide acid **68**.

Thiol ester **69** was prepared by Yamada thiol esterification of acid **68** and polypeptide thiol **60** (Scheme 8) (48). The vicinal azide moiety was then incorporated to yield **71**. Treatment of **71** with Ph_3P in anhydrous THF effected thiazoline formation by the intramolecular *S-aW* process to deliver C35 *O*-TBS ether **73**. Under these optimized conditions, no complications were observed.

Macrolactam Formation. Completion of the total synthesis of **1** from thiazoline **73** would have required only two further operations: amide formation between proline amine and *iso*-leucine carboxylate residues and removal of the C35 hydroxyl silyl-protecting group (Scheme 8). The proline *tert*-butyl carbamate moiety was cleaved first in a two-step fashion that avoided strongly acidic conditions (47) and was previously established at the stage of carbamate **65** (Scheme 7). This process involved conversion of the *tert*-butyl carbamate into the corresponding *tert*-butyldimethylsilyl carbamate with TBSOTf at rt followed by selective desilylative carbamate fragmentation with tetra-*n*-butylammonium fluoride (TBAF) to generate the free amine **75**. Thereafter, the methyl ester of **75** was saponified to carboxylic acid **77**. Amino acid **77** was then subjected to PyAOP-mediated amide formation (49) to yield cyclodepsipeptide **79** in good yield. Cyclopdepsipeptide **79** was presumably

differentiated from apratoxin A only by the presence of the C35 *O*-TBS group. However, extensive attempts to cleave the C35 *O*-TBS ether at this ultimate stage of the projected total synthesis were uniformly unsuccessful. Whereas the TBS ether was resistant to cleavage under mild conditions that maintained the sensitive functionality of **1**, typical and more forcing conditions led to degradation. Thus, the similar, yet more labile TES ether was selected for protection of the C35 hydroxyl group in place of the resistant TBS ether.

Attempts to exchange the C35 *O*-TBS ether with a TES ether after thiazoline formation were unsuccessful because of the incompatibility of the highly substituted thiazoline moiety to the reaction conditions for TBS ether cleavage with TBAF or HF-pyridine. Alternatively, introduction of a C35 *O*-TES group at the stage of the aldol adduct **65** also proved to be problematic due to silyl ether cleavage during formation of the C33 carboxylic acid (compare **67**) under various reaction conditions. Hence, the C35 *O*-TBS ether was exchanged for a TES ether (**72**) after formation of the thiol ester but before thiazoline assembly (Scheme 8). Subsequent thiazoline formation proceeded smoothly throughout the *S-aW* process by α-azido-thiol ester **72** to generate thiazoline **74**. Removal of the prolyl *tert*-butyl carbamate followed by *iso*-leucyl ester saponification provided amino carboxylate **78**. PyAOP-mediated macrolide formation from **78** proceeded smoothly to provide depsipeptide **80**. In contrast to the recalcitrant C35 *O*-TBS ether **79**, the TES group of **80** was removed uneventfully with HF-pyridine to finally deliver synthetic **1**. Moreover, synthetic **1** was determined to be identical with an authentic sample of the natural product apratoxin A both spectroscopically and chromatographically.

Conclusion

The total synthesis of the cyclodepsipeptide apratoxin A was achieved by the conjunction of triamide **60** and ketide-prolyl ester **68**. These two advanced intermediates were developed specifically to accommodate the unique synthetic challenges encountered in the evolution of the total synthesis effort. First, the sensitive, highly functionalized thiazoline moiety was prone to side-chain dehydration on liberation of an amine moiety from masked α-aminothiol esters (Scheme 3). Hence, the essential thiazoline nitrogen functionality was introduced into the modified cysteine residue as an azide moiety (**71**, Scheme 8) subsequent to thiol esterification with the ketide domain carboxylate. This azide allowed the use of a uniquely mild, late-stage intramolecular Staudinger reduction–aza-Wittig process to successfully assemble the functionalized thiazoline motif. Second, the failure to achieve macrolide closure by lactonization of the proline carboxylic acid with the hindered C39 hydroxyl (**52**, Scheme 5) dictated a revision of the fragment-coupling strategy. For this, the proline ester was incorporated into the polyketide domain at the outset of the total synthesis (**62**, Scheme 7) and triamide **60** was deployed for macrolide closure by sequential thiazoline and *N*-methyl-*iso*-leucine-proline amide formation (Scheme 8). Finally, the inability to cleave the C35 *O*-TBS ether without inducing extensive molecular degradation prompted the replacement of the TBS group with the appropriately labile TES at the stage of thiol ester **71**. Thereafter, thiazoline formation, macrolide closure, and scission of the final protecting group proceeded satisfactorily to deliver **1**. This study documents the total synthesis of apratoxin A. In addition to corroborating the structure of the natural product and defining an unexpected modular assembly approach to **1** and its structural variants, this work also highlights the challenges of natural products total synthesis as a stimulus for innovation. Extension of the defined synthetic entry to the generation of apratoxin analogs with improved therapeutic potential and probes to establish the distinct mode of action should be explored.

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