Graphical Abstract

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Apratoxin A (1)

● = structural diversity positions

The Apratoxin Marine Natural Products: Isolation, Structure Determination, and Asymmetric Total Synthesis

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ABSTRACT

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Isolation and Structural Determination

Apratoxins A-H and apratoxin A sulfoxide (**1-9**, Fig. 1) comprise a family of cyclic depsipeptides isolated from the *Lyngbya* species of cyanobacteria.¹⁻⁸ The first member of the family to be discovered, apratoxin A (**1**), was isolated in 2001 by Moore, Paul and co-workers'.¹ It was isolated from the marine cyanobacterium *Lyngbya majuscula* from Finger's Reef, Apra Harbor, Guam. Structure determination revealed that apratoxin A (**1**) was composed of discrete polyketide and polypeptide domains, joined via an amide and ester linkage. The polypeptide domain contains three methylated L-amino acid residues (*O*-methyltyrosine, *N*-methylalanine, *N*-methylisoleucine), one L-proline residue, and a modified D-cysteine residue, and the polyketide domain contains four stereogenic centers (C-34, C-35, C-37, and C-39). Apratoxin A exhibited potent *in vitro* cytotoxicity against KB and LoVo cancer cell lines, with IC₅₀ values of 0.52 nM and 0.38 nM, respectively.

Apratoxins B (**2**) and C (**3**) were isolated in 2002 from the same *Lyngbya* sp. of cyanobacterium as apratoxin A, following an expanded geographical search around the island of Guam and the surrounding Palauan waters.² Apratoxin B (**2**) was found in organisms from the same collection site as apratoxin A, whereas apratoxin C (**3**) was produced by specimens collected from Short Dropoff, Palau. Structure determination revealed these new apratoxins to be very similar to apratoxin A, differing only in the degree of methylation; apratoxin B lacks *N*-methylation of the isoleucine residue, and apratoxin C possesses an isopropyl rather than a *t*-butyl group at C-39. Apratoxin B displayed potent *in vitro* cytotoxicity against KB and LoVo cancer cell lines, with IC₅₀ values of 21.3 nM and 10.8 nM, respectively. Apratoxin C showed stronger *in vitro* cytotoxicity than apratoxin B against KB and LoVo cancer cell lines, with IC₅₀ values of 1.0 nM and 0.73 nM, respectively.

Apratoxin D (**4**) was isolated in 2008 by Gutiérrez and co-workers' from samples of *Lyngbya majuscula* and *Lyngbya*

sordida, off the coast of Papua New Guinea.³ Interestingly, apratoxin D was found to have the same amino acid sequence as apratoxins A and C, but possessed a new polyketide moiety, 3,7-dihydroxy-2,5,8,10,10-pentamethylundecanoic acid, which was longer than the other apratoxins by an acetate group. Apratoxin D showed potent *in vitro* cytotoxicity against H-460 human lung cancer cells with an IC₅₀ value of 2.6 nM.

Apratoxin E (**5**) was isolated shortly after apratoxin D by Luesch and co-workers' from the cyanobacterium *Lyngbya bouillonii*.⁴ Apratoxin E was found to possess an identical polypeptide domain to that of apratoxin A. However, it is unsaturated at the C-34–C-35 bond, and lacks a methyl group at C-34. In addition, the modified cysteine residue is fully saturated at C-28–C-29 and lacks a C-28 methyl group. Apratoxin E exhibited strong cytotoxicity when tested for activity against several human cancer cell lines: HT29 (IC₅₀ of 21 nM), HeLa (IC₅₀ of 72 nM), and U2OS osteosarcoma cells (IC₅₀ of 59 nM).

Apratoxins F (**6**) and G (**7**) were isolated in 2010 by Gerwick and co-workers' from samples of *Lyngbya bouillonii* collected from Palmyra Atoll in the Central Pacific.⁵ The polyketide moiety of apratoxins F and G was found to match that of apratoxin A. In both apratoxins F and G, the polypeptide portions contain an *N*-methylated alanine in place of the proline residue present in apratoxin A. Apratoxin G had one additional difference, namely, the *N*-methylisoleucine was replaced with *N*-methylvaline. Apratoxins F and G were the first apratoxins isolated to possess significant structural difference in the peptide sequence. Apratoxin F and G displayed potent *in vitro* cytotoxicity against H-460 lung cancer cells with an IC₅₀ of 2 nM and 14 nM, respectively. Additionally, apratoxin F showed potent activity against HCT-116 cell lines (IC₅₀ of 36 nM).

Apratoxin H (**8**) and apratoxin A sulfoxide (**9**) were isolated by McPhail and co-workers' in 2013 from the cyanobacterium *Moorea producers* obtained from the Red Sea.⁶ Apratoxin H and apratoxin A sulfoxide were found to retain the same polyketide

Figure 1. The apratoxins.

moiety as apratoxin A. Apratoxin H only differed from apratoxin A in that a pipecolic acid residue was present instead of a proline residue. Apratoxin A sulfoxide differed in degree of oxidation in the modified cysteine residue; the sulfur of the thiazoline is oxidized to the sulfoxide. Apratoxin H exhibited potent cytotoxicity towards H-460 lung cancer cells with an IC_{50} value of 3.4 nM, while apratoxin A sulfoxide showed a vast decline in cytotoxicity against H-460 lung cancer cells (IC_{50} of 89.9 nM).

The slight structural differences in the apratoxin family hints at mixed biogenetic origins for this class of compounds. This variation is common among cyanobacteria, which often produce more than one member of a certain class of compounds due to relaxed specificity of biosynthetic enzymes during precursor-biosynthesis.^{4,10}

Total Synthesis Apratoxin A

Five total syntheses of apratoxin A have been reported: one by Forsyth,^{11,12} two by Takahashi and Doi,^{7b,13} one by Ma,^{7a} and one by Doi.^{7d} As outlined in Scheme 1, apratoxin A could logically be disconnected to yield tetrapeptide **10** and the thiazoline containing polyketide **11**. However, one strategy common to all apratoxin A syntheses, based on merging the peptide and polyketide domains, is the early incorporation of the proline residue as the C-39 ester (cf. **13**). This strategy was used in anticipation of a higher yielding amide formation between the isoleucine carboxylate of tripeptide **12a-c** and the proline amine moiety of **13**, in comparison to the coupling between the proline carboxylate of **10** and the hindered C-39 hydroxyl of **11**.^{7a,b,d,11-13} Also common to the five syntheses of apratoxin A is the late stage assembly of the thiazoline moiety, which is oxidatively sensitive and potentially prone to unwanted side reactions. While

tripeptides **12a-c** can theoretically be accessed in a relatively straightforward way using standard peptide synthesis procedures, the polyketide and thiazoline portions of the structure present a significant synthetic challenge, which necessitated the development of new strategies for their synthesis.

The first total synthesis of apratoxin A was conducted by Chen and Forsyth.^{11,12} Their strategy for constructing the sensitive thiazoline moiety was a very interesting one-pot Staudinger reduction/intramolecular aza-Wittig reaction of an α -azido thioester **15** (Scheme 2). Exposure of **15** to Ph_3P generated a phosphinimine from the azide *in situ*, which could then undergo the aza-Wittig reaction with the adjacent carbonyl of the thioester to generate the thiazoline.¹⁴ This strategy for thiazoline formation required an advanced intermediate containing the essential α -azido thioester (**15**), which was constructed from triamide **16** and polyketide **17**.

As outlined in Scheme 3, the synthesis of **17** began with esterification¹⁵ of compound **18**¹⁶ with acrylic acid to yield diene **19**. Ring closing metathesis¹⁷ of **19** gave an α,β -unsaturated δ -valeryl lactone, which upon methyl cuprate addition¹⁸ set the C-37 stereocenter of **20**. The lactone was then reductively opened, and the corresponding primary alcohol was TBS-protected to give **21**. Esterification of **21** with *N*-Boc-L-proline under Yamaguchi conditions,¹⁹ followed by TBS group removal and oxidation of the resulting primary alcohol with TPAP and NMO, afforded aldehyde **24**. To set the C-34 and C-35 stereocenters, a Paterson *anti*-selective aldol reaction²⁰ of aldehyde **24** with (*R*)-2-benzoyloxy-3-pentanone (**25**) provided β -hydroxy ketone **26**. Compound **26** was TBS protected and the benzoyloxy ketone was cleaved in a two step manner to give carboxylic acid **17**.

Scheme 1. Retrosynthetic analysis of apratoxin A.

Scheme 2. Forsyth's retrosynthetic pathway towards apratoxin A.

Scheme 3. Synthesis of fragment 17.

The required polypeptide fragment, triamide **12a**, was assembled beginning with isoleucine derivative **28** (Scheme 4). Compound **28** was deprotected on nitrogen and coupled with *N*-Boc-*N*-methyl-L-alanine (**29**) to yield diamide **30**. Boc removal, followed by a PyAOP-mediated²¹ condensation with tyrosine derivative **31** gave triamide **12a**.

Scheme 4. Synthesis of triamide **12a**.

The precursor to the sensitive thiazoline moiety, cysteine analog **32**, was synthesized from a Wittig reaction between (*S*)-glyceraldehyde acetonide (**33**) and methyl 2-(triphenylphosphoranylidene)-propionate (**34**) (Scheme 5). Cleavage of the acetonide from the product (**35**) gave diol **36** in quantitative yield. Subsequent protection of the primary alcohol with TBSCl, followed by protection of the secondary alcohol with PMB trichloroacetimidate furnished **38**. Finally, hydrolysis of the methyl ester afforded cysteine analog **32**.

Scheme 5. Synthesis of cysteine analog **32**.

Completion of the first total synthesis of apratoxin A is illustrated in Scheme 6. To carry out the intended Staudinger reduction/aza-Wittig strategy for thiazoline formation, triamide **12a** had to be condensed with modified cysteine analog **32** to form **39**, which was then converted to thiol **16** in a stepwise fashion.²² Thioester formation²³ between **16** and **17**, followed by deprotection of the C-30 hydroxyl and treatment with diphenylphosphoryl azide under Mitsunobu conditions, yielded azido thioester **44**. At this stage, the TBS group was changed to a TES group to give **15**. [In their first attempt at completing the total synthesis of apratoxin A, Chen and Forsyth were able to advance **44** to the cyclized form (cf. **48** with TBS in place of TES). However, unfortunately all attempts at removal of the TBS group were unsuccessful.] Thiazoline formation from **15** via a Staudinger reduction/intramolecular aza-Wittig reaction was then carried out using Ph₃P to give compound **14**.¹⁴ Conversion of compound **14** to silyl carbamate **45**,²⁴ followed by selective desilylation with TBAF, gave compound **46**. Saponification of the isoleucine methyl ester gave **47**. The total synthesis was concluded with macrolactamization of **47** to afford the cyclic depsipeptide (**48**), which was subsequently deprotected to yield apratoxin A (**1**) in 28 steps for the longest linear sequence and overall yield of 3.1%.

Scheme 6. Completion of the synthesis of apratoxin A

Scheme 7. Thiazoline formation via tandem deprotection/cyclodehydration.

In their efforts towards apratoxin A, Takahashi and Doi,^{7b,11} Ma,^{7a} and Doi^{7d} assembled the thiazoline unit using a tandem deprotection/cyclodehydration strategy (50 $\rightarrow$ 49, Scheme 7). This strategy required the coupling of carboxylic acid 17 with modified cysteine residue 51a-e to form trityl protected thiol amide 50.

Takahashi, Doi and co-workers¹³ approached the synthesis of apratoxin A with fragment 17 as the initial target. The synthesis started with compound 52 (Scheme 8), the product of a proline-catalyzed aldol reaction²⁵ between acetone and pivalaldehyde. PMB protection, followed by subsequent vinylation²⁶ and acetylation provided 53. Palladium (II)-catalyzed isomerization of allylic acetate 53 (*E/Z* = 9:1) and removal of the acetyl group gave alcohol 54, which was then hydrogenated with Ru(OAc)₂[(*S*)-binap] under 100 atm of hydrogen²⁷ to set the C-37 stereocenter (55) in >95:5 *dr*. Alcohol 55 was then oxidized to the aldehyde under Swern conditions, and, taking inspiration from the Forsyth synthesis,^{11,12} was then subjected to a Paterson²⁰ *anti*-aldol reaction to set stereocenters C-34 and C-35. The resulting *anti*-aldol product was TBS protected to give 56. Removal of the PMB group with DDQ, followed by Yamaguchi coupling¹⁹ with *N*-Boc-L-Pro-OH, provided compound 27. Removal of the benzoate functionality and oxidative cleavage of the resulting α -hydroxy ketone provided acid 17, as previously reported.^{11,12}

Scheme 8. Takahashi and Doi's synthesis of compound 17.

To prepare modified cysteine residue 51a, Takahashi, Doi and co-workers' converted the corresponding *S*-Trt-*N*-Boc-D-cysteine (57) to its Weinreb amide, reduced that to the corresponding aldehyde with DIBAL, and then subjected the resulting aldehyde to a Wittig olefination to afford 58 (Scheme 9). Conversion of the ethyl ester to an allyl ester, followed by selective removal of the *N*-Boc group using TMSOTf, 2,6-lutidine, and MeOH, provided modified cysteine residue 51a.

Scheme 9. Preparation of modified cysteine residue **51a**.

Having polyketide **17** and modified cysteine residue **51a** in hand, focus was turned towards making the sensitive thiazoline ring. Coupling of **17** and **51a** was followed by stepwise conversion of product **50** into the corresponding Troc carbonate (**59**) (Scheme 10). Thiazoline formation was then carried out using Kelly's conditions ($\text{Ph}_3\text{P}(\text{O})/\text{Tf}_2\text{O}$)²⁸ to furnish **49**. The Troc group was then removed with $\text{Zn-NH}_4\text{OAc}$, and subsequent conversion of the allyl ester to the acid by treatment with $\text{Pd}(\text{PPh}_3)_4/\text{N-methylaniline}$ ²⁹ afforded compound **61**.

Scheme 10. Takahashi and Doi's synthesis of **61**.

The final portion of the synthesis included incorporation of tripeptide **12b** to thiazoline **61** and closing the macrocycle. Tripeptide **12b** was prepared by sequential coupling of *N*-methyl-

L-isoleucine allyl ester with *N*-Boc-*N*-methyl-L-alanine and *N*-Fmoc-*O*-methyl-L-tyrosine using HATU/*i*-Pr₂NEt and Et₃NH in acetonitrile. **12b** was then coupled to fragment **61** providing compound **62** (Scheme 11). Cleavage of the allyl ester²⁹ of **62**, followed by removal of the Fmoc group, afforded cyclization precursor **63**, which was treated with HATU/*i*-Pr₂NEt to promote macrolactamization yielding apratoxin A (**1**) in 25 steps for the longest linear sequence and overall yield of 4.6%.

Scheme 11. Takahashi and Doi's completion of apratoxin A.

Soon after, Takahashi, Doi and co-workers' published a second generation route to apratoxin A.^{7b} The second generation synthesis began like the first with the same proline-catalyzed aldol reaction²⁵ (cf. **52**, Scheme 8), PMB protection, and vinylation reaction to form **64** (Scheme 12). In the first generation synthesis, the tertiary alcohol of **64** was protected as an acetate, and olefin isomerization was achieved using $\text{PdCl}_2(\text{MeCN})_2$. In the present case, the isomerization was carried out directly on allylic alcohol **64** using catalytic triphenylsilyl perhenhate.³⁰ Under these conditions **64** was smoothly converted to the corresponding TMS protected allylic alcohol as a 1:1 mixture of *E/Z* olefins, which then gave allylic alcohols **54** upon treatment with Et₃N and K₂CO₃ in MeOH. An interesting feature of this reaction is the use of *N,O*-bis(trimethylsilyl)-acetamide, which selectively silylates the primary allylic alcohol isomer over the tertiary one, thereby shifting the equilibrium towards the desired isomer. Ruthenium-mediated asymmetric hydrogenation,²⁷ followed by Swern oxidation, afforded aldehyde **65**. The second generation synthesis avoided the Paterson *anti*-aldol reaction²⁰ in an effort to allow direct incorporation of the Troc and Fmoc groups, which are needed for thiazoline formation. Instead, Roush's crotylation³¹ was utilized to set the C-34 and C-35 stereogenic centers. In the event, crotylation of aldehyde **65** with (*E*)-crotylboronate (**66**) yielded **67** in excellent yield and diastereoselectivity (Scheme 12). Two protecting group manipulations followed by installation of the proline residue via Yamaguchi esterification¹⁹ gave **69** in 92% yield over three steps. Oxidative cleavage of olefin **69** afforded acid **70**. With acid **70** in

hand, construction of the thiazoline precursor was carried out by coupling acid **70** with modified cysteine species **51a** to afford **59**. From thiazoline precursor **59**, the second generation synthesis then follows Takahashi, Doi and co-workers' first generation synthetic strategy (Scheme 10).¹³

Scheme 12. Takahashi and Doi's second generation synthesis of thiazoline precursor **59**.

Ma and co-workers' synthesis of apratoxin A^{7a} employed the same tandem deprotection/cyclodehydration strategy for thiazoline formation as the Takahashi, Doi, and co-workers' synthesis.^{7b,13} Therefore, the initial target for the synthesis was fragment **71**, which contains all four stereocenters of the polyketide portion of apratoxin A. As illustrated in Scheme 13, the synthesis of

fragment **71** began with the same proline-catalyzed aldol product²⁵ (**52**) used by Takahashi, Doi and co-workers'.^{7b,13} Alcohol **52** was TBS protected, the resulting ketone reduced, and then subjected to elimination via its mesylate to give TBS-protected alcohol **72**. This compound was converted to α,β -unsaturated lactone **73** in a stepwise manner via ozonolysis, addition of $\text{LiCH}_2\text{CO}_2\text{Et}$ to the resulting aldehyde, cyclization, and elimination. Lactone **73** was then treated with a methyl cuprate¹⁸ to form **20**, thus setting the C-37 stereocenter.^{11,12} The lactone was reduced, and the resulting diol was protected as the diacetate. Selective deprotection of the masked primary alcohol then was followed by oxidation to provide aldehyde **74**. To set the C-34 and C-35 stereocenters, aldehyde **74** was subjected to an *anti*-aldol reaction with Oppolzer's sultam³² (**75**) to give **76**. Notably, eight equivalents of TiCl_4 were required in the *anti*-aldol reaction to ensure high yield. Treatment of **76** with LiAlH_4 removed both the auxiliary and the acetyl group yielding a triol, which was protected with 2,2-dimethoxypropane to provide alcohol **77**. The Fmoc-protected proline was installed via Yamaguchi esterification,¹⁹ as with the other syntheses,^{7b,11-13} giving **78**. The diol was liberated, and the primary alcohol was selectively oxidized to the aldehyde using the bulky chloro oxammonium salt which was generated from TEMPO and NaOCl . The resulting aldehyde was subsequently oxidized to acid **71**.³³

Scheme 13. Ma's synthesis of fragment **71**.

The next key step of the synthesis was incorporation of the thiazoline moiety to polyketide **71**. To begin, modified cysteine unit **51b** was prepared according to Takahashi, Doi, and co-workers' synthesis¹³ with two exceptions: they started with *N*-Fmoc protected cysteine instead of *N*-Boc-protected cysteine, and they used the prenyl, rather than the allyl ester (Scheme 9). Modified cysteine residue **51b** was deprotected on nitrogen and coupled to carboxylic acid **71** to afford thiazoline precursor **79** (Scheme 14). Formation of the thiazoline was accomplished with Kelly's conditions,²⁸ which provided **80**.

Scheme 14. Ma's synthesis of thiazoline **80**.

To complete the synthesis of apratoxin A, the required tripeptide unit (**12c**) needed to be synthesized and connected to fragment **80**. The synthesis of tripeptide **12c** began with deprotection on nitrogen of Fmoc-*N*-Me-L-Ala-OBn (**81**) and subsequent coupling of tyrosine derivative **82** (Scheme 15). The benzyl group was then removed via hydrogenolysis to afford dipeptide **83**. BEP-mediated³⁴ coupling of dipeptide **83** with Fmoc-*N*-Me-L-Ile-OTMSE (**84**) provided tripeptide **12c**.

Scheme 15. Synthesis of tripeptide **12c**.

With tripeptide **12c** in hand, the prenyl group of thiazoline **80** was removed,³⁵ and the resulting acid was then coupled with tripeptide **12c** to give protected cyclization precursor **85** (Scheme 16). Removal of Fmoc and TMSE protecting groups, macrolactamization, and acyl group removal resulted in apratoxin A in 27 steps for the longest linear sequence and overall yield of 0.8%.

Scheme 16. Ma's completion of apratoxin A.

Doi's synthesis of apratoxin A^{7d} differed from the previous syntheses^{7a,7b,11-13} in that a solid phase peptide synthesis (SPPS) approach was used. Two possible retrosynthetic pathways were considered (Scheme 17). For route A, thiazoline formation would be performed after macrolactamization of **86**, due to the ease of epimerization of the C-34 position and potential for dehydration under weakly acidic conditions. For route B, thiazoline formation would be conducted prior to the peptide coupling step, with macrolactamization as the terminal step.

SPPS of route A was performed by use of the Fmoc strategy using a trityl linker. To begin, commercially available trityl alcohol SynPhase Lanterns³⁵ (**88**) was chlorinated with AcCl. Fmoc-protected *N*-methyl-L-isoleucine (**89**) was then connected to the resin using Hunig's base to afford **92**. The peptide synthesis continued by first removal of the Fmoc group of **92** (Scheme 18) with 20% piperidine/DMF and subsequent acylation with *N*-Fmoc-*N*-Me-L-Ala-OH (**90**) by using PyBroP³⁶ and Hunig's base. After Fmoc cleavage from the dipeptide, coupling with tyrosine derivative (**91**) was performed using the same conditions as the previous acylation, to give **93**. Removal of the Fmoc group from tripeptide **93** allowed for a subsequent coupling with **51c** (made according to Takahashi, Doi and co-workers' synthesis¹³, see Scheme 9). The transformation was mediated by DIC/HOBt to afford the polymer supported tetrapeptide **94** in 93% overall yield from intermediate **92**.

Scheme 17. Doi's retrosynthetic analysis for SPPS of apratoxin A.

Scheme 18. Synthesis of fragment **94**.

Assembly of the thiazoline unit began with Fmoc deprotection of **70**, made according to Takahashi, Doi, and co-workers' second generation synthesis^{7b} (see Scheme 12), and HATU/Hunig's base-mediated peptide coupling with polymer supported **94**, to give **95** (Scheme 19). Removal of the Fmoc group and subsequent removal of the polymer support was achieved by

using 20% 2,6-dimethylpiperidine/DMF, followed by 30% HFIPA³⁸/CH₂Cl₂, to afford **96**. Macrolactamization of **96** was achieved by using a solution of HATU/Hunig's base. Thiazoline ring formation was then completed by Kelly's method²⁸ and removal of the Troc group yielded apratoxin A in 15 steps for the longest linear sequence and 6% overall yield.

In Doi's alternative route to apratoxin A, formation of polymer-supported tripeptide **93** was identical to route A. Fmoc cleavage of polymer-supported tripeptide **93**, followed by coupling with the thiazoline-containing **61** (made according to Takahashi, Doi and co-workers' second generation synthesis,^{7b} see Scheme 12), and removal of the Fmoc group and polymer support afforded cyclization precursor **87** (Scheme 20). Macrolactamization resulted in a mixture of apratoxin A and its C-34 epimer (85:15) in 53% overall yield from **92**, a common intermediate.

Scheme 19. Doi's completion of apratoxin A by route A.

Scheme 20. Doi's completion of apratoxin A by route B.

Apratoxin C

The first asymmetric total synthesis of apratoxin C (**3**) was completed by Doi and co-workers³⁹ in 2014. Apratoxin C contains only one structural difference from apratoxin A: an isopropyl instead of a *t*-butyl group is present in the polyketide moiety. The synthetic strategy for apratoxin C paralleled the second generation synthesis of apratoxin A by Doi, Takahashi, and co-workers'.^{7b}

The initial target for apratoxin C was acid **97** (Scheme 21). The synthesis began with a D-proline catalyzed aldol reaction²⁵ between acetone and isobutyraldehyde (**98**) to afford alcohol **99**. Subsequent alcohol protection with PMB trichloro-acetimidate and Grignard addition with vinyl magnesium bromide gave **101**. Rhenium-catalyzed isomerization³⁰ of the olefin (**101**) gave **102** as a 1:1 mixture of *E/Z* alkenes. Asymmetric hydrogenation²⁷ of **102** furnished **103** as a single diastereomer, and subsequent oxidation of primary alcohol **103** afforded aldehyde **104**. The C-34 and C-35 stereocenters were set by Roush's crotylation³¹ using (*E*)-crotylboronate (**66**), which afforded a 9:1 mixture of **105** and its diastereomer. Troc protection of the resulting secondary alcohol (**105**) and DDQ-mediated removal of the PMB group set the stage for addition of the proline ring using Yamaguchi's conditions,¹⁹ giving **107**. The synthesis of acid **97** was completed by oxidative cleavage of the terminal alkene and subsequent oxidation of the resulting aldehyde.

Scheme 21. Doi's synthesis of fragment **97**.

The synthesis of apratoxin C was completed by nitrogen deprotection of modified cysteine **51e**, followed by coupling to acid **97**, giving **108** (Scheme 22). Thiazoline formation was accomplished using Kelly's conditions²⁸ and subsequent removal of the Troc group produced thiazoline **109**. Removal of the allyl group allowed for coupling with tripeptide **12b** to furnish **110**. Removal of the protecting groups at the *N*- and *C*-terminus allowed for macrolactamization affording apratoxin C in 19 steps for the longest linear sequence and overall yield of 2.0%.

As shown in Scheme 24, the synthesis began with the preparation of aldehyde **113**. This strategy relied on an α,α -bisalkylation of an ACC hydrazone to control both the regio- and diastereoselectivity of the alkylation, setting the C-37 stereocenter.⁴⁷ Starting from α -benzyloxyacetone (**116**), condensation with camphor derived ACC auxiliary **117** gave hydrazone **115** in 80% yield. In order to obtain the desired (*R*)-configuration at C-37, the hydrazone was first methylated, followed by allylation to give **114** as a single diastereomer, in 83% yield over two steps. Auxiliary removal using $\text{BF}_3 \cdot \text{OEt}_2$ proceeded smoothly, leaving only deoxygenation of the ketone and oxidation of the allyl moiety to complete fragment **113**. Reduction of the ketone with LiAlH_4 and formation of the xanthate set the stage for Barton-McCombie deoxygenation,⁴⁸ followed by Johnson-Lemieux oxidation to give aldehyde **113** in 8 steps and 36% overall yield.

Scheme 24. Synthesis of aldehyde **113**.

Apratoxin D

Similar to the prior syntheses of apratoxin A, apratoxin D could be disconnected into polyketide **111** and tripeptide **12b** (Scheme 23). Again, early incorporation of the proline residue would avoid a late stage esterification with the hindered tertiary C-39 hydroxyl. Since the synthesis of tripeptide **12b** was known,^{7b,13} the target became polyketide **111**. **111** could be broken into polyketide **112** and cysteine-derived intermediate **51e**, which would be coupled during late stage construction of the sensitive thiazoline moiety. The C-39–C-40 stereochemistry would be set using an Evans *syn*-aldol addition,⁴¹ and a subsequent Paterson *anti*-aldol²⁰ would set the C-34 and C-35 centers. The neopentyl group could be introduced via a copper-catalyzed tosylate displacement.⁴² This strategy would require intermediate **113**, which would carry the C-37 stereocenter, set using asymmetric ACC (amino cyclic carbamate) α,α -

With aldehyde **113** in hand, the focus now turned to preparation of carboxylic acid **112** (Scheme 25). Thus, **113** was engaged in an Evans *syn*-aldol addition,⁴¹ followed by installation of a TES-group and reductive auxiliary removal, to yield **122**. At this stage, the *t*-Bu group was installed. After numerous failed attempts, a method introduced by Terao and Kambe for the cuprate-based alkylation of various electrophiles was used.⁴² Tosylation of the primary alcohol followed by copper-catalyzed addition of *t*-BuMgCl gave compound **123** in good yield, which was subsequently transformed to alcohol **124** over three steps.¹⁹ Alcohol **124** was then oxidized to the corresponding aldehyde, and this was used in a Paterson *anti*-aldol²⁰ with ketone **25**, thus setting the C-34 and C-35 stereocenters. The product of this reaction (**125**) was then subjected to TBS-protection, benzoate hydrolysis, and oxidation to give carboxylic acid **112** in good overall yield.

Having completed the synthesis of acid **112**, all that remained was construction of the thiazoline moiety, peptide coupling, and macrocyclization to complete the synthesis of apratoxin D. Synthesis of thiazoline **111** began with Boc group removal from

51e and HATU-mediated coupling with **112**, which produced **126** in good yield (Scheme 26). At this stage, the Boc protecting group on the proline residue and the TBS group on the C-37 hydroxyl were exchanged for Fmoc and Troc groups, respectively, to facilitate the final macrocyclization event, giving compound **127**. Thiazoline formation was then undertaken by using Kelly's procedure²⁸ to give **128**. Removal of the Troc group, as well as deprotection of the carboxylic acid, gave **111**, setting the stage for the final peptide coupling and macrocyclization events.

To complete the synthesis of apratoxin D, **111** was coupled with tripeptide **12b**^{7b,13} using HATU and Hunig's base, giving **129** in very good yield (Scheme 27). Conversion of allyl ester **129** to the corresponding carboxylic acid using Pd(PPh₃)₄ and *N*-methylalanine²⁹ was followed by removal of the Fmoc group from the proline residue and HATU-mediated macrolactamization, giving apratoxin D in 33 steps for the longest linear sequence and overall yield of 1.3%.

Scheme 25. Synthesis of acid **112**.

Scheme 26. Synthesis of thiazoline **111**.

Scheme 27. Coltart's completion of apratoxin D.

Scheme 28. Luesch's retrosynthesis of apratoxin F.

Apratoxin F

The first asymmetric total synthesis of apratoxin F (**6**) was completed by Luesch and co-workers' in 2011.⁴⁹ Apratoxin F contains only one structural difference from apratoxin A: the proline ring is replaced by an *N*-methyl alanine residue. The synthesis of apratoxin F was undertaken as part of a SAR study with multiple apratoxin analogs. Like the previous syntheses,^{7a,b,d,11-13,39,40} Luesch chose the retrosynthetic pathway in which the macrocycle was disconnected into tripeptide **12b** and thiazoline polyketide **130**, with the latter having an *N*-methyl alanine residue where proline previously was (Scheme 28).

The synthesis of apratoxin F began with formation of olefin **68** according to Takahashi, Doi and co-workers' second generation synthesis of apratoxin A.^{7b} DDQ-mediated removal of the PMB protecting group and subsequent Yamaguchi esterification¹⁹ with Fmoc-*N*-Me-L-Ala-OH provided ester **131** (Scheme 29). Acid **132** was obtained by oxidation of **131** with $\text{OsO}_4/\text{oxone}/\text{NaIO}_4$.⁵⁰ Modified cysteine residue (**51a**) was constructed in the same manner as previous synthetic work.¹³ It was then coupled with acid **132** to afford thiazoline precursor **133**. Thiazoline formation proceeded via Kelly's method,²⁸ and the Troc group was then cleaved to give polyketide **134**. Removal of the allyl group was accomplished via $\text{Pd(PPh}_3)_4/N\text{-methylaniline}$ ²⁹ affording acid **130**, which was then coupled to tripeptide **12b**, to give **135**. To finish the synthesis of apratoxin F, the allyl²⁹ and Fmoc protecting groups were removed to give the macrolactamization

precursor **136**. Macrocyclization proceeded with HATU/Hunig's base under dilute conditions to provide apratoxin F (**6**) in 20 steps for the longest linear sequence and an overall yield of 4.6%.

Scheme 29. Luesch's completion of apratoxin F.

Conclusion

The extremely promising biological activity of the apratoxins, as well as the limited amounts available via isolation from natural sources, make them very compelling lead compounds for the development of anti-tumor drugs via asymmetric total synthesis. This review details the isolation and cytotoxicity on various cell lines from the naturally isolated apratoxins A-H and apratoxin A sulfoxide, and synthetic routes developed for apratoxins A, C, D, and F for the apratoxin family.

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