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Total Synthesis of the Cyclodepsipeptide Apratoxin A and Its Analogues and Assessment of Their Biological Activities

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Abstract: A novel total synthesis of apratoxin A is described, with key steps including the assembly of its ketide segment through a D-proline-catalyzed direct aldol reaction and Oppolzer's *anti* aldol reaction and the preparation of its thiazoline unit in a biomimetic synthesis. An oxazoline analogue of apratoxin A has also been elaborated by a similar approach. This compound has a potency against HeLa cell proliferation only slightly lower than that of apratoxin A, whilst a C(40)-demethylated oxazoline analogue of apratoxin A displays a much lower cytotoxicity and the C(37)-epimer and C(37) demethylation product of this new analogue are inactive. These results suggest that the two methyl groups at C(37) and C(40) and the stereochemistry at C(37) are essential for the potent cellular activity of the oxazoline analogue of apratoxin A. Further biological analysis revealed that both synthetic apratoxin A and its oxazoline analogue inhibited cell proliferation by causing cell cycle arrest in the G1 phase.

Keywords: aldol reaction · cell cycle · cyclic depsipeptides · cytotoxicity · total synthesis

Introduction

Natural products and analogues showing potent cytotoxic effects are important sources of new anticancer drugs.[1] Since resistance often occurs, and not all tumor cells are equally sensitive to a given drug, discovery of new compounds with potent cytotoxicity and novel modes of action is crucial. Apratoxin A (**1**, Scheme 1) is a cyclodepsipeptide isolated from the marine cyanobacterium *Lyngbya majuscula* by Moore and co-workers in 2001.[2] This compound was shown to be cytotoxic against the LoVo and KB cancer cell lines, exhibiting in vitro IC_{50} values ranging from 0.36 to 0.52 nM and being the most cytotoxic among several cyclodepsipeptides discovered in the marine cyanobacterium.[2] Preliminary biological studies have indicated that apratoxin A affects neither microtubule polymerization dynamics nor topoisomerase I, but further investigation of its mode of action has been hampered by the scarcity of the natural material. In addition, apratoxin A was poorly tolerated in mice, probably due to its intrinsic toxicity to normal cells and lack of selectivity for tumor cell lines.[2] One possible solution to this problem is to carry out structure–activity relationship (SAR) studies through synthesis and testing of new analogues of apratoxin A. In fact, the different cytotoxicity patterns displayed by its new family members, apratoxins B (**2**) and C (**3**), suggest that the cytotoxicities of different members of this family of natural products are highly dependent on their structures.[3] It was reported that **3** exhibits in vitro IC_{50} cytotoxicity values almost identical to those of **1** whilst **2** is significantly less cytotoxic than **1**. These observations, together with the intriguing structure of apratoxin A, have attracted considerable synthetic attention, which culminated in the first total synthesis of apratoxin A by Forsyth and Chen.[4a,b] Here we report a novel synthetic route to apratoxin A, which also enabled the synthesis of a number of analogues for preliminary SAR and mechanistic studies.[5,6]

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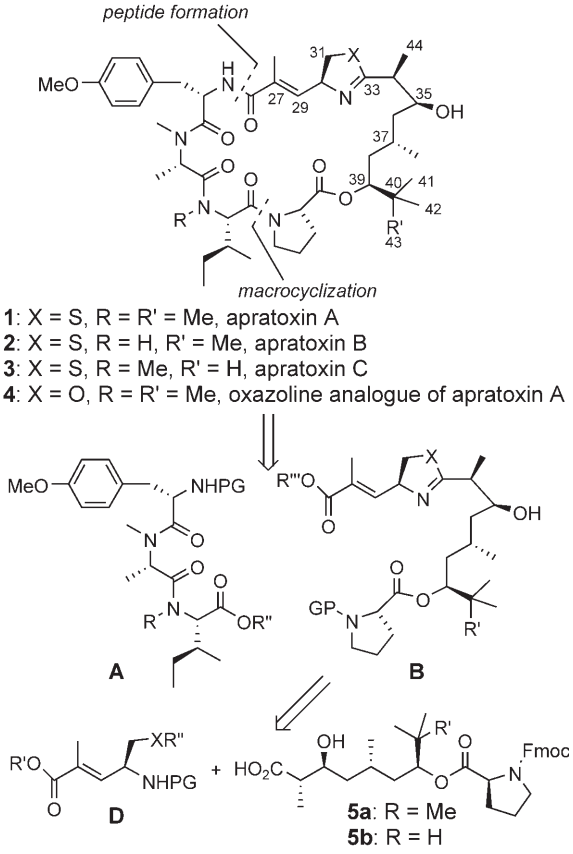
Supporting information for this article (experimental procedures for preparation of the intermediates for assembling **39**–**41**, and copies of ^{1}H NMR spectra for compounds **1**, **4**, and **39**–**41**) is available on the WWW under http://www.chemeurj.org/ or from the author.

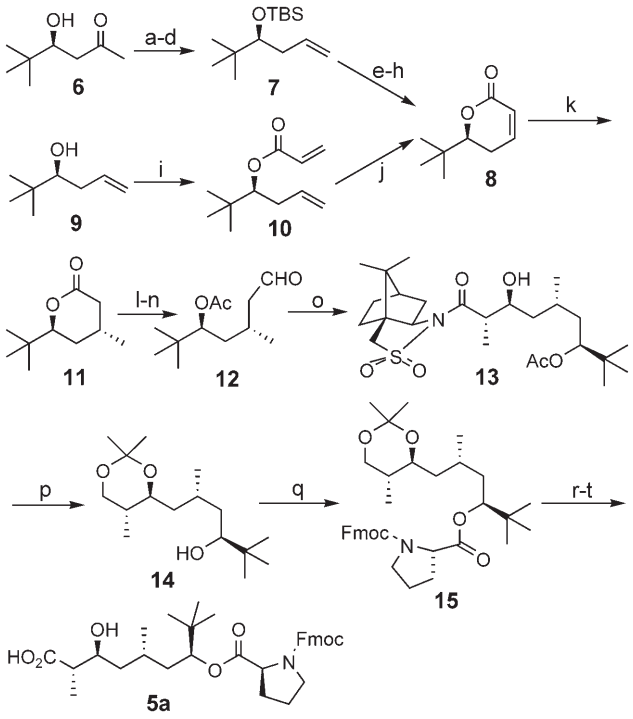


Scheme 1. Structures of apratoxins and an oxazoline analogue and their retrosynthetic analysis.

Results and Discussion

As outlined in Scheme 1, our synthetic strategy was based on the retrosynthetic degradation of apratoxin A into a tripeptide part (**A**) and a thiazoline unit (**B**). Further bond disconnection of **B** gave a cysteine-derived α,β-unsaturated ester (**D**) and an L-proline-based carboxylic acid (**5a**). One challenge for the assembly of intermediate **B** was the installation of its β-hydroxy-2,4-disubstituted thiazoline unit, as most existing methods for thiazoline ring elaboration are forbidden to this unstable moiety. Indeed, Forsyth and Chen's success in the total synthesis of apratoxin A was highly dependent on their newly developed methodology for preparing thiazoline rings.[4c] This problem prompted us to consider replacing the thiazoline ring with a more conveniently accessible oxazoline ring in further SAR studies.[7,8] Thus, if the modified compound **4** still retained potent cytotoxicity, not only would we have determined the importance of the thiazoline moiety for the cytotoxicity of apratoxin A, but we would also have facilitated future SAR studies.

The synthesis of apratoxin A (**1**) and its oxazoline analogue **4** began with the construction of their common proline-ketide ester **5a** as outlined in Scheme 2. As the starting material we chose β-hydroxy ketone **6** (>99% *ee*), a D-proline-catalyzed direct aldol reaction product that could be prepared on a large scale from trimethylacetaldehyde and



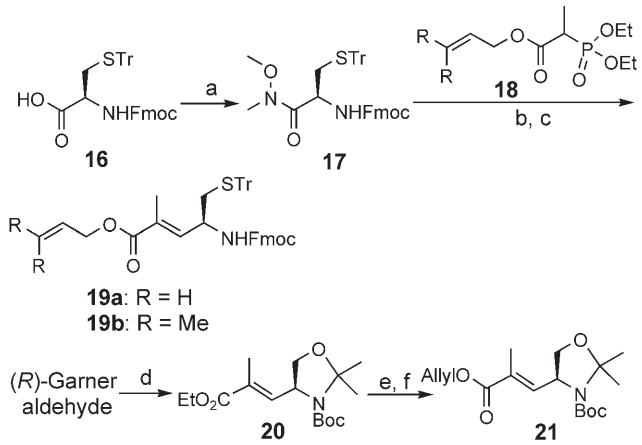
Scheme 2. Synthesis of the acid **5a**. a) TBSCl, imidazole; b) $NaBH_4$, MeOH; c) MsCl, Et_3N; d) *t*BuOK, 71% yield over four steps; e) O_3, Me_2S; f) $LiCH_2CO_2Et$; g) 40% HF; h) MsCl, Et_3N, 45% yield over four steps; i) CH_2=CHCOCl, Et_3N, 90%; j) 10 mol% Grubbs' catalyst, 80%; k) $Me_2(CuCN)Li_2$, 94%; l) $LiAlH_4$, THF; m) AcCl, pyridine, then K_2CO_3, MeOH; n) Dess–Martin oxidation, 69% yield over three steps; o) *N*-Propionylsultam, Et_2BOTf, *i*Pr_2NEt, then $TiCl_4$, 90%; p) $LiAlH_4$, THF, then DMP, PPTs, 67%; q) Fmoc-L-proline, 2,4,6-trichlorobenzoyl chloride, DMAP, 90%; r) TsOH, MeOH; s) TEMPO, NaClO; t) NaH_2PO_4, $NaClO_2$, 81% yield over three steps. TBS = *tert*-butyldimethylsilyl. DMAP = 4-dimethylaminopyridine; TEMPO = 2,2,6,6-tetramethylpiperidinyl-1-oxyl.

acetone.[9] Protection of **6** with TBSCl, followed by reduction of the ketone moiety with $NaBH_4$, produced an alcohol, which was subjected to elimination through its mesylate to afford allyl ether **7**. Ozonolysis of **7** provided an aldehyde, which was condensed with $LiCH_2CO_2Et$ at −78 °C, followed by cyclization and elimination, to give the α,β-unsaturated lactone **8**. We should note that this lactone was initially prepared by a shorter route, which employed asymmetric allylboration[10] of trimethylacetaldehyde and ring-closing metathesis as the key steps,[11] but that in the asymmetric allylboration step it was found that the desired product **9** was difficult to separate from the resulting isopinocampheol either by distillation or by column chromatography. This drawback, together with the high cost of Grubbs' first-generation catalyst for conversion of **10** into **8**, forced us to abandon this alternative route to **8**.

Methylcupration[12] of **8** with $Me_2Cu(CN)Li_2$ gave **11** in 94% yield as a single diastereomer as determined by ^{1}H NMR spectroscopy. Reduction of the lactone **11** with LAH, followed by protection of the resulting diol with acetic chloride, afforded a diester, which was treated with 1.2 equivalents of K_2CO_3 in methanol to liberate the pri-

mary alcohol selectively. The alcohol was then subjected to Dess–Martin oxidation to yield aldehyde **12**. For further carbon chain elongation, Oppolzer's methodology[13] for preparing *anti*-diols from bornanesultam-derived boryl enolates appeared particularly attractive. To this end, treatment of *N*-propionylsultam with Et_2BOTf resulted in a *Z*-enolate, which was treated with the aldehyde **12** in the presence of $TiCl_4$ to afford "*anti*"-aldol **13** in 90% yield as the only detectable diastereomer. It is noteworthy that use of eight equivalents of $TiCl_4$ was necessary to ensure the high yield. Next, removal of both the chiral auxiliary and the acyl group by LAH reduction gave a triol, which was protected with DMP to furnish alcohol **14** in 67% yield. Esterification of **14** with *N*-Fmoc-L-proline by Yamaguchi's procedure[14] produced **15** in 90% yield. Finally, treatment of **15** with TsOH in methanol to liberate its diol moiety, and subsequent selective oxidation of the resulting primary alcohol with the hindered chloro oxammonium salt generated from TEMPO/NaClO, afforded an aldehyde, which was subsequently oxidized with $NaClO_2$ to provide the desired acid **5a**.

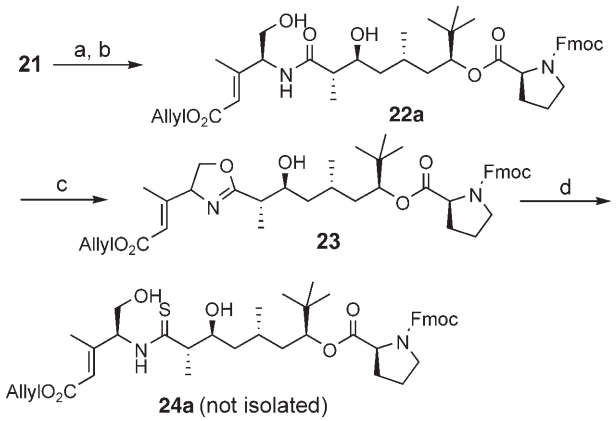
For installation of the modified cysteine or serine units in **1** or **4**, α,β-unsaturated esters **19** and **21** were prepared as depicted in Scheme 3. Weinreb amide **17** was synthesized



Scheme 3. Assembly of the α,β-unsaturated esters **19** and **21**. a) HNMeOMe, HATU, iPr_2NEt, 78%; b) $LiAlH_4$, THF; c) **18**, *n*-BuLi, then **17**, 30% yield over two steps; d) $Ph_3P{=}C(Me)CO_2Et$, 100%; e) aq. NaOH; f) allyl alcohol, EDCI, DMAP, 74% yield over two steps. HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide, EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

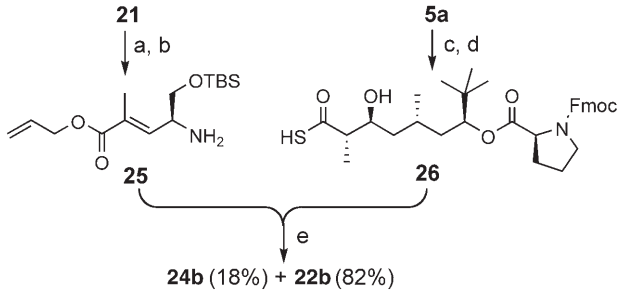
from protected D-cysteine, and reduction of **17** with LAH afforded an aldehyde, which was directly subjected to a Wittig–Horner reaction to provide **19**. Ethyl ester **20**, on the other hand, was obtained in quantitative yield from (*R*)-Garner aldehyde. Hydrolysis of **20** and esterification with allyl alcohol yielded **21**, in which the allyl ester is removable under neutral conditions.[15]

With the α,β-unsaturated esters **19** and **21** in hand, our next tasks were to connect them with the acid **5a**, and the subsequent installation of a thiazoline or oxazoline ring bearing an α,β-unsaturated ester side chain. Treatment of **21** with trifluoroacetic acid/water (3:1) liberated the amine, which was coupled with the acid **5a** in the presence of HATU and DIPEA to provide amide **22a** in 90% yield (Scheme 4). Cyclodehydration[16] of **15** with 2.5 equivalents



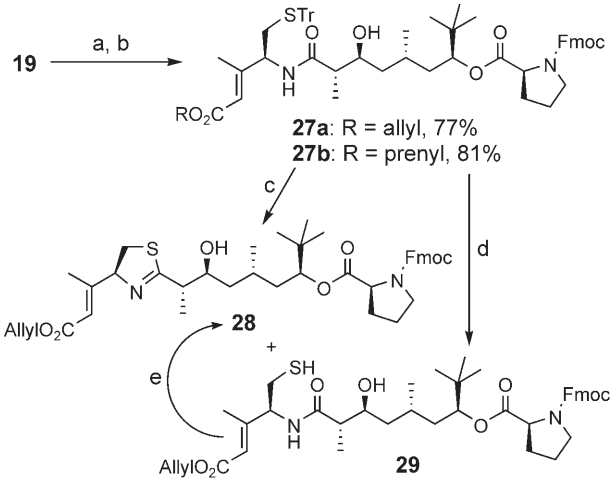
Scheme 4. Attempts to construct thioamide **24a**. a) CF_3CO_2H, H_2O, CH_2Cl_2; b) **5a**, HATU, iPr_2NEt, 90% yield over two steps; c) DAST, CH_2Cl_2, −78°C, 86%; d) H_2S, Et_3N, MeOH. DAST = (diethylamino)sulfur trifluoride.

of DAST in methylene chloride at −78°C proceeded smoothly, delivering oxazoline **23** in 86% yield. Formation of the required thiazoline intermediate, however, proved challenging as expected. Many existing methods were tried without success for this special case: Wipf's thiolysis/cyclodehydration strategy,[17] for example, failed to give thioamide **24a** because of Michael addition of H_2S to the α,β-unsaturated ester moiety. To solve this problem, we also tried to prepare **24a** through in situ generation of active thiono ester by a procedure reported by Hoeg-Jensen and co-workers.[18] Unfortunately, although this method did give some **24b**, the coupling yield was low, with the major product being amide **22b** (Scheme 5).



Scheme 5. Synthesis of thioamide **22b**. a) CF_3CO_2H, H_2O, CH_2Cl_2; b) TBSCl, iPr_2NEt, 90% yield over two steps; c) C_2F_5OH, EDCI, CH_2Cl_2; d) H_2S, iPr_2NEt, MeOH, 56% yield over two steps; e) PyBOP, iPr_2NEt, 30%. PyBOP = benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.

Having failed in the above attempts, we turned our attention to the assembly of the thiazoline intermediate by a tandem deprotection/cyclodehydration strategy reported by Kelly and co-workers.[19] Deprotection of **19** liberated amines, which were then condensed with **5a** in the presence of HATU to afford amides **27** (Scheme 6). Initially, five

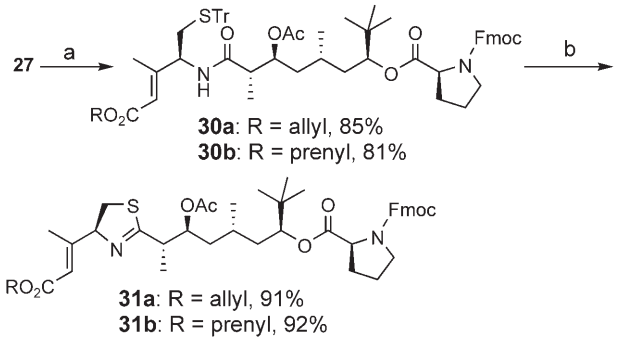


Scheme 6. Elaboration of the thiazoline intermediate **28**: a) Et_2NH, MeCN; b) **5a**, HATU, iPr_2NEt; c) 1.5 equiv $TiCl_4$, 15%; d) CF_3CO_2H, Et_3SiH, CH_2Cl_2; e) 1.5 equiv $TiCl_4$, 68%.

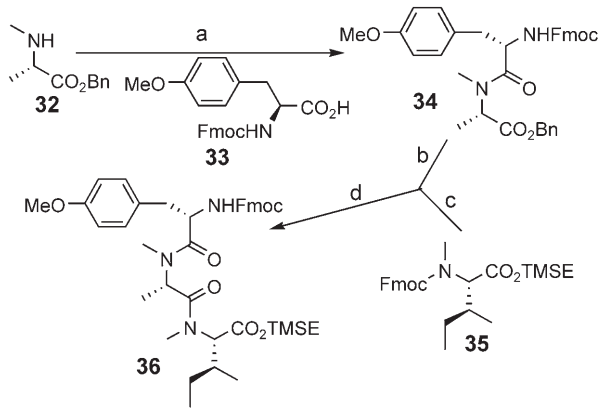
equivalents of $TiCl_4$ were used to induce deprotection/cyclodehydration, but this gave a complex mixture as determined by TLC, with no desired product **28**. We then tried the use of varying amounts of $TiCl_4$ and found that when 1.5 equivalents of $TiCl_4$ were used, thiazoline **28** could be isolated in less than 15% yield, together with the deprotection product **29** in about 30% yield. As a result, we decided to run the deprotection reaction first, followed by cyclodehydration. Treatment of **27a** with TFA thus produced thiol **29**, which was treated with 1.5 equivalents of $TiCl_4$ to provide **28** in 68% yield. Unfortunately, this route to the key thiazoline intermediate was found to be suitable only for small-scale preparations, and much lower yields were obtained if this reaction was run on scales over 100 mg. The underlying cause of this difference remains unclear; attempts to switch the Lewis acids from $TiCl_4$ to PCl_5 and $F_3B{\cdot}OEt_2$ failed to give any desired product **28**.

At that point Kelly's new report on biomimetic synthesis of thiazolines appeared, offering a potential solution to the problem,[20] and we were pleased to find that treatment of **30**, the acylation products of **27**, with Ph_3PO/Tf_2O provided thiazoline intermediates **31** in excellent yields (Scheme 7).

For the synthesis of the highly methylated tripeptide fragment, 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP), an efficient coupling reagent for hindered peptide synthesis developed by Xu and Li,[21] was employed. As shown in Scheme 8, BEP-mediated coupling of **32** with *N*-Fmoc-*O*-methyl-L-tyrosine (**33**) gave dipeptide **34** in 55% yield. Pd/C-catalyzed hydrogenolysis of **34** afforded an acid, which



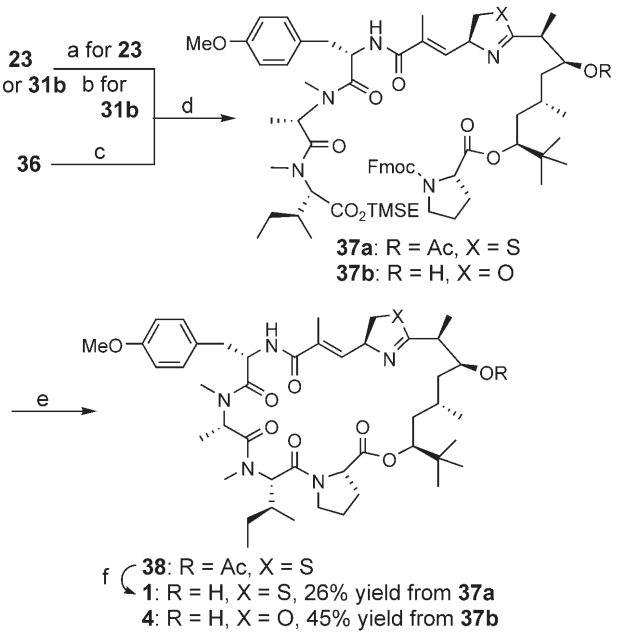
Scheme 7. Assembly of the thiazoline intermediate **31**: a) AcCl, DMAP; b) Ph_3PO, Tf_2O, 0°C.



Scheme 8. Preparation of the tripeptide **36**. a) BEP, iPr_2NEt, 55%; b) Pd/C, H_2, EtOAc; c) Et_2NH, MeCN; d) BEP, iPr_2NEt, 60% yield from **34**. BEP = 2-bromo-1-ethyl-pyridinium tetrafluoroborate.

was connected with a liberated amine from **35** to furnish tripeptide **36** in 60% yield.

With the required coupling components in hand, we followed our strategy to create the macrocycle. As outlined in Scheme 9, the allyl protecting group in **23** was removed with the aid of $[Pd(PPh_3)_4]$ catalysis in the presence of *N*-methylaniline to give an acid,[15] which was coupled with a liberated amine from **36** to provide **37b** in 59% yield. The utilization of *N*-methylaniline was essential, as other bases employed (such as morphine) caused the simultaneous cleavage of the *N*-Fmoc protecting group during the deprotection reaction. This procedure was unsuccessful for deallylation of thiazoline **31a**, however, so **31b**, a prenyl-protected intermediate,[22] was selected for further conversion. To our delight, treatment of **31b** with TMSOTf produced a good yield of the desired deprotection product, which was connected with the liberated amine from **36** to afford amide **37a**. Cleavage of the Fmoc and TMSE protecting groups in **37** was accomplished by treatment with TBAF/THF to give cyclization precursors. Macrocyclization of masked amino acids **37a** and **37b** with HATU/DIPEA in dilute methyl chloride afforded **38** and **4**, respectively, and removal of the acyl group in **38** with KCN in ethanol afforded apratoxin A.



Scheme 9. Assembly of apratoxin A and its oxazoline analogue. a) [Pd(PPh_3)$_4$]; b) TMSOTf; c) Et_2NH; d) HATU, iPr_2NEt, 50% yield for **37a** from **31b**, 59% yield for **37b** from **23**; e) TBAF, THF, then HATU, iPr_2NEt, CH_2Cl_2 (0.002 M); f) KCN, EtOH, 50 °C. TBAF = tetrabutylammonium fluoride.

The synthetic apratoxin A (**1**) and its oxazoline analogue **4** were tested for cytotoxicity against human cervical cancer cell line HeLa. Both compounds exhibited potent inhibitory effects on the proliferation of HeLa, with IC_{50} values of 2.2 and 9.7 nM, respectively (Table 1). Further analysis revealed

Table 1. Apratoxin A and its analogues inhibit HeLa cell proliferation.

Compound	Potency (IC_{50}, nM)
1	2.2 ± 0.2
4	9.7 ± 1.4
39	920 ± 18
40	>10000
41	>10000

that both synthetic apratoxin A and its oxazoline analogue inhibited cell proliferation by causing cell cycle arrest in the G1 phase (Figure 1), so the cell cycle distribution of HeLa cells was followed by fluorescence-activated cell sorting (FACS) after staining of cells with the DNA-intercalating dye propidium iodide. Upon treatment with either compound for 24 h, more HeLa cells accumulate at the G1 phase of the cell cycle, with corresponding decreases in cells at the S or G2/M phases of the cell cycle (Table 2 and Figure 1 B, C, vs. A). Prolonged treatment of HeLa cells with the compounds for 48 h resulted in more pronounced cell cycle arrest in G1 (Figure 1 E, F, vs. D). The cell cycle effect of apratoxin A and its analogue suggests that they are likely to work by specifically interfering with the function of a target required for cell cycle progression of HeLa and probably other cells.[23]

These results also indicated that the replacement of the thiazoline ring of apratoxin A with an oxazoline ring had only a marginal effect on potency, which prompted us to design and synthesize more simplified analogues of apratoxin A containing the more accessible oxazoline ring for a preliminary structure–activity relationship study. To this end, the apratoxin C oxazoline analogue **39**, its C(37)-epimer **40**, and the C(37)-demethylated analogue **41** (Scheme 10), became our new synthetic targets. These could be assembled from more conveniently available aldehydes **42**, by the same process as used for elaborating **4**.

As shown in Scheme 11, we synthesized the requisite aldehydes **42a** and **42b** from ketone **43**, another product of a D-proline-catalyzed direct aldol reaction,[9] as the starting material. Protection of **43** with TBSCl, followed by a Peterson reaction, provided olefin **44** as an inseparable mixture of *cis* and *trans* isomers.[24] Treatment of **44** with 40% HF in MeCN afforded *trans* olefin **45** and lactone **46**, and hydrogenation and subsequent LAH reduction of **45** gave a separable mixture of alcohol **47a** and its epimer. The latter compound was also obtained from **46** as a single product by the same reaction sequence. Protection of **47** with acetyl chloride produced diesters, which were selectively hydrolyzed, and the resulting primary alcohols were then oxidized to furnish **42** in 81% yield. For construction of aldehyde **42c**, ring-opening of epoxide **48** with a lithium reagent generated from 1-benzyloxyprop-2-yne with the assistance of boron trifluoride diethyl etherate was conducted to give alcohol **49**. After **49** had been masked with an acetyl group, hydrogenation was carried out to provide alcohol **50**, and Dess–Martin oxidation of **50** furnished **42c** in 93% yield. By the same procedure used for preparing **4** from aldehyde **12**, the three aldehydes **42a–c** were converted into **39**–**41**, respectively.

We next determined the biological activities of analogues **39**, **40**, and **41** in the HeLa cell proliferation assay. Surprisingly, **39** was almost 100 times less active than its structurally closely related analogue **4** (see Table 1), indicating that the methyl group at C(40) is important for the biological activity in oxazoline analogues of apratoxin A. This result is distinct from what has been observed in the natural apratoxin family, because apratoxins A and C have almost identical potencies.[3] This difference might be the result of a conformation change caused by some subtle structural variation, whilst it is noteworthy that in Wipf's report, replacement of thiazoline with oxazoline resulted in a significant decrease in cytotoxicity in SAR studies of lissoclinamide 7.[25] In addition, when the stereochemistry at C(37) was inverted, the resulting analogue **40** was inactive even at 10000 nM, whilst a similar loss of activity was seen when the C(37) methyl group was removed in analogue **41**. Together, these results suggest that the two methyl groups at C(37) and C(40), as well as the stereochemistry at C(37), are essential for the cellular activity of the oxazoline analogue of apratoxin A, probably because of their intimate involvement in binding

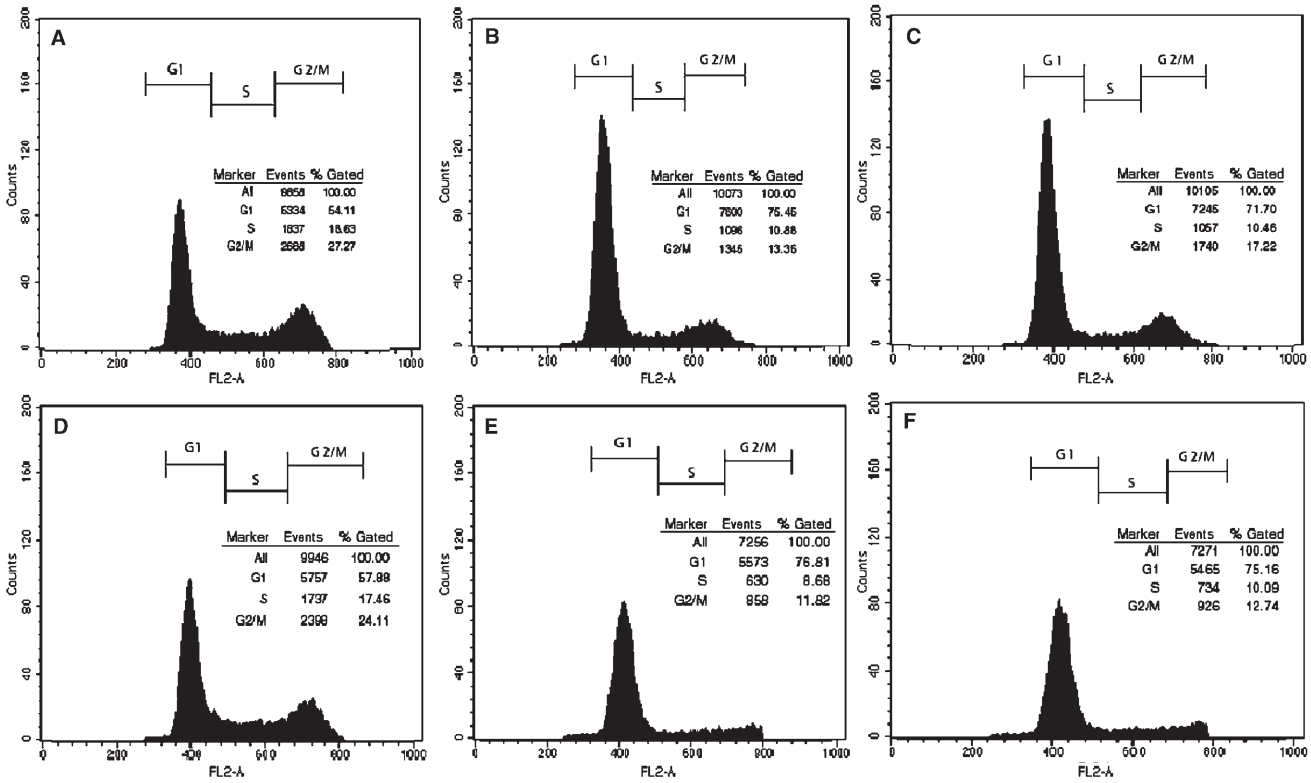


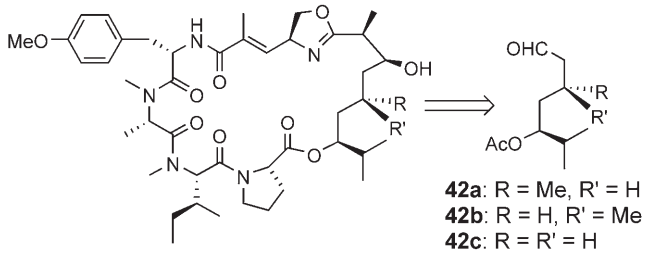
Figure 1. Cell cycle effects of apratoxin A and its oxazoline analogue. A representative HeLa cell cycle profile is shown with 24 h treatment of vehicle control (**A**). Significant increases in G1 cell populations were observed for **4** (**B**) and **1** (**C**) with corresponding decreases in both S phase and G2/M phase cell populations. When cells were incubated for 48 h, the profile of vehicle remains the same (**D**), whereas only G1 cell populations remained for **4** (**E**) and **1** (**F**) treatments. 50 nM final concentrations of either compound were used.

Table 2. Percentages of cell populations in G1, S, and G2/M phases when treated with compounds **1** and **4**.[a]

Compound	G1	S	G2/M
DMSO	54.11 (57.88)	18.83 (17.46)	27.27 (24.11)
1	71.70 (75.16)	10.46 (10.09)	17.22 (12.74)
4	75.45 (76.81)	10.88 (8.68)	13.35 (11.82)

[a] Treatment with 50 nM compounds for 24 h. The data in parentheses were obtained by treatment of these compounds for 48 h.

to the molecular target responsible for cell cycle inhibition by apratoxin A.



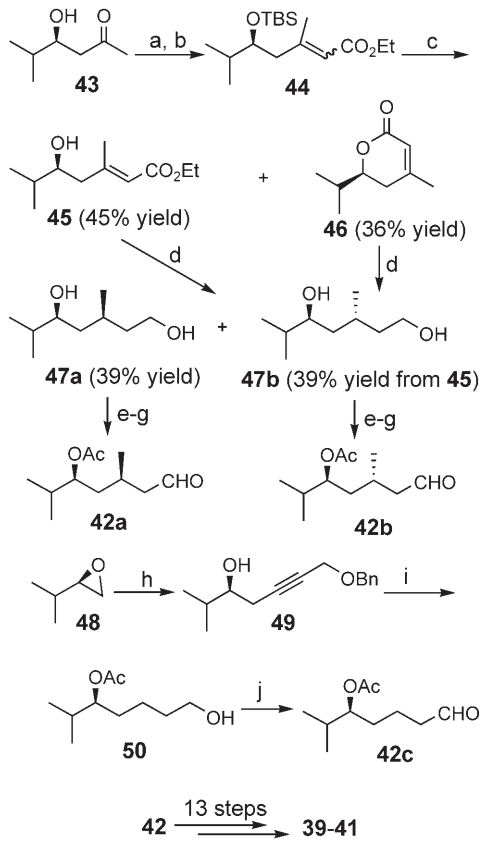
42a: R = Me, R' = H
42b: R = H, R' = Me
42c: R = R' = H

39: R = Me, R' = H, oxazoline analogue of apratoxin C
40: R = H, R' = Me
41: R = R' = H

Scheme 10. Structures of apratoxin C analogues and their retrosynthetic analysis.

Conclusion

In summary, we have devised a new synthetic route for the total synthesis of apratoxin A. Novel elements of the synthesis include the concise assembly of its polyketide acid segment through two asymmetric aldol reactions and the elaboration of its thiazoline unit by Kelly's biomimetic method. Several oxazoline analogues were elaborated by a similar approach, and compound **4** was found to be nearly as potent as apratoxin A in inhibiting proliferation of HeLa cells. Preliminary investigations revealed that both apratoxin A and the analogue **4** inhibit cell proliferation by blocking the progression of cell cycle at the G1 phase. Since the oxazoline analogue **4** could be more easily assembled, it may serve as a more expedient probe with which to explore the biological function of apratoxin A. Moreover, the use of the synthetic analogues of apratoxin A identified essential structural elements that are important for the biological activity, which should guide future design and synthesis of bioprobes of apratoxin A for the identification of its molecular target and for the improvement of its biological activity.



Scheme 11. Synthesis of oxazoline analogues **39**–**41**. a) TBSCl, imidazole; b) $TMSCH_2CO_2Et$, LDA, −78 °C, 90 % yield over two steps; c) 40 % HF, MeCN; d) Pd/C, H_2, then $LiAlH_4$; e) AcCl, pyridine; f) Aq. K_2CO_3, MeOH; g) Dess–Martin oxidation, 80 % yield over three steps; h) *n*BuLi, 3-benzyloxyprop-1-yne, then $F_3B{\cdot}OEt_2$, 96 %; i) AcCl, pyridine, then Pd/C, H_2, 86 %; j) Dess–Martin oxidation, 93 %.

Experimental Section

Compound 7: Imidazole (12.2 g, 179 mmol) and *tert*-butyldimethylsilyl chloride (14.8 g, 91 mmol) were added to a stirred solution of **6** (8.5 g, 59 mmol) in DMF (12 mL). The resulting solution was stirred for 24 h, and was then quenched with methanol (25 mL) and water (200 mL). The mixture was extracted with ethyl acetate, the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo, and the resulting oil was purified by chromatography (elution with ethyl acetate/petroleum ether 1:10) to give 15 g of silyl ether **7**.

$NaBH_4$ (4.4 g, 116 mmol) was added at 0 °C to a solution of the above silyl ether (15 g, 58 mmol) in methanol (150 mL) and the resulting solution was allowed to stir for 0.5 h. The solution was concentrated and then diluted with water (200 mL), the mixture was extracted with ethyl acetate, and the organic layers were washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo to give the crude alcohol. This was dissolved in dry CH_2Cl_2 (200 mL), after which Et_3N (16 mL, 116 mmol) and MsCl (9 mL, 116 mmol) were added successively at 0 °C under nitrogen atmosphere. Two hours later the reaction was quenched with brine and the organic layer was washed with water, dried over Na_2SO_4, and concentrated to give the crude mesylate.

*t*BuOK (21.3 g, 189 mmol) was added to a solution of the above mesylate in toluene (200 mL), and the resulting mixture was heated at reflux for 1 h and then allowed to cool to room temperature. After the reaction had been quenched with water, the organic layer was separated, the aqueous layer was extracted with *n*-heptane, and the combined organic layers were washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo. The residual oil was purified by chromatography (elution with pure petroleum ether) to give **7** (10 g, 71 % yield from **6**) as a pale yellow oil. $[\alpha]_D^{20} = +3.8$ (c = 12.2 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.90 (m, 1H), 5.03 (m, 2H), 3.35 (m, 1H), 2.41 (m, 1H), 2.20 (m, 1H), 0.92 (s, 9H), 0.88 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H) ppm; IR (film): $\tilde{\nu}_{max}$ = 2958, 2931, 1394, 1363 cm^{-1}; EI-MS: m/z: 227 $[M-CH_3]^+$, 185 $[M-t\text{-Bu}]^+$; elemental analysis calcd (%) for $C_{14}H_{30}SiO$: C 69.35, H 12.47; found: C 68.88, H 12.21.

Compound 8: A solution of **7** (6 g, 25 mmol) in dichloromethane (100 mL) was cooled to −78 °C and treated with ozone until the solution became blue in color. The excess ozone was removed in a stream of N_2, the resulting mixture was allowed to warm to room temperature, and then Me_2S (4 mL) was added. The solution was kept at 40 °C for 24 h and was then cooled and washed with water. The separated organic layer was dried over Na_2SO_4 and concentrated in vacuo to give the aldehyde as a yellow oil, which was used in the next step without further purification.

n-Butyllithium (1.6 M in hexane, 15.6 mL, 25 mmol) was added dropwise at 0 °C under N_2 to a stirred solution of diisopropylamine (3.5 mL, 25 mmol) in dry THF (80 mL). The solution was stirred for 15 min, the temperature was lowered to −78 °C, and dry ethyl acetate (2.5 mL, 25.5 mmol) was added dropwise. The solution was stirred for 20 min and a solution of above crude aldehyde in THF was added slowly. One hour later, the solution was allowed to warm to room temperature and quenched with saturated NH_4Cl solution. The separated organic layer was washed with brine and water, dried over Na_2SO_4, and concentrated to give the crude alcohol, which was dissolved in acetonitrile containing a suitable amount (5–30 %) of aqueous HF (40 %). The resulting solution was stirred at room temperature until the starting material had disappeared, as monitored by TLC. The solution was concentrated in vacuo, diluted with ethyl acetate, and washed with saturated $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated to give the crude hydroxy lactone.

Et_3N (7 mL, 50 mmol), followed by MsCl (4.9 mL, 50 mmol), were added to a solution of the above lactone in dichloromethane (50 mL). The resulting solution was stirred for 2 h and was then quenched with water and separated. The aqueous layer was extracted three times with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The resulting oil was purified by chromatography (elution with ethyl acetate/petroleum ether 1:4) to give **8** (1.75 g, 45 % yield from **7**). $[\alpha]_D^{20} = -129$ (c = 0.51 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.95 (m, 1H), 6.05 (m, 1H), 4.11 (m, 1H), 2.40 (m, 2H), 1.03 (m, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.77, 145.44, 121.09, 85.22, 33.78, 25.42, 24.53 ppm; IR (film): $\tilde{\nu}_{max}$ = 3474, 1719, 1482, 1383 cm^{-1}; EI-MS: m/z: 153 $[M-H]^+$, 149; HRMS: m/z: calcd for $C_9H_{14}O_2$: 154.0994; found 154.1006 $[M]^+$.

Compound 11: Methyllithium (1.6 M solution in diethyl ether, 33 mL, 52.8 mmol) was added under nitrogen at −78 °C to a suspension of CuCN (2.4 g, 26.4 mmol) in dry ether (100 mL). The mixture was stirred for 2 h at this temperature, after which a solution of **8** (2.7 g, 17.8 mol) in ether was added slowly. The resulting mixture was stirred for 1 h and was then quenched with aqueous $FeCl_3$ solution, the organic layer was washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo, and the residual oil was purified by chromatography (elution with ethyl acetate/petroleum ether 1:10) to give **11** (2.84 g, 94 %). $[\alpha]_D^{20} = +46.5$ (c = 0.3 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.00 (dd, J = 11.7, 3.9 Hz, 1H), 2.55 (m, 1H), 2.20 (m, 2H), 1.80 (m, 1H), 1.53 (m, 1H), 1.10 (d, J = 6.0 Hz, 3H), 1.00 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.07, 83.57, 36.89, 33.84, 29.70, 25.33, 23.95, 21.11 ppm; IR (film): $\tilde{\nu}_{max}$ = 1733, 1481, 1398, 1367, 1073 cm^{-1}; EI-MS: m/z: 170 $[M]^+$, 155, 127, 43; HRMS: m/z: calcd for $C_{10}H_{18}O_2$: 170.1307; found 170.1309 $[M]^+$.

Compound 12: A solution of the lactone **11** (2.6 g, 15.3 mmol) in dry THF (10 mL) was added dropwise under nitrogen to a boiling THF (70 mL) suspension of $LiAlH_4$ (1.0 g, 30 mmol). After having been heated at reflux for 1 h, the reaction mixture was allowed to cool to room temperature and quenched by sequential slow addition of water (1 mL), aqueous NaOH solution (15 %, 1 mL), and water (1 mL). After stirring for 2 h, the white solid was filtered and the filtrate was concentrated to give the crude diol, which was dissolved in dichloromethane

(50 mL). Pyridine (5 mL, 62 mmol) was added at 0 °C to this solution, followed by AcCl (4.4 mL, 62 mmol). The solution was allowed to stir overnight and was then quenched with water and separated, the aqueous layer was extracted three times with CH_2Cl_2, the combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was dissolved in methanol (40 mL) and water (10 mL), after which K_2CO_3 (2.2 g, 15.9 mmol) was added. The resulting mixture was stirred for 5 h and then concentrated in vacuo to remove the methanol, and the resulting mixture was diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated to give the monoalcohol.

Dess–Martin reagent (7.1 g, 16.8 mmol) was added at 0 °C to a solution of the above alcohol in dry CH_2Cl_2 (60 mL). The mixture was stirred for 3 h and quenched with sat. $Na_2S_2O_3$, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo, and the resulting oil was purified by chromatography (elution with ethyl acetate/petroleum ether 1:10) to give **12** (2.3 g, 69% yield from **8**). $[\alpha]_D^{20} = -16$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.7$ (t, $J = 1.2$ Hz, 1H), 4.79 (dd, $J = 7.8$, 3.6 Hz 1H), 2.67 (dd, $J = 16.2$, 3.6 Hz, 1H), 2.21–2.10 (m, 1H), 2.08 (s, 3H), 2.01–1.92 (m, 1H), 1.51 (m, 2H), 0.97 (d, $J = 6.6$ Hz, 3H), 0.89 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 202.5$, 171.2, 78.2, 49.4, 36.6, 34.6, 25.9, 25.1, 21.2, 21.0 ppm; IR (film): $\tilde{\nu}_{max} = 2877$, 1733, 1710, 1481, 1373 cm^{-1}; EI-MS: m/z: 171 $[M-Ac]^+$, 153, 113, 43; HRMS: m/z: calcd for $C_{10}H_{19}O_2$: 171.1385; found 171.1385 $[M-Ac]^+$.

Compound 13: CF_3SO_3H (1.3 mL, 15 mmol) was added at room temperature under nitrogen to Et_3B (1.0 M solution in hexane, 15 mL, 15 mmol) and the mixture was stirred at 40 °C until gas evolution had ceased. A solution of *N*-propionylsultam (1.7 g, 6.2 mmol) in CH_2Cl_2 (10 mL) and DIPEA (2.7 mL, 15.9 mmol) were added successively at −10 °C and the stirring was continued for 30 min. The resulting boryl enolate solution was cooled to −78 °C, and a precooled (−78 °C) solution of the aldehyde **12** (2.66 g, 12.4 mmol) and $TiCl_4$ (5.5 mL, 50 mmol) in CH_2Cl_2 (80 mL) was added by cannula. The resulting mixture was stirred for 2 h and quenched with saturated NH_4Cl. After the water phase had been extracted with CH_2Cl_2, the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:5) to give **13** as a white solid (2.8 g, 90%). $[\alpha]_D^{20} = -70.5$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.78$ (dd, $J = 10.8$, 1.8 Hz, 1H), 3.92 (dd, $J = 7.8$, 5.1 Hz, 1H), 3.71 (m, 1H), 3.52 (q, $J = 13.8$ Hz, 2H), 3.15 (t, $J = 6.6$ Hz, 1H), 2.33 (d, $J = 10.2$ Hz), 2.18 (m, 1H), 2.12 (t, $J = 6.9$ Hz, 1H), 2.05 (s, 3H), 1.91 (m, 3H), 1.65 (s, 3H), 1.51–1.30 (m, 4H), 1.24 (s, 3H), 1.20 (d, $J = 6.9$ Hz, 3H), 0.98 (s, 3H), 0.92 (d, $J = 6.6$ Hz, 3H), 0.88 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 175.1$, 171.6, 78.1, 73.1, 65.4, 53.2, 48.3, 47.8, 46.5, 44.8, 40.8, 38.4, 37.5, 34.3, 33.0, 26.5, 26.0, 25.2, 21.1, 20.9, 20.6, 19.9, 13.9 ppm; IR (KBr): $\tilde{\nu}_{max} = 3528$, 2962, 1727, 1696, 1481 cm^{-1}; ESI-MS: m/z: 486.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{25}H_{43}NO_6S$: C 61.82, H 8.92, N 2.88; found: C 61.94, H 9.20, N 2.62.

Compound 14: A solution of **13** (2.8 g, 5.8 mmol) in dry THF (5 mL) was added under nitrogen to a suspension of $LiAlH_4$ (0.5 g, 15 mmol) in THF (30 mL) at reflux. After having been heated at reflux for 0.5 h, the reaction mixture was allowed to cool to room temperature and quenched by slow sequential addition of water (0.5 mL), aqueous NaOH (15%, 0.5 mL), and water (0.5 mL). The white solid was filtered and the filtrate was concentrated to give the crude triol.

PPTs (200 mg) was added to a solution of the above triol in 2,2-dimethoxypropane (30 mL). The resulting mixture was stirred for 24 h and was then diluted with ether and washed with water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, and the residual oil was purified by chromatography (elution with ethyl acetate/petroleum ether 1:8) to give **14** (1.05 g, 67% yield from **13**). $[\alpha]_D^{20} = -60$ ($c = 0.88$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.79$ (dd, $J = 12.0$, 5.1 Hz, 1H), 3.50 (t, $J = 11.4$ Hz, 2H), 3.35 (d, $J = 9.0$ Hz, 1H), 2.41 (m, 1H), 1.83 (m, 1H), 1.51 (m, 1H), 1.45 (s, 3H), 1.40 (s, 3H), 1.39–1.28 (m, 3H), 0.98 (d, $J = 6.9$ Hz, 3H), 0.90 (s, 9H), 0.75 (d, $J = 6.6$ Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 98.2$, 75.8, 73.9, 66.0, 38.7, 38.2, 34.6, 34.4, 29.5, 26.1, 25.7, 21.5, 19.0, 12.7 ppm; IR (film): $\tilde{\nu}_{max} = 3497$, 2955, 2871, 1464 cm^{-1}; EI-MS: m/z: 257 $[M-CH_3]^+$, 215, 157, 59; HRMS: m/z: calcd for $C_{15}H_{29}O_3$: 257.2117; found 257.2134 $[M-CH_3]^+$.

Compound 15: 2,4,6-Trichlorobenzoyl chloride (0.17 mL, 1.08 mmol) was added under nitrogen to a suspension of *N*-Fmoc-L-proline (250 mg, 0.74 mmol) in benzene (1 mL), followed by DIPEA (0.18 mL, 1.08 mmol). A solution of **14** (200 mg, 0.74 mmol) in benzene (0.5 mL) was added to the resulting solution, after which DMAP (180 mg, 1.5 mmol) was added in one portion. The mixture was stirred overnight, diluted with ethyl acetate, and washed with water, the organic layer was dried over Na_2SO_4 and concentrated in vacuo, and the residual oil was purified by chromatography (elution with ethyl acetate/petroleum ether 1:10) to give **15** as a white foam (391 mg, 90%). $[\alpha]_D^{20} = -71$ ($c = 1.07$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.77$ (m, 2H), 7.61 (m, 2H), 7.45–7.25 (m, 4H), 4.92 (m, 1H), 4.58–4.48 (m, 1H), 4.44–4.25 (m, 2H), 4.20 (m, 1H), 3.80–3.41 (m, 6H), 2.41–2.15 (m, 2H), 2.12–1.91 (m, 2H), 1.80–1.53 (m, 2H), 1.41 (s, 3H), 1.38–1.32 (m, 3H), 1.25 (s, 3H), 1.23–1.10 (m, 1H), 1.00–0.95 (m, 1H), 0.87 (s, 9H), 0.83–0.67 (m, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 172.0$, 154.3, 144.1, 143.9, 141.2, 127.6, 127.0, 125.1, 119.8, 97.8, 79.5, 72.4, 72.2, 67.6, 67.3, 66.1, 59.4, 47.1, 46.8, 46.2, 38.8, 37.8, 34.9, 34.4, 31.0, 29.6, 25.8, 24.2, 23.2, 20.2, 18.9, 12.6 ppm; IR (film): $\tilde{\nu}_{max} = 1710$, 1417, 1198 cm^{-1}; ESI-MS: m/z: 592 $[M+H]^+$, 609 $[M+NH_4]^+$; HRMS: m/z: calcd for $C_{36}H_{49}NO_6Na$: 614.3452; found 614.3457 $[M+Na]^+$.

Compound 5a: TsOH (129 mg, 0.676 mmol) was added to a solution of **15** (200 mg, 0.338 mmol) in methanol (5 mL). The resulting solution was stirred overnight and concentrated to remove the solvent, and the residue was diluted with ethyl acetate and washed sequentially with sat. $NaHCO_3$, brine, and water. The organic layer was dried over Na_2SO_4 and concentrated to give the crude diol.

2,2,6,6-Tetramethylpiperidinyloxyl (10 mg) and KBr (10 mg) were added at 0 °C to a two-phase mixture of the above crude diol product in CH_2Cl_2 (4 mL) and saturated $NaHCO_3$ (2 mL). The mixture was stirred vigorously, and NaClO (0.1 M solution in water, 4 mL, 0.40 mmol) was added. Stirring was continued for 1 h and the reaction mixture was quenched with saturated $Na_2S_2O_3$ and extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated to give the crude aldehyde.

A solution of $NaClO_2$ (123 mg, 1.35 mmol) and NaH_2PO_4 (81 mg, 0.676 mmol) in H_2O (1.5 mL) was added to a solution of the above crude aldehyde in *t*-BuOH (3 mL) and 2-methylbutene (1.5 mL). The mixture was stirred for 1 h and extracted with ethyl acetate, the organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:1) to give **5a** as a colorless solid (155 mg, 81% yield from **15**). $[\alpha]_D^{20} = -49.5$ ($c = 1.15$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.77$ (d, $J = 7.2$ Hz, 2H), 7.62 (dd, $J = 10.8$, 7.2 Hz, 2H), 7.41 (t, $J = 7.5$ Hz, 2H), 7.33 (t, $J = 7.5$ Hz, 2H), 4.92 (d, $J = 10.5$ Hz, 1H), 4.44 (dd, $J = 9.9$, 6.6 Hz, 1H), 4.38–4.31 (m, 2H), 4.29 (d, $J = 6.6$ Hz, 1H), 3.82 (m, 1H), 3.71–3.61 (m, 1H), 3.57–3.49 (m, 1H), 2.45 (t, $J = 7.2$ Hz, 1H), 2.35–2.25 (m, 1H), 2.11–1.91 (m, 4H), 1.72 (m, 2H), 1.35–1.25 (m, 3H), 1.19 (d, $J = 9.0$ Hz, 3H), 0.99 (d, $J = 6.3$ Hz, 3H), 0.91 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 178.77$, 172.53, 155.15, 144.03, 143.74, 141.31, 142.28, 127.67, 127.15, 127.10, 125.24, 125.14, 119.91, 78.34, 70.56, 67.77, 59.49, 47.12, 46.65, 46.56, 38.84, 37.38, 34.72, 29.91, 29.72, 26.01, 25.96, 24.80. 24.50, 20.32, 20.18, 13.41 ppm; IR (KBr): $\tilde{\nu}_{max} = 3477$ (br), 2967, 1741, 1687, 1453 cm^{-1}; ESI-MS: m/z: 566 $[M+H]^+$; HRMS: m/z: calcd for $C_{33}H_{43}NO_7Na$: 588.2932; found 588.2908 $[M+Na]^+$.

Compound 17: Weinreb amine (1.35 g, 13.8 mmol), EDCI (2.91 g, 15.2 mmol), HOBt (2.05 g, 15.2 mmol), and DIPEA (4.7 g, 27.6 mmol) were added successively to a solution of acid **16** (8.08 g, 13.8 mmol) in dry CH_2Cl_2 (20 mL). The solution was stirred overnight and was then washed with brine, the organic layers were dried over Na_2SO_4 and concentrated, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:5) to give **17** (6.76 g, 78%). $[\alpha]_D^{20} = +13.1$ ($c = 2.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.78$ (d, $J = 6.9$ Hz, 2H), 7.62 (m, 2H), 7.40 (m, 9H), 7.33–7.18 (m, 10H), 5.40 (d, $J = 9.0$ Hz, 1H), 4.80 (m, 1H), 4.19 (m, 2H), 4.10 (m, 1H), 3.65 (s, 3H),

3.19 (s, 3H), 2.62 (dd, J = 4.5, 12.3 Hz, 1H), 2.46 (dd, J = 7.8, 12.0 Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.72, 144.36, 143.83, 143.71, 141.21, 141.18, 129.52, 127.88, 127.62, 127.03, 126.99, 126.72, 125.14, 125.12, 119.86, 67.02, 66.84, 61.49, 50.15, 47.04, 33.95 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3303, 3058, 2939, 1723, 1660, 1446 cm^{-1}; ESI-MS: m/z: 651 $[M+Na]^+$; HRMS: m/z: calcd for $C_{39}H_{36}N_2O_4SNa$: 651.2288; found 651.2284 $[M+Na]^+$.

Compound 19b: A solution of **17** (0.7 g, 1.1 mmol) in dry THF (5 mL) was cooled to −78 °C under nitrogen, and $LiAlH_4$ (0.08 g, 2.1 mmol) was then added. After the solution had been stirred for 2 h, water (0.1 mL) was added carefully at −78 °C. After the reaction mixture had been allowed to warm to room temperature, it was quenched by sequential slow addition of aqueous NaOH (15%, 0.1 mL) and water (0.1 mL). The white solid was filtered off and the filtrate was concentrated to give the crude aldehyde.

n-Butyllithium (1.6 M in hexane, 0.66 mL, 1.06 mmol) was added dropwise at −78 °C under N_2 to a stirred solution of **18** (293 mg, 1.05 mmol) in dry toluene (2 mL). The resulting solution was stirred for 0.5 h, after which a solution of the above crude aldehyde in toluene (2 mL) was added. After it had been stirred at −78 °C for 1 h, the solution was allowed to warm to room temperature and was then quenched with saturated NH_4Cl solution. The separated organic layer was washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:10) to give **19b** (220 mg, 30% yield from **17**). $[\alpha]_D^{20}$ = −5.9 (c = 0.8 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.70 (m, 2H), 7.58 (m, 2H), 7.45–7.20 (m, 19H), 6.4 (m, 1H), 5.38 (m, 1H), 4.80–4.60 (m, 3H), 4.40 (m, 3H), 2.45 (m, 2H), 1.78 (s, 3H), 1.72 (s, 3H), 1.25 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.47, 155.34, 144.39, 143.76, 141.23, 139.09, 138.72, 129.51, 127.94, 127.62, 126.98, 126.83, 124.94, 119.89, 118.68, 67.17, 66.62, 64.72, 61.78, 47.16, 36.12, 31.52, 25.70, 22.59, 19.17, 18.01, 14.06, 12.83 ppm; IR (film): $\tilde{\nu}_{max}$ = 2934, 1706, 1450 cm^{-1}; ESI-MS: m/z: 716 $[M+Na]^+$; HRMS: m/z: calcd for $C_{45}H_{43}NO_4SNa$: 716.2805; found 716.2799 $[M+Na]^+$.

Compound 20: A solution of (*R*)-Garner aldehyde (1.15 g, 5.0 mmol) in dry CH_2Cl_2 (10 mL) was slowly added at 0 °C to a solution of 2-triphenylphosphoranylidene-propionic acid ethyl ester (2.0 g, 5.5 mmol) in dry CH_2Cl_2 (25 mL). The resulting solution was allowed to warm to room temperature and stirred for 12 h, and the solution was concentrated and then purified by chromatography (elution with ethyl acetate/petroleum ether 1:10) to give **20** (1.72 g, 100%): $[\alpha]_D^{20}$ = +14.4 (c = 1.1 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.64 (d, J = 7.9 Hz, 1H), 4.62 (m, 1H), 4.21 (m, 2H), 4.16–4.11 (m, 1H), 3.70 (dd, J = 3.6, 8.9 Hz, 1H), 1.89 (s, 3H), 1.63 (s, 3H), 1.54 (s, 3H), 1.41 (s, 9H), 1.30 (t, J = 7.1 Hz, 3H) ppm; IR (film): $\tilde{\nu}_{max}$ = 1741, 1698, 1657 cm^{-1}; EI-MS: m/z: 298 $[M-CH_3]^+$, 199, 198, 156, 126, 110, 109, 57, 41; elemental analysis calcd (%) for $C_{16}H_{27}NO_5$: C 61.31, H 8.68, N 4.47; found: C 61.04, H 8.37, N 4.70.

Compound 21: Sodium hydroxide (0.1 g, 2.5 mmol) was added to a solution of **20** (0.4 g, 1.3 mmol) in methanol (4 mL) and water (1 mL). The resulting solution was warmed to 50 °C, stirred for 4 h, and concentrated in vacuo before addition of water and acidification to pH 2 with HCl (1 N). The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:1) to give the acid (0.36 g).

This acid was dissolved in dry CH_2Cl_2 (3 mL), and allyl alcohol (89 mg, 1.5 mmol), DMAP (24 mg, 0.2 mmol), and EDCI (0.37 mg, 1.9 mmol) were then added successively. The solution was stirred for 2 h and was then diluted with CH_2Cl_2 and washed with brine, the organic layers were dried over Na_2SO_4 and concentrated, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:20) to give **21** (308 mg, 74%). $[\alpha]_D^{20}$ = +22.5 (c = 1.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.70 (d, J = 5.1 Hz, 1H), 6.00 (m, 1H), 5.30 (m, 2H), 4.80–4.60 (m, 3H), 4.15 (t, J = 7.5 Hz, 1H), 3.70 (dd, J = 3.3, 8.7 Hz, 1H), 1.95 (d, J = 14.4 Hz, 3H), 1.65 (s, 3H), 1.55 (s, 3H), 1.45 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.9, 140.9, 132.1, 117.6, 94.2, 80.0, 67.4, 65.0, 55.1, 28.1, 27.1, 26.0, 24.8, 23.9, 12.3 ppm; IR (film): $\tilde{\nu}_{max}$ = 1702, 1388, 1366 cm^{-1}; ESI-MS: m/z: 326 $[M+H]^+$; HRMS: m/z: calcd for $C_{17}H_{27}NO_5Na$: 348.1781; found 348.1789 $[M+Na]^+$.

Compound 22: The acid **21** (300 mg, 0.92 mmol) was dissolved in CF_3COOH (0.6 mL) and water (0.2 mL) and the resulting mixture was stirred for 1 h and diluted with toluene (2 mL). The mixture was concentrated to dryness, and the residue was then redissolved in toluene (2 mL) and concentrated to dryness again.

The acid **5a** (430 mg, 0.76 mmol), HATU (525 mg, 1.38 mmol), and DIPEA (0.4 mL, 2.4 mmol) were sequentially added to a solution of the above residue in dry CH_2Cl_2 (3 mL). The resulting mixture was stirred for 24 h, and the mixture was then concentrated in vacuo and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:1) to give **22** as a colorless solid (505 mg, 90% yield). $[\alpha]_D^{20}$ = −16.1 (c = 0.66 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.2 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.3 (t, J = 7.2 Hz, 2H), 6.60 (m, 2H), 5.90–5.81 (m, 1H), 5.31–5.15 (m, 2H), 4.92 (d, J = 11.1 Hz, 1H), 4.75 (m, 1H), 4.61–4.45 (m, 3H), 4.38–4.25 (m, 3H), 3.62 (m, 3H), 3.51–3.42 (m, 1H), 3.34 (dd, J = 11.4, 6.3 Hz, 1H), 2.31–2.22 (m, 1H), 2.15–2.05 (m, 1H), 2.01–1.92 (m, 3H), 1.89 (s, 3H), 1.71–1.52 (m, 3H), 1.43–1.21 (m, 3H), 1.12 (d, J = 7.2 Hz, 3H), 0.95 (d, J = 6.3 Hz, 3H), 0.91 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 176.00, 172.26, 167.05, 155.40, 143.91, 143.71, 141.22, 137.66, 132.20, 130.71, 127.71, 127.65, 127.10, 127.03, 125.10, 119.92, 119.87, 118.06, 78.63, 71.49, 67.67, 65.35, 64.10, 59.39, 50.34, 47.56, 47.16, 46.54, 40.17, 38.56, 37.15, 34.70, 29.87, 25.88, 24.84. 24.24, 20.27, 14.54, 12.94 ppm; ESI-MS: m/z: 733 $[M+H]^+$; HRMS: m/z: calcd for $C_{42}H_{56}N_2O_9Na$: 755.3878; found 755.3895 $[M+Na]^+$.

Compound 23: A solution of amido alcohol **22** (150 mg, 0.2 mmol) in CH_2Cl_2 (3 mL) was cooled at −78 °C under nitrogen and DAST (25 μL, 0.2 mmol) was slowly added. One hour later, further DAST (25 μL, 0.2 mmol) was added. The solution was stirred for another one hour, and then DAST (12 μL, 0.1 mmol) was again added. The solution was stirred for a further 1 h and quenched with NH_4OH (4 M, 1.0 mL) at −78 °C. The aqueous layer was then extracted with $CHCl_3$, the combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 2:1) to give the oxazoline product **23** (130 mg, 90% yield). $[\alpha]_D^{20}$ = −79.0 (c = 1.1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, J = 7.2 Hz, 2H), 7.65 (d, J = 6.1 Hz, 2H), 7.43–7.25 (m, 4H), 6.68 (d, J = 8.4 Hz, 1H), 5.90 (m, 1H), 5.20 (q, J = 13.5 Hz, 2H), 4.90 (m, 2H), 4.62 (dd, J = 6.0, 15.0 Hz, 1H), 4.56–4.40 (m, 1H), 4.37 (dd, J = 3.0, 8.4 Hz, 3H), 4.30–4.20 (m, 2H), 3.83 (t, J = 8.1 Hz, 1H), 3.78–3.70 (m, 1H), 3.69–3.60 (m, 2H), 3.58–3.48 (m, 2H), 2.50 (t, J = 6.3 Hz, 1H), 2.30–2.20 (m, 1H), 2.10–1.92 (m, 3H), 1.90 (s, 3H), 1.50–1.40 (m, 1H), 1.30–1.20 (m, 3H), 0.97 (d, J = 6.3 Hz, 3H), 0.88 (s, 9H), 0.80 (t, J = 6.6 Hz, 3H) ppm; IR (KBr): $\tilde{\nu}_{max}$ = 2959, 2929, 1712, 1452, 1421 cm^{-1}; ESI-MS: m/z: 715 $[M+H]^+$; HRMS: m/z: calcd for $C_{42}H_{55}N_2O_8$: 715.3953; found 715.3920 $[M+H]^+$.

Compound 34: Fmoc-*N*-Me-Ala-OBn (**32**, 0.7 g, 1.7 mmol) was dissolved in MeCN (2 mL) and Et_2NH (1 mL). The resulting solution was stirred for 15 min and concentrated to dryness, the residue was dissolved in dry CH_2Cl_2 (3 mL), and the acid **33** (0.7 g, 1.7 mmol) and BEP (0.8 g, 2.9 mmol) were then added successively. DIPEA (0.5 mL, 2.9 mmol) was added to this solution at 0 °C, the resulting mixture was stirred for 24 h and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:4) to give the dipeptide product **34** (60% yield from **32**). $[\alpha]_D^{20}$ = −20.8 (c = 3.5 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.77 (d, J = 7.5 Hz, 2H), 7.57 (t, J = 5.7 Hz, 2H), 7.45–7.25 (m, 9H), 7.08 (d, J = 8.7 Hz, 2H), 6.76 (d, J = 8.4 Hz, 2H), 5.61 (d, J = 8.7 Hz, 1H), 5.18 (m, 2H), 4.85 (m, 1H), 4.40–4.10 (m, 4H), 3.75 (s, 3H), 3.00 (m, 1H), 2.88 (s, 3H), 2.80 (m, 1H), 1.42 (d, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.50, 170.99, 158.42, 155.52, 143.76, 143.72, 141.14, 135.40, 130.51, 128.47, 128.29, 128.09, 127.81, 127.69, 127.54, 126.90, 125.06, 124.99, 119.81, 113.71, 66.82, 55.00, 52.82, 52.08, 47.03, 37.72, 31.43, 14.02 ppm; IR (film): $\tilde{\nu}_{max}$ = 3298, 3065, 2945, 2837, 1739, 1718, 1647, 1513, 1451 cm^{-1}; ESI-MS: m/z: 593 $[M+H]^+$; HRMS: m/z: calcd for $C_{36}H_{37}N_2O_6$: 593.2646; found 593.2649 $[M+H]^+$.

Compound 36: A solution of the above dipeptide **34** in ethyl acetate was hydrogenated over 10% palladium on charcoal at room temperature and 1 atm for 12 h. After the mixture had been filtered through Celite, the filtrate was concentrated to give the crude acid.

Fmoc-*N*-Me-Ile-OTMSE (**35**, 0.1 g, 0.2 mmol) was dissolved in MeCN (2 mL) and Et_2NH (1 mL). The solution was stirred for 15 min and concentrated to dryness, and the residue was dissolved in dry CH_2Cl_2 (3 mL), after which the above acid (0.1 g, 0.2 mmol) and BEP (0.12 g, 0.44 mmol) were added successively. DIPEA (0.1 mL, 0.6 mmol) was added at 0°C to this resulting solution, the resulting mixture was stirred for 24 h and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:4) to give **36** as a pale yellow solid (90 mg, 60% overall yield). $[\alpha]_D^{20} = -36$ ($c = 1.23$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.81$ (m, 2H), 7.62 (m, 2H), 7.51–7.32 (m, 4H), 7.15 (t, J = 8.4 Hz, 2H), 6.81 (d, J = 7.8 Hz, 2H), 5.50 (m, 1H), 4.95 (m, 2H), 4.51–4.15 (m, 5H), 3.82 (s, 3H), 3.40 (m, 1H), 3.05 (m, 1H), 3.01 (s, 3H), 2.93–2.82 (m, 1H), 2.82 (s, 3H), 2.01 (m, 1H), 1.31 (m, 4H), 1.01–0.81 (m, 9H), 0.05 (s, 9H) ppm; IR (KBr): $\tilde{\nu}_{max} = 2950$, 2940, 1729, 1512 cm^{-1}; ESI-MS: m/z: 730 $[M+H]^+$.

Compound 37b: $[Pd(PPh_3)_4]$ (20 mg, 0.017 mmol) was added under nitrogen to a solution of the oxazoline **23** (120 mg, 0.17 mmol) in dry THF (3 mL), followed by *N*-methylaniline (40 μL, 0.37 mmol). The resulting solution was stirred for 2 h and concentrated, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 2:3) to give the crude acid.

Tripeptide **36** (130 mg, 0.18 mmol) was dissolved in MeCN (2 mL) and Et_2NH (1 mL). The solution was stirred for 15 min and concentrated to dryness. After the residue had been dissolved in dry CH_2Cl_2 (3 mL), the above acid (95 mg, 0.14 mmol), HATU (80 mg, 0.21 mmol), and DIPEA (0.07 mL, 0.41 mmol) were added successively, the resulting mixture was stirred for 48 h at room temperature and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 2:3) to give **37b** as a pale yellow solid (96 mg, 59% yield). $[\alpha]_D^{20} = -63.3$ ($c = 1.38$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.75$ (d, $J = 7.8$ Hz, 2H), 7.58 (d, $J = 7.5$ Hz, 2H), 7.41–7.32 (m, 4H), 7.07 (m, 2H), 6.78 (m, 2H), 6.51 (d, $J = 7.8$ Hz, 1H), 6.15 (d, $J = 8.7$ Hz, 1H), 5.42 (m, 1H), 5.21 (m, 1H), 4.95–4.81 (m, 3H), 4.52–4.11 (m, 7H), 3.75 (s, 3H), 3.71–3.52 (m, 3H), 3.15 (m, 1H), 3.03–2.96 (m, 1H), 2.95 (s, 3H), 2.91–2.82 (m, 2H), 2.71 (s, 3H), 2.52 (m, 1H), 2.11–1.91 (m, 4H), 1.82 (s, 3H), 1.81–1.62 (m, 3H), 1.45 (m, 1H), 1.31–1.22 (m, 5H), 1.05–0.92 (m, 12H), 0.90 (s, 9H), 0.86–0.78 (m, 4H), 0.06 (s, 9H) ppm; ESI-MS: m/z: 1164 $[M+H]^+$; HRMS: m/z: calcd for $C_{65}H_{94}N_5O_{12}Si$: 1164.6663; found 1164.6687 $[M+H]^+$.

The oxazoline analogue of apratoxin A (4): TBAF (1.0 M solution in THF, 50 μL, 50 μmol) was added to a solution of **37b** (20 mg, 17.2 μmol) in dry THF (1 mL). The resulting solution was stirred overnight and concentrated to dryness, and the residue was dissolved in toluene (2 mL) and concentrated to dryness. This residue was dissolved in dry CH_2Cl_2 (9 mL, 0.002 M), and HATU (20 mg, 52.6 μmol) and DIPEA (25 μL, 143 μmol) were then added successively. The resulting mixture was stirred for three days at room temperature and concentrated in vacuo, and the residue was purified by chromatography (elution with pure ethyl acetate) to give **4** as a colorless solid (7 mg, 45% yield). $[\alpha]_D^{20} = -117.5$ ($c = 0.2$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.15$ (d, $J = 8.7$ Hz, 2H), 6.80 (d, $J = 8.2$ Hz, 2H), 6.21 (d, $J = 9.3$ Hz, 1H), 6.01 (d, $J = 9.3$ Hz, 1H), 5.25 (d, $J = 11.5$ Hz, 1H), 5.05 (td, $J = 10.2$, 5.6 Hz, 1H), 4.97 (d, $J = 11.6$ Hz, 1H), 4.81 (m, 1H), 4.70 (d, $J = 10.6$ Hz, 1H), 4.38 (m, 1H), 4.20 (m, 1H), 3.78 (s, 3H), 3.70–3.55 (m, 3H), 3.28 (br q, $J = 6.7$ Hz, 1H), 3.15 (d, $J = 3.8$ Hz, 1H), 3.10 (d, $J = 11.7$ Hz, 1H), 2.85 (dd, $J = 12.7$, 4.6 Hz, 1H), 2.81 (s, 3H), 2.75 (s, 3H), 2.65 (d, $J = 13.5$ Hz, 1H), 2.33 (m, 1H), 2.25 (m, 1H), 2.15 (m, 1H), 2.05 (m, 1H), 1.92 (s, 3H), 1.90–1.75 (m, 3H), 1.45 (m, 1H), 1.31 (m, 1H), 1.26 (m, 1H), 1.25 (s, 3H), 1.10 (m, 1H), 1.07 (d, $J = 6.9$ Hz, 3H), 1.01 (t, $J = 7.0$ Hz, 3H), 0.99 (d, $J = 6.4$ Hz, 3H), 0.96 (m, 1H), 0.95 (d, $J = 7.6$ Hz, 3H), 0.87 (m, 9H) ppm; ESI-MS: m/z: 824 $[M+H]^+$; HRMS: m/z: calcd for $C_{45}H_{70}N_5O_9$: 824.5168; found 824.5160 $[M+H]^+$.

Compound 39: $[\alpha]_D^{20} = -113.3$ ($c = 0.055$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.08$ (d, $J = 7.5$ Hz, 2H), 6.79 (d, $J = 7.5$ Hz, 2H), 6.50 (dd, $J = 12.1$, 8.1 Hz, 1H), 6.11 (m, 1H), 5.43 (dd, $J = 7.0$, 2.0 Hz, 1H), 5.15 (d, $J = 11.1$ Hz, 1H), 5.05 (m, 1H), 4.94 (m, 1H), 4.84 (dd, $J = 8.5$, 2.9 Hz, 1H), 4.54 (dd, $J = 8.3$, 3.5 Hz, 1H), 4.35 (dt, $J = 8.6$, 3.0 Hz, 1H), 4.22 (t, $J = 6.0$ Hz, 1H), 4.02 (m, 1H), 3.77 (s, 3H), 3.70 (m, 1H), 3.66 (m, 1H), 3.61 (m, 1H), 3.06 (m, 1H), 3.01 (s, 3H), 2.91 (m, 1H), 2.85 (m, 1H), 2.81 (s, 3H), 2.41 (m, 1H), 2.30 (m, 1H), 2.25 (m, 1H), 2.12 (m, 1H), 2.05 (m, 1H), 1.95 (m, 1H), 1.87 (m, 1H), 1.86 (s, 3H), 1.81 (m, 1H), 1.69 (m, 1H), 1.58 (m, 1H), 1.37 (m, 1H), 1.22 (m, 1H), 1.16 (d, $J = 7.1$ Hz, 3H), 1.09 (d, $J = 7.2$ Hz, 3H), 0.97 (d, $J = 6.6$ Hz, 3H), 0.93 (m, 1H), 0.91 (m, 3H), 0.86 (m, 6H), 0.90 (m, 3H) ppm; ESI-MS: m/z: 810.3 $[M+H]^+$; HRMS: m/z: calcd for $C_{44}H_{68}N_5O_9$: 810.5012; found 810.5017 $[M+H]^+$.

Compound 40: $[\alpha]_D^{20} = -74.7$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.15$ (m, 2H), 6.80 (d, $J = 8.1$ Hz, 2H), 6.40 (d, $J = 10.0$ Hz, 1H), 6.25 (d, $J = 8.1$ Hz, 1H), 5.25 (m, 1H), 5.20–5.10 (m, 3H), 5.00 (d, $J = 11.2$ Hz, 1H), 4.80 (m, 1H), 4.77 (s, 1H), 4.32 (m, 1H), 4.00 (m, 1H), 3.75 (s, 3H), 3.70–3.60 (m, 3H), 3.50 (m, 1H), 3.15 (d, $J = 13.3$ Hz, 1H), 3.10 (d, $J = 8.6$ Hz, 1H), 3.00 (m, 1H), 2.75 (s, 3H), 2.67 (d, $J = 8.1$ Hz, 1H), 2.58 (s, 3H), 2.56 (m, 1H), 2.25–2.05 (m, 3H), 1.93 (s, 3H), 1.90–1.75 (m, 3H), 1.45 (m, 1H), 1.28–1.13 (m, 4H), 1.10–0.75 (m, 20H) ppm; ESI-MS: m/z: 810 $[M+H]^+$; HRMS: m/z: calcd for $C_{44}H_{68}N_5O_9$: 810.5012; found 810.5019 $[M+H]^+$.

Compound 41: $[\alpha]_D^{20} = -39.4$ ($c = 0.11$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.12$ (d, $J = 8.2$ Hz, 2H), 6.80 (d, $J = 8.2$ Hz, 2H), 6.49 (dd, $J = 15.3$, 8.6 Hz, 1H), 6.12 (d, $J = 8.6$ Hz, 2H), 5.43 (dd, $J = 8.6$, 2.0 Hz, 1H), 5.16 (d, $J = 10.9$ Hz, 1H), 4.94 (m, 1H), 4.86 (dd, $J = 8.5$, 2.9 Hz, 1H), 4.82 (d, $J = 8.2$ Hz, 1H), 4.52 (dd, $J = 6.8$, 3.5 Hz, 1H), 4.38 (t, $J = 8.0$ Hz, 1H), 4.02 (m, 1H), 3.90 (m, 1H), 3.81 (s, 3H), 3.77 (m, 1H), 3.68 (m, 1H), 3.55 (m, 1H), 3.40 (m, 1H), 3.06 (m, 1H), 3.01 (s, 3H), 2.90 (m, 1H), 2.86 (m, 1H), 2.81 (s, 3H), 2.40 (m, 1H), 2.34 (m, 1H), 2.20 (m, 1H), 2.09 (m, 1H), 1.94 (m, 1H), 1.88 (m, 1H), 1.87 (s, 3H), 1.82 (m, 1H), 1.62 (m, 1H), 1.54 (m, 1H), 1.47 (m, 1H), 1.45 (m, 1H), 1.39 (m, 1H), 1.20 (m, 1H), 1.13 (d, $J = 7.1$ Hz, 3H), 1.10 (d, $J = 7.5$ Hz, 3H), 0.96 (d, $J = 6.2$ Hz, 3H), 0.92 (m, 1H), 0.88 (m, 3H), 0.86 (m, 6H) ppm; ESI-MS: m/z: 796.5 $[M+H]^+$; HRMS: m/z: calcd for $C_{43}H_{66}N_5O_9$: 796.4855; found 796.4862 $[M+H]^+$.

Compound 27b: A solution of **19b** (200 mg, 0.29 mmol) in MeCN (2 mL) and Et_2NH (1 mL) was stirred for 15 min and was then concentrated to dryness. After the residue had been dissolved in dry CH_2Cl_2 (3 mL), the acid **5a** (160 mg, 0.29 mmol), HATU (164 mg, 0.43 mmol), and DIPEA (0.10 mL, 0.58 mmol) were added successively. The resulting mixture was stirred for 12 h at room temperature and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:3) to give **27b** (237 mg, 81% yield). $[\alpha]_D^{20} = -22.7$ ($c = 0.3$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.77$ (d, $J = 6.6$ Hz, 2H), 7.60 (t, $J = 6.9$ Hz, 2H), 7.50–7.15 (m, 19H), 7.00 (d, $J = 13.5$ Hz, 1H), 6.41 (d, $J = 9.3$ Hz, 1H), 5.30 (m, 1H), 4.90 (d, $J = 9.6$ Hz, 1H), 4.70–4.40 (m, 5H), 4.30–4.20 (m, 2H), 3.75–3.45 (m, 4H), 2.45 (m, 1H), 2.35–2.05 (m, 5H), 1.95 (m, 3H), 1.71 (s, 3H), 1.66 (s, 3H), 1.61 (s, 3H), 1.50–1.30 (m, 3H), 1.15 (d, $J = 6.9$ Hz, 3H), 0.9 (m, 12H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 175.26$, 172.09, 167.51, 155.12, 144.56, 144.50, 144.06, 143.68, 141.22, 139.35, 130.19, 129.55, 127.92, 127.84, 127.63, 127.09, 127.01, 126.76, 126.64, 125.10, 125.03, 119.89, 118.86, 78.52, 71.05, 67.68, 61.58, 59.52, 47.19, 47.10, 46.39, 46.18, 40.42, 37.17, 36.03, 34.85, 34.79, 29.83, 29.80, 25.85, 25.68, 25.65, 25.40, 24.19, 19.99, 17.97, 15.10, 12.93 ppm; IR (film): $\tilde{\nu}_{max} = 3322$, 2962, 2928, 1712, 1684, 1450 cm^{-1}; ESI-MS: m/z: 1041 $[M+Na]^+$; HRMS: m/z: calcd for $C_{63}H_{74}N_2O_8SNa$: 1041.5058; found 1041.5061 $[M+Na]^+$.

Compound 30b: DIPEA (40 μL, 0.24 mmol) was added at 0°C to a solution of **27b** (120 mg, 0.18 mmol) in dichloromethane (2 mL), followed by AcCl (15 μL, 0.21 mmol). After the solution had been stirred for 0.5 h, DIPEA (40 μL, 0.24 mmol) and AcCl (15 μL, 0.21 mmol) were again added. The solution was stirred for 1 h and then concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:3) to give **30b** (100 mg, 81% yield). $[\alpha]_D^{20} = -40.3$ ($c = 0.7$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.78$ (d, $J = 6.6$ Hz, 2H), 7.64 (m, 2H), 7.55–7.15 (m, 19H), 6.39 (m, 1H), 5.35 (m, 1H), 5.05 (m, 1H), 4.90–4.10 (m, 9H), 3.70–3.50 (m, 2H), 2.55–2.30 (m,

4H), 2.10 (s, 3H), 1.98 (s, 3H), 1.85–1.65 (m, 9H), 1.50–1.25 (m, 5H), 1.10 (d, J = 4.5 Hz, 3H), 0.90 (s, 9H), 0.85 (d, J = 5.4 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.19, 172.00, 167.41, 154.59, 154.12, 144.41, 144.31, 143.80, 143.51, 141.17, 138.98, 129.47, 127.83, 127.56, 126.96, 126.92, 126.85, 126.72, 126.69, 124.99, 124.95, 119.83, 119.76, 118.70, 79.66, 79.09, 72.90, 67.31, 61.64, 60.23, 59.57, 59.23, 47.16, 47.04, 46.18, 44.88, 34.71, 29.87, 25.98, 25.74, 25.72, 25.62, 24.12, 23.21, 20.90, 20.78, 20.33, 17.92, 14.09, 13.30, 12.85 ppm; IR (film): $\tilde{\nu}_{max}$ = 3300, 1711, 1421 cm^{-1}; ESI-MS: m/z: 1061 $[M+H]^+$, 1083 $[M+Na]^+$; HRMS: m/z: calcd for $C_{65}H_{76}N_2O_9SNa$: 1083.5164; found 1083.5166 $[M+Na]^+$.

Compound 31b: Trifluoromethanesulfonic anhydride (120 μL, 0.73 mmol) was added at 0 °C under nitrogen to a solution of triphenylphosphine oxide (410 mg, 1.47 mmol) in dry dichloromethane (5 mL). After the mixture had been stirred for 0.5 h, a solution of **30b** (195 mg, 0.18 mmol) in dry dichloromethane (2 mL) was added, the resulting solution was stirred for 1 h, and then sodium hydrogen carbonate solution (5%) was added. The aqueous layer was extracted with $CHCl_3$, the combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:3) to give **31b** (135 mg, 92% yield). $[\alpha]_D^{20}$ = −56.1 (c = 0.4 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.77 (d, J = 6.6 Hz, 2H), 7.64 (m, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.32 (m, 2H), 6.72 (m, 1H), 5.35 (m, 1H), 5.15 (m, 2H), 4.80 (d, J = 8.4 Hz, 1H), 4.63 (d, J = 7.2 Hz, 2H), 4.56–4.20 (m, 5H), 3.70–3.38 (m, 4H), 3.00 (m, 2H), 2.09 (s, 3H), 2.05 (s, 3H), 1.75 (s, 3H), 1.71 (s, 3H), 1.45–1.25 (m, 10H), 0.90 (m, 12H) ppm; IR (film): $\tilde{\nu}_{max}$ = 2961, 1737, 1711, 1618, 1452 cm^{-1}; ESI-MS: m/z: 801 $[M+H]^+$, 823 $[M+Na]^+$; HRMS: m/z: calcd for $C_{46}H_{61}N_2O_8S$: 801.4143; found 801.4145 $[M+H]^+$.

Compound 37a: TMSOTf (13 μL, 62.5 μmol) was added under nitrogen to a solution of **31b** (50 mg, 62.5 μmol) in dry dichloromethane (2 mL). The resulting solution was stirred for 1 h and concentrated to give the crude acid.

Tripeptide **36** (57 mg, 78 μmol) was dissolved in MeCN (1 mL) and Et_2NH (0.5 mL), and the solution was stirred for 15 min and concentrated to dryness. After the residue had been dissolved in dry CH_2Cl_2 (2 mL), HATU (80 mg, 0.21 mmol), a solution of the above acid in dry CH_2Cl_2 (1 mL), and DIPEA (27 μL, 156 μmol) were added successively, the resulting mixture was stirred overnight at room temperature and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 1:1) to give **37a** (38 mg, 50% yield). $[\alpha]_D^{20}$ = −100.9 (c = 1.0 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (m, 2H), 7.66 (m, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.12 (d, J = 7.5 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 6.58 (m, 1H), 6.32 (m, 1H), 5.43 (m, 1H), 5.30–5.05 (m, 3H), 4.94 (d, J = 13.2 Hz, 1H), 4.82 (m, 1H), 4.60–4.05 (m, 7H), 3.80 (s, 3H), 3.62 (m, 2H), 3.42 (m, 1H), 3.10 (m, 1H), 3.01 (s, 3H), 2.90 (m, 3H), 2.75 (s, 3H), 2.30 (m, 1H), 2.12 (s, 3H), 2.07 (s, 3H), 2.00–1.90 (m, 6H), 1.50–1.20 (m, 5H), 1.10–0.70 (m, 25H), 0.07 (s, 9H) ppm; ESI-MS: m/z: 1244 $[M+Na]^+$; HRMS: m/z: calcd for $C_{67}H_{95}N_5O_{12}SSiNa$: 1244.6359; found 1244.6348 $[M+Na]^+$.

Apratoxin A (1): TBAF (1.0 M solution in THF, 130 μL, 130 μmol) was added to a solution of **37a** (40 mg, 32.8 μmol) in dry THF (2 mL). The resulting solution was stirred overnight and concentrated to dryness, the residue was dissolved in toluene (2 mL), was again concentrated to dryness, and was redissolved in dry CH_2Cl_2 (16 mL, 0.002 M), and HATU (25 mg, 65.5 μmol) and DIPEA (28 μL, 164 μmol) were then added successively. The resulting mixture was stirred for 3 days at room temperature and concentrated in vacuo, and the residue was purified by chromatography (elution with ethyl acetate/petroleum ether 4:1) to give **38** (10 mg).

This product was dissolved in ethanol (95%, 2 mL), and KCN (0.7 mg, 11 μmol) was added. The solution was warmed to 50 °C, stirred overnight, and then concentrated in vacuo and purified by chromatography (elution with pure ethyl acetate) to give **1** (7 mg, 26% yield from **37a**). $[\alpha]_D^{20}$ = −159 (c = 0.45 in MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.20 (d, J = 8.3 Hz, 2H), 6.80 (d, J = 8.2 Hz, 2H), 6.40 (d, J = 9.3 Hz, 1H), 6.05 (d, J = 9.3 Hz, 1H), 5.28 (m, 1H), 5.21 (d, J = 11.5 Hz, 1H), 5.05 (m, 1H), 5.00 (dd, J = 10.2, 5.6 Hz, 1H), 4.69 (d, J = 10.6 Hz, 1H), 4.23 (m, 1H), 4.19 (t, J = 7.3 Hz, 1H), 3.78 (s, 3H), 3.66 (m, 1H), 3.54 (m, 1H), 3.45 (t, J = 6.9 Hz, 1H), 3.25 (m, 1H), 3.14 (dd, J = 3.8 Hz, 1H), 3.10 (dd, J = 11.7 Hz, 1H), 2.83 (dd, J = 12.7, 4.6 Hz, 1H), 2.79 (s, 3H), 2.72 (s, 3H), 2.60 (m, 1H), 2.19 (m, 3H), 2.10 (m, 1H), 1.95 (s, 3H), 1.85 (m, 2H), 1.80 (m, 1H), 1.55 (m, 1H), 1.22 (m, 1H), 1.20 (m, 4H), 1.10 (m, 1H), 1.05 (d, J = 6.9 Hz, 3H), 0.96 (m, 1H), 0.95 (m, 6H), 0.91 (m, 3H), 0.87 (s, 9H) ppm; ESI-MS: m/z: 862 $[M+Na]^+$; HRMS: m/z: calcd for $C_{45}H_{70}N_5O_8S$: 840.4940; found 840.4931 $[M+H]^+$.

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