

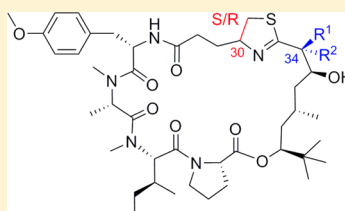
# Improved Total Synthesis and Biological Evaluation of Potent Apratoxin S4 Based Anticancer Agents with Differential Stability and Further Enhanced Activity

Qi-Yin Chen,<sup>†,‡</sup> Yanxia Liu,<sup>†,‡</sup> Weijing Cai,<sup>†</sup> and Hendrik Luesch<sup>\*,†,‡</sup>

<sup>†</sup>Department of Medicinal Chemistry and <sup>‡</sup>Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville, Florida, United States

## S Supporting Information

**ABSTRACT:** Apratoxins are cytotoxic natural products originally isolated from marine cyanobacteria that act by preventing cotranslational translocation early in the secretory pathway to downregulate receptor levels and inhibit growth factor secretion, leading to potent antiproliferative activity. Through rational design and total synthesis of an apratoxin A/E hybrid, apratoxin S4 (**1a**), we have previously improved the antitumor activity and tolerability in vivo. Compound **1a** and newly designed analogues apratoxins S7–S9 (**1b–d**), with various degrees of methylation at C34 (**1b,c**) or epimeric configuration at C30 (**1d**), were efficiently synthesized utilizing improved procedures. Optimizations have been applied to the synthesis of key intermediate aldehyde **7** and further include the application of Leighton's silanes and modifications of Kelly's methods to induce thiazoline ring formation in other crucial steps of the apratoxin synthesis. Apratoxin S9 (**1d**) exhibited increased activity with subnanomolar potency. Apratoxin S8 (**1c**) lacks the propensity to be deactivated by dehydration and showed efficacy in a human HCT116 xenograft mouse model.



Apratoxin	IC <sub>50</sub> (nM) cell viability	IC <sub>50</sub> (nM) VEGF-A secretion
S4 (30S,34-Me)	1.43	0.32
S7 (30S,34-H <sub>2</sub> )	1.25	0.30
S8 (30S,34-Me <sub>2</sub> )	1.99	0.47
S9 (30R,34-Me)	0.69	0.12

## INTRODUCTION

Apratoxins are potent cytotoxins derived from marine cyanobacteria.<sup>1</sup> Because of their biological activity and intriguing structures, they have been subject to several total syntheses and SAR studies.<sup>2</sup> We recently established that apratoxins prevent cotranslational translocation and thereby downregulate various receptors, including receptor tyrosine kinases (RTKs), and inhibit trafficking of other secretory molecules, including growth factors that act on RTKs.<sup>2a,3</sup> RTKs such as epidermal growth factor receptors and corresponding ligands such as vascular endothelial growth factor A (VEGF-A) individually are validated drug targets, which resulted in the approval of small molecules and antibodies against these proteins for colorectal cancer and other cancers.<sup>4</sup> The combined indirect inhibition of both classes of molecules by apratoxins has proven very powerful and an alternative to the specific targeting of selected secretory proteins in cancers that rely on autocrine loops<sup>2a</sup> such as colorectal cancer.<sup>5</sup> Apratoxin A was rigorously profiled and shown to possess broad-spectrum yet differential in vitro activity;<sup>6</sup> however, it also showed irreversible toxicity in vivo and was not well tolerated.<sup>2a</sup> Our initial investigation of the structure–activity relationships indicated that the irreversible toxicity may be an off-target effect rather than mechanism-based and may be related to the presence of the Michael acceptor in the molecule that might be prone to nonspecific addition of cellular nucleophiles.<sup>2a</sup> Indeed, in vitro experiments showed that Michael addition at C29 of apratoxin A can occur with thiol-containing compounds such as glutathione, cysteine, and *N*-acetylcysteine (Supporting In-

formation, Scheme S1).<sup>7</sup> Unfortunately, the only natural apratoxin without that  $\alpha,\beta$ -unsaturated carbonyl system has been apratoxin E, which, however, exerts much reduced activity, presumably due to the dehydration of the tertiary alcohol at C35.<sup>1c</sup> Therefore, we designed and synthesized apratoxin S4 (**1a**), which represents a hybrid of the natural products apratoxins A and E, containing the hydroxy group as in apratoxin A that is important for potent activity and lacking the Michael acceptor as in apratoxin E (Figure 1).<sup>2a</sup> Compound **1a** showed greater potency and efficacy in a colorectal tumor xenograft model than the parent compound (apratoxin A) and was much better tolerated in vivo, providing evidence that the apratoxin scaffold can serve as a new modality to treat cancer but also validating the mechanism of action as a new therapeutic strategy.<sup>2a</sup>

In our previous work,<sup>2a</sup> some fragments of **1a** were prepared with modified methods that paralleled those by other groups.<sup>2b–d</sup> To enable the large-scale synthesis of **1a** for rigorous preclinical assessment, several steps needed to be improved or replaced due to their low efficiency or harsh operation. An improved synthesis would also provide easier access to additional apratoxin analogues, which we designed based on earlier observations with the goal of improving metabolic stability and reducing complexity of the molecule. In apratoxin A, the chiral center at C34 bears the risk of epimerization; however, the C34 epimer has the same potency

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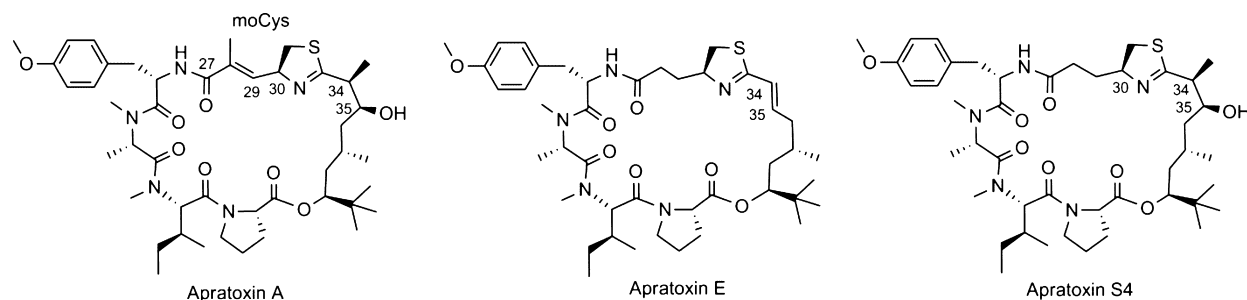
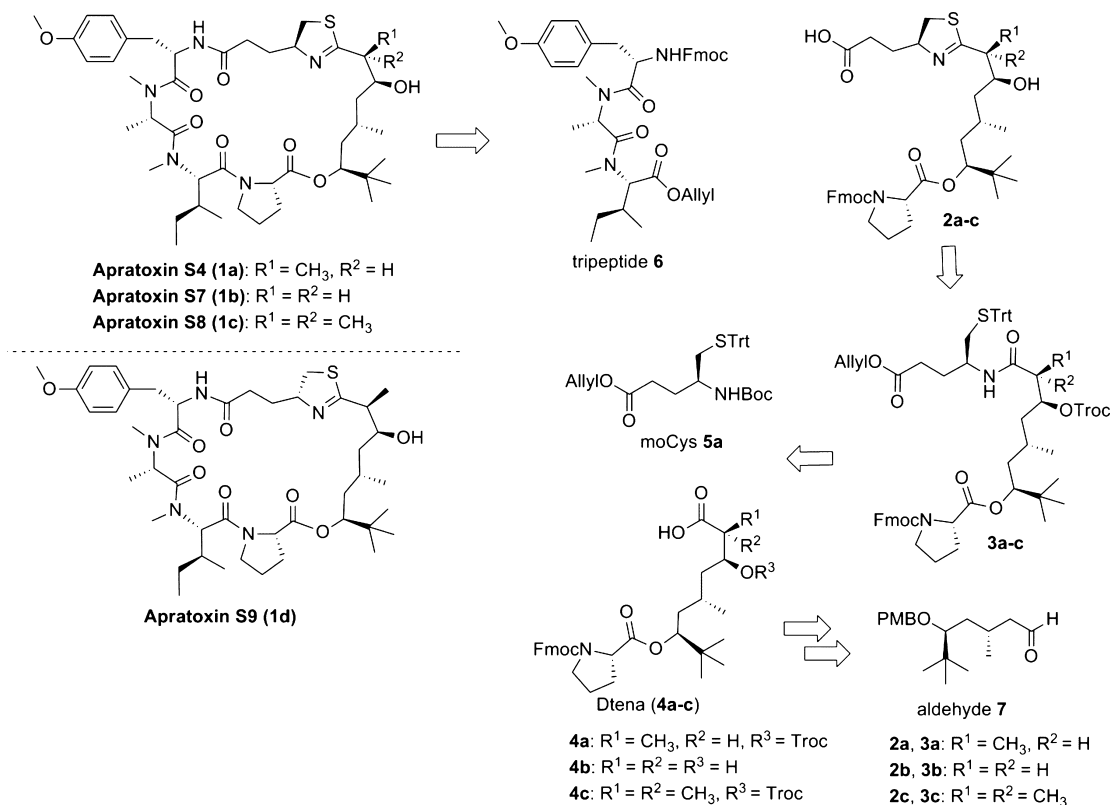


Figure 1. Natural apratoxins A and E and synthetic apratoxin S4.

### Scheme 1. Retrosynthetic Analysis of 1a and Analogues 1b–d



as apratoxin A<sup>2c,e</sup> and the same holds true for **1a** and 34-*epi*-**1a** (apratoxin S6).<sup>2a</sup> It is also known that the hydroxy group at C35 in apratoxin A is sensitive toward acid-induced dehydration, leading to the double bond between C34 and C35,<sup>1b,2c</sup> and the product has greatly decreased activity,<sup>1b,c</sup> thus presenting a potential major deactivation pathway for **1a** as well. On the basis of the above, it was necessary to explore and investigate analogues at C34: (1) nonmethyl-C34, to remove the chiral center, thereby simplifying the molecule (apratoxin S7, **1b**), and (2) *gem*-dimethyl-C34, to remove the chiral center to simplify the molecule and reduce the possibility to dehydrate to form a conjugated system with the thiazoline (apratoxin S8, **1c**). Both analogues were expected to retain potent activity based on our previous results with 34-*epi*-**1a**.<sup>2a</sup> To continue our systematic SAR investigation in the nonpeptide portions, we also aimed to explore the synthesis and activity of 30-*epi*-**1a** (apratoxin S9, **1d**).

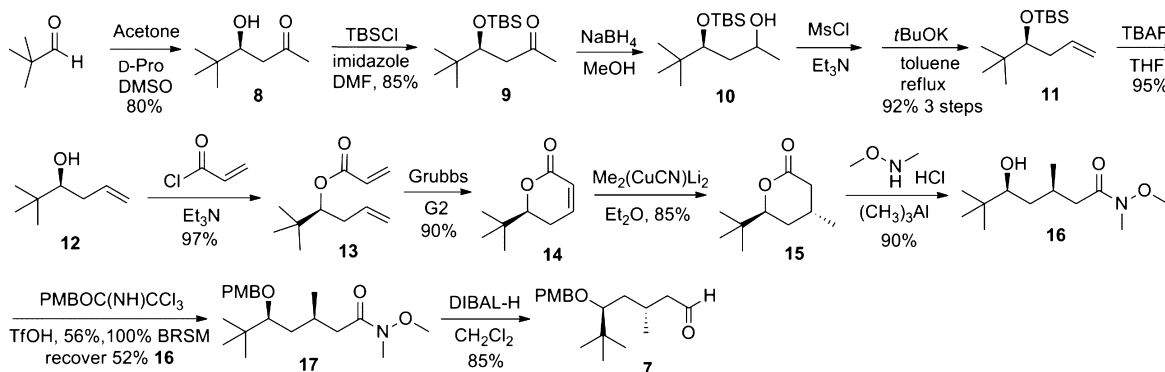
Here we show the details of an improved synthesis of **1a** and the synthesis of analogues with different C34 methylation status (**1b,c**) and configuration of C30 (**1d**). During the development

of this improved synthesis, several new reactions were applied, key reactions were modified to meet different substrate requirements, and many steps were optimized, resulting in an efficient synthesis that enables the generation of large-scale quantities of apratoxins for preclinical evaluation. We studied the SAR by subjecting all new synthetic apratoxins to a multidimensional assay platform, measuring cell growth, receptor tyrosine kinase receptor (RTK) levels, and growth factor secretion. We also investigated the in vitro stability of these compounds and tested the compound with the least propensity to be deactivated via dehydration (**1c**) for in vivo antitumor efficacy.

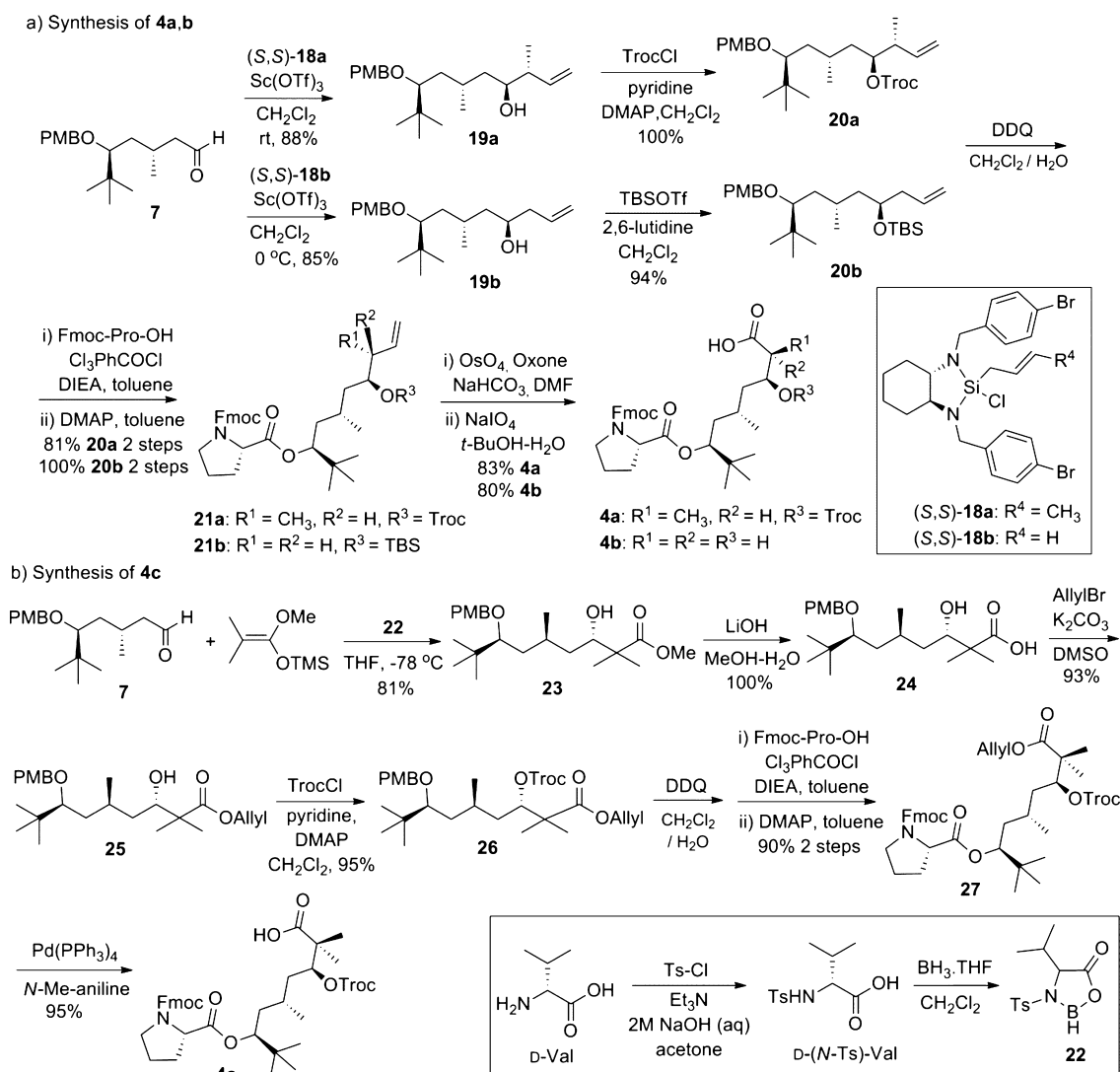
## RESULTS AND DISCUSSION

**Synthesis.** The main strategies and procedures in the synthesis of all analogues we designed paralleled those of reported procedures but with notable modifications.<sup>2a–d</sup> On the basis of the retrosynthetic analysis depicted in Scheme 1, **1a** and its analogues **1b–d** can be synthesized from two parts: tripeptide **6** and thiazole-containing long chain aliphatic acid **2**.

Scheme 2. Synthesis of Aldehyde 7



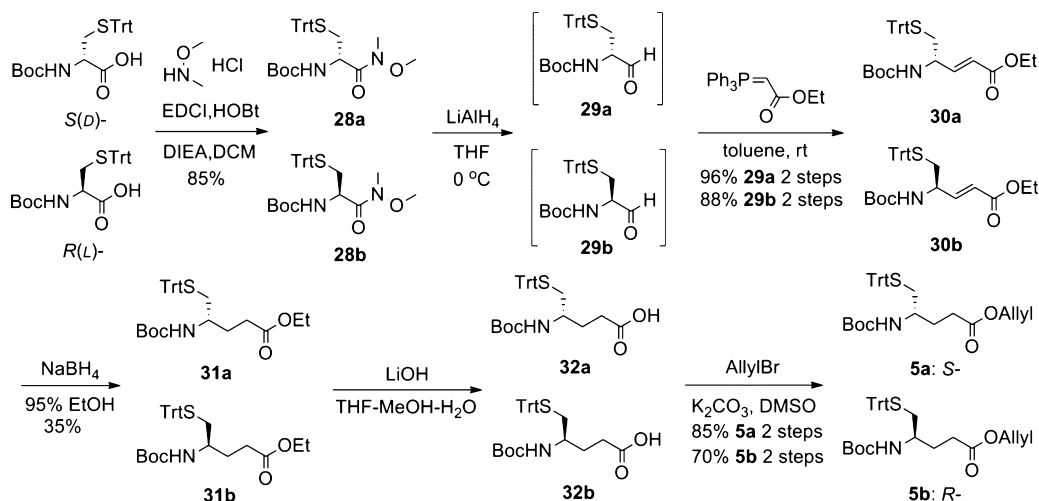
Scheme 3. Synthesis of 4a–c



The key step for the synthesis of each analogue is the formation of the thiazoline ring in **2** from the open-chain precursor **3**. Experiments below showed that different degrees of methylation at C34 or different configuration of C30 affected the ring formation to different extents. Compound **3** can be disconnected into modified cysteine **5** and proline ester based carboxylic acid **4**, which in turn can be synthesized from aldehyde **7** through a series of known reactions.

Initially, we prepared aldehyde **7**,<sup>2a–d</sup> a public and key intermediate. Scheme 2 details the steps which were improved from published procedures<sup>2b–h</sup> to meet practical large-scale production.  $\beta$ -Hydroxy ketone **8** was prepared via D-proline catalyzed aldol reaction of pivalaldehyde with acetone. After protection with TBS to **9**, reduction with NaBH<sub>4</sub> to **10**, and elimination through mesylate, **8** was transformed into allyl TBS ether **11**. The cleavage of the TBS from **11** afforded allyl

Scheme 4. Synthesis of 5a and 5b



alcohol **12**, which is the most critical step in the preparation of **12** due to its volatile property.<sup>8</sup> The solvent (Et<sub>2</sub>O and THF) in **12**-containing fractions can be removed by distillation using a Vigreux fractionation column for small-scale reactions under normal pressure; however, for large-scale reactions, this was not feasible and we used the combination of cooling-concentration method under ambient reduced pressure (see Supporting Information, Figure S1) and Vigreux fraction concentration. Even though our reaction sequence consisted of more steps to prepare **12** than Brown's method using IPC<sub>2</sub>BOMe,<sup>2d</sup> the latter caused purification problems which we did not encounter utilizing our optimized method.<sup>2g,h</sup> We found that this is indeed a practical route to the large-scale production of **12**, which we could easily achieve on 10 g scale.

According to published procedures<sup>2d</sup> with minor modifications, aldehyde **7** was prepared starting from allyl alcohol **12** through formation of acryloyl ester **13**, Grubbs' catalyst-effected RCM reaction (**14**), methylation with Me<sub>2</sub>(CuCN)Li<sub>2</sub> (**15**), Weinreb amide formation (**16**), protection of hydroxy group with PMB (**17**), and reduction with DIBAL-H (**7**). The yield was low (10–25%) when we used *p*-methoxybenzyl bromide (PMBBr) to protect the hydroxy group of **16** in the presence of NaH/*tetra-n*-butyl-ammonium iodide (TBAI) in THF or NaH in DMF. Fortunately, we found that the PMBOC(NH)CCl<sub>3</sub>/TfOH method<sup>2b</sup> was effective in smoothly converting **16** into PMB ether in moderate yield (56%). Furthermore, unreacted starting material **16** was recovered quantitatively and could be used in the next cycle.

It is noteworthy that, to shorten the synthetic route, we also tried to apply a Grignard reaction to the *syn*-cyclic sulfate derivative of **8** to afford aldehyde **7** (Supporting Information, Scheme S2).<sup>9a</sup> Using this strategy, **8** should be stereoselectively reduced to the *syn*-diol. We tried both Et<sub>3</sub>BOMe/NaBH<sub>4</sub><sup>9a</sup> and DIBAL-H<sup>9b</sup> as reducing systems. When we performed small-scale reactions (<2 g), we obtained adequate results; however, for large-scale reactions (>4 g), both methods provided unsatisfactory results: low yields, low diastereoselectivity, and most of the product was obtained as an inseparable mixture. We speculate that an extremely slow rate of addition of reducing reagents is crucial for the success of this reaction.<sup>9b</sup>

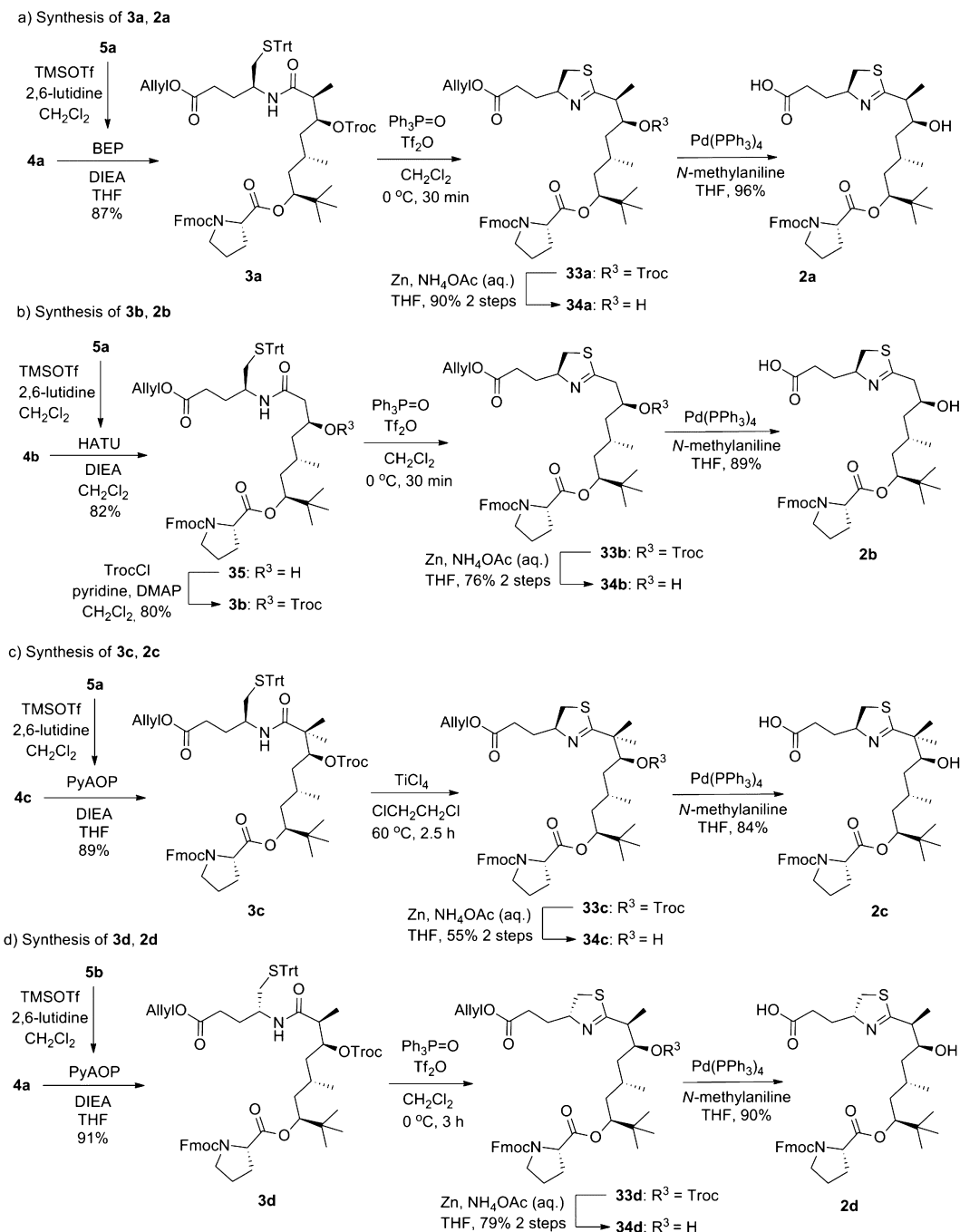
With aldehyde **7** in hand, we prepared proline ester-based aliphatic acid **4a–c** using modified published procedures<sup>2a–c</sup> (Scheme 3). C34-Methyl **4a** and C34-nonmethyl **4b** were

synthesized utilizing a similar strategy (Scheme 3a). In our previous work, we constructed the C34(Me)–C35(OH) chiral unit of **19a** through Roush's crotylation with Roush's (*E*)-crotylborate at –78 °C in toluene<sup>2a</sup> following published procedures.<sup>2c,10</sup> However, Roush's (*E*)-crotylborate is not commercially available, it is laborious to purify crude Roush's (*E*)-crotylborate prepared in the lab by distillation and, if crude product was used without purification, the impurity reduced the yield to as low as 55%. Here we now applied Leighton's silanes **18a,b** instead of Roush's crotylborate to construct the C34–C35 units in **19a,b**. Leighton's silanes **18a** and **18b** are commercially available and inexpensive; they are solid and easy to handle.<sup>11</sup> Compound **19a** and **19b** were obtained smoothly in high yields when aldehyde **7** was treated with **18a** and **18b** along with Sc(OTf)<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> at 0 °C, respectively. Compound **19a** was converted into Troc ester **20a** with 2,2,2-trichloroethoxycarbonyl chloride (TrocCl) in the presence of pyridine and DMAP. The Troc ester of **19b** was not stable and partly dehydrated to form a conjugate double bond, which led to low yield and caused problems with purification. Therefore, we protected hydroxy group **19b** with TBS and smoothly obtained the stable TBS ether **20b**.

The removal of the PMB group and subsequent esterification with Fmoc-Pro-OH by Yamaguchi's method<sup>2b,c,12</sup> provided prolyl ester **21a** and **21b**. The combination of oxidants, OsO<sub>4</sub>/oxone and NaIO<sub>4</sub>, was effective to oxidize **21a** to carboxylic acid **4a**; however, when **21b** was exposed to the same conditions, the TBS group was cleaved simultaneously and gave carboxylic acid **4b** with a free hydroxy group. The cleavage of the TBS group probably resulted from the acidic property of oxone which is a triple salt 2KHSO<sub>5</sub>·KHSO<sub>4</sub>·K<sub>2</sub>SO<sub>4</sub>. Both **4a** and **4b** were obtained in good yields (83% and 80%, respectively).

The enantioselective chiral borane-mediated aldol reaction developed by Kiyooka<sup>13</sup> was used to construct the β-hydroxy-α,α-dimethyl acid part of C34-*gem*-dimethyl acid **4c** (Scheme 3b). Treatment of aldehyde **7** with methyl trimethylsilyl dimethylketene acetal at –78 °C in the presence of chiral oxazaborolidinone **22** (derived from D-Val) provided (*S*)-β-hydroxy ester **23**. With **23** in hand, we first tried to protect the hydroxy with the Troc group and then hydrolyze the methyl ester with LiOH. However, we found that TrocO-**23** was not compatible to the LiOH aq solution during hydrolysis in which

Scheme 5. Synthesis of 3a–d and Further 2a–d



a complex mixture including Troc-cleaved acid was generated. We therefore hydrolyzed **23** with LiOH in the MeOH–H<sub>2</sub>O solution to give  $\beta$ -hydroxy acid **24** smoothly. We planned to couple **24** with modified cysteine (moCys) unit **5a** first and then protect the hydroxy group with Troc and finally remove PMB to form proline ester. However, we found the esterification from this reaction sequence provided a very low yield and caused difficulty in the purification. Therefore, we adjusted the reaction sequence to esterify with Fmoc-Pro-OH first and then install the moCys unit. Hydroxy acid **24** was converted into allyl ester **25** with allylBr in the presence of K<sub>2</sub>CO<sub>3</sub> in 93% yield. Similarly to the preparation of **21a,b**, hydroxy ester **25** was protected with Troc and the PMB group was removed followed by esterification with Fmoc-Pro-OH to

provide prolyl ester **27**, which was transformed into the carboxylic acid **4c** by treatment with Pd<sup>0</sup> catalyst and *N*-methylaniline. The yield for each step was greater than 90%.

The synthesis of modified cysteine unit **5a** and its enantiomer **5b** was achieved by following published procedures<sup>2a</sup> with minor modifications as depicted in Scheme 4. The Weinreb amide **28** from *S*- (or *R*-) *N*-Boc-Cys(*S*-Trt)-OH was reduced to amino aldehyde **29**, which was selectively converted into chain-extended modified cysteine (*E*)-**30** by Wittig olefination. During this conversion, the choice of reductant was of importance because  $\alpha$ -amino aldehydes are easily racemized.<sup>14</sup> The method using lithium aluminum hydride (LiAlH<sub>4</sub>) in THF at 0 °C decreased the racemization rate compared with that using DIBAL-H in toluene at –78 °C in



this preparation.<sup>2g,14–16</sup> The reduction with  $\text{LiAlH}_4$  probably proceeds through a stable lithium-chelated intermediate.<sup>14a,b</sup> Subsequent reduction of the conjugated double bond in **30a** and **30b** using  $\text{NaBH}_4$  in 95% ethanol afforded saturated compounds **31a** and **31b**, respectively, but only in 35% yield for both of them.<sup>2a</sup> To increase this yield, we also attempted hydrogenation with  $\text{RhCl}(\text{PPh}_3)_3/\text{H}_2$  in toluene at 50 °C.<sup>17</sup> This reaction, however, gave a similar yield but also posed problems during the chromatographic purification of the product, which was considered a greater problem. Fortunately, our apratoxin synthesis is convergent and this low yield is still not detrimental because it is not part of the longest linear sequence. Finally, ethyl esters **31** were transformed into allyl ester **5** through hydrolysis and subsequent alkylation.

Using modified reported procedures,<sup>2a–c</sup> **3a–d** and **2a–d** were synthesized as depicted in Scheme 5. The *N*-Boc groups in **4a–c** were selectively removed with TMSOTf in the presence of 2,6-lutidine and subsequent coupling of deprotected **4a–c** with **5a** or **5b** gave **3a–d**. First, we screened several coupling reagents for the preparation of **3a** (Table 1,

**Table 1. Screening of Coupling Reagents for Preparation of **3a**, **3c**, and **3d** (% Yield)**

entry	EDCI (%)	HATU (%)	BOP (%)	BEP (%)	PyAOP (%)
1 ( <b>4a</b> → <b>3a</b> )	63	50	80	87	<i>a</i>
2 ( <b>4c</b> → <b>3c</b> )	<i>a</i>	20	<i>a</i>	47 <sup>b</sup>	89
3 ( <b>4d</b> → <b>3d</b> )	<i>a</i>	<i>a</i>	62	<i>a</i>	91

<sup>a</sup>Coupling reagent was not tried. <sup>b</sup><sup>1</sup>H NMR is complex.

entry 1). The coupling reaction with BEP gave the highest yield of 87%. Even though HATU did not provide a good yield in the preparation of **3a**, the yield was as high as 82% when it was used in the preparation of **3b**.

Acknowledging that it may be difficult to prepare **3c** due to the steric hindrance of  $\alpha$ -gem-dimethyl in acid **4c**, we also screened coupling reagents for its preparation (Table 1, entry 2): PyAOP gave the highest yield of 89% and surprisingly the BEP coupling product gave a complex proton NMR as well as low yield while HATU gave an even lower yield of 20%. Considering the similarity of **3d** to **3a** and the problem with BEP to obtain **3c**, we used BOP as coupling reagent but only obtained product in moderate yield (62%); however, PyAOP provided a high yield of 91% (Table 1, entry 3).

With **3a–d** in hand, we synthesized thiazoline-based acids **2a–d** (Scheme 5). The formation of thiazoline ring is the key step in the preparation **2a–d**. Replicating published procedures<sup>2a–c</sup> using Kelly's method,<sup>18a</sup>  $\text{Ph}_3\text{P}=\text{O}/\text{Tf}_2\text{O}$  induced thiazoline ring formation smoothly for **3a,b** to give **33a,b** in  $\text{CH}_2\text{Cl}_2$  at 0 °C within 30 min. However, for **3d**, it took 3 h to consume all starting material and to finally form cyclized compound **33d**. For gem-dimethyl **3c**, it was surprising to us that  $\text{Ph}_3\text{P}=\text{O}/\text{Tf}_2\text{O}$  was unable to induce thiazoline formation effectively (Table 2), despite extended reaction time or increased reaction temperature. Solely the reaction at 60 °C for 24 h gave 29% of **33c** (Table 2, entry 5). Under other conditions, we only detected trace amounts of product (Table 2, entries 1–4). Then we turned to Kelly's  $\text{TiCl}_4$  method<sup>18b</sup> using conditions as shown in Table 2. For  $\text{TiCl}_4$  mediated thiazoline formation, at 25–40 °C in 5–40 h, only 30–34% yield was obtained (entries 6–7); however, the yield improved to 72% when the reaction temperature was increased to 60 °C

**Table 2. Exploring the Conditions of Thiazoline Formation of **33c** from **3c****

entry	reagents <sup>a</sup>	solvent	temp (°C)	time (h) <sup>c</sup>	yield (%) <sup>d</sup>
1	$\text{Ph}_3\text{PO}/\text{Tf}_2\text{O}$	$\text{CH}_2\text{Cl}_2$	0	0.5	trace
2	$\text{Ph}_3\text{PO}/\text{Tf}_2\text{O}$	$\text{CH}_2\text{Cl}_2$	0	24	trace
3	$\text{Ph}_3\text{PO}/\text{Tf}_2\text{O}$	$\text{CH}_2\text{Cl}_2$	25	24	trace
4	$\text{Ph}_3\text{PO}/\text{Tf}_2\text{O}$	$(\text{ClCH}_2)_2$	60	6	trace
5	$\text{Ph}_3\text{PO}/\text{Tf}_2\text{O}$	$(\text{ClCH}_2)_2$	60	15	29
6	$\text{TiCl}_4$	$\text{CH}_2\text{Cl}_2$	25	5	34
7	$\text{TiCl}_4$	$\text{CH}_2\text{Cl}_2$	25–40 <sup>b</sup>	24–40 <sup>b</sup>	30
8	$\text{TiCl}_4$	$(\text{ClCH}_2)_2$	60	2.5	72

<sup>a</sup>Reactions were carried out using  $\text{Ph}_3\text{P}=\text{O}$  (8 equiv) and  $\text{Tf}_2\text{O}$  (4 equiv) for entries 1–5.  $\text{TiCl}_4$  (5 equiv) for entries 6–8 (10 mg scale reactions) or 2.5–3.0 equiv  $\text{TiCl}_4$  used for >30 mg scale reactions).

<sup>b</sup>This reaction first was carried out at 25 °C for 24 h, when large amounts of starting material were still found by TLC and MS, and then this reaction was heated under reflux for another 16 h. <sup>c</sup>Reactions were monitored by MS, the bands of starting material and product on TLC were very close. <sup>d</sup>Products were isolated using preparative TLC plates.

(Table 2, entry 8). Thiazoline-containing intermediates **33a–d** were immediately treated with  $\text{Zn-NH}_4\text{OAc}$  to remove the Troc group to avoid elimination of the *O*-Troc group, providing **34a–d** in 55–90% yield. In the preparation of **34a–b**, the percentage of their dehydration products was: **dehyd-34b** = 15.5%  $\gg$  **dehyd-34d** trace > **dehyd-34a** trace  $\gg$  **dehyd-34c** = 0. **Dehyd-34b** was isolated, and **dehyd-34a** and **dehyd-34d** were detected by MS. The removal of allyl esters of **34a–d** using  $\text{Pd}(\text{PPh}_3)_4/\text{N}$ -methylaniline provided acids **2a–d**.

With **2a–d** in hand, we synthesized the final targets **1a–d** (Scheme 6). Fmoc-protected tripeptide **6**<sup>2a–c</sup> was treated with  $\text{Et}_2\text{NH}$  in MeCN to liberate the corresponding amine and then coupled with acids **2a–d** to provide **36a–d** in yields of 71–95%. PyAOP was chosen as the coupling reagent in the preparation of **36a,c,d** with acceptable results.<sup>2e,f</sup> However, for C34-nonmethyl-acid **2b** (Table 3), when PyAOP or HATU were used as coupling reagents, product **36b** was obtained in only low yield (10–35%) along with dedhydration product (**dehyd-36b**) (5–20% isolated yield). One of the reasons for the low yield of **36b** was that starting material **2b** was consumed by intramolecular dehydration (via elimination to form a double bond at C34–C35 or intramolecular cyclization to form lactone between COOH and C35-OH of **2b**). When we used DEPBT, a specific reagent for amine coupling,<sup>19</sup> the desired product **36b** was obtained in 72% yield and 13% of **dehyd-36b** was isolated, while only trace amount of intramolecular dehydration compound of **2b** was detected.

The cleavage of *O*-allyl ester by  $\text{Pd}(\text{PPh}_3)_4/\text{N}$ -methylaniline and the removal of Fmoc by  $\text{Et}_2\text{NH}/\text{MeCN}$  from linear precursors **36a–d** gave unmasked reactive precursors, which were then macrocyclized with PyAOP or DEPBT in diluted solution. Subsequent purification by semipreparative HPLC afforded final targets **1a–d** in yields of 60%, 25%, 70%, and 45%, respectively. Along with **1b** and **1d**, there were 10% and 5% dehydrated cyclized compounds isolated, respectively. The yields of cyclization and final total yields for the longest linear sequence from pivalaldehyde are summarized in Table 4. The most optimized yield (5.0%) was obtained for **1a**. The lower yields of **1b** and **1d** resulted mainly from their dehydration propensity at C35 during the formation of the thiazoline ring and macrocyclization steps. However, the lower total yield of **1c**

Scheme 6. Synthesis of Targets 1a–d

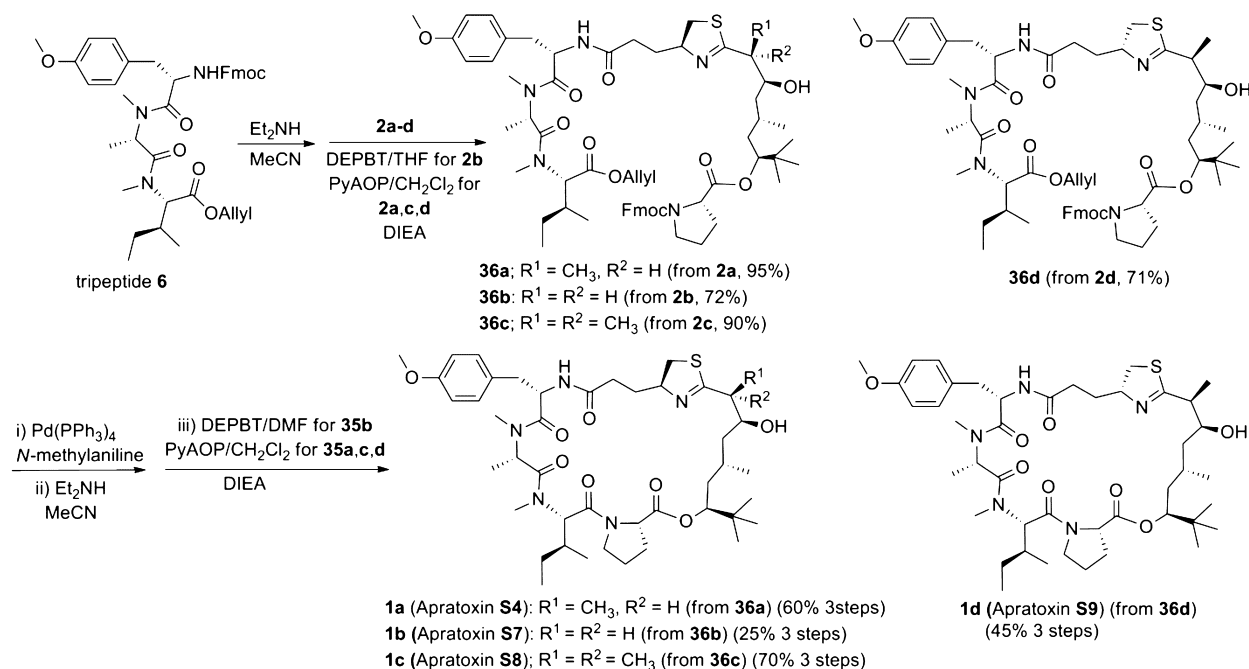


Table 3. Screening of Coupling Reagents for Reaction of 2b and 6 to Form 36b

entry	coupling reagent	yield of 36b (%)	yield of dehyd-36b (%)	ratio 2b to dehyd-2b
1	HATU	10	5	1:1
2	PyAOP	35	20	3:1
3	DEPBT	72	13	20:1

Table 4. Summary of Yields of Final Products 1a–d

	apratoxin S4 (1a)	apratoxin S7 (1b)	apratoxin S8 (1c)	apratoxin S9 (1d)
yield <sup>a</sup> of cyclization (%)	60.0 <sup>c</sup>	25.0	70.0	45.0
total yield <sup>b</sup> (%)	5.0 <sup>d</sup>	1.2	3.0	2.4

<sup>a</sup>Yield over 3 steps. <sup>b</sup>Based on the reaction sequence: pivalaldehyde → 7 → 4 → 3 → 2 → 1. <sup>c</sup>Previous yield<sup>2a</sup> was 52%. <sup>d</sup>Previous yield<sup>2a</sup> was 0.8%.

is due to the lower efficiency of the thiazoline ring formation, presumably as a result of steric hindrance of *gem*-dimethyl at C34. Nevertheless, the prevention of dehydration of *gem*-dimethyl in the formation of the thiazoline ring (34c) and the best yield (70%) during macrocyclization (1c) supported the utility and synthetic accessibility of the *gem*-dimethyl analogue at C34.

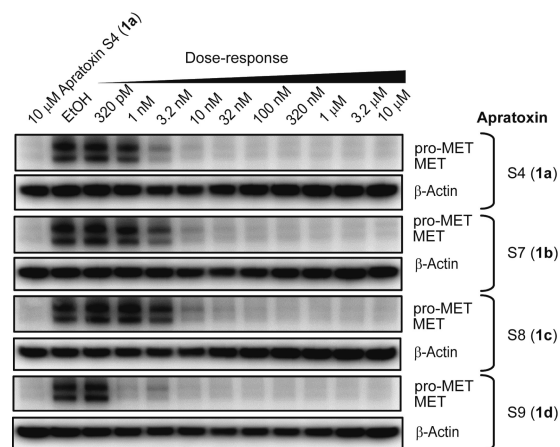
**Structure–Activity Relationships.** Compounds 1b–d retained potent activity compared with 1a in all biological assays. As hypothesized at the onset of this study and consistent with our previous data that the cytotoxic activity is independent of the configuration of methyl-C34, we found that the nonmethylated compound (1b) as well as the *gem*-dimethyl analogue (1c) have similar antiproliferative activity between 1 and 2 nM (Table 5). This indicates that the complexity of apratoxins by eliminating one chiral center (C34) can be somewhat reduced. We have previously shown that apratoxins inhibit cotranslational translocation of secretory molecules, including receptors and growth factors,<sup>2a,3</sup> and therefore we

Table 5. Activities of Synthetic Apratoxins on HCT116 Cell Viability and VEGF-A Secretion

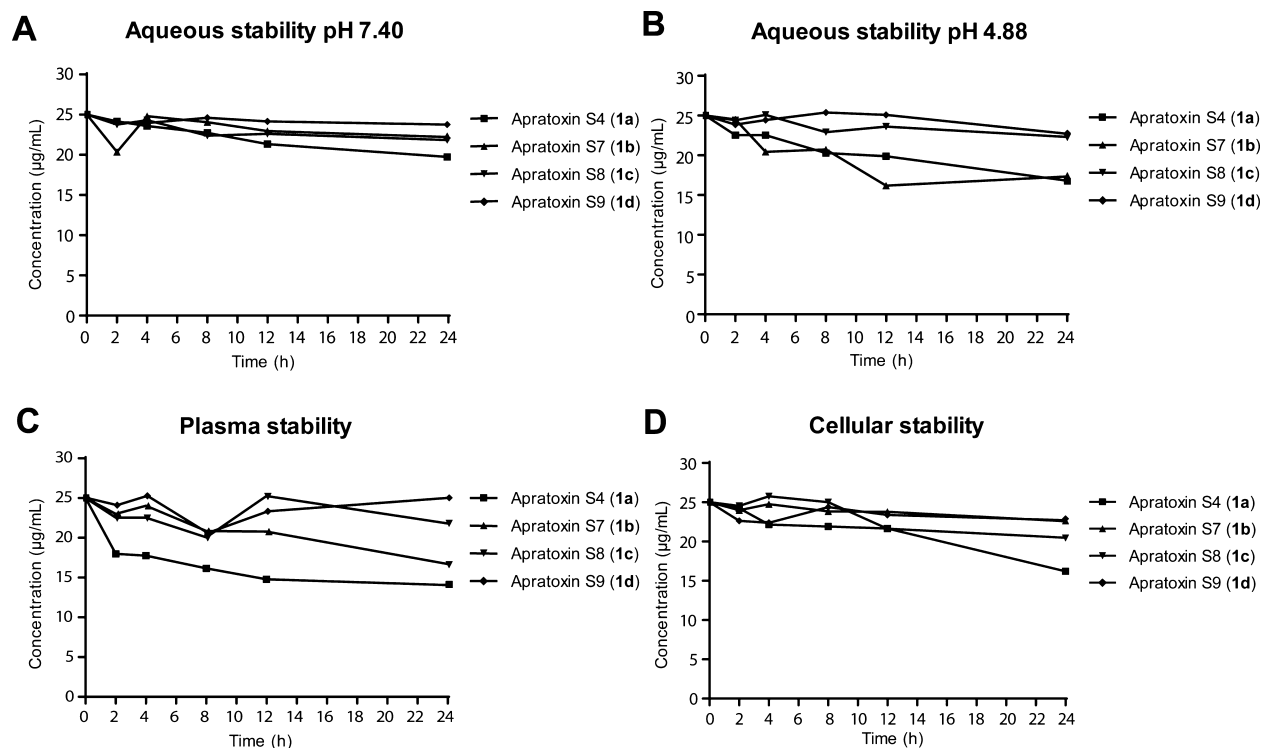
apratoxin	IC <sub>50</sub> (nM) <sup>a</sup> cell viability	IC <sub>50</sub> (nM) <sup>b</sup> VEGF-A secretion
S4 (1a)	1.43	0.32
S7 (1b)	1.25	0.30
S8 (1c)	1.99	0.47
S9 (1d = C30- <i>epi</i> -1a)	0.69	0.12

<sup>a</sup>Determined after 48 h (*n* = 4). <sup>b</sup>Determined after 12 h (*n* = 3).

measured representative key members of these protein classes. The low-nanomolar antiproliferative activity is paralleled by a similar potency in reducing levels of met proto-oncogene receptor (MET) (Figure 2), representative for receptor tyrosine kinases (RTKs) overexpressed in cancers. We also measured secretion of the angiogenic VEGF-A, which is potentially inhibited even at subnanomolar concentrations by 1b and 1c



**Figure 2.** SAR for synthetic apratoxins by immunoblot analysis for RTK (MET) levels.



**Figure 3.** In vitro stability of apratoxins under various conditions. Apratoxins were incubated as indicated and extracted with ethyl acetate, subjected to LC-MS and monitored by using compound-specific MRM mode with harmine as internal standard. (A) Stability in aqueous solution, pH 7.40. (B) Stability in aqueous solution, pH 4.88. (C) Stability in mouse serum. (D) Cellular stability upon exposure to HCT116 protein lysate (0.7 mg/mL).

**Table 6. Microsomal Stability Studies<sup>a</sup>**

time (min)	apratoxin S4 (1a)		apratoxin S7 (1b)		apratoxin S8 (1c)		apratoxin S9 (1d)	
	microsomes only	microsomes + NADPH	microsomes only	microsomes + NADPH	microsomes only	microsomes + NADPH	microsomes only	microsomes + NADPH
0	100	100	100	100	100	100	100	100
3	87.05 ± 1.63	39.55 ± 1.06	66.60 ± 12.30	54.95 ± 4.74	74.10 ± 9.62	37.20 ± 1.27	83.10 ± 0.42	51.40 ± 8.06
5	70.80 ± 23.48	32.20 ± 16.12	60.50 ± 2.26	18.70 ± 1.41	77.45 ± 3.18	17.30 ± 2.55	71.40 ± 1.27	17.80 ± 0.28
15	87.20 ± 2.83	10.07 ± 1.46	76.55 ± 9.55	52.40 ± 2.40	63.45 ± 3.61	8.28 ± 2.86	45.25 ± 5.30	3.55 ± 0.25
30	73.85 ± 15.49	9.58 ± 1.03	54.80 ± 1.56	6.10 ± 1.96	74.75 ± 8.70	4.83 ± 1.05	56.05 ± 1.06	3.73 ± 0.25
60	87.10 ± 1.41	10.00 ± 0.71	54.50 ± 0.00	5.41 ± 1.09	81.25 ± 1.91	3.55 ± 0.91	48.45 ± 0.92	6.06 ± 2.40
120	55.25 ± 12.09	6.02 ± 2.11	53.85 ± 9.40	4.30 ± 0.82	70.05 ± 0.78	5.01 ± 0.23	56.25 ± 0.21	5.63 ± 0.18

<sup>a</sup>Assays were done in triplicate. Values are expressed as % remaining. Mean values are shown ± SD.

with IC<sub>50</sub> values of 300 and 470 pM, respectively. These effects are comparable to the activity of **1a** (Table 5). We had been unable to predict the biological consequences of inverting the C30 configuration of the thiazoline ring. Interestingly, the 30-*epi*-**1a**, compound **1d**, showed superior, picomolar (subnanomolar) potency in all three assays, outperforming the other apratoxins by 2–3-fold (Table 5, Figure 2).

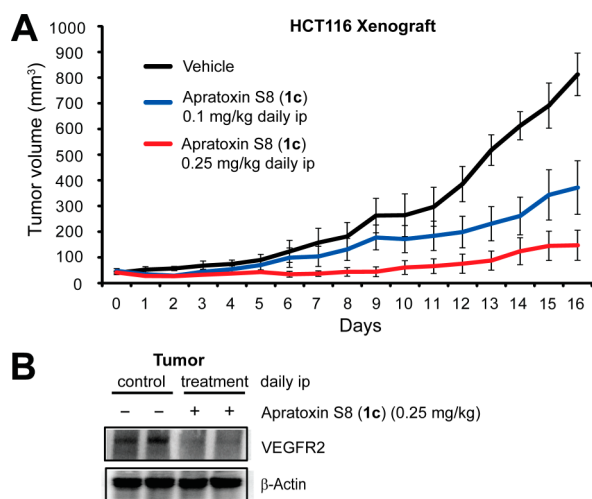
**In Vitro Stability.** Compounds **1a–d** were remarkably stable ( $t_{1/2} > 24$  h) under aqueous conditions at physiological (pH 7.4) and lysosomal pH (4.88) (Figures 3A,B) and possessed excellent plasma and cellular stability (Figures 3C,D). Under these conditions, the C34–C35 dehydrated and hydrated compounds were probably also not interconverting because we also did not observe any hydration at C28–C29 of the conjugated system in apratoxin A. We had been concerned about the tendency of apratoxins **A**, **1a**, **1b**, and **1d** to dehydrate during synthesis as well as upon prolonged exposure to acidic organic solvents (e.g., chloroform),<sup>1b,c</sup> which

could represent a major deactivation pathway for the apratoxins because the dehydrated compounds possess much reduced activity. While an issue during synthesis (see above) and in organic solvents, however, this appeared to not be a major concern in our biological in vitro systems. Nevertheless, **1a** and **1b** were generally somewhat less stable than **1c**, as expected (Figure 3), especially under acidic conditions (Figure 3B). The stability of **1d** was also slightly enhanced compared with that of **1a**, suggesting that the configuration at C30 may affect the tendency to dehydrate (possibly by changing the macrocycle conformation), assuming that dehydration is indeed the mode of primary apratoxin modification. In the synthesis, however, the dehydration propensity at C35 during the formation of the thiazoline ring and macrocyclization steps was slightly greater for **1d** compared with **1a**. Microsomal metabolism of all apratoxins was strongly accelerated by NADPH and stability was found to be low ( $t_{1/2} < 5$  min, Table 6), which may suggest that certain apratoxin biotransformation products could also



retain activity, considering that **1a** was extremely potent and active in vivo as well.<sup>2a</sup>

**In Vivo Activity of 1c.** Even though **1c** had slightly lower activity than our other synthetic apratoxins, it was still very potent in vitro and had the potential advantage that it cannot dehydrate to form a conjugated system. Therefore, we tested **1c** for efficacy in the same HCT116 xenograft mouse model as previously performed for **1a**<sup>2a</sup> and administered **1c** at the previous efficacious dose for **1a** (0.25 mg/kg, ~5 µg/mouse) and a 2.5-fold lower dose (0.1 mg/kg, ~2 µg/mouse) daily via intraperitoneal (ip) injection for 16 days. There was a clear dose-dependent tumor growth inhibition (Figure 4A). The



**Figure 4.** Dose-dependent in vivo activity of **1c** using a HCT116 xenograft mouse model. (A) Subcutaneous tumor-bearing mice were injected daily ip with **1c** ( $n = 7$ ) or vehicle ( $n = 6$ ), and tumor volumes were monitored over time to assess efficacy. Error bars indicate SEM (B) At the end of the efficacy study, tumors were analyzed by immunoblot analysis for levels of an RTK.

higher dose strongly retarded the tumor growth, paralleling the effects of **1a**.<sup>2a</sup> The lower dose showed a weaker yet pronounced effect (Figure 4A); however, the tumor growth could only be inhibited by about 50% using 0.1 mg/kg. It is noteworthy that we did not observe signs of toxicity based on lack of both weight loss and abnormal behavior. Although VEGFR2 expression in HCT116 xenografts was found to be low (consistent with literature data<sup>20</sup>), at the higher dose of **1c**, there was a detectable reduction in VEGFR2 expression levels, indicating target engagement in vivo (Figure 4B).

## CONCLUSION

Apratoxin S4 (**1a**) along with its hitherto unknown C30 epimer (**1d**) and two analogues with achiral C34 (**1b,c**) were synthesized using improved procedures. Most steps have been further optimized compared to our previous apratoxin S4 synthesis. The innovative points in the synthesis are: (1) preparation of homoallylic alcohol **12** from its TBS ether directly working up by cooling-concentration, which is critical to the scale up of aldehyde **7**, (2) Leighton's silanes were introduced to efficiently prepare  $\beta$ -hydroxy acid **4**, one key segment of composing apratoxins, (3)  $\text{LiAlH}_4$  was used instead of DIBAL-H to prepare amino aldehyde in the preparation of moCys part to greatly decrease the racemization, (4) Kelly's thiazoline formation methods (both  $\text{Ph}_3\text{P}=\text{O}/\text{TiF}_2\text{O}$  and  $\text{TiCl}_4$ ) were developed to meet different substrate require-

ments, and (5) the optimized steps are efficient for the synthesis of **1a** and viable to generate **1c** and **1d**. As hypothesized, SAR studies demonstrated that **1b** and **1c** retain activity compared with **1a**. Unexpectedly, we identified **1d** as the most potent apratoxin to date, exhibiting 2–3-fold improved in vitro activity, even though **1d** still has the potential to be deactivated by dehydration. However, compound **1d** was found to be stable during repurifications and stability tests. The propensity of apratoxins to dehydrate to form a C34–C35 double bond is decreased with each methylation at C34; this is possibly a reason why the methylene-C34 has not been isolated from cyanobacteria but only the dehydrated form, apratoxin E (Figure 1),<sup>1c</sup> which could have been formed enzymatically or nonenzymatically. While dehydration appears to not be a problem during in vitro incubations and stability studies, it may lead to apratoxin deactivation in vivo so that the corresponding gem-dimethyl compound **1c** was further characterized and shown to possess in vivo antitumor efficacy. The low microsomal stability combined with the potent in vivo activity may suggest that apratoxin biotransformation products could play a role in the overall activity, and future experiments are aimed at characterizing the in vivo biotransformation products. Overall, our study further corroborates that the development of this class of anticancer agents is warranted.

## EXPERIMENTAL SECTION

**Synthesis. General Procedures.** All commercial reagents were used without further purification unless otherwise noted. Solvents were purified according to the guidelines in ref 21. Tetrahydrofuran (THF) and diethyl ether ( $\text{Et}_2\text{O}$ ) were distilled from sodium chips in the presence of a small amount of benzophenone;  $\text{CH}_2\text{Cl}_2$  and toluene were distilled from  $\text{CaH}_2$ ; MeCN,  $N,N$ -dimethylformamide (DMF) were dried with 4 Å molecular sieves (MS) and MeOH dried with 3 Å MS; 4 M hydrochloric acid (HCl) solution in ethyl acetate was prepared by dissolving HCl gas (yielding by dropping aqueous hydrochloric acid (34%) to concentrated sulfuric acid (98%)) to ethyl acetate. All reactions were performed in heat-gun dried flasks (400 °C under reduced pressure) under an inert atmosphere of anhydrous Ar unless otherwise noted. Thin layer chromatography was performed on EMD silica gel 60 Å  $\text{F}_{254}$  glass plates and preparative thin layer chromatography was performed on Whatman silica gel 60 Å  $\text{F}_{254}$  glass plates (layer thick 1000 µm). Flash column chromatography was performed with Fisher 170–400 mesh silica gel. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 400 MHz, Bruker Avance II 600, Bruker Avance III 600 MHz, or Agilent VNMR 600 MHz spectrometer as indicated in the data list. Chemical shifts for proton nuclear magnetic resonance ( $^1\text{H}$  NMR) spectra are reported in parts per million relative to the signal residual  $\text{CDCl}_3$  at 7.26 ppm. Chemical shifts for carbon nuclear magnetic resonance ( $^{13}\text{C}$  NMR) spectra are reported in parts per million relative to the center line of the  $\text{CDCl}_3$  triplet at 77.16 ppm. The abbreviations s, d, dd, ddd, dddd, t, q, br, and m stand for the resonance multiplicity singlet, doublet, doublet of doublets, doublet of doublet of doublets, doublet of doublet of doublet of doublets, triplet, quartet, broad and multiplet, respectively. Optical rotation was measured on a Perkin-Elmer 341 polarimeter (Na D line) using a microcell of 1 dm path length. High resolution mass spectra (HRMS) data were obtained using an Agilent-LC-TOF mass spectrometer with an APCI/ESI multimode ion source detector. LR-MS data was obtained using a 3200 QTrap triple quadrupole mass spectrometer and detection by electrospray ionization-MS in the positive ion mode. The purity (>95%) of all tested compounds was determined by HPLC analysis (Shimadzu) equipped with a Phenomenex Ultracarb ODS column (250 mm × 10 mm, 5 µm) and a Shimadzu SPD-M20A detector at 200/220 nm wavelength. The mobile phase was a MeCN/ $\text{H}_2\text{O}$  mixture. All tested compounds were at least 95% pure.

(*S*)-2,2-Dimethyl-5-hexen-3-ol (**12**).<sup>2d-h,8</sup> The solution of tetra-*n*-butylammonium fluoride trihydrate (TBAF) (82.07 g, 260.116 mmol) in THF (220 mL) was added to the mixture of compound **11** (21.0 g, 86.705 mmol) and 4 Å molecular sieves (22 g, predried at 450 °C under reduced pressure 1 h) in anhydrous THF (300 mL) at 0 °C. Then the reaction mixture was stirred at room temperature overnight and filtered through a small pad of Celite (washed with diethyl ether). The filtrate was quenched with 200 mL of water, extracted with diethyl ether (300 mL × 3), washed with brine (200 mL × 2), dried over anhydrous MgSO<sub>4</sub>, concentrated with cooling/condensing fraction distillation system under moderate vacuum, and further concentrated by Vigreux fraction distillation column. The concentrated mixture was purified by column chromatography eluted by 3–5% diethyl ether in pentane. The eluted product fractions were also concentrated by cooling/condensing fraction distillation system under moderate vacuum to give product **12** and further distilled by Vigreux fraction distillation column to provide product **12** (10.5 g, 95%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.86 (dddd, *J* = 14.4, 10.4, 8.8, 6.0 Hz, 1H), 5.14 (m, 2H), 3.25 (dd, *J* = 10.4, 2.0 Hz, 1H), 2.39–2.33 (m, 1H), 1.98 (ddd, *J* = 13.6, 9.6, 9.6 Hz, 1H), 0.91 (s, 9H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 136.7, 117.9, 78.2, 36.7, 34.7, 25.9 ppm.

(3*R*,5*S*)-5-(4-Methoxy-benzoyloxy)-3,6,6-trimethylheptanoic Acid Methoxy-methyl-amide (**17**).<sup>2d</sup> 4-Methoxybenzyl-2,2,2-trichloroacetimidate (3.7 mL, 17.8 mmol) and trifluoromethane sulfonic acid (TfOH) (7.9 μL, 0.089 mmol) was added sequentially to the solution of **16** (2.05 g, 8.9 mmol) in THF (20 mL) at 0 °C. The resulting mixture was stirred at room temperature overnight and was then diluted with ethyl acetate (20 mL), quenched with saturated NaHCO<sub>3</sub> (20 mL), extracted with ethyl acetate (20 mL × 3), dried with anhydrous MgSO<sub>4</sub>, and evaporated in vacuo. Hexane (100 mL) was added to the residue, which resulted in the precipitation of a white solid (2,2,2-trichloroacetimidate). The solid was filtered off, and the filtrate was concentrated and purified by column chromatography (eluted by 20–50% ethyl acetate in hexane) to give product **17** (1.74 g, 56%, 100% BRSM) (recovered starting material 1.08 g and used in next cycle).

(3*R*,4*S*,6*S*,8*S*)-8-(4-Methoxybenzyloxy)-3,6,9,9-tetramethyldec-1-en-4-ol (**19a**).<sup>2a,c</sup> To the solution of **7** (234.1 mg, 0.801 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) was added (*S,S*)-trans EZ-CrotylMix (mixture of **18a** and Sc(OTf)<sub>3</sub>) (943 mg, 1.602 mmol) at room temperature. This mixture was stirred vigorously at the same temperature for 2.5 h, and then it was treated with 12 mL of Et<sub>2</sub>O and 12 mL of 1N aq HCl. After this quenched mixture was stirred at room temperature for 30 min, it was filtered off to remove precipitated solid, extracted with Et<sub>2</sub>O (15 mL × 3), washed with saturated NaHCO<sub>3</sub> (20 mL × 2) and brine (20 mL), dried with anhydrous MgSO<sub>4</sub>, evaporated in vacuo, and purified by chromatography column (eluted by 3.5–4.5% ethyl acetate in hexane) to give product **19a** (245.7 mg, 88%) as a colorless oil.

(4*S*,6*S*,8*S*)-8-(4-Methoxybenzyloxy)-6,9,9-tetramethyldec-1-en-4-ol (**19b**). To the solution of **7** (203.3 mg, 0.696 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (7 mL) was added (*S,S*)-**18b** (772.1 mg, 1.392 mmol) and Sc(OTf)<sub>3</sub> (28.5 mg, 0.058 mmol) at 0 °C. This mixture was stirred vigorously at the same temperature for 2.0 h, and then it was treated with 12 mL of Et<sub>2</sub>O and 12 mL of 1N aq HCl. Afterwards, the quenched mixture was stirred at room temperature for 30 min, filtered to remove the precipitate, extracted with Et<sub>2</sub>O (15 mL × 3), washed with saturated NaHCO<sub>3</sub> (20 mL × 2) and brine (20 mL), dried with anhydrous MgSO<sub>4</sub>, evaporated in vacuo, and purified by column chromatography (eluted by 4.2% ethyl acetate in hexane) to give product **19b** (197.6 mg, 85%) as a colorless oil; [α]<sub>D</sub><sup>20</sup> –57.8 (*c* 0.32, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.30 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 5.89–5.79 (m, 1H), 5.17–5.13 (m, 2H), 4.63 (d, *J* = 10.4 Hz, 1H), 4.52 (d, *J* = 10.4, 1H), 3.79 (s, 3H), 3.79–3.74 (m, 1H), 3.11 (dd, *J* = 9.2, 2.4 Hz, 1H), 2.30–2.24 (m, 1H), 2.21–2.14 (m, 1H), 2.00–1.90 (br m, 1H), 1.65 (ddd, *J* = 13.6, 10.6, 2.8 Hz, 1H), 1.46 (ddd, *J* = 14.0, 8.8, 4.0 Hz, 1H), 1.35 (ddd, *J* = 14.0, 9.2, 2.4 Hz, 1H), 1.09 (ddd, *J* = 13.6, 10.4, 2.0 Hz, 1H), 0.97 (d, *J* = 6.4 Hz, 3H), 0.94 (s, 9H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 159.0, 135.0, 131.7, 129.2, 118.1, 113.7, 85.3, 74.3, 68.4, 55.3, 43.5, 43.3, 39.8, 36.2, 26.6,

21.1 ppm. HRMS (ESI) *m/z* calcd for C<sub>21</sub>H<sub>34</sub>O<sub>3</sub>Na (M + Na)<sup>+</sup> 357.2400, found 357.2409.

*tert*-Butyl [(4*S*,6*S*,8*S*)-8-(4-Methoxybenzyloxy)-6,9,9-tetramethyldec-1-en-4-yloxy]dimethyl-silane (**20b**). To the solution of **19b** (178.6 mg, 0.534 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) were added 2,6-lutidine (310 μL, 2.672 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (368.2 μL, 1.603 mmol) at 0 °C under Ar. After being stirred at the same temperature for 1.5 h, the reaction was quenched with MeOH (5 mL) and saturated aq NH<sub>4</sub>Cl (7 mL), extracted with ethyl acetate (10 mL × 4), washed with brine (10 mL × 2), dried with anhydrous MgSO<sub>4</sub>, evaporated in vacuo, and purified by column chromatography (eluted by 3.6% ethyl acetate in hexane) to give product **20b** (224.4 mg, 94%) as a colorless oil; [α]<sub>D</sub><sup>20</sup> –49.0 (*c* 0.31, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.28 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.86–5.76 (m, 1H), 5.07–5.03 (m, 2H), 4.65 (d, *J* = 10.8 Hz, 1H), 4.45 (d, *J* = 10.8 Hz, 1H), 3.86–3.80 (m, 1H), 3.80 (s, 3H), 3.08 (dd, *J* = 7.2, 3.6 Hz, 1H), 2.31–2.20 (m, 2H), 1.87 (br m, 1H), 1.66 (ddd, *J* = 13.2, 9.2, 2.8 Hz, 1H), 1.46–1.34 (m, 2H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.91 (s, 9H), 0.86 (s, 9H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 159.0, 135.2, 131.9, 128.9, 117.0, 113.7, 85.6, 73.9, 69.8, 55.4, 43.9, 43.4, 40.5, 36.2, 26.8, 26.6, 26.1, 21.0, 18.2, –4.1, –4.3 ppm. HRMS (ESI) *m/z* calcd for C<sub>27</sub>H<sub>48</sub>O<sub>3</sub>SiNa (M + Na)<sup>+</sup> 471.3265, found 471.3264.

**Compound 21a,b.** To a solution of **20** (0.806 mmol) in the mixture of CH<sub>2</sub>Cl<sub>2</sub> (5.0 mL) and H<sub>2</sub>O (0.5 mL) was added 2,3-dichloro-5,6-dibenzoquinone (DDQ) (220 mg, 0.968 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h, quenched with saturated aqueous NaHCO<sub>3</sub> (10 mL), and filtered in vacuo. The organic layer was separated and the water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL × 3). The organic phase was combined and washed with brine (20 mL × 2), dried with anhydrous MgSO<sub>4</sub>, filtered, and concentrated in vacuo. This residue was used for the next reaction without further purification.

To the suspended solution of Fmoc-Pro-OH (549.5 mg, 1.629 mmol) in toluene (5.0 mL) were added *N,N*-diisopropylethylamine (DIEA) (435 μL, 2.5 mmol) and 2,4,6-trichlorobenzoyl chloride (390 μL, 2.5 mmol) at room temperature under argon, and the mixture was stirred at the same temperature for 30 min. Then the crude alcohol in toluene (3.0 mL) and DMAP (350 mg, 2.862 mmol) were added to the above mixture at 10 °C. After being stirred at room temperature for 3 h, the reaction mixture was quenched and extracted with diethyl ether (10 mL × 3). The combined organic layer was washed with saturated NH<sub>4</sub>Cl (15 mL × 2), saturated NaHCO<sub>3</sub> (15 mL × 2), and brine (15 mL), dried over MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluted by 10% ethyl acetate in hexane) to give ester **21**.

*Pyrrolidine-1,2-dicarboxylic Acid*-(2*S*)-2-[(4*S*,6*S*,8*S*)-8-*tert*-Butyl-4-(*tert*-butyldimethylsilyl-oxy)-6-dimethyloct-1-en-8-yl]ester 1-(9*H*-Fluoren-9-ylmethyl)ester (**21b**) (from **20b**). Yield 100%; [α]<sub>D</sub><sup>20</sup> –66.4 (*c* 0.11, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 7.78–7.75 (m, 2 H), 7.67–7.55 (m, 2H), 7.42–7.38 (m, 2H), 7.33–7.30 (m, 2H), 5.85–5.71 (m, 1H), 5.05–4.99 (m, 2H), 4.80 (dd, *J* = 8.4, 2.0 Hz, 1H), 4.53–4.40 (m, 2H), 4.35–4.17 (m, 2H), 3.81–3.71 (m, 1H), 3.70–3.62 (m, 1H), 3.60–3.50 (m, 1H), 2.32–2.15 (m, 3H), 2.14–1.93 (m, 3H), 1.62–1.49 (m, 3H), 1.39–1.30 (m, 1H), 1.20–1.03 (m, 1H), 0.95 (d, *J* = 6.4 Hz, 2H), 0.87 (s, 9 H), 0.87 (s, 9 H), 0.82 (d, *J* = 6.4 Hz, 1H), 0.05–0.02 (m, 6H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 172.2, 172.1, 154.7, 154.5, 144.3, 144.2, 144.1, 143.8, 141.4, 141.4, 141.3, 135.6, 135.3, 127.8, 127.8, 127.2, 127.1, 127.1, 125.5, 125.3, 125.2, 120.0, 116.9, 116.8, 80.6, 80.4, 77.5, 77.2, 76.8, 70.2, 70.0, 67.8, 67.5, 59.8, 59.6, 47.4, 47.0, 46.4, 44.2, 44.0, 42.6, 42.5, 39.3, 39.1, 35.2, 35.1, 31.3, 30.1, 27.1, 27.0, 26.1, 26.0, 26.0, 24.4, 23.4, 20.9, 20.6, 18.2 ppm. HRMS (ESI) *m/z* calcd for C<sub>39</sub>H<sub>57</sub>NO<sub>5</sub>SiNa (M + Na)<sup>+</sup> 670.3898, found 670.3920.

**Compound 4a,b.** To the solution of **21** (0.646 mmol) in DMF (6.0 mL) were added Oxone (1.588 g, 2.583 mmol), NaHCO<sub>3</sub> (217.0 mg, 2.583 mmol), and OsO<sub>4</sub> (2.5% solution in *tert*-BuOH) (81 μL, 6.5 μmol) at room temperature. After being stirred at the same temperature for 15 h, the reaction mixture was diluted with water (4



mL) and *tert*-BuOH (7.5 mL), and then NaIO<sub>4</sub> (276.3 mg, 1.292 mmol) was added. The reaction mixture was stirred at room temperature for an additional 5 h and poured into aqueous HCl (1 M for **4a**, 0.5 M for **4b** to pH 1; 25 mL) and CH<sub>2</sub>Cl<sub>2</sub> (25 mL). The water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL × 3). The combined CH<sub>2</sub>Cl<sub>2</sub> layer was washed with 10 wt % Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (50 mL × 3) and brine (50 mL × 1), dried over MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give product **4** as a white solid (**4a** eluted by 17–25% ethyl acetate in hexane; **4b** eluted by 2.5–10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>).

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-[(1S,3S,5S)-1-*tert*-Butyl-6-carboxy-5-hydroxy-3-methylhept-1-yl]ester 1-(9H-fluoren-9-ylmethyl)ester (**4b**)** (from **21b**). Yield 80%; [ $\alpha$ ]<sub>D</sub><sup>20</sup> –46.2 (c 0.119, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, mixture of rotamers, major):  $\delta$  7.75 (d, *J* = 7.6 Hz, 2H), 7.62–7.55 (m, 2H), 7.39 (dd, *J* = 7.6, 7.4 Hz, 2H), 7.33–7.29 (m, 2H), 5.40 (br, 1H), 4.91 (d, *J* = 10.8 Hz, 1H), 4.42–4.38 (m, 1H), 4.34–4.29 (m, 1H), 4.12–4.06 (m, 1H), 3.68–3.62 (m, 1H), 3.52–3.47 (m, 1H), 2.44–2.43 (m, 2H), 2.33–2.23 (m, 1H), 2.08–1.91 (m, 3H), 1.86–1.80 (m, 1H), 1.75 (dd, *J* = 13.6, 2.4 Hz, 1H), 1.66 (dd, *J* = 14.4, 2.0 Hz, 1H), 1.33–1.26 (m, 1H), 1.04–0.98 (m, 1H), 0.98 (d, *J* = 6.4 Hz, 3H), 0.89 (s, 9H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, mixture of rotamers, major):  $\delta$  174.6, 172.4, 155.4, 144.0, 143.7, 127.9, 127.8, 127.3, 127.2, 125.3, 125.2, 120.1, 120.1, 78.5, 68.0, 66.0, 59.6, 47.2, 46.7, 42.7, 41.7, 37.2, 34.8, 30.0, 26.1, 25.0, 24.6, 20.6 ppm. HRMS (ESI) *m/z* calcd for C<sub>32</sub>H<sub>41</sub>NO<sub>7</sub>Na (M + Na)<sup>+</sup> 574.2775, found 574.2970.

**Methyl (3S,5S,7S)-3-Hydroxy-7-(4-methoxybenzyloxy)-2,2,5,8,8-pentamethylnonanoate (**23**)**. To the solution of *N*-tosyl-D-Val-OH (145.6 mg, 0.537 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (7 mL) at 0 °C under argon was added the solution of BH<sub>3</sub> in THF (1M, 537  $\mu$ L, 0.537 mmol) dropwise. The resulting mixture was stirred at room temperature for 20 min and then cooled to –78 °C. Aldehyde **7** (157.0 mg, 0.537 mmol) and methyl trimethylsilyl dimethylketene acetal (120.0  $\mu$ L, 0.591 mmol) were added successively to the above mixture at –78 °C. After being stirred at –78 °C for 3 h, the reaction mixture was quenched with buffer (pH 7; 8 mL) and then it was warmed to room temperature and another 5 mL of buffer (pH 7) was added. The quenched reaction mixture was extracted with Et<sub>2</sub>O (25 mL × 3), washed with saturated NaHCO<sub>3</sub> (15 mL × 2) and brine (15 mL × 2), dried with anhydrous MgSO<sub>4</sub>, and purified by column chromatography (eluted by 8.5% ethyl acetate in hexane) to give product **23** as a colorless oil (171.5 mg, 81%); [ $\alpha$ ]<sub>D</sub><sup>20</sup> –55.8 (c 0.12, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.29 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 4.63 (d, *J* = 10.4 Hz, 1H), 4.50 (d, *J* = 10.4 Hz, 1H), 3.77 (s, 3H), 3.74 (br m, 1H), 3.69 (s, 3H), 3.09 (dd, *J* = 9.2, 2.4 Hz, 1H), 2.47 (br, 1H), 2.03–1.95 (br m, 1H), 1.58–1.45 (m, 2H), 1.37–1.30 (m, 1H), 1.18 (s, 6H), 1.03–1.00 (m, 1H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.93 (s, 9H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  178.3, 159.0, 131.6, 129.2, 113.7, 85.2, 74.4, 74.2, 55.3, 51.9, 47.3, 39.7, 38.2, 36.2, 26.8, 26.6, 22.1, 20.9, 20.6 ppm. HRMS (ESI) *m/z* calcd for C<sub>23</sub>H<sub>38</sub>O<sub>5</sub>Na (M + Na)<sup>+</sup> 417.2611, found 417.2628.

**(3S,5S,7S)-3-Hydroxy-7-(4-methoxybenzyloxy)-2,2,5,8,8-pentamethylnonanoic Acid (**24**)**. To the solution of **23** (223.8 mg, 0.568 mmol) in the mixture of THF–MeOH–H<sub>2</sub>O (7 mL–3.5 mL–0.7 mL) was added aq LiOH (119.1 mg in 2.8 mL H<sub>2</sub>O, 2.838 mmol). After being stirred at room temperature for 5 h, the reaction mixture was diluted with 10 mL of water, acidified by addition of aq HCl (2M) to pH 2, extracted with ethyl acetate (10 mL × 4), washed with brine (10 mL × 2), dried with anhydrous MgSO<sub>4</sub>, and purified by column chromatography (eluted by 8.5–17% acetone in hexane) to give **24** (225 mg, 100%) as a white solid; [ $\alpha$ ]<sub>D</sub><sup>20</sup> –52.3 (c 0.26, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.30 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 4.63 (d, *J* = 10.4 Hz, 1H), 4.53 (d, *J* = 10.4 Hz, 1H), 3.80 (d, *J* = 13.0, 1H), 3.78 (s, 3H), 3.13 (dd, *J* = 8.0, 2.0 Hz, 1H), 1.80 (br m, 1H), 1.62–1.49 (m, 2H), 1.40–1.34 (m, 1H), 1.21 (s, 3H), 1.19 (s, 3H), 1.09 (dd, *J* = 12.4, 12.4), 0.98 (d, *J* = 6.4 Hz, 3H), 0.95 (s, 9H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  182.9, 159.0, 131.4, 129.3, 113.7, 85.2, 74.3, 74.3, 55.3, 47.1, 39.4, 37.9, 36.2, 26.8, 26.6, 22.3, 20.8, 20.2 ppm. HRMS (ESI) *m/z* calcd for C<sub>22</sub>H<sub>36</sub>O<sub>5</sub>Na (M + Na)<sup>+</sup> 403.2455, found 403.2472.

**Allyl (3S,5S,7S)-3-Hydroxy-7-(4-methoxybenzyloxy)-2,2,5,8,8-pentamethylnonanoate (**25**)**. To the solution of compound **24** (222.4 mg, 0.585 mmol) in dimethyl sulfoxide (DMSO) (5.0 mL) were added K<sub>2</sub>CO<sub>3</sub> (242.5 mg, 1.754 mmol) and allyl bromide (AllylBr) (99  $\mu$ L, 1.170 mmol) at room temperature. After being stirred at the same temperature overnight, the reaction was quenched with water (10 mL) and extracted with ethyl acetate (20 mL × 4). The combined organic layer was washed with brine (10 mL × 5), filtered, concentrated in vacuo, and purified by silica gel column chromatography (eluted by 6.5% ethyl acetate in hexane) to give product **25** (228.6 mg, 93%) as a colorless oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> –56.0 (c 0.175, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.30 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 5.96–5.86 (m, 1H), 5.35–5.22 (m, 2H), 4.64 (d, *J* = 10.4 Hz, 1H), 4.60 (d, *J* = 5.6 Hz, 2H), 4.50 (d, *J* = 10.4 Hz, 1H), 3.78 (br m, 1H), 3.77 (s, 3H), 3.10 (dd, *J* = 8.0, 2.0 Hz, 1H), 2.54 (br, 1H), 2.0 (br m, 1H), 1.61–1.55 (m, H), 1.49 (ddd, *J* = 17.6, 9.6, 4.0 Hz, 1H), 1.37–1.31 (m, 1H), 1.21 (s, 6H), 1.05–0.98 (m, 1H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.94 (s, 9H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  177.3, 158.9, 132.0, 131.5, 129.1, 118.2, 113.6, 85.0, 74.3, 74.2, 65.2, 55.2, 47.3, 39.7, 38.1, 36.1, 26.6, 26.5, 21.9, 20.9, 20.7 ppm. HRMS (ESI) *m/z* calcd for C<sub>25</sub>H<sub>40</sub>O<sub>5</sub>Na (M + Na)<sup>+</sup> 443.2768, found 443.2769.

**Allyl (3S,5S,7S)-3-(2,2,2-Trichloroethoxycarbonyloxy)-7-(4-methoxybenzyloxy)-2,2,5,8,8-pentamethylnonanoate (**26**)**. To the solution of **25** (220.0 mg, 0.523 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5.0 mL) at 0 °C under Ar were added 4-dimethylaminopyridine (DMAP) (1.3 mg, 10.5  $\mu$ mol), pyridine (423  $\mu$ L, 5.234 mmol), and 2,2,2-trichloroethoxycarbonyl chloride (Troc-Cl) (554.5 mg/360.0  $\mu$ L, 2.617 mmol). After being stirred at 0 °C for 10 min and room temperature for 1 h, the reaction was quenched with water (10 mL) and extracted with ethyl acetate (15 mL × 4). The combined organic layer was washed with saturated NaHCO<sub>3</sub> and brine, dried with MgSO<sub>4</sub>, concentrated in vacuo, and purified by silica gel column chromatography (2.5–10–15% ethyl acetate in hexane) to give **26** (295.1 mg, 95.0%); [ $\alpha$ ]<sub>D</sub><sup>20</sup> –7.6 (c 0.37, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.27 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 5.96–5.86 (m, 1H), 5.37–5.23 (m, 3H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.59 (d, *J* = 5.6 Hz, 2H), 4.55 (d, *J* = 10.8 Hz, 1H), 4.49 (d, *J* = 10.8 Hz, 1H), 4.30 (d, *J* = 12.0 Hz, 1H), 3.78 (s, 3H), 3.05 (dd, *J* = 9.6, 2.0 Hz, 1H), 1.99–1.93 (m, 1H), 1.75 (br m, 1H), 1.50 (ddd, *J* = 17.2, 9.2, 3.0 Hz, 1H), 1.35–1.29 (m, 1H), 1.27 (s, 3H), 1.25 (s, 3H), 1.08–1.01 (m, 4H), 0.92 (s, 9H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  174.8, 159.0, 154.4, 131.9, 131.5, 128.7, 118.6, 113.7, 94.6, 85.0, 80.9, 76.6, 74.4, 65.6, 55.3, 47.0, 39.8, 36.8, 36.1, 26.5, 26.4, 22.4, 20.8, 19.7 ppm. HRMS (ESI) *m/z* calcd for C<sub>28</sub>H<sub>41</sub>Cl<sub>3</sub>O<sub>7</sub>Na (M + Na)<sup>+</sup> 617.1810, found 617.1832.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-[(1S,3S,5S)-1-*tert*-Butyl-6-allyloxycarbonyl-5-(2,2,2-trichloroethoxycarbonyloxy)-3,6-dimethylhept-1-yl]ester 1-(9H-Fluoren-9-ylmethyl)ester (**27**)**. To a solution of **26** (291.5 mg, 0.491 mmol) in a mixture of CH<sub>2</sub>Cl<sub>2</sub> (5.0 mL) and H<sub>2</sub>O (0.5 mL) was added 2,3-dichloro-5,6-dibenzoquinone (DDQ) (133.6 mg, 0.589 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h, quenched with saturated aqueous NaHCO<sub>3</sub> (6 mL), and filtered in vacuo. The organic layer was separated, and the water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (15 mL × 4). The organic phase was combined and washed with brine (3 × 15 mL), dried with anhydrous MgSO<sub>4</sub>, filtered, and concentrated in vacuo. This residue was used for the next reaction without further purification.

To the suspended solution of Fmoc-Pro-OH (334.3 mg, 0.991 mmol) in toluene (3.0 mL) were added *N,N*-diisopropylethylamine (DIEA) (0.26 mL, 1.49 mmol) and 2,4,6-trichlorobenzoyl chloride (233  $\mu$ L, 1.491 mmol) at room temperature under argon, which was stirred at the same temperature for 10 min. Then the crude alcohol in toluene (1.5 mL) and DMAP (212.8 mg, 1.742 mmol) were added to the above mixture. After being stirred at room temperature overnight, the reaction mixture was quenched with water and extracted with diethyl ether (20 mL × 4). The combined organic layer was washed with saturated NH<sub>4</sub>Cl (20 mL × 2), saturated NaHCO<sub>3</sub> (20 mL × 2), and brine (20 mL), dried over MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluted by 10% ethyl acetate in hexane) to give ester **27** (360.8 mg, 90.4%) as a

colorless oil;  $[\alpha]_D^{20}$   $-58.9$  ( $c$  0.124,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.77–7.75 (m, 2H), 7.67–7.62 (m, 2H), 7.40 (dd,  $J$  = 7.4, 7.2 Hz, 2H), 7.34–7.29 (m, 2H), 6.00–5.79 (m, 1H), 5.34–5.12 (m, 3H), 4.97 (d,  $J$  = 12.0 Hz, 0.6H), 4.87 (d,  $J$  = 12.0 Hz, 0.4H), 4.78 (d,  $J$  = 10.0 Hz, 1H), 4.69 (d,  $J$  = 12.0 Hz, 0.4H), 4.64 (d,  $J$  = 12.0 Hz, 0.6H), 4.59–4.16 (m, 6H), 3.67–3.61 (m, 1H), 3.59–3.48 (m, 1H), 2.38–2.28 (m, 0.6H), 2.24–2.16 (m, 0.4H), 2.15–2.06 (m, 1H), 2.04–1.93 (m, 2H), 1.92–1.80 (m, 1H), 1.55–1.48 (m, 1H), 1.46–1.42 (m, 1H), 1.38–1.29 (m, 1H), 1.25–1.19 (m, 6H), 1.15–1.07 (m, 0.6H), 1.00–0.96 (m, 1.6H), 0.89 (s, 5.4H), 0.86 (s, 3.6H), 0.67 (d,  $J$  = 6.4 Hz, 1.8H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  175.0, 175.0, 172.9, 172.4, 154.7, 154.5, 154.3, 154.2, 144.4, 144.3, 144.0, 143.9, 141.4, 141.4, 141.3, 141.3, 132.0, 131.9, 127.8, 127.2, 127.1, 125.5, 125.4, 125.3, 125.2, 120.0, 120.0, 118.6, 94.9, 94.8, 80.8, 80.6, 79.8, 79.5, 77.1, 76.9, 68.0, 67.5, 65.7, 65.6, 59.9, 59.4, 47.3, 47.3, 47.2, 47.1, 46.5, 38.2, 38.0, 37.3, 36.8, 34.9, 34.8, 34.7, 31.7, 31.4, 30.1, 26.9, 26.7, 25.9, 25.9, 25.4, 23.5, 22.8, 22.0, 22.0, 20.5, 20.4, 19.8, 19.7, 14.2 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{40}\text{H}_{50}\text{Cl}_3\text{NO}_9\text{Na}$  ( $M + \text{Na}$ ) $^+$  816.2443, found 816.2420.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-[(1S,3S,5S)-1-tert-Butyl-6-carboxy-5-(2,2,2-trichloroethoxycarbonyloxy)-3,6-dimethylhept-1-yl]ester 1-(9H-Fluoren-9-ylmethyl)ester (4c).** To a solution of **27** (349.3 mg, 0.440 mmol) in THF (8 mL) were added  $\text{Pd}(\text{PPh}_3)_4$  (76.3 mg, 0.066 mmol) and *N*-methyl aniline (144.3  $\mu\text{L}$ , 1.321 mmol) at room temperature under argon. This reaction was protected with aluminum foil. After being stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo and purified by column chromatography (eluted by acetone/hexane 1:3) to give acid **4c** (354.1 mg, 95%) as a pale-yellow solid;  $[\alpha]_D^{20}$   $-51.1$  ( $c$  0.131,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  9.90 (br, 1H), 7.77–7.74 (m, 2H), 7.68–7.63 (m, 2H), 7.42–7.38 (m, 2H), 7.34–7.29 (m, 2H), 5.23 (d,  $J$  = 10.4 Hz, 0.4H), 5.13 (d,  $J$  = 10.4 Hz, 0.6H), 5.01 (d,  $J$  = 12.0 Hz, 0.6H), 4.90 (d,  $J$  = 12.0 Hz, 0.4H), 4.85–4.79 (m, 1H), 4.70 (d,  $J$  = 12.0 Hz, 0.4H), 4.63 (d,  $J$  = 12.0 Hz, 0.6H), 4.52–4.17 (m, 4H), 3.67–3.48 (m, 1H), 2.37–2.27 (m, 0.6H), 2.24–2.19 (m, 0.4H), 2.16–2.10 (m, 1H), 2.01–1.83 (m, 3H), 1.78–1.69 (m, 0.4H), 1.62–1.49 (m, 1.6H), 1.43–1.35 (m, 1H), 1.31–1.20 (m, 6H), 1.09–1.00 (m, 1H), 1.01 (d,  $J$  = 6.4 Hz, 1.2 H), 0.94 (s, 5.4H), 0.87 (s, 3.6H), 0.69 (d,  $J$  = 6.4 Hz, 1.8H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  181.1, 180.7, 172.8, 172.3, 154.8, 154.4, 154.2, 144.3, 144.1, 143.9, 143.7, 141.3, 141.3, 141.2, 141.2, 135.1, 135.0, 135.0, 130.6, 128.1, 128.1, 128.0, 127.7, 127.1, 127.1, 127.0, 125.5, 125.3, 125.2, 125.1, 120.0, 119.9, 94.9, 94.8, 80.5, 80.3, 79.8, 79.4, 77.0, 76.8, 68.0, 67.6, 59.8, 59.3, 47.2, 47.1, 46.9, 46.4, 38.1, 37.5, 37.1, 36.7, 34.9, 34.7, 34.6, 34.5, 31.6, 31.3, 30.0, 29.1, 29.1, 26.7, 26.5, 25.9, 25.3, 24.3, 23.5, 22.7, 22.2, 21.9, 20.8, 20.4, 19.9, 19.6, 19.4, 18.8, 14.2, 11.5 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{37}\text{H}_{46}\text{Cl}_3\text{NO}_9\text{Na}$  ( $M + \text{Na}$ ) $^+$  776.2130, found 776.2149.

**Compound 30a<sup>2a-c</sup> and 30b.** To the solution of Weinreb amide **28** (2.505 g, 4.945 mmol) in THF (50 mL) was added  $\text{LiAlH}_4$  (234.6 mg, 6.182 mmol) in one portion at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was quenched with 0.2N aq  $\text{KHSO}_4$  (30 mL) and extracted with  $\text{Et}_2\text{O}$  (50 mL  $\times$  3). The combined organic layer was washed with 1N aq HCl (30 mL  $\times$  3) and brine (30 mL  $\times$  3), dried with anhydrous  $\text{MgSO}_4$ , and evaporated in vacuo to give the crude aldehyde **29**, which was used in the next step without further purification. To the crude aldehyde **28** in toluene (30 mL) was added  $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$  (3.101 g, 8.901 mmol) at 0 °C under Ar. After being stirred at room temperature for 3 h, the reaction mixture was concentrated in vacuo and purified by column chromatography (eluted by 12–17% ethyl acetate in hexane) to give product(s) **30** as a colorless oil.

**Ethyl (R,E)-4-tert-Butoxycarbonylamino-5-(triphenylmethylthio)-penta-2-enoate (30b).** Yield 88%, 2 steps;  $[\alpha]_D^{20}$   $+6.0$  ( $c$  0.20,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.44–7.42 (m, 6H), 7.31–7.27 (m, 6H), 7.24–7.20 (m, 3H), 6.72 (dd,  $J$  = 15.6, 4.4 Hz, 1H), 5.84 (d,  $J$  = 15.6 Hz, 1H), 4.80 (d,  $J$  = 8.4 Hz, 1H), 4.29 (br, 1H), 4.17 (q,  $J$  = 7.2 Hz, 2H), 2.53–2.41 (m, 2H), 1.45 (s, 9H), 1.27 (t,  $J$  = 7.2 Hz, 3H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  169.9, 154.7, 146.6, 144.3, 129.5, 128.0, 126.8, 121.5, 79.7, 67.0, 60.4, 50.5, 36.2,

28.5, 14.2 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{31}\text{H}_{35}\text{NO}_4\text{SNa}$  ( $(M + \text{Na})^+$  540.2210, found 540.2223.

**Compound 31a<sup>2a</sup> and 31b.** To the solution of compound **30** (3.379 g, 6.532 mmol) in 95% EtOH (35 mL) was added  $\text{NaBH}_4$  (247.1 mg, 6.532 mmol) at room temperature. The resulting mixture was stirred at room temperature for 30 h and then quenched with water (50 mL) and extracted with  $\text{Et}_2\text{O}$  (50 mL  $\times$  4). The combined organic layer was dried with anhydrous  $\text{MgSO}_4$ , evaporated in vacuo, and purified by column chromatography (eluted with the mixture of  $\text{CH}_2\text{Cl}_2$ /hexane/acetone from 50:148:2  $\rightarrow$  50:98:2  $\rightarrow$  50:48:2) to give product **31** (1.187 g, 35%) as a thick colorless oil.

**Ethyl (R)-4-tert-Butoxycarbonylamino-5-(triphenylmethylthio)-pentanoate (31b).**  $[\alpha]_D^{20}$   $+4.5$  ( $c$  0.20,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.42–7.40 (m, 6H), 7.30–7.27 (m, 6H), 7.23–7.19 (m, 3H), 4.49 (d,  $J$  = 9.2 Hz, 1H), 4.10 (q,  $J$  = 7.2 Hz, 2H), 3.64 (br, 1H), 2.34 (br m, 2H), 2.21 (t,  $J$  = 7.6 Hz, 2H), 1.78–1.59 (m, 2H), 1.43 (s, 9H), 1.23 (t,  $J$  = 7.2 Hz, 3H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  173.3, 155.3, 144.7, 129.7, 128.0, 126.8, 79.4, 66.7, 60.5, 49.6, 37.2, 31.1, 29.7, 28.5, 14.3 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{31}\text{H}_{37}\text{NO}_4\text{SNa}$  ( $M + \text{Na}$ ) $^+$  542.2336, found 542.2331.

**Compound 5a<sup>2a</sup> and 5b.** To the solution of **31** (1.045g, 2.013 mmol) in 95% ethanol (7.5 mL) was added aq NaOH (1M, 4.0 mL) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was diluted with water (10 mL), acidified with 1–0.5 M aq HCl to pH 4–5, and extracted with diethyl ether (20 mL  $\times$  3). The combined organic layer was dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuo to give crude acid **32**, which was used in the next step without further purification. To the above crude acid **32** solution in DMSO (15 mL) was added  $\text{K}_2\text{CO}_3$  (556.5 mg, 4.027 mmol) and allyl bromide (255.5  $\mu\text{L}$ , 3.020 mmol) at room temperature. After being stirred at room temperature 5 h, the reaction was quenched with water (30 mL) and extracted with ethyl acetate (50 mL  $\times$  3). The combined organic layer was washed with brine, filtered, and concentrated in vacuo, and the residue was purified by column chromatography on silica gel (eluted by 10% ethyl acetate in hexane) to give product **5** as a colorless oil.

**Allyl (R)-4-tert-Butoxycarbonylamino-5-(triphenylmethylthio)-pentanoate (5b).** Yield 0.75 g, 70%;  $[\alpha]_D^{20}$   $+10.0$  ( $c$  0.16,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.42–7.41 (m, 6H), 7.31–7.27 (m, 6H), 7.23–7.20 (m, 3H), 5.95–5.85 (m, 1H), 5.32–5.21 (m, 2H), 4.55 (d,  $J$  = 5.6 Hz, 2H), 4.49 (d,  $J$  = 8.8 Hz, 1H), 3.65 (br m, 1H), 2.34 (br m, 2H), 2.26 (t,  $J$  = 7.6 Hz, 2H), 1.80–1.62 (m, 2H), 1.44 (s, 9H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  172.9, 155.3, 144.7, 132.2, 129.6, 128.1, 126.8, 118.3, 79.4, 66.7, 65.3, 49.5, 37.2, 31.0, 29.7, 28.5 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{32}\text{H}_{37}\text{NO}_4\text{SNa}$  ( $(M + \text{Na})^+$  554.2336, found 554.2341.

**General Procedure A: Synthesis of 3a,c,d and 34.** To the solution of **5** (169.6 mg, 0.319 mmol) in  $\text{CH}_2\text{Cl}_2$  (4 mL) were added 2,6-lutidine (556  $\mu\text{L}$ , 4.790 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) (694  $\mu\text{L}$ , 3.832 mmol) dropwise at room temperature under argon. After being stirred at the same temperature for 1.5 h, the reaction mixture was quenched with MeOH (6 mL) and water (10 mL) at 0 °C and extracted with  $\text{CH}_2\text{Cl}_2$  (15 mL  $\times$  4). The combined organic layer was washed with brine, dried over anhydrous  $\text{MgSO}_4$ , and evaporated in vacuo to give the crude free amine of **5**, which was used in next step without further purification.

To the crude free amine of **5** (1.3 equiv) in  $\text{CH}_2\text{Cl}_2$  (4 mL) were added corresponding acid **4** (based in Scheme 5) (0.245 mmol, 1 equiv), coupling reagent (CR, 0.368 mmol, 1.5 equiv), and DIEA (140  $\mu\text{L}$ , 0.735 mmol, 3.0 equiv) at room temperature or 0 °C. After being stirred at room temperature for 2.5–24 h, the resulting reaction mixture was evaporated in vacuo and purified by column chromatography, eluting with ethyl acetate in hexane to give product **3a,c,d** or **34** as a colorless oil.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-[(1S,3S,5S,6S)-6-[(1S)-3-Allyloxycarbonyl-1-(triphenylmethylthio)methyl-propylcarbamoyl]-1-tert-butyl-5-(2,2,2-trichloroethoxy-carbonyloxy)-3-methylhept-1-yl]ester 1-(9H-Fluoren-9-ylmethyl)ester (3a).**<sup>2a</sup> From **4a**, **5a**; CR BEP added at 0 °C; reaction time 2.5 h; yield 245.6 mg, 87%).



**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S)-6-((1S)-3-Allyloxycarbonyl-1-(triphenylmethylthio)methyl-propylcarbamoyle)-1-tert-butyl-5-hydroxy-3-methylhex-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (35).** From **4b**, **5a**; CR HATU added at rt, reaction time 3.0 h; yield 193.8 mg, 82%;  $[\alpha]_D^{20}$  -31.2 (c 0.24, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 7.74 (d, J = 7.6 Hz, 2H), 7.63–7.54 (m, 2H), 7.41–7.36 (m, 8H), 7.30–7.15 (m, 11H), 6.69 (d, J = 8.8 Hz, 0.84H), 6.06 (d, J = 8.4 Hz, 0.16H), 5.92–5.77 (m, 1H), 5.30–5.15 (m, 2H), 4.89 (d, J = 11.2 Hz, 0.84H), 4.79 (d, J = 10.4 Hz, 0.16H), 4.53–4.44 (m, 2H), 4.39–4.29 (m, 2H), 4.23–4.16 (m, 1H), 4.01 (br m, 1H), 3.95–3.85 (m, 1H), 3.79 (br, 1H), 3.66–3.61 (m, 1H), 3.58–3.44 (m, 1H), 2.40–2.15 (m, 6H), 2.11–2.01 (m, 3H), 1.98–1.88 (m, 2H), 1.85–1.75 (m, 2H), 1.71–1.60 (m, 3H), 1.55–1.47 (m, 1H), 1.40–1.30 (m, 1H), 1.04–0.97 (m, 1H), 0.96 (d, J = 6.8 Hz, 2.52H), 0.88 (s, 9H), 0.77 (d, J = 6.8 Hz, 0.48H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 172.7, 172.3, 172.0, 155.2, 144.8, 144.7, 144.0, 143.9, 141.4, 132.3, 129.7, 128.1, 128.0, 127.8, 127.3, 127.2, 126.9, 126.8, 125.2, 125.2, 120.1, 120.1, 118.3, 78.7, 67.7, 66.6, 66.3, 65.2, 59.6, 47.9, 47.3, 46.6, 44.3, 42.9, 42.2, 41.4, 38.0, 37.1, 36.8, 34.9, 34.6, 31.7, 31.3, 31.0, 30.0, 29.8, 29.3, 26.1, 25.2, 24.5, 23.5, 22.8, 20.7, 14.3 ppm. HRMS (ESI) *m/z* calcd for C<sub>59</sub>H<sub>68</sub>N<sub>2</sub>O<sub>8</sub>Na (M + Na)<sup>+</sup> 987.4589, found 987.4628.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S)-6-((1S)-3-Allyloxycarbonyl-1-(triphenylmethylthio)methyl-propylcarbamoyle)-1-tert-butyl-3,6-dimethyl-5-(2,2,2-trichloroethoxycarbonyloxy)hept-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (3c).** From **4c**, **5a**; CR PyAOP added at rt, reaction time 24 h; yield 254.3 mg, 89%;  $[\alpha]_D^{20}$  -48.5 (c 0.20, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 7.78–7.75 (m, 2H), 7.67 (d, J = 7.6 Hz, 0.6H), 7.62 (d, J = 7.2 Hz, 1.4H), 7.41–7.38 (m, 8H), 7.34–7.27 (m, 8H), 7.22–7.20 (m, 3H), 6.02 (d, J = 8.0 Hz, 0.4H), 5.96 (d, J = 8.4 Hz, 0.6H), 5.92–5.80 (m, 1H), 5.30–5.18 (m, 2H), 5.13 (d, J = 10.0 Hz, 0.4H), 5.04 (d, J = 10.0 Hz, 0.6H), 4.95 (d, J = 12.0 Hz, 0.6H), 4.83 (d, J = 12.0 Hz, 0.4H), 4.79–4.75 (m, 1H), 4.70 (d, J = 12.0 Hz, 0.4H), 4.66 (d, J = 12.0 Hz, 0.6H), 4.52–4.34 (m, 4.4H), 4.28–4.23 (m, 1H), 4.20–4.16 (m, 0.6H), 3.99–3.87 (m, 1H), 3.67–3.45 (m, 2H), 2.36–2.27 (m, 2.6H), 2.24–2.16 (m, 2.4H), 2.12 (br m, 1H), 2.01–1.90 (m, 2H), 1.85–1.74 (m, 3H), 1.53–1.44 (m, 2H), 1.37–1.27 (m, 1H), 1.19–1.16 (m, 6H), 1.06–1.00 (m, 1H), 0.97 (d, J = 6.4 Hz, 1.8H), 0.88 (s, 5.4H), 0.85 (s, 3.6H), 0.69 (d, J = 6.4 Hz, 1.2H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 174.2, 172.7, 172.6, 172.2, 154.6, 154.3, 154.2, 154.1, 144.5, 144.5, 144.3, 144.1, 143.9, 143.7, 141.3, 141.2, 132.1, 132.0, 129.5, 128.0, 127.7, 127.1, 127.0, 126.8, 125.4, 125.3, 125.1, 125.1, 120.0, 119.9, 118.3, 94.8, 94.8, 81.6, 81.4, 79.6, 79.4, 79.3, 76.7, 67.8, 67.4, 66.5, 65.2, 59.8, 59.3, 48.3, 47.2, 47.0, 46.8, 46.3, 38.1, 37.8, 36.8, 36.3, 36.3, 34.8, 34.7, 34.5, 31.6, 31.3, 30.8, 30.0, 26.8, 26.5, 25.8, 25.8, 25.3, 24.4, 23.4, 23.1, 22.7, 20.4, 20.3, 20.1, 14.2 ppm. HRMS (ESI) *m/z* calcd for C<sub>64</sub>H<sub>73</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>10</sub>Na (M + Na)<sup>+</sup> 1189.3944, found 1189.3964.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S,6S)-6-((1R)-3-Allyloxycarbonyl-1-(triphenylmethylthio)methyl-propylcarbamoyle)-1-tert-butyl-3-methyl-5-(2,2,2-trichloro-ethoxycarbonyloxy)hept-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (3d).** From **4a**, **5b**; CR PyAOP added at rt, reaction time 24 h; yield 257.0 mg, 91%;  $[\alpha]_D^{20}$  -40.0 (c 0.05, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 7.87–7.78 (m, 2H), 7.71–7.67 (dd, J = 8.4, 8.0 Hz, 2H), 7.51–7.46 (m, 8H), 7.40–7.28 (m, 11H), 6.17 (d, J = 8.4 Hz, 0.6H), 6.02–5.92 (m, 1H), 5.51 (d, J = 8.4 Hz, 0.4H), 5.40–5.30 (m, 2H), 5.10 (ddd, J = 7.6, 7.4, 7.2 Hz, 1H), 4.97–4.80 (m, 2.4H), 4.75 (d, J = 12.0 Hz, 0.6H), 4.62–4.30 (m, 6H), 4.00 (br m, 1H), 3.74–3.55 (m, 2H), 2.60–2.57 (m, 0.4H), 2.42–2.23 (m, 6.6H), 2.06 (br m, 2H), 1.98–1.86 (m, 1H), 1.82–1.70 (m, 3H), 1.66–1.58 (m, 2H), 1.55–1.30 (m, 1H), 1.24 (d, J = 6.8 Hz, 1.8H), 1.20 (d, J = 7.2 Hz, 1.2H), 1.07 (d, J = 6.0 Hz, 1.8H), 0.98 (s, 3.6H), 0.96 (s, 5.4H), 0.88 (d, J = 6.4 Hz, 1.2H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 172.8, 172.8, 172.6, 172.5, 172.4, 172.3, 154.9, 154.5, 153.7, 153.7, 144.6, 144.5, 144.2, 144.0, 143.8, 141.4, 141.2, 132.2, 129.8, 129.6, 128.1, 128.0, 127.8, 127.3, 127.2, 127.1, 126.9, 126.9, 125.7, 125.5, 125.2, 120.0, 118.6, 118.5, 79.5, 79.2, 79.0, 78.7, 76.7, 68.0, 67.5, 66.8, 66.7, 65.4, 65.4, 59.8, 59.5, 48.1, 47.3, 47.3, 47.1, 46.4, 45.7, 45.4, 38.3, 37.6, 37.1, 36.6, 36.5, 35.1, 34.8, 34.8, 31.7, 31.4, 29.9,

30.1, 29.8, 29.2, 29.2, 26.2, 26.1, 26.0, 25.9, 25.4, 24.4, 23.5, 22.8, 20.6, 20.2, 14.3, 14.0, 13.8 ppm. HRMS (ESI) *m/z* calcd for C<sub>63</sub>H<sub>71</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>10</sub>Na (M + Na)<sup>+</sup> 1175.3787, found 1175.3805.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S)-6-((1S)-3-Allyloxycarbonyl-1-(triphenylmethylthio)methyl-propylcarbamoyle)-1-tert-butyl-3-methyl-5-(2,2,2-trichloroethoxycarbonyloxy)hex-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (3b).** To the solution of compound **35** (166.3 mg, 0.172 mmol) and pyridine (140 μL, 1.742 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5.0 mL) were added 2,2,2-trichloroethoxycarbonyl chloride (Troc-Cl) (182.6 mg/118.7 μL, 0.862 mmol) and 4-dimethylaminopyridine (DMAP) (0.36 mg, 2.93 μmol) at 0 °C. After being stirred at the same temperature for 1 h, the reaction was quenched with water (15 mL). The water layer was extracted with ethyl acetate (20 mL × 4). The combined organic layer was washed with 5% NaHCO<sub>3</sub> (20 mL × 2) and brine (20 mL), dried with MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by chromatography column on silica gel (20–30% ethyl acetate in hexane) to give **3b** (156.5 mg, 80%);  $[\alpha]_D^{20}$  -45.0 (c 0.111, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 7.77–7.74 (m, 2H), 7.67 (d, J = 7.6 Hz, 0.4H), 7.60 (d, J = 7.6 Hz, 1.6H), 7.41–7.37 (m, 8H), 7.33–7.17 (m, 11H), 6.13 (d, J = 8.4 Hz, 0.6H), 5.92–5.80 (m, 1H), 5.62 (d, J = 8.4 Hz, 0.4H), 5.30–5.18 (m, 2.4H), 4.85–4.80 (m, 1.2H), 4.77 (d, J = 12.0 Hz, 0.4H), 4.61 (d, J = 12.0 Hz, 0.4H), 4.54 (d, J = 12.0 Hz, 0.6H), 4.53–4.45 (m, 3H), 4.40–4.30 (m, 1.4H), 4.26–4.18 (m, 1.6H), 3.93–3.82 (m, 1H), 3.66–3.60 (m, 1H), 3.58–3.45 (m, 1H), 2.54–2.56 (m, 4H), 2.22–2.10 (m, 3H), 2.00–1.85 (m, 3H), 1.75–1.60 (m, 2H), 1.55–1.24 (m, 3H), 0.99 (d, J = 6.4 Hz, 1.8H), 0.88 (s, 3.6H), 0.87 (s, 5.4H), 0.80 (d, J = 6.4 Hz, 1.2 H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 172.8, 172.7, 172.7, 172.3, 168.7, 168.3, 154.9, 154.4, 153.6, 153.5, 144.6, 144.6, 144.2, 144.1, 143.9, 143.8, 141.3, 141.3, 132.2, 132.1, 129.6, 128.0, 128.0, 127.8, 127.8, 127.1, 126.9, 126.8, 125.5, 125.4, 125.2, 120.0, 120.0, 118.4, 118.3, 94.8, 94.8, 79.5, 79.1, 76.7, 76.6, 75.3, 74.8, 67.8, 67.5, 65.3, 65.2, 59.8, 59.5, 48.4, 47.3, 47.0, 46.5, 41.6, 41.0, 39.8, 39.4, 38.1, 37.7, 36.4, 36.3, 35.0, 34.7, 31.7, 31.3, 29.8, 29.1, 25.9, 25.9, 25.4, 24.4, 23.4, 22.7, 20.8, 20.7, 14.2 ppm. HRMS (ESI) *m/z* calcd for C<sub>62</sub>H<sub>69</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>10</sub>Na (M + Na)<sup>+</sup> 1161.3631, found 1161.3634.

**General Procedure B: Synthesis of 34a,b,d.** To the solution of triphenylphosphine oxide (223.3 mg, 0.802 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was added dropwise trifluoromethanesulfonic anhydride (Tf<sub>2</sub>O) (68 μL, 0.401 mmol) at 0 °C under argon. After being stirred at the same temperature for 10 min, compound **3** (0.100 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) was added at 0 °C. The reaction mixture was stirred at the same temperature for 30 min for **3a/3b** and 3 h for **3d**. This reaction was monitored by mass spectrometry and was quenched with saturated NaHCO<sub>3</sub> (6 mL) at 0 °C when starting material disappeared. The aqueous layer was extracted with ethyl acetate (10 mL × 4), washed with brine (10 mL), dried with MgSO<sub>4</sub>, filtered, and concentrated in vacuo to give Troc protected thiazoline intermediate **33a, b, or d**. The residue was used in the next step immediately without further purification.

The above residue **33** was dissolved in THF (4 mL), and then aqueous NH<sub>4</sub>OAc (1 M, 1.0 mL) and zinc powder (freshly activated with 1 M aqueous HCl) (100 mg) were added at room temperature. After being stirred at the same temperature for 30 min, ethyl acetate (5 mL) and brine (5 mL) and were added to the reaction mixture. The aqueous layer was extracted with ethyl acetate (5 mL × 4). The combined organic layer was dried with MgSO<sub>4</sub>, filtered, concentrated in vacuo, and purified by column chromatography on silica gel (eluted by ethyl acetate/hexane 1:3, v/v) to give thiazoline ring product **34a,b, or d** as a colorless oil.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S)-6-((5S)-5-(2-Allyloxycarbonyl)ethyl)-4,5-dihydro-thiazol-2-yl)-1-tert-butyl-5-hydroxy-3-methylhex-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (34b).** Yield 53.2 mg, 76%;  $[\alpha]_D^{20}$  -78.0 (c 0.10, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, mixture of rotamers): δ 7.74 (d, J = 7.6 Hz, 2H), 7.63 (dd, J = 5.6, 6.0 Hz, 1.6 H), 7.56 (d, J = 7.6 Hz, 0.4H), 7.37 (dd, J = 7.2, 7.2 Hz, 2H), 7.32–7.27 (m, 2H), 5.93–5.83 (m, 1H), 5.30–5.19 (m, 2H), 4.90 (d, J = 10.8 Hz, 0.8H), 4.83 (d, J = 9.6 Hz, 0.2H), 4.58–4.51 (m, 2H), 4.46–4.34 (m, 3.8H), 4.28–4.18 (m, 1.2H), 3.97 (br m,

1H), 3.68–3.61 (m, 2H), 3.57–3.47 (m, 1H), 3.33 (dd,  $J = 10.4$ , 8.8 Hz, 0.2H), 3.24 (dd,  $J = 10.4$ , 8.8 Hz, 0.8H), 2.88 (dd,  $J = 10.0$ , 8.4 Hz, 0.2H), 2.81 (dd,  $J = 10.4$ , 8.4 Hz, 0.8H), 2.55–2.48 (m, 4H), 2.29–2.19 (m, 1H), 2.08–1.80 (m, 5H), 1.75–1.58 (m, 2H), 1.48–1.25 (m, 2H), 1.04–0.97 (m, 1H), 0.96 (d,  $J = 6.4$  Hz, 2.4H), 0.87 (s, 9H), 0.79 (d,  $J = 6.4$  Hz, 0.6H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  172.9, 172.9, 172.8, 172.3, 169.2, 169.1, 154.9, 154.3, 144.3, 144.1, 144.0, 143.8, 141.3, 141.3, 141.2, 132.2, 132.2, 127.6, 127.6, 127.1, 127.0, 127.0, 125.4, 125.3, 125.3, 125.2, 79.5, 78.5, 76.0, 75.8, 67.8, 67.7, 67.2, 66.7, 65.2, 65.1, 59.5, 47.2, 47.1, 47.0, 46.5, 42.9, 42.2, 42.0, 41.9, 38.0, 37.9, 37.4, 34.8, 34.7, 34.6, 31.6, 31.5, 31.4, 31.2, 30.4, 29.9, 26.0, 25.9, 25.7, 25.3, 25.1, 24.5, 23.3, 22.7, 20.6, 20.5, 14.2 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{40}\text{H}_{52}\text{N}_2\text{O}_7\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  727.3387, found 727.3399.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S,6S)-6-((5R)-5-(2-Allyloxycarbonyl-ethyl)-4,5-dihydro-thiazol-2-yl)-1-tert-butyl-5-hydroxy-3-methylhept-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (34d).** Yield 57.0 mg, 79%;  $[\alpha]_{\text{D}}^{20} -22.5$  (c 0.12,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.76 (d,  $J = 7.2$  Hz, 2H), 7.63 (dd,  $J = 6.6$ , 6.4 Hz, 1.7 H), 7.57 (d,  $J = 7.2$  Hz, 0.3H), 7.39 (dd,  $J = 7.2$ , 7.2 Hz, 2H), 7.30 (dd,  $J = 7.4$ , 7.2 Hz, 2H), 5.94–5.85 (m, 1H), 5.31–5.20 (m, 2H), 4.88 (d,  $J = 10.0$  Hz, 0.7H), 4.81 (d,  $J = 8.8$  Hz, 0.3H), 4.59–4.51 (m, 2.3H), 4.45–4.33 (m, 3H), 4.31–4.18 (m, 1.7H), 3.82–3.72 (m, 0.7H), 3.68–3.62 (m, 1.3H), 3.58–3.49 (m, 1.3H), 3.35–3.24 (m, 1H), 2.90–2.85 (m, 0.3H), 2.81 (dd,  $J = 10.8$ , 7.6 Hz, 0.7H), 2.72–2.65 (m, 0.7H), 2.61–2.44 (m, 2.3H), 2.34–2.18 (m, 1.7H), 2.10–1.89 (m, 4.4H), 1.82 (m, 0.7H), 1.70–1.57 (m, 2H), 1.37–1.29 (m, 1H), 1.19 (d,  $J = 7.0$  Hz, 0.9H), 1.17 (d,  $J = 7.0$  Hz, 2.1H), 1.06–1.00 (m, 1H), 0.96 (d,  $J = 6.4$  Hz, 2.1H), 0.87 (s, 9H), 0.79 (d,  $J = 6.4$  Hz, 0.9H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  174.2, 173.1, 173.0, 172.8, 172.5, 155.0, 154.5, 144.4, 144.3, 144.1, 143.9, 141.4, 141.4, 141.3, 132.3, 132.3, 128.0, 127.7, 127.4, 127.2, 127.1, 125.6, 125.4, 125.3, 125.3, 121.0, 120.0, 118.4, 118.3, 79.6, 78.6, 77.4, 76.2, 75.7, 71.5, 71.5, 70.7, 67.9, 67.8, 65.3, 65.3, 59.7, 59.7, 47.3, 47.2, 47.1, 46.6, 46.0, 45.3, 40.4, 39.3, 38.2, 37.8, 37.4, 37.3, 34.8, 34.7, 31.7, 31.5, 31.3, 30.3, 30.2, 30.1, 29.8, 26.1, 26.1, 25.9, 25.5, 25.3, 24.6, 23.4, 20.7, 20.6, 16.3, 15.8 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{41}\text{H}_{54}\text{N}_2\text{O}_7\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  741.3544, found 741.3567.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S)-6-((5S)-5-(2-Allyloxycarbonyl-ethyl)-4,5-dihydro-thiazol-2-yl)-1-tert-butyl-3,6-dimethyl-5-hydroxyhept-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (34c).** To the solution of **3c** (97.8 mg, 0.084 mmol) in 1,2-dichloroethane (5 mL) was added  $\text{TiCl}_4$  (1 M in  $\text{CH}_2\text{Cl}_2$ , 0.294 mL, 0.293 mmol, 3.5 equiv) at room temperature. The resulting solution was heated to 60 °C and stirred at this temperature for 2.5 h. This reaction was monitored by mass spectrometry. When the starting material was consumed completely, the reaction was cooled to 0 °C and quenched with saturated aq  $\text{NaHCO}_3$  (7 mL), stirred at room temperature for another 10 min, extracted with ethyl acetate (10 mL  $\times$  4), dried over anhydrous  $\text{MgSO}_4$ , filtered, and evaporated in vacuo to give crude intermediate **33c**, which was used in the next step without further purification.

The above crude **33c** was dissolved in THF (4 mL), and then aqueous  $\text{NH}_4\text{OAc}$  (1 M, 1.0 mL) and zinc powder (freshly activated with 1 M aqueous HCl) (80 mg) were added at room temperature. After being stirred at the same temperature for 30 min, to the reaction were added ethyl acetate (5 mL) and brine (5 mL). The aqueous layer was extracted with ethyl acetate (7 mL  $\times$  4). The combined organic layer was dried with  $\text{MgSO}_4$ , filtered, concentrated in vacuo, and purified by column chromatography on silica gel (eluted by ethyl acetate/hexane 1:3, v/v) to give thiazoline ring product **34c** (101.3 mg, 55%) as a colorless oil;  $[\alpha]_{\text{D}}^{20} -80.8$  (c 0.12,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.76–7.74 (m, 2H), 7.66–7.61 (m, 1.7H), 7.57 (d,  $J = 7.2$  Hz, 0.3H), 7.39 (dd,  $J = 7.4$ , 7.2 Hz, 2H), 7.31–7.28 (m, 2H), 5.94–5.84 (m, 1H), 5.31–5.19 (m, 2H), 4.88 (dd,  $J = 11.6$ , 1.6 Hz, 0.7H), 4.81 (dd,  $J = 11.6$ , 1.6 Hz, 0.3H), 4.58–4.54 (m, 2H), 4.52–4.38 (m, 3H), 4.35–4.19 (m, 2H), 3.66–3.59 (m, 2H), 3.58–3.48 (m, 2H), 3.30–3.23 (m, 1H), 2.86–2.81 (m, 1H), 2.59–2.46 (m, 2H), 2.36–2.19 (m, 1H), 2.12–1.91 (m, 5H), 1.78 (br m, 1H), 1.67–1.56 (m, 2H), 1.37–1.27 (m, 2H), 1.22 (s,

0.9H), 1.19–1.18 (m, 5.1H), 0.96 (d,  $J = 6.4$  Hz, 2.1H), 0.87 (s, 9H), 0.79 (d,  $J = 6.8$  Hz, 0.9H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  178.8, 178.3, 173.1, 173.0, 172.9, 172.5, 154.9, 154.4, 144.4, 144.3, 144.0, 143.9, 141.4, 141.3, 141.2, 132.3, 127.7, 127.7, 127.1, 127.1, 125.5, 125.4, 125.3, 125.2, 120.0, 118.3, 118.3, 79.4, 78.6, 76.0, 75.8, 75.0, 74.9, 67.9, 67.7, 65.2, 65.2, 59.7, 59.7, 47.3, 47.2, 47.0, 46.5, 45.9, 45.6, 38.0, 37.8, 37.0, 37.0, 36.9, 36.6, 34.8, 34.6, 31.6, 31.4, 31.3, 30.3, 30.2, 30.0, 29.8, 26.1, 25.8, 25.3, 24.5, 24.3, 23.7, 23.4, 23.1, 20.6, 20.5 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{42}\text{H}_{56}\text{N}_2\text{O}_7\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  755.3700, found 755.3718.

**General Procedure C: Synthesis of Compounds 2a–d.** To a solution of **34** (0.054 mmol) in THF (1.5 mL) were added  $\text{Pd}(\text{PPh}_3)_4$  (7.1 mg, 0.005 mmol) and *N*-methylaniline (0.015 mL, 0.136 mmol) at room temperature under argon. This reaction was protected with aluminum foil. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated in vacuo and purified by preparative TLC (20 cm  $\times$  20 cm plate) to give acid **2**.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S)-6-((5S)-5-(2-Carboxylethyl)-4,5-dihydro-thiazol-2-yl)-1-tert-butyl-5-hydroxy-3-methylhex-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (2b).** Yield 31.8 mg, 89%;  $[\alpha]_{\text{D}}^{20} -90.0$  (c 0.04,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.74 (d,  $J = 7.6$  Hz, 2H), 7.63 (d,  $J = 7.2$  Hz, 1.8H), 7.57 (d,  $J = 7.6$  Hz, 0.2H), 7.38 (dd,  $J = 7.6$ , 7.6 Hz, 2H), 7.32–7.28 (m, 2H), 6.22 (br, 1H), 4.90 (d,  $J = 10.0$  Hz, 0.8H), 4.84 (d,  $J = 9.2$  Hz, 0.2H), 4.53–4.34 (m, 3.8H), 4.29–4.17 (m, 1.2H), 4.00–3.92 (m, 1H), 3.68–3.61 (m, 1H), 3.57–3.45 (m, 1H), 3.35 (dd,  $J = 10.8$ , 8.8 Hz, 0.2H), 3.23 (dd,  $J = 10.8$ , 8.4 Hz, 0.8H), 2.91 (dd,  $J = 10.8$ , 8.4 Hz, 0.2H), 2.80 (dd,  $J = 10.8$ , 8.4 Hz, 0.8H), 2.60–2.43 (m, 4H), 2.35–2.17 (m, 1H), 2.08–1.82 (m, 5H), 1.74–1.59 (m, 2H), 1.48–1.25 (m, 2H), 1.03–0.96 (m, 1H), 0.96 (d,  $J = 6.4$  Hz, 2.4H), 0.87 (s, 9H), 0.77 (d,  $J = 6.4$  Hz, 0.6H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  176.7, 176.5, 173.1, 172.5, 171.8, 170.5, 155.1, 154.5, 144.3, 144.1, 144.0, 143.8, 141.4, 141.4, 141.3, 141.3, 127.7, 127.7, 127.2, 127.1, 127.1, 125.5, 125.4, 125.3, 120.0, 79.8, 75.7, 75.3, 67.9, 67.8, 67.4, 67.0, 59.6, 47.2, 47.2, 47.0, 46.6, 42.7, 42.3, 42.1, 41.9, 38.2, 38.1, 38.0, 37.4, 36.8, 34.8, 34.7, 32.0, 31.7, 31.3, 30.1, 30.0, 30.0, 26.1, 26.0, 25.7, 25.1, 24.6, 23.4, 20.7, 20.5 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{37}\text{H}_{48}\text{N}_2\text{O}_7\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  687.3074, found 687.3070.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S)-6-((5S)-5-(2-Carboxylethyl)-4,5-dihydro-thiazol-2-yl)-1-tert-butyl-3,6-dimethyl-5-hydroxyhept-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (2c).** Yield 31.2 mg, 84%;  $[\alpha]_{\text{D}}^{20} -57.7$  (c 0.052,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.76 (d,  $J = 7.6$  Hz, 2H), 7.65–7.58 (m, 2H), 7.39 (dd,  $J = 7.4$ , 7.2 Hz, 2H), 7.32–7.28 (m, 2H), 4.88 (d,  $J = 12.0$ , 0.7H), 4.80 (d,  $J = 12.0$  Hz, 0.3H), 4.51 (dd,  $J = 8.8$ , 2.8 Hz, 0.3H), 4.46 (d,  $J = 7.2$  Hz, 0.3H), 4.43 (d,  $J = 7.2$  Hz, 0.7H), 4.41–4.32 (m, 2H), 4.30–4.25 (m, 1.7H), 3.65–3.56 (m, 2H), 3.53–3.45 (m, 1H), 3.30 (dd,  $J = 11.0$ , 8.4 Hz, 0.3H), 3.19 (dd,  $J = 11.0$ , 8.4 Hz, 0.7H), 2.88 (dd,  $J = 10.8$ , 10.8 Hz, 1H), 2.70–2.53 (m, 2H), 2.37–2.14 (m, 1H), 2.08–1.90 (m, 5H), 1.79 (br m, 1H), 1.68–1.60 (m, 2H), 1.35–1.26 (m, 2H), 1.26 (s, 0.9H), 1.19 (s, 2.1H), 1.18 (s, 3H), 0.95 (d,  $J = 6.6$  Hz, 2.1H), 0.87 (s, 9H), 0.74 (d,  $J = 6.6$  Hz, 0.9H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  182.4, 180.5, 175.7, 175.5, 173.2, 172.7, 155.1, 154.7, 144.5, 144.2, 144.1, 143.8, 141.5, 141.4, 141.3, 127.8, 127.8, 127.2, 127.1, 127.1, 125.5, 125.4, 125.4, 125.3, 120.0, 79.7, 78.6, 76.0, 75.8, 75.4, 75.1, 68.1, 67.9, 59.7, 59.6, 47.3, 47.2, 47.1, 46.6, 46.5, 45.8, 38.0, 37.8, 37.5, 37.4, 37.1, 36.3, 34.8, 34.6, 33.4, 32.7, 32.7, 31.7, 31.3, 30.2, 30.0, 29.8, 29.7, 26.1, 25.6, 25.1, 24.9, 24.7, 23.8, 23.7, 23.5, 22.8, 20.7, 20.4, 14.3 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{39}\text{H}_{52}\text{N}_2\text{O}_7\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  715.3387, found 715.3397.

**Pyrrolidine-1,2-dicarboxylic Acid (2S)-2-((1S,3S,5S,6S)-6-((5R)-5-(2-Carboxylethyl)-4,5-dihydro-thiazol-2-yl)-1-tert-butyl-5-hydroxy-3-methylhept-1-yl)ester 1-(9H-Fluoren-9-ylmethyl)ester (2d).** Yield 33.0 mg, 90%;  $[\alpha]_{\text{D}}^{20} -60.0$  (c 0.04,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.75 (d,  $J = 7.6$  Hz, 2H), 7.67–7.57 (m, 2H), 7.39 (dd,  $J = 7.4$ , 7.2 Hz, 2H), 7.30 (dd,  $J = 7.6$ , 7.4 Hz, 2H), 5.90 (br, 1H), 4.91 (dd,  $J = 12.8$ , 12.4 Hz, 0.7H), 4.86–4.82 (m, 0.3H), 4.54–4.50 (m, 0.3H), 4.45–4.40 (m, 1H), 4.38–4.33 (m, 1.7H), 4.30–4.27 (m, 1.3H), 4.25–4.16 (m, 0.7H), 3.82–3.80 (m, 0.7H),

3.75–3.70 (m, 0.3H), 3.64 (br m, 1H), 3.51–3.49 (m, 1H), 3.36–3.30 (m, 0.3H), 3.26–3.15 (m, 0.7H), 2.91–2.85 (m, 0.3H), 2.84–2.72 (m, 0.7H), 2.68–2.50 (m, 3H), 2.35–2.21 (m, 1H), 2.21–2.15 (m, 0.3H), 2.05–1.89 (m, 5H), 1.84–1.79 (m, 0.7H), 1.75–1.70 (m, 1H), 1.66–1.60 (m, 1H), 1.54–1.45 (m, 0.7H), 1.45–1.40 (m, 0.3H), 1.19–1.16 (m, 3H), 1.03–1.00 (m, 1H), 0.96 (d,  $J = 6.0$  Hz, 2.1H), 0.88 (s, 9H), 0.74 (d,  $J = 6.0$  Hz, 0.9H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  177.8, 177.6, 175.9, 173.4, 173.4, 173.3, 172.7, 155.1, 155.1, 154.6, 144.4, 144.4, 144.2, 144.2, 144.2, 144.0, 143.9, 141.4, 141.4, 141.3, 141.3, 128.0, 128.0, 127.8, 127.8, 127.5, 127.2, 127.1, 125.5, 125.4, 125.4, 125.3, 121.0, 121.0, 120.0, 120.0, 80.0, 80.0, 79.2, 78.7, 77.4, 76.2, 76.0, 75.6, 75.5, 71.8, 70.8, 70.4, 68.0, 67.9, 67.9, 67.7, 67.5, 59.7, 59.6, 47.3, 47.3, 47.1, 46.6, 46.0, 45.9, 45.4, 40.7, 39.7, 39.4, 39.2, 38.1, 38.0, 37.8, 37.6, 37.0, 34.8, 34.7, 32.9, 32.9, 32.3, 31.7, 31.3, 31.1, 30.2, 30.0, 29.8, 29.4, 29.2, 28.7, 26.1, 26.0, 25.8, 25.4, 25.2, 25.1, 24.6, 23.4, 22.8, 20.8, 20.8, 20.6, 20.5, 16.6, 16.0, 14.3, 14.2 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{38}\text{H}_{50}\text{N}_2\text{O}_7\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  701.3231, found 701.3253.

**General Procedure D: Synthesis of Cyclic Precursors 36a–d.** To a solution of Fmoc protected tripeptide **6**<sup>2a–c</sup> (28.1 mg, 0.042 mmol) in MeCN (1.2 mL) was added diethylamine (0.6 mL) at room temperature. After being stirred at the same temperature for 30 min, the reaction mixture was evaporated in vacuo, then azeotroped with toluene and  $\text{CH}_2\text{Cl}_2$  two times, respectively, and dried under reduced pressure for 1 h to give the free amine tripeptide, which was used in the next coupling reaction without further purification.

The above crude free amine tripeptide was dissolved in  $\text{CH}_2\text{Cl}_2$  (THF for **2b**) (2 mL). To this solution were added acid **2** (0.028 mmol), corresponding coupling reagent (0.056 mmol) (PyAOP for **2a,c,d** and DEPBT for **2b**), and DIEA (0.014 mL, 0.083 mmol) at room temperature. After being stirred at the same temperature for 15–24 h, the reaction mixture was concentrated in vacuo and purified by preparative TLC plate (developed by acetone/hexane (2:3, v/v)) to give the precursor **36a–d** as a colorless oil.

**Cyclic Precursor 36b.** Yield 22.0 mg, 72%;  $[\alpha]_{\text{D}}^{20}$  –99.3 ( $c$  0.14,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.77–7.74 (m, 2H), 7.64–7.62 (m, 1.7H), 7.56 (d,  $J = 7.6$  Hz, 0.3H), 7.39 (dd,  $J = 7.6, 7.4$  Hz, 2H), 7.32–7.26 (m, 2H), 7.09 (d,  $J = 8.0$  Hz, 2H), 6.76 (d,  $J = 8.0$  Hz, 2H), 6.63 (d,  $J = 8.0$  Hz, 0.3H), 6.49 (br m, 0.3H), 6.34–6.27 (m, 1H), 6.20–6.12 (m, 0.3H), 5.92–5.83 (m, 0.7H), 5.54–5.47 (m, 0.3H), 5.42–5.35 (m, 0.7H), 5.31–5.17 (m, 2.7H), 5.11–5.06 (m, 0.3H), 4.94–4.85 (m, 1.7H), 4.62–4.50 (m, 2H), 4.47–4.35 (m, 2.3H), 4.27 (t,  $J = 7.2$  Hz, 1H), 4.20–4.16 (m, 1.4H), 3.74 (s, 3H), 3.72–3.64 (m, 1.3H), 3.60–3.54 (m, 1H), 3.26–3.07 (m, 1H), 3.02–2.90 (m, 4H), 2.83–2.77 (m, 2H), 2.77–2.60 (m, 4H), 2.50–2.46 (m, 0.7H), 2.39–2.19 (m, 4H), 2.18–1.90 (m, 6.3H), 1.87–1.78 (m, 1H), 1.73 (br m, 1H), 1.61 (br m, 0.7H), 1.55–1.49 (m, 1H), 1.42–1.38 (m, 1H), 1.27–1.21 (m, 3H), 1.00–0.84 (m, 19.1H), 0.68 (d,  $J = 6.4$  Hz, 0.9H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  172.7, 172.1, 172.0, 171.9, 171.6, 171.6, 170.8, 158.7, 154.8, 154.4, 144.3, 144.2, 144.1, 143.9, 141.4, 141.3, 131.8, 131.4, 130.5, 130.4, 128.2, 128.2, 127.8, 127.8, 127.2, 127.1, 127.1, 126.4, 125.5, 125.4, 125.3, 120.1, 118.8, 114.0, 79.6, 79.1, 77.4, 75.9, 75.7, 67.9, 67.7, 66.1, 65.5, 60.6, 59.6, 59.4, 55.3, 50.4, 49.8, 49.2, 47.3, 47.1, 46.6, 38.7, 38.2, 37.9, 37.2, 37.1, 36.8, 34.8, 34.5, 33.7, 33.4, 32.1, 31.7, 31.5, 31.1, 31.0, 30.7, 30.2, 29.8, 29.6, 29.5, 28.8, 26.1, 25.1, 24.6, 23.5, 22.8, 21.1, 20.7, 16.2, 15.9, 14.5, 14.4, 14.3, 11.8, 10.7 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{61}\text{H}_{83}\text{N}_5\text{O}_{11}\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  1116.5702, found 1116.5742.

**Cyclic Precursor 36c.** Yield 28.3 mg, 90%;  $[\alpha]_{\text{D}}^{20}$  –130.0 ( $c$  0.10,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.76–7.73 (m, 2H), 7.65–7.59 (m, 2H), 7.40–7.36 (m, 2H), 7.31–7.26 (m, 2H), 7.10–7.08 (m, 2H), 6.85 (d,  $J = 8.4$  Hz, 0.3H), 6.77–6.74 (m, 2H), 6.65 (d,  $J = 8.0$  Hz, 0.7H), 5.92–5.82 (m, 1H), 5.37 (dddd,  $J = 6.8, 6.8, 6.8, 6.8$  Hz, 1H), 5.31–5.20 (m, 2H), 5.20–5.14 (m, 1H), 4.93–4.87 (m, 1.7H), 4.81 (d,  $J = 10.8$  Hz, 0.3H), 4.62–4.51 (m, 3H), 4.46–4.41 (m, 2H), 4.33–4.24 (m, 3H), 3.74 (s, 3H), 3.68–3.62 (m, 1.3H), 3.60–3.48 (m, 1.7H), 3.18 (ddd,  $J = 18.8, 10.8, 8.8$  Hz, 1H), 3.03–2.95 (m, 4H), 2.82–2.73 (m, 2H), 2.68 (s, 2.1H), 2.65 (s, 0.9H), 2.43–2.17 (m, 4H), 2.15–1.86 (m, 5H), 1.85–1.75 (m, 2H), 1.70–

1.55 (m, 2.3H), 1.50–1.45 (m, 0.7H), 1.35–1.17 (m, 13H), 0.97–0.91 (m, 6H), 0.91–0.82 (m, 14.1H), 0.76 (d,  $J = 6.4$  Hz, 0.9H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  178.8, 178.3, 173.0, 172.7, 172.3, 172.2, 171.9, 171.6, 171.6, 171.4, 170.8, 169.7, 158.6, 154.9, 154.6, 144.6, 144.3, 144.0, 143.9, 141.4, 141.4, 141.3, 131.8, 130.5, 130.5, 130.4, 128.4, 128.3, 127.8, 127.7, 127.1, 125.5, 125.4, 125.4, 125.3, 120.0, 120.0, 118.8, 113.9, 79.4, 78.7, 77.4, 76.1, 75.7, 75.2, 75.0, 68.0, 67.7, 66.1, 65.5, 64.6, 60.5, 59.8, 59.6, 55.3, 53.6, 50.4, 49.8, 49.3, 47.3, 47.2, 47.1, 46.6, 45.9, 45.4, 38.1, 37.9, 37.0, 36.6, 34.8, 34.7, 34.5, 33.7, 33.5, 33.3, 32.0, 31.7, 31.3, 31.0, 31.0, 30.9, 30.6, 30.1, 29.8, 26.1, 25.6, 25.4, 25.1, 24.6, 24.0, 23.7, 23.5, 22.8, 20.8, 20.7, 20.6, 16.2, 15.9, 15.0, 14.9, 14.4, 14.3, 11.7, 10.7 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{63}\text{H}_{87}\text{N}_5\text{O}_{11}\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  1144.6015, found 1144.6041.

**Cyclic Precursor 36d.** Yield 22.0 mg, 71%;  $[\alpha]_{\text{D}}^{20}$  –66.0 ( $c$  0.05,  $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  7.77–7.74 (m, 2H), 7.65–7.60 (m, 2H), 7.39 (dd,  $J = 7.6, 7.2$  Hz, 2H), 7.30 (dd,  $J = 7.2, 6.8$  Hz, 2H), 7.10–7.07 (m, 2H), 6.78–6.75 (m, 2H), 5.92–5.81 (m, 1H), 5.39–5.35 (m, 1H), 5.31–5.21 (m, 2H), 5.18–5.11 (m, 1H), 4.93–4.81 (m, 2H), 4.62–4.53 (m, 2H), 4.46–4.21 (m, 5H), 3.81–3.69 (m, 4H), 3.69–3.60 (m, 1H), 3.58–3.49 (m, 1H), 3.22–3.18 (m, 1H), 3.03–2.88 (m, 4H), 2.82–2.76 (m, 3H), 2.71–2.57 (m, 4H), 2.34–2.21 (m, 4H), 2.11–1.79 (m, 8H), 1.71 (br m, 1H), 1.64–1.58 (m, 1H), 1.34–1.18 (m, 5H), 0.97–0.78 (m, 21H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , mixture of rotamers):  $\delta$  175.1, 174.6, 173.2, 172.9, 172.7, 172.6, 172.3, 172.2, 172.0, 172.0, 171.9, 171.9, 171.8, 171.6, 171.5, 171.4, 170.8, 169.7, 158.7, 158.6, 155.1, 155.0, 154.5, 144.5, 144.4, 144.3, 144.2, 144.1, 144.0, 143.9, 141.4, 141.4, 141.3, 131.8, 130.5, 128.5, 128.3, 128.0, 127.8, 127.1, 125.5, 125.4, 125.4, 125.3, 120.0, 118.8, 114.0, 113.9, 79.7, 78.8, 77.4, 76.2, 75.9, 75.6, 71.8, 70.9, 70.3, 67.9, 67.8, 67.6, 66.1, 65.5, 60.5, 59.8, 59.7, 59.7, 55.3, 50.4, 49.9, 47.3, 47.2, 47.1, 46.6, 45.8, 45.2, 45.0, 40.8, 39.8, 39.6, 38.1, 37.8, 37.7, 37.3, 37.3, 34.8, 34.7, 33.5, 33.4, 32.1, 31.7, 31.4, 31.0, 31.0, 30.7, 30.6, 30.1, 30.1, 29.8, 26.1, 25.7, 25.4, 25.2, 25.1, 24.6, 23.4, 22.8, 20.7, 20.6, 16.8, 16.4, 15.9, 14.4, 14.4, 14.3, 13.9, 11.8, 11.6, 10.7 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{62}\text{H}_{85}\text{N}_5\text{O}_{11}\text{SNa}$  ( $\text{M} + \text{Na}$ ) $^+$  1130.5859, found 1130.5905.

**General Procedure E: Synthesis of 1a–d.** To a solution of cyclic precursor **36** (11.6  $\mu\text{mol}$ ) in THF (1.0 mL) were added  $\text{Pd}(\text{PPh}_3)_4$  (2.7 mg, 2.32  $\mu\text{mol}$ ) and *N*-methylaniline (6.3  $\mu\text{L}$ , 58.0  $\mu\text{mol}$ ) at room temperature under argon. This reaction was protected with aluminum foil. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated in vacuo and purified by preparative TLC plate (developed with  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  1:9, v/v) to give the free acid cyclic precursor.

To the solution of free acid cyclic precursor in MeCN (1.5 mL) was added *N,N*-diethylamine (0.75 mL). After being stirred at room temperature for 30 min, the reaction mixture was evaporated in vacuo, azeotroped with toluene (three times) and  $\text{CH}_2\text{Cl}_2$  (two times), and then dried under reduced pressure for 1 h to give the unmasked precursor as a foam solid. Then the unmasked precursor was dissolved in  $\text{CH}_2\text{Cl}_2$  (DMF for **36b**) (20 mL). To this solution were added DIEA (20.0  $\mu\text{L}$ , 0.116 mmol) and corresponding coupling reagent (34.8  $\mu\text{mol}$ ) at 0  $^\circ\text{C}$  (PyAOP for **36a,c,d** and DEPBT for **36b**). After being stirred at 0  $^\circ\text{C}$  for 30 min, the reaction was allowed to warm up to room temperature and stirred for additional 15 h. Then the reaction was concentrated in vacuo and purified by semipreparative reversed-phase HPLC (Phenomenex Ultracarb, ODS 250 mm  $\times$  10 mm, 5  $\mu\text{m}$ , 3.0 mL/min, UV detection at 200/220 nm) using an isocratic system of 80% aqueous MeCN for 30 min, 80–100% MeCN for 30–40 min, and 100% MeCN for 40–60 min to afford **1a–d**.

**Apratoxin S7 (1b).** Yield 2.3 mg, 25% in 3 steps;  $[\alpha]_{\text{D}}^{20}$  –106.2 ( $c$  0.024,  $\text{CH}_2\text{Cl}_2$ );  $t_{\text{R}} = 17.5$  min.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , mixture of rotamers, major and minor (6/4)):  $\delta$  7.14 (d,  $J = 8.4$  Hz, 1.2H), 7.10 (d,  $J = 8.4$  Hz, 0.8H), 6.80 (d,  $J = 8.4$  Hz, 0.8H), 6.79 (d,  $J = 8.4$  Hz, 1.2H), 6.17 (d,  $J = 9.0$  Hz, 0.4H), 5.78 (d,  $J = 10.2$  Hz, 0.6H), 5.26 (d,  $J = 11.4$  Hz, 0.6H), 5.20–5.16 (m, 0.4H), 5.15 (ddd,  $J = 15.0, 10.2, 4.8$  Hz, 0.6H), 4.96 (dd,  $J = 12.6, 2.4$  Hz, 0.6H), 4.89 (d,  $J = 11.4$  Hz, 0.4H), 4.87 (dd,  $J = 12.6, 2.4$  Hz, 0.4H), 4.64 (q,  $J = 6.6$  Hz, 0.4H), 4.60 (d,  $J = 10.8$  Hz, 0.6H), 4.38–4.31 (m, 1.2H), 4.24–4.19 (m, 1H), 4.11–4.08 (m, 0.4H), 4.05 (br m, 0.4H), 4.00–3.95 (m, 0.6H), 3.90



(br m, 0.4H), 3.77 (s, 1.8H), 3.77 (s, 1.2H), 3.70–3.66 (m, 0.6H), 3.65–3.61 (m, 0.4H), 3.39 (dd,  $J = 10.8, 8.4$  Hz, 0.6H), 3.32 (dd,  $J = 10.8, 8.4$  Hz, 0.4H), 3.29 (q,  $J = 6.6$  Hz, 0.6H), 3.09 (dd,  $J = 12.0, 11.4$  Hz, 1H), 3.05–3.01 (m, 1H), 2.94 (dd,  $J = 12.6, 4.2$  Hz, 0.4H), 2.88 (s, 1.2H), 2.80 (s, 1.8H), 2.77 (dd,  $J = 12.6, 4.8$  Hz, 0.6H), 2.74 (s, 1.8H), 2.64–2.58 (m, 1.8H), 2.52 (dd,  $J = 13.5, 11.4$  Hz, 0.6H), 2.45–2.32 (m, 1.6H), 2.31–2.23 (m, 1.6H), 2.15–1.96 (m, 2.4H), 1.96–1.83 (m, 3.6H), 1.81–1.69 (m, 4H), 1.62 (ddd,  $J = 13.8, 9.6, 3.6$  Hz, 1H), 1.56–1.51 (m, 1H), 1.31–1.25 (m, 1H), 1.23 (d,  $J = 7.2$  Hz, 1.8H), 1.21–1.17 (m, 0.4H), 1.09 (d,  $J = 6.6$  Hz, 1.2H), 1.03 (t,  $J = 6.6$  Hz, 1.8H), 1.03 (d,  $J = 6.6$  Hz, 1.8H), 1.00 (d,  $J = 6.6$  Hz, 1.2H), 0.98 (d,  $J = 6.6$  Hz, 1.2H), 0.98–0.92 (m, 0.6H), 0.87 (s, 9H), 0.84 (t,  $J = 7.2$  Hz, 1.2H), 0.57 (d,  $J = 6.6$  Hz, 1.2H) ppm.  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ , mixture of rotamers, major and minor):  $\delta$  172.7, 172.1, 171.9, 171.2, 170.7, 170.5, 170.3, 170.2, 170.0, 168.6, 158.8, 158.7, 130.7, 130.6, 128.7, 128.5, 114.2, 114.0, 78.1, 77.6, 76.2, 75.8, 68.6, 67.3, 60.7, 59.9, 59.5, 58.1, 57.9, 57.4, 55.5, 55.4, 54.1, 50.9, 49.8, 47.8, 45.0, 43.9, 42.9, 41.0, 39.8, 38.8, 37.6, 37.5, 37.4, 37.0, 36.9, 34.1, 34.1, 33.2, 33.0, 31.5, 30.6, 30.6, 29.3, 29.3, 29.0, 26.2, 26.2, 25.7, 25.6, 25.3, 25.2, 24.8, 20.9, 19.9, 15.1, 14.3, 14.1, 14.1, 10.0, 10.0 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{43}\text{H}_{67}\text{N}_5\text{O}_8\text{Na}$  ( $M + \text{Na}$ ) $^+$  836.4603, found 836.4607.

**Apratoxin 58 (1c).** Yield 6.8 mg, 70% in 3 steps;  $[\alpha]_{\text{D}}^{20} -59.5$  ( $c$  0.037,  $\text{CH}_2\text{Cl}_2$ );  $t_{\text{R}} = 26.2$  min.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , mixture of rotamers, major and minor (7/3)):  $\delta$  7.14 (d,  $J = 8.4$  Hz, 0.6H), 7.12 (d,  $J = 8.4$  Hz, 1.4H), 6.80 (d,  $J = 8.4$  Hz, 1.4H), 6.78 (d,  $J = 8.4$  Hz, 0.6H), 6.18 (d,  $J = 9.0$  Hz, 0.7H), 5.73 (d,  $J = 10.2$  Hz, 0.3H), 5.27 (d,  $J = 11.4$  Hz, 0.3H), 5.18–5.11 (m, 1H), 4.96 (d,  $J = 12.0$  Hz, 0.3H), 4.89 (d,  $J = 10.8$  Hz, 1H), 4.60 (q,  $J = 6.0$  Hz, 0.7H), 4.45–4.37 (m, 1H), 4.35–4.31 (m, 0.7H), 4.15 (t,  $J = 8.4$  Hz, 0.3H), 4.10–4.06 (m, 0.7H), 3.87 (d,  $J = 10.8$  Hz, 0.7H), 3.76 (s, 3H), 3.70 (t,  $J = 10.8$  Hz, 0.7H), 3.63–3.59 (m, 1H), 3.34 (dd,  $J = 10.8, 8.4$  Hz, 0.3H), 3.31 (q,  $J = 6.6$  Hz, 0.3H), 3.24 (dd,  $J = 10.8, 8.4$  Hz, 0.7H), 3.12 (dd,  $J = 12.6, 11.4$  Hz, 0.3H), 3.09–3.04 (m, 1.4H), 2.95 (dd,  $J = 12.6, 3.6$  Hz, 0.7H), 2.88 (s, 2.1H), 2.87 (s, 0.9H), 2.76 (s, 0.9H), 2.75–2.73 (m, 0.3H), 2.58 (s, 2.1H), 2.41 (ddd,  $J = 13.2, 13.2, 4.8$  Hz, 0.7H), 2.36–2.26 (m, 2.3H), 2.23–2.18 (m, 1H), 2.14–2.10 (br m, 0.7H), 2.08–2.02 (m, 1.3H), 1.98–1.91 (m, 3.3H), 1.89–1.82 (m, 2.3H), 1.78–1.73 (m, 3.3H), 1.60–1.56 (m, 0.7H), 1.51 (m, 1H), 1.29–1.23 (m, 2.8H), 1.17 (s, 0.9H), 1.16 (s, 2.1H), 1.07–1.06 (m, 5.1H), 1.03 (t,  $J = 7.2$  Hz, 0.9H), 0.98 (d,  $J = 6.6$  Hz, 3H), 0.87 (s, 9H), 0.83 (t,  $J = 7.2$  Hz, 2.1H), 0.49 (d,  $J = 6.6$  Hz, 2.1H) ppm.  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ , mixture of rotamers, major and minor):  $\delta$  179.6, 178.4, 172.2, 172.0, 172.0, 171.8, 170.9, 170.4, 170.3, 170.2, 170.1, 169.8, 158.9, 158.6, 130.7, 130.6, 128.7, 128.5, 114.2, 113.9, 77.7, 75.8, 75.3, 74.8, 73.9, 60.8, 60.0, 59.3, 57.8, 57.4, 55.5, 55.4, 54.2, 50.9, 49.8, 47.8, 46.7, 46.5, 40.0, 38.2, 38.0, 37.6, 37.3, 37.0, 36.0, 35.2, 35.1, 34.9, 34.6, 34.1, 33.7, 31.7, 31.4, 30.8, 30.8, 30.7, 29.8, 29.5, 29.3, 28.9, 26.3, 26.3, 25.8, 25.6, 25.6, 25.3, 25.0, 24.2, 22.8, 20.7, 20.3, 18.7, 18.3, 14.9, 14.6, 14.3, 14.1, 14.1, 10.7, 10.0 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{45}\text{H}_{71}\text{N}_5\text{O}_8\text{Na}$  ( $M + \text{Na}$ ) $^+$  864.4916, found 864.4918.

**Apratoxin 59 (1d).** Yield 4.3 mg, 45% in 3 steps;  $[\alpha]_{\text{D}}^{20} -82.6$  ( $c$  0.023,  $\text{CH}_2\text{Cl}_2$ );  $t_{\text{R}} = 24.8$  min.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , mixture of rotamers, major and minor (6/4)):  $\delta$  7.13 (d,  $J = 9.0$  Hz, 1.2H), 7.13 (d,  $J = 9.0$  Hz, 0.8H), 6.82 (d,  $J = 9.0$  Hz, 0.8H), 6.79 (d,  $J = 9.0$  Hz, 1.2H), 6.23 (d,  $J = 8.4$  Hz, 0.4H), 5.86 (d,  $J = 9.6$  Hz, 0.6H), 5.23 (d,  $J = 11.4$  Hz, 0.6H), 5.19 (ddd,  $J = 10.5, 10.5, 4.8$  Hz, 0.6H), 5.05 (ddd,  $J = 10.8, 8.4, 4.2$  Hz, 0.4H), 4.95 (dd,  $J = 13.2, 3.0$  Hz, 0.6H), 4.88 (dd,  $J = 12.6, 3.6$  Hz, 0.4H), 4.82 (d,  $J = 11.4$  Hz, 0.4H), 4.73 (d,  $J = 11.4$  Hz, 0.6H), 4.59 (q,  $J = 6.6$  Hz, 0.4H), 4.23 (qd,  $J = 8.4, 4.8$  Hz, 0.4H), 4.36–4.34 (m, 0.4H), 4.31–4.23 (m, 1H), 4.23 (t,  $J = 7.8$  Hz, 0.6H), 4.19 (d,  $J = 10.2$  Hz, 0.4H), 4.13–4.09 (m, 0.4H), 3.77 (s, 1.8H), 3.77 (s, 1.2H), 3.69–3.64 (m, 0.6H), 3.64–3.60 (m, 0.4H), 3.55–3.51 (m, 0.4H), 3.50 (dd,  $J = 10.8, 7.8$  Hz, 0.4H), 3.46 (qd,  $J = 11.1, 3.6$  Hz, 0.6H), 3.38 (dd,  $J = 10.8, 7.8$  Hz, 0.6H), 3.26 (q,  $J = 6.6$  Hz, 0.6H), 3.09–2.98 (m, 1.4H), 2.94 (s, 1.2H), 2.80–2.76 (m, 1.6H), 2.74 (s, 1.8H), 2.74 (s, 1.8H), 2.70 (dd,  $J = 11.4, 11.4$  Hz, 0.6H), 2.63–2.57 (m, 2.2H), 2.44 (ddd,  $J = 16.8, 5.4, 3.0$  Hz, 0.6H), 2.41–2.34 (m, 1H), 2.30–2.22 (m, 1.2H), 2.20–2.17 (m, 0.4H), 2.16–2.10 (m, 1H), 2.09–2.03 (m, 1.4H), 1.98–1.84 (m, 2.4H), 1.79 (td,  $J = 13.2, 3.6$  Hz, 0.6H), 1.75–1.61 (m, 4.4H), 1.58–1.52 (m, 0.6H), 1.49

(td,  $J = 11.4, 3.6$  Hz, 0.6H), 1.33 (ddd,  $J = 13.8, 11.1, 3.0$  Hz, 0.4H), 1.29–1.26 (m, 1.4H), 1.23 (d,  $J = 7.2$  Hz, 1.8H), 1.12–1.09 (m, 2.2H), 1.07 (d,  $J = 6.6$  Hz, 1.8H), 1.05 (d,  $J = 7.2$  Hz, 1.8H), 1.01 (t,  $J = 7.2$  Hz, 1.8H), 0.97–0.95 (m, 3.6H), 0.92 (m, 0.4H), 0.87 (s, 5.4H), 0.87 (s, 3.6H), 0.85 (t,  $J = 7.2$  Hz, 1.2H), 0.43 (d,  $J = 6.6$  Hz, 1.2H) ppm.  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ , mixture of rotamers, major and minor):  $\delta$  175.3, 174.9, 172.6, 172.2, 171.9, 171.4, 171.1, 170.7, 170.5, 170.5, 170.3, 170.0, 158.9, 158.7, 130.7, 130.5, 128.7, 128.4, 114.3, 114.0, 78.0, 77.6, 75.5, 74.9, 72.5, 72.1, 60.7, 59.8, 59.3, 58.3, 56.9, 55.5, 55.4, 53.8, 51.6, 49.6, 49.0, 47.8, 47.5, 40.0, 39.4, 38.3, 38.0, 37.8, 37.6, 37.5, 37.1, 36.8, 35.2, 35.2, 34.2, 34.0, 33.2, 32.5, 31.6, 31.1, 30.8, 30.5, 29.3, 29.2, 28.8, 26.2, 25.8, 25.7, 25.3, 25.2, 24.8, 24.7, 20.6, 20.1, 16.5, 16.4, 14.6, 14.3, 14.0, 14.0, 10.1, 9.9 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{44}\text{H}_{69}\text{N}_5\text{O}_8\text{Na}$  ( $M + \text{Na}$ ) $^+$  850.4759, found 850.4773.

**Biological Material and Methods. Cell Culture.** Human colon adenocarcinoma HCT116 cells were purchased from ATCC (Manassas, VA) and cultured in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) at 37 °C humidified air and 5%  $\text{CO}_2$ .

**Cell Viability Assay (MTT).** HCT116 cells were seeded at a density of  $1 \times 10^4$  cells per well in 96-well plates. Then 24 h later, the cells were treated with various concentrations of apratoxins or solvent control (1% EtOH). After 48 h of incubation, cell viability was detected using MTT according to the manufacturer's instructions (Promega, Madison, WI).

**Measurement of VEGF-A Production.** HCT116 cells ( $1 \times 10^4$  cells per well) were seeded in 96-well plates and one day later treated with various concentrations of apratoxins or solvent control. After 12 h of incubation, culture supernatants were collected for detection of VEGF-A by using an alphaLISA kit (PerkinElmer, Waltham, MA) following the manufacturer's instructions. Briefly, acceptor bead and anti-VEGF antibody were incubated first with the supernatants for 60 min, donor beads were added later and incubated for another 30 min, and then VEGF-A levels were detected using Envision (PerkinElmer).

**Immunoblot Analysis.** HCT116 cells were seeded in 6-well plates at a density of  $4 \times 10^5$  cells and the next day treated with various concentrations of apratoxins or solvent control (1% EtOH). Then 24 h later, whole cell lysates were collected using PhosphoSafe buffer (EMD Chemicals, Inc., Gibbstown, NJ). The protein concentration was measured with the BCA Protein Assay kit (Thermo Fisher Scientific, Rockford, IL). Lysates containing equal amounts of protein were separated by SDS polyacrylamide gel electrophoresis (4–12%), transferred to polyvinylidene difluoride membranes, probed with primary and secondary antibodies, and detected with the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific). Anti-Met, anti-VEGFR2, and secondary antimouse antibodies were from Cell Signaling Technology, Inc. (Danvers, MA).

**In Vivo Efficacy Studies.** Three–five week old female nude mice ( $nu/nu$ ) were obtained from Charles River Laboratory (Wilmington, MA). One  $\times 10^6$  HCT116 cells in a volume of 100  $\mu\text{L}$  of sterile saline were injected subcutaneously on the left rear flank of a nude mouse to establish tumors. Tumor dimensions were measured using calipers every day, and tumor volumes were calculated using the formula  $W^2 \times L \times 0.5$ , where width ( $W$ )  $\leq$  length ( $L$ ). Tumors with a starting volume bigger than 100  $\text{mm}^3$  were excluded from the analysis. Mice were injected intraperitoneally with the doses of 2  $\mu\text{g}/\text{mouse}$  (0.1  $\text{mg}/\text{kg}$ ), 5  $\mu\text{g}/\text{mouse}$  (0.25  $\text{mg}/\text{kg}$ ) of 1c or solvent (DMSO) control every day (25  $\mu\text{L}$ ) until the tumor size in one dimension reached 15 mm and tumor tissue was harvested on the following day. Finally, 50 mg of tumor tissue was sonicated in PhosphoSafe lysis buffer (EMD chemicals, Inc.) and used for immunoblot analysis described as the above. All studies were carried out under the protocol approved by the Institutional Animal Care and Use Committee at the University of Florida.

**Metabolite Analyses. Materials and General Procedures.** HPLC-MS was done on a 3200 QTRAP (Applied Biosystems) equipped with a Shimadzu (Kyoto, Japan) UFLC System. Mouse serum and harmine were purchased from Sigma-Aldrich. Pooled CD1 mouse liver (female) microsomes were purchased from XenoTech, LCC (Lenexa, KS) with protein concentrations of 0.5  $\text{mg}/\text{mL}$ .



HCT116 cell lysates were prepared with PhosphoSafe lysis buffer (Novagen, San Diego, CA). Protein concentration was determined by using the BCA Assay. Analysis was carried out similarly as previously described.<sup>22</sup>

**Sample Preparation.** Stock solutions of **1a–d** were prepared by dissolving the compounds in ethanol to give a 1 mg/mL solution. Aliquots of this stock solution were then obtained to afford a 40 µg/mL solution in acetonitrile. Serial dilution of the 40 µg/mL solution in acetonitrile gave standard solutions with concentrations of 25, 12.5, 2.5, 1.25, 0.25, 0.125, 0.025, and 0.0125 µg/mL. A 1 mg/mL stock solution of the internal standard harmine was prepared in ethanol, which subsequently was used to prepare a 100 µg/mL solution with ethanol. An aliquot of the 100 µg/mL harmine solution was diluted to 35 ng/mL with ethyl acetate to serve as the working internal standard solution.

**Plasma Stability.** In vitro plasma stability of **1a–d** was done by using a modification of a published method.<sup>23</sup> First, 10 µL of apratoxins (25 µg/mL) were added to 100 µL of mouse serum, and the solution was then vortex-mixed for 15 s and incubated for 0.25 min to 24 h. At the end of each incubation period, 400 µL of ethyl acetate was added to each tube, followed by 200 µL of harmine to quench the reaction and extract remaining apratoxin. Samples were further incubated in a thermomixer at 27 °C (750 rpm, 5 min) and later centrifuged for 5 min at 1643g. The ethyl acetate layer was collected and evaporated to dryness under nitrogen. Samples were reconstituted in 50 µL of acetonitrile. A volume of 10 µL of the reconstituted solution was injected into the HPLC-MS system.

**Microsomal Stability.** The stability of **1a–d** in the presence of mouse microsomes was determined by using an adaptation of a published procedure.<sup>24</sup> In brief, microsomes were added to prewarmed phosphate buffer (100 mM, pH 7.4) at 37 °C. Apratoxins (3 µL) were added to the microsomal preparation followed by NADPH cofactor solution (1.3 mM NADP, 3.3 mM glucose 6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM MgCl<sub>2</sub>). The reaction was allowed to proceed for 3 min to 2 h at 37 °C (Thermomixer, 1050 rpm). The reaction was quenched by addition of ethyl acetate and subsequently spiked with harmine. The zero time point was defined by denaturing the microsomes with ethyl acetate before the addition of apratoxins. Incubation of apratoxins with microsomes alone was also performed following the same procedure to determine NADPH-independent metabolism. The final concentration of the incubation mixture contained 0.5 mg/mL protein concentration and 1 µM apratoxins.

**Cellular Stability.** Aliquots of HCT116 cell lysates were diluted with 25 mM Tris-HCl buffer, pH 8.0, to give a final reaction volume of 100 µL and protein concentration of 0.7 mg/mL. Cell lysate solutions were incubated with 10 µL of apratoxins (25 µg/mL) for 0.25 min to 24 h. Remaining apratoxins were extracted from the reaction solution at the end of the incubation periods with ethyl acetate using the same procedure as described for the plasma stability assay.

**Aqueous Stability.** The stability of **1a–d** in aqueous solution was determined in 100 mM phosphate buffer, pH 4.88, 100 mM phosphate buffer, pH 7.4. Portions of each solution (100 µL) were spiked with 10 µL of apratoxin solution (25 µg/mL) and allowed to incubate for 0.25 min to 24 h. The reaction was quenched at the end of each time point, and the remaining apratoxins were extracted with ethyl acetate, as in the plasma stability study.

**HPLC-MS Parameters.** Analysis of **1a–d** was done by using HPLC-MS [column, Onyx Monolithic C18 (3.0 mm × 100 mm), Phenomenex (Torrance, CA); solvent, water (solvent A) acetonitrile (solvent B); flow rate, 0.5 mL/min; detection by electrospray ionization–MS in positive ion mode (MRM scan)]. A stepwise gradient elution was used starting at 60% B and 40% A, then increasing to 80% B at 5 min and maintained at this condition for 5 min. Parameters were optimized before analysis by using direct syringe infusion. The retention times (*t<sub>R</sub>*, min; MRM ion pair) of the analytes and internal standard are as follows: harmine (2.2; 213.1 → 169.9), **1a** (4.1; 828.5 → 432.2), **1b** (4.05; 814.5 → 418.2), **1c** (5.2; 842.5 → 446.2), **1d** (4.9; 828.5 → 432.2). Compound-dependent parameters used were as follows: apratoxins, declustering potential (DP) 51,

entrance potential (EP) 12, collision energy (CE) 45, collision cell exit potential (CXP) 6, collision cell entrance potential (CEP) 32; and harmine, DP 56.0, EP 4.5, CE 44.0, CXP 5, CEP 16.0. Source gas parameters used were as follows: curtain gas, 15.0; collision gas low, ionspray voltage 5500; temperature, 600.0; ion source gas 1 50.0; ion source gas 2 60.0.

**Data Analysis.** Calibration curves for **1a–d** in the presence of mouse serum, HCT116 cell lysates, and aqueous solutions were generated by least-squares linear regression analysis of the analyte peak area and internal standard peak area ratio against the nominal concentration of the standard solutions. A linear regression analysis was performed, and the concentration of remaining apratoxins at each time point was determined through interpolation for plasma, cellular, and aqueous stability experiments. The amount of remaining apratoxins with microsome incubation was determined from the peak area ratio of apratoxins at *t<sub>x</sub>* (3 min to 2 h) and *t<sub>0</sub>*. All calculations were done by using Analyst 1.4.2 (Applied Biosystems) Quantitate Mode.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Preparation procedures and characterizations for some known compounds, cooling concentration equipment for the preparation of **12**, Michael adduct formation of apratoxin A with thiol-containing compounds, trial route to synthesize **7** from diol derivative of **8**, <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra for new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Phone: +1-352-273-7738. Fax: +1-352-273-7741. E-Mail: [luesch@cop.ufl.edu](mailto:luesch@cop.ufl.edu).

### Notes

The authors declare the following competing financial interest(s): Hendrik Luesch is co-founder of Oceanix Pharmaceuticals, Inc., which is negotiating licenses for patent applications related to the subject matter.

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## ■ ABBREVIATIONS USED

BCA, bicinechonic acid; BEP, 2-bromo-1-ethyl-pyridinium tetrafluoroborate; BOP, (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate; BRSM, based on recovered starting material; CEP, collision cell entrance potential; CXP, collision cell exit potential; CE, collision energy; DEPBT, 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one; DIEA, *N,N*-diisopropylethylamine; DP, declustering potential; EDCI, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride; EGFR, epidermal growth factor receptor; EP, entrance potential; *epi*, epimer; G2, generation 2; GSH, glutathione; HATU, 1-[bis-(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxid hexafluorophosphate; HCT116, human colorectal carcinoma cell line; HOBt, 1-Hydroxybenzotriazole; ip, intraperitoneal; IPC, diisopinocampheyl; LiAlH<sub>4</sub>, lithium aluminum hydride; moCys, modified cysteine; MET, met proto-oncogene (hepatocyte growth factor receptor); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAC, *N*-acetyl-L-cysteine; PyAOP, (7-azabenzotriazol-1-yloxy)-

tripyrrrolidinophosphonium hexafluorophosphate; RTK, receptor tyrosine kinase; SEM, standard error of the mean; TBAI, tetrabutylammonium iodide; TMSOTf, trimethylsilyl trifluoromethanesulfonate; TfOH, trifluoromethanesulfonic acid; Troc, 2,2,2-trichloroethoxycarbonyl; Trt, trityl/triphenylmethyl; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

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