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Synthesis and cell cycle inhibition of the peptide enamide natural products terpeptin and the aspergillamides

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Abstract—Total syntheses of the peptide enamide natural products terpeptin and aspergillamides A and B are reported. An oxidative decarboxylation–elimination protocol is employed to construct the indolic enamide moiety. Unambiguous stereochemical assignment of (−)-terpeptin is accomplished by synthesis of all possible stereochemical analogues. Select compounds have been evaluated in cell cycle inhibitor assays which show that the natural amino acid configuration of terpeptin has the most potent inhibitory activity.

1. Introduction

The enamide (*N*-acyl-1-amino-alkene) is a functional group in which a carbon–carbon double bond occurs next to the nitrogen atom of an amide moiety. Enamides have been studied extensively as synthons in organic synthesis, and used prominently in the preparation of heterocycles[1] and in asymmetric synthesis.[2] However, little is known about the role of the enamide moiety in biologically active natural products.[3] A class of bioactive enamide natural products that has attracted our attention is the linear and cyclic peptide enamides, representative members (**1**–**6**) which are shown in Figure 1.[4] Recently, we reported the synthesis of peptide enamides, including the indolic enamide[5] natural product chondriamide C (**1**), using a tandem oxidative decarboxylation/elimination protocol.[6] Interestingly,

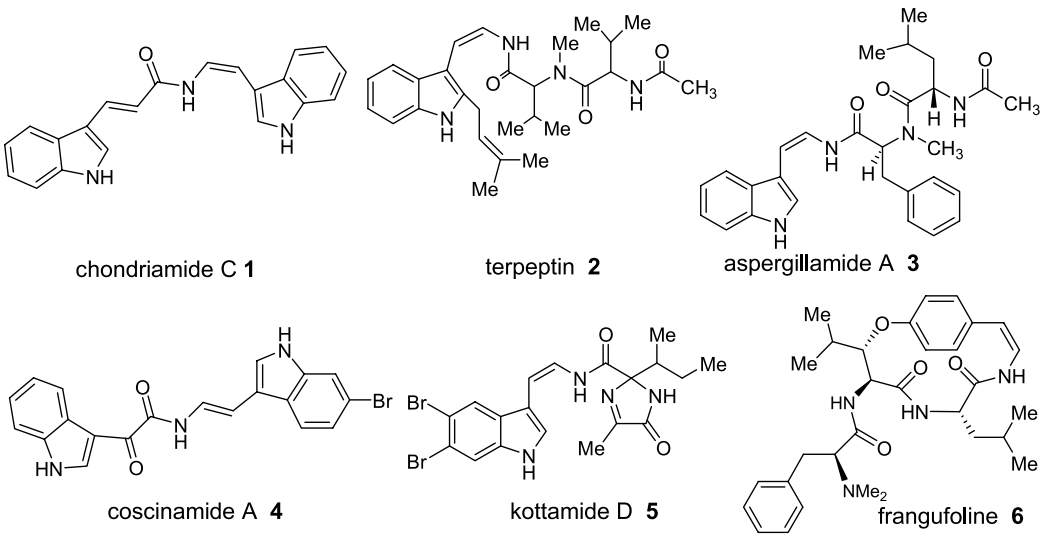

Figure 1. Representative peptide enamide natural products.

Keywords: indolic enamide; terpeptin; aspergillamides; oxidative decarboxylation–elimination; cell cycle inhibitor; peptide.
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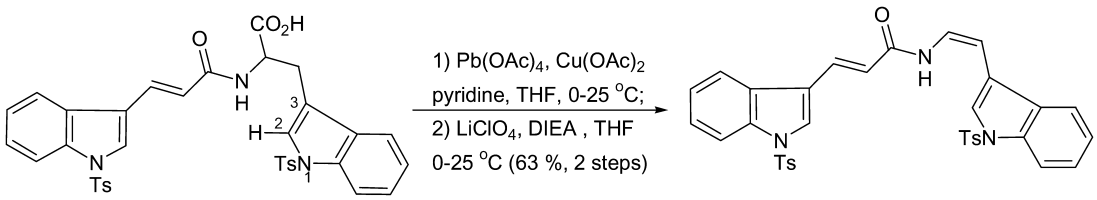


Figure 2. Oxidative decarboxylation elimination of a tryptophan-derived substrate.[6]

substrates containing C-terminal tryptophans (Fig. 2) were clearly distinct from other C-terminal peptides and formed *Z*-enamides with high selectivity.

Terpeptin (**2**) is a novel peptide recently isolated from *Aspergillus terreus* by Osada and co-workers which inhibits the cell cycle at G2/M phase (minimum inhibitory concentration 62.5 μM).[4b] A related molecule aspergillamide A (**3**) and the corresponding *E* enamide stereoisomer (aspergillamide B) were isolated from the genus *Aspergillus* and showed modest in vitro cytotoxicity toward the human carcinoma cell line HCT-116.[4c] To extend our studies towards the synthesis of indolic enamide-containing natural products, we targeted terpeptin and aspergillamides A and B for synthesis. In particular, we wished to determine the influence of the 2-prenyl tryptophan on the stereochemical outcome of the oxidative decarboxylation–elimination process (cf. Fig. 2). Herein, we report total syntheses of terpeptin and aspergillamides A and B employing the tandem oxidative decarboxylation elimination of C-terminal tryptophan-containing peptides. Cell cycle inhibition of aspergillamide A, terpeptin, and stereochemical analogues will also be described.

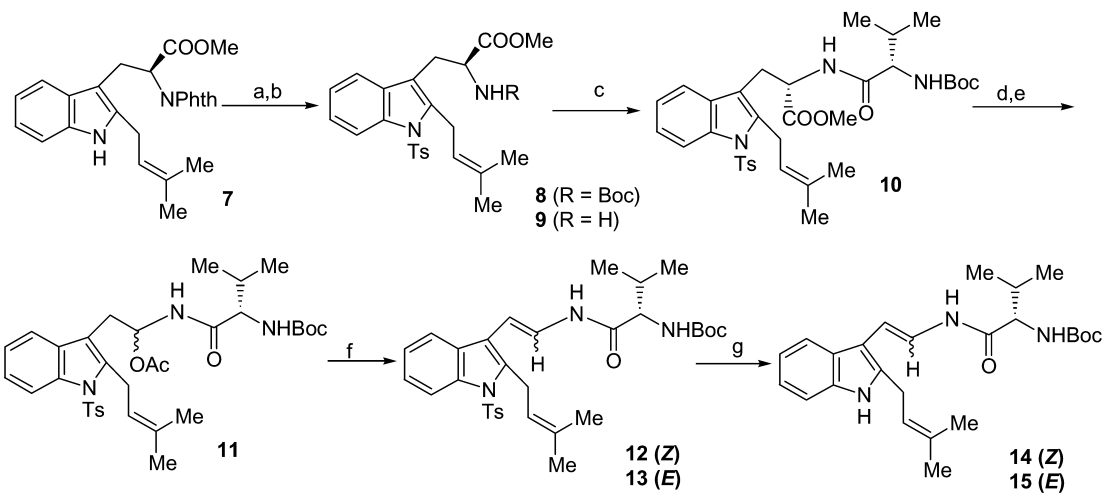
2. Results and discussion

Studies towards synthesis of terpeptin and a suitable model system to examine the critical oxidative decarboxylation–elimination step were initiated with the readily available 2-prenyl indole **7**[7] (Scheme 1). In line with our previous studies of the oxidative decarboxylation of C-terminal tryptophans, we employed a tosyl group (‘*Nin*-Ts’) for indole protection. Phthalimide deprotection of **7**,[7] followed by Boc-protection, afforded tryptophan derivative **8**. After considerable experimentation, we found that *Nin*-tosylation of **8** could be accomplished in satisfactory yield with NaOH and NBu_4HSO_4 as a phase-transfer catalyst.[8] Subsequent deprotection of the Boc group was carried out using 10% aqueous HF in CH_3CN.[9] Dipeptide **10** was prepared without loss of optical purity using EDC/HOBt. After hydrolysis of the methyl ester, compound **10** was readily converted to *N*,*O*-acetal **11** (1:1 mixture of diastereomers) using $Pb(OAc)_4$.[6] At this point, this intermediate was subjected to a screen of elimination conditions including variation of base, solvent and temperature (Table 1). In most instances, elimination preferentially afforded the *E* enamide as a major isomer (cf. entries 1–5). The highest *Z*–*E* selectivity was obtained using the polymer-supported base 1,5,7-triazabicyclo [4.4.0]dec-5-ene polystyrene (PS-TBD)[10] (CH_2Cl_2, 0°C) which afforded a 1.5:1 ratio of *Z* enamide **12** and *E* enamide **13** (entry 6). The model study was completed by detosylation of **12** and **13** using Mg/MeOH[11] to afford indolic enamides **14** and **15**.

Table 1. Evaluation of conditions for elimination of *N*,*O*-acetal **11**

Entry	Base	Additive	Temperature (°C)	Solvent	*Z*–*E*	Yield (%)
1	DIEA[a]	$LiClO_4$	0–rt	THF	1:5	65
2	DBU[b]	–	−78	THF	1:2	60
3	DBU[b]	–	0	THF	1:2	55
4	DBU[b]	–	0	CH_2Cl_2	1:2	55
5	PS-TBD[a]	–	0	THF	1:1.5	60
6	PS-TBD[a]	–	0	CH_2Cl_2	1.5:1	62

[a] 2 equiv.
[b] 1.5 equiv.



Scheme 1. Model studies towards the synthesis of terpeptin. *Reagents and conditions*: (a) (1) $NH_2NH_2{\cdot}H_2O$, 2:1 CH_2Cl_2–MeOH; (2) $(Boc)_2O$, Et_3N, DMF, 45°C, 90%. (b) NaOH, TsCl, CH_2Cl_2, cat. NBu_4HSO_4, reflux, 63%. (c) (1) 10% HF, CH_3CN, 81%; 2. HOBT, EDC, DIEA, DMF, 0–25°C, Boc-*L*-Val-OH, 95%. (d) LiOH, THF–H_2O=2:1, rt, 95%. (e) $Pb(OAc)_4$,$Cu(OAc)_2$, pyridine,THF, 0°C–rt. (f) conditions (Table 1). (g) Mg, MeOH, rt, 0°C–rt, 95%.

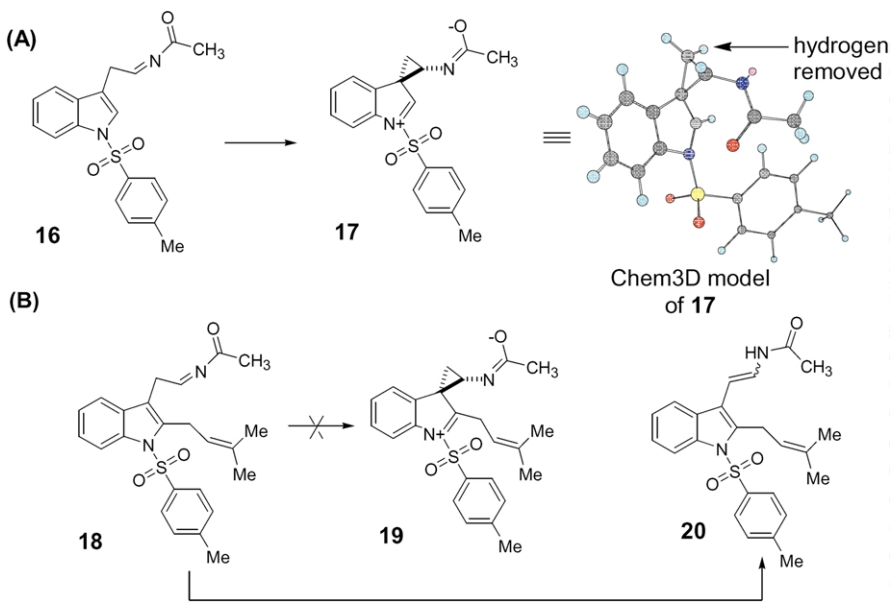
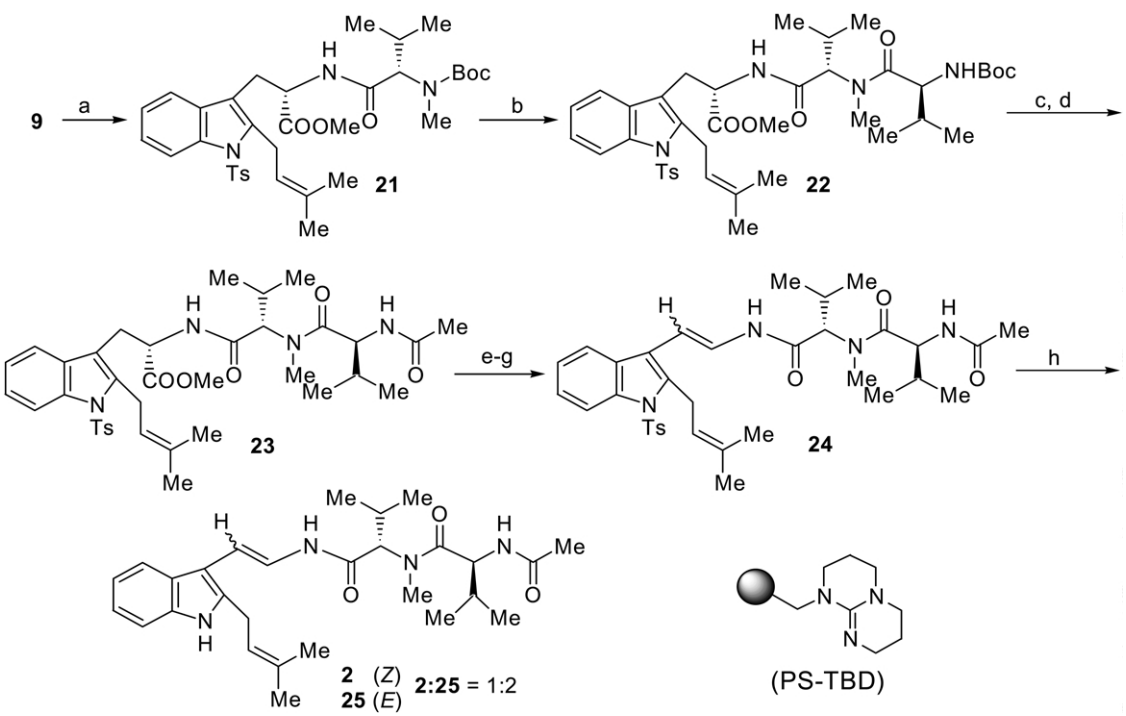
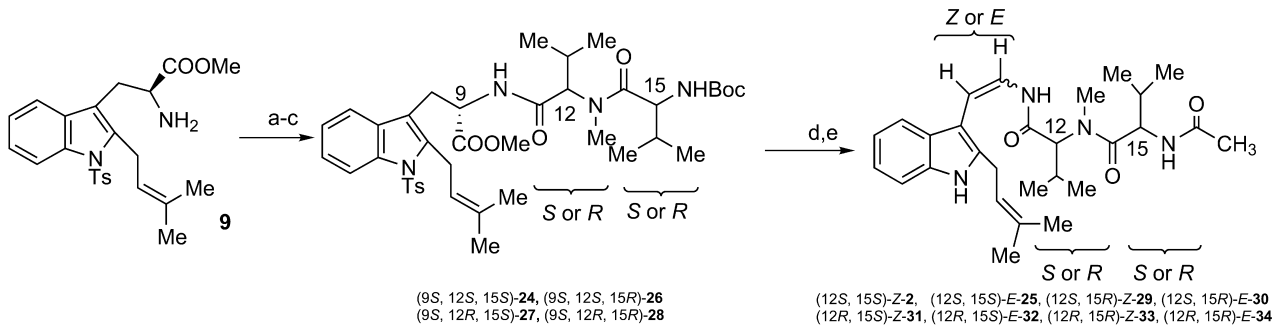


Figure 3. Possible involvement of spiroindolinium intermediates for tryptophan substrates.

We previously reported that C-terminal tryptophans with the 2-position unsubstituted (cf. Fig. 2) form *Z*-enamides with high selectivity.[6] To explain this result, we proposed the transformation of a reactive *N*-acylimine to a spirocyclopropylindolinium intermediate.[12] Figure 3(A) illustrates the transformation of simplified *N*-acylimine **16** to the putative spirocyclopropylindolinium intermediate **17**. The Chem3D model shows that the negatively charged amide anion is positioned directly above the positively charged iminium ion to form a contact ion pair for maximum electrostatic stabilization. Formation of the *Z*-enamide may be explained by preferential elimination of the indicated, less hindered hydrogen of **17**. However we have found that 2-prenyl substitution of the indole substantially reduces the *Z*-enamide selectivity (cf. **11→12**). The observed reduction in selectivity may be explained (Fig. 3(B)) by the inability of *N*-acylimine **18** to form a spirocyclopropylindolinium intermediate such as **19**, and formation of the enamide product **20** directly via isomerization of **18**.



Scheme 2. Stepwise synthesis of terpeptin. *Reagents and conditions*: (a) HOBT, EDC, DIEA, DMF, 0–25°C, Boc-NMe-*L*-Val-OH, 96%. (b) (1) 10% HF, CH_3CN, 90%; (2) HATU, DIEA, DMF, 0–25°C, Boc-*L*-Val-OH, 94%. (c) (1) TMSOTf, 2,6-lutidine; (2) MeOH. (d) Et_3N, Ac_2O, CH_2Cl_2, 89% from **22**. (e) LiOH, 2:1 THF/H_2O, rt, 95%. (f) $Pb(OAc)_4$, $Cu(OAc)_2$, pyridine, THF, 0°C–rt. (g) PS-TBD, CH_2Cl_2, 0°C. (h) Mg, MeOH, rt, 0°C–rt, 43% from **23**.



(9*S*, 12*S*, 15*S*)-**24**, (9*S*, 12*S*, 15*R*)-**26**
(9*S*, 12*R*, 15*S*)-**27**, (9*S*, 12*R*, 15*R*)-**28**

(12*S*, 15*S*)-*Z*-**2**, (12*S*, 15*S*)-*E*-**25**, (12*S*, 15*R*)-*Z*-**29**, (12*S*, 15*R*)-*E*-**30**
(12*R*, 15*S*)-*Z*-**31**, (12*R*, 15*S*)-*E*-**32**, (12*R*, 15*R*)-*Z*-**33**, (12*R*, 15*R*)-*E*-**34**

Scheme 3. Parallel synthesis of stereochemical analogues of terpeptin: *Reagents and conditions*: (a) Boc-NMe-*L*-Val-OH or Boc-NMe-*D*-Val-OH, HOBt, EDC, DIEA, DMF. (b) 10% HF, MeCN, rt. (c) Boc-*L*-Val-OH or Boc-*D*-Val-OH, HATU, DIEA, CH_2Cl_2. (d) (1) TMSOTf, 2,6-lutidine then MeOH; (2) Et_3N, Ac_2O, CH_2Cl_2. (e) (1) LiOH, 2:1 THF/H_2O, rt; (2) $Pb(OAc)_4$, $Cu(OAc)_2$, pyridine, THF, 0°C–rt; (3) PS-TBD, CH_2Cl_2, 0°C; (4) Mg, MeOH, 0°C–rt.

Based on the model studies, we continued with efforts to prepare terpeptin with the natural *L* configuration at both stereogenic centers. Initial attempts to prepare terpeptin following a convergent route by coupling of amine **9** and the dipeptide Ac-*L*-Val-NMe-*L*-Val-OH led to substantial racemization, presumably due to the formation of an oxazolone intermediate.[13] After optimization experiments to minimize racemization, we changed to a stepwise synthesis approach. Dipeptide **21** was prepared from condensation of compound **9** and commercially available *N-tert*-butyloxycarbonyl-*N*-methyl-*L*-valine (Scheme 2). Treatment of dipeptide **21** with 10% aqueous HF followed by a HATU-mediated[14] coupling with *N-tert*-butoxycarbonyl-*L*-valine led to the formation of tripeptide **22**. Interestingly, treatment of **22** with the 10% aqueous HF led to substantial decomposition and formation of numerous products. In contrast, Boc deprotection of **22** with TMSOTf and 2,6-lutidine,[15] followed by the acetylation of the primary amine, cleanly afforded the desired tripeptide **23** (89%, two steps). Ester hydrolysis of **23**, followed by treatment with $Pb(OAc)_4$, afforded the crude *N*,*O*-acetals (1:1 mixture of diastereomers), which were subjected to elimination (2 equiv. PS-TBD, CH_2Cl_2, 0°C). *Nin*-Ts removal (Mg powder/MeOH) cleanly afforded terpeptin **2** and its *E* isomer **25** (**2**–**25**=1:2). Although the ^1H and ^{13}C NMR spectra of **2** were identical to those derived from the natural product,[4b] we observed a substantial difference between the published rotation of natural terpeptin ($[\alpha]_D$= −135.2°, *c*=0.1, $CHCl_3$) and synthetic **2** ($[\alpha]_D$=−39.9°, *c*=0.43, distilled $CHCl_3$)[16] which led to further studies to prepare terpeptin stereoisomers to confirm the stereochemistry of the natural product and evaluate their biological properties.

We employed parallel synthesis to construct a small library of all possible stereochemical analogues of terpeptin (Scheme 3).[17] Construction of tripeptide precursors was accomplished using liquid–liquid and solid-phase extraction and polymer-supported scavengers.[18] Treatment of amine **9** with EDC, HOBt, and *L* or *D N-tert*-butyloxycarbonyl-*N*-methyl valine, respectively, afforded two dipeptides which were subjected to simple $NaHCO_3$ workup and Boc deprotection using aqueous HF. After reaction workup (aq. $NaHCO_3$) amide bond formation was achieved using excess HATU[14] and *L* or *D N-tert*-butoxycarbonyl valine. Scavenging of the excess reagents[19] using polystyrene–diethylenetriamine (PS–DETA),[20] followed by solid-phase extraction (SPE) of the four tripeptide stereoisomers using silica gel, provided the desired tripeptides **24** and **26**–**28**. Boc deprotection (TMSOTf, 2,6 lutidine)[15] and acetylation of the primary amine afforded all four precursors to stereochemical analogues of terpeptin. Subjection of these

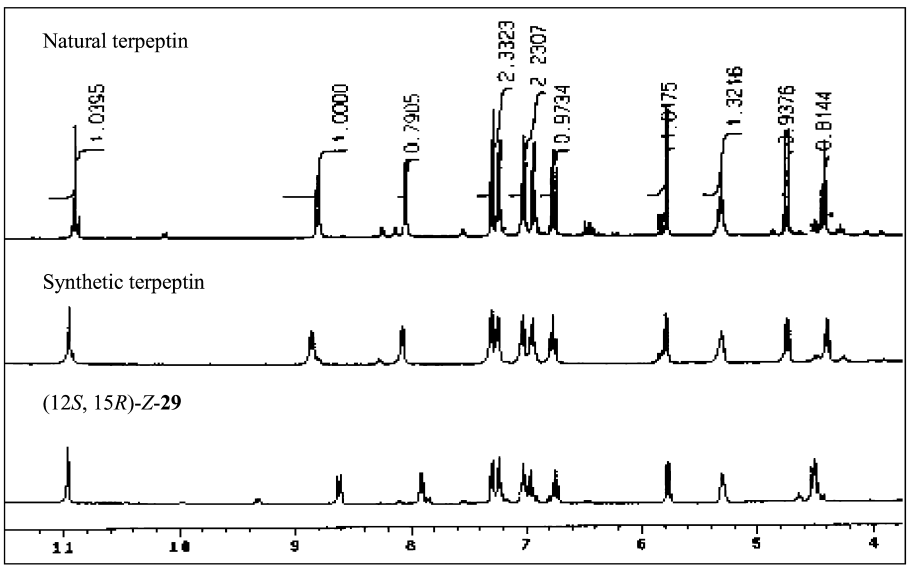


Figure 4. ^1H NMR spectral comparison of natural terpeptin, synthetic terpeptin and (12*S*,15*R*)-*Z*-**29**. (400 MHz, DMSO-d_6).

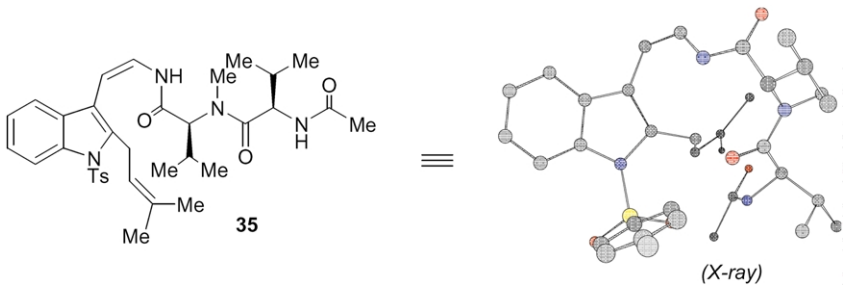


Figure 5. X-Ray structure of *Nin*-Ts-(12*S*,15*R*)-**35**.

compounds individually to methyl ester hydrolysis, oxidative decarboxylation–elimination, and *Nin*-Ts deprotection afforded the terpeptin isomers **2**, **25**, and **29**–**34**. A single X-ray crystal structure analysis of *Nin*-Ts enamide **35** (Fig. 5)[21] confirmed that the synthesis protocol proceeds to form indolic enamides with preservation of amino acid stereochemistry. Interestingly, due to the interaction between the prenyl group and the peptide sidechain, the indole ring and the enamide have a dihedral angle of −52.2° instead of sharing the same plane. This distortion may activate the enamide moiety and profoundly effect the bioactivity of this class of compounds (vide infra).

Comparison of ^{1}H NMR spectra of terpeptin stereoisomers indicated that only the (12*S*,15*S*)-*Z*-**2** and (12*R*,15*R*)-*Z*-**33** showed identical ^{1}H NMR spectrum to natural terpeptin (Fig. 4). Optical rotations (Table 2) of **2** and **33** were found to be −39.9 and +39.9°, respectively (distilled $CHCl_3$). Considering the natural product had a reported negative rotation (−135.2°), we reasoned that natural product terpeptin was the (12*S*,15*S*)-*Z*-**2** compound which contains the natural *L* configuration at both amino acid-derived stereocenters. The difference in optical rotations between natural and synthetic terpeptin may also be explained by the different impurity profiles as evidenced by comparison of ^{1}H NMR spectra (Fig. 4).

Terpeptin stereoisomers **2**, **25**, and **29**–**34** differ only in their enamide geometry and stereochemistry at two asymmetric centers. To further understand the role of the stereochemical diversity on the hydrophobicity of the compounds, we measured the retention times of **2**, **25**, **29**, and **30** using reverse phase HPLC (C_8).[22] As shown in Figure 6, the stereochemistry has a significant effect on the overall hydrophobicity of the compounds. In general, *E* isomers are more polar than the *Z* isomers, and compounds with the natural *L* amino acid configurations are more polar than the *D* stereoisomers. This preliminary data further supports the notion that stereochemical variation can tune the overall hydrophobicity of molecules which, in turn, may influence their pharmacological properties.

The methodology developed in the terpeptin synthesis was next applied to the synthesis of the indole-containing peptide enamide natural products aspergillamides A and B (Scheme 4). Tryptophan derivative **36** was prepared from readily available *L*-tryptophan methyl ester hydrochloride by amine protection with $(Boc)_2O$ and *Nin*-tosylation using our optimized conditions (cf. Scheme 1). After Boc removal using TFA, Boc-*N*-methyl-*L*-phenylalanine was coupled to the primary amine to provide dipeptide **37**. Following Boc removal, HATU-mediated[14] coupling afforded the Boc-protected tripeptide, which was subjected to TFA deprotection and acetylation of the terminal amine to afford tripeptide **38** without any observable racemization. After hydrolysis of the methyl ester, oxidative decarboxylation of the resulting acid afforded a mixture of *N*,*O*-acetals (1:1 d.r.) which were subjected to elimination using PS-TBD resin. In this case, a 20:1 *Z* to *E* ratio was obtained, which was anticipated based on our previous studies (cf. Fig. 2). Enamide **39** was isomerized efficiently to *E* isomer **40** using KI/AcOH.[23] Further deprotection of **39** and **40** using Mg powder/MeOH afforded aspergillamides A (**3**) and B (**41**) respectively. Synthetic **3** and **41** were confirmed to be identical to natural aspergillamides A and B by mass spectral analysis and comparison of ^{1}H and ^{13}C NMR

Table 2. Optical rotations of terpeptin and stereoisomers

Stereoisomers	(12*S*,15*S*)-*Z*-**2**	(12*S*,15*S*)-*E*-**25**	(12*S*,15*R*)-*Z*-**29**	(12*S*,15*R*)-*E*-**30**	(12*R*,15*S*)-*Z*-**31**	(12*R*,15*S*)-*E*-**32**	(12*R*,15*R*)-*Z*-**33**	(12*R*,15*R*)-*E*-**34**
Concentration	0.43	1.0	0.50	1.0	0.52	1.0	0.43	1.0
$[\alpha]_D$	−39.9°	−97.2°	+45.6°	+87.6°	−42.6°	−86.8°	+39.9°	+95.2°

All rotations were done in distilled $CHCl_3$.

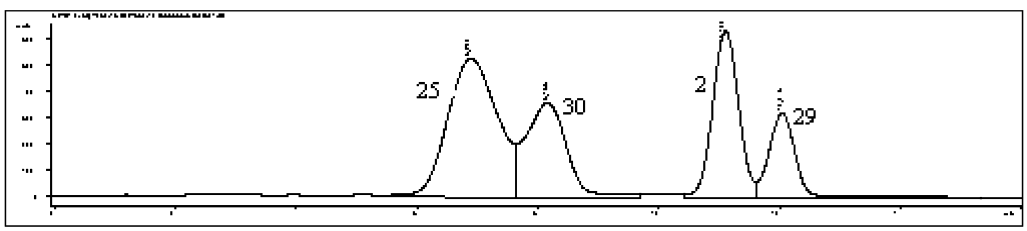
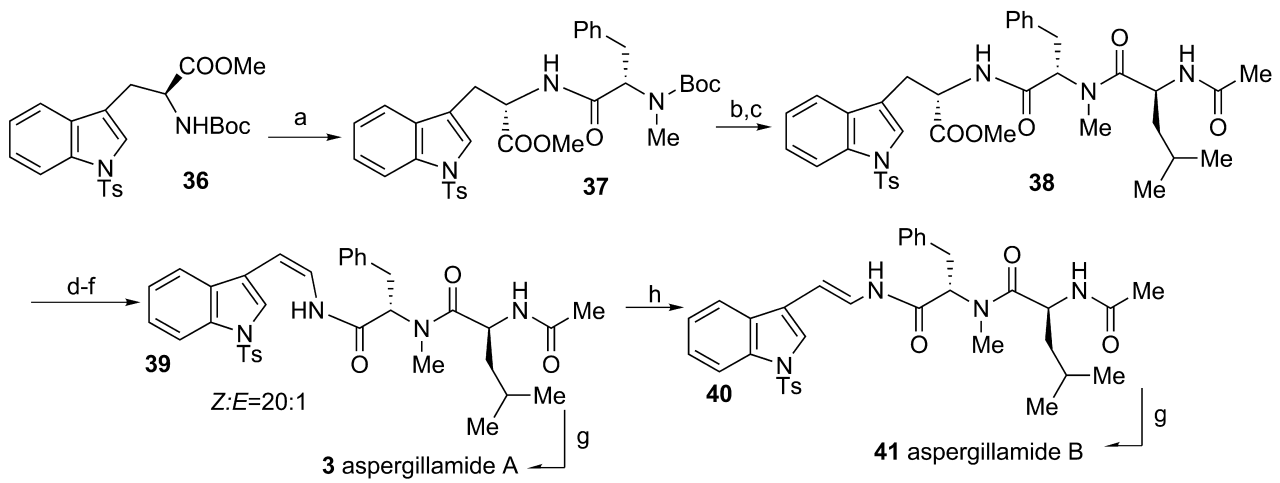


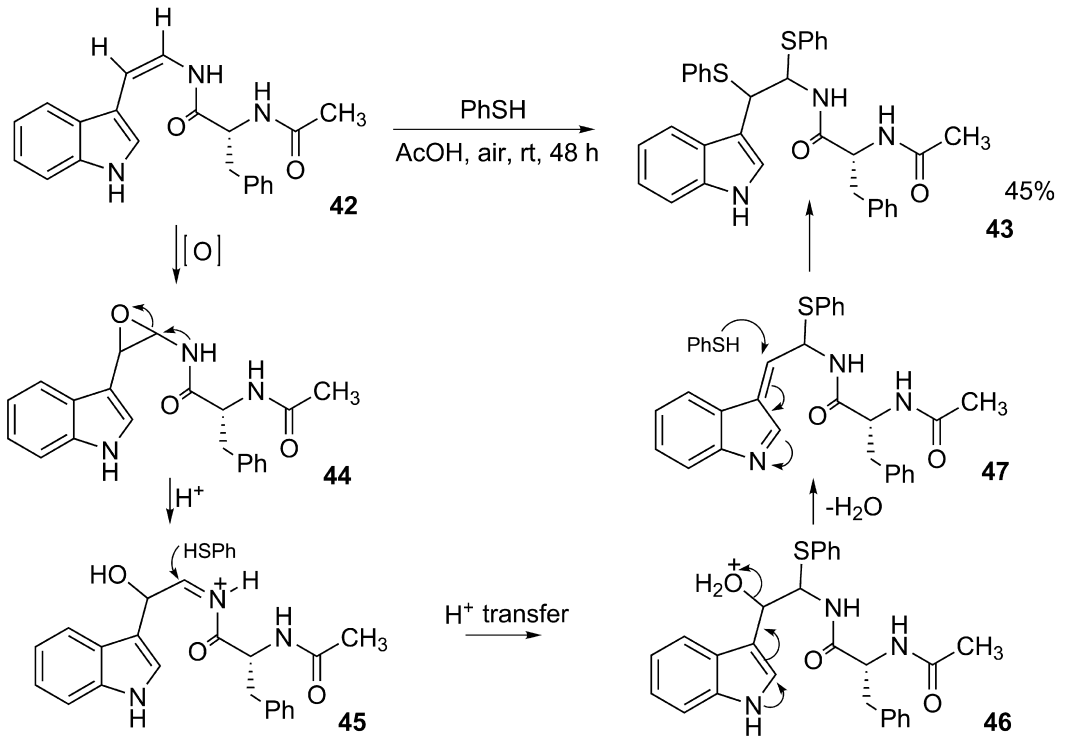
Figure 6. Reverse phase HPLC trace of (12*S*,15*S*)-*E*-**25**, (12*S*,15*R*)-*E*-**30**, (12*S*,15*S*)-*Z*-**2** and (12*S*,15*R*)-*Z*-**29**, reading from left to right. (1:1 MeCN/H_2O, HP. Eclipse XDB-C_8 column (5 μm, 4.6×150 mm) with UV detection (280 nm)).



Scheme 4. Synthesis of aspergillamides A and B. *Reagents and conditions*: (a) (1) 2:1 CH_2Cl_2, TFA; (2) HOBt, EDC, DIEA, DMF,0–25°C, Boc-*L*-NMePhe-OH, 90% for two steps. (b) (1) 2:1 CH_2Cl_2, TFA; (2) HATU, DIEA, DMF, 0–25°C, Boc-*L*-Leu-OH; (3) 2:1 CH_2Cl_2, TFA. (c) Et_3N, Ac_2O, CH_2Cl_2, 93% for three steps. (d) LiOH, 2:1 THF/H_2O, rt. (e) $Pb(OAc)_4$, $Cu(OAc)_2$, pyridine,THF, 0°C–rt. (f) PS-TBD, CH_2Cl_2, 0°C. (g) Mg, MeOH, rt, 0°C–rt, 42% from **38**. (h) KI, AcOH, 72%.

spectra. As in the case for terpeptin, we also experienced difficulty with optical rotation measurements of **3**. For example, synthetic **3** had an $[\alpha]_D=-39.3°$ (c=1.0, distilled MeOH), and natural **3** has a reported $[\alpha]_D=-26.2°$ (c=3.05, MeOH). We were unable to prepare homogeneous solutions of synthetic **3** for optical rotation measurements at the concentration reported for the natural material.[24]

Enamides are stable under neutral or basic conditions, but with Brønsted acids afford rate-determining protonation on carbon leading to hydrolysis of the double bond to form carbonyl compounds and amides.[25] The resulting intermediate after protonation is a reactive *N*-acyliminium ion which may react with a range of nucleophiles including oxygen, sulfur, or π-based nucleophiles.[26] Recent reports have highlighted the possibility that enamide-containing natural products may inhibit their molecular targets using this pathway.[27] Accordingly, we examined the chemical reactivity of model *Z*-indole enamide **42** to acidic conditions. Incubation of **42** with 2 M HCl in MeOH led to rapid decomposition, and with thiophenol (4 equiv.) and pH=1 buffer (25°C) led to no reaction. We were intrigued by a literature report suggesting that the *Z*-enamide containing cyclopeptide alkaloid frangufoline (**6**, Fig. 1) undergoes enamide epoxidation in the presence of air to produce a reactive epoxide which is further hydrolyzed to acyclic products.[28] Interestingly, treatment of model **42** with thiophenol or thiophenol derivatives (e.g.



Scheme 5. Possible reaction pathway for bis-thioether adduct **43**.

Table 3. Effect of terpeptin, its stereoisomers, and aspergillamide A on the cell growth in tsFT210 cells

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)[a]
(12*S*,15*S*)-*Z*-**2**[b]	20	(12*S*,15*R*)-*Z*-**29**	61
(12*S*,15*S*)-*E*-**25**	>100	(12*S*,15*R*)-*E*-**30**	>100
(12*S*,15*S*)-Ts-*Z*-**24**	100	(12*R*,15*R*)-*Z*-**31**	71
(12*S*,15*S*)-Ts-*E*-**24**	>100	(12*R*,15*S*)-*E*-**32**	>100
Z-**3**[c]	60	(12*R*,15*R*)-*Z*-**33**	100
		(12*R*,15*R*)-*E*-**30**	>100

Exponentially growing tsFT210 cells were treated with test compounds at 32°C for 48 h. Cell viability was measured using the color reagent, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt, WST-8™.

[a] Concentration that causes 50% growth inhibition.

[b] Terpeptin.

[c] Aspergillamide A.

4-bromothiophenol) under aerobic conditions using acetic acid as solvent led to the production of bis-thioether adduct **43** in 45% yield (5:1 mixture of diastereomers, stereochemistry unassigned, Scheme 5). This transformation only occurred in the presence of oxygen and did not proceed efficiently under anaerobic conditions. The reaction of the corresponding *E* isomer of **42** with thiophenol under these reaction conditions gave similar results. A possible reaction pathway for the formation of **43** is shown in Scheme 5. Oxidation of **42** affords epoxide **44**, which is ring-opened to iminium ion **45**. Thiol addition to **45**[27] followed by protonation of the alcohol, leads to intermediate **46**. Dehydration of **46**[29] affords an intermediate **47** which suffers addition of a second equivalent of thiol to afford **43**.

In order to rule out the possible involvement of diphenyl disulfide (PhSSPh) in the reaction process, a control experiment was carried out. Treatment of **42** with diphenyl disulfide led to recovered starting material which indicates that thiol oxidation is not required for the formation of **43**. These results underscore the potential for activation of enamide moieties by oxidation and subsequent covalent attachment to their biological targets.

The effects of compounds, terpeptin (*Z*-**2**), terpeptin stereoisomers (*E*-**25**, *Z*-**29**, *E*-**30**, *Z*-**31**, *E*-**32**, *Z*-**33**, and *E*-**34**), *Nin*-Ts terpeptin analogs (*E*-**24** and *Z*-**24**) and aspergillamide A (*Z*-**3**) on cell growth and cell cycle control in tsFT210 cells were examined. tsFT210 cells, temperature-sensitive *cdc2* mutant cells that were isolated from the mouse mammary carcinoma cell line FM3A, exponentially grow at the permissive temperature (32°C), but the cells arrest at G2-M boundary at the non-permissive temperature (39°C) due to inactivation of $p34^{cdc2}$. After a 4 h-incubation at 32°C, a significant fraction of the cells have progressed through mitosis into G1 phase.[30]

As shown in Table 3, terpeptin (*Z*-**2**) and aspergillamide A (*Z*-**3**) inhibited cell growth at IC_{50} values of 20 and 60 μM, respectively. In addition, *Z*-enamide, *Z*-**29**, *Z*-**31**, and *Z*-**33** exhibited moderate inhibitory activity with IC_{50} values of 61, 71, and 100 μM, respectively. However, the IC_{50} values of *E*-**25**, an *E*-indole enamide stereoisomer of terpeptin, as well as all the *E*- stereoisomers, *E*-**30**, *E*-**32**, and *E*-**34**, were >100 μM irrespective of the amino acid stereochemistry. Moreover, tosylation of the indole nitrogen substantially

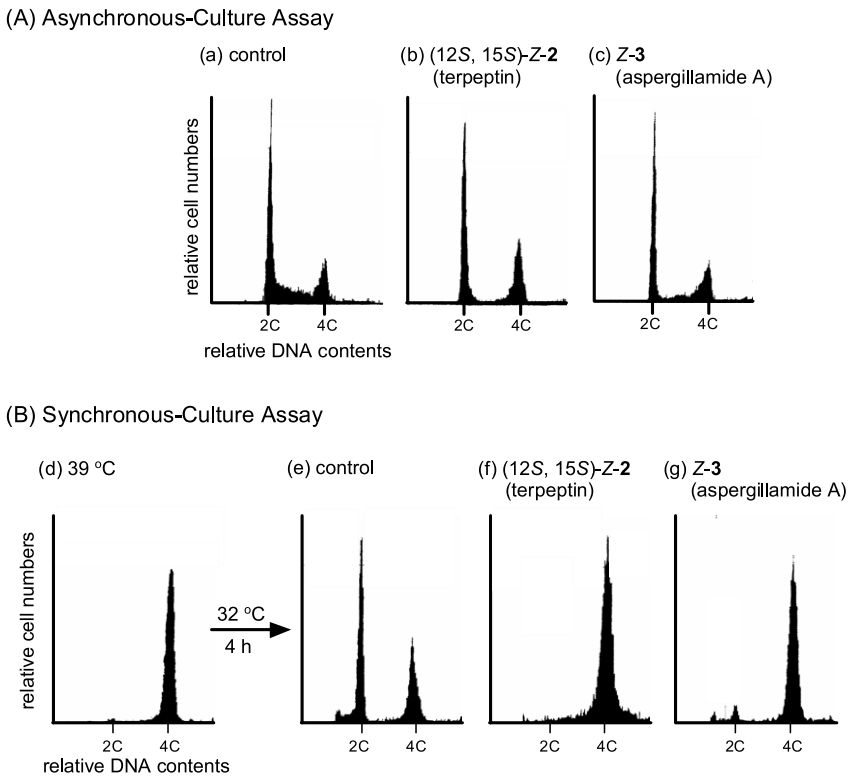


Figure 7. Cell cycle assay. (A) Exponentially growing tsFT210 cells were treated with test compounds (none (a), 30 μM of (12*S*,15*S*)-*Z*-**2** (b), and 30 μM of aspergillamide A (c)) for 20 h, and the distribution of DNA contents was determined by flow cytometry. (B) tsFT210 cells were synchronized in G2 phase by incubation at 39°C for 17 h (d). After release from G2 arrest by shifting cells to 32°C, the cells were incubated for 4 h in the absence (e) or presence of 30 μM of (12*S*,15*S*)-*Z*-**2** (f) and 30 μM of aspergillamide A (g). The distribution of DNA contents was determined by flow cytometry.

decreased cell growth inhibition. These results suggest that the *Z*-enamide configuration and the amino acid stereochemistry in terpeptin play a significant role in cell growth inhibition. Terpeptin and aspergillamide A arrested the cell cycle at the G1 and G2/M phase as cells treated with these compounds had both 2C and 4C DNA contents in the asynchronous-culture assay (Fig. 7(A)). Moreover, in the synchronous-culture assay (Fig. 7(B)), terpeptin *Z*-**2** and aspergillamide A *Z*-**3** were found to accumulate cells in G2 phase judging from the cell size and the cell staining (data not shown). Our studies establish for the first time that aspergillamide A also inhibits G2/M progression and illustrate the importance of the *Z*-indole enamide for cell cycle inhibition. Further studies aimed at elucidating the molecular mechanism by which terpeptin and aspergillamide A arrest the cell cycle are currently under investigation.

3. Conclusion

In summary, we have achieved the total synthesis of the indolic enamides terpeptin and aspergillamides A and B. Unambiguous stereochemical assignment of terpeptin was accomplished by preparation of all possible stereochemical analogues. Cell cycle inhibition studies show that terpeptin with the natural amino acid configuration is the most potent cell cycle inhibitor. Further studies on enamide and related natural products are in progress and will be reported in due course.

4. Experimental

4.1. General experimental procedures

^{1}H NMR spectra were recorded on a 400 MHz spectrometer at ambient temperature with $CDCl_3$ as the solvent unless otherwise stated. ^{13}C NMR spectra were recorded on a 75.0 MHz spectrometer (unless otherwise stated) at ambient temperature. Chemical shifts are reported in parts per million relative to chloroform (^{1}H, δ 7.24; ^{13}C, δ 77.23). Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (app=apparent, par obsc=partially obscure, ovrlp=overlapping, s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet) and coupling constants. All ^{13}C NMR spectra were recorded with complete proton decoupling. Infrared spectra were recorded on a Nicolet Nexus 670 FT-IR spectrophotometer. Low and high-resolution mass spectra were obtained in the Boston University Mass Spectrometry Laboratory using a Finnegan MAT-90 spectrometer. Optical rotations were recorded on an AUTOPOL III digital polarimeter at 589 nm, and are recorded as $[\alpha]_D$ (concentration in grams/100 mL solvent). HPLC analysis was performed on an Agilent 1100 series (HP. Eclipse XDB-C_8, 5 μm, 4.6×150 mm). Analytical thin layer chromatography was performed on 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 200–400 mesh silica gel (Scientific Absorbents, Inc.). Preparative HPLC was performed using a Waters 600 preparative HPLC (Waters Symmetry C_{18} column, 5 μm, 19×50 mm). Parallel synthesis of compound **22** and stereoisomers were performed using a Quest 210 synthesizer (Argonaut Technologies, Foster City, CA.). Sonication was performed using an AQUASONIC 50T ultrasonic cleaner. Yields refer to chromatographically and spectroscopically pure materials, unless otherwise stated. PS-TBD was obtained from Argonaut Technologies (Foster City, CA). PS–Diethylenetriamine resin was prepared according to the literature procedure.[20] All other reagents were used as supplied by Sigma-Aldrich, Novabiochem, Bachem and Lancaster Synthesis. Methylene chloride, toluene, hexane, chloroform and benzene and 1,2-dichloroethane were distilled from calcium hydride; tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone ketyl prior to use. All reactions were carried out in oven-dried glassware under an argon atmosphere unless otherwise noted.

4.1.1. Prenyl tryptophan (8). A mixture of 2-prenyl-Boc-*L*-Trp-OMe (1.563 g, 4.05 mmol), NaOH (486 mg, 12.15 mmol), NBu_4HSO_4 (1.375 g, 4.05 mmol) and 30 mL freshly distilled CH_2Cl_2 were refluxed for 10 min, then TsCl (2.32 g, 12.15 mmol) was added. The reaction mixture was refluxed for 1 h, and then diluted with EtOAc. The organic layer was washed with sat. $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification on silica gel (3% EtOAc in CH_2Cl_2) provided 1.37 g (2.55 mmol, 63%) of **8** as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, 1H, J=7.6 Hz), 7.54 (d, 2H, J=7.6 Hz), 7.37 (d, 1H, J=7.2 Hz), 7.24–7.14 (m, 4H), 5.17 (m, 1H), 4.91 (d, 1H, J=3.2 Hz), 4.50 (m, 1H), 3.74–3.62 (m, 2H), 3.48 (s, 3H), 3.12–3.00 (m, 2H), 2.31 (s, 3H), 1.74 (s, 3H), 1.36 (s, 9H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 172.5, 155.1, 144.7, 138.9, 136.9, 136.2, 133.0, 131.7, 130.61, 130.55, 130.2, 129.8, 126.5, 125.8, 124.5, 123.8, 121.9, 118.7, 116.0, 115.5, 80.2, 53.5, 52.4, 28.5, 27.7, 25.9, 25.6, 21.8, 18.3; IR (thin film) ν_{max} 3392, 2976, 2927, 1744, 1715, 1597, 1498, 1453, 1366, 1259, 1172, 1121, 1093, 1064, 1021 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{29}H_{37}N_2O_6S$: 541.2374, found: 541.2351; $[\alpha]_D^{23}$=+17.7° (c=1.1, CH_2Cl_2).

4.1.2. Dipeptide (10). Prenyl tryptophan **8** (950 mg, 1.76 mmol) was dissolved in 30 mL CH_3CN and 6 mL 48% HF was added at rt. After stirring for 8 h, solid $NaHCO_3$ was added until no further gas evolution was observed. The reaction mixture was extracted with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification on silica gel (100% EtOAc) afforded 723 mg (1.55 mmol, 88%) of the free amine. To the free amine (150 mg, 0.341 mmol) was added HOBt (1-hydroxybenzotriazole, 50.8 mg, 0.375 mmol), EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 71.7 mg, 0.375 mmol), Boc-*L*-Val-OH (88.9 mg, 0.409 mmol) followed by 1 mL DMF. The mixture was cooled to 0°C and DIEA (*N*,*N*-disopropylethylamine, 218 μL, 1.228 mmol) was added. The reaction was stirred for 6 h (0°C to rt), then diluted with EtOAc. The organic layer was washed with sat. $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification on silica gel (30% EtOAc in hexane) provided 200 mg (0.31 mmol, 92%) of **10** as a pale yellow solid. Mp 58.0–60.5°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, 1H, J=7.6 Hz), 7.54 (d, 2H, J=7.6 Hz), 7.37 (d, 1H, J=7.2 Hz), 7.23–7.13 (m, 4H), 6.44 (d, 1H, J=7.2 Hz), 5.11 (m, 1H), 5.01 (d, 1H, J=8.4 Hz), 4.76 (dt, 1H, J=6.8, 14 Hz), 3.84

(m, 1H), 3.73–3.62 (m, 2H), 3.12–3.00 (m, 2H), 2.28 (s, 3H), 2.05–1.95 (m, 1H), 1.71 (s, 3H), 1.62 (s, 3H), 1.40 (s, 9H), 0.84–0.72 (m, 6H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 171.9, 171.3, 155.7, 144.6, 138.7, 136.7, 136.2, 132.8, 130.4, 129.8, 126.4, 124.5, 123.8, 121.7, 118.5, 115.6, 115.3, 79.9, 60.5, 59.8, 52.4, 52.3, 31.1, 28.4, 27.5, 25.8, 25.5, 21.6, 19.2, 18.2, 17.6; IR (thin film) ν_{max} 3320, 2967, 2930, 1745, 1684, 1655, 1521, 1453, 1366, 1174, 1121 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{34}H_{45}N_3O_7S$: 639.2978, found: 639.2968; $[\alpha]_D^{23}=+4.6°$ (c=0.5, CH_2Cl_2).

4.1.3. *Z*-Enamide (12). Dipeptide **10** (200 mg, 0.31 mmol) was dissolved in 2 mL THF and $LiOH{\cdot}H_2O$ (26.3 mg, 0.62 mmol) in 1 mL H_2O was added. After stirring for 1 h at rt, 2 mL 1N HCl was added and the reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was treated with $Cu(OAc)_2$ (17.1 mg, 0.0934 mmol) and pyridine (50.3 μL, 0.344 mmol) in 2 mL distilled THF at 0°C. $Pb(OAc)_4$ (152.7 mg, 0.344 mmol) was added under N_2. The resulting solution was warmed to rt and stirred for a further 2 h, then diluted with 10 mL 3% Et_3N/EtOAc. The mixture was eluted through a polypropylene cartridge containing 6 mL silica gel, washed with 20 mL 3% Et_3N/EtOAc, and concentrated in vacuo. The crude *N*,*O*-acetal was used directly without further purification in the next step. The crude product was dissolved in 7.5 mL distilled CH_2Cl_2, then treated with 230 mg (2.6 mmol/g, 0.60 mmol) PS-TBD resin. The mixture was stirred at 0°C for 1 h, then filtered and concentrated in vacuo. Purification on silica gel (30% EtOAc, 3% Et_3N/hexanes) provided **12** (48.3 mg, 0.083 mmol) and **13** (30.2 mg, 0.052 mmol) as pale yellow solids (43% three steps).

Compound **12**. Mp 46.5–49.0°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, 1H, J=8.8 Hz), 7.67 (d, 2H, J=8.4 Hz), 7.36–7.18 (m, 5H), 7.10 (dd, 1H, J=9.2, 11.2 Hz), 5.65 (d, 1H, J=7.2 Hz), 5.17–5.14 (m, 1H), 4.80 (d, 1H, J=8 Hz), 3.75 (m, 1H), 3.68 (d, 2H, J=6 Hz), 2.32 (s, 3H), 2.07–2.03 (m, 1H), 1.67 (s, 3H), 1.63 (s, 3H), 1.30 (s, 9H), 0.88 (d, 3H, J=6.8 Hz), 0.81 (d, 3H, J=6.8 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 169.2, 155.7, 145.0, 138.5, 136.8, 136.3, 133.5, 130.0, 128.6, 126.7, 124.8, 124.1, 123.9, 121.2, 119.4, 115.5, 115.0, 101.4, 80.3, 60.0, 30.9, 29.9, 28.3, 26.5, 25.8, 21.8, 19.4, 18.3, 17.6; IR (thin film) ν_{max} 3408, 3323, 2970, 2929, 1697, 1658, 1491, 1453, 1369, 1261, 1173, 1092 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{32}H_{41}N_3O_5S$: 580.2847, found: 580.2885; $[\alpha]_D^{23}=+5.5°$ (c=1.0, CH_2Cl_2).

4.1.4. *E*-Enamide (13). Mp 78.5–81.5°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, 1H, J=8 Hz), 7.88 (d, 1H, J=10.8 Hz), 7.63 (d, 1H, J=7.2 Hz), 7.57 (d, 2H, J=7.2 Hz), 7.41 (dd, 1H, J=11.2, 15.8 Hz), 7.28–7.20 (m, 1H), 7.14 (d, 2H, J=7.6 Hz), 6.13 (d, 1H, J=14.8 Hz), 5.10 (m, 1H), 4.99 (m, 1H), 3.95 (t, 1H, J=8 Hz), 3.77 (d, 2H, J=5.6 Hz), 2.30 (s, 3H), 2.23 (m, 1H), 1.74 (s, 3H), 1.66 (s, 3H), 1.43 (s, 9H), 0.99 (d, 3H, J=6.8 Hz), 0.95 (d, 3H, J=6.8 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 171.4, 169.5, 156.4, 144.7, 137.4, 136.9, 136.2, 133.0, 129.8, 128.7, 126.6, 124.5, 123.9, 121.8, 119.7, 117.1, 104.6, 80.5, 60.6, 60.4, 30.9, 29.9, 28.5, 25.8, 21.7, 21.2, 19.5, 18.4, 18.2; IR (thin film) ν_{max} 3296, 2969, 2929, 1675, 1652, 1517, 1452, 1367, 1173, 1124, 1090 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{32}H_{41}N_3O_5S$: 580.2847, found: 580.2853; $[\alpha]_D^{23}=-15.8°$ (c=1.0, CH_2Cl_2).

4.1.5. (9*S*,12*S*)-Dipeptide (21). A mixture of **9** (530 mg, 1.20 mmol), HOBt (179 mg, 1.325 mmol), EDC (253.3 mg, 1.325 mmol), Boc-NMe-*L*-Val-OH (308 mg, 1.33 mmol) and 5 mL CH_2Cl_2 was cooled to 0°C and DIEA (766 μL, 4.32 mmol) was added. The reaction mixture was stirred for 6 h (0°C to rt), then diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to afford 780 mg (1.20 mmol, 100%) of **21** as pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, 1H, J=8.4 Hz), 7.57 (d, 2H, J=8.4 Hz), 7.43 (d, 1H, J=6.8 Hz), 7.24–7.14 (m, 4H), 6.68 (m, 1H), 5.15 (m, 1H), 4.66 (m, 1H), 4.00 (d, 1H, J=8.8 Hz), 3.78 (dd, 1H, J=6.0, 15.6 Hz), 3.68 (dd, 1H, J=7.2, 16.4 Hz), 3.34 (m, 1H), 3.16–2.94 (m, 2H), 2.64 (s, 3H), 2.30 (s, 3H), 2.23–2.17 (m, 1H), 1.72 (s, 3H), 1.64 (s, 3H), 1.45 (s, 9H), 0.86–0.80 (m, 6H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 172.0, 170.6, 144.7, 138.7, 136.7, 136.4, 132.8, 130.4, 129.8, 126.6, 124.5, 123.9, 121.7, 118.6, 115.8, 115.4, 80.6, 64.9, 52.5, 30.4, 30.0, 28.6, 27.6, 26.4, 25.9, 25.6, 21.7, 19.8, 18.7, 18.3; IR (thin film) ν_{max} 3349, 2966, 2928, 1745, 1683, 1453, 1367, 1175, 1153 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{35}H_{47}N_3O_7S$: 654.3215, found: 654.3292; $[\alpha]_D^{23}=-47.2°$ (c=0.77, CH_2Cl_2). The (9*S*,12*R*) stereoisomer of **21** was prepared following a similar procedure.

4.1.6. (9*S*,12*S*,15*S*)-Tripeptide (22). (9*S*,12*S*)-Dipeptide **21** (780 mg, 1.20 mmol) was dissolved in 20 mL CH_3CN and 4 mL 48% HF was added at rt. After stirring for 8 h, solid $NaHCO_3$ was added until no further gas evolution was observed. The reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude free amine, HATU (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide, 465 mg, 1.223 mmol), Boc-*L*-Val-OH (268 mg, 1.223 mmol) and 5 mL CH_2Cl_2 were dissolved in a 5 mL reaction vessel on the Quest 210 synthesizer and DIEA (778 μL, 4.39 mmol) was added. The reaction was agitated for 24 h, then 500 mg (3.0 mmol/g, 1.5 mmol) of PS–diethylenetriamine resin was added and the reaction mixture was agitated for an additional 24 h. The reaction vessel was drained, washed with CH_2Cl_2 (2×3 mL), and the solvent collected. Filtration of the eluent through a SiO_2 column (4 g) with 20 mL 1:1 EtOAc/hexane, and concentration in vacuo provided 763 mg (1.02 mmol, 85% for two steps) of **22** as a pale yellow solid. Mp 84.5–86.0°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, 1H, J=7.6 Hz), 7.58 (d, 2H, J=8.4 Hz), 7.44 (d, 1H, J=6.4 Hz), 7.26–7.12 (m, 4H), 6.56 (d, 1H, J=6.8 Hz), 5.16–5.14 (m, 2H), 4.58–4.52 (m, 2H), 3.77 (dd, 1H, J=5.6, 15.6 Hz), 3.67 (dd, 1H, J=6.0, 16.0 Hz), 3.33 (s, 3H), 3.07–2.87 (m, 5H), 2.30 (s, 3H), 2.26–2.22 (m, 1H), 1.86–1.78 (m, 1H), 1.72 (s, 3H), 1.66 (s, 3H), 1.39 (s, 9H), 1.06–0.76 (m, 12H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 174.0, 171.9, 169.9, 156.1, 144.7, 138.5, 136.7, 136.5, 133.0, 130.0, 129.9, 124.6, 123.8, 121.7, 118.5, 115.3, 79.8, 62.5, 55.59, 55.55, 52.6, 52.3, 31.3, 30.9, 29.9, 31.4, 30.9, 28.5, 27.6, 26.0, 25.9, 25.4,

21.719.6, 19.4, 18.5, 18.3, 17.9; IR (thin film) ν_{max} 3309, 2966, 2931, 2874, 1741, 1710, 1629, 1507, 1454, 1367, 1175 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{40}H_{56}N_4O_8S$: 753.3899, found: 753.3906; $[\alpha]_D^{23}=-74.9°$ (c=1.0, CH_2Cl_2). The (9*S*,12*S*,15*R*), (9*S*,12*R*,15*R*), (9*S*,12*R*,15*S*) stereoisomers of **22** were prepared following a similar procedure.

4.1.7. (9*S*,12*S*,15*S*)-Tripeptide (23). (9*S*,12*S*,15*S*)-Tripeptide **22** (690 mg, 0.92 mmol) was dissolved in 5 mL CH_2Cl_2 at rt, and 376 μL (3.22 mmol) 2,6-lutidine and 416 μL (2.30 mmol) TMSOTf were added consecutively. After stirring for 1 h at rt, 4 mL sat. NH_4Cl was added and the reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was dissolved in 10 mL MeOH and stirred for 2 h and concentrated in vacuo. The resulting product was dissolved in 10 mL CH_2Cl_2 and Et_3N (629 μL, 4.60 mmol) was added. After stirring for 10 min, Ac_2O (257 μL, 2.76 mmol) was added. The mixture was stirred for 2 h and then concentrated in vacuo. Purification on silica gel (50% EtOAc in CH_2Cl_2) provided 570 mg (0.82 mmol, 89%) of **23** as a pale yellow solid. Mp 70.0–73.0°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, 1H, J=7.2 Hz), 7.60 (d, 2H, J=8.8 Hz), 7.43–7.42 (m, 1H), 7.32–7.13 (m, 4H), 6.62 (d, 1H, J=6 Hz), 6.22 (d, 1H, J=8.8 Hz), 5.17 (m, 1H), 4.73 (dd, 1H, J=6.8, 8.8 Hz), 4.57–4.46 (m, 2H), 3.78–3.67 (m, 2H), 3.38 (s, 3H), 3.10–2.91 (m, 5H), 2.31 (s, 3H), 2.28–2.20 (m, 1H), 1.99 (s, 3H), 1.80–1.77 (m, 1H), 1.72 (s, 3H), 1.66 (s, 3H), 0.97–0.69 (m, 12H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 173.5, 172.2, 172.15, 172.08, 171.8, 171.1, 170.1, 170.0, 144.8, 138.6, 136.7, 136.6, 133.2, 132.9, 129.9, 129.8, 126.6, 124.6, 124.4, 123.8, 121.8, 118.6, 118.5, 115.2, 115.1, 103.1, 66.0, 63.0, 52.7, 52.4, 31.7, 31.5, 31.2, 29.9, 27.4, 25.98, 25.90, 25.8, 25.6, 25.5, 23.5, 21.7, 20.4, 19.7, 19.6, 19.3, 18.7, 18.5, 18.46, 18.36, 17.7; IR (thin film) ν_{max} 3300, 2964, 2929, 1740, 1679, 1628, 1531, 1453, 1368, 1176 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{37}H_{50}N_4O_7S$: 695.3480, found: 695.3528; $[\alpha]_D^{23}=-74.3°$ (c=0.66, CH_2Cl_2). The (9*S*,12*S*,15*R*), (9*S*,12*R*,15*R*), (9*S*,12*R*,15*S*) stereoisomers of **23** were prepared following a similar procedure.

4.1.8. *Nin*-Ts (12*S*,15*S*)-terpeptin (24-*Z*). (9*S*,12*S*,15*S*)-Tripeptide **23** (570 mg, 0.82 mmol) was dissolved in 6 mL THF and $LiOH{\cdot}H_2O$ (69 mg, 1.6 mmol) in 3 mL H_2O was added. After stirring for 1 h, 4 mL 1N HCl was added and the reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was treated with $Cu(OAc)_2$ (44.7 mg, 0.246 mmol) and pyridine (131 μL, 1.64 mmol) in 6 mL distilled THF at 0°C and $Pb(OAc)_4$ (436 mg, 0.984 mmol) was added under N_2. The resulting solution was warmed to rt and stirred for 2 h, then diluted with 3% Et_3N/EtOAc (10 mL). The mixture was eluted through a 6 mL polypropylene cartridge containing SiO_2 (4 g), washed with 20 mL 3% Et_3N/EtOAc and concentrated in vacuo. The crude *N*,*O*-acetal was used directly without further purification in the next step. The crude product was dissolved in 15 mL of distilled CH_2Cl_2, then treated with 628 mg PS-TBD resin (2.6 mmol/g, 1.64 mmol). The mixture was stirred at 0°C for 1 h, then filtered and concentrated in vacuo. Purification using a Waters Preparative HPLC (Waters Symmetry C_{18}, 5 μm, 19×50 mm, 60% MeCN in water) provided 83 mg (0.131 mmol) of **24**-*Z* and 174 mg (0.274 mmol) **24**-*E* as pale yellow solids (49%, three steps).

Compound **24**-*Z*. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 1H, J=8.4 Hz), 7.72 (d, 2H, J=8.4 Hz), 7.65 (d, 1H, J=11.2 Hz), 7.29–7.15 (m, 5H), 7.08 (dd, 1H, J=9.2, 11.2 Hz), 6.05 (d, 1H, J=8.8 Hz), 5.63 (d, 1H, J=9.2 Hz), 5.22 (m, 1H), 4.67 (dd, 1H, J=7.2, 9.2 Hz), 4.37 (d, 1H, J=11.2 Hz), 3.74 (dd, 1H, J=7.2, 16.4 Hz), 3.65 (dd, 1H, J=6.4, 15.6 Hz), 2.98 (s, 3H), 2.33 (s, 3H), 2.30–2.22 (m, 5H), 1.94 (s, 3H), 1.64 (s, 3H), 0.92 (d, 1H, J=6.4 Hz), 0.76 (d, 1H, J=6.4 Hz), 0.65 (d, 1H, J=6.4 Hz), 0.60 (d, 1H, J=6.8 Hz); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 173.5, 170.0, 167.2, 144.8, 138.2, 137.0, 136.3, 133.5, 129.9, 129.0, 126.8, 124.6, 124.2, 123.6, 121.3, 119.3, 115.1, 101.5, 62.9, 53.5, 31.1, 30.8, 26.3, 25.53, 25.45, 23.1, 21.5, 19.5, 19.0, 18.3, 18.0, 17.1; IR (thin film) ν_{max} 3287, 2963, 2926, 1696, 1637, 1487, 1452, 1370, 1265, 1172, 1091 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{35}H_{46}N_4O_5S$: 634.3189, found: 634.3154; $[\alpha]_D^{23}=-2.0°$ (c=0.10, CH_2Cl_2). The *Nin*-Ts (12*S*,15*R*)-*Z*, *Nin*-Ts (12*R*,15*S*)-*Z*, *Nin*-Ts (12*R*,15*R*)-*Z* stereoisomers of **24**-*Z* were prepared following the same procedure.

4.1.9. *Nin*-Ts (12*S*,15*S*)-terpeptin (24-*E*). ^{1}H NMR (400 MHz, $CDCl_3$) (1:1 mixture of rotamer) δ 9.32 (d, 0.5H, J=6.4 Hz), 8.19 (d, 1H, J=8.4 Hz), 8.01 (d, 1H, J=6.4 Hz), 7.65–7.55 (m, 3H), 7.41–7.31 (m, 1H), 7.26–7.19 (m, 2H), 7.13–7.09 (m, 2H), 6.85 (d, 0.5H, J=7.6 Hz), 6.31 (d, 0.5H, J=14.8 Hz), 6.16 (d, 0.5H, J=8.8 Hz), 6.10 (d, 0.5H, J=14.8 Hz), 5.09 (m, 1H), 4.82–4.76 (m, 1H), 4.60 (d, 0.5H, J=11.2 Hz), 4.19 (d, 0.5H, J=10.4 Hz), 3.79–3.73 (m, 2H), 3.09 (s, 1.5H), 2.92 (s, 1.5H), 2.54–2.43 (m, 0.5H), 2.38–2.32 (m, 0.5H), 2.29 (s, 1.5H), 2.27 (s, 1.5H), 2.22–2.15 (m, 1H),2.01–1.99 (m, 3H), 1.75–1.63 (m, 6H), 1.02–0.81 (m, 12H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 174.5, 172.7, 172.4, 170.5, 167.2, 165.9, 145.1, 145.07, 137.7, 137.3, 137.2, 136.5, 130.12, 130.09, 126.90, 126.87, 124.9, 124.8, 124.4, 124.3, 122.1, 122.0, 120.2, 120.0, 117.6, 117.2, 115.6, 105.4, 105.0, 67.0, 63.4, 54.3, 54.2, 31.8, 31.5, 31.2, 30.0, 29.4, 27.3, 25.92, 25.86, 25.7, 23.5, 21.8, 20.5, 19.9, 19.6, 18.7, 18.53, 18.47, 17.9; IR (thin film) ν_{max} 3296, 2962, 1650, 1451, 1370, 1293, 1174, 1089 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{35}H_{46}N_4O_5S$: 634.3189, found: 634.3230; $[\alpha]_D^{23}=-76.0°$ (c=0.10, CH_2Cl_2). The *Nin*-Ts (12*S*,15*R*)-*E*, *Nin*-Ts (12*R*,15*S*)-*E*, *Nin*-Ts (12*R*,15*R*)-*E* stereoisomers of **24**-*E* were prepared following the same procedure.

4.1.10. *Nin*-Ts (12*S*,15*R*)-*Z*-terpeptin (35). Prepared from the (9*S*,12*S*,15*R*) stereoisomer of **23** following the procedure reported above for **24**-*Z*. White solid. Mp 153.0–154.0°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, 1H, J=8.4 Hz), 7.81 (d, 2H, J=8.0 Hz), 7.76 (d, 1H, J=10.8 Hz), 7.26–7.13 (m, 5H), 7.08 (dd, 1H, J=9.2, 11.2 Hz), 6.07 (d, 1H, J=8.8 Hz), 5.63 (d, 1H, J=9.2 Hz), 5.27 (m, 1H), 4.82 (dd, 1H, J=5.6, 9.2 Hz), 4.21 (d, 1H, J=11.2 Hz), 3.76 (dd, 1H, J=5.6, 15.6 Hz), 3.55 (dd, 1H, J=7.2, 15.6 Hz), 3.03 (s, 3H), 2.32 (s, 3H), 1.89 (s, 3H), 1.60 (d, 6H, J=5.2 Hz), 0.92–0.76 (m, 12H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 173.7, 170.0, 166.9, 144.7, 138.2, 136.7, 136.2, 133.4, 129.8,

129.3, 129.1, 128.3, 126.9, 124.4, 124.3, 123.5, 121.3, 119.3, 115.2, 114.9, 101.5, 63.7, 53.8, 31.5, 31.1, 29.6, 26.3, 2.6, 25.5, 22.9, 21.4, 19.5, 18.6, 17.9, 16.9; IR (thin film) ν_{max} 3407, 3319, 2966, 2929, 2874, 1693, 1640, 1493, 1452, 1493, 1452, 1370, 1712, 1091, 910, 732 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{35}H_{47}N_4O_5S$: 635.3269, found: 635.3286; $[\alpha]_D^{23}=+74.0°$ (c=0.10, CH_2Cl_2).

4.1.11. (12*S*,15*S*)-Terpeptin (2). 100 mg (4.17 mmol) of Mg powder was mixed with 1 mL MeOH. The reaction mixture was sonicated until evolution of H_2 was observed and then cooled to 0°C. **24**-*Z* (20 mg, 0.032 mmol) in 1 mL MeOH was added. After the mixture was stirred for 2 h at 0°C, 2 mL sat. NH_4Cl was added and the reaction mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification on silica gel (3% Et_3N, 50% EtOAc in CH_2Cl_2) provided 14.4 mg (0.030 mmol, 95%) of **2** as a pale yellow solid. ^1H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.83 (d, 1H, J=10.4 Hz), 8.05 (d, 1H, J=8 Hz), 7.30 (d, 1H, J=8 Hz), 7.23 (d, 1H, J=7.6 Hz), 7.03 (t, 1H, J=7.2 Hz), 6.94 (m, 1H), 6.77 (t, 1H, J=9.6 Hz), 5.78(d, 1H, J=9.2 Hz), 5.30 (m, 1H), 4.74 (d, 1H, J=10.8 Hz), 4.41 (t, 1H, J=8.4 Hz), 3.05 (s, 3H), 2.13 (m, 1H), 1.85 (m, 1H), 1.77 (s, 3H), 1.67 (s, 3H), 1.64 (s, 3H), 0.85–0.65 (m, 12H); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ 173.0, 169.3, 168.0, 136.9,135.8, 132.6, 127.2, 121.1, 120.9, 120.6, 118.9, 118.6, 111.0, 105.4, 104.3, 60.6, 55.0, 54.1, 30.5, 30.0, 26.2, 25.7, 25.5, 22.1, 19.0, 18.6, 18.5, 18.4, 17.7; IR (thin film) ν_{max} 3300, 2963, 2927, 1657, 1626, 1546, 1493, 1460 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{41}N_4O_3$: 481.3180, found: 481.3178; $[\alpha]_D^{23}=-39.9°$ (c=0.43, distilled $CHCl_3$); $[\alpha]_D^{23}=-42.9°$ (c=0.77, distilled MeOH); natural $[\alpha]_D^{23}=-135.2°$ (c=0.1, distilled $CHCl_3$).

4.1.12. (12*S*,15*S*)-*E*-Terpeptin (25). Prepared from **24**-*E* following the same procedure reported for **2**. ^1H NMR (400 MHz, $CDCl_3$) (1:1 mixture of rotamers) δ 9.14 (d, 0.5H, J=10 Hz), 7.98–7.95 (m, 1.5H), 7.75–7.71 (m, 1H), 7.38 (dd, 1H, J=10.8, 14.8 Hz), 7.26–7.24 (m, 1H), 7.13–7.06 (m, 2H), 6.71 (d, 0.5H, J=9.6 Hz), 6.50 (d, 0.41H, J=14.8 Hz), 6.31 (d, 0.52H, J=14.8 Hz), 6.19 (d, 0.5H, J=8.8 Hz), 5.31–5.27 (m, 1H), 4.86–4.79 (m, 1H), 4.63 (d, 0.5H, J=7.2 Hz), 4.19 (d, 0.5H, J=10.8 Hz), 3.50–3.46 (m, 2H), 3.12 (s, 1.5H), 2.97 (s, 1.5H), 2.57–2.48 (m, 0.5H), 2.42–2.33 (m, 0.5H), 2.07 (s, 1H), 2.00 (s, 2H), 1.77 (s, 3H), 1.72 (s, 3H), 1.03–0.83 (m, 12H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 174.0, 172.3, 171.9, 170.1, 168.1, 166.4, 165.0, 135.9, 135.3, 126.5, 121.7, 121.6, 120.2, 120.1, 119.9, 119.6, 119.57, 119.5, 119.4, 110.6, 110.5, 107.5, 107.3, 107.0, 66.7, 63.0, 60.4, 53.9, 53.8, 31.5, 31.1, 30.7, 29.6, 29.0, 27.0, 25.7, 25.34, 25.30, 23.2, 23.1, 20.0, 19.5, 19.4, 19.2, 18.3, 17.8, 17.5; IR (thin film) ν_{max} 3291, 2965, 2929, 1652, 1624, 1558, 1458 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{41}N_4O_3$: 481.3180, found: 481.3178; $[\alpha]_D^{23}=-97.2°$ (c=1.0, distilled $CHCl_3$); $[\alpha]_D^{23}=-140°$ (c=0.9, distilled MeOH).

4.1.13. (12*S*,15*R*)-*Z*-Terpeptin (29). Prepared from the (12*S*,15*R*) stereoisomer of **24**-*Z* following the procedures reported for **2**. ^1H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 1H), 8.63 (d, 1H, J=10.4 Hz), 7.92 (d, 1H, J=7.2 Hz), 7.30 (d, 1H, J=7.6 Hz), 7.24 (d, 1H, J=7.6 Hz), 7.04–6.91 (m, 2H), 6.76 (t, 1H, J=10.4 Hz), 5.79 (d, 1H, J=9.2 Hz), 5.31 (m, 1H), 4.53–4.47 (m, 2H), 3.07 (s, 3H), 2.18 (m, 1H), 1.85 (m, 1H), 1.74 (s, 3H), 1.66 (s, 3H), 1.63 (s, 3H), 0.90–0.54 (m, 12H); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ 173.2, 169.9, 168.0, 137.3, 136.0, 133.0, 127.3, 121.3, 121.0, 119.3, 118.7, 111.2, 105.5, 104.5, 62.2, 55.1, 52.9, 31.1, 30.1, 26.4, 25.9, 22.2, 19.4, 19.3, 19.0, 18.0, 17.8; IR (thin film) ν_{max} 3312, 2966, 2929, 2874, 1739, 1653, 1542, 1496, 1373, 1244 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{41}N_4O_3$: 481.3180, found: 481.3207; $[\alpha]_D^{23}=+45.6°$ (c=0.5, $CHCl_3$).

4.1.14. (12*S*,15*R*)-*E*-Terpeptin (30). Prepared from the (12*S*,15*R*) stereoisomer of **24**-*E* following the procedures reported for **2**. ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, 1H, J=10.4 Hz), 8.03 (s, 1H), 7.75–7.73 (m, 1H), 7.38 (d, 1H, J=6.4 Hz), 7.27–7.23 (m, 1H), 7.12–7.06 (m, 2H), 6.39 (d, 1H, J=14.8 Hz), 6.29 (d, 1H, J=8 Hz), 5.31 (m, 1H), 4.82 (dd, 1H, J=6.8 Hz), 4.60 (d, 1H, J=10.8 Hz), 3.48 (d, 2H, J=7.2 Hz), 3.10 (s, 3H), 2.44–2.35 (m, 1H), 2.00 (s, 3H), 1.76 (s, 3H), 1.71 (s, 3H), 1.04–0.85 (m, 12H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 174.1, 170.4, 166.2, 135.8, 135.6, 135.4, 128.0, 127.9, 127.3, 126.5, 121.5, 120.1, 119.60 119.56, 110.5, 107.6, 107.0, 63.3, 54.4, 31.3, 31.1, 29.6, 25.9, 25.6, 25.3, 23.1, 19.9, 19.5, 18.7, 17.8, 17.3; IR (thin film) ν_{max} 3293, 2966, 2930, 2874, 1652, 1553, 1460, 1373, 1314, 1221, 1094 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{41}N_4O_3$: 481.3180, found: 481.3144; $[\alpha]_D^{23}=+87.6°$ (c=1.0, $CHCl_3$).

4.1.15. (12*R*,15*S*)-*Z*-Terpeptin (31). Prepared from the (12*R*,15*S*) stereoisomer of **24**-*Z* following the procedures reported for **2**. ^1H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.63 (d, 1H, J=10.4 Hz), 7.92 (d, 1H, J=7.2 Hz), 7.30 (d, 1H, J=7.6 Hz), 7.24 (d, 1H, J=7.6 Hz), 7.04–6.91 (m, 2H), 6.76 (t, 1H, J=10.4 Hz), 5.79 (d, 1H, J=9.2 Hz), 5.30 (m, 1H), 4.53–4.47 (m, 2H), 3.07 (s, 3H), 2.18 (m, 1H), 2.02–2.15 (m, 1H), 1.87–1.82 (m, 1H), 1.74 (s, 3H), 1.66 (s, 3H), 1.63 (s, 3H), 0.90–0.54 (m, 12H); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ 173.0, 169.4, 168.0, 137.1, 135.9, 132.7, 127.2, 122.1, 121.3, 120.74, 120.70, 119.0, 118.6, 111.0, 105.3, 104.1, 63.4, 61.9, 54.3, 52.9, 31.0, 30.0, 26.3, 25.7, 25.5, 22.3, 22.1, 19.23, 19.16, 18.9, 18.7, 17.9, 17.7; IR (thin film) ν_{max} 3310, 2966, 2929, 1653, 1635, 1541, 1496, 1458, 1093 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{41}N_4O_3$: 481.3180, found: 481.3172; $[\alpha]_D^{23}=-42.6°$ (c=0.52, $CHCl_3$).

4.1.16. (12*R*,15*S*)-*E*-Terpeptin (32). Prepared from the (12*R*,15*S*) stereoisomer of **24**-*E* following the procedures reported for **2**. ^1H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 1H, J=10.0 Hz), 8.00 (s, 1H), 7.75–7.73 (m, 1H), 7.38 (dd, 1H, J=10.8, 14.8 Hz), 7.26–7.23 (m, 1H), 7.13–7.07 (m, 2H), 6.39 (d, 1H, J=14.8 Hz), 6.26 (d, 1H, J=8.4 Hz), 5.31–5.27 (m, 1H), 4.82 (dd, 1H, J=6, 8.4 Hz), 4.59 (d, 1H, J=10.8 Hz), 3.49 (d, 2H, J=7.2 Hz), 3.10 (s, 3H), 2.44–2.35 (m, 1H), 2.00 (s, 3H), 1.77 (s, 3H), 1.71 (s, 3H), 1.04–0.85 (m, 12H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 174.1, 170.3, 166.2, 135.8, 135.6, 135.4, 126.6, 121.6, 120.1, 119.65 119.56, 110.5, 107.6, 107.0, 63.4, 54.4, 31.3, 31.1, 29.6, 25.9, 25.6, 25.3, 23.2, 19.9, 19.5, 18.8, 17.8, 17.3; IR (thin film) ν_{max} 3303, 2967, 2930, 2875, 1634, 1552, 1461, 1373, 1313, 1217, 1093 cm^{-1}; CIHRMS $[M+H]^+$ calculated for

$C_{28}H_{41}N_4O_3$: 481.3180, found: 481.3146; $[\alpha]_D^{23}=-86.8°$ (c=1.0, $CHCl_3$).

4.1.17. (12*R*,15*R*)-*Z*-Terpeptin (33). Prepared from the (12*R*,15*R*) stereoisomer of **24**-*Z* following the procedures reported for **2**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 8.82 (d, 1H, J=10.4 Hz), 8.05 (d, 1H, J=8 Hz), 7.30 (d, 1H, J=8 Hz), 7.23 (d, 1H, J=7.6 Hz), 7.03 (t, 1H, J=7.2 Hz), 6.94 (t, 1H, J=7.2 Hz), 6.77 (t, 1H, J=9.6 Hz), 5.79 (d, 1H, J=9.2 Hz), 5.30 (m, 1H), 4.73 (d, 1H, J=10.8 Hz), 4.40 (t, 1H, J=8.4 Hz), 3.05 (s, 3H), 2.14–2.07 (m, 1H), 1.77 (s, 3H), 1.67 (s, 3H), 1.64 (s, 3H), 0.85–0.65 (m, 12H); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ 173.0, 171.8, 169.3, 168.0, 137.0, 136.6, 135.9, 132.6, 127.2, 121.1, 120.9, 120.6, 118.9, 118.6, 111.0, 105.4, 104.3, 64.3, 60.6, 55.0, 54.2, 30.5, 30.0, 26.2, 25.7, 25.5, 22.1, 19.0, 18.6, 18.5, 18.4, 17.7; IR (thin film) ν_{max} 3301, 2965, 2930, 1658, 1626, 1546, 1493, 1093 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{41}N_4O_3$: 481.3180, found: 481.3174; $[\alpha]_D^{23}=+39.9°$ (c=0.43, $CHCl_3$).

4.1.18. (12*R*,15*R*)-*E*-Terpeptin (34). Prepared from the (12*R*,15*R*) stereoisomer **24**-*E* following the procedures reported for **2**. ^{1}H NMR (400 MHz, $CDCl_3$) (1:1 mixture of rotamers) δ 9.17 (d, 0.5H, J=9.6 Hz), 8.02–7.99 (m, 1.5H), 7.74–7.70 (m, 1H), 7.38–7.31 (m, 1H), 7.26–7.24 (m, 1H), 7.13–7.05 (m, 2H), 6.88 (d, 0.5H, J=9.6 Hz), 6.50 (d, 0.42H, J=14.8 Hz), 6.31 (d, 0.52H, J=14.8 Hz), 6.19 (d, 0.5H, J=8.8 Hz), 5.30–5.27 (m, 1H), 4.86–4.79 (m, 1H), 4.64 (d, 0.5H, J=7.2 Hz), 4.20 (d, 0.5H, J=10.8 Hz), 3.49–3.45 (m, 2H), 3.12 (s, 1.5H), 2.98 (s, 1.5H), 2.55–2.47 (m, 0.5H), 2.42–2.34 (m, 0.5H), 2.25–2.15 (m, 0.5H), 2.07 (s, 1H), 1.99 (s, 2H), 1.76 (s, 3H), 1.71 (s, 3H), 1.03–0.83 (m, 12H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 174.0, 172.3, 171.9, 170.0, 166.4, 165.0, 135.9, 135.8, 135.5, 135.4, 126.5, 126.4, 121.6, 121.5, 120.2, 120.1, 119.9, 119.6, 119.57, 119.5, 119.4, 110.6, 110.5, 107.8, 107.5, 107.3, 107.0, 66.7, 63.0, 53.9, 53.8, 31.4, 31.1, 30.7, 29.6, 29.0, 27.0, 25.6, 25.4, 25.3, 23.1, 23.0, 20.0, 19.5, 19.4, 19.2, 18.3, 17.8, 17.5,; IR (thin film) ν_{max} 3291, 2967, 2931, 2875, 1653, 1558, 1458, 1373, 1302, 1218, 1093 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{28}H_{40}N_4O_3$: 480.3100, found: 480.3062; $[\alpha]_D^{23}=+95.2°$ (c=1.0, $CHCl_3$).

4.1.19. *Nin*-Ts tryptophan (36). Tryptophan methyl ester hydrochloride (500 mg, 1.96 mmol) was dissolved in 2 mL CH_2Cl_2, and Et_3N (1.4 mL, 9.8 mmol) and $(Boc)_2O$ (642 mg, 2.94 mmol) were consecutively added at rt. After stirring for 1 h, the reaction mixture was concentrated in vacuo. Purification on silica gel (30% EtOAc in hexane) provided 620 mg (1.96 mmol, 100%) of MeO-Trp-NHBoc as a white solid. The product was treated with 392 mg (9.8 mmol) NaOH, 665 mg (1.96 mmol) NBu_4HSO_4, and 10 mL of freshly distilled CH_2Cl_2. After refluxing for 10 min, 1.12 g (5.88 mmol) TsCl was added. The reaction mixture was refluxed for a further 1 h, cooled to rt, and then diluted with EtOAc. The organic layer was washed with sat. $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification on silica gel (30% EtOAc in hexane) provided 806 mg (1.71 mmol, 87%) of **36** as a pale yellow solid. Mp 43.5–48.0°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, 1H, J=8 Hz), 7.71 (d, 2H, J=8 Hz), 7.44 (d, 1H, J=8 Hz), 7.33 (s, 1H), 7.30–7.19 (m, 4H), 5.04 (d, 1H, J=7.2 Hz), 4.61 (m, 1H), 3.61 (s, 3H), 3.24 (dd, 1H, J=5.6, 14.4 Hz), 3.16 (dd, 1H, J=5.2, 14.8 Hz), 2.32 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 172.2, 155.1, 145.0, 135.23, 135.16, 131.0, 130.0, 126.9, 125.0, 124.5, 123.3, 119.6, 117.4, 113.8, 80.2, 53.7, 52.5, 28.4, 27.9, 21.7; IR (thin film) ν_{max} 3395, 3008, 2979, 2931, 1743, 1711, 1598, 1502, 1447, 1367, 1172, 1123 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{24}H_{29}N_2O_6S$: 473.1748, found: 473.1717; $[\alpha]_D^{23}=+37.4°$ (c=0.81, CH_2Cl_2).

4.1.20. Dipeptide (37). 432 mg (0.915 mmol) of **36** was dissolved in 10 mL CH_2Cl_2 and 5 mL TFA was added at rt. After stirring for 1 h, volatiles were removed by evaporation using a N_2 stream. To the crude product was added HOBt (136.5 mg, 1.01 mmol), EDC (193 mg, 1.01 mmol), HO-(*L*)-Phe-NMeBoc (282 mg, 1.01 mmol) followed by 5 mL of DMF. The mixture was cooled to 0°C and DIEA (810 μL, 4.57 mmol) was added. The reaction was stirred for 6 h (0°C to rt), then diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layers was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification on silica gel (30% EtOAc in hexane) provided 521 mg (0.82 mmol, 90% for two steps) of **37** as a pale yellow solid. Mp 46.0–49.0°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, 1H, J=8.4 Hz), 7.72 (d, 2H, J=8.4 Hz), 7.47 (d, 1H, J=7.6 Hz), 7.33–7.11 (m, 10H), 6.71 (m, 0.5H), 6.41 (m, 0.5H), 4.85 (m, 2H), 3.65 (s, 1H), 3.58 (s, 2H), 3.30–3.16 (m, 4H), 2.94–2.77 (m, 2H), 2.31 (s, 3H), 1.31 (s, 9H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 171.6, 170.8, 170.5, 156.5, 155.0, 145.1, 145.0, 137.8, 137.5, 135.2, 135.0, 130.8, 130.0, 129.0, 128.5, 126.9, 126.6, 125.0, 124.3, 123.4, 119.4, 117.2, 113.8, 80.6, 61.5, 60.0, 52.9, 52.5, 52.4, 34.0, 31.1, 30.3, 28.2, 27.6, 21.6; IR (thin film) ν_{max} 3320, 2967, 2930, 1745, 1684, 1655, 1521, 1453, 1366, 1174, 1121 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{34}H_{40}N_3O_7S$: 634.2589, found: 634.2561; $[\alpha]_D^{23}=-27.7°$ (c=0.65, CH_2Cl_2).

4.1.21. Tripeptide (38). Dipeptide **37** (160 mg, 0.253 mmol) was dissolved in 3.2 mL CH_2Cl_2 and 1.6 mL TFA was added at rt. After stirring for 30 min, volatiles were evaporated with a N_2 stream. To the crude product was added HATU (134.2 mg, 0.353 mmol), Boc-*L*-Leu-OH, and 1 mL DMF. The reaction mixture was cooled to 0°C and DIEA (208 μL, 1.18 mmol) was added. The reaction was stirred for 6 h (0°C to rt), then diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layers was dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting compound was dissolved in 2 mL CH_2Cl_2 and Et_3N (145 μL, 1.06 mmol) was added followed by Ac_2O (59.3 μL, 0.636 mmol). The reaction was stirred for 1 h and then concentrated in vacuo. Purification on silica gel (50% EtOAc in CH_2Cl_2) provided 158 mg (0.235 mmol, 93% for three steps) of **38** as a pale yellow solid. Mp 58.5–62.0°C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, 1H, J=6.8 Hz), 7.94–7.90 (m, 1H), 7.80 (d, 1H, J=8.4 Hz), 7.75 (d, 1H, J=8.4 Hz), 7.47–7.42 (m, 1H), 7.37 (s, 1H), 7.30–7.07 (m, 9H), 6.67 (d, 0.5H, J=6.8 Hz), 6.29 (d, 1H, J=8.8 Hz), 5.95 (m, 1H), 5.02 (t, 0.5H, J=7.6 Hz), 4.85–4.71 (m, 2H), 4.38–4.33 (m, 1H), 3.65 (s, 1.8H), 3.53 (s, 1.1H), 3.33–2.95 (m, 4H), 2.91 (s, 1.2H), 2.61 (s, 1.8H), 2.32 (s, 3H), 1.91 (s, 1.2H), 1.79 (s, 1.8H), 1.36 (m, 1H), 0.91–0.58 (m, 6H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 173.9, 173.7, 172.0, 171.8, 171.5, 171.3, 170.2, 169.7, 169.5, 173.9,

173.7, 171.9, 171.8, 171.4, 171.3, 170.2, 169.7, 145.1, 144.9, 137.8, 137.0, 135.3, 135.2, 135.0, 130.8, 130.6, 130.0, 129.6, 129.2, 129.0, 128.7, 127.1, 127.0, 126.9, 125.1, 124.9, 124.7, 124.2, 123.3, 119.44, 119.36, 118.2, 113.8, 113.7, 62.6, 60.5, 60.1, 53.2, 52.5, 52.3, 48.0, 47.4, 41.3, 37.9, 34.0, 33.9, 32.9, 29.4, 27.4, 26.8, 24.8, 24.1, 23.3, 23.1, 22.5, 21.7, 21.6, 21.2, 20.4, 14.3; IR (thin film) ν_{max} 3266, 3027, 2957, 2930, 2870, 1746, 1633, 1448, 1369, 1279, 1214, 1174, 1123, 1096 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{37}H_{45}N_4O_7S$: 689.3011, found: 689.3011; $[\alpha]_D^{23}=-78.6°$ (c=0.83, CH_2Cl_2).

4.1.22. Ts-aspergillamide A (39). Tripeptide **38** (137 mg, 0.199 mmol) was dissolved in 2 mL THF and $LiOH{\cdot}H_2O$ (16.7 mg, 0.398 mmol) in 1 mL H_2O was added. After stirring for 1 h, 2 mL 0.1N HCl was added and the reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was mixed with $Cu(OAc)_2$ (10.8 mg, 0.60 mmol) and pyridine (31.7 μL, 0.398 mmol) in 1 mL distilled THF was added at 0°C. $Pb(OAc)_4$ (97 mg, 0.219 mmol) was next added as a solid under nitrogen. The resulting solution was allowed to warm to rt and stirred for 2 h, then diluted with 10 mL 3% Et_3N/EtOAc. The mixture was eluted through a polypropylene cartridge containing silica gel (4 g), washed with 20 mL 3% Et_3N/EtOAc and concentrated in vacuo. The crude *N*,*O*-acetal was dissolved in 3 mL distilled CH_2Cl_2, and then treated with 90.1 mg (2.58 mmol/g, 0.235 mmol) PS-TBD resin. The mixture was stirred at 0°C for 1 h, then filtered and concentrated in vacuo. Crude ^1H NMR (400 MHz) analysis showed a 20:1 ratio of **39** and its *E* isomer. Purification on silica gel (50% EtOAc, 3% Et_3N/hexanes) provided 62.5 mg (0.099 mmol, 50% yield) of **39** as a pale yellow solid. Mp 55.0–58.0°C ^1H NMR (400 MHz, acetone-d_6, 1:1 mixture of rotamers) δ 9.72 (d, 0.5H, J=9.6 Hz), 8.99 (d, 0.5H, J=9.6 Hz), 8.08–7.93 (m, 2.5H), 7.67 (d, 0.5H, J=6.4 Hz), 7.61 (d, 0.5H, J=7.6 Hz), 7.56 (d, 0.5H, J=8 Hz), 7.37–7.18 (m, 9H), 6.99–6.94 (m, 1H), 5.90 (d, 0.5H, J=9.6 Hz), 5.82 (d, 0.5H, J=9.6 Hz), 5.33–5.27 (m, 1H), 4.97–4.92 (m, 0.5H), 4.46–4.41 (m, 0.5H), 3.40–3.16 (m, 2H), 2.33–2.32 (m, 3H), 2.06–2.04 (m, 3H), 0.89–0.67 (m, 6H), −0.017 (m, 1H); ^{13}C NMR (75.0 MHz, acetone-d_6) δ 175.4, 174.0, 172.3, 170.2, 168.9, 168.7, 146.5, 146.4, 138.9, 138.3, 136.0, 135.7, 135.3, 131.3, 131.0, 130.7, 130.0, 129.8, 129.3, 128.1, 127.6, 127.5, 126.1, 125.7, 125.2, 124.4, 124.25, 124.20, 124.09, 124.06, 123.5, 120.7, 120.5, 118.0, 117.8, 114.3, 114.2, 102.4, 99.8, 62.9, 60.5, 55.7, 49.0, 48.7, 47.8, 41.7, 38.8, 35.4, 34.0, 33.0, 25.4, 24.8, 23.6, 23.5, 22.8, 21.8, 21.5, 20.9; IR (thin film) ν_{max} 3398, 3282, 2958, 2927, 1694, 1652, 1498, 1449, 1372, 1173, 1136, 1092 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{35}H_{40}N_4O_5S$: 628.2719, found: 628.2706; $[\alpha]_D^{23}=+51.4°$ (c=0.50, CH_2Cl_2).

4.1.23. Aspergillamide A (3). 100 mg (4.2 mmol) of Mg powder was suspended in 1 mL of anhydrous MeOH. After sonication of the reaction mixture until hydrogen gas evolution was observed, the reaction was cooled to 0°C and 37 mg of **39** in 1 mL MeOH was added. After the mixture was stirred for 2 h at 0°C, the reaction was quenched by addition of 2 mL sat. NH_4Cl and the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification on silica gel (3% Et_3N, 50% EtOAc in CH_2Cl_2) provided 23 mg (0.049 mmol, 83%) of **3** as a pale yellow solid. ^1H NMR (400 MHz, acetone-d_6, 1:1 mixture of rotamers) δ 10.57 (d, 0.5H, J=16.4 Hz), 9.21 (d, 0.5H, J=9.6 Hz), 8.47 (d, 0.5H, J=10 Hz), 7.61–7.5 (m, 2H), 7.43–7.03 (m, 8.5H), 6.82–6.76 (m, 1H), 6.03 (d, 0.5H, J=9.2 Hz), 5.97 (d, 0.5H, J=9.6 Hz), 5.40–5.36 (m, 0.5H), 5.25 (t, 0.5H, J=8.8 Hz), 4.92–4.86 (m, 0.5H), 3.39–3.29 (m, 1H), 2.95 (s, 1.5H), 1.84 (s, 1.5H), 1.50 (s, 1.5H), 0.87–0.62 (m, 6H), 0.112 (m, 1H); ^{13}C NMR (75.0 MHz, acetone-d_6) δ 174.7, 174.2, 172.1, 170.2, 168.31, 168.27, 139.0, 138.6, 137.1, 130.7, 130.0, 129.8, 129.3, 127.6, 127.4, 125.0, 123.9, 122.9, 122.6, 120.3, 120.2, 119.5, 119.3, 112.4, 112.2, 105.2, 103.4, 62.7, 60.4, 48.3, 47.4, 41.7, 39.2, 35.5, 34.2, 33.1, 25.4, 24.8, 23.5, 22.7, 21.9, 21.0,; IR (thin film) ν_{max} 3292, 2956, 2925, 2854, 1632, 1538, 1494, 1099 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{35}N_4O_3$: 475.2711, found: 475.2723; $[\alpha]_D^{23}=-39.3°$, c=1.00, MeOH. (Natural **3** $[\alpha]_D^{23}=-26.2°$, c=3.05, MeOH).[4c]

4.1.24. Ts-aspergillamide B (40). Ts-aspergillamide A **39** (50 mg, 0.08 mmol) was treated with KI (20 mg, mmol) and 3 mL AcOH at rt for 24 h, and the reaction mixture was concentrated in vacuo. Crude ^1H NMR (400 MHz) analysis indicated a 14:1 *E*–*Z* ratio of indolic enamides. Purification using a Waters Preparative HPLC (Waters Symmetry C_{18}, 5 μm, 19×50 mm, 80% MeOH in H_2O) provided 36 mg (0.058 mmol, 72%) of Ts-aspergillamide B **40** as a white solid. Mp 111.5–113.5°C ^1H NMR (400 MHz, acetone-d_6, 5:1 mixture of rotamers, major rotamer is reported) δ 10.51 (d, 1H, J=10.0 Hz), 8.05 (d, 1H, J=8.0 Hz), 7.90 (m, 2H), 7.84 (d, 1H, J=10.0 Hz), 7.77 (s, 1H), 7.72–7.54 (m, 2H), 7.40–7.21 (m, 9H), 6.58 (d, 1H, J=14.0 Hz), 5.04 (dd, 1H, J=3.6, 11.2 Hz), 4.62–4.56 (m, 1H), 3.31–3.08 (m, 2H), 2.33 (s, 3H), 2.01 (s, 3H), 1.64 (m, 1H), 1.24 (m, 1H), 0.89 (d, 2H, J=6.8 Hz), 0.72 (d, 3H, J=8.4 Hz), 0.68 (d, 3H, J=8.4 Hz), −0.15 (m, 1H); ^{13}C NMR (75.0 MHz, acetone-d_6) δ 174.1, 173.0, 168.7, 168.1, 146.7, 139.7, 136.8, 136.2, 131.2, 131.0, 130.5, 130.0, 129.5, 128.4, 128.1, 127.9, 127.6, 126.1, 125.8, 124.8, 123.5, 123.3, 121.33, 121.27, 121.0, 104.4, 103.7, 64.2, 63.6, 49.5, 48.2, 46.8, 41.3, 38.9, 36.2, 35.1, 34.6, 25.4, 24.9, 23.7, 23.6, 22.8, 22.7, 22.2, 21.5, 20.7, 9.0; IR (thin film) ν_{max} 3256, 3364, 2956, 2927, 2870, 1689, 1632, 1558, 1496, 1446, 1373, 1308, 1175, 968 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{35}H_{40}N_4O_5S$: 628.2719, found: 628.2779; $[\alpha]_D^{23}=-97.9°$ (c=0.29, CH_2Cl_2).

4.1.25. Aspergillamide B (41). 100 mg (4.2 mmol) of Mg powder was suspended in 1 mL anhydrous MeOH. After sonication of the reaction mixture until hydrogen gas evolution was observed, the reaction was cooled to 0°C and 36 mg of **40** in 1 mL MeOH was added. After the mixture was stirred for 2 h at 0°C, the reaction was quenched by addition of 2 mL sat. NH_4Cl and the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification on silica gel (3% Et_3N, 50% EtOAc in CH_2Cl_2) provided 22 mg (0.049 mmol, 80%) of Aspergillamide B **41** as a white solid. ^1H NMR (400 MHz, acetone-d_6, 5:1 mixture of rotamers, major rotamer is reported) δ 10.28 (bs, 1H), 10.24 (d, 1H, J=9.6 Hz),

7.81–7.73 (m, 2H), 7.54–7.07 (m, 11H), 6.69 (d, 1H, J=14.8 Hz), 5.02 (dd, 1H, J=3.6, 10.8 Hz), 4.63 (m, 1H), 3.31 (dd, 1H, J=3.6, 14.6 Hz), 3.15 (dd, 1H, J=10.8, 14.6 Hz), 2.89(s, 3H), 1.98 (s, 3H), 1.64 (s, 1H), 1.19(s, 1H), 0.89–0.68 (m, 6H), −0.13 (m, 1H); ^{13}C NMR (75.0 MHz, acetone-d_6) δ 174.1, 172.8, 170.2, 167.4, 139.8, 138.6, 131.0, 130.4, 130.0, 129.5, 127.9, 127.5, 126.7, 124.1, 123.9, 122.9, 121.4, 121.3, 120.6, 120.5, 113.9, 112.8, 108.1, 107.6, 63.6, 49.3, 48.0, 41.5, 39.0, 35.2, 34.8, 25.4, 24.9, 23.7, 23.6, 22.7, 22.2, 20.7; IR (thin film) ν_{max} 3272, 3060, 2960, 2870, 1653, 1635, 1558, 1497, 1338, 1180, 1086, 946 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{28}H_{35}N_4O_3$: 475.2711, found: 475.2683; $[\alpha]_D^{23}$=−113.7° (c=0.41, distilled MeOH); The optical rotation of natural **41** was not reported.[4c]

4.1.26. Indolic enamide (42). Prepared from **36** and Boc-*L*-Phe-OH following a similar procedure for the preparation of the aspergillamides. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (bs, 1H), 7.92 (d, 1H, J=10.8 Hz), 7.42 (d, 1H, J=7.6 Hz), 7.35 (d, 1H, J=8.4 Hz), 7.27–7.05 (m, 6H), 6.92 (d, 1H, J=2.0 Hz), 6.84 (dd, 1H, J=9.2, 10.8 Hz), 6.17 (d, 1H, J=8.0 Hz), 4.71 (dd, 1H, J=7.6, 14.0 Hz), 3.16 (dd, 1H, J=6.0, 13.6 Hz), 3.07 (dd, 1H, J=8.0, 14.0 Hz), 2.93 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 170.5, 163.4, 136.4, 135.9, 129.4, 128.9, 127.2, 122.8, 122.4, 120.2, 119.3, 119.0, 111.4, 110.8, 103.9, 54.6, 37.8, 22.9; IR (thin film) ν_{max} 3283, 3061, 2926, 2854, 1658, 1536, 1454, 1372, 1266, 1106, 1043 cm^{-1}; CIHRMS $[M+H]^+$ calculated for $C_{21}H_{22}N_3O_2$: 348.1714, found: 348.1680; $[\alpha]_D^{23}$=+3.3° (c=0.42, CH_2Cl_2).

4.1.27. Bis-thioether (43). Model compound **42** (20 mg, 0.058 mmol) was treated with 4-bromothiophenol (43.5 mg, 0.23 mmol) and AcOH (2 mL) for 48 h at rt open to the air. The reaction mixture was concentrated and purified on silica gel (50% EtOAc in hexane) to afford 18.6 mg (0.026 mmol, 45%) of **43** (5:1 mixture of diastereomers). Further purification using a Waters Preparative HPLC (Waters Symmetry C_{18}, 5 μm, 19×50 mm, 20% MeCN in water) provided 12 mg (0.017 mmol) of the major diastereomer **43** as a white solid. Mp 186.0–189.0°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (bs, 1H), 7.48 (d, 1H, J=8.0 Hz), 7.36–7.09 (m, 16H), 6.99 (d, 1H, J=2.4 Hz), 6.52 (d, 1H, J=10.0 Hz), 5.86 (dd, 1H, J=5.6, 9.6 Hz), 4.76 (d, 1H, J=6.0 Hz), 4.44 (dd, 1H, J=7.6, 14.8 Hz), 3.01 (dd, 1H, J=7.2, 13.2 Hz), 2.89 (dd, 1H, J=6.8, 14.0 Hz), 1.77 (s, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 170.1, 136.3, 126.0, 134.9, 134.0, 133.4, 132.0, 131.97, 129.4, 128.8, 127.2, 126.3, 124.3, 122.8, 122.2, 121.9, 120.4, 119.1, 111.6, 111.2, 61.7, 54.2, 49.5, 37.2, 22.9; IR (thin film) ν_{max} 3433, 3282, 2923, 1644, 1539, 1472, 1382, 1085, 1068, 1009, 818 cm^{-1}; CIHRMS $[M]^+$ calculated for $C_{28}H_{35}N_4O_3$: 721.0068, found: 721.0133; $[\alpha]_D^{23}$=+81.5° (c=0.24, CH_2Cl_2).

4.2. Biological section

4.2.1. Cell culture. A temperature-sensitive *cdc2* mutant cell line, tsFT210, isolated from the mouse mammary carcinoma cell line FM3A, was a kind gift from Dr. F. Hanaoka (RIKEN).[30a] tsFT210 cells were maintained in RPMI 1640 with 10% fetal calf serum (FCS) at the permissive temperature of 32°C.

4.2.2. Proliferation assay. Exponentially growing tsFT210 cells were treated with the test compounds at 32°C for 48 h. The cell number was evaluated by the subsequent color reaction. 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetra-zolium, monosodium salt, WST-8™ (Nakalai Tesque, Kyoto, Japan) was added, and the cells were further incubated for 4 h at 37°C. The absorbance (A_{450}) of each well was measured using a Wallac 1420 multilabel counter (Amersham Biosciences).

4.2.3. Cell cycle analysis. In the synchronous-culture assay, cells were seeded at a density of 1×10^5 cells/mL in 0.5 mL into a 24-well plate and were pre-incubated at 39°C for 17 h to arrest at the G2-M boundary. Next, 5 μL of each sample solution was added, and the cells were incubated at 32°C for 4 h. After incubation, morphological characteristics of the cells were examined by microscopic observation. The cells were subjected to flow cytometric analysis as described below to confirm the DNA contents in cells. Flow cytometric analysis was performed essentially as described in previous reports.[30] The harvested cells were stained with solution containing 50 μg/ml propidium iodide, 0.1% sodium citrate, and 0.2% NP-40 and analyzed for DNA contents using a flow cytometer (Coulter Co., Hialeah, FL).

4.2.4. Cell staining. Cells were treated with 0.55% of KCl for 20 min, fixed in Carnoy's solution, and placed on a wet glass slide. The chromosomes and intact nuclei were stained with 1 mg/mL of Hoechst 33258, and examined using fluorescent microscopy (Olympus).

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