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Total Synthesis of the Proposed Microcyclamides MZ602 and MZ568

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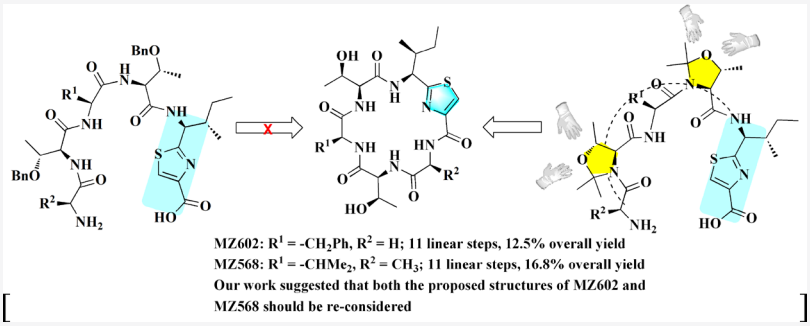

ABSTRACT: The first convergent total synthesis for the proposed structures of microcyclamides MZ602 (**1**) and MZ568 (**2**) has been accomplished in 11 linear steps with 12.5 and 16.8% overall yield, respectively. Key features of the syntheses include a one-pot cascade reaction to construct core Boc-L-Ile-Thz-OAllyl fragment **5**, and a removable pseudoproline ($\Psi^{Me,Me}$ pro) inducer assisted cyclization of thiazole-containing all-L linear peptides. The spectral data (^{1}H NMR, ^{13}C NMR, and HRMS) of synthetic MZ602 (**1**) were quite similar to those of the proposed natural microcyclamide MZ602, except to an opposite sign of the optical rotation value. Surprisingly, the synthetic MZ568 (**2**) presented large discrepancies in characteristic spectral data from those of the reported natural product, although the absolute configuration of key intermediate **36** was unambiguously determined by single-crystal X-ray analysis in our work. These findings revealed that the proposed structures of natural microcyclamides MZ602 and MZ568 required revision.

INTRODUCTION

With the outbreak of cyanobacteria blooms worldwide, the potential risks of their secondary metabolites to environmental safety and human health have become an ever-growing concern.[1] These metabolites were found not only to be associated with the off-flavors in aquatic animals or contamination of drinking water but also to be responsible for a range of lethal and sublethal effects in plants and animals.[1a,2] For example, very recently, the mass death of ~350 African elephants in Botswana was suspected to be associated with the contamination of toxic secondary metabolites of cyanobacteria.[3]

Microcyclamides MZ602 and MZ568 were two cyclic hexapeptide-type cyanobacteria secondary metabolites isolated from the freshwater *Microcystis* sp. bloom in 2010 by Carmeli and co-workers (Figure 1).[4] In a limited biological screening, microcyclamide MZ602 exhibited a mild inhibitory activity against Molt4 cells (20% cell growth inhibition at 83 μM) and a moderate inhibitory activity against chymotrypsin (IC_{50} = 75 μM). Compared to MZ602, microcyclamide MZ568 showed a more potent inhibitory activity against Molt4 cells with cell growth inhibition of 36% at 1.8 μM, but not active on chymotrypsin. In addition, there are more than 50 hexapeptides that were characterized from these metabolites (didmolamides,[5] balgacyclamides,[6a] venturamides,[6b] etc.), and many of them exhibited engaging biological activities,

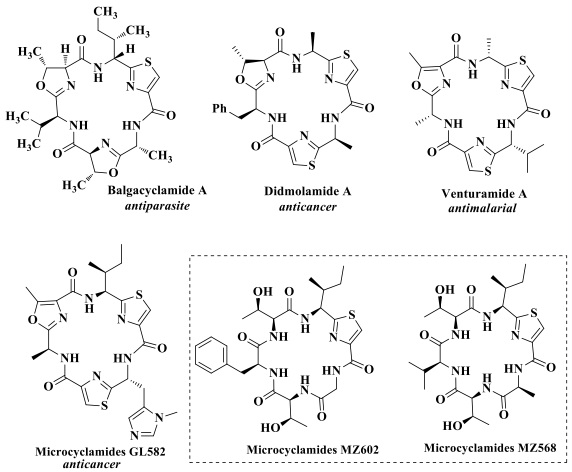

Figure 1. Representative bioactive cyanobacterial metabolites.

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such as antineoplastic activity,[5] antiparasitic activity,[6] and antimicrobial activity.[7]

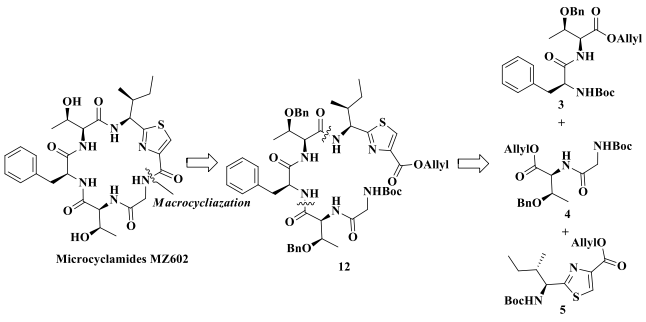
The structures of microcyclamides MZ602 and MZ568 were deduced on the basis of extensive spectroscopic data (1D and 2D NMR, UV), and the absolute configurations of amino acid residues were established as all-L (L-Thr, L-Phe, L-Ala, L-Val, and L-Ile) by using the classical Marfey's method.[4] Unlike other hexapeptide-type metabolites containing two or more five-membered heterocycles, microcyclamides MZ602 and MZ568 are structurally unusual and possess only one thiazole ring in the skeleton (Figure 1). Although much effort has been devoted to the preparation of heterocycle-containing cyclopeptides, to the best of our knowledge, few total synthesis of these single thiazole-containing all-L cyclic peptides have been reported.[8] Intrigued by their unique structures and potential biological activities, we are interested in developing an efficient synthetic procedure to access heterocycle-containing cyclic hexapeptides and wish to synthesize microcyclamides MZ602 and MZ568, for the first time, to confirm their absolute structures.

Cyclization of the small linear peptide, especially to all-L linear peptide precursor, remains a notoriously difficult task.[9] The challenges come from the fact that amide bonds preferred to exist in *S*-transoid conformation due to their strong π-character, and thus enhanced the rigidity of linear peptide. It turns out that the cyclization of these peptides suffers several difficulties, such as poor yield, cyclodimerization, or partial epimerization.[10] Although some turn-promoting residues, such as L-proline, L-glycine, *N*-alkylated amino acids, and even unnatural D-amino acid derivatives were proven to be useful for the macrocyclization, and many studies revealed that it is the sequence of linear peptide precursor that plays the key role in forming of the target cyclic structure.[9]

RESULTS AND DISCUSSION

Our first retrosynthetic analysis of all-L cyclic hexapeptide microcyclamide MZ602 is outlined in Scheme 1. To minimize

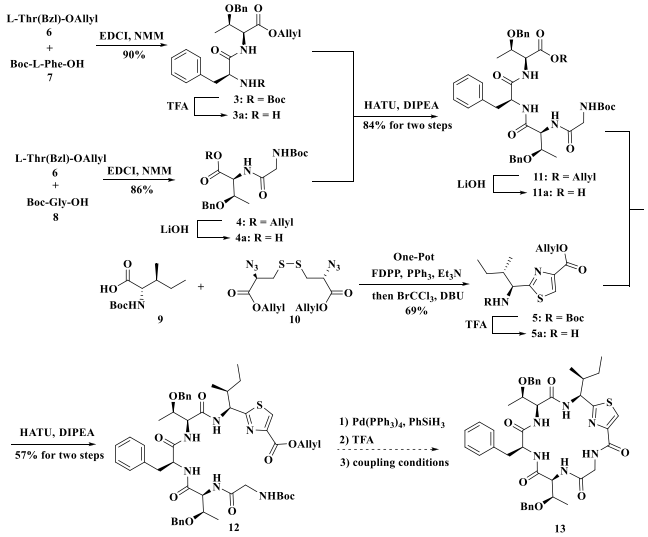
Scheme 1. Retrosynthetic Analysis of Microcyclamide MZ602



the influence of steric hindrance and epimerization during macrocyclization, the smaller glycine and thiazole unit were chosen as the N- and C-terminal residues, respectively. Linear precursor **12** could be achieved by a convergent synthetic procedure from fragments **3**, **4**, and **5**. The key building block Boc-L-Ile-Thz-Oallyl (**5**) could be obtained from commercially available Boc-L-Ile-OH using a one-pot cascade reaction previously established in our laboratory.[11] Dipeptides **3** and **4** could be readily obtained by coupling reaction from amino acid residues L-Thr(Bzl)-OAllyl, Boc-L-Phe-OH, and Boc-Gly-OH.

As seen in Scheme 2, our approach commenced with the preparation of dipeptide **3**, which was synthesized in 90% yield

Scheme 2. Route A Towards MZ602 Synthesis



from commercially available Boc-L-Phe-OH and L-Thr(Bzl)-OAllyl using EDCI/NMM as coupling agents. On the other hand, dipeptide **4** was obtained in 86% yield by coupling reaction of Boc-Gly-OH and L-Thr(Bzl)-OAllyl. However, partial epimerization of tetrapeptide **11** was observed in EDCI/NMM or PyBop/DMAP promoted coupling reactions. When the coupling agents HATU/DIPEA were applied, desired tetrapeptide **11** was obtained in 84% yield. Next, the one-pot cascade thiazole formation reaction was investigated. As expected, the reaction proceeded smoothly following our reported procedure,[11] and the desired thiazole **5** was achieved in 69% yield from Boc-L-Ile-OH **9** and β-azido disulfide diallyl ester **10** by sequential treatment with 8.0 equiv PPh_3, 4.0 equiv pentafluorophenyl diphenylphosphinate (FDPP), 10.0 equiv Et_3N, 12.0 equiv DBU, and 10.0 equiv $BrCCl_3$. Hydrolysis of tetrapeptide **11** and subsequent coupling with the free amine **5a** generated from thiazole **5**, afforded the linear peptide **12** in 57% yield over three steps. Unfortunately, sequential removal of the allyl and Boc groups in **12** by LiOH and trifluoroacetic acid (TFA), followed by macrolactamization did not achieve the desired cyclic peptide **13** under different coupling conditions (see Table 1).

We speculated that the possible reason behind this hard formation of **13** could be ascribed to the all-L configuration of constituent amino acids and the rigidity of the thiazole unit. This frustration prompted us to change the synthetic strategy to prepare the desired microcyclamide MZ602. It is well

Table 1. Macrolactamization Conditions

entry	coupling conditions	**13** (%)
1	HATU, HOBt, DCM/DMF (1/1)	trace[a]
2	FDPP, DIPEA, CH_3CN	trace[a]
3	PyBop, HOBt, DIPEA, DCM	ND[b]
4	DPPA, Et_3N, DCM	ND[b]

[a]Detected by LC–MS. [b]ND means no desired product.

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documented that the pseudoproline ($\Psi^{Me,Me}$ pro) derived from threonine, serine, or cysteine might induce a significant effect on *cis*-conformer amide bond formation and substantially increase the head-to-tail cyclization efficiency in many cyclic peptides.[9,10] Since MZ602 contain two threonine residues in the skeleton, we envisaged that two embedded $\Psi^{Me,Me}$ pro in the linear peptide precursor would improve the *cis*/*trans* amide bond ratio and eventually increase the head-to-tail cyclization yield (Figure 2). In this context, a new selective protection strategy (Cbz/Boc/$\Psi^{Me,Me}$ pro) was established and applied to prepare MZ602 (Scheme 3).

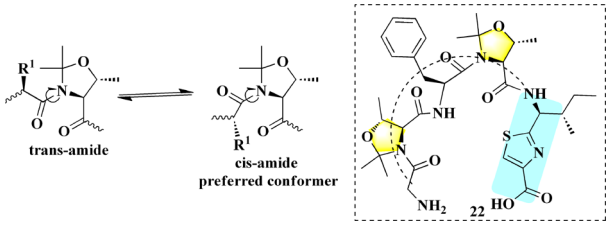
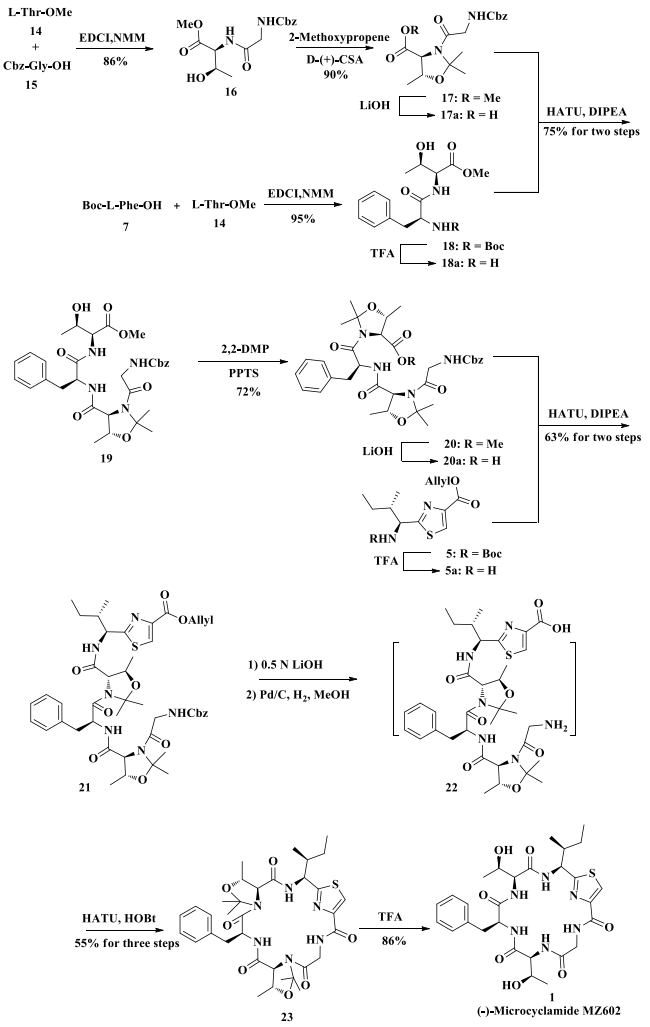


Figure 2. Pseudoproline-assisted *Cis*/*Trans* Conformer Equilibrium.

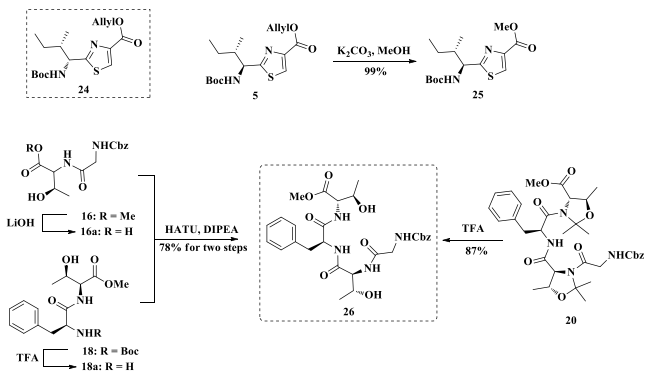
Scheme 3. Route B Towards MZ602 Synthesis



The new synthetic approach began with the preparation of dipeptide **16**, which was achieved from commercial available L-Thr-OMe and Cbz-Gly-OH in 86% yield. $\Psi^{Me,Me}$ Pro-containing dipeptide **17** was prepared in 90% yield upon treatment of **16** with 3.0 equiv of 2-methoxypropene and catalytic amount of (+)-camphorsulfonic acid. Coupling reaction of Boc-L-Phe-OH with L-Thr-OMe in the presence of EDCI/DIPEA rendered dipeptide **18** in 95% yield. Notably, the spectral features of the synthesized dipeptides **16** and **18** were in full consistent with those of reported previously.[10,13] Next, the dipeptide **17** was saponified with LiOH and then coupled with N-deprotected **18a** to give the mono-$\Psi^{Me,Me}$ pro-containing tetrapeptide **19** in 75% yield. Di-$\Psi^{Me,Me}$ pro **20** was obtained from **19** in 72% yield under reflux overnight with 2,2′-dimethoxypropene (2,2′-DMP), and pyridinium *p*-toluenesulfonate (PPTS) at 80 °C. Hydrolysis of **20**, followed by coupling with N-deprotected thiazole fragment **5a**, utilizing the HATU/DIPEA promoter to give linear macrolactam precursor **21** in 63% yield. With **21** in hand, its allyl and Cbz groups was sequentially removed and then subjected to macrolactamization using HATU/HOBt agents under diluted conditions (0.001 M). To our delight, this type of the $\Psi^{Me,Me}$ pro strategy works well, and the desired cyclic peptide **23** was obtained in 55% yield over three steps. Finally, removal of two $\Psi^{Me,Me}$ pro groups on treatment with 30% TFA in DCM at 0 °C accomplished **1** in 86% yield. All spectroscopic features (^{1}H NMR, ^{13}C NMR, and HMBC) of synthetic compound **1** were quite similar to those of the isolated MZ602 (see Supporting Information, Table S1), except for the optical rotation {observed for **1**: $[\alpha]_D^{22}$ −24.3 (*c* 0.7, MeOH), lit.:[4] $[\alpha]_D^{24}$ + 53 (*c* 0.06, MeOH)}.

With this unexpected result, we re-examined the absolute structure of our synthetic thiazole **5** and di-$\Psi^{Me,Me}$ pro-containing tetrapeptide **20** (Scheme 5). First, the fragment Boc-D-*allo*-Ile-Thz-OAllyl **24** was synthesized using the similar procedure as described in the preparation of its diastereomer **5**. By comparison of ^{1}H NMR spectra between **24** and **5**, we concluded that there was no epimerization at the α-stereogenic center of intermediate **5** using our previously reported thiazole-formation method[11a] (see Supporting Information, Figure S8). Second, methyl ester **25** was prepared quantitatively by transesterification of **5** in the presence of K_2CO_3/MeOH. The spectroscopic data of **25** were in full agreement with those reported in the literature.[12] Third, the di-$\Psi^{Me,Me}$ pro group of **20** was cleaved with 30% TFA in DCM to generate tetrapeptide Cbz-Gly-L-Thr-L-Phe-L-Thr-OMe (**26**) in 87% yield. Alternatively, a direct coupling of dipeptides **16a** and **18a** also generated **26** in 78% yield (Scheme 4). As expected, the physical and spectroscopic data of **26** obtained from two

Scheme 4. Synthesis of Thiazoles 24, 25, and Tetrapeptide 26



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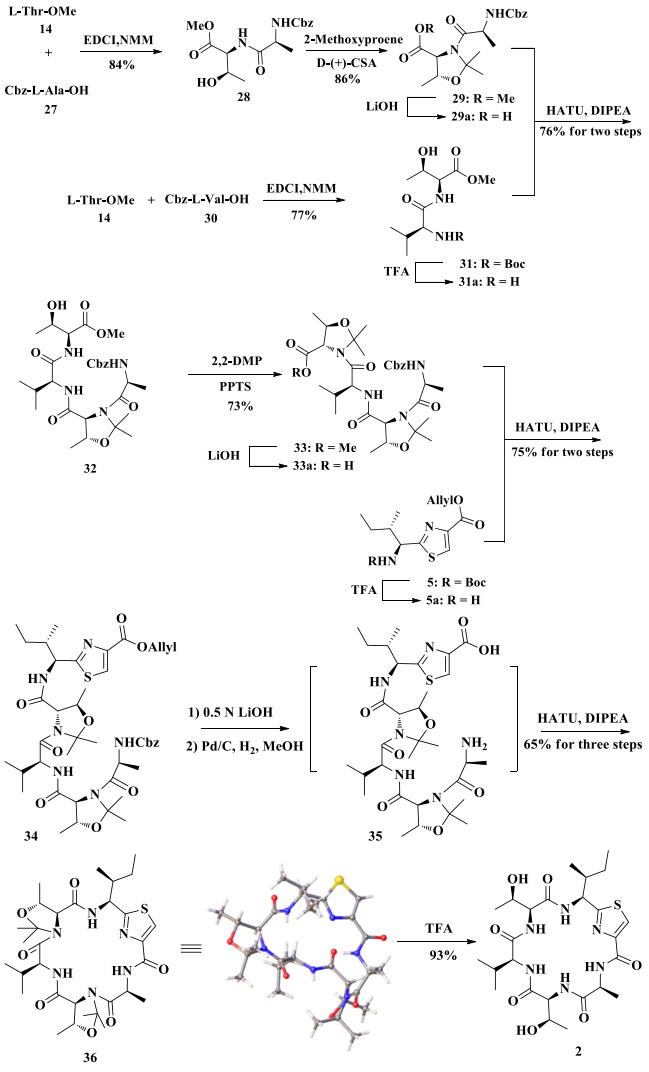
different synthetic methods were in full agreement with each other. This demonstrated that no epimerization occurred during the di-$\Psi^{Me,Me}$ pro deprotection process. All of the above evidences support that our synthetic **1** is in a right structure of cyclo-[L-Thr-L-Phe-L-Thr-Gly-L-Ile(Thz)].

However, the true structure of natural microcyclamide MZ602 is still shrouded in mystery. The literature search revealed that MZ568 and MZ602 were isolated and elucidated in the same metabolites family, and MZ602 presents just two different amino acid units than MZ568. The amino acids in microcyclamide MZ568 were also determined as all-L using the same Marfey's analysis procedures, as reported for MZ602. We believed that a total synthesis of microcyclamide MZ568 would provide some extra clues on the structure determination of natural product MZ602.

Following the similar synthetic procedure to MZ602, dipeptides **28** and **31** were respectively obtained in 84 and 77% yields from commercially available Boc-L-Val-OH, L-Thr-OMe, and Boc-L-Ala-OH (Scheme 5). The spectral features of synthetic dipeptides **28** and **31** were identical to those reported previously.[14,15] Pseudoproline dipeptide **29** was achieved in 86% yield from **28** on treatment with 3.0 equiv 2-methoxypropene and 0.1 equiv D-(+)-CSA. Removal of the Boc group on **31**, followed by coupling with the corresponding acid **29a** in the presence of HATU/DIPEA, generated tetrapeptide **32** in 76% yield. Compound **32** was then converted into di-$\Psi^{Me,Me}$ pro **33** under DMP/PPTS conditions. Saponification of **33**, followed by coupling with thiazole fragment **5a**, gave the linear peptide **34** in 55% yield over three steps. Sequential removal of Boc and allyl ester on **34** (→ **35**), then subjected to macrocyclization to afford the desired cyclic peptide **36** in 65% yield. Treatment of **36** with TFA for 15 h furnished **2** in 95% yield.

Scheme 5. Route Towards the Total Synthesis of MZ568



Surprisingly, the ^{1}H and ^{13}C NMR spectral data of the synthetic **2** were significantly different from those of the reported MZ568 (see Supporting Information, Table S2), as well as the optical rotation value {observed for compound **2**: $[\alpha]_D^{25}$ −260 (*c* 0.1, MeOH), lit.:[4] $[\alpha]_D^{24}$ −225 (*c* 0.03, MeOH)}. Luckily, the prism-shaped crystals of **36**, that is the direct precursor for **2**, was obtained from hexane and ethyl acetate. X-ray analysis unambiguously confirmed the absolute all-L configuration of amino acid residues in our synthetic compound. These results indicated that the structure of natural microcyclamide MZ568 requires further revision.

To unravel the structure mystery of microcyclamides MZ602 and MZ568, we decided to recheck the Marfey's method, which was used to determine the absolute configuration of natural MZ602 and MZ568.[4] As reported, this method included four steps: (1) acid degradation (6 N HCl) of the natural microcyclamides MZ602 and MZ568, (2) derivatization with Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, i.e., FDAA), (3) HPLC analysis of different FDAA-derivatized analytes at 340 nm, and (4) comparison with the standard samples to determine the configuration of the amino acids in an original natural product. The recent studies, however, revealed that the classical Marfey's method had failed to distinguish the FDAA derivative of L-isoleucine from L-*allo*-isoleucine, and failed to distinguish the FDAA derivative of L-threonine from L-*allo*-threonine as well.[16] Both microcyclamides MZ602 and MZ568 happen to contain one Ile and two Thr residues in their skeleton, and there were no comparison results among the analytes, the standard L-*allo*-Ile-FDAA and L-allo-Thr-FDAA derivatives in the published work.[4]

Based on these findings, we believed that there might be one or more L-*allo*-Thr or L-*allo*-Ile residue presented in the structures of microcyclamides MZ568 and MZ602, instead of L-Thr or L-Ile. In this context, it might have to prepare 16 isomers (MZ602: 2^3 = 8 isomers, MZ568: 2^3 = 8 isomers) to unambiguously determine the structures of natural microcyclamides MZ568 and MZ602.

SUMMARY

Two all-L cyclic hexapeptides cyclo-[L-Thr-L-Phe-L-Thr-Gly-L-Ile(Thz)] (**1**) and cyclo-[L-Thr-L-Val-L-Thr-L-Ala-L-Ile(Thz)] (**2**), as proposed structures for microcyclamides MZ602 and MZ568, were synthesized for the first time via a convergent synthetic route from commercial available amino acids in 11 longest linear steps with good yields. Synthetic **1** was found to be quite similar in all respects to the isolated microcyclamide MZ602, except for the optical rotation. The spectral data of synthetic **2** were found to be significantly different with the natural microcyclamide MZ568. Our findings suggested that the proposed structures of natural microcyclamides MZ602 and MZ568 need to be revised both. The highlights of our synthetic method include one-pot cascade thiazole-formation

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reaction and removable pseudoprolines ($\Psi^{Me,Me}$ pro) assisted cyclization strategy. Further studies toward other possible isomers of micrcocyclamides MZ602 and MZ568, and other cyanobacteria secondary metabolites (balgacyclamides A-C, aerucyclamides A-D) are currently in progress in our lab.

EXPERIMENTAL SECTION

General Experimental Details. Unless other indicated, all reagents were purchased from commercial corporations. All reactions were performed in oven-dried glassware under standard conditions. Flash chromatography (FC) was performed using silica gel (200−300 meshes). High-resolution mass spectrometry data were acquired using a Q-TOF analyzer. Optical rotations were recorded on a Rudolph Polarimeter Autopol 111. Melting points were analyzed on a Melt-Temp II capillary melting point apparatus. ^{1}H NMR, ^{13}C NMR, and HMBC were measured on 400 MHz/100 MHz Bruker spectrometers or JEOL JNM-ECZ400S spectrometers (NMR in $CDCl_3$ with TMS as an internal standard). Chemical shifts (δ) are given in ppm relative to residual solvent (usually chloroform: δ 7.26 for ^{1}H NMR or 77.23 for proton decoupled ^{13}C NMR; DMSO; δ 2.50 for ^{1}H NMR or 39.53 for proton decoupled ^{13}C NMR; MeOH; δ 3.31 for ^{1}H NMR or 49.03 for proton decoupled ^{13}C NMR), and coupling constants (J) in Hz. Signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), ddt (doublet of doublets of triplets), dq (doublet of quartets), br s (broad singlet), and m (multiplet). β-azido disulfide **10** was prepared according to our previous report[11a].

General Procedure for the Peptide Coupling Reaction Method A. Under anhydrous conditions, *N*-methylmorpholine (NMM) (2.2 mmol) was added to a solution of N-protected amino acid (1.0 mmol), EDCI (1.5 mmol), and HOBt (1.2 mmol) in 15 mL THF. After stirring at 0 °C for 10 min, a solution of amine (1.0 mmol), NMM (1.2 mmol) in 10 mL DMF was added into the mixture. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was then quenched by H_2O and extracted with EtOAc. The combined organic phase was washed with saturated brine, dried (Na_2SO_4), and evaporated. The crude compound was purified by flash chromatography to give the desired peptide.

Method B. HATU (1.5 mmol) was added to the solution of N-protected substrate (1.0 mmol) and DIPEA (2.0 mmol) in 20 mL CH_2Cl_2. After stirring at room temperature for 10 min, amine (1.0 mmol) was added into the reaction mixture and stirred overnight. The reaction was quenched by saturated aqueous NH_4Cl and extracted with 30 mL CH_2Cl_2 three times. The combined organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The crude compound was purified by flash chromatography to give the desired coupled peptide.

General Procedure for Hydrolysis Method C. LiOH aqueous (0.5 N, 8 mL) was added dropwise to a solution of methyl ester or allyl ester (1 mmol) in MeOH (10 mL)/THF (10 mL). The reaction mixture was stirred at room temperature until the reaction was complete, as judged by TLC (typically 3 to 5 h). The reaction mixture was diluted with H_2O and acidified to pH 3 by the 10% aqueous solution of citric acid. The mixture was extracted with EtOAc. The combined organic phase was washed with saturated brine, dried (Na_2SO_4), and concentrated under reduced pressure to give the crude carboxylic acid.

General Procedure for Removal of Cbz Group Method D. A 0.1 mmol catalytic Pd/C (10 mol %) was added to a solution of the Cbz-protected peptide (1 mmol) in MeOH (20 mL), The flask was evacuated and then filled with H_2(g) and the procedure was repeated three times, then the mixture was stirred under H_2(g) at room temperature for 5 h. After which, the contents of the flask were filtered through a pad of Celite. The Celite was then washed several times (CH_2Cl_2/MeOH/NH_3(aq) 90:9:1), the organic fractions were combined, and the solvent was removed by rotary evaporation to give the desired free amine.

General Procedure for Removal of Boc Group Method E. A 6 mL trifluoroacetic acid was added to a solution of the Boc-protected peptide (1 mmol) in 15 mL CH_2Cl_2. The reaction mixture was stirred at room temperature until the reaction was complete, as judged by TLC (typically 3 h). The solvent was removed by rotary evaporation with toluene to give the desired free amine.

General Procedure for the Formation of Pseudoproline($\Psi^{Me,Me}$ pro) Method F. 2-Methoxypropene (3.0 mmol) was added to a cooled solution of a substrate (1.0 mmol) in 20 mL CH_2Cl_2. Then, D-(+)-camphorsulfonic acid (D-(+)-CSA) (10 mol %) was slowly added into the solution. The solution immediately became red, and the reaction mixture was stirred at 0 °C for further 5 h. The reaction was quenched and diluted with 10 mL of CH_2Cl_2, and washed with 20 mL $NaHCO_3$(aq). The organic fraction was dried over $MgSO_4$, filtered, and concentrated, and the residue was purified by flash chromatography to give the desired $\Psi^{Me,Me}$ pro-containing product.

Method G. 2,2-Dimethoxypropane (6.0 mmol) and PPTS (0.3 mmol) were added to a solution of the threonine-containing substrate (1 mmol) in 15 mL toluene at room temperature. The reaction mixture was heated to 80 °C using oil bath and stirred overnight. The solution was cooled to room temperature and CH_2Cl_2 (20 mL) was added. Then, the solution was washed with 20 mL $NaHCO_3$(aq) and extracted with CH_2Cl_2. The combined organic phase was washed with saturated brine, dried (Na_2SO_4), and concentrated, and the residue was purified by flash chromatography to give the desired $\Psi^{Me,Me}$ pro-containing product.

*Cbz-Gly-L-Thr-OMe (**16**).*[10] The reaction was performed according to the method A using Cbz-Gly-OH **15** (1.5 g, 7.2 mmol) and NH_2-L-Thr-OMe **14** (1.0 g, 7.2 mmol) for 18 h, and the crude mixture was purified using EtOAc/petroleum ether = 4:1 as an eluent to give compound **16** as a white solid (2.0 g, 86%). Data are consistent with a previously characterized compound.[10] mp 101−105 °C, $[\alpha]_D^{25}$ = −3.6 (*c* 1.0, CH_3OH). [Lit.[10] $[\alpha]_D$ = −5.2 (*c* 1.0, CH_3OH)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (m, 5H), 7.11 (s, 1H), 5.79 (br, s, 1H), 5.11 (s, 2H), 4.58 (d, *J* = 8.0 Hz, 1H), 4.31 (s, 1H), 3.94 (s, 2H), 3.73 (s, 3H), 2.74 (s, 1H), 1.17 (s, 3H). HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{15}H_{20}N_2O_6Na$ 347.1214; found 347.1212.

*Cbz-Gly-L-Thr($\psi^{Me,Me}$Pro)-OMe (**17**).*[10] According to the method F, condensation of the dipeptide **16** (130 mg, 0.4 mmol) with 2-methoxypropene, and the crude mixture was purified using EtOAc/petroleum ether = 1:2 as an eluent to give the desired product **17** (131 mg, 90%) as a colorless oil. Data are consistent with a previously characterized compound.[10] $[\alpha]_D^{25}$−40.0 (*c* 5.5, $CHCl_3$). [Lit.[10] $[\alpha]_D$ = −27.73 (*c* 4.4, $CHCl_3$)]. ^{1}H NMR (400 MHz, DMSO) δ: 7.35 (m, 5H), 5.03 (s, 2H), 4.56 (d, *J* = 5.2 Hz, 1H), 4.34 (m, 1H), 3.83 (dd, *J* = 16.8, 5.6 Hz, 1H), 3.75 (s, 3H), 3.65 (m, 1H), 3.43 (dd, *J* = 16.8, 6.4 Hz, 1H), 1.58 (s, 3H), 1.44 (s, 3H), 1.36* (d, *J* = 6.0. Hz, 3H) (*minor rotamer); HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{18}H_{24}N_2O_6Na$ 387.1532; found 387.1530.

*Boc-L-Phe-L-Thr-OMe (**18**).* The reaction was performed according to the method A using compound **7** (2.2 g, 8.3 mmol) and compound **14** (1.1 g, 8.3 mmol) as substrates and stirred for 18 h. The crude mixture was purified using EtOAc/petroleum ether = 1:1 as an eluent to give compound **18** as a glassy solid (3.0 g, 95%). $[\alpha]_D^{25}$−5.3 (*c* 1.0, $CHCl_3$). [Lit.[13] $[\alpha]_D^{29}$ = −6.6 (*c* 1.0, $CHCl_3$)].[#] ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.21−7.26 (m, 5H), 6.75−6.80 (m, 1H), 5.07−5.11 (m, 1H), 4.57 (dd, *J* = 8.8, 2.0 Hz, 1H), 4.37−4.41 (m, 1H), 4.28 (dd, *J* = 6.4, 2.0 Hz, 1H), 3.72 (s, 1H), 3.13 (dd, *J* = 14.0, 6.4 Hz, 1H), 3.05 (dd, *J* = 13.2, 6.4 Hz, 1H), 2.57 (br. s, 1H), 1.39 (s, 9H), 1.16 (d, *J* = 6.4 Hz, 3H); ^{13}C{1H} NMR (100 MHz) δ: 171.7, 171.0, 155.5, 136.4, 129.3, 128.5, 126.7, 80.3, 66.2, 57.2, 55.8, 52.5, 37.8, 28.2, 19.7; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{19}H_{28}N_2O_6Na$ 403.1845; found 403.1856.

*Cbz-Gly-L-Thr($\psi^{Me,Me}$Pro)-L-Phe-L-Thr-OMe (**19**).* Following the general method C, **17a** was obtained from **17** (327 mg, 0.9 mmol) and used for the next step without purification. Following the general method E, **18a** was obtained from **18** (342 mg, 0.9 mmol) and used for the next step without purification. Following the general method B, the desired tetrapeptide **19** (416 mg, 75% for two steps) was obtained as a glassy solid. EtOAc/petroleum ether 1:1 (v/v) was used as an eluent. $[\alpha]_D^{25}$−31.0 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO) δ: 8.69 (d, *J* = 8.4 Hz, 1H), 8.33 (d, *J* = 9.6 Hz, 1H), 8.32 (s,

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1H), 7.42−7.27 (m, 6H), 7.13 (t, J = 7.2 Hz, 2H), 7.00 (t, J = 7.2 Hz, 1H), 5.07 (d, J = 4.8 Hz, 1H), 5.07−4.99 (m, 2H),4.88 (t, J = 12.0 Hz, 1H), 4.31 (dd, J = 8.4, 3.2 Hz, 1H), 4.19−4.10 (m, 1H), 4.01 (d, J = 7.2 Hz, 1H), 3.96−3.87 (m, 1H), 3.63 (s, 3H), 3.11 (td, J = 18.0, 4.0 Hz, 2H), 2.77 (t, J = 10.8 Hz,1H), 1.50 (s, 3H), 1.40 (s, 3H), 1.33 (d, J = 6.4 Hz, 3H), 1.07 (d, J = 6.4 Hz, 3H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ: 171.6[#], 168.9[#], 157.5[#], 137.7, 135.8, 129.1, 128.5, 128.3, 128.1, 127.9, 126.8[#], 94.7[#], 73.2[#], 67.5, 60.4, 58.7[#], 55.3[#], 52.6, 43.5, 38.6, 28.5, 25.0, 19.8, 18.4 ([#]minor rotamer); mp75−76 °C; ESI-HRMS calcd for ([M + Na]$^+$), found. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{31}H_{40}N_4O_9Na$ 635.2693; found 635.2657.

*Cbz-Gly-L-Thr($\psi^{Me,Me}$Pro)-L-Phe-L-Thr($\psi^{Me,Me}$Pro)-OMe (**20**).* According to the method G, the tetrapeptide **19** (122 mg, 0.2 mmol) reacted with 2,2-dimethoxypropane to give the desired pseudoproline protected tetrapeptide **20** (94 mg, 72%) as a colorless oil. EtOAc/petroleum ether (2:3, v/v) was used as an eluent. $[\alpha]_D^{25}$−20.9 (c 3.5, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO) δ: 8.90 (d, J = 8.8 Hz, 1H), 7.38−7.15 (m, 10H), 5.02 (q, J = 12.4 Hz, 2H), 4.55 (q, J = 8.0 Hz, 1H), 4.18−4.13 (m, 2H), 4.06 (t, J = 6.0 Hz, 1H),3.87 (d, J = 6.0 Hz, 1H), 3.66 (s, 3H), 3.40 (dd, J = 16.8, 6.0 Hz, 1H), 3.04 (dd, J = 16.8, 6.0 Hz, 1H), 2.96−2.83 (m, 2H), 1.54 (s, 3H), 1.45 (s, 3H), 1.43 (s, 3H), 1.40 (s, 3H), 1.34 (d, J = 6.0 Hz, 3H), 1.10 (d, J = 6.0 Hz, 3H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ: 170.6, 168.9, 167.8, 166.0, 156.2, 136.5, 135.6, 129.5, 128.7, 128.5, 128.0, 127.4, 97.3, 97.1, 75.9, 74.3, 66.8, 65.6, 60.4, 54.0, 53.4, 43.9, 40.7, 26.6, 26.0, 23.9, 23.4, 19.9, 19.1; HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{34}H_{44}N_4O_9Na$ 675.3006; found 675.2979.

*Cbz-Gly-L-Thr($\psi^{Me,Me}$Pro)-L-Phe-L-Thr($\psi^{Me,Me}$Pro)-L-Ile-Thz-OAllyl (**21**).* Following the general method C, **20a** was obtained from **20** (260 mg, 0.4 mmol) and used for the next step without purification. Following the general method E, **5a** was obtained from **5** (142 mg, 0.4 mmol) and used for the next step without purification. Following the general method B, the desired tetrapeptide **21** (220 mg, 63% for two steps) was obtained as a colorless oil. EtOAc/petroleum ether (1:1, v/v) was used as an eluent. $[\alpha]_D^{25}$−84.7 (c 0.5, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.29 (s, 1H), 7.42−7.14 (m, 10H), 6.05 (ddt, J = 16.8, 10.8, 1.2 Hz, 1H), 5.40 (dd, J = 16.8, 1.2 Hz, 1H), 5.27 (dd, J = 10.8, 1.2 Hz, 1H), 5.13−5.07 (m, 2H), 5.04 (d, J = 6.0 Hz, 1H), 4.80 (d, J = 6.0 Hz, 2H), 4.65−4.61 (m, 1H), 4.25−4.18 (m, 1H), 4.13 (d, J = 7.6 Hz, 1H), 3.97 (t, J = 6.4 Hz, 1H), 3.81 (d, J = 6.4 Hz, 1H), 3.61 (d, J = 16.8 Hz, 1H), 3.23 (d, J = 16.8 Hz, 1H), 3.07−2.94 (m, 2H), 2.11 (br s, 1H), 1.77−1.68 (m, 2H), 1.62 (s, 3H), 1.48 (s, 3H), 1.45 (s, 3H), 1.43 (d, J = 6.4 Hz, 3H), 1.24 (d, J = 6.4 Hz, 3H), 0.99 (t, J = 6.4 Hz, 3H), 0.90 (t, J = 6.4 Hz, 3H); ^{13}C{1H} NMR (100 MHz, CD_3OD) δ: 173.5, 170.7, 170.3, 169.8, 168.5, 162.3, 158.7, 147.3, 138.2, 137.5, 133.4, 130.7, 129.7, 129.4, 129.0, 128.9, 128.2, 118.8, 98.1, 97.9[#], 77.3, 76.9, 67.8[#], 67.2, 66.8, 58.4[#], 55.4[#], 44.6[#], 40.6, 39.9, 27.2, 27.0, 26.7, 24.2, 19.9, 19.1[#], 15.8[#], 12.1[#] ([#]minor rotamer); HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{45}H_{58}N_6O_{10}SNa$ 897.3833; found 897.3803.

*Cyclo-[Gly-L-Thr($\psi^{Me,Me}$Pro)-L-Phe-L-Thr($\psi^{Me,Me}$Pro)-L-Ile-Thz] (**23**).* Following the method C and method D, linear peptide **21** (88 mg, 0.1 mmol) generate the intermediate **22**. Next, **22** was added to a solution of HATU (76 mg, 0.2 mmol) and HOBt (14 mg, 0.1 mmol) in CH_2Cl_2/DMF (3/1, 100 mL) at room temperature. The mixture (0.001 M) was stirred until TLC showed complete consumption of the starting material (∼24 h). The reaction was quenched by saturated NH_4Cl (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phase was washed with H_2O, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 30:1) to give compound **23** as colorless oil (38 mg, 55% for three steps). $[\alpha]_D^{25}$−86.5 (c 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.26 (s, 1 H), 8.17 (br. s, 1H),7.29−7.27 (m, 2H), 7.20−6.95 (m, 5H), 5.12 (d, J = 16.8 Hz, 1H), 4.73−4.68 (m, 1H), 4.43−4.37 (m, 1H), 4.34−4.27 (m, 2H), 3.65 (d, J = 16.8 Hz, 1H), 3.44 (s, 1H), 3.18−3.07 (m, 2H), 2.96 (dd, J = 14.8, 5.6 Hz, 1H), 2.65−2.55 (m, 1H), 1.72 (s, 2H), 1.66−1.62 (m, 12H), 1.57−1.49 (m, 3H), 0.95−0.82 (m, 9H); ^{13}C{1H} NMR (100 MHz, CD_3OD) δ: 171.9, 170.3, 169.5, 168.7, 168.4, 136.5, 130.6, 130.1, 129.6, 129.0, 128.2, 98.8, 78.4, 77.4, 69.2, 68.6, 67.3, 66.3, 57.0, 38.0, 37.4, 35.7, 28.7, 26.9, 25.1, 24.1, 22.0, 19.4, 16.7, 10.0; HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{34}H_{46}N_6O_7SNa$ 705.3046; found 705.3052.

*Synthetic Microcyclamide MZ602 (**1**).* To a solution of compound **23** (10 mg, 0.01 mmol) in CH_2Cl_2 (5 mL) was added TFA (1.5 mL) at 0 °C. Then, the mixture was warmed to room temperature and stirred until TLC showed complete consumption of the starting material (∼14 h). The organic phase was concentrated under reduced pressure, and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 10:1) to give **1** as a glassy solid (7.6 mg, 86%). {Observed for compound **1**:$[\alpha]_D^{22}$−24.3 (c 0.7, MeOH), lit.[4] microcyclamide MZ602: $[\alpha]_D^{24}$ + 53 (c 0.06, MeOH)}; ^{1}H NMR (400 MHz, DMSO) δ: 8.53 (t, J = 5.6 Hz, 1H), 8.22 (s, 1 H), 8.15 (d, J = 7.2 Hz, 1H), 8.09 (d, J = 7.6 Hz, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.74 (d, J = 8.8 Hz, 1H), 7.30−7.16 (m, 5H), 5.16 (br s, 1H), 5.11 (dd, J = 8.4, 5.6 Hz, 1H), 4.84 (br s, 1H), 4.49 (m, 1H), 4.16 (m, 1H), 4.02−3.82 (m, 5H), 3.32 (m, 1H), 2.87 (dd, J = 14.0, 10.8 Hz, 1H), 2.05−1.97 (m, 1H), 1.46−1.39 (m, 1H), 1.15−1.06 (m, 1H), 1.09 (d, J = 6.4, 3H), 0.87 (d, J = 6.8, 3H), 0.86 (t, J = 7.2, 3H), 0.82 (d, J = 6.0, 3H); ^{13}C{1H} NMR (100 MHz, DMSO) δ: 172.0, 170.6, 169.9, 169.1, 168.8, 161.1, 148.8, 138.0, 129.2, 128.2, 126.4, 124.0, 66.1, 65.3, 61.0, 60.7, 55.1, 54.4, 43.6, 39.2, 37.2, 24.6, 20.5, 20.2, 15.7, 11.8; HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{28}H_{38}N_6O_7SNa$ 625.2420; found 625.2380.

*Boc-L-Phe-L-Thr(Bzl)-OAllyl (**3**).* The reaction was performed according to the method A using compound **7** (2.0 g, 7.5 mmol) and compound **6** (1.8 g, 7.5 mmol) as substrates and stirred for 18 h. The crude mixture was purified by flash column chromatography to give compound **3** as a colorless oil (3.4 g, 90%). EtOAc/petroleum ether (1:1, v/v) was used as an eluent. $[\alpha]_D^{25}$ + 28.9 (c 4.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34−7.25 (m, 5H), 7.23−7.20 (m, 5H), 6.62 (d, J = 9.6 Hz, 1H), 5.87−5.77 (m, 1H), 5.28 (dq, J = 17.2, 1.6 Hz, 1H), 5.21 (dq, J = 10.4, 1.2 Hz, 1H), 5.04 (br s, 1H), 4.65 (dd, J = 9.2, 2.4 Hz, 1H), 4.59 (dt, J = 6.0, 1.2 Hz, 1H), 4.53 (d, J = 12.0 Hz, 2H), 4.44 (br s, 1H), 4.35 (d, J = 11.6 Hz, 1H), 4.13 (qd, J = 6.4, 2.4 Hz, 1H), 3.14 (dd, J = 14.0, 6.0 Hz, 1H), 3.05 (dd, J = 12.8, 6.4 Hz, 1H), 1.39 (s, 9H), 1.16 (d, J = 6.4 Hz, 3H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ: 171.9, 169.9, 155.3, 137.8, 136.6, 131.6, 129.6, 129.5, 129.4, 128.7, 128.6, 128.4, 127.9, 127.8, 127.0, 119.0, 80.1, 74.3, 70.9, 66.1, 56.8, 55.7, 38.3, 28.3, 16.2; HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{28}H_{36}N_2NaO_6$ 519.2471; found 519.2466.

*Boc-Gly-L-Thr(Bzl)-OAllyl (**4**).* The reaction was performed according to the method A using Boc-Gly-OH **8** (2.0 g, 11.4 mmol) and L-Thr(Bzl)-OAllyl **6** (2.8 g, 11.4 mmol) as substrates, and stirred overnight. The crude mixture was purified by flash column chromatography to give compound **4** as a colorless oil (4.0 g, 86%). EtOAc/petroleum ether (1:1, v/v) was used as an eluent. $[\alpha]_D^{25}$−15.2 (c 3.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34−7.23 (m, 5H), 6.91 (d, J = 9.2 Hz, 1H), 5.82 (ddt, J = 10.4, 5.6, 1.2 Hz, 1H), 5.40 (br. s, 1H), 5.28 (dd, J = 16.8, 1.2 Hz, 1H),5.10 (d, J = 10.4 Hz, 1H), 4.70 (dd, J = 9.2, 2.0 Hz, 1H), 4.60 (dd, J = 13.2, 5.6 Hz, 1H), 4.56 (d, J = 12.0 Hz, 1H), 4.51 (dd, J = 13.2, 5.6 Hz, 1H), 4.36 (d, J = 12.0 Hz, 1H), 4.17 (dq, J = 6.0, 2.0 Hz, 1H), 3.86 (dq, J = 16.8, 5.6 Hz, 2H), 1.53 (s, 9H), 1.31 (d, J = 6.0 Hz, 3H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ: 170.2, 170.1, 156.0, 137.8, 131.6, 128.4, 127.8, 118.9, 80.0, 74.2, 70.7, 66.1, 56.6, 44.2, 28.3, 16.1; ESI-HRMS calcd for $C_{21}H_{30}N_2O_6Na$ ([M + Na] $^+$) 429.2002, found 429.2011. HRMS (ESI) m/z: [M + Na]$^+$ calcd for $C_{21}H_{30}N_2O_6Na$ 429.2002; found 429.2011.

*Boc-L-Ile-Thz-OAllyl (**5**).* To a solution of compound **9** (115 mg, 0.5 mmol) in CH_2Cl_2 (10 mL) was added pentafluorophenyl diphenylphosphinate (FDPP) (200 mg, 0.5 mmol) and triethylamine (TEA) (125 mg, 1.0 mmol) at room temperature. After stirring for 15 min, **10** (40 mg, 0.125 mmol) and PPh_3 (270 mg, 1.0 mmol) was added into the solution and heated to reflux for further 5 h away from light. After cooling to 0 °C, 1,8-diazabicycloundec-7-ene (DBU) (228 mg, 1.5 mmol) and bromotrichloromethane (248 mg, 1.25 mmol) were introduced via spyringe over 5 min and stirred for further 30 min at room temperature. The solvent was quenched with saturated NH_4Cl solution and extracted with DCM (20 mL × 3). The

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combined organic layer was washed with brine and dried over anhydrous Na_2SO_4, filtered, concentrated in vacuo (below 45 °C), and purified by flash column chromatography (EtOAc/petroleum ether, 1:5) to give compound **5** as a colorless oil (61 mg, 69% yield). $[\alpha]_D^{25}$−18.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.05 (s, 1H), 5.95 (ddt, *J* = 8.4, 4.4, 0.8 Hz, 1H), 5.35 (s, 1H), 5.33 (dd, *J* = 14.0, 0.8 Hz, 1H), 5.21 (dd, *J* = 8.4, 0.8 Hz, 1H), 4.87 (br s, 1H), 4.77 (d, *J* = 4.4 Hz, 2H), 2.09 (br s, 1H), 1.36 (s, 9H), 1.11−1.09 (m, 1H), 0.87−0.81 (m, 6H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) *δ*: 173.1, 160.9, 155.4, 146.9, 132.0, 127.2, 118.8, 80.0, 66.9, 57.5, 39.7, 28.3, 24.5, 15.8, 11.5; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{17}H_{26}N_2NaO_4S$ 377.1511; found 377.1522.

Boc-Gly-L-Thr(Bzl)-L-Phe-L-Thr(Bzl)-OAllyl ***(11)***. Following the general method C, **4a** was obtained from **4** (1.1 g, 2.7 mmol) and used for the next step without purification. Following the general method E, **3a** was obtained from **3** (1.3 g, 2.7 mmol) and used for the next step without purification. Following the general method B, the desired tetrapeptide **11** (1.7 g, 84% for two steps) was obtained as a glassy solid. EtOAc/petroleum ether (1:1, v/v) was used as an eluent. $[\alpha]_D^{25}$ + 10.1 (*c* 5.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.39−7.15 (m, 15H), 5.86−5.72 (m, 1H), 5.47 (dt, *J* = 22.0, 4.0 Hz 1H), 5.31−5.24 (m, 1H), 5.22−5.16 (m, 1H), 4.99−4.91 (m, 1H), 4.71 (t, *J* = 10.0 Hz, 1H),4.60−4.37 (m, 6H), 4.30 (d, *J* = 12.0 Hz, 1H), 4.17−4.12 (m, 1H), 3.89−3.75 (m, 1H), 3.64−3.73 (m, 1H), 3.13 (d, *J* = 12.0 Hz, 1H), 2.88 (dq, *J* = 20.0, 12.0 Hz, 1H), 1.40 (s, 9H), 1.09−0.97 (m, 6H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) *δ*: 171.7, 170.1, 169.6, 169.4, 156.2, 138.1, 137.9, 136.7, 131.8, 129.5, 129.4, 128.5, 128.4, 128.0, 127.9, 126.9, 118.9, 80.0, 75.2, 74.1, 71.3, 70.8, 66.1, 56.8, 56.0, 54.5, 44.2, 38.5, 28.4, 28.3, 16.1, 14.9; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{41}H_{52}N_4O_9Na$ 767.3632; found 767.3625.

Boc-Gly-L-Thr(Bzl)-L-Phe-L-Thr(Bzl)-L-Ile-Thz-OAllyl ***(12)***. Following the general method C, **11a** was obtained from **11** (521 mg, 0.7 mmol) and used for the next step without purification. Following the general method E, **5a** was obtained from **5** (248 mg, 0.7 mmol) and used for the next step without purification. Following the general method B, the desired hexapeptide **12** (375 mg, 57% yield for two steps) was obtained as a glassy solid. EtOAc/petroleum ether (1:1, v/v) was used as an eluent. $[\alpha]_D^{25}$ −11.2 (*c* 4.0, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 8.25 (s, 1H), 7.28−7.19 (m, 18H), 6.00 (ddt, *J* = 16.4, 10.8, 6.4 Hz, 1H), 5.37 (dd, *J* = 16.4, 1.6 Hz, 1H), 5.27 (dd, *J* = 10.8, 1.6 Hz, 1H), 5.17 (d, *J* = 8.0 Hz, 1H), 4.80 (dd, *J* = 8.0, 1.6 Hz, 1H), 4.76 (d, *J* = 6.0 Hz, 2H), 4.59−4.53 (m, 3H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.45−4.31 (m, 4H), 4.10−3.99 (m, 2H), 3.70 (d, *J* = 4.4 Hz, 2H), 3.17 (dd, *J* = 13.6, 4.4 Hz, 1H), 2.97 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.11−2.05 (m, 1H), 1.56−1.50 (m, 1H), 1.41 (s, 9H), 1.13 (d, *J* = 6.4 Hz, 3H), 1.11 (d, *J* = 6.4 Hz, 3H), 0.88−0.82 (m, 6H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) *δ*: 172.3, 170.9, 169.9, 169.9, 169.3, 161.0, 146.6, 137.9, 137.8, 132.0, 129.1, 128.8, 128.5, 128.4, 128.2, 128.0, 127.9, 127.6, 127.2, 118.9, 80.6, 74.1, 73.4, 71.7, 71.5, 65.9, 57.0, 56.7, 55.2, 39.1, 37.5, 32.0, 30.0, 29.8, 29.4, 28.4, 27.3, 15.8, 15.4, 14.2, 11.4; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{50}H_{64}N_6O_{10}SNa$ 963.4302; found 963.4297.

Boc-D-allo-Ile-Thz-OAllyl ***(24)***. Following the same procedure as for compound **5**, compound **24** was prepared in the solution as colorless oil (56 mg, 64% yield). $[\alpha]_D^{20}$−10.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.08 (s, 1H); 6.01 (ddt, *J* = 8.8, 4.8, 1.2 Hz, 1H), 5.38 (dd, *J* = 14.0, 1.2 Hz, 1H), 5.27 (dd, *J* = 8.4, 1.2 Hz, 1H), 5.24 (s, br, 1H), 5.04 (s, br, 1H), 4.82 (d, *J* = 4.4 Hz, 2H), 2.23−2.25 (m, 1H), 1.46−1.48 (m, 2H), 1.44 (s, 9H), 0.94 (t, *J* = 6.0 Hz, 3H), 0.82 (d, *J* = 5.6 Hz, 3H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) *δ*: 174.6, 161.1, 155.6, 147.1, 132.0, 127.2, 119.0, 80.3, 66.1, 56.6, 39.7, 28.4, 26.5, 14.0, 11.7; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{17}H_{26}N_2O_4SNa$ 377.1511; found 377.1520.

Boc-L-Ile-Thz-OMe ***(25)***[11a,12]. Compound **5** (35 mg, 0.1 mmol) was dissolved in 5 mL MeOH, then K_2CO_3 (69 mg, 0.5 mmol) was added into the solution and stirred for 3 h. The reaction mixture was diluted with 15 mL H_2O and extracted with 15 mL EtOAc three times. The combined organic phase was washed with saturated brine, dried (Na_2SO_4), and concentrated, and the residue was purified by flash chromatography to give the desired **25** in 32 mg (99% yield). Data are consistent with a previously characterized compound.[11a,12] $[\alpha]_D^{20}$−18.2 (c 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.04 (s, 1H), 5.31 (d, *J* = 8.4 Hz, 1H), 4.88 (s, br, 1H), 3.87 (s, 3H), 2.08−2.26 (m, 1H), 1.37 (s, 9H), 1.05−1.12 (m, 1H), 0.81−0.87 (m, 6H); HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{15}H_{24}N_2O_4SNa$ 351.1349; found 351.1346.

Cbz-Gly-L-Thr-L-Phe-L-Thr-OMe ***(26)***. Following the general method C and B, compound **26** was obtained from **16** (97 mg, 0.3 mmol) and **18** (114 mg, 0.3 mmol) as a colloidal solid (134 mg, 78% yield); $[\alpha]_D^{25}$−37.2 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 7.36−7.18 (m, 10H), 5.11 (s, 2H), 4.73 (dd, *J* = 8.8, 5.6 Hz, 1H), 4.43 (d, *J* = 3.6 Hz, 1H), 4.30 (d, *J* = 4.0 Hz, 1H), 4.25 (m, 1H), 4.09 (m, 1H), 3.83 (d, *J* = 1.2 Hz, 2H), 3.71 (s, 3H), 3.24 (dd, *J* = 13.6, 5.2 Hz, 1H), 2.98 (dd, *J* = 14.0, 8.8 Hz, 1H), 1.15 (d, *J* = 6.4 Hz, 3H), 1.10 (d, *J* = 6.4 Hz, 3H); ^{13}C{1H} NMR (100 MHz, CD_3OD) *δ*: 172.4, 171.3, 170.9, 170.8, 157.8, 137.1, 136.7, 129.0, 128.2, 128.1, 127.7, 127.5, 126.4, 67.2, 66.8, 66.6, 58.2, 54.7, 51.5, 43.7, 37.0, 18.9, 18.5; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{28}H_{36}N_4O_9Na$ 595.2374; found 595.2375.

Cbz-L-Ala-L-Thr-OMe ***(28)***.[14] The reaction was performed according to the method A using compound **27** (2.0 g, 8.9 mmol) and compound **14** (1.2 g, 8.9 mmol) as substrates and stirred overnight. The crude mixture was purified using EtOAc/petroleum ether = 1:1 as an eluent to give compound **28** (2.5 g, 84%) as a white solid. Data are consistent with a previously characterized compound.[14] mp 128−131 °C; $[\alpha]_D^{25}$−14.8 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.83 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.36−7.12 (m, 5H), 5.03 (d, *J* = 1.6 Hz, 2H), 4.29 (dd, *J* = 8.4, 2.8 Hz, 1H), 4.20 (m, 1H), 4.14 (m, 1H), 3.62 (s, 3H), 1.23 (d, *J* = 7.2 Hz, 3H), 1.06 (d, *J* = 6.4 Hz, 3H); HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{16}H_{22}N_2O_6Na$ 361.1376; found 361.1374.

Cbz-L-Ala-L-Thr(ψ^{Me,Me}Pro)-OMe ***(29)***. According to the method F, the reaction of the dipeptide **28** (203 mg, 0.6 mmol) with 2-methoxypropene/D-(+)-CSA and the crude mixture was purified using EtOAc/petroleum ether = 1:2 as eluent to give the desired pseudoproline protected dipeptide **29** (195 mg, 86%) as a colorless oil. $[\alpha]_D^{25}$−75.2 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.35−7.28 (m, 5H), 5.69, 5.50* (d, *J* = 7.6 Hz, 1H), 5.08 (m, 2H), 4.68*, 4.31 (m, 1H), 4.14 (m, 1H), 4.13*, 4.06 (d, *J* = 6.0 Hz, 1H), 3.76, 3.73* (s, 3H), 1.80*, 1.63 (s, 3H), 1.67*, 1.58 (s, 3H), 1.46, 1.39* (d, *J* = 6.4 Hz, 3H), 1.37*, 1.29 (d, *J* = 6.8 Hz, 3H) (*minor rotamer); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) *δ*: 170.5$^{\#}$, 169.7, 155.3$^{\#}$, 136.6$^{\#}$, 128.5, 128.1, 128.0, 97.2$^{\#}$, 75.0$^{\#}$, 72.9, 66.7$^{\#}$, 65.6, 53.4$^{\#}$, 49.5$^{\#}$, 26.4, 23.8, 19.9$^{\#}$, 18.5 ($^{\#}$minor rotamer); HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{19}H_{26}N_2O_6Na$ 401.1689; found 401.1683.

Boc-L-Val-L-Thr-OMe ***(31)***.[15] The reaction was performed according to the method A using compound **30** (1.5 g, 6.9 mmol) and compound **14** (0.9 g, 6.9 mmol) as substrates, and stirred for 18 h. The crude mixture was purified using EtOAc/petroleum ether = 1:2 as an eluent to give compound **31** (1.8 g, 77%) as a colorless oil. Data are consistent with a previously characterized compound.[15] $[\alpha]_D^{25}$−39.2 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.06 (d, *J* = 8.0 Hz, 1H), 5.31 (d, *J* = 8.0 Hz, 1H), 4.60 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.33 (dd, *J* = 6.4, 2.4 Hz, 1H), 3.94 (t, *J* = 8.8 Hz, 1H), 3.73 (s, 3H), 2.07−2.02 (m, 1H), 1.40 (s, 9H), 1.17 (d, *J* = 6.8 Hz, 3H), 0.97−0.92 (m, 6H); HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{15}H_{28}N_2O_6Na$ 355.1845; found 355.1843.

Cbz-L-Ala-L-Thr(ψ^{Me,Me}Pro)-L-Val-L-Thr-OMe ***(32)***. Following the general method C, **29a** was obtained from **29** (227 mg, 0.6 mmol) and used for the next step without purification. Following the general method E, **31a** was obtained from **31** (202 mg, 0.6 mmol) and used for the next step without purification. Following the general method B, the desired hexapeptide **32** (264 mg, 76% for two steps) was obtained as a glassy solid. EtOAc/petroleum ether (2:1, v/v) was used as an eluent. $[\alpha]_D^{25}$−110.8 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) *δ*: 7.34−7.27 (m, 5H), 5.06 (s, 2H), 4.48 (d, *J* = 2.8 Hz, 1H), 4.30−4.09 (m, 5H), 3.73 (s, 3H), 3.35 (s, 1H), 2.15 (s, 1H), 1.62 (s, 6H), 1.41 (s, 3H), 1.32 (d, *J* = 6.8 Hz, 3H), 1.15 (d, *J* = 6.4 Hz, 3H), 1.04 (t, *J* = 8.0 Hz, 6H); ^{13}C{1H} NMR (100 MHz, CD_3OD) *δ*: 172.6, 171.0, 169.8, 156.2, 136.9, 128.2, 127.7, 127.5,

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96.9, 76.1, 67.1, 66.2, 60.3, 57.9, 51.5, 49.5, 29.9, 25.5, 19.0, 18.5, 17.3; HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{28}H_{42}N_4O_9Na$ 601.2849; found 601.2850.

*Cbz-L-Ala-L-Thr($\psi^{Me,Me}$Pro)-L-Val-L-Thr($\psi^{Me,Me}$Pro)-OMe (**33**).* According to the method G, tetrapeptide **32** (175 mg, 0.3 mmol) reacted with 2,2-dimethoxypropane to give the desired di-pseudoproline protected tetrapeptide **33** (135 mg, 73%) as a colorless oil. EtOAc/petroleum ether (1:1, v/v) was used as an eluent. $[\alpha]_D^{25}$−124.8 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33−7.28 (m, 5H), 5.87 (s, 1H), 5.03 (m, 2H), 4.49 (m, 1H), 4.19 (m, 2H), 4.13 (t, J = 6.8 Hz, 1H), 4.07 (m, 2H), 3.92 (m, 1H), 3.76 (s, 3H), 1.96 (m, 1H), 1.68 (d, J = 5.2 Hz, 3H), 1.65 (s, 3H), 1.64 (d, J = 6.4 Hz, 3H), 1.53 (s, 3H), 1.45 (d, J = 5.6 Hz, 3H), 1.32 (t, J = 7.2 Hz, 6H), 0.93 (d, J = 5.2 Hz, 6H); ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ: 170.6, 169.9, 169.6, 168.3, 154.9#, 136.7#, 128.5, 127.9, 97.2, 74.8, 68.4, 66.2, 57.5#, 53.6, 53.3, 49.8, 48.2, 33.5#, 26.1#, 19.9, 18.9, 18.5 (#minor rotamer); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{31}H_{46}N_4O_9Na$ 641.3162; found 641.3153.

*Cbz-L-Ala-L-Thr($\psi^{Me,Me}$Pro)-L-Val-L-Thr($\psi^{Me,Me}$Pro)-L-Ile-Thz-OAllyl (**34**).* Following the general method C, **33a** was obtained from **33** (100 mg, 0.2 mmol) and used for the next step without purification. Following the general method E, **5a** was obtained from **5** (43 mg, 0.2 mmol) and used for the next step without purification. Following the general method B, the desired hexapeptide **34** (118 mg, 75% for two steps) was obtained as a colorless oil. EtOAc/petroleum ether (1:1, v/v) was used as an eluent. $[\alpha]_D^{25}$−182.8 (c 1.0, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.32 (d, J = 19.2 Hz, 1H), 7.33−7.28 (m, 5H), 6.09−6.00 (m, 1H), 5.41 (m, 1H), 5.28 (m, 1H), 5.11 (d, J = 5.2 Hz, 1H), 5.04 (m, 2H), 4.81 (d, J = 5.6 Hz, 2H), 4.33 (d, J = 7.2 Hz, 1H), 4.24 (m, 2H), 4.15 (m, 1H), 4.08 (m, 2H), 2.09 (m, 2H), 1.80 (s, 1H), 1.75 (s, 1H), 1.62 (d, J = 7.6 Hz, 6H), 1.53 (s, 3H), 1.49 (d, J = 6.4 Hz, 3H), 1.40 (d, J = 5.6 Hz, 3H), 1.34 (q, J = 4.4 Hz, 6H), 1.02 (d, J = 6.8 Hz, 3H), 0.98 (d, J = 6.4 Hz, 3H), 0.94 (d, J = 7.2 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H); ^{13}C{1H} NMR (100 MHz, CD_3OD) δ: 172.3, 170.5, 169.4, 168.8, 161.0, 146.1, 132.2, 128.1, 127.7, 127.5, 117.5, 96.6, 75.9, 75.6, 66.6, 65.5, 57.9, 57.1, 56.3, 49.7, 38.6, 32.4, 29.4, 25.9, 25.3, 25.2, 23.1, 22.5, 18.2, 18.0, 17.8, 16.9, 14.2, 10.8; HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{42}H_{60}N_6O_{10}SNa$ 863.3989; found 863.3984.

*Cyclo-[L-Ala-L-Thr($\psi^{Me,Me}$Pro)-L-Val-L-Thr($\psi^{Me,Me}$Pro)-L-Ile-Thz] (**36**).* Following the method C and method D, linear peptide **34** (126 mg, 0.15 mmol) generates the intermediate **35**. Next, **35** was added to the solution of HATU (114 mg, 0.3 mmol) and HOBt (20 mg, 0.15 mmol) in CH_2Cl_2/DMF (3/1, 150 mL) at room temperature. The mixture (0.001 M) was stirred until TLC showed complete consumption of the starting material (∼24 h). The reaction was quenched by saturated NH_4Cl (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phase was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 30:1) to give compound **36** as a glassy solid (63 mg, 65% for three steps). $[\alpha]_D^{25}$−74.8 (c 1.0, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.12 (s, 1H), 5.01 (q, J = 8.0 Hz, 1H), 4.57 (s, 1H), 4.32 (m, 2H), 4.09 (d, J = 2.0 Hz, 1H), 4.05 (d, J = 7.2 Hz, 1H), 3.82 (d, J = 2.8 Hz, 1H), 2.55 (s, 1H), 2.13 (m, 1H), 1.86 (s, 3H), 1.73 (s, 3H), 1.71 (s, 3H) 1.64 (s, 3H), 1.59 (m, 2H), 1.46 (d, J = 2.8 Hz, 3H), 1.44 (d, J = 2.4 Hz, 3H), 1.39 (d, J = 6.8 Hz, 3H), 0.95 (t, J = 7.2 Hz, 3H), 0.83 (dd, J = 6.8, 5.2 Hz, 6H), 0.75 (d, J = 6.8 Hz, 3H); ^{13}C{1H} NMR (100 MHz, CD_3OD) δ: 171.1, 170.1, 169.1, 168.7, 161.2, 123.8, 98.5, 97.8, 78.3, 76.6, 67.6, 66.9, 57.1, 31.7, 30.5, 30.1, 29.4, 29.4, 29.1, 27.1, 26.4, 25.5, 25.2, 21.5, 18.2, 16.4, 15.3, 9.3; HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{31}H_{48}N_6O_7SNa$ 671.3203; found 671.3197.

*Synthetic Microcyclamide MZ568 (**2**).* To a solution of compound **36** (26 mg, 0.04 mmol) in CH_2Cl_2 (5 mL) was added TFA (1.5 mL) at 0 °C. Then, the mixture was warmed to room temperature and stirred until TLC showed complete consumption of the starting material (∼14 h). The organic phase was concentrated under reduced pressure, and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 10:1) to give **2** as a glassy solid (21 mg, 93%). {Observed for compound **2**: $[\alpha]_D^{25}$−260 (c 0.1, MeOH), lit.[4] microcyclamide MZ568: $[\alpha]_D^{24}$−225 (c 0.03, MeOH)}; ^{1}H NMR (400 MHz, DMSO) δ: 8.50 (d, J = 10.0 Hz, 1H), 8.17 (s, 1H), 7.97 (d, J = 9.2 Hz, 1H), 7.94 (d, J = 5.6 Hz, 1H), 7.59 (d, J = 7.6 Hz, 2H), 5.04 (d, J = 3.2 Hz, 1H), 5.02 (s, 1H), 5.00−4.97 (m, 1H), 4.57 (dd, J = 10.0, 7.2 Hz, 1H), 4.30 (d, J = 3.2 Hz, 1H), 4.27 (d, J = 2.8 Hz, 1H), 4.15 (q, J = 6.0 Hz, 1H), 3.84 (t, J = 5.2 Hz, 1H), 3.79 (dd, J = 7.6, 5.6 Hz, 1H), 2.18−2.09 (m, 2H), 1.57−1.50 (m, 1H), 1.45 (d, J = 7.2 Hz, 3H), 1.14−1.07 (m, 1H), 1.13 (d, J = 6.0 Hz, 3H), 1.08 (d, J = 6.4 Hz, 3H), 0.93 (d, J = 6.8 Hz, 6H), 0.86 (t, J = 7.6 Hz, 3H), 0.79 (d, J = 6.8 Hz, 3H); ^{13}C{1H} NMR (100 MHz, DMSO) δ: 171.9, 171.2, 170.8, 169.6, 167.8, 161.2, 150.1, 123.5, 67.8, 65.2, 62.0, 59.9, 57.6, 53.8, 49.9, 38.4, 29.6, 24.8, 21.0, 19.9, 19.1, 17.8, 17.6, 15.4, 10.9; HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{25}H_{40}N_6O_7SNa$ 591.2571; found 591.2572.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c02541.

X-ray crystallographic data for **36**; comparison of spectroscopic data of our synthetic natural products with isolation reports; reference; data of control experiments; and copies of NMR spectra of synthesized compounds **1**−**4**, **11**, **12**, **16**−**21**, **23**, **28**, **29**, **31**−**34**, and **36** (PDF)

FAIR Data and including the primary NMR FID files for compounds **1**−**5**, **11**, **12**, **16**−**21**, **23**−**26**, **28**, **29**, **31**−**34**, and **36** (ZIP)

Accession Codes

CCDC 2038866 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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