

Total Synthesis and Structural Revision of Cyclotetrapeptide Asperterrestide A

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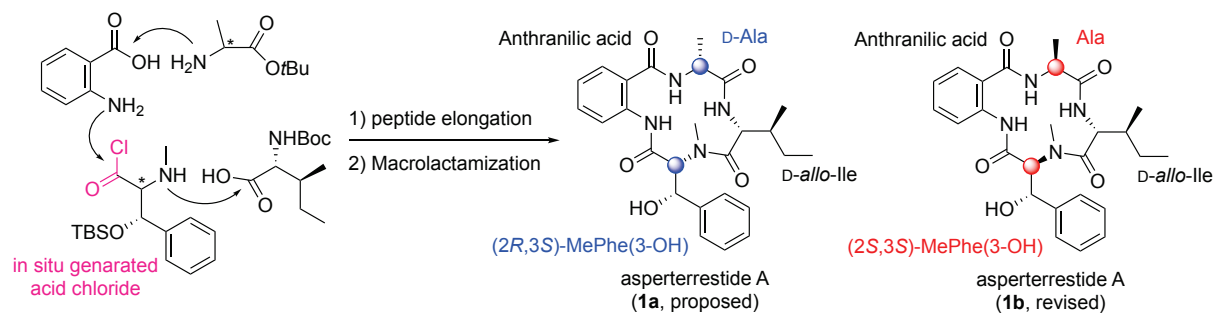
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Abstract Graphic



Abstract

The structural revision of cyclotetrapeptide asperterrestide A has been achieved based on the total synthesis and molecular modeling. For these studies, (2*R*,3*S*)-MePhe(3-OH) and (2*S*,3*S*)-MePhe(3-OH) suitably protected for peptide synthesis, were prepared via a stereoselective reduction of a ketone precursor, derived from L- or D-serine, using L-Selectride or DIBAL-H. The synthesis of the proposed structure of asperterrestide A (**1a**) was accomplished by solution-phase synthesis of a linear precursor followed by macrolactamization. The NMR spectra of our synthetic **1a** were not identical to those reported for the natural compound. Molecular modeling studies suggested that the correct structure (**1b**) was one in which the stereochemistry at the α -positions of the Ala and MePhe(3-OH) residues is the opposite to that of the proposed structure. This was confirmed by the total synthesis of **1b** and its subsequent structural characterization.

INTRODUCTION

Naturally occurring cyclopeptides exhibit unique biological activities and often possess nonproteinogenic amino acid residues: β -amino acids, D-amino acids, *N*-methylamino acids, and other highly functionalized amino acids.^{1,2} Among them, anthranilic acid (Ant)-containing peptides are known to be attractive three-dimensional scaffolds of peptidomimetics because anthranilic acid is one of the most rigid β -amino acids and gives conformational rigidity to the corresponding cyclic and even linear peptides.^{3–9}

Asperterrestide A (**1**), a cyclotetrapeptide isolated from a fermentation broth of marine-derived fungal strain *Aspergillus terreus* SCSGAF0162 in 2013,¹⁰ was reported to consist of four nonproteinogenic amino acids: D-Ala, Ant, (2*R*,3*S*)-MePhe(3-OH), and D-Ile or D-*allo*-Ile (Figure 1). The stereochemistry at the α -positions of Ala and Ile was determined by chiral-phase HPLC analysis and Marfey's methods after acidic degradation, whereas the D-Ile or D-*allo*-Ile residue was not differentiated in the original report. Asperterrestide A (**1**) exhibits cytotoxicity for human carcinoma cell lines with IC₅₀ values of 6.4 μ M against U937 and 6.2 μ M against MOLT4 and inhibits influenza virus strains with IC₅₀ values of 15 μ M against A/WSN/33 (H1N1) and 8.1 μ M against A/Hong Kong/8/68 (H3N2), respectively. Because Ant-containing 13-membered cyclotetrapeptides are rare in nature¹¹ compared with cyclopenta-^{12–14} and cyclohexapeptides,^{15–17} it is worthwhile to reveal the three-dimensional macrocyclic conformation of **1** for the design of novel peptidomimetics. Herein, we report the total synthesis of asperterrestide A (**1**) via solution-phase peptide elongation, followed by macrolactamization and its structural revision based on the molecular modeling and NMR analysis of the synthesized peptides.

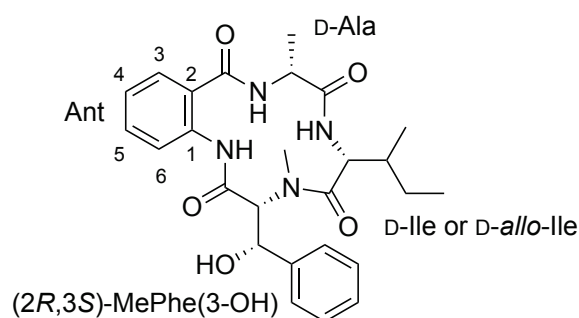
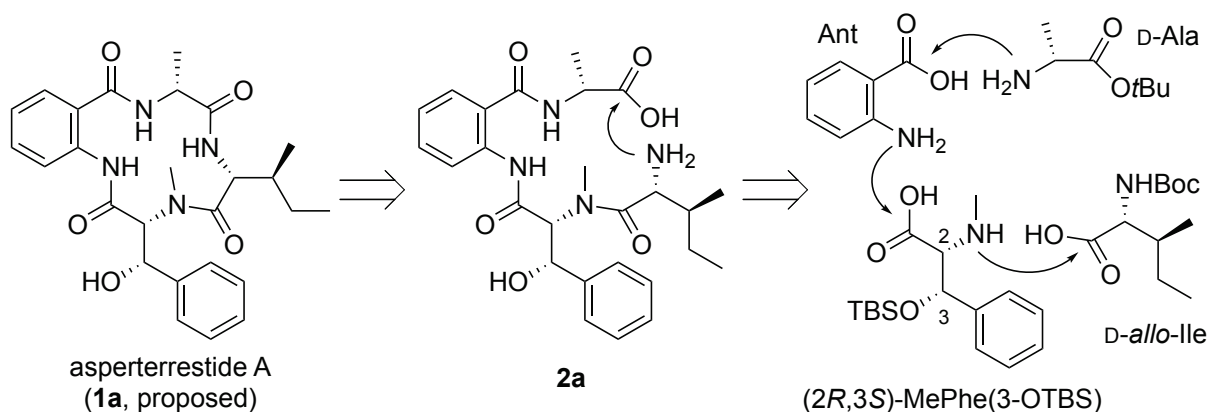


Figure 1. Reported structure of asperterrestide A (**1**)

RESULTS AND DISCUSSION

There have been recent reports for the structure elucidation of ambiguous configuration of enantiomeric isoleucine residues to be *D-allo*-Ile in cyclic peptide natural products¹⁸⁻²⁰ and it would be likely produced by the epimerization of L-Ile at the α -position in secondary metabolites not that at both α - and β -positions, though some examples having a *D*-Ile residue as a component are in literature.^{21,22} Cyclo-[*D-allo*-Ile-(2*R*,3*S*)-MePhe(3-OH)-Ant-D-Ala] (**1a**), thus, was speculated to be the structure of natural asperterrestide A. Our retrosynthetic analysis for **1a** is illustrated in Scheme 1. The macrolactamization is a key step for the total synthesis of **1a**. The cyclization site should be carefully selected considering i) the nucleophilicity of N-terminus and ii) possible side reactions after the activation of C-terminus. Aromatic amines involved in resonance and sterically hindered *N*-methyamines are generally inappropriate as the N-terminus of the macrolactamization site due to low nucleophilicity. Besides, the activated carboxylic acid group of an Ant residue might induce benzoxazinone formation during the cyclization process.^{7, 23, 24} Therefore, we planned the macrolactamization between N-terminus *D-allo*-Ile and C-terminus D-Ala of the linear tetrapeptide **2a**. Furthermore, we anticipated that the conformationally rigid Ant residue would be turn-inducing and support the access of both reaction sites. The cyclization precursor **2a** would be provided by peptide elongation using four amino acids: *D-allo*-Ile, (2*R*,3*S*)-MePhe(3-OH), Ant and D-Ala.

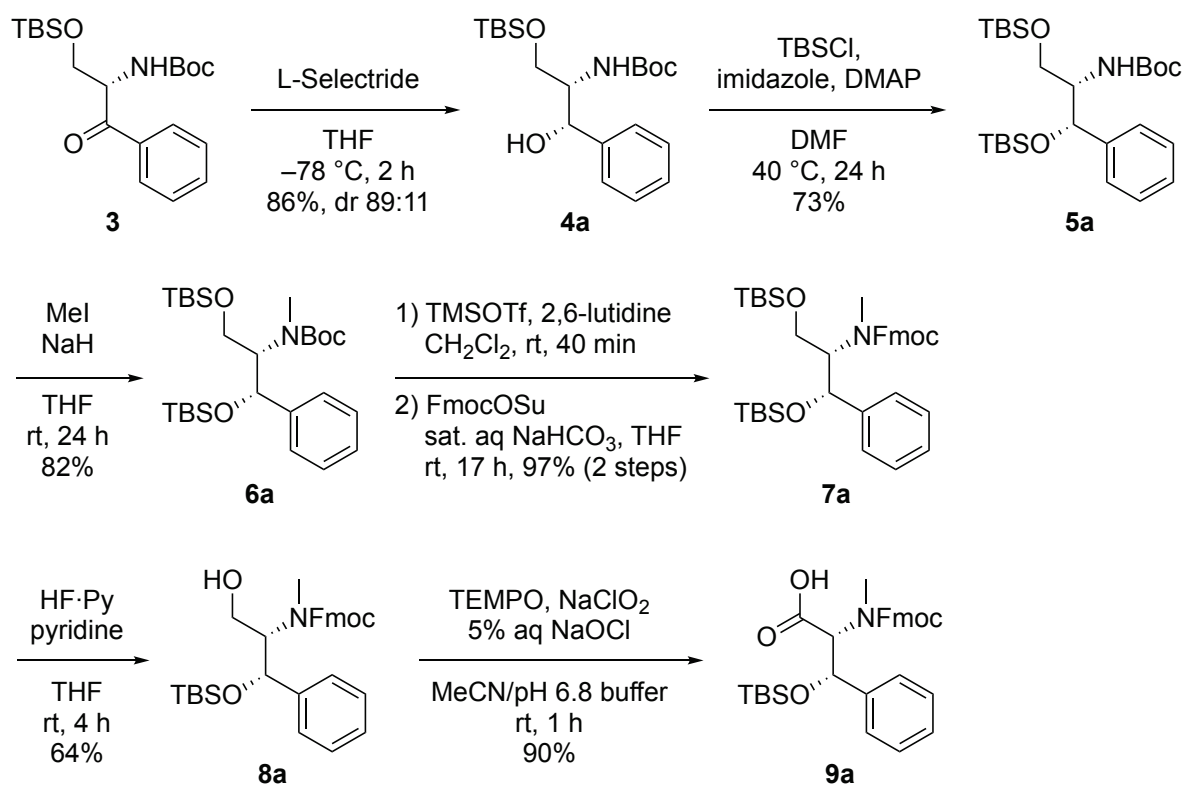
Scheme 1. Retrosynthetic analysis of the proposed structure of asperterrestide A (**1a**)



Our study began from the stereoselective synthesis of a nonstandard (2*R*,3*S*)-MePhe(3-OH) residue. Structurally similar β -hydroxy-*p*-iodo-*N*-methylphenylalanine derivatives bearing *anti* configurations have been synthesized by Boger and coworkers via asymmetric epoxidation or dihydroxylation of cinnamic acid derivatives;²⁵ however, there are no reports describing the synthesis of *N*-methylated *syn* derivatives. The nucleophilic addition of alkyl metal species to the Garner's aldehyde is one of the reliable methods to give facile and stereoselective access to 2-amino-1,3-dihydroxypropyl substructures.²⁶ However, highly reactive organometallic species react with the Garner's aldehyde to give a mixture of *syn* and *anti* products with low diastereomeric ratios (dr) due to a competition between steric (Felkin-Anh) and chelation effects. Therefore, two additional steps (oxidation of the hydroxy group to the ketone followed by stereoselective reduction of the ketone back to the alcohol) are required to give the *syn* or *anti* product in high dr.^{27–29} Thus, a step-economical and stereoselective approach to (2*R*,3*S*)-MePhe(3-OH) is desirable.

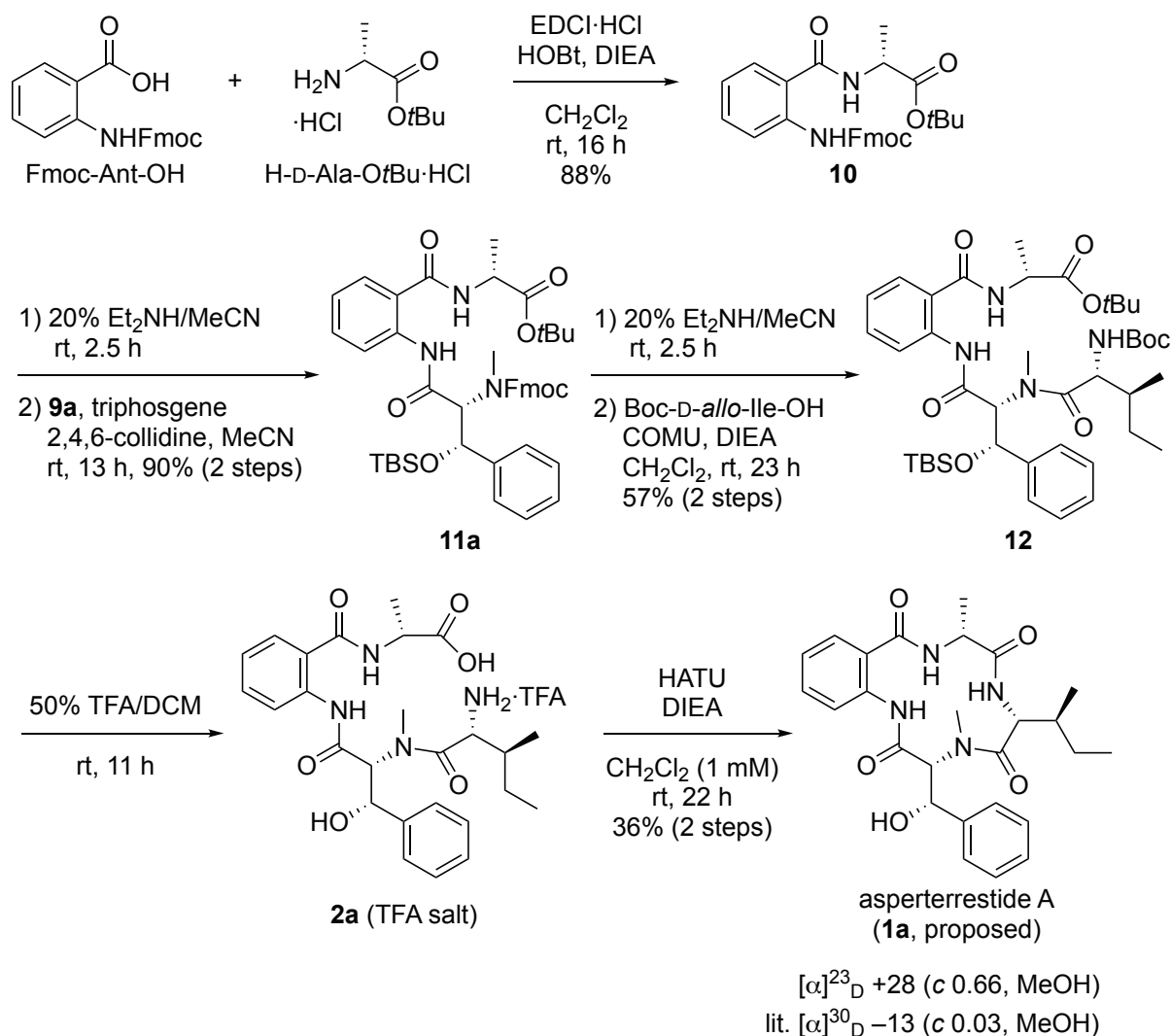
Our synthetic approach to Fmoc-(2*R*,3*S*)-MePhe(3-OTBS)-OH (**9a**) is shown in Scheme 2. In initial studies for the synthesis of **9a**, we found that the late-stage *N*-methylation of β -hydroxyphenylalanine derivatives had poor reproducibility due to side reactions such as β -elimination of the hydroxy group under acidic and basic conditions. Therefore, we chose to introduce a methyl group on the nitrogen in the absence of a carboxyl group. Starting from commercially available Boc-L-Ser-OH, the phenyl ketone **3** was prepared according to the reported

procedures by Appella and coworkers.³⁰ The stereoselective reduction of the ketone **3** via the Felkin-Anh model successfully proceeded with L-Selectride, providing *syn*-enriched **4a** in 86% yield (*syn:anti* = 89:11). After the TBS-protection of the resulting alcohol in **4a**, the methylation of the amino group in **5a** using excess amounts of NaH and MeI³¹ furnished the *N*-methylcarbamate **6a**. The Boc group in **6a** was removed by TMSOTf/2,6-lutidine^{32, 33} without losing acid-labile TBS groups, and the subsequent protection with an Fmoc group gave the Fmoc amine **7a** in 97% yield over two steps. The selective cleavage of the primary TBS ether in **7a** using HF·pyridine was then carried out. The *syn*-product and minor *anti*-diastereomer were separated by silica gel column chromatography in this step, and the alcohol **8a** was obtained in 64% yield as a single diastereomer. Finally, the oxidation of the primary alcohol using TEMPO/NaOCl/NaClO₂³⁴ furnished the desired carboxylic acid **9a**.



With synthetic **9a** in hand, compound **1a** was prepared as outlined in Scheme 3. H-D-Ala-O*t*Bu and Fmoc-Ant-OH were coupled with EDCI·HCl/HOBt to give the dipeptide **10** in 88% yield. The benzoxazinone formation was not observed under this coupling condition. After removal of the Fmoc group in **10** with 20% Et₂NH/MeCN, we performed the acylation of the low-nucleophilic Ant amine with sterically hindered carboxylic acid **9a**. The in situ generated amino acid chloride of **9a** using triphosgene/2,4,6-collidine^{35–38} is useful for the acylation of the aromatic amine, and the desired tripeptide **11a** was obtained in 90% yield without epimerization at the α-position. The Fmoc group in **11a** was removed in the same manner as described above, and we next attempted the coupling between the sterically hindered *N*-methylamine and bulky Boc-D-*allo*-Ile-OH. After trials of several coupling reagents (HATU/DIEA, PyBroP/DIEA, triphosgene/2,4,6-collidine, and COMU/DIEA), we found that COMU completed the coupling reaction to provide the tetrapeptide **12** in 57% yield. The concomitant removal of the Boc, *tert*-butyl, and TBS groups in **12** with 50% TFA in CH₂Cl₂ furnished the cyclization precursor **2a** as its TFA salt. Subsequent macrolactamization of **2a** was performed using HATU/DIEA under high dilution conditions (1 mM). No epimerization at the α-position of the Ala residue was observed during the cyclization process, and the resulting crude cyclopeptide was purified by normal-phase silica gel column chromatography and preparative thin-layer chromatography to isolate the proposed structure of asperterrestide A (**1a**) in 36% yield from **12**.

Scheme 3. Synthesis of the proposed structure of asperterrestide A (1a)



The ^1H and ^{13}C NMR spectroscopic data of synthetic **1a**, however, are not identical to those of natural asperterrestide A, as shown in Figure 2 and Table S1 (Supporting Information). In particular, the chemical shifts and coupling constants on the main chain (NH, NMe, CH_α and C=O) are hardly matched between natural **1** and synthetic **1a**. Besides, NOESY correlations between MePhe(3-OH) H_β / MePhe(3-OH) NMe, and Ala H_α / Ile NH, which were observed in the natural compound, were not found in synthetic **1a** (see Supporting Information). We suspected that the stereochemistry at the α -carbons in natural asperterrestide A is different from the proposed structure of **1a** because the sign of the specific rotation was mismatched (see Scheme 3). The HPLC analysis of acid hydrolysates of natural **1** reported by Qi¹⁰ was reexamined, and it was found that the absolute configuration

of the Ala residue was unclear. Chiral HPLC analysis with authentic L-Ala and D-Ala did not show clear differences in their retention times. 1-Fluoro-2,4-dinitrophenyl-5-alanine-amide (FDAA) derivatives of the acid hydrolysates might suggest containing both L-Ala and D-Ala on the HPLC chromatogram. Because the epimerization of the α -position during hydrolysis process was indicated, it is not possible to prove the absolute configuration of the Ala residue. In addition, the stereochemistry of the α -position of (2*R*,3*S*)-MePhe(3-OH) is ambiguous. The stereochemistry of the β -position is assigned to be *S* using the modified Mosher's ester method for the natural compound. On the other hand, there is no spectroscopic evidence that a large coupling constant ($^3J_{\text{H,H}} = 10.0$ Hz) between α - and β -protons of the MePhe(3-OH) residue suggests a *syn* arrangement in conformationally constrained cyclotetrapeptides because the relative configuration was deduced in reference to previously reported β -hydroxy-Tyr-containing cyclooctapeptides³⁹ and other acyclic fragments ($^3J_{\text{H,H}} = 3.5\text{--}4.8$ Hz for *anti* and 4.6–6.6 Hz for *syn*, respectively).^{40–42} Jung's group has recently reported that a large coupling constant ($^3J_{\text{H,H}} = 9.5$ Hz) between α - and β -protons of the MePhe(3-OH) residue was observed in a 12-membered cyclotetrapeptide named tentoxin B.⁴³ The absolute configuration was assigned to be (2*S*, 3*S*) by the modified Mosher's method followed by the comparison of the lowest energy-conformer obtained by molecular modeling to the X-ray crystallographic structure of its dehydroxy analog. These analytical reports by Jung and the fact that our synthetic *syn*-product **1a** showed a small coupling constant ($^3J_{\text{H,H}} = 2.5$ Hz) strongly support the relative configuration of natural **1** to be *anti*. Therefore, the absolute configuration in the MePhe(3-OH) should be (2*S*,3*S*), not (2*R*,3*S*). In summary, we deduced the absolute configuration of the natural asperterrestide A to be cyclo-[D-*allo*-Ile-(2*S*,3*S*)-MePhe(3-OH)-Ant-Ala] **(1b)** or cyclo-[D-*allo*-Ile-(2*S*,3*S*)-MePhe(3-OH)-Ant-D-Ala] **(1c)** (Figure 3).

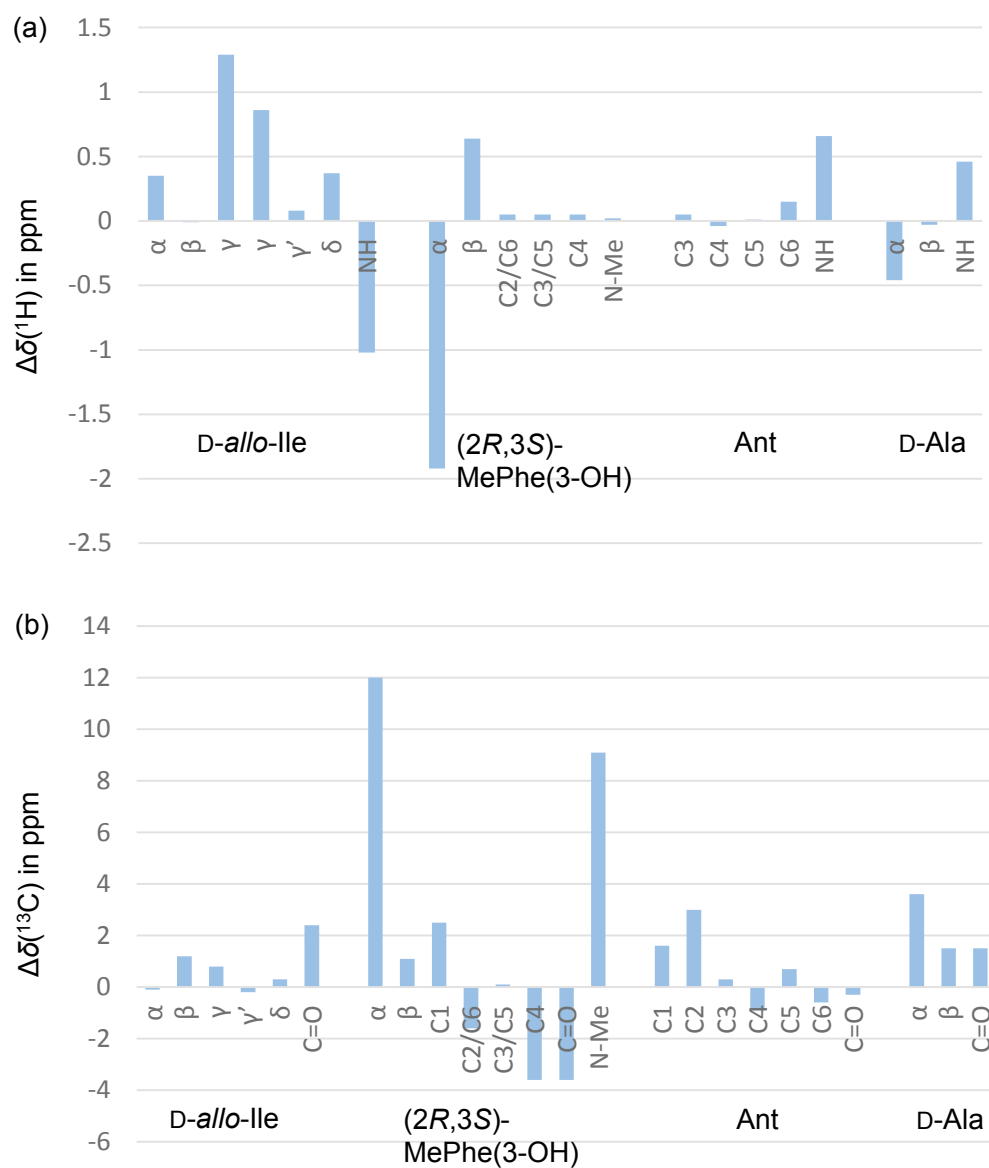


Figure 2. (a) $\Delta\delta(^1\text{H})$, and (b) $\Delta\delta(^{13}\text{C})$ values of constituting four amino acids in synthetic **1a** in CDCl_3 , $\Delta\delta = \delta_{\text{synthetic } \mathbf{1a}} (\text{ppm}) - \delta_{\text{natural } \mathbf{1}} (\text{ppm})$. Table S1 (Supporting Information) summarizes NMR spectroscopic data of **1a**.

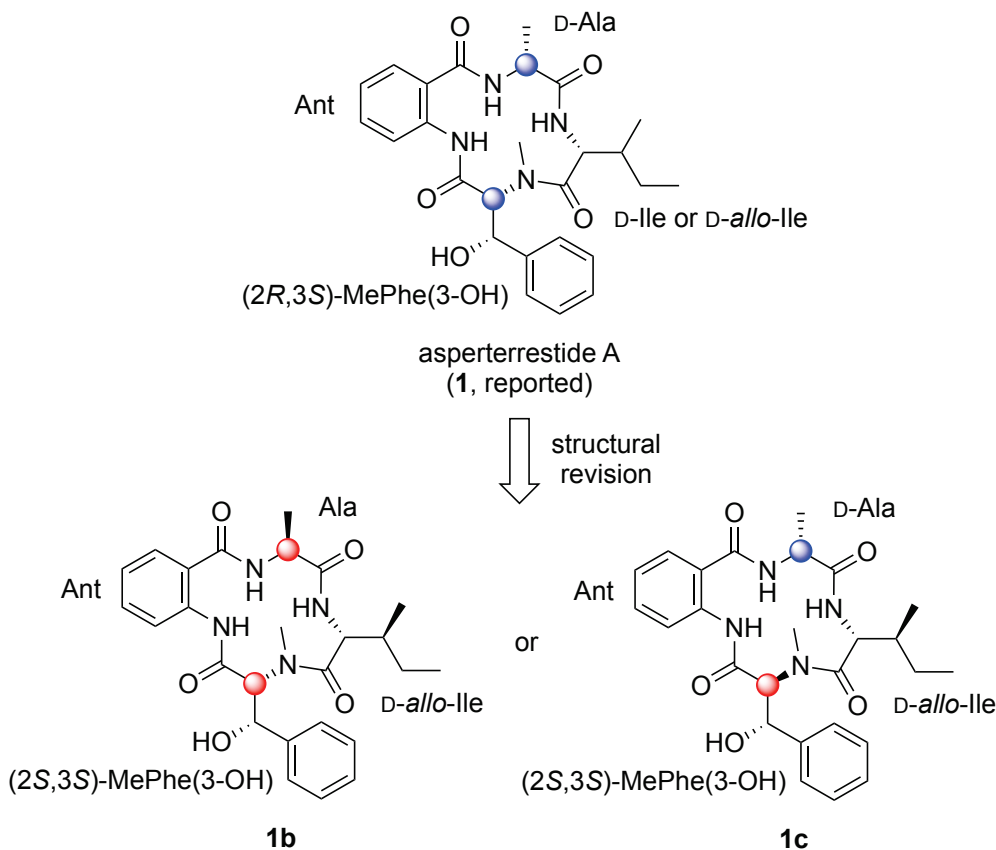


Figure 3. Putative structures of natural asperterrestide A (**1**)

To narrow down the putative candidates of natural asperterrestide A, we conducted molecular modeling studies using the MacroModel program (Maestro Version 10.1.018).^{44–46} The correlation between the observed NMR spectroscopic data and calculated values by the molecular modeling was verified using our synthetic **1a** prior to putative **1b** and **1c**. The *sec*-butyl group in the Ile residue was replaced with an isopropyl group to simplify the calculation; thus, cyclo-[D-Val-(2*R*,3*S*)-MePhe(3-OH)-Ant-D-Ala] (**1d**) was selected as a model cyclotetrapeptide of **1a** (Figure 4a). A conformational search was performed using 10,000 steps of torsional sampling based on the mixed-torsional/low-mode sampling method. An OPLS-2005 force field was applied, and the calculation was conducted without solvents. The obtained lowest-energy conformer of **1d** is shown in Figure 4b. The calculated dihedral angles in **1d** were then converted to coupling constants based on the Karplus equation.⁴⁷ Focused on $J_{\text{H,H}}$ coupling constants involved in the main chain, the calculated values in **1d** were

found to be in good agreement with those observed in our synthetic **1a**, not the natural product (Table S9, Supporting Information). Theoretical distances between proximal protons in **1d** also fit the result of NOE correlations observed in **1a**. Among five NOE correlations observed in the natural **1**, the calculated distances in **1d** between MePhe(3-OH) H_β / MePhe(3-OH) NMe (4.36 Å) and Ala H_α / Val NH (3.55 Å) seem to be long to get NOE correlations as well as **1a** (Table S10, Supporting Information). These main chain-derived correlations should be important to define the three-dimensional structures of conformationally constrained cyclopeptides. Therefore, we concluded that the molecular modeling using the model tetrapeptide is a reliable and adoptable method for estimating the stereochemistry of natural asperterrestide A.

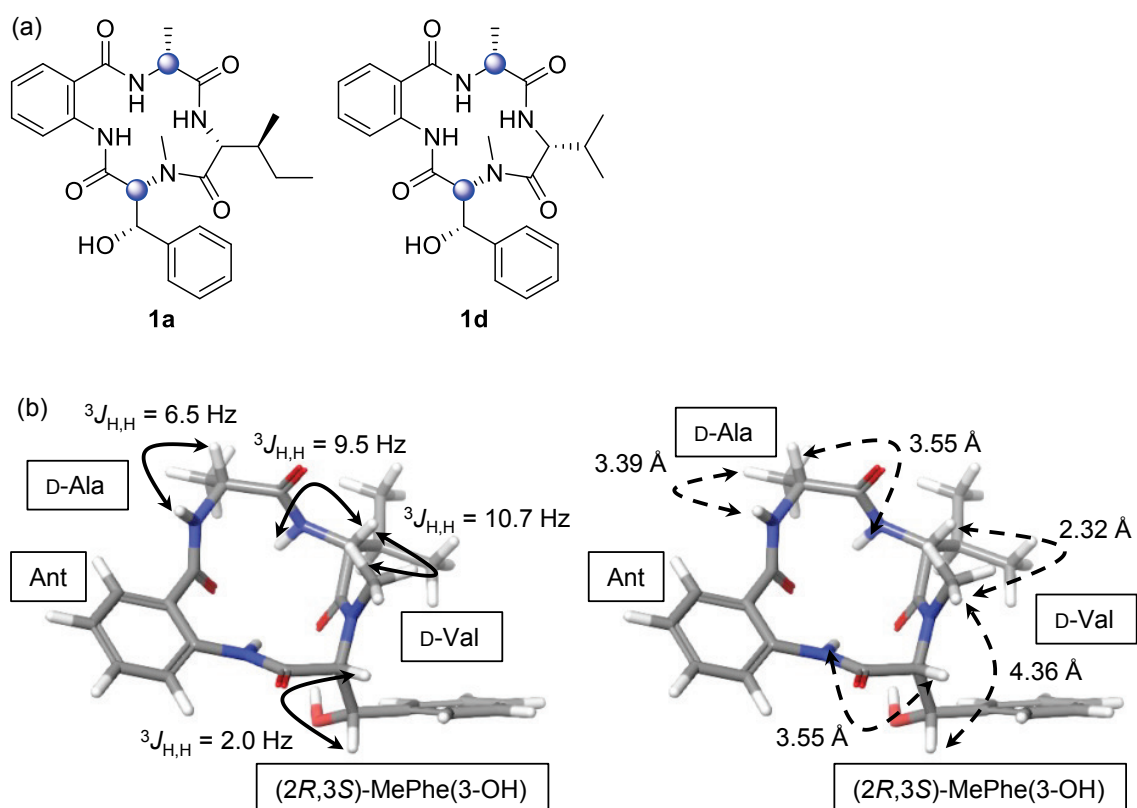


Figure 4. (a) Chemical structures of the synthetic **1a** and its model tetrapeptide **1d**. (b) The lowest-energy conformer of **1d**. The calculated distinctive coupling constants (plain, left) and theoretical distances between proximal protons (dashed, right) are shown in double-headed arrows. Tables S9 and S10 (Supporting Information) summarize calculated data of **1d**.

According to the procedure mentioned above, the molecular modeling of our putative structures of asperterrestide A was carried out. Conformational searches of cyclo-[D-Val-(2*S*,3*S*)-MePhe(3-OH)-Ant-Ala] (**1e**) and cyclo-[D-Val-(2*S*,3*S*)-MePhe(3-OH)-Ant-D-Ala] (**1f**) (Figure 5a) that are corresponding model tetrapeptides of **1b** and **1c** gave the lowest-energy conformers as shown in Figures 5b and 5c. The calculated dihedral angles and distances between proximal protons are summarized in Tables S11 and S12 (Supporting Information), respectively. Focused on the MePhe(3-OH), the calculated $^3J_{\text{H,H}}$ coupling constants between MePhe(3-OH) H $_{\alpha}$ / MePhe(3-OH) H $_{\beta}$ in both **1e** and **1f** ($^3J_{\text{H,H}}$ = 9.5 Hz, respectively) are as large as that of the natural **1** ($^3J_{\text{H,H}}$ = 10.0 Hz). In addition, all the theoretical distances of the protons involved in NMe and H $_{\alpha}$ of the MePhe(3-OH) residue in **1e** and **1f** are relatively short within 3.0 Å, satisfying the NOE observation in the natural compound. These calculated results strongly supported our suggestion that the absolute configuration of the MePhe(3-OH) residue in the natural **1** should be (2*S*,3*S*). The calculated values related to the Ala residue in **1e** are also in good agreement with those of the natural **1**, whereas relatively small $^3J_{\text{H,H}}$ coupling constants between Ala NH / Ala H $_{\alpha}$ ($^3J_{\text{H,H}}$ = 6.5 Hz) and long distances between Ile NH / Ala H $_{\alpha}$ (3.55 Å) and Ala NH / Ala H $_{\beta}$ (3.39 Å) were found in D-Ala-containing **1f**. Therefore, the component in the natural product was speculated to be L-Ala, not D-Ala. Considering these results, we argued that cyclo-[D-Val-(2*S*,3*S*)-MePhe(3-OH)-Ant-Ala] (**1e**) would follow the stereochemistry of natural asperterrestide A, and the stereochemistry of natural asperterrestide A should be corresponding to cyclo-[D-*allo*-Ile-(2*S*,3*S*)-MePhe(3-OH)-Ant-Ala] (**1b**).

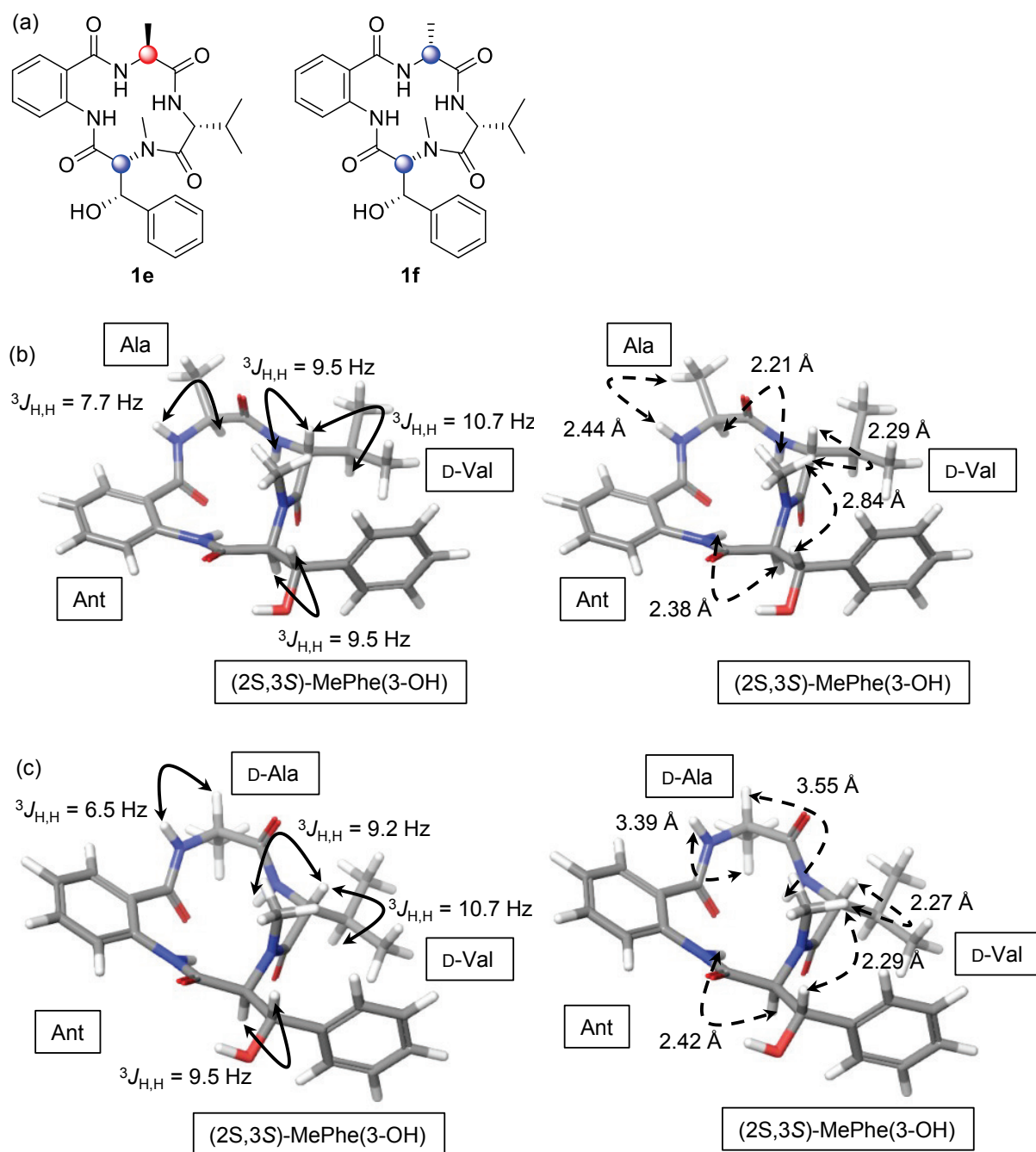
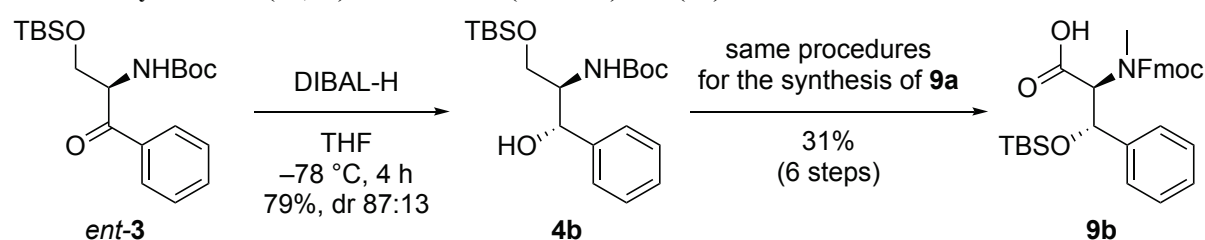


Figure 5. (a) Chemical structures of model tetrapeptides **1e** and **1f**. (b) The lowest-energy conformer of **1e**. (c) The lowest-energy conformer of **1f**. The calculated distinctive coupling constants (plain, left) and theoretical distances between proximal protons (dashed, right) are shown in double-headed arrows. Tables S11 and S12 (Supporting Information) summarize calculated data of **1e** and **1f**.

To validate our revised structure of natural asperterrestide A, it was necessary to prepare (2*S*,3*S*)-MePhe(3-OH) (Scheme 4). Conditions had to be developed such that the stereoselective reduction of the ketone *ent*-**3**, prepared from Boc-D-Ser-OH, provided *anti* product **4b** instead of the previously obtained *syn* product **4a**. After investigation of several reductants (DIBAL-H and LiAlH(*O*tBu)₃)⁴⁸ and conditions (solvents and temperatures), the chelation-controlled reduction of *ent*-**3** was found to be successful using DIBAL-H to afford *anti*-enriched **4b** in 79% yield with good stereoselectivity (*anti*:*syn* = 87:13). Further conversions, including *N*-methylation and oxidation, were conducted according to the established procedure as that of **9a** (see Experimental Section), providing the desired (2*S*,3*S*)-**9b**. We herein established the synthetic route for both *syn* and *anti* diastereomers of **9** by simply switching the reductants for the phenylketone **3**.

Scheme 4. Synthesis of (2*S*,3*S*)-Fmoc-MePhe(3-OTBS)-OH (9b**)**



The synthesis of the revised structure of asperterrestide A (**1b**) was carried out as illustrated in Scheme 5. In situ generation of the amino acid chloride of **9b** using triphosgene/2,4,6-collidine was also adopted for the acylation of the corresponding aromatic amine of *ent*-**10** regardless of the relative configuration of the MePhe(3-OH) residue, and the tripeptide **11b** was obtained in 88% yield. In the next peptide elongation, we initially attempted the synthesis of the tetrapeptide from **11b** similar to the preparation of **12**. However, the coupling of the corresponding *N*-methylamine of **11b** with Boc-D-*allo*-Ile-OH did not proceed under various coupling conditions (HATU/DIEA, PyBroP/DIEA, triphosgene/2,4,6-collidine, and COMU/DIEA) but recovered the *N*-methylamine. Therefore, we decided to remove the TBS group in **11b** before the peptide

elongation. Exposure of the β -hydroxyl group would not only help the approach of activated carboxylic acid due to less-steric bulkiness but also give another pathway, such as *O*-to-*N* acyl transfer, as well. The TBS group in **11b** was removed by HF·pyridine to afford the alcohol **13** in 76% yield. After removal of the Fmoc group in **13**, the coupling between the resulting *N*-methylamine and Boc-D-*allo*-Ile-OH was investigated, and the details are summarized in Table 1. The best results were obtained using COMU/DIEA, which gave the tetrapeptide **14** in a 45 % yield along with a small amount of the *O*-acylated compound **15**, after separation by silica gel column chromatography followed by preparative TLC (entry 1). The combined yield of **14** and **15** was improved using highly reactive acid chlorides generated from triphosgene/2,4,6-collidine, whereas the *O*-acylation was prior to the *N*-acylation to give both the amide **14** in 28% yield and the ester **15** in 34% yield, respectively (entry 2). The facts that the *O*-to-*N* acyl transfer from isolated **15** to **14** did not proceed even under harsh conditions (DMSO, 180 °C under microwave irradiation, 5 min \times 6 times) indicated that the amide bond in **14** was directly formed without going through the ester **15**. DMT-MM^{49, 50} gave only **14** as the product; however, the yield was very low (12%, entry 3).

Although the yield of the tetrapeptide **14** was disappointing, we continued the synthesis to determine the absolute configuration of **1b** (Scheme 5). The revised structure of asperterrestide A (**1b**) was obtained by treating **14** with TFA/DCM, followed by cyclization of the resulting TFA salt **2b** using HATU/DIEA under high dilution conditions, and subsequent purification (41% yield over two steps). The epimer at the Ala residue was not observed in the crude cyclization product.

Scheme 5. Synthesis of the revised structure of asperterrestide A (**1b**)

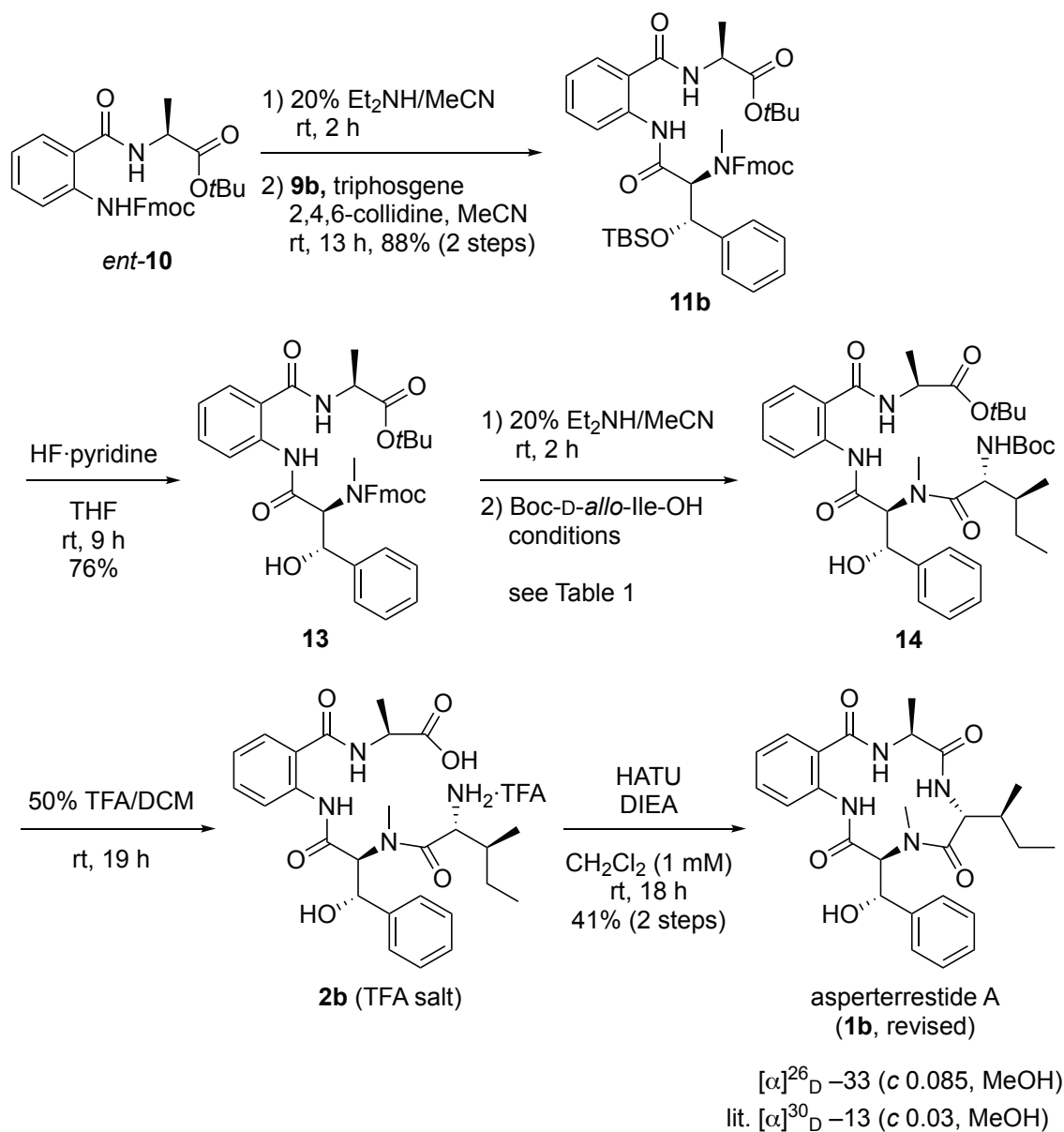
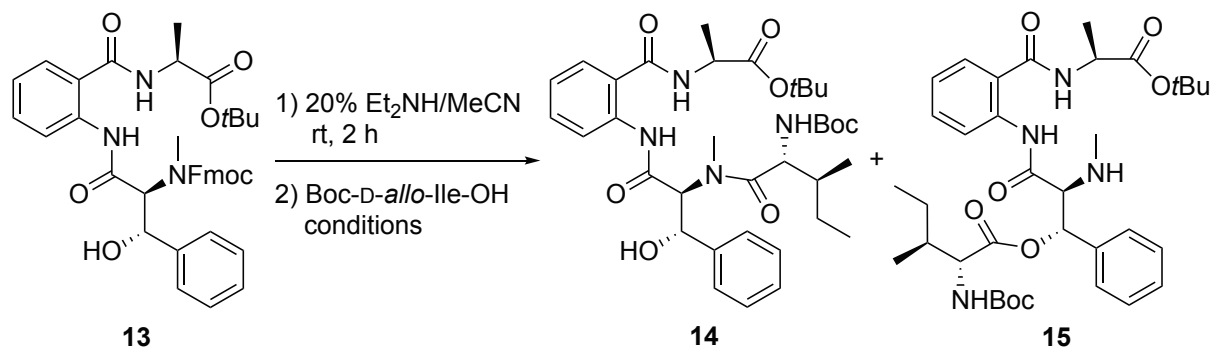


Table 1. Peptide elongation from the tripeptide 13 to the tetrapeptide 14



Entry	Reagents	Temp. (°C)	Time (h)	Product ^a (Yield%)	Ratio of 14/15 ^b
1 ^c	COMU, DIEA	rt	26	14 (45), 15 (7)	88:12
2 ^d	triphosgene 2,4,6-collidine	rt	19	14 (28), 15 (34)	38:62
3 ^e	DMT-MM·nH ₂ O	60	48	14 (12)	>95:5

^a Isolated yield. ^b The ratio was determined by crude ¹H NMR. ^c Performed in DMF. ^d Performed in MeCN. ^e Performed in MeOH.

The ¹H and ¹³C NMR spectroscopic data of synthetic **1b** are in good agreement with those of the natural compound, except for the chemical shift of concentration-dependent amide proton of the Ala and Ile residues, as shown in Figure 6 and Table S2 (Supporting Information). Large ³J_{H,H} coupling constants between MePhe(3-OH) H_α / MePhe(3-OH) H_β (³J_{H,H} = 9.5 Hz) and Ala H_α / Ala NH (³J_{H,H} = 7.9 Hz) were observed in synthetic **1b** as well as those of the natural **1** (³J_{H,H} = 10.0 and 8.0 Hz, respectively). In addition, NOE correlations between MePhe(3-OH) H_β / MePhe(3-OH) NMe and Ala H_α / Ile NH were observed in **1b**, which were not found in **1a** (see Supporting Information). It should be noted that the spectroscopic data observed in the synthetic **1b** were fully satisfied with not only those of the natural compound but also those suggested by the molecular modeling using the model tetrapeptide **1e**. We thus concluded the absolute configuration of natural **1** to be cyclo-[D-*allo*-Ile-(2*S*,3*S*)-MePhe(3-OH)-Ant-Ala].

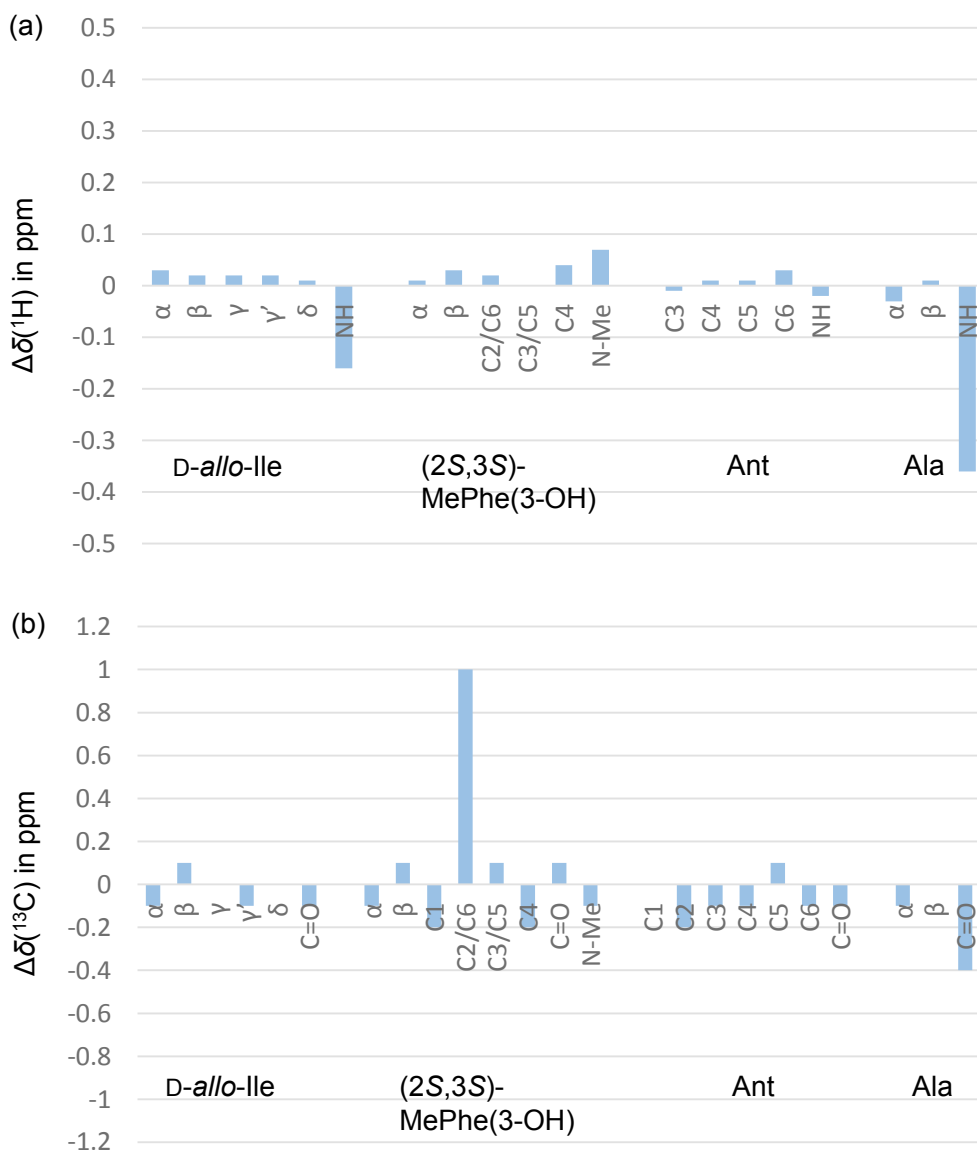


Figure 6. (a) $\Delta\delta(^1\text{H})$, and (b) $\Delta\delta(^{13}\text{C})$ values of constituting four amino acids in synthetic **1b** in CDCl_3 , $\Delta\delta = \delta_{\text{synthetic } \mathbf{1b}} (\text{ppm}) - \delta_{\text{natural } \mathbf{1}} (\text{ppm})$. Table S2 (Supporting Information) summarizes NMR spectroscopic data of **1b**.

The cytotoxicity of the synthetic **1a** and **1b** against three selected cancer cell lines (U937, MOLT4 and A549) was evaluated by CellTiter-Glo2.0 assay after 72 h treatments, and their IC_{50} values are depicted in Table 2. The synthetic **1a**, the proposed structure of asperterrestide A, did not show sufficient potency against all three cancer cell lines, thereby it is clear that the structure of **1a** is not that of the natural compound. On the other hand, the

synthetic **1b** exhibited potent activity against U937 and MOLT4 cells with the IC₅₀ values of 5.6 and 18.1 μ M, respectively, which are comparable with those of the natural compound.¹⁰ No cytotoxicity of **1b** against A549 cells might also be similar as the value evaluated for the natural compound was not indicated.⁵¹ These results also suggested that the structure of asperterrestide A should be **1b**. It should be noted that the stereochemistry of the main chain is essential for the potent activity in the constrained cyclotetrapeptides.

Table 2. IC₅₀ values of cytotoxicity of natural and synthetic asperterrestides against cancer cell lines

Cancer cell line tissue	IC ₅₀ (μ M) ^a			
	Natural 1 ^b	Synthetic 1a (proposed)	Synthetic 1b (revised)	Mitomycin C ^c
U937 blood	6.4	>20	5.6	0.067
MOLT4 blood	6.2	>20	18.1	0.031
A549 lung	not indicated	>20	>20	0.19

^a The IC₅₀ values were determined by derivation of the best-fit dose response line of triplicate experiments. ^b

Data extracted from ref 10. ^c Mitomycin C was evaluated as a control.

CONCLUSION

In summary, we have demonstrated the structural revision of natural asperterrestide (**1**) based on the total synthesis and molecular modeling. Suitably protected D-*syn*-N-methyl- β -hydroxyphenylalanine **9a** was prepared as a single isomer via the stereoselective reduction of the phenyl ketone **3**. The established synthetic route was adopted to synthesize L-*anti*-N-methyl- β -hydroxyphenylalanine **9b** by selecting the reductants for the phenyl ketone *ent*-**3**. During the peptide elongation process to the tetrapeptide **12**, the use of the in situ generated amino acid chloride of **9a** and COMU was found to be useful for the acylation of low-nucleophilic aromatic amine and sterically hindered N-methylamine, respectively. We achieved the first total synthesis of the proposed structure of asperterrestide (**1a**) by macrolactamization of the cyclization precursor **2a**; however, the discrepancy of the ¹H

and ^{13}C NMR spectroscopic data indicated that the stereochemistry of the natural compound is incorrect from the proposed structure of **1a**. The absolute configuration of natural asperterrestide A was then deduced to be cyclo-[D-*allo*-Ile-(2*S*,3*S*)-MePhe(3-OH)-Ant-Ala] (**1b**) or cyclo-[D-*allo*-Ile-(2*S*,3*S*)-MePhe(3-OH)-Ant-D-Ala] (**1c**) based on the reexamination of the reported HPLC analysis of acid hydrolysates of the natural product and the analysis of NMR spectroscopic data of synthetic **1a**. We next conducted the molecular modeling of structurally simplified D-Val-containing asperterrestides **1d-1f** on the MacroModel program to narrow down the putative candidates of natural asperterrestide A. Comparison between synthetic **1a** and corresponding **1d** revealed that $^3J_{\text{H,H}}$ coupling constants and NOE correlations observed are in good agreement with those calculated in the lowest-energy conformer of the corresponding model tetrapeptide. Because the theoretical values of L-Ala-containing **1e** are more consistent with those observed in the natural **1** than D-Ala-containing **1f**, we estimated the stereochemistry of natural asperterrestide A to be corresponding **1b**. The synthesis of **1b** was then performed to validate our speculation according to the established synthetic procedures of **1a**. In contrast to the synthesis of the tetrapeptide **12**, the *anti*-configuration of the MePhe(3-OTBS) residue was revealed to interrupt the acylation of the *N*-methylamine by the steric repulsion of the TBS group. Thus, the desired tetrapeptide **14** was provided after removal of the TBS group in **11b**. We accomplished the total synthesis of the revised structure of asperterrestide A (**1b**) by the deprotection followed by macrolactamization, and the ^1H and ^{13}C NMR spectroscopic data of synthetic **1b** are identical with those of natural **1**. Therefore, we concluded the absolute configuration of natural asperterrestide A to be cyclo-[D-*allo*-Ile-(2*S*,3*S*)-MePhe(3-OH)-Ant-Ala]. It is noteworthy that the $^3J_{\text{H,H}}$ coupling constant and NOE observation of **1b** were fully satisfied with not only the reported data of the natural **1** but the results obtained by the molecular modeling of the model cyclotetrapeptide **1e** as well. These results strongly suggest that molecular modeling is an efficient and reliable tool to predict three-dimensional structures of conformationally constrained cyclotetrapeptides. The evaluation of cytotoxicity

of synthetic **1a** and **1b** against three selected cancer cell lines revealed that the stereochemistry of the main chain is important for the potent activity. A structure-activity relationship study based on the three-dimensional structure of **1b** and its derivatives is underway.

EXPERIMENTAL SECTION

General Techniques. All commercially available reagents were purchased from commercial suppliers, and used as received. Dry THF and CH₂Cl₂ (Kanto Chemical Co.) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina column. All reactions in solution-phase were monitored by thin-layer chromatography (TLC) carried out on Merck silica gel plates (0.2 mm, 60F-254) with UV light, and visualized by *p*-anisaldehyde H₂SO₄-EtOH solution or phosphomolybdic acid-EtOH solution or ninhydrin-AcOH-1-BuOH solution. Silica gel 60N (Kanto Chemical Co. 100–210 μm) was used for column chromatography, and Merck silica gel plate (2.0 mm, 60F-254) was used for preparative thin-layer chromatography. ¹H NMR spectra (400 and 600 MHz) and ¹³C NMR spectra (100 and 150 MHz) were recorded on JEOL JNM-AL400 and JEOL JNM-ECA600 spectrometers in the indicated solvent. Chemical shifts (δ) are reported in units parts per million (ppm) relative to the signal for internal TMS (0.00 ppm for ¹H) for solutions in CDCl₃. NMR spectral data are reported as follows: chloroform (7.26 ppm for ¹H) or chloroform-*d* (77.0 ppm for ¹³C), and dimethyl sulfoxide (2.50 ppm for ¹H) or dimethyl sulfoxide-*d*₆ (39.5 ppm for ¹³C) when internal standard is not indicated. Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), dd (double doublet), dt (double triplet), dq (double quartet), td (triple doublet), brs (broad singlet), brd (broad doublet) and *J* (coupling constants in Hertz). High-resolution mass spectra were measured on Thermo Scientific™ Exactive™ Plus Orbitrap Mass Spectrometer (for ESI). IR spectra were recorded on a JASCO FTIR-4100. Only the strongest and/or structurally important absorption are

reported as the IR data afforded in wavenumbers (cm^{-1}). Optical rotations were measured on a JASCO P-1010 polarimeter. Melting points were measured with Round Science Inc. RFS-10, and are not corrected. Analytical HPLC was performed using Daicel Chiralpak OD-H.

Fmoc-Ant-OH. To a suspension of H-Ant-OH (3.50 g, 25.5 mmol) in dioxane (80 mL) were added a solution of Na_2CO_3 (13.5 g, 128 mmol) in water (16 mL) and FmocCl (7.26 g, 28.1 mmol) at 0 °C. After being stirred at room temperature for 23 h, the reaction mixture was concentrated in vacuo. The aqueous layer was washed with Et_2O , acidified with 3 M aqueous HCl until pH 2, and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was suspended in Et_2O . The precipitate was filtered, washed with Et_2O , and dried under vacuum to afford Fmoc-Ant-OH (7.13 g, 78%) as a white solid. mp 188–189 °C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.0 (brs, 1H), 8.11 (d, 1H, $J = 8.1$ Hz), 7.96 (d, 1H, $J = 7.7$ Hz), 7.91 (d, 2H, $J = 7.4$ Hz), 7.68 (d, 2H, $J = 7.4$ Hz), 7.40–7.46 (m, 3H), 7.34 (t, 2H, $J = 7.4$ Hz), 7.02 (t, 1H, $J = 7.7$ Hz), 4.44 (d, 2H, $J = 4.4$ Hz), 4.33 (t, 1H, $J = 4.4$ Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, $\text{DMSO}-d_6$) δ 170.0, 152.8, 143.7, 140.74, 140.73, 132.7, 131.3, 127.7, 127.1, 125.0, 121.4, 120.2, 118.8, 117.8, 66.1, 46.5; IR (neat) 2948, 1738, 1665, 1586, 1523, 1211 cm^{-1} ; HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{22}\text{H}_{18}\text{NO}_4$ 360.1230; Found 360.1229.

tert-Butyl (*S*)-(3-((*tert*-butyldimethylsilyl)oxy)-1-oxo-1-phenylpropan-2-yl)-carbamate (**3**).²⁶ To a solution of Boc-Ser-OH (5.00 g, 24.5 mmol) in dry CH_2Cl_2 (80 mL) were added $\text{MeNH}(\text{OMe})\cdot\text{HCl}$ (2.39 g, 24.5 mmol), NMM (2.8 mL, 25.7 mmol) and EDCI·HCl (4.74 g, 24.8 mmol) at –15 °C under an argon atmosphere. After being stirred at the same temperature for 2 h, the reaction mixture was quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 . The combined organic layers were washed with saturated aqueous NaHCO_3 and brine, dried over MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was suspended in hexane. The precipitate was filtered, washed

with hexane, and dried under vacuum to afford the Weinreb amide (5.43 g, 89%) as a white solid.

To a solution of the alcohol (4.80 g, 19.3 mmol) in dry CH_2Cl_2 (60 mL) were added imidazole (2.63 g, 38.6 mmol) and TBSCl (3.21 g, 21.3 mmol) at 0 °C under an argon atmosphere. After being stirred at room temperature for 1.5 h, the reaction mixture was quenched with water at 0 °C. The organic layer was separated, washed three times with 10% aqueous citric acid, saturated aqueous NaHCO_3 and brine, dried over MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 19:1) to afford the TBS ether (6.95 g, 99%) as a yellowish oil.

A solution of PhMgBr (1.0 M in THF) was prepared from PhBr (5.5 mL, 52.8 mmol), Mg tuning (2.57 g, 106 mmol), catalytic amount of I_2 , and dry THF (53 mL). To a solution of the Weinreb amide (6.00 g, 16.5 mmol) in dry THF (80 mL) was added above solution of PhMgBr dropwise at 0 °C under an argon atmosphere. After being stirred at room temperature for 3 h, the reaction mixture was quenched with 1 M aqueous HCl at 0 °C. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 19:1) to afford the phenyl ketone **3** (4.89 g, 78%) as a yellowish oil. The spectroscopic data of **3** were in good agreement with those reported in the literature.³⁰ $[\alpha]_{\text{D}}^{28} +37$ (c 1.0, CHCl_3).

tert-Butyl ((1*S*,2*S*)-3-((*tert*-butyldimethylsilyl)oxy)-1-hydroxy-1-phenylpropan-2-yl)carbamate (**4a**). To a solution of the ketone **3** (2.00 g, 5.27 mmol) in dry THF (20 mL) was added a solution of L-Selectride (1.0 M in THF, 11.6 mL, 11.6 mmol) dropwise at -78 °C under an argon atmosphere. After being stirred at the same temperature for 2 h, the reaction mixture was quenched with 1 M aqueous Rochelle salt at 0 °C. The mixture was stirred at room temperature. The organic layer was separated, and the aqueous layer was extracted twice with

EtOAc. The combined organic layers were washed with saturated aqueous NaHCO_3 and brine, dried over Na_2SO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 19:1) to afford the alcohol **4a** (1.73 g, 86%, dr 89:11) as a yellowish oil. The diastereomeric ratio of **4a** was determined by chiral column OD-H (eluted with hexane/*i*PrOH = 9:1; flow rate: 0.5 mL/min; retention time: 7.8 min for C1 diastereomer of **4a**, 8.3 min for **4a**). $[\alpha]_{\text{D}}^{28} +38$ (*c* 1.0, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.24–7.37 (m, 5H), 5.16 (d, 1H, J = 6.4 Hz), 5.00 (d, 1H, J = 3.2 Hz), 3.66–3.84 (m, 4H), 1.37 (s, 9H), 0.93 (s, 9H), 0.08 (s, 6H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ 156.2, 141.2, 128.2, 127.5, 126.1, 79.6, 74.8, 64.9, 56.5, 28.3, 25.8, 18.6, –5.57, –5.60; IR (neat) 3443, 2955, 2929, 1696, 1497, 1171, 838 cm^{-1} ; HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{20}\text{H}_{36}\text{NO}_4\text{Si}$ 382.2408; Found 382.2405.

tert-Butyl ((5*S*,6*S*)-2,2,3,3,9,9,10,10-octamethyl-5-phenyl-4,8-dioxo-3,9-disila-undecan-6-yl)-carbamate (**5a**).

To a solution of the alcohol **4a** (1.60 g, 4.19 mmol) in DMF (20 mL) were added imidazole (856 mg, 12.6 mmol), DMAP (512 mg, 4.19 mmol) and TBSCl (948 mg, 6.29 mmol) at 0 °C under an argon atmosphere. After being stirred at 40 °C for 23 h, the reaction mixture was quenched with water. The aqueous layer was extracted twice with Et_2O . The combined organic layers were washed three times with brine, dried over Na_2SO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 29:1) to afford the TBS ether **5a** (1.53 g, 73%) as a yellowish oil. $[\alpha]_{\text{D}}^{24} +37$ (*c* 1.0, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , 4:1 rotamer mixture) δ 7.20–7.33 (m, 5.0H), 5.01 (d, 1.0H, J = 2.7 Hz), 4.83 (d, 0.8H, J = 9.3 Hz), 4.66 (d, 0.2H, J = 8.8 Hz), 3.64–3.70 (m, 0.8H), 3.49–3.59 (m, 2.2H), 1.34 (s, 7.2H), 1.21 (s, 1.8H), 0.93 (s, 9.0H), 0.90 (s, 9.0H), 0.08 (s, 3.0H), 0.07 (s, 3.0H), 0.05 (s, 3.0H), –0.15 (s, 3.0H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3 , 4:1 rotamer mixture) δ 155.5, 142.7, 142.5, 127.9, 127.1, 126.3, 79.3, 78.9, 72.0, 71.5, 62.2, 61.8, 59.9, 58.3, 28.4, 28.0, 25.9, 18.2, 18.1, –4.6, –5.2, –5.3, –5.4; IR (neat) 3445, 1954, 1929,

1721, 1492, 1101, 838 cm^{-1} ; HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{26}\text{H}_{50}\text{NO}_4\text{Si}_2$ 496.3273; Found 496.3272.

tert-Butyl methyl((5*S*,6*S*)-2,2,3,3,9,9,10,10-octamethyl-5-phenyl-4,8-dioxo-3,9-disilaundecan-6-yl)-carbamate (**6a**). To a solution of the amine **5a** (1.40 g, 2.82 mmol) in dry THF (15 mL) were added MeI (1.4 mL, 22.6 mmol) and NaH (60% in mineral, 338 mg, 8.46 mmol) at 0 °C under an argon atmosphere, and the mixture was stirred until H_2 evolution was completed. Then, same amount of MeI (1.4 mL, 22.6 mmol) and NaH (60% in mineral, 338 mg, 8.46 mmol) were added to above mixture at 0 °C. After being stirred at room temperature for 24 h, the reaction was quenched with water. The organic layer was separated, and the aqueous layer was extracted twice with Et_2O . The combined organic layers were washed with brine, dried over Na_2SO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 19:1) to afford the *N*-methylamine **6a** (1.18 g, 82%) as a colorless oil, which was solidified under vacuum. mp 56–57 °C; $[\alpha]_D^{24} +43$ (c 1.0, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , 3:2 rotamer mixture) δ 7.21–7.32 (m, 5.0H), 4.94 (brs, 0.6H), 4.90 (d, 0.4H, $J = 4.8$ Hz), 4.17 (brs, 0.6H), 3.58–3.77 (m, 2.4H), 2.92 (s, 1.8H), 2.91 (s, 1.2H), 1.38 (s, 3.6H), 1.18 (s, 5.4H), 0.89 (s, 5.4H), 0.88 (s, 5.4H), 0.85 (s, 7.2H), 0.03 (s, 7.2H), –0.04 (s, 1.8H), –0.26 (s, 1.8H), –0.32 (s, 1.2H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3 , 3:2 rotamer mixture) δ 156.0, 142.6, 142.5, 128.00, 127.97, 127.4, 127.2, 127.0, 126.6, 78.9, 78.8, 74.5, 73.4, 62.2, 61.2, 28.4, 28.1, 25.8, 25.7, 18.03, 18.01, 17.9, –4.6, –4.7, –5.31, –5.38, –5.42, –5.48, –5.54, –5.6; IR (neat) 2955, 2929, 1697, 1152, 837 cm^{-1} ; HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{52}\text{NO}_4\text{Si}_2$ 510.3429; found 510.3425.

(9*H*-Fluoren-9-yl)methyl

methyl((5*S*,6*S*)-2,2,3,3,9,9,10,10-octamethyl-5-phenyl-4,8-dioxo-3,9-disila-undecan-6-yl)carbamate (**7a**). To a solution of the *N*-Boc amine **6a** (1.10 g, 2.16 mmol) in dry CH_2Cl_2 (15 mL) were added 2,6-lutidine (2.0 mL,

17.3 mmol) and TMSOTf (1.6 mL, 8.63 mmol) at 0 °C under an argon atmosphere. After being stirred at room temperature for 40 min, the reaction mixture was quenched with brine and MeOH at 0 °C. The organic layer was separated, and the aqueous layer was extracted twice with CH₂Cl₂. The combined organic layers were washed with 10% aqueous citric acid and brine, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo, and the crude amine was used for next reaction without further purification.

To a solution of the crude amine in dry THF (8.0 mL) were added saturated aqueous NaHCO₃ (8.0 mL) and FmocOSu (729 mg, 2.16 mmol, 1.0 equiv) at 0 °C. After being stirred at room temperature for 17 h, the reaction mixture was quenched with 10% aqueous citric acid. The aqueous layer was extracted with Et₂O. The organic layer was washed with brine, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 29:1) to afford the *N*-Fmoc amine **7a** (1.32 g, 97% in 2 steps) as a colorless oil. $[\alpha]_D^{25} +41$ (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 1:1 rotamer mixture) δ 7.74–7.78 (m, 2.0H), 7.58–7.62 (m, 1.5H), 7.52 (d, 0.5H, *J* = 7.1 Hz), 7.23–7.42 (m, 9.0H), 5.02 (d, 0.5H, *J* = 6.8 Hz), 4.89 (brs, 0.5H), 4.04–4.37 (m, 4.0H), 3.60–3.66 (m, 2.0H), 3.05 (s, 3.0H), 0.85–0.89 (m, 18.0H), 0.03 (s, 3.0H), 0.04 (brs, 3.0H), –0.02 (s, 3.0H), –0.27 (brs, 1.5H), –0.28 (s, 1.5H); ¹³C{¹H} NMR (100 MHz, CDCl₃, 1:1 rotamer mixture) δ 157.0, 144.5, 144.4, 144.2, 144.0, 142.2, 141.3, 141.2, 128.13, 128.07, 127.6, 127.5, 126.94, 126.92, 126.8, 125.19, 125.15, 125.0, 119.9, 73.8, 73.1, 67.2, 62.9, 61.2, 60.9, 47.34, 47.30, 25.8, 25.7, 18.1, 17.9, –4.6, –5.3, –5.6; IR (neat) 2953, 2928, 1700, 1254, 1100, 836 cm^{–1}; HRMS (ESI-TOF) *m/z*: [M+Na]⁺ Calcd for C₃₇H₅₃NO₄Si₂Na 654.3405; Found 654.3403.

(9H-Fluoren-9-yl)methyl

((1S,2S)-1-((tert-butyldimethylsilyl)oxy)-3-hydroxy-1-phenylpropan-2-yl)(methyl)carbamate (8a). To a solution of the TBS ether **7a** (1.10 g, 1.74 mmol) in dry THF (15 mL) were added pyridine (1.4 mL, 17.4 mmol) and HF·pyridine (950 μ L, 52.2 mmol) at 0 °C under an argon atmosphere, and the mixture was stirred for 30 min at

the same temperature. After being stirred at room temperature for 4 h, the reaction mixture was poured into saturated aqueous NaHCO₃ at 0 °C. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with 10% aqueous citric acid and brine, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (eluted with hexane/EtOAc = 4:1) to afford the alcohol **8a** (575 mg, 64%) as a white amorphous solid and a mixture of **8a** and its diastereomer (150 mg, 17%) as a white amorphous solid. R_f: 0.23 for **8a**, 0.29 for C1 diastereomer of **8a** (hexane/EtOAc = 2:1); mp 55–56 °C; [α]_D²⁵ +44 (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 3:2 rotamer mixture) δ 7.76–7.79 (m, 2.0H), 7.57–7.60 (m, 2.0H), 7.20–7.60 (m, 8.6H), 7.12 (brs, 0.4H), 5.06 (d, 0.6H, *J* = 6.8 Hz), 4.64 (brs, 0.4H), 4.44 (dd, 0.6H, *J* = 10.4, 6.8 Hz), 4.34 (brs, 0.4H), 4.26 (dd, 0.6H, *J* = 10.4, 6.8 Hz), 4.18 (t, 1.0H, *J* = 6.8 Hz), 4.11 (brs, 0.4H), 3.88 (brs, 0.4H), 3.55–3.66 (m, 1.6H), 3.43 (brs, 0.4H), 2.94–3.00 (m, 3.6H), 0.84 (s, 5.4H), 0.80 (brs, 3.6H), –0.02 (s, 3H), –0.30 (s, 1.8H), –0.36 (brs, 1.2H); ¹³C{¹H} NMR (100 MHz, CDCl₃, 3:2 rotamer mixture) δ 157.3, 144.1, 143.9, 141.8, 141.6, 141.4, 141.3, 141.23, 141.17, 128.20, 128.15, 127.74, 127.71, 127.62, 127.59, 127.14, 127.0, 126.9, 126.7, 126.5, 125.1, 125.0, 119.9, 73.9, 73.2, 67.4, 61.1, 60.2, 47.24, 47.20, 25.7, 25.6, 17.84, 17.80, –4.8, –5.2; IR (neat) 3444, 2954, 2929, 1684, 1451, 758 cm^{–1}; HRMS (ESI-TOF) *m/z*: [M+Na]⁺ Calcd for C₃₁H₃₉NO₄SiNa 540.2541; Found 540.2538.

(2*R*,3*S*)-*Fmoc-MePhe*(3-*OTBS*)-*OH* (**9a**). To a solution of the alcohol **8a** (500 mg, 966 μ mol) in dry MeCN (4.0 mL) and pH 6.8 buffer (4.0 mL) were added TEMPO (30.2 mg, 193 μ mol), NaClO₂ (80% grade, 328 mg, 2.90 mmol) and 5% aqueous NaOCl (287 μ L, 193 μ mol) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was quenched with 10% aqueous Na₂S₂O₃, and acidified with 10% aqueous citric acid. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was recrystallized from Et₂O/hexane to afford the carboxylic acid **9a** (461 mg, 90%) as a white solid. mp

167–168 °C; $[\alpha]_{\text{D}}^{25} +40$ (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 2:1 rotamer mixture) δ 7.72–7.76 (m, 2.0H), 7.23–7.44 (m, 11.0H), 5.50 (d, 0.7H, *J* = 4.6 Hz), 5.33 (d, 0.3H, *J* = 4.9 Hz), 5.21 (d, 0.7H, *J* = 4.6 Hz), 4.93 (d, 0.3H, *J* = 4.9 Hz), 4.24–4.32 (m, 1.0H), 4.00–4.16 (m, 2.0H), 3.15 (s, 2.0H), 3.10 (s, 1.0H), 0.89 (s, 6.0H), 0.85 (s, 3.0H), 0.07 (s, 2.0H), 0.04 (s, 1.0H), –0.18 (s, 2.0H), –0.25 (s, 1.0H); ¹³C{¹H} NMR (100 MHz, CDCl₃, 2:1 rotamer mixture) δ 174.3, 174.2, 157.2, 156.1, 144.1, 143.7, 141.3, 141.2, 140.3, 140.1, 128.3, 128.2, 128.0, 127.7, 127.6, 127.1, 127.02, 126.98, 126.7, 126.4, 125.2, 125.0, 124.93, 124.86, 119.9, 75.0, 74.8, 68.0, 67.7, 65.0, 64.3, 47.12, 47.06, 25.71, 25.66, 18.0, 17.9, –4.5, –4.6, –5.48, –5.51; IR (neat) 3021, 2954, 2930, 1703, 1253, 758 cm^{–1}; HRMS (ESI-TOF) *m/z*: [M+Na]⁺ Calcd for C₃₁H₃₇NO₅SiNa 554.2333; Found 554.2328.

Fmoc-Ant-D-Ala-OtBu (**10**). To a solution of H-D-Ala-OtBu·HCl (1.00 g, 5.50 mmol) in dry CH₂Cl₂ (22 mL) were added DIEA (1.1 mL, 6.06 mmol), Fmoc-Ant-OH (2.18 g, 6.06 mmol), HOBT (819 mg, 6.06 mmol) and EDCI·HCl (1.16 g, 6.06 mmol) at 0 °C under an argon atmosphere. After being stirred at room temperature for 16 h, the reaction mixture was quenched with 1 M aqueous HCl. The organic layer was separated, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous NaHCO₃ and brine, dried over MgSO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 6:1) to afford the dipeptide **10** (2.36 g, 88%) as a white amorphous solid. mp 122–123 °C; $[\alpha]_{\text{D}}^{27} -8.1$ (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 10.7 (s, 1H), 8.35 (d, 1H, *J* = 7.9 Hz), 7.76 (d, 2H, *J* = 7.4 Hz), 7.66 (d, 2H, *J* = 7.4 Hz), 7.55 (dd, 1H, *J* = 7.9, 1.3 Hz), 7.46 (dt, 1H, *J* = 7.9, 1.3 Hz), 7.40 (t, 2H, *J* = 7.4 Hz), 7.32 (t, 2H, *J* = 7.4 Hz), 7.04 (d, 1H, *J* = 7.9 Hz), 6.91 (d, 1H, *J* = 6.9 Hz), 4.66 (quin, 1H, *J* = 6.9 Hz), 4.42 (d, 2H, *J* = 7.4 Hz), 4.30 (t, 1H, *J* = 7.4 Hz), 1.53 (d, 3H, *J* = 6.9 Hz), 1.51 (s, 9H); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 172.1, 168.2, 153.6, 143.9, 141.2, 139.9, 132.8, 127.7, 127.1, 126.8, 125.3, 122.0, 120.0, 119.9, 119.5, 82.5, 67.3, 49.1, 47.0, 27.9, 18.6; IR (neat) 3336, 2979, 1733, 1646, 1590, 1522, 1450, 1215, 757 cm^{–1}; HRMS (ESI-TOF) *m/z*:

[M+Na]⁺ Calcd for C₂₉H₃₀N₂O₅Na 509.2047; Found 509.2036.

Fmoc-(2*R*,3*S*)-*MePhe*(3-*OTBS*)-*Ant-D-Ala-OtBu* (**11a**). To a solution of the *N*-Fmoc amine **10** (150 mg, 308 μmol) in dry MeCN (2.4 mL) was added Et₂NH (0.6 mL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in vacuo. The resulting residue was azeotroped three times with toluene, and dried under vacuum. The crude amine was used for next reaction without further purification.

To a solution of the acid **9a** (246 mg, 462 μmol) in dry MeCN (1.5 mL) were added triphosgene (45.7 mg, 154 μmol) and 2,4,6-collidine (124 μL, 924 μmol) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 5 min, a suspension of the crude amine in dry MeCN (1.5 mL) was added to the above mixture at 0 °C. After being stirred at room temperature for 13 h, the reaction mixture was quenched with 10% aqueous citric acid, and concentrated in vacuo. The aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with 10% aqueous citric acid, saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 6:1) to give desired product containing a small amount of impurities. Further purification by column chromatography on silica gel (eluted with toluene/EtOAc = 14:1) afforded the tripeptide **11a** (215 mg, 90% in 2 steps) as a white amorphous solid. mp 88–89 °C; [α]_D²³ +63 (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 3:2 rotamer mixture) δ 11.72 (s, 0.4H), 11.71 (s, 0.6H), 8.65 (d, 0.6H, *J* = 8.3 Hz), 8.61 (d, 0.4H, *J* = 8.3 Hz), 7.72–7.78 (m, 2H), 7.66 (d, 0.8H, *J* = 7.3 Hz), 7.17–7.56 (m, 12.2H), 7.05–7.11 (m, 1.0H), 6.81 (d, 0.6H, *J* = 7.0 Hz), 6.78 (d, 0.4H, *J* = 7.0 Hz), 5.72 (d, 0.6H, *J* = 4.7 Hz), 5.57 (d, 0.4H, *J* = 5.4 Hz), 5.26 (d, 0.6H, *J* = 4.7 Hz), 5.04 (d, 0.4H, *J* = 5.4 Hz), 4.46 (quin, 0.4H, *J* = 7.0 Hz), 4.17–4.40 (m, 3.2H), 4.08 (dd, 0.4H, *J* = 10.1, 7.0 Hz), 3.37 (s, 1.8H), 3.22 (s, 1.2H), 1.45 (s, 3.6H), 1.44 (s, 5.4H), 1.21 (d, 3H, *J* = 7.0 Hz), 0.88 (s, 5.4H), 0.84 (s, 3.6H), 0.13 (s, 1.8H), 0.10 (s, 1.2H), –0.15 (s, 1.8H),

−0.20 (s, 1.2H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3 , 3:2 rotamer mixture) δ 172.2, 167.8, 167.7, 167.6, 167.4, 157.3, 156.3, 144.3, 144.2, 144.10, 144.06, 141.5, 141.4, 141.1, 139.41, 139.35, 132.71, 132.68, 128.2, 128.0, 127.6, 127.53, 127.52, 127.49, 127.46, 126.98, 126.96, 126.94, 126.91, 126.6, 126.5, 125.4, 125.3, 125.2, 125.1, 123.0, 122.9, 121.12, 121.09, 119.9, 119.80, 119.79, 119.7, 82.4, 82.3, 74.0, 73.4, 68.1, 66.8, 66.7, 48.9, 48.8, 47.2, 47.1, 33.5, 27.9, 25.8, 25.7, 18.41, 18.37, 18.0, 17.9, −4.5, −4.6, −5.2, −5.3; IR (neat) 3339, 2954, 2929, 1693, 1518, 1448, 114, 757 cm^{-1} ; HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{45}\text{H}_{56}\text{N}_3\text{O}_7\text{Si}$ 778.3882; Found 778.3880.

Boc-D-allo-Ile-(2R,3S)-MePhe(3-OTBS)-Ant-D-Ala-OtBu (12). To a solution of the *N*-Fmoc amine **11a** (150 mg, 193 μmol) in dry MeCN (1.6 mL) was added Et_2NH (0.4 mL) at 0 $^\circ\text{C}$ under an argon atmosphere. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in vacuo. The resulting residue was azeotroped three times with toluene, and dried under vacuum. The crude amine was used for next reaction without further purification.

To a solution of the crude amine in dry CH_2Cl_2 (2.0 mL) were added Boc-*D-allo-Ile*-OH (66.9 mg, 289 μmol), DIEA (101 μL , 579 μmol) and COMU (124 mg, 289 μmol) at 0 $^\circ\text{C}$ under an argon atmosphere. After being stirred at room temperature for 23 h, the reaction mixture was quenched with 10% aqueous citric acid. The organic layer was separated, and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with saturated aqueous NaHCO_3 and brine, dried over Na_2SO_4 , and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 6/1) to give desired product containing a small amount of impurities. Further purification by column chromatography on silica gel (eluted with toluene/EtOAc = 9:1) afforded the tetrapeptide **12** (85.0 mg, 57% in 2 steps) as a white amorphous solid. mp 84–85 $^\circ\text{C}$; $[\alpha]_D^{24} +80$ (c 1.0, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , 3:1 rotamer mixture) δ 11.7 (s, 0.25H), 11.4 (s, 0.75H), 8.71 (d, 0.25H, J = 8.3 Hz), 8.63 (d, 0.75H, J =

8.3 Hz), 7.17–7.59 (m, 7.0H), 7.03–7.10 (m, 1.25H), 6.80 (d, 0.75H, $J = 7.1$ Hz), 5.77 (d, 0.25H, $J = 3.2$ Hz), 5.70 (d, 0.75H, $J = 4.4$ Hz), 5.61 (d, 0.75H, $J = 4.4$ Hz), 5.53 (d, 0.25H, $J = 10.3$ Hz), 4.90 (d, 0.75H, $J = 10.4$ Hz), 4.70 (d, 0.25H, $J = 3.2$ Hz), 4.52–4.66 (m, 1.75H), 3.67 (dd, 0.25H, $J = 10.3, 6.6$ Hz), 3.37 (s, 2.25H), 3.15 (s, 0.75H), 1.98–2.04 (m, 0.75H), 1.64–1.71 (m, 1.0H), 1.42–1.51 (m, 21.0H), 0.97 (t, 2.25H, $J = 7.4$ Hz), 0.85 (s, 2.25H), 0.84 (s, 6.75H), 0.62–0.79 (m, 4.0H), 0.50–0.58 (m, 0.25H), 0.13 (s, 0.75H), 0.08 (s, 2.25H), –0.14 (s, 0.75H), –0.19 (s, 2.25H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3 , 3:1 rotamer mixture) δ 173.6, 172.9, 172.3, 172.1, 170.0, 167.7, 167.5, 166.8, 155.44, 155.41, 141.5, 141.2, 140.0, 139.4, 133.1, 132.8, 128.4, 128.0, 127.6, 127.5, 126.73, 126.67, 126.61, 126.5, 122.94, 122.86, 121.3, 121.0, 119.8, 118.8, 82.7, 82.5, 79.3, 28.9, 74.34, 74.26, 67.3, 64.0, 53.7, 52.8, 48.9, 48.8, 36.8, 36.5, 34.4, 34.3, 28.4, 27.9, 27.0, 26.6, 25.8, 18.8, 18.6, 18.02, 17.96, 14.2, 13.6, 11.9, –4.6, –5.3; IR (neat) 3346, 2961, 2931, 1711, 1649, 1517, 1449, 1158, 756 cm^{-1} ; HRMS (ESI-TOF) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{41}\text{H}_{64}\text{N}_4\text{O}_8\text{SiNa}$ 791.4386; Found 791.4377.

The proposed structure of asperterrestide A (1a). To a solution of the *N*-Boc amine **12** (60.0 mg, 78.0 μmol) in dry CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL) at 0 $^\circ\text{C}$ under an argon atmosphere. After being stirred at room temperature for 11 h, the reaction mixture was concentrated in vacuo. The resulting residue was azeotroped three times with toluene, and dried under vacuum. The crude amine·TFA salt **2a** was used for next reaction without further purification.

To a solution of the crude linear tetrapeptide **2a** in dry CH_2Cl_2 (78 mL) were added DIEA (136 μL , 780 μmol) and HATU (89.0 mg, 234 μmol) at 0 $^\circ\text{C}$ under an argon atmosphere. After being stirred at room temperature for 22 h, the reaction mixture was quenched with 1 M aqueous HCl, and concentrated in vacuo. The aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with 1 M aqueous HCl, saturated aqueous NaHCO_3 and brine, dried over MgSO_4 , and filtered. The filtrate was concentrated in vacuo, and CHCl_3 was added to the resulting residue. The suspension was filtered to remove white solid impurities. The filtrate was

concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/EtOAc = 1:1) to give desired product containing a small amount of impurities. Further purification by preparative TLC (eluted with toluene/EtOAc = 2:1) afforded the proposed structure of asperterrestide A (**1a**) (13.3 mg, 36% in 2 steps) as a white solid. mp 175–176 °C; $[\alpha]_D^{23} +28$ (*c* 0.66, MeOH); ^1H NMR (600 MHz, CDCl_3) δ 9.83 (s, 1H), 8.32 (d, 1H, *J* = 8.1 Hz), 7.47–7.50 (m, 1H), 7.44 (d, 2H, *J* = 7.3 Hz), 7.41 (dd, 1H, *J* = 7.7, 1.4 Hz), 7.38 (t, 2H, *J* = 7.3 Hz), 7.31 (t, 1H, *J* = 7.3 Hz), 7.11 (td, 1H, *J* = 7.7, 0.9 Hz), 6.92 (brs, 1H), 6.16 (d, 1H, *J* = 9.5 Hz), 5.79 (brs, 1H), 4.66 (t, 1H, *J* = 9.5 Hz), 4.06 (dq, 1H, *J* = 7.3, 3.8 Hz), 3.94 (brs, 1H), 3.84 (d, 1H, *J* = 2.6 Hz), 2.77 (s, 3H), 1.73–1.75 (m, 1H), 1.56–1.61 (m, 1H), 1.40 (d, 3H, *J* = 7.3 Hz), 1.13–1.18 (m, 1H), 0.91 (t, 3H, *J* = 7.4 Hz), 0.87 (d, 3H, *J* = 6.8 Hz); $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, CDCl_3) δ 174.9, 172.8, 170.0, 166.5, 141.2, 136.7, 132.2, 128.6, 127.8, 125.91, 125.88, 123.7, 123.6, 122.9, 72.9, 72.5, 53.8, 53.7, 40.5, 37.3, 25.5, 16.3, 14.3, 11.1; IR (neat) 3317, 3202, 2963, 1698, 1622, 1516, 1444, 755 cm^{-1} ; HRMS (ESI-TOF) *m/z*: $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{26}\text{H}_{32}\text{N}_4\text{O}_5\text{Na}$ 503.2265; Found 503.2258.

tert-Butyl (*R*)-(3-((*tert*-butyldimethylsilyl)oxy)-1-oxo-1-phenylpropan-2-yl)-carbamate (*ent*-**3**).²⁶ By following the procedure described above for **3**, the amidation of Boc-D-Ser-OH (4.20 g, 20.5 mmol) afforded the Weinreb amide (4.56 g, 90%). The silylation of the alcohol (4.80 g, 19.3 mmol) afforded the TBS ether (7.02 g, quant). The 1,2-addition to the Weinreb amide (6.66 g, 18.4 mmol) afforded the phenyl ketone *ent*-**3** (5.98 g, 86%). The spectroscopic data of *ent*-**3** were in good agreement with those reported in the literature except for the sign of the specific rotation.³⁰ $[\alpha]_D^{23} -29$ (*c* 1.1, CHCl_3).

tert-Butyl ((1*S*,2*R*)-3-((*tert*-butyldimethylsilyl)oxy)-1-hydroxy-1-phenylpropan-2-yl)carbamate (**4b**). To a solution of the ketone *ent*-**3** (1.70 g, 4.48 mmol) in dry THF (45 mL) was added a solution of DIBAL-H (1.0 M in hexane, 14.8 mL, 14.8 mmol) dropwise at –78 °C under an argon atmosphere. After being at the same temperature for 4 h, the reaction mixture was quenched with MeOH at –78 °C and 1 M aqueous Rochelle salt at

0 °C. The mixture was stirred at room temperature for 4 h. The organic layer was separated, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 6:1) to afford the alcohol **4b** (1.35 g, 79%, dr 87:13) as a colorless oil. The diastereomeric ratio of **4b** was determined by chiral column OD-H (eluted with Hexane/IPA = 9:1; flow rate: 0.5 mL/min; retention time: 8.0 min for **4b**, 8.7 min for C1 diastereomer of **4b**). [α]_D²⁴ -6.0 (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.36–7.37 (m, 4H), 7.26–7.30 (m, 1H), 5.35 (d, 1H, *J* = 6.8 Hz), 4.93 (dd, 1H, *J* = 7.8, 3.2 Hz), 4.12 (d, 1H, *J* = 7.8 Hz), 3.82–3.85 (m, 1H), 3.67 (brs, 2H), 1.45 (s, 9H), 0.91 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 155.6, 141.5, 128.3, 127.3, 125.7, 79.5, 76.3, 63.0, 55.2, 28.3, 25.8, 18.1, -5.7; IR (neat) 3444, 3060, 2940, 2888, 2586, 1697, 1497, 1371, 836 cm⁻¹; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₂₀H₃₆NO₄Si 382.2408; Found 382.2399.

tert-Butyl ((5*S*,6*R*)-2,2,3,3,9,9,10,10-octamethyl-5-phenyl-4,8-dioxa-3,9-disila-undecan-6-yl)-carbamate (**5b**).

By following the procedure described above for **5a**, the silylation of the alcohol **4b** (1.35 g, 3.54 mmol) afforded the TBS ether **5b** (1.67 g, 95%) as a yellowish oil. [α]_D²⁷ +9.5 (*c* 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 4:1 rotamer mixture) δ 7.21–7.34 (m, 5.0H), 4.87 (brd, 0.8 H, *J* = 5.2 Hz), 4.69 (brs, 0.2H), 4.59 (brd, 0.8 H, *J* = 8.0 Hz), 4.40 (brs, 0.2H), 3.77–3.82 (m, 1.8H), 3.58 (brs, 0.2H), 3.48–3.56 (m, 1.0H), 1.34 (s, 7.2H), 1.25 (s, 1.8H), 0.88 (s, 18.0H), 0.01–0.03 (m, 9.0H), -0.19 (s, 3.0H); ¹³C{¹H} NMR (100 MHz, CDCl₃, 4:1 rotamer mixture) δ 155.4, 141.6, 127.9, 127.2, 126.8, 78.8, 73.8, 72.0, 61.0, 58.3, 58.0, 28.3, 28.0, 25.89, 25.87, 25.80, 18.2, 18.1, -4.8, -5.2, -5.4, -5.5; IR (neat) 3457, 2955, 2930, 2886, 2858, 1721, 1496, 1173, 837, 777 cm⁻¹; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₂₆H₅₀NO₄Si₂ 496.3273; Found 496.3260.

tert-Butyl methyl((5*S*,6*R*)-2,2,3,3,9,9,10,10-octamethyl-5-phenyl-4,8-dioxa-3,9-disilaundecan-6-yl)-carbamate

(**6b**). By following the procedure described above for **6a**, *N*-methylation of the amine **5b** (900 mg, 1.82 mmol) afforded the *N*-methylamine **6b** (842 mg, 91%) as a colorless oil. $[\alpha]_D^{27} +13$ (*c* 0.85, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 3:2 rotamer mixture) δ 7.20–7.34 (m, 5.0H), 4.95 (brs, 0.6H), 4.59 (brs, 0.4H), 3.86–4.23 (m, 3.0H), 2.80 (s, 1.2H), 2.59 (s, 1.8H), 1.34 (s, 5.4H), 1.25 (brs, 3.6H), 0.86 (s, 18.0H), –0.04–0.02 (s, 9.0H), –0.27–0.33 (m, 3.0H); ¹³C{¹H} NMR (100 MHz, CDCl₃, 3:2 rotamer mixture) δ 142.5, 127.9, 127.7, 127.5, 127.2, 126.8, 126.7, 78.7, 74.2, 73.58, 73.57, 65.8, 63.0, 60.6, 60.3, 28.3, 28.2, 25.84, 25.80, 25.74, 25.71, 18.11, 18.07, 17.99, 17.94, 15.2, –4.62, –4.65, –5.18, –5.24, –5.44, –5.49, –5.51; IR (neat) 2956, 2930, 2889, 2858, 1695, 1472, 1254, 1157, 1065, 861 cm^{–1}; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₂₇H₅₂NO₄Si₂ 510.3429; Found 510.3417.

(9H-Fluoren-9-yl)methyl

methyl((5S,6R)-2,2,3,3,9,9,10,10-octamethyl-5-phenyl-4,8-dioxo-3,9-disila-undecan-6-yl)carbamate (**7b**). By following the procedure described above for **7a**, *N*-Fmoc protection of the *N*-Boc amine **6b** (754 mg, 1.65 mmol) afforded the *N*-Fmoc amine **7b** (758 mg, 65% in 2 steps) as a colorless oil. $[\alpha]_D^{28} +8.0$ (*c* 0.82, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 3:2 rotamer mixture) δ 7.75–7.77 (m, 2.6H), 7.20–7.45 (m, 9.8H), 7.08 (brs, 0.6H), 5.04 (brs, 0.6H), 4.53 (brs, 0.4H), 3.60–4.56 (m, 6.0H), 2.87 (s, 1.2H), 2.79 (s, 1.8H), 0.85–0.89 (m, 18.0H), –0.03–0.04 (m, 9.0H), –0.27 (s, 1.8H), –0.33 (s, 1.2H); ¹³C{¹H} NMR (100 MHz, CDCl₃, 3:2 rotamer mixture) δ 144.2, 144.14, 144.12, 142.3, 141.8, 141.33, 141.27, 141.19, 128.0, 127.9, 127.6, 127.55, 127.50, 127.1, 127.0, 126.91, 126.90, 126.7, 126.5, 125.22, 125.19, 124.9, 119.9, 119.8, 67.1, 62.8, 60.7, 60.6, 60.1, 48.2, 47.4, 47.1, 25.85, 25.80, 25.75, 25.71, 18.14, 18.08, 17.98, 17.93, –4.65, –4.73, –5.1, –5.3, –5.5, –5.6; IR (neat) 3065, 3030, 2954, 2929, 2890, 2857, 1702, 1471, 1254, 1089, 836 cm^{–1}; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₃₇H₅₄NO₄Si₂ 632.3586; Found 632.3570.

(9H-Fluoren-9-yl)methyl

((1*S*,2*S*)-1-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-1-phenylpropan-2-yl)(methyl)carbamate (**8b**). By following the procedure described above for **8a**, desilylation of the TBS ether **7b** (738 mg, 1.17 mmol) afforded the alcohol **8b** (350 mg, 58%), its diastereomer (46.0 mg, 8%) and a mixture of **8b** and its diastereomer (94.0 mg, 16%) as a colorless oil, respectively. R_f : 0.29 for **8b**, 0.23 for C1 diastereomer of **8b** (hexane/EtOAc = 2:1); $[\alpha]_D^{29} +8.0$ (c 0.98, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 2:1 rotamer mixture) δ 7.74–7.79 (m, 2.0H), 7.58 (t, 0.7H, J = 8.2 Hz), 7.15–7.48 (m, 9.6H), 6.86 (d, 0.7H, J = 6.0 Hz), 5.13 (d, 0.7H, J = 8.4 Hz), 4.28–4.56 (m, 2.3H), 4.15–4.18 (m, 1.0H), 4.04 (brs, 1.3H), 4.39 (dd, 0.5H, J = 7.1, 10.7 Hz), 4.32 (dd, 0.5H, J = 7.1, 10.7 Hz), 4.15–4.19 (m, 0.6H), 4.04 (brs, 1.0H), 3.62 (brs, 1.3H), 2.88 (brs, 0.7H), 2.71 (brs, 0.7H), 2.56 (s, 2.0H), 0.87 (s, 6.0H), 0.84 (s, 3.0H), 0.05 (2.0H), –0.11 (s, 1.0H), –0.25 (s, 2.0H), –0.41 (s, 1.0H); ¹³C{¹H} NMR (100 MHz, CDCl₃, 2:1 rotamer mixture) δ 156.8, 144.1, 143.9, 143.8, 142.3, 141.5, 141.3, 128.2, 128.0, 127.7, 127.6, 127.2, 127.1, 126.98, 126.96, 126.6, 126.4, 125.0, 124.6, 120.1, 120.0, 119.9, 75.2, 73.5, 68.2, 67.3, 66.2, 61.9, 47.5, 47.2, 35.3, 25.73, 25.71, 18.0, 17.9, –4.7, –4.8, –5.2, –5.3; IR (neat) 3446, 3066, 3034, 2954, 2929, 2891, 2857 1682, 1451, 1252, 740 cm^{–1}; HRMS (ESI-TOF) m/z : $[M+H]^+$ Calcd for C₃₁H₄₀NO₄Si 518.2721; Found 518.2708.

(2*S*,3*S*)-Fmoc-MePhe(3-OTBS)-OH (**9b**). By following the procedure described above for **9a**, the oxidation of the alcohol **8b** (300 mg, 579 μ mol) afforded the carboxylic acid **9b** (270 mg, 88%) as a white solid. mp 79–80 °C; $[\alpha]_D^{25} -15$ (c 0.97, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 2:1 rotamer mixture) δ 7.72–7.78 (m, 2.0H), 7.55–7.60 (m, 0.7H), 7.19–7.42 (m, 9.7H), 7.07 (d, 0.7H, J = 6.4 Hz), 5.36 (d, 0.7H, J = 9.0 Hz), 4.90 (d, 0.3H, J = 9.6 Hz), 4.66 (brd, 0.3H, J = 8.0 Hz), 4.55 (d, 0.7H, J = 9.0 Hz), 4.47 (dd, 0.3H, J = 10.2, 5.8 Hz), 4.23–4.32 (m, 1.3H), 4.07–4.17 (m, 1.4H), 2.81 (s, 1.0H), 2.68 (s, 2.0H), 0.84 (s, 6.0H), 0.83 (s, 3.0H), 0.08 (s, 2.0H), 0.02 (s, 1.0H), –0.24 (s, 2.0H), –0.32 (s, 1.0H); ¹³C{¹H} NMR (100 MHz, CDCl₃, 2:1 rotamer mixture) δ 173.9, 172.8, 156.7, 155.4, 143.9, 143.8, 143.7, 143.6, 141.31, 141.26, 140.2, 139.6, 128.5, 128.4, 128.3, 128.2, 127.67, 127.63, 127.11, 127.08, 127.06, 126.99, 125.04, 124.97, 124.8, 120.0, 119.9, 73.3, 73.1, 68.0, 67.5, 67.2, 64.4,

47.1, 46.9, 34.4, 25.6, 17.94, 17.89, -4.6, -5.2, -5.3; IR (neat) 3371, 3065, 2954, 2929, 2895, 2856, 1710, 1681, 1451, 1252, 1091, 838 cm^{-1} ; HRMS (ESI-TOF) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{31}\text{H}_{37}\text{NO}_5\text{SiNa}$ 554.2333; Found 554.2320.

Fmoc-Ant-Ala-OtBu (**ent-10**). By following the procedure described above for **10**, the acylation of H-Ala-OtBu-HCl (1.00 g, 5.50 mmol) afforded the dipeptide **ent-10** (2.55 g, 95%) as a white amorphous solid. mp 123–124 °C; $[\alpha]_D^{25} +8.2$ (c 1.0, CHCl_3).

Fmoc-(2S,3S)-MePhe(3-OTBS)-Ant-Ala-OtBu (**11b**). By following the procedure described above for **11a**, the acylation of the *N*-Fmoc amine **ent-10** (274 mg, 563 μmol) afforded the tripeptide **11b** (383 mg, 88% in 2 steps) as a white amorphous solid. mp 88–89 °C; $[\alpha]_D^{25} +22$ (c 0.92, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , 3:2 rotamer mixture) δ 11.7 (s, 0.4H, s), 11.5 (s, 0.6H), 8.69 (d, 1.0H, $J = 8.3$ Hz), 7.75 (d, 0.8H, $J = 7.6$ Hz), 7.73 (d, 1.2H, $J = 7.6$ Hz), 7.10–7.63 (m, 14H), 6.89 (d, 0.4H, $J = 7.0$ Hz), 6.84 (d, 0.6H, $J = 7.0$ Hz), 5.30 (d, 0.6H, $J = 9.3$ Hz), 5.24 (d, 0.4H, $J = 9.3$ Hz), 5.06 (d, 0.6H, $J = 9.3$ Hz), 4.94 (d, 0.4H, $J = 9.3$ Hz), 4.62 (quin, 0.6H, $J = 7.0$ Hz), 4.32–4.47 (m, 1.8H), 4.07 (t, 0.6H, $J = 7.2$ Hz), 3.92–4.01 (m, 1.0H), 2.96 (s, 1.2H), 2.93 (s, 1.8H), 1.44–1.46 (m, 10.8H), 1.36 (d, 1.2H, $J = 7.0$ Hz), 0.76 (s, 3.6H), 0.71 (s, 5.4H), 0.04 (s, 1.2H), 0.02 (s, 1.8H), -0.22 (s, 1.2H), -0.23 (s, 1.8H); $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3 , 3:2 rotamer mixture) δ 172.4, 172.1, 168.5, 167.8, 167.7, 156.3, 155.7, 144.5, 144.2, 144.1, 143.8, 141.22, 141.17, 141.11, 141.07, 140.7, 139.7, 139.4, 132.8, 132.5, 128.22, 128.16, 128.1, 127.6, 127.5, 127.4, 127.3, 127.1, 127.0, 126.9, 126.84, 126.78, 126.7, 125.6, 125.3, 125.1, 125.0, 123.0, 122.9, 121.6, 121.5, 120.4, 119.9, 119.84, 119.80, 119.7, 82.5, 82.4, 73.4, 72.9, 68.3, 67.7, 66.1, 48.9, 47.1, 47.0, 31.5, 27.93, 27.89, 25.54, 25.49, 18.7, 18.6, 17.91, 17.87, -4.7, -4.8, -5.26, -5.30; IR (neat) 3339, 3066, 2951, 2929, 2888, 2856, 1691, 1650, 1523, 1444, 1146 cm^{-1} ; HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{45}\text{H}_{56}\text{N}_3\text{O}_7\text{Si}$ 778.3882; Found 778.3863.

Fmoc-(2S,3S)-MePhe(3-OH)-Ant-Ala-OtBu (**13**). To a solution of the TBS ether **11b** (100 mg, 129 μmol) in

dry THF (2.6 mL) was added HF·pyridine (234 μ L, 12.9 mmol, 100 equiv) at 0 °C under an argon atmosphere, and the mixture was stirred for 30 min at the same temperature. After being stirred at room temperature for 9 h, the reaction mixture was poured into saturated aqueous NaHCO₃ at 0 °C. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with 10% aqueous citric acid and brine, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (eluted with toluene/EtOAc = 9:1) to afford the alcohol **13** (65.0 mg, 97.9 μ mol, 76%) as a white amorphous solid. mp 68–69 °C; $[\alpha]_D^{24}$ –64 (*c* 0.81, CHCl₃); ¹H NMR (400 MHz, CDCl₃, 2:1 rotamer mixture) δ 12.1 (s, 0.7H), 11.9 (s, 0.3H), 8.67 (d, 0.7H, *J* = 8.4 Hz), 8.48 (d, 0.3H, *J* = 8.4 Hz), 7.72–7.76 (m, 2.0H), 7.10–7.57 (m, 14.0H), 6.87 (d, 0.7H, *J* = 6.8 Hz), 6.85 (d, 0.3H, *J* = 6.9 Hz), 5.39 (dd, 0.7H, *J* = 8.8, 2.0 Hz), 4.98 (d, 0.7H, *J* = 2.0 Hz), 4.64–4.76 (m, 1.6H), 4.53 (quin, 0.3H, *J* = 6.9 Hz), 4.16–4.33 (m, 3.7H), 2.80 (s, 2.0H), 2.61 (s, 1.0H), 1.47 (s, 3.0H), 1.39 (s, 6.0H), 1.32 (d, 1.0H, *J* = 6.9 Hz), 1.29 (d, 2.0H, *J* = 7.2 Hz); ¹³C{¹H} NMR (100 MHz, CDCl₃, 2:1 rotamer mixture) δ 172.2, 172.0, 170.9, 170.3, 167.7, 167.6, 156.3, 155.1, 144.0, 143.9, 143.83, 143.80, 141.5, 141.3, 141.2, 141.1, 139.7, 139.6, 139.1, 138.9, 132.9, 132.8, 128.41, 128.36, 128.32, 128.2, 127.7, 127.62, 127.57, 127.3, 127.04, 127.01, 126.97, 126.93, 126.8, 126.7, 125.4, 125.2, 124.7, 124.4, 123.4, 121.54, 121.47, 120.1, 120.0, 119.91, 119.87, 119.83, 82.6, 82.5, 73.0, 72.4, 68.1, 67.7, 66.6, 48.9, 48.8, 47.2, 27.93, 27.86, 18.6, 18.5; IR (neat) 3444, 3323, 2956, 2929, 2893, 2851, 1707, 1665, 1519, 1455, 1167, 847 cm^{–1}; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₃₉H₄₂N₃O₇ 664.3017; Found 664.3001.

Boc-D-allo-Ile-(2S,3S)-MePhe(3-OH)-Ant-Ala-OtBu (14) and the ester 15. By following the procedure described above for **12**, deFmoc of the *N*-Fmoc amine **13** (52.0 mg, 78.3 μ mol, 1.0 equiv) was performed. The resulting crude amine was used for next reaction without further purification.

To a solution of the crude amine in dry DMF (0.2 mL) was added Boc-D-*allo*-Ile-OH (54.0 mg, 235 μ mol), DIEA (82.0 μ L, 470 μ mol) and COMU (111 mg, 259 μ mol) at 0 °C under an argon atmosphere. The mixture

was stirred at room temperature for 26 h. After workup procedure described above for **12**, the resulting residue was purified by column chromatography on silica gel (eluted with hexane/EtOAc = 2:1) to give a mixture of the amide **14** and the ester **15**. Further purification by preparative TLC (eluted with hexane/EtOAc = 2:1) afforded the amide **14** (23.1 mg, 45% in 2 steps) as a white amorphous solid and the ester **15** (3.6 mg, 7% in 2 steps) as a pale yellow oil, respectively. Amide **14**: mp 59–60 °C; $[\alpha]_D^{24} -15$ (c 0.56, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 11.7 (s, 1H), 8.62 (d, 1H, *J* = 8.0 Hz), 7.55 (d, 1H, *J* = 8.0 Hz, c), 7.50 (t, 1H, *J* = 8.0 Hz), 7.39–7.40 (m, 2H), 7.29–7.35 (m, 4H), 7.13 (t, 1H, *J* = 8.0 Hz), 6.93 (d, 1H, *J* = 6.8 Hz), 5.37 (d, 1H, *J* = 9.1 Hz), 5.33 (d, 1H, *J* = 9.1 Hz), 4.90 (brs, 1H), 4.69 (brs, 1H), 4.57 (quin, 1H, *J* = 6.8 Hz), 4.45 (dd, 1H, *J* = 9.2, 2.1 Hz), 2.83 (s, 3H, k), 1.64 (brs, 1H), 1.49 (s, 9H, l), 1.45 (d, 3H, *J* = 6.8 Hz), 1.42 (s, 9H), 1.18–1.26 (m, 1H, n), 0.95–1.01 (m, 1H), 0.80 (t, 3H, *J* = 7.2 Hz), 0.41 (d, 3H, *J* = 6.5 Hz); ¹³C{¹H} NMR (150 MHz, CDCl₃) δ 173.1, 172.2, 170.2, 167.5, 155.8, 139.3, 139.0, 132.9, 128.54, 128.49, 127.2, 126.7, 123.4, 121.4, 120.0, 82.7, 79.1, 72.4, 53.2, 49.0, 45.8, 37.0, 28.3, 28.0, 26.9, 18.7, 13.4, 11.9, 8.6; IR (neat) 3423, 3355, 2972, 2929, 1715, 1647, 1600, 1589, 1521, 1448, 1160, 756 cm⁻¹; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₃₅H₅₁N₄O₈ 655.3701; Found 655.3685. Ester **15**: $[\alpha]_D^{22} +8.7$ (c 0.13, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ 11.4 (s, 1H), 8.54 (d, 1H, *J* = 7.7 Hz), 7.49 (d, 1H, *J* = 7.9 Hz), 7.46 (t, 1H, *J* = 7.9 Hz), 6.79 (d, 1H, *J* = 6.6 Hz), 6.19 (d, 1H, *J* = 5.4 Hz), 4.58 (quin, 1H, *J* = 6.4 Hz), 4.45 (dd, 1H, *J* = 9.3, 2.3 Hz), 3.63 (brd, 1H, *J* = 5.4 Hz), 2.46 (s, 3H), 1.92–1.97 (m, 1H), 1.52 (s, 9H), 1.45 (d, 3H, *J* = 6.6 Hz), 1.40 (s, 9H), 1.34–1.42 (m, 1H), 1.12–1.21 (m, 1H), 0.89 (t, 3H, *J* = 7.2 Hz), 0.73 (d, 3H, *J* = 6.6 Hz); ¹³C{¹H} NMR (150 MHz, CDCl₃) δ 172.2, 171.3, 169.6, 167.6, 155.9, 138.4, 136.0, 132.5, 128.44, 128.36, 127.0, 126.8, 123.2, 121.7, 121.4, 82.6, 79.6, 69.9, 56.9, 49.0, 37.5, 35.4, 29.7, 28.3, 28.0, 26.2, 18.7, 14.3, 11.7; IR (neat) 2961, 2928, 2882, 2861, 1701, 1513, 1471, 1246, 1156, 1088, 837 cm⁻¹; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₃₅H₅₁N₄O₈ 655.3701; Found 655.3682.

The revised structure of Asperterrestide A (1b). By following the procedure described above for **1a**, the

deprotection and macrolactamization of the linear tetrapeptide **14** (10.0 mg, 15.3 μ mol) afforded the revised structure of asperterrestide A (**1b**) (3.0 mg, 41% in 2 steps) as a white solid. mp 141–142 °C; $[\alpha]_D^{26}$ –33 (*c* 0.085, MeOH) [lit.¹⁰ $[\alpha]_D^{30}$ –13 (*c* 0.03, MeOH)]; ¹H NMR (600 MHz, CDCl₃) δ 9.15 (s, 1H), 8.20 (brd, 1H, *J* = 7.9 Hz), 7.49 (td, 1H, *J* = 7.9, 1.4 Hz), 7.41–7.42 (m, 2H), 7.29–7.37 (m, 4H), 7.16 (td, 1H, *J* = 7.9, 1.0 Hz), 7.02 (d, 1H, *J* = 10.0 Hz), 6.12 (d, 1H, *J* = 7.9 Hz), 5.77 (d, 1H, *J* = 9.5 Hz), 5.18 (dd, 1H, *J* = 9.5, 3.2 Hz), 4.47–4.52 (m, 1H), 4.34 (t, 1H, *J* = 10.0 Hz), 2.82 (s, 3H), 1.73–1.80 (m, 1H), 1.44 (d, 3H, *J* = 6.9 Hz), 0.81 (d, 3H, *J* = 6.5 Hz), 0.55 (t, 3H, *J* = 7.4 Hz), 0.26–0.37 (m, 2H); ¹³C{¹H} NMR (150 MHz, CDCl₃) δ 172.4, 170.9, 170.2, 169.2, 138.5, 135.1, 131.6, 128.6, 128.5, 127.4, 126.5, 125.5, 124.4, 123.4, 71.5, 60.8, 53.8, 50.0, 36.2, 31.3, 24.7, 14.8, 14.4, 10.8; IR (neat) 3286, 2961, 2925, 2871, 2856, 1676, 1644, 1602, 1584, 1450, 1045, 758 cm^{–1}; HRMS (ESI-TOF) *m/z*: [M+H]⁺ Calcd for C₂₆H₃₃N₄O₅ 481.2445; Found 481.2433.

General Procedure of Molecular Modeling. Conformational searches of **1d–f** were performed by a MacroModel program on Maestro Version 10.1.018.^{44–46} Initial coordinates of a macrocycle were generated by Mixed torsional/Low-mode sampling (Torsional sampling options: extended; Use 200 steps per rotatable bond; Energy window for saving structures: 42 kJ·mol^{–1}) with 10,000 steps and were energy minimized using an OPLS-2005 force field without solvent to provide possible conformers. 3D structures, calculated potential energies, relative energies, and the atomic coordinates of the lowest-energy conformers are summarized in Figures S1–S3 and Tables S3–S8 in the Supporting Information.

Evaluation of cytotoxicity of the synthetic 1a and 1b against cancer cell lines. A panel of three cancer cell lines were obtained and cultured as summarized in Table S13 (Supporting Information). The cytotoxicity of synthetic asperterrestides **1a** and **1b** against the cancer cell lines was assayed by measuring the amount of ATP in the cells using CellTiter-Glo (Promega, Madison, WI) as reported previously.^{52, 53} In brief, the cells were incubated in 384-well plates at a density of 500 cells/ well with a medium volume of 40 μ L for 24 h at 37 °C

under 5% CO₂. The cells were then treated with 0.1 μL of compound solutions at final concentration ranges of 20 μM to 1 nM (10-point dose) using an Echo 555 Liquid Handler (Labcyte, San Jose, CA). The vehicle solvent (DMSO) was used as a control at a maximum concentration of 0.1%. After a 72 h incubation at 37 °C under 5% CO₂, 10 μL of CellTiter-Glo reagent solution was added to the medium, and the plate was mixed with a plate mixer and incubated for 10 min at 30 °C. The luminescence was measured using an EnSpire 2300 (PerkinElmer). Absorbance for the control well (C) and test well (T) was measured along with that for the test well at time 0 (T₀). Cell growth inhibition (% growth) by each concentration of the drug was calculated as $100[(T - T_0)/(C - T_0)]$, and the IC₅₀ values were analyzed using Morphit software (The Edge Software Consultancy, Guildford, U.K.).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxxxxxxxxx.

¹H and ¹³C NMR for synthetic Fmoc-Ant-OH, **1a-b**, **3**, *ent*-**3**, **4a-b**, **5a-b**, **6a-b**, **7a-b**, **8a-b**, **9a-b**, **10**, *ent*-**10**, **11a-b**, **12**, **13**, **14** and **15**, NOESY spectra for **1a-b**, comparison of NMR spectroscopic data between natural **1** and synthetic **1a-b**, conformers obtained by molecular mechanics calculation for **1d-f**, correlation between observed NMR spectroscopic data and calculated values by molecular modeling, and biological assay data for **1a-b** (PDF)

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Prof. Motoki Takagi at Fukushima Medical University for evaluation of cytotoxicity against cancer cell lines. This work was supported by JSPS KAKENHI, Grant no. JP15H05837 (Grant-in-Aid for Scientific Research on Innovative Areas: Middle Molecular Strategy). This work was partially supported by the Platform Project for Supporting in Drug Discovery and Life Science Research from AMED under Grant Number JP18am0101095 and JP18am0101100.

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