Regular Article

Diastereoselective Total Synthesis and Structural Confirmation of Surugamide F

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Surugamide F is a linear decapeptide (1) isolated along with the cyclic octapeptides surugamides A–E (2–6), from a marine-derived *Streptomyces* species. The linear peptide 1 is produced by two nonribosomal peptide synthetases (NRPSs) encoded in adjacent open reading frames, which are further flanked by an additional pair of NRPS genes responsible for the biosyntheses of the cyclic peptides 2–6. While the cyclic peptides 2–6 were identified to be cathepsin B inhibitors, the biological activity of the new metabolite 1 still remained unclear. In order to elucidate its unique biosynthetic pathway and biological activity in detail, we planned to develop an efficient synthetic route toward 1. Here we report the diastereoselective total synthesis of 1, utilizing 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid-phase peptide synthesis. During this study, we found that the structural correction of 1 was required, due to the mislabeling of the commercially obtained 3-amino-2-methylpropionic acid, and the true structure of 1 was corroborated by the chemical synthesis and chromatographic comparison.

Key words marine natural product; structural elucidation; total synthesis; peptide

The marine-derived actinomycete *Streptomyces* sp. JAMM992 produces surugamides A–E (**2**–**6**, Fig. 1b), along with surugamide F (**1**, Fig. 1a). The cyclic octapeptides **2**–**6** were initially isolated and identified to be cathepsin B inhibitors. Subsequently, **1** was identified as a new linear decapeptide[1] from the same *Streptomyces* strain,[2,3] although its biological activity has not been evaluated yet. The draft genome sequence encodes four successive genes *surA*, *surB*, *surC* and *surD*, which are clustered and annotated as nonribosomal peptide synthetases (NRPSs) with 18 A domains in total. The mutation experiment revealed that *surA* and *surD* at both ends are responsible for the biosyntheses of **2**–**6**. The other genes, *surB* and *surC*, which are flanked by *surA* and *surD* are responsible for the biosynthesis of the structurally unrelated peptide **1**. Since its intercalated NRPS gene architecture is unprecedented, the new marine natural product **1** attracted interest in terms of not only its unidentified biological activities but also the biosynthetic mechanisms of **1** in relation to the cyclic peptides **2**–**6**.

In the preceding study, we unveiled the structure of the linear peptide **1** by the combination of NMR, LC-MS/MS, and Marfey's analyses.[4,5] The structure of peptide **1** was determined to possess four D-amino acids and a 3-amino-2-methylpropionic acid (AMPA). Furthermore, the planar structure and stereochemistries of all amino acids except for AMPA, were validated by chemical synthesis.[6,7] Specifically, peptide **1** was chemically constructed by 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid-phase peptide synthesis using racemic Fmoc-AMPA (**9**) as a building block, to yield the diastereomers (Chart 1a), which were separated by reversed-phase HPLC. The stereochemically pure peptides were chromatographically and spectroscopically compared with natural **1** by LC-MS (Chart 1b) and NMR, respectively. For the structural elucidation of AMPA, natural **1** was subjected to total hydrolysis (6 M HCl, 110°C, 4 h), and then the corresponding hydrolysates were treated with N^{α}-(2,4-dinitro-5-fluorophenyl)-L-valinamide (FDNP-Val). The FDNP-Val derivative of AMPA (C, Chart 1c) was chromatographically compared to the authentic samples, which were obtained from commercially available, optically active AMPAs. As a result, the stereochemistry of AMPA in natural **1** was reported to be the (*S*) form (Chart 1d).

We then turned our attention to the development of an efficient synthetic strategy for **1a** because the previous synthetic scheme generates an equal amount of the diastereomer **1b**, which is difficult to separate from **1a**. Concurrently, the reagent supplier notified us about the mislabeling of the optically pure 3-amino-2-methylpropionic acids,[8] which were utilized as the standards for the stereochemical assignment in the previous study (Chart 1). Thus, the stereochemistry of AMPA required a structural correction. Herein, we report the first diastereoselective total synthesis of surugamide F (**1**), as well as the confirmation of the true structure of **1** by chemical synthesis. The details of the structural correction of **1** are also described.

At the outset of the diastereoselective total synthesis of **1**, the requisite building blocks **9a** and **9b** were prepared (Chart 2). The chiral **14a** was synthesized from **11a**, by using Evans' asymmetric alkylation[9] and azide reduction as key reactions. Protecting group manipulation of **14a** with trifluoroacetic acid (TFA), followed by Fmoc-Cl, led to **9a**.

The enantiomeric counterpart **9b** was also synthesized from **11b**, in the same manner. At this stage, the absolute configuration of **14b** was confirmed by the Kusumi method[10,11] (Chart 3).

With the building blocks in hand, we commenced the solid-phase peptide syntheses of **1a** and **1b** from Fmoc-D-alanine-loaded Wang-resin **7** (Chart 4a). The Fmoc group of **7** was de-

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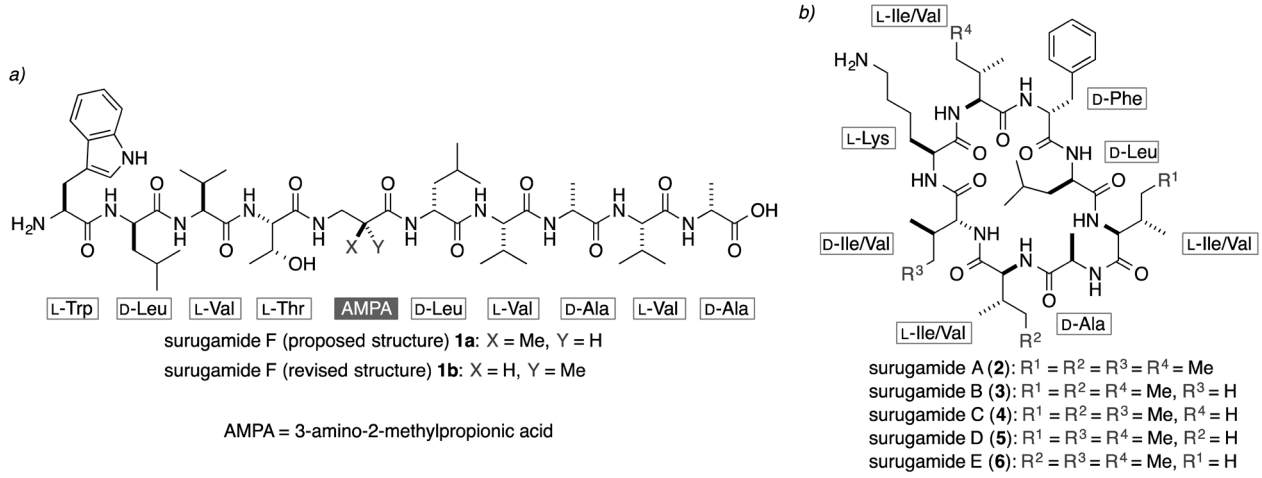
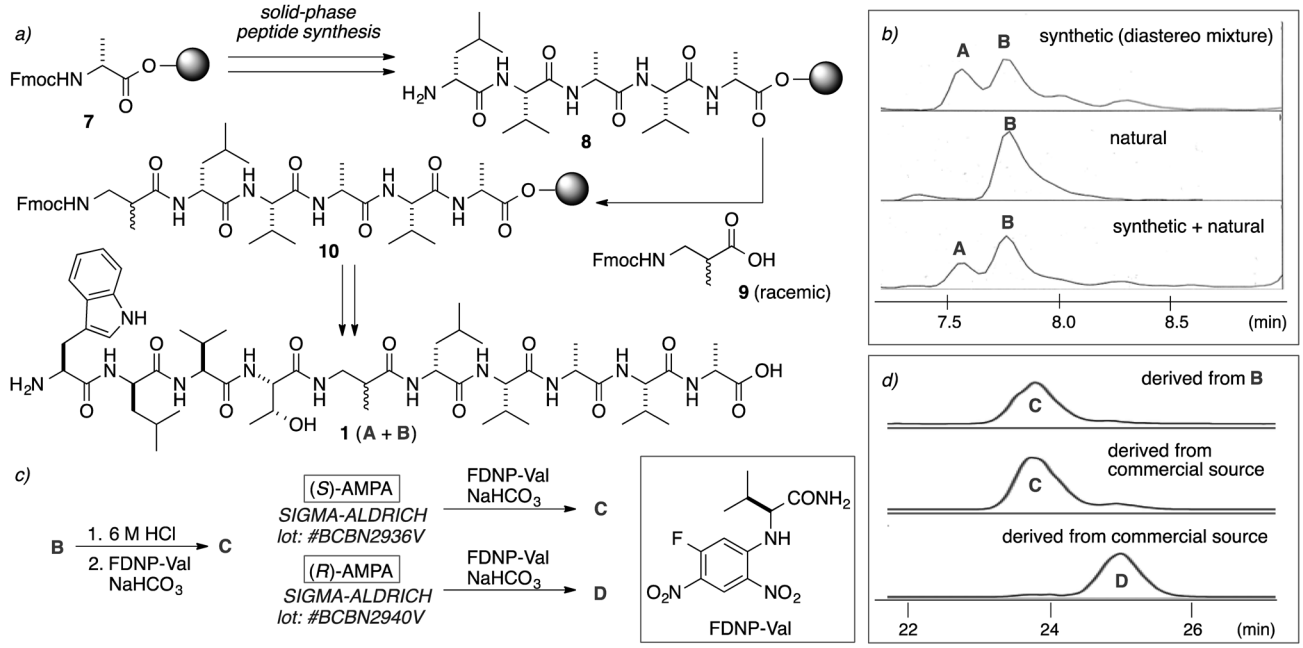


Fig. 1. (a) Structure of Surugamide F (**1**); (b) Structures of Surugamides A–E (**2**–**6**)



(a) Previous structural confirmation of **1** by chemical synthesis. (b) LC-MS charts of synthetic and natural **1**. (c) Marfey's analysis of AMPA. (d) LC-MS charts of the Marfey's analysis of AMPA.

Chart 1.

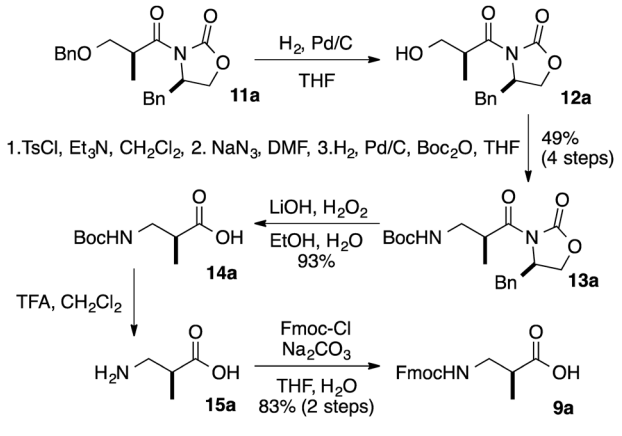


Chart 2. Synthesis of Chiral AMPA

tached by a treatment with piperidine, and then four rounds of *N*,*N*′-diisopropylcarbodiimide (DIC)/Oxyma[12]-mediated amide coupling[13] and piperidine-promoted N^{α}-deprotection were applied to **18**, leading to the resin-bound pentapeptide **8**. The amine **8** was separately coupled with **9a** and **9b** to afford **10a** and **10b**, and then Fmoc-based solid phase peptide synthesis was re-applied to **10a** and **10b** for the syntheses of the resin-bound decapeptides **20a** and **20b**. Finally, the treatment of **20a** and **20b** with TFA–*i*-Pr_3SiH–H_2O (=90 : 5 : 5) simultaneously achieved the global deprotection of the protecting groups and the cleavage from the Wang resin, thus releasing crude **1a** and **1b** into the solution. After octadecyl silica (ODS)-HPLC purification, **1a** and **1b** were obtained in 36% yield and 38% yields in 20 steps, respectively. The synthesized **1a** and **1b** were then chromatographically compared with the natural **1** (Chart 4b), confirming the true structure of the natural surugamide F (**1**),

as depicted in **1b**.

In summary, the diastereoselective total synthesis of surugamide F (**1**) was achieved, utilizing Fmoc-based solid-phase peptide synthesis was achieved. During this study, we found that the structural correction of **1** was required, and we also corroborated the true structure of **1** by the chemical synthesis. Based on the established synthetic pathway to **1**, detailed biological and biosynthetic studies of **1** are currently underway and will be reported in due course.

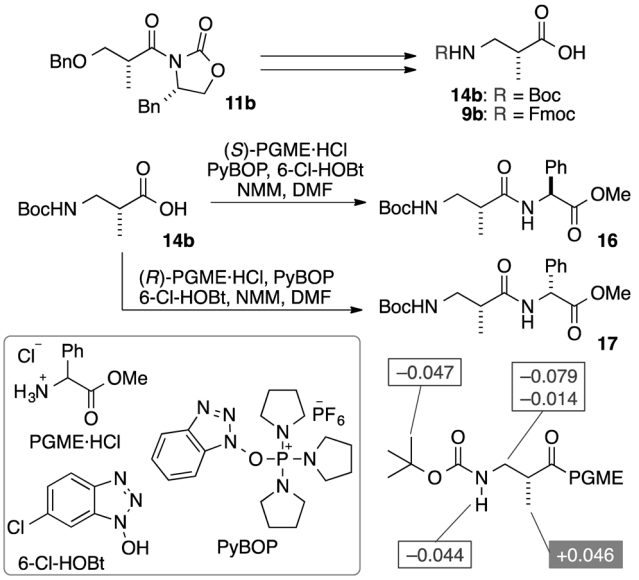


Chart 3. Structural Confirmation of Chiral AMPA

Experimental

General Methods ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL ECA 500 spectrometer (500 MHz for ^{1}H-NMR). Chemical shifts are denoted in δ (ppm) relative to residual solvent peaks as internal standards ($CDCl_3$, ^{1}H δ 7.26, ^{13}C δ 77.0). Electrospray ionization (ESI)-MS spectra were recorded on a Thermo Scientific Exactive mass spectrometer or a SHIMADZU LCMS-2020 spectrometer. Specific rotations were recorded on a JASCO P-1030 polarimeter. HPLC experiments were performed with a SHIMADZU HPLC system equipped with an LC-20AD intelligent pump. All reagents were used as supplied unless otherwise stated. Analytical TLC was performed using E. Merck Silica gel 60 F_{254} precoated plates. Column chromatography was performed using 40–50 μm Silica Gel 60N (Kanto Chemical Co., Inc., Japan).

Procedure for Solid-Phase Peptide Synthesis (SPPS) Step 1: To the solution of carboxylic acid (4 eq) were added DIC (4 eq, 0.50 M in NMP) and Oxyma (4 eq, 0.50 M in *N*,*N*-dimethylformamide (DMF)). After 2 min of pre-activation, the mixture was injected into the reaction vessel. The resulting mixture was stirred for 30 min at 37°C.

Step 2: The resin in the reaction vessel was washed with DMF (×3) and CH_2Cl_2 (×3).

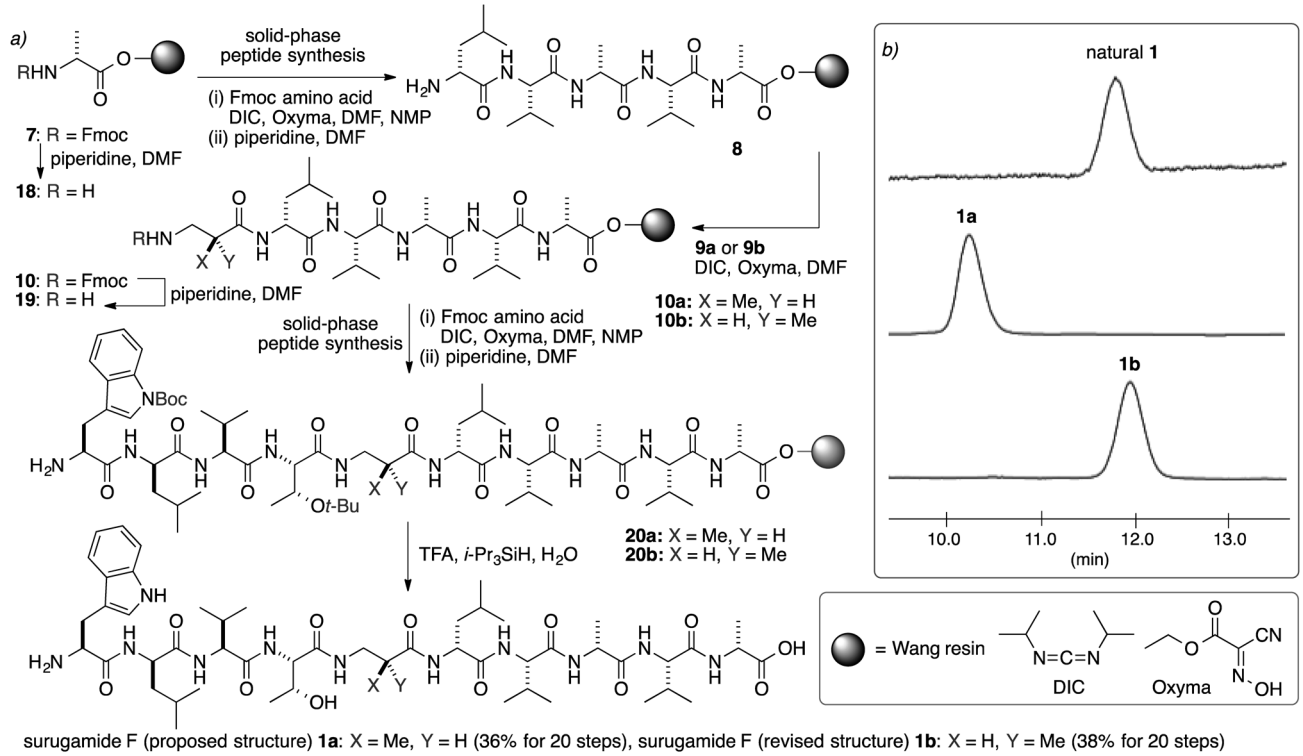
Step 3: The Fmoc group of the solid supported peptide was removed with a 20% piperidine/DMF solution (10 min, room temperature).

Step 4: The resin in the reaction vessel was washed with DMF (×3) and CH_2Cl_2 (×3).

Amino acids were condensed onto the solid support by repeating Steps 1–4.

Carbamate **13a**

To a solution of **11a** [CAS 203454-44-8] (162 mg,



surugamide F (proposed structure) **1a**: X = Me, Y = H (36% for 20 steps), surugamide F (revised structure) **1b**: X = H, Y = Me (38% for 20 steps)

(a) Diastereoselective total synthesis of **1**. (b) LC-MS charts of synthetic and natural **1**.

Chart 4.

0.457 mmol) in tetrahydrofuran (THF) (10 mL) was added Pd/C (10% on carbon, 80.2 mg). The mixture was exposed to a hydrogen atmosphere at room temperature. After stirring overnight, the resulting mixture was filtered and concentrated to give **12a**, which was used in the next reaction without further purification.

To a solution of **12a** in CH_2Cl_2 (4 mL) at room temperature were added Et_3N (0.13 mL, 0.93 mmol), *N*,*N*-dimethyl-4-aminopyridine (DMAP) (13.9 mg, 0.114 mmol), and *p*-TsCl (109 mg, 0.569 mmol). After stirring overnight, saturated aqueous NH_4Cl was added to the reaction mixture. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give the crude tosylate, which was used in the next reaction without further purification.

To a solution of the above tosylate in DMF (4 mL) at room temperature was added NaN_3 (132 mg, 2.03 mmol). After stirring at 40°C for 2 d, saturated aqueous $NaHCO_3$ was added to the reaction mixture. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give the crude azide, which was used in the next reaction without further purification.

To a solution of the above azide in THF (8 mL) were added Boc_2O (0.19 mL, 0.83 mmol) and Pd/C (10% on carbon, 80.7 mg). The mixture was exposed to a hydrogen atmosphere at room temperature. After stirring overnight, the resulting mixture was filtered and concentrated. The residue was purified by column chromatography (EtOAc–hexane=30 : 70) to afford **13a** (80.4 mg, 49% for 4 steps) as a colorless solid: $[\alpha]_D^{26}$ −51.7 (*c*=2.00, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.30 (dd, 2H, *J*=7.5, 7.5 Hz), 7.23 (t, 1H, *J*=7.5 Hz), 7.16 (d, 2H, *J*=7.5 Hz), 4.94 (br, 1H), 4.63 (m, 1H), 4.14 (m, 2H), 3.86 (m, 1H), 3.43 (m, 1H), 3.34 (m, 1H), 3.25 (d, 1H, *J*=13.0 Hz), 2.75 (dd, 1H, *J*=10.0, 13.3 Hz), 1.39 (s, 9H), 1.16 (d, 3H, *J*=7.5 Hz); ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 175.6, 155.9, 153.2, 135.5, 129.6, 129.1, 128.4, 127.4, 79.3, 66.3, 55.5, 43.2, 38.9, 38.0, 28.5, 14.8; high resolution (HR)-MS (ESI) Calcd for $C_{19}H_{26}N_2O_5Na^+$ $[M+Na]^+$ 385.1734. Found 385.1734.

13b: $[\alpha]_D^{25}$ +57.0 (*c*=2.00, MeOH).

Acid **14a**

To a solution of **13a** (201 mg, 0.555 mmol) in THF (10 mL) at 0°C were added 30% aqueous H_2O_2 (0.18 mL) and $LiOH \cdot H_2O$ (36.8 mg, 1.61 mmol) in H_2O (3 mL). After stirring at 0°C for 2.5 h, saturated aqueous Na_2SO_3 and saturated aqueous $NaHCO_3$ were added to the reaction mixture. After stirring at room temperature for 2 h, the mixture was evaporated to remove the THF. The aqueous solution was washed with CH_2Cl_2 (twice), acidified with 1 M HCl (to pH 3), and then extracted with EtOAc (3 times). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give **14a** [CAS 190897-47-3] (105 mg, 93%).

Acid **9a**

TFA (3 mL) was added to a solution of **14a** (105 mg, 0.519 mmol) in CH_2Cl_2 (7 mL) at room temperature. After stirring at room temperature for 2 h, the resulting mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

Fmoc-Cl (167 mg, 0.646 mmol) in THF (2 mL) was added to a solution of the above amine, in THF (6 mL) and 10% aqueous Na_2CO_3 (9 mL) at 0°C. After stirring overnight at room temperature, the mixture was evaporated to remove the THF. The aqueous solution was washed with Et_2O (twice), acidified with 1 M HCl (to pH 2), and then extracted with EtOAc (3 times). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated to give **9a** [CAS 211682-15-4] (140 mg, 83%).

Peptide **20a**

The Fmoc-D-Ala-Wang-resin **7** (82.4 mg, 0.0503 mmol) in a Libra tube (HiPep Laboratories) was suspended in a 20% piperidine/DMF solution. After stirring at room temperature for 10 min, the reaction mixture was washed with DMF (×3) and CH_2Cl_2 (×3) to afford the amine **18**, as the resin-bound form. This resin was swelled in DMF for 1 h, and then subjected to 9 cycles [Fmoc-L-Val-OH, Fmoc-D-Ala-OH, Fmoc-L-Val-OH, Fmoc-D-Leu-OH, **9a**, Fmoc-L-Thr(*t*-Bu)-OH, Fmoc-L-Val-OH, Fmoc-D-Leu-OH, and Fmoc-L-Trp(Boc)-OH] of the SPPS protocol, to afford the peptide **20a** as the resin-bound form.

Peptide **1a**

To peptide **20a** was added a mixture of TFA–H_2O–*i*-Pr_3SiH=90 : 5 : 5 (1.0 mL). After stirring for 30 min, the reaction mixture was filtered, and then washed with a mixture of TFA–H_2O–*i*-Pr_3SiH=90 : 5 : 5 (1.0 mL). The filtrate was diluted with Et_2O (20 mL) and centrifuged (4°C, 3500 G, 5 min), and then the Et_2O was removed by decantation. The obtained crude peptide **1a** was purified by reversed phase HPLC (COSMOSIL 5C_{18}-MS-II 10×250 mm) with MeOH–H_2O (=65 : 35) containing 0.05% TFA to afford **1a**[1)] (18.9 mg, 36% from **7**). $[\alpha]_D^{16}$ −6.6 (*c*=0.05, MeOH).

Peptide **20b**

The Fmoc-D-Ala-Wang-resin **7** (86.9 mg, 0.0530 mmol) in a Libra tube (HiPep Laboratories) was suspended in a 20% piperidine/DMF solution. After stirring at room temperature for 10 min, the reaction mixture was washed with DMF (×3) and CH_2Cl_2 (×3) to afford the amine **18**, as the resin-bound form. This resin was swelled in DMF for 1 h, and then subjected to 9 cycles [Fmoc-L-Val-OH, Fmoc-D-Ala-OH, Fmoc-L-Val-OH, Fmoc-D-Leu-OH, **9b**, Fmoc-L-Thr(*t*-Bu)-OH, Fmoc-L-Val-OH, Fmoc-D-Leu-OH, and Fmoc-L-Trp(Boc)-OH] of the SPPS protocol, to afford the peptide **20b** as the resin-bound form.

Peptide **1b**

Peptide **20b** was combined with a mixture of TFA–H_2O–*i*-Pr_3SiH=90 : 5 : 5 (1.0 mL). After stirring for 30 min, the reaction mixture was filtered, and then washed with a mixture of TFA–H_2O–*i*-Pr_3SiH=90 : 5 : 5 (1.0 mL). The filtrate was diluted with Et_2O (20 mL) and centrifuged (4°C, 3,500 G, 5 min), and then the Et_2O was removed by decantation. The obtained crude peptide **1a** was purified by reversed phase HPLC (COSMOSIL 5C_{18}-AR-II 10×250 mm), with MeOH–H_2O (=60 : 40 to 100 : 0 for 40 min) containing 0.05% TFA, to afford **1b**[1] (20.3 mg, 38% from **7**). $[\alpha]_D^{16}$ −30.8 (*c*=0.05, MeOH).

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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