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 initially isolated and 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-fluorenylmethylcarbonyl (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. JMM992 produces surugamides A–E (2–6), 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 from the same Streptomyces strain, 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 non-ribosomal 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 Marley’s analyses. The structure of peptide 1 was determined to possess four α-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. Specifically, peptide 1 was chemically constructed by 9-fluorenylmethylcarbonyl (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 Nα-(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 1 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, 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 and azide reduction as key reactions. Protecting group manipulation of 14a with trifluoroacetic acid (TFA), followed by Fmoc-CI, 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 (Chart 3).

With the building blocks in hand, we commenced the solid-phase peptide syntheses of 1a and 1b from Fmoc-α-alanine-loaded Wang-resin 7 (Chart 4a). The Fmoc group of 7 was de-
attached by a treatment with piperidine, and then four rounds of $N,N'$-diisopropylcarbodiimide (DIC)/Oxyma 12)-mediated amide coupling and piperidine-promoted $N\alpha$-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-PrSiH–H2O ($\equiv$ 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 (I) was achieved, utilizing Fmoc-based solid-phase peptide synthesis was achieved. During this study, we found that the structural correction of I was required, and we also corroborated the true structure of I by the chemical synthesis. Based on the established synthetic pathway to I, detailed biological and biosynthetic studies of I are currently underway and will be reported in due course.

Experimental

General Methods  
'H- and 13C-NMR spectra were recorded on a JEOL ECA 500 spectrometer (500MHz for 'H-NMR). Chemical shifts are denoted in δ (ppm) relative to residual solvent peaks as internal standards (CDCl3, 'H δ 7.26, 13C 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 F254 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 CH2Cl2 (×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 CH2Cl2 (×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,
To a solution of 12a in CH₂Cl₂ (4 mL) at room temperature were added Et₂N (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 NH₄Cl 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 MgSO₄, 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 NaN₃ (132 mg, 2.03 mmol). After stirring, the resulting mixture was washed with brine, dried over MgSO₄, filtered, and concentrated to afford the amine 12a, which was used in the next reaction without further purification.

To a solution of 12a in CH₂Cl₂ (4 mL) at room temperature were added 30% aqueous H₂O₂ (0.18 mL) and LiOH·H₂O (36.8 mg, 1.61 mmol) in H₂O (3 mL). After stirring overnight at room temperature, the reaction mixture was washed with Et₂O (20 mL) and centrifuged (4°C, 3500 G, 5 min), and then the Et₂O was removed by decantation. The obtained crude peptide 1a was purified by reversed phase HPLC (COSMOsil SC 5μ-MS-II 10×250 mm) with MeOH–H₂O–HCl (to pH 2) as the resin-bound form. This resin was swelled in DMF (1h), and then subjected to 9 cycles [Fmoc-L-Val-OH, Fmoc-D-Ala-OH, Fmoc-L-Val-OH, Fmoc- WARRANTY-Oh, Fmoc-Val-OH, Fmoc-Val-OH, Fmoc-Val-OH, Fmoc-Val-OH, and Fmoc-Val-OH] of the SPPS protocol, to afford the peptide 20a as the resin-bound form.

To peptide 20a was added a mixture of TFA–H₂O–i-PrSiH=90:5:5 (1.0 mL). After stirring for 30 min, the reaction mixture was filtered, and then washed with a mixture of TFA–H₂O–i-PrSiH=90:5:5 (1.0 mL). The filtrate was diluted with Et₂O (20 mL) and centrifuged (4°C, 3500 G, 5 min), and then the Et₂O was removed by decantation. The obtained crude peptide 1a was purified by reversed phase HPLC (COSMOsil SC 5μ-MS-II 10×250 mm) containing 0.05% TFA, to afford 1a (18.9 mg, 36% from 7). [α]D° -6.6 (c=0.05, MeOH).

To peptide 20b was combined with a mixture of TFA–H₂O–i-PrSiH=90:5:5 (1.0 mL). After stirring for 30 min, the reaction mixture was filtered, and then washed with a mixture of TFA–H₂O–i-PrSiH=90:5:5 (1.0 mL). The filtrate was diluted with Et₂O (20 mL) and centrifuged (4°C, 3500 G, 5 min), and then the Et₂O was removed by decantation. The obtained crude peptide 1b was purified by reversed phase HPLC (COSMOsil SC 5μ-AR-II 10×250 mm), containing 0.05% TFA, to afford 1b (20.3 mg, 38% from 7). [α]D° -30.8 (c=0.05, MeOH).

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References