Instructions for use

Title

Diastereoselective Total Synthesis and Structural Confirmation of Surugamide F

Author(s)

Kuranaga, Takefumi; Fukuba, Atsuki; Ninomiya, Akihiro; Takada, Kentaro; Matsunaga, Shigeki; Wakimoto, Toshiyuki

Citation

Chemical & pharmaceutical bulletin, 66(6): 637-641

Issue Date

2018-06

Doc URL

http://hdl.handle.net/2115/70954

Type

article

File Information

WoS_84955_Wakimoto.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP
Diastereoselective Total Synthesis and Structural Confirmation of Surugamide F

Takefumi Kuranaga,*a Atsuki Fukuba,a Akihiro Ninomiya,b Kentaro Takada,b Shigeki Matsunaga,ba and Toshiyuki Wakimoto*a,b

Faculty of Pharmaceutical Sciences, Hokkaido University; Kita-12, Nishi-6, Kita-ku, Sapporo 060–0812, Japan; and
Graduate School of Agricultural and Life Sciences, The University of Tokyo; 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan.

Received January 31, 2018; accepted March 7, 2018.© 2018 The Pharmaceutical Society of Japan

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. Subsequently, 1 was identified as a new linear decapptide (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 Marley’s analyses.5,6) The structure of peptide 1 was determined to possess four ε-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.5,6) Specifically, peptide 1 was chemically constructed by 9-fluorenylmethyl-oxycarbonyl (Fmoc)-based solid-phase peptide synthesis using racemic Fmoc-AMPA (9a) 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 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,9) 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, using Evans’ asymmetric alkylation9) 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 method10) (Chart 3).

With the building blocks in hand, we commenced the solid-phase peptide syntheses of 1a and 1b from Fmoc-δ-alanine-loaded Wang-resin 7 (Chart 4a). The Fmoc group of 7 was de-
tached by a treatment with piperidine, and then four rounds of N,N'-diisopropylecarboximidate (DIC)/Oxyma 12)-mediated amide coupling13) and piperidine-promoted Nα-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-Pr₃SiH–H₂O (=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),

Chart 1. Synthesis of Chiral AMPA
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 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.

Experimental

General Methods 1H- and 13C-NMR spectra were recorded on a JEOL ECA 500 spectrometer (500MHz for 1H-NMR). Chemical shifts are denoted in δ (ppm) relative to residual solvent peaks as internal standards (CDCl3, 1H 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 60F$_{254}$ pre-coated plates. Column chromatography was performed using 40–50µm 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, 0.50M in NMP) and Oxyma (4 eq, 0.50M 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,
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 CH₂Cl₂ (4 mL) at room temperature were added Et₃N (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 Na₂SO₃ (20.1 mg, 0.19 mL, 0.83 mmol) and Pd/C (10% on carbon, 80.2 mg). The mixture was exposed to air and stirred at 0°C for 2.5 h, saturated aqueous NaHCO₃ was added to the reaction mixture. 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 afford the crude amine, 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 Et₂O (20 mL) and centrifuged (4°C, 3,500 G, 5 min), and then the Et₂O was removed by decantation. The obtained crude peptide 1a was purified by reversed phase HPLC (COSMOSIL 5C₁₈-AR-II 100 × 25 mm) with MeOH–H₂O=95:5 containing 0.05% TFA to afford 1b (18.9 mg, 36% from 7). [α]D₂⁰ = −6.6 (c = 0.05, MeOH).

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 times) and CH₂Cl₂ (3 times) 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–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 S₅₁₈-MS-II 100 × 250 mm) with MeOH–H₂O=65:35 containing 0.05% TFA to afford 1b (18.9 mg, 36% from 7). [α]D₂⁰ = −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 times) and CH₂Cl₂ (3 times) 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–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 S₅₁₈-AR-II 100 × 250 mm) with MeOH–H₂O=60:40 to 100:0 for 40 min) containing 0.05% TFA, to afford 1b (20.3 mg, 38% from 7). [α]D₂⁰ = −30.8 (c = 0.05, MeOH).

Acknowledgments

This work was partly supported by the Takeda Science Foundation, the Astellas Foundation for Research on Metabolic Disorders, the Naito Foundation, the SUNBOR GRANT, the NOASTEC Foundation, the Akira- yama Life Science Foundation, and Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (JSPS KAKENHI Grant Numbers JP16703511, JP15547389, JP15597834, and JP14506930).
Conflict of Interest  The authors declare no conflict of interest.

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

References
1) Ninomiya A., Katsuyama Y., Kuranaga T., Miyazaki M., Nogi Y., Okada S., Wakimoto T., Ohnishi Y., Matsunaga S., Takada K., ChemBioChem, 17, 1709–1712 (2016).
2) Takada K., Ninomiya A., Naruse M., Sun Y., Miyazaki M., Nogi Y., Okada S., Matsunaga S., J. Org. Chem., 78, 6746–6750 (2013).
3) Mohimani H., Gurevich A., Mikheenko A., Garg N., Nothias L.-F., Ninomiya A., Takada K., Dorrestein P. C., Pevzner P. A., Nat. Chem. Biol., 13, 30–37 (2017).
4) Marfey P., Carlsberg Res. Commun., 49, 591–596 (1984).
5) Bhushan R., Brückner H., J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 879, 3148–3161 (2011).
6) Structural revision of scarce peptide by chemical synthesis, see: Kuranaga T., Sesoko Y., Sakata K., Maeda N., Hayata A., Inoue M., J. Am. Chem. Soc., 135, 5467–5474 (2013).
7) Structural revision of scarce peptide by chemical synthesis, see: Kuranaga T., Mutoh H., Sesoko Y., Goto T., Matsunaga S., Inoue M., J. Am. Chem. Soc., 137, 9443–9451 (2015).
8) Ninomiya A., Katsuyama Y., Kuranaga T., Miyazaki M., Nogi Y., Okada S., Wakimoto T., Ohnishi Y., Matsunaga S., Takada K., ChemBioChem, 18, 1770 (2017).
9) Evans D. A., Connell B. D., J. Am. Chem. Soc., 125, 10899–10905 (2003).
10) Nagai Y., Kusumi T., Tetrahedron Lett., 36, 1853–1856 (1995).
11) Yabuuchi T., Kusumi T., J. Org. Chem., 65, 397–404 (2000).
12) Subirós-Funosas R., Prohens R., Barbas R., El-Faham A., Albericio F., Chem. Eur. J., 15, 9394–9403 (2009).
13) Kuranaga T., Enomoto A., Tan K., Fujita K., Wakimoto T., Org. Lett., 19, 1366–1369 (2017).