Tetrahedron 67 (2011) 9067–9071

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of tokaramide A, a cysteine protease inhibitor from marine sponge *Theonella aff. mirabilis*

Hiroyuki Konno [a,*], Kazuto Nosaka [b], Kenichi Akaji [c]

[a] Department of Biochemical Engineering, Graduate School of Science and Technology, Yamagata University, Yonezawa, Yamagata 992-8510, Japan
[b] Department of Chemistry, Hyogo Collage of Medicine, Nishinomiya, Hyogo 663-8501, Japan
[c] Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan

ARTICLE INFO

Article history:
Received 30 August 2011
Received in revised form 23 September 2011
Accepted 24 September 2011
Available online 1 October 2011

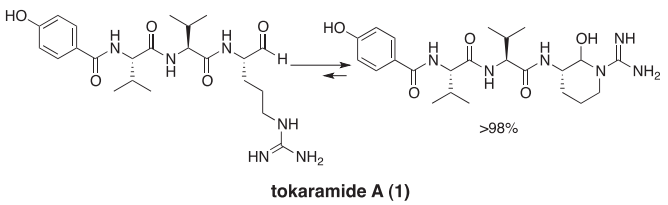
Keywords:
Tokaramide A
Cysteine protease inhibitor
Marine sponge
Unusual amino acid
Cathepsin B

ABSTRACT

The first synthesis of tokaramide A (**1**) is described. Tokaramide A (**1**) was synthesized via the peptide with a side-chain unprotected arginine residue on Weinreb AM resin by reductive cleavage.

1. Introduction

Tokaramide A (**1**) was isolated by Fusetani et al. from the marine sponge *Theonella aff. mirabilis* and was shown to highly inhibit cathepsin B with an IC_{50} value of 29 ng/mL.[1] Cathepsin B, the most extensively studied enzyme of the group, is not only a lysosomal cysteine protease that plays a role in a large number of physiological processes, but is also known to be involved in various disease stages, such as inflammation, trauma, muscular dystrophy, and tumors.[2] In particular, its possible roles in cancer metastasis are of major concern in cancer chemotherapy as cathepsin B inhibitors are potential anticancer drugs.[3] Moreover, Hook's group reported that cathepsin B cleaved the β-secretase site of the wild-type amyloid precursor protein (APP) in the brain to produce amyloid beta peptide, whose aggregation causes Alzheimer's disease.[4] Tokaramide A (**1**) is composed of two L-valine (Val), L-argininal (Argal), and *p*-hydroxybenzoyl (HBA) residues and is believed to inhibit the enzyme by forming a tetrahedral hemithioacetate between the aldehyde of the argininal residue and the thiolate of the enzyme's active site. Recently, peptide aldehyde has been used as an enzyme inhibitor, a probe of peptide structure–function relationships, and as a precursor for the preparation of other compounds.[5] As part of our program for the design and synthesis of cysteine protease inhibitors,[6] we herein describe the first synthesis of tokaramide A (**1**) (Fig. 1).



tokaramide A (1)

Fig. 1. Structure of tokaramide A (**1**) from marine sponge.

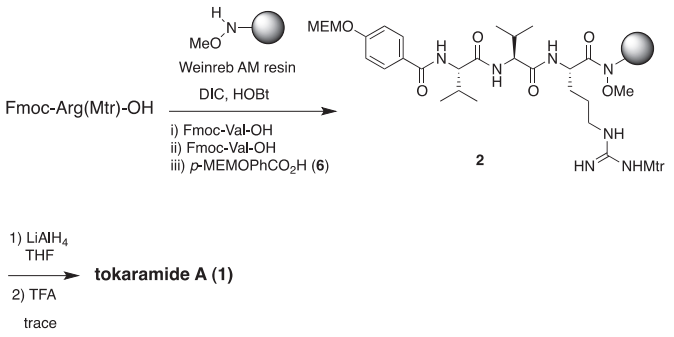
2. Results and discussion

We first examined the construction of C-terminus aldehyde via the reduction of Weinreb amide on a solid support. Although several methods for solid-phase synthesis of peptide aldehyde have been reported,[7] the reactions of Weinreb-type amides on solid support have more potential to enable the synthesis of aldehyde and ketone libraries using commercially available Fmoc-amino acids.[8] The condensation/deprotection procedure was repeated for the introduction of Fmoc-Arg(Mtr)–OH, Fmoc-Val–OH, and *p*-MEMOPhCO$_2$H (**6**) to give the tripeptide resin (**2**). Reduction of **2**

* Corresponding author. Tel./fax: +81 238 26 3131; e-mail address: konno@yz.yamagata-u.ac.jp (H. Konno).

0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.09.116

with $LiAlH_4$ in THF and deprotection with TFA afforded tokaramide A (**1**) as a cyclized hemi-aminal structure in a trace amount of yield (Scheme 1).



Scheme 1.

In order to confirm the discouraging results of solid-phase synthesis, it was attempted to synthesize the required peptide aldehyde by solution-phase peptide synthesis using Fmoc chemistry. Fmoc-Arg(Mtr)–OH was coupled with *N*,*N*-methoxymethylamine in the presence of BOP[9]/HOBt to give Weinreb amide (**3**) in 90% yield. Sequential addition of the two valine residues gave protected tripeptide (**5**). After removal of the Fmoc group with 20% piperidine in MeCN, coupling of *p*-$MEMOPhCO_2H$ (**6**) using HATU[10]/HOAt[11] afforded Weinreb amide-containing peptide (**7**) in 90% yield. Reduction of Weinreb amide (**7**) with $LiAlH_4$ in THF gave the desired hemi-aminal (**8**) in 60% yield. Finally, the hemi-aminal (**8**) was treated with TFA; however, the ^1H NMR and mass spectra of the major product were not identical to those recorded for tokaramide A (**1**) as a cyclic aminal structure and the determined structure **9** (Scheme 2).

a Friedel–Crafts-type reaction between the 2,4,6-trimethyl-4-methoxyphenyl-sulfonyl (Mtr) protecting group and aldehyde (Scheme 3). The initial attack of the electron-rich aromatic ring (**10**) on the aldehyde could occur either before or after desulfonation to form transient intermediate **11** followed by **12** with mass 134 Da higher than tokaramide A (**1**). Subsequently dehydration, cyclization of the guanidine group, and elimination of the aromatic ring results in the proposed modified peptide **9** with mass 16 Da smaller than tokaramide A (**1**). It shall be assumed to give the precursor of the compound **9** as a mixture in the ratio 4:3 via the elimination of the α-hydrogen in the C-terminal residue. TFA-mediated deprotection in the presence of several scavengers was also not effective.

For these reasons, TFA mediated the deprotection of **7** and following purification by RP-HPLC gave tripeptide (**13**) in 55% yield. Subsequently, reduction of **13** with $LiAlH_4$ in THF afforded tokaramide A (**1**) as a cyclic structure in moderate yield (Scheme 4).

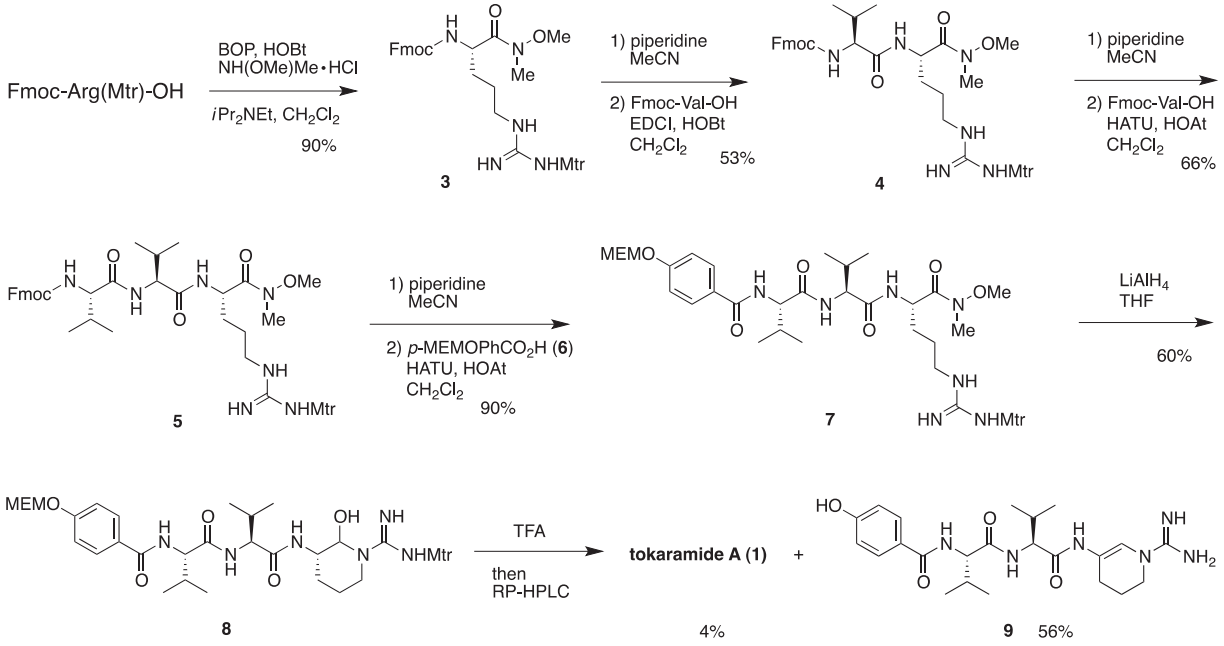
As an alternative route, tripeptide (**2**) was prepared using Fmoc-Arg(Mtr)–OH on Weinreb AM resin. Deprotection of **2** and subsequent cleavage from resin with $LiAlH_4$ gave tokaramide A (**1**) as a cyclized hemi-aminal structure in 12% overall yield (Scheme 5).

Thus, we have completed the first synthesis of tokaramide A (**1**) as a cyclic hemi-aminal structure on Weinreb AM resin. Peptide C-terminus Weinreb amide with a side-chain unprotected arginine residue could be easily converted to peptide aldehyde to avoid the side reaction. The method will be used in solid-phase synthesis for investigation of cysteine protease inhibitors in our laboratory.

3. Experimental

3.1. General

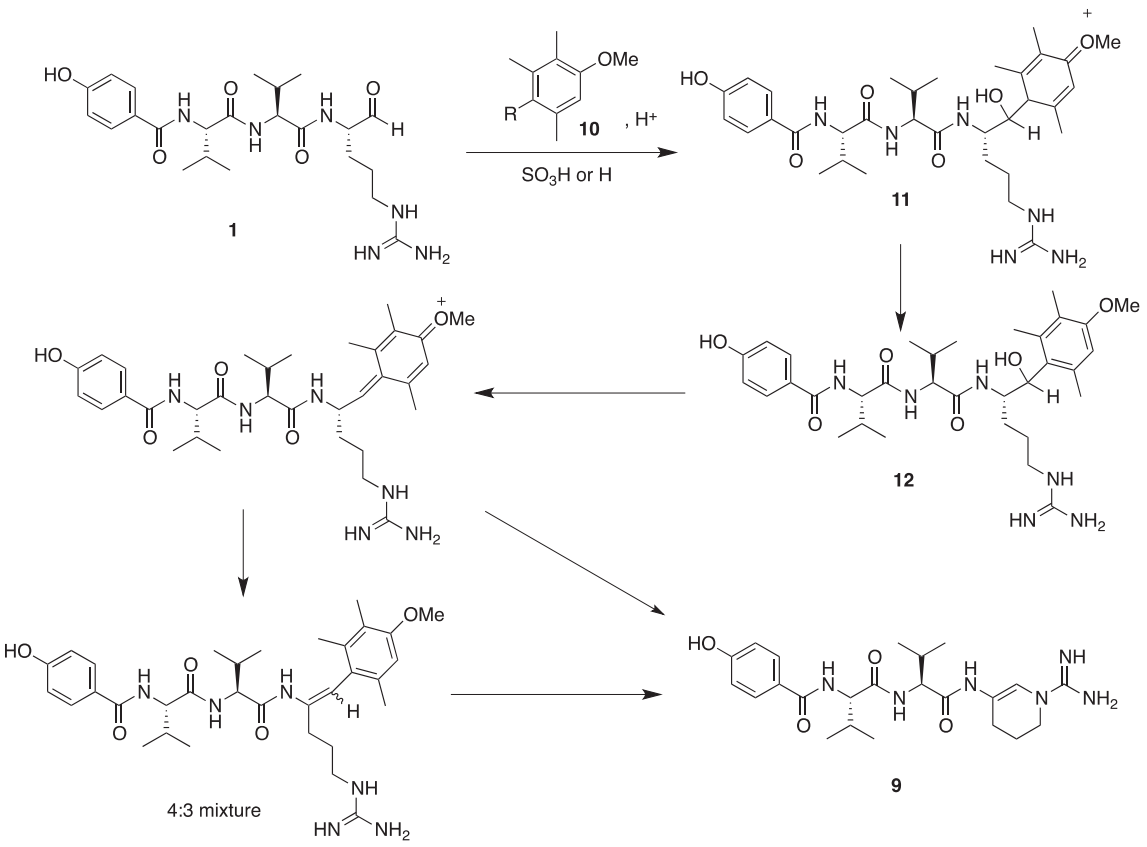
Solvents were reagent grade and dried prior to use. Fmoc-amino acid derivatives and solid supports were obtained from Nova-



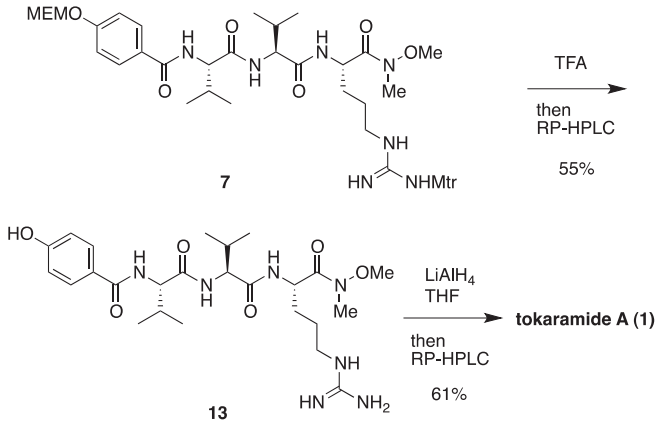
Scheme 2.

In addition to these anticipated issues, an expected problem arose when the tripeptide (**8**) was cleaved, namely, the observation of two abundant by-products with masses 134 Da greater and 16 Da smaller than the desired product.[12] The side reaction occurred independently of the cleavage cocktail and the abundance of the by-products increased over time. This unique structure likely results in

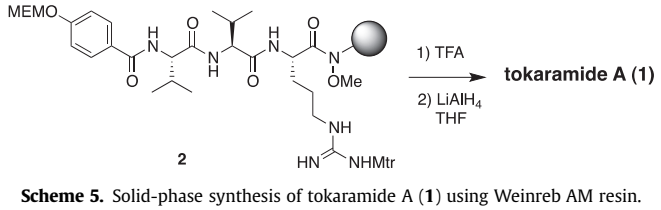
biochem (Merck) or Watanabe Chemical, and were used without further purification. Optical rotations were measured with a JASCO DIP-7 spectrometer, and IR spectra were measured with a Horiba FT-710 infrared spectrometer. ^1H and ^{13}C NMR spectra were measured with a JEOL JMN-400 or Bruker AM-300 spectrometer. Chemical shifts (ppm) were relative to tetramethylsilane as an



Scheme 3. Proposed mechanism for the formation of byproduct **9**.



Scheme 4. Solution phase synthesis of tokaramide A (**1**).



Scheme 5. Solid-phase synthesis of tokaramide A (**1**) using Weinreb AM resin.

internal standard. Mass spectra were recorded by Bruker autoflex II (MALDI-TOFMS) and JEOL JMS-HX211A (FABMS) instruments. HPLC was carried out on a Cosmosil 5C18 AR-II column (4.6×150 mm or 10×250 mm), which was eluted with MeCN in 0.1% aqueous TFA and detected at OD 220 nm.

3.2. Synthesis

*3.2.1. p-MEMOPhCO$_2$H (**6**).* To a solution of *p*-hydroxybenzaldehyde (50.0 g, 409 mmol) in CH_2Cl_2 (700 ml) were added *N*,*N*-diisopropylethylamine (107 ml, 614 mmol) and MEMCl (51.4 ml, 450 mmol) at 0 °C. The mixture was warmed to room temperature and stirred for 10 h. Reaction mixture was partitioned between H_2O (500 ml) and CH_2Cl_2 (500 ml). The organic layer was washed with brine (300 ml), dried over $MgSO_4$, filtered, and concentrated in vacuo. To a solution of the crude residue in acetone (500 ml) was added Jones' reagent at 0 °C. After 30 min, the mixture was added to Celite, filtered using a Celite pad, and concentrated in vacuo. The residue was crystallized (hexane/AcOEt) to give carboxylic acid (92.0 g, quant.) as a white solid. IR (film) ν_{max} cm^{-1}: 2928, 2888, 1687, 1610, 1517, 1419, 1303, 1267, 1172, 1149, 958, 846, 771, 667, 549. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.38 (3H, s), 3.56 (2H, t, J=4.6 Hz), 3.84 (1H, t, J=4.6 Hz), 5.34 (2H, s), 7.11 (2H, d, J=9.0 Hz), 8.06 (2H, d, J=8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 59.1, 68.0, 71.6, 93.1, 115.8, 122.8, 132.3, 161.4, 171.8. FABHRMS $[M+H]^+$ calcd for $C_{11}H_{15}O_5$: 227.0919, found: 227.0923.

*3.2.2. Fmoc-Arg(Mtr)–N(OMe)Me (**3**).* To a solution of Fmoc-Arg(Mtr)–OH (5.00 g, 8.21 mmol) in CH_2Cl_2 (50 ml) were added NH(OMe)Me·HCl (1.20 g, 12.3 mmol), BOP (5.44 g, 12.3 mmol), HOBt (1.66 g, 12.3 mmol), and *N*,*N*-diisopropylethylamine (4.29 ml, 24.6 mmol) at room temperature. After stirring for 2 h, the reaction mixture was poured into H_2O and extracted with AcOEt. Drying over $MgSO_4$ and subsequent evaporation in vacuo gave crude Weinreb amide **3**, which was chromatographed over silica gel (CH_3Cl/MeOH=9:1) to give **3** (4.81 g, 7.39 mmol, 90%) as a colorless oil. $[\alpha]_D^{22}$ −1.8 (*c* 4.0, $CHCl_3$). IR (film) ν_{max} cm^{-1}: 3421, 2942, 1716, 1637, 1120, 838, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.61

(3H, m), 1.74 (2H, m), 2.10 (3H, s), 2.59 (1H, s), 2.61 (3H, s), 2.69 (3H, s), 3.18 (3H, s), 3.30 (1H, m), 3.71 (2H, s), 3.71–3.77 (1H, s), 3.80 (3H, s), 4.17 (1H, t, *J*=6.8 Hz), 4.37 (2H, m), 4.70 (1H, br s), 5.77 (1H, d, *J*=8.1 Hz), 6.03 (2H, s), 6.50 (1H, s), 7.28 (2H, t, *J*=6.1 Hz), 7.36 (2H, t, *J*=7.6 Hz), 7.56 (2H, t, *J*=6.1 Hz), 7.76 (2H, d, *J*=7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.9, 18.3, 24.1, 36.6, 36.7, 40.9, 47.1, 55.4, 67.1, 77.2, 111.7, 119.9, 124.7, 125.1, 127.1, 127.69, 127.73, 133.7, 136.5, 138.5, 141.2, 143.6, 143.8, 156.6, 158.3, 183.9, 220.2. MALDI-TOFMS $[M+H]^+$ calcd for $C_{33}H_{42}N_5O_7S$: 652.273, found: 652.342.

*3.2.3. Fmoc-Val-Arg(Mtr)–N(OMe)Me (**4**).* To a solution of Fmoc-Arg(Mtr)–N(OMe)Me (**3**) (300 mg, 0.460 mmol) in MeCN (5 ml) was added piperidine (1 ml) at room temperature. After stirring for 30 min, the reaction mixture was evaporated to remove piperidine and the residue was poured into Et_2O. The mixture was decanted and the solvent was removed to give crude H–Arg(Mtr)–N(OMe)Me. To a solution of crude H–Arg(Mtr)–N(OMe)Me in CH_2Cl_2 (5 ml) were added Fmoc-Val–OH (312 mg, 0.624 mmol), EDCI (175 mg, 0.920 mmol), HOBt (124 mg, 0.920 mmol), and *N*,*N*-diisopropylethylamine (0.160 ml, 0.920 mmol) at room temperature. After stirring for 2 h, the reaction mixture was poured into H_2O and extracted with AcOEt. Drying over $MgSO_4$ and subsequent evaporation in vacuo gave crude dipeptide **4**, which was chromatographed over silica gel (CH_3Cl/MeOH=9:1) to give **4** (180 mg, 0.240 mmol, 53%) as a colorless oil. $[\alpha]_D^{22}$ −10.4 (*c* 2.0, $CHCl_3$). IR (film) ν_{max} cm^{-1}: 3423, 3320, 1710, 1650, 1120, 844, 757. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (3H, d, *J*=8.1 Hz), 0.93 (3H, d, *J*=7.3 Hz), 1.53 (3H, m), 1.74 (2H, m), 2.08 (3H, s), 2.58 (3H, s), 2.65 (3H, s), 2.79 (1H, s), 3.17 (3H, s), 3.72 (1H, s), 3.77 (3H, s), 4.18 (1H, m), 4.37–4.40 (1H, m), 4.95–5.79 (1H, br s), 6.30 (2H, s), 6.48 (2H, s), 7.25 (2H, m), 7.35 (2H, q, *J*=7.6 Hz), 7.54 (2H, t, *J*=7.6 Hz), 7.72 (2H, dd, *J*=4.4, 3.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.8, 17.7, 18.2, 19.1, 23.9, 25.1, 29.4, 31.3, 36.55, 36.59, 38.4, 40.5, 46.9, 47.0, 55.2, 59.9, 61.4, 67.0, 77.3, 111.5, 120.0, 124.4, 124.9, 126.9, 127.5, 133.8, 136.2, 138.3, 141.0, 141.1, 143.5, 143.7, 156.4, 156.5, 158.1, 171.8. MALDI-TOFMS $[M+H]^+$ calcd for $C_{38}H_{52}N_6O_8S$: 751.349, found 751.355.

*3.2.4. Fmoc-Val-Val-Arg(Mtr)–N(OMe)Me (**5**).* To a solution of Fmoc-Val-Arg(Mtr)–N(OMe)Me (**4**) (660 mg, 0.878 mmol) in MeCN (5 ml) was added piperidine (1 ml) at room temperature. After stirring for 30 min, the reaction mixture was evaporated to remove piperidine and the residue was poured into Et_2O. Deprotected residue was decanted and the solvent was removed to give crude H–Val-Arg(Mtr)–N(OMe)Me without further purification. To a solution of crude amine in CH_2Cl_2 (5 ml) were added Fmoc-Val–OH (600 mg, 1.77 mmol), HATU (600 mg, 1.58 mmol), HOAt (200 mg, 1.77 mmol), and *N*,*N*-diisopropylethylamine (0.300 ml, 1.72 mmol) at room temperature. After stirring for 2 h, the reaction mixture was poured into H_2O and extracted with AcOEt. Drying over $MgSO_4$ and subsequent evaporation in vacuo gave crude tripeptide **5**, which was chromatographed over silica gel (CH_3Cl/MeOH=9:1) to give **5** (490 mg, 576 mmol, 66%) as a colorless oil. $[\alpha]_D^{22}$ −21.4 (*c* 1.5, $CHCl_3$). IR (film) ν_{max} cm^{-1}: 3286, 2962, 1716, 1623, 1450, 1241, 1029, 757, 740. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87 (6H, d, *J*=6.8 Hz), 0.93 (6H, br s), 1.52 (2H, br s), 1.76 (1H, br s), 1.98 (1H, br s), 2.03 (1H, m), 2.11 (3H, s), 2.59 (3H, s), 2.67 (3H, s), 3.16 (4H, br s), 3.68 (3H, s), 3.80 (3H, s), 4.05 (1H, m), 4.16 (1H, m) 4.27 (1H, m), 4.40 (2H, m), 5.01 (1H, br s), 6.07 (1H, br s), 6.25 (2H, br s), 6.50 (1H, s), 6.92 (1H, br s), 7.25 (2H, m), 7.35 (2H, m), 7.55 (2H, t, *J*=8.1 Hz), 7.57 (1H, br s), 7.72 (2H, d, *J*=7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.9, 18.3, 18.4, 18.7, 19.1, 19.2, 24.1, 24.9, 29.8, 30.9, 31.2, 32.1, 47.1, 55.4, 58.7, 60.7, 61.7, 67.1, 77.3, 111.7, 119.9, 124.7, 125.2, 127.0, 127.6, 133.7, 136.4, 138.4, 141.2, 143.8, 143.9, 156.4, 156.7, 158.3, 172.4. MALDI-TOFMS $[M+H]^+$ calcd for $C_{43}H_{60}N_7O_9S$: 850.417, found, 850.275.

*3.2.5. (MEM)HBA-Val-Val-Arg(Mtr)–N(OMe)Me (**7**).* To a solution of Fmoc-Val-Val-Arg(Mtr)–N(OMe)Me (**5**) (660 mg, 1.05 mmol) in MeCN (10 ml) was added piperidine (2 ml) at room temperature. After stirring for 30 min, the reaction mixture was evaporated to remove piperidine and the residue was poured into Et_2O. Deprotected residue was decanted and the solvent was removed to give crude H–Val-Val-Arg(Mtr)–N(OMe)Me without further purification. To a solution of crude H–Val-Val-Arg(Mtr)–N(OMe)Me in CH_2Cl_2 (10 ml) were added *p*-MEMOPhCO$_2$H (**6**) (356 mg, 1.58 mmol), HATU (599 mg, 1.58 mmol), HOAt (214 mg, 1.58 mmol), and *N*,*N*-diisopropylethylamine (0.150 ml, 1.58 mmol) at room temperature. After stirring for 2 h, the reaction mixture was poured into H_2O and extracted with AcOEt. Drying over $MgSO_4$ and subsequent evaporation gave crude tripeptide **7**, which was chromatographed over silica gel (CH_3Cl/MeOH=9:1) to give **7** (790 mg, 0.945 mmol, 90%) as a colorless oil. $[\alpha]_D^{22}$ −16.2 (*c* 0.4, $CHCl_3$). IR (film) ν_{max} cm^{-1}: 3311, 1635, 1558, 1234, 1120, 955, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.81 (3H, d, *J*=6.8 Hz), 0.84 (3H, d, *J*=6.6 Hz), 0.97 (3H, d, *J*=6.8 Hz), 0.99 (3H, d, *J*=6.6 Hz), 1.61 (4H, m), 1.80 (1H, m), 1.94 (1H, m), 2.11 (3H, s), 2.26 (1H, m), 2.60 (3H, s), 2.67 (3H, s), 3.18 (3H, br s), 3.36 (5H, br s), 3.54 (2H, m), 3.71 (3H, s), 3.80 (2H, m), 3.81 (3H, s), 4.39 (1H, t, *J*=8.1 Hz), 4.44 (1H, t, *J*=8.1 Hz), 5.02 (1H, br s), 5.29 (1H, s), 6.30 (1H, br s), 6.51 (1H, s), 7.02 (2H, d, *J*=8.8 Hz), 7.12 (1H, br s), 7.34 (1H, br s), 7.61 (1H, br s), 7.79 (2H, d, *J*=8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.0, 18.4, 18.6, 18.8, 19.2, 19.4, 24.1, 24.9, 29.9, 30.67, 30.7, 55.5, 59.0, 60.0, 67.9, 71.6, 76.7, 77.3, 93.2, 111.7, 115.8, 124.7, 127.2, 129.3, 133.9, 136.5, 138.6, 156.5, 158.4, 160.0, 167.5, 172.4. MALDI-TOFMS $[M+H]^+$ calcd for $C_{39}H_{63}N_7O_{11}S$: 836.423, found, 836.429.

*3.2.6. (MEM)HBA-Val-Val-Argal(Mtr) (**8**).* To a solution of tripeptide **7** (14 mg, 0.017 mmol) in THF (1 ml) was added $LiAlH_4$ (5 mg, 0.13 mmol) at 0 °C. After stirring for 1 h, the mixture was added to H_2O and 1 M NaOH. The crude residue was filtered through a Celite pad and evaporated in vacuo. The residue was purified by chromatography on silica gel to give **8** (8 mg, 0.010 mmol, 60%) as a yellow oil. $[\alpha]_D^{22}$ −15.0 (*c* 0.2, MeOH). IR (film) ν_{max} cm^{-1}: 3318, 2962, 2927, 1708, 1631, 1504, 1234, 1120, 991, 752. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87 (3H, d, *J*=6.8 Hz), 0.89 (3H, d, *J*=7.1 Hz), 0.96 (3H, d, *J*=8.3 Hz), 0.98 (3H, d, *J*=7.1 Hz), 1.35 (4H, m), 1.71 (2H, m), 2.10 (3H, s), 2.24 (1H, m), 2.57 (3H, s), 2.65 (3H, s), 3.13 (1H, m), 3.36 (3H, s), 3.53 (2H, m), 3.70 (1H, m), 3.82 (5H, m), 3.96 (1H, br s), 4.26 (1H, br s), 4.54 (1H, br s), 5.28 (2H, s), 5.52–5.62 (1H, br s), 6.51 (1H, s), 6.65 (1H, br s), 6.81 (1H, br s), 6.99–7.22 (1H, br s), 7.02 (2H, d, *J*=9.0 Hz), 7.80 (2H, d, *J*=8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.0, 18.2, 18.4, 18.8, 19.3, 19.4, 24.1, 24.2, 29.8, 30.5, 31.0, 55.5, 59.1, 59.7, 67.96, 68.0, 71.6, 71.7, 77.3, 86.3, 93.2, 111.8, 116.0, 125.0, 129.1, 129.4, 136.8, 138.6, 158.7, 165.4, 199.9. FABHRMS $[M+H]^+$ calcd for $C_{37}H_{57}N_6O_{10}S$: 777.3857, found: 777.3866.

*3.2.7. HBA-Val-Val-Argal [tokaramide A (**1**)] and compound **9**.* A solution of **8** (8 mg, 0.010 mmol) in TFA was stirred for 2 h. The reaction mixture was evaporated to remove TFA. The residue was purified by preparative RP-HPLC (CH_3CN/H_2O) to give tokaramide A (**1**) (0.2 mg, 0.0004 mmol, 4%) and **9** (4 mg, 0.009 mmol, 56%) as a colorless oil. Tokaramide A (**1**): ^{1}H NMR (300 MHz, CD_3OD) δ: 0.97 (3H, d, *J*=6.3 Hz), 0.98 (3H, d, *J*=6.6 Hz), 1.02 (3H, d, *J*=6.9 Hz), 1.03 (3H, d, *J*=6.6 Hz), 1.62–1.77 (4H, m), 2.05 (1H, m), 2.16 (1H, m), 3.20 (2H, m), 3.89 (1H, m), 4.16 (1H, m), 4.34 (1H, t, *J*=8.4 Hz), 4.70 (1H, m), 6.84 (2H, d, *J*=8.7 Hz), 7.73 (2H, d, *J*=8.7 Hz), 8.00 (1H, m), 8.01 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.1, 29.1, 31.1, 39.6, 55.3, 59.4, 95.9, 114.8, 124.9, 129.3, 159.4, 162.3, 169.3, 176.1. MALDI-TOFMS $[M+H]^+$ calcd for $C_{23}H_{36}N_6O_5$: 477.283, found: 477.388. Compound **9**: IR (film) ν_{max} cm^{-1}: 3398, 2966, 2924, 1662, 1203, 1138, 847, 723. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.04 (12H, m), 2.05 (2H, m), 2.17 (1H, m), 2,32 (1H, m), 2.34 (2H, t, *J*=5.7 Hz), 3.59 (2H, m), 4.21

(1H, t, *J*=7.8 Hz), 4.37 (1H, t, *J*=8.4 Hz), 6.85 (2H, d, *J*=9.0 Hz), 7.40 (1H, s), 7.75 (2H, d, *J*=9.0 Hz), 8.03 (1H, d, *J*=8.1 Hz), 8.21 (1H, d, *J*=8.1 Hz). MALDI-TOFMS $[M+H]^+$ calcd for $C_{23}H_{34}N_5O_5$: 459.272, found: 459.258.

*3.2.8. HBA-Val-Val-Arg–N(OMe)Me (**13**).* A solution of **7** (17 mg, 0.022 mmol) in TFA (1 ml) was stirred for 2 h. The reaction mixture was evaporated to remove TFA. The residue was purified by preparative RP-HPLC (CH_3CN/H_2O) to give **13** (10 mg, 0.018 mmol, 82%) as a white powder. $[\alpha]_D^{22}$ −12.7 (*c* 0.6, $CHCl_3$). IR (film) ν_{max} cm^{-1}: 3316, 2921, 1630, 1458, 1298, 1201, 985, 849, 750. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.00 (12H, m), 1.67 (4H, m), 2.08 (1H, m), 2.17 (1H, m), 2.70 (2H, m), 3.33 (3H, s), 3.82 (3H, s), 4.21 (1H, m), 4.35 (1H, m), 4.85 (1H, m), 6.85 (2H, d, *J*=8.4 Hz), 7.50 (1H, br s), 7.74 (2H, d, *J*=8.4 Hz), 7.99 (1H, br s), 8.09 (1H, br s), 8.31 (1H, br s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.0, 19.4, 19.7, 19.9, 26.1, 29.8, 31.8, 35.4, 37.0, 41.9, 60.4, 61.3, 116.17, 126.1, 130.5, 158.7, 162.3, 163.7, 170.2, 173.6, 173.7, 174.2, 174.3. MALDI-TOFMS $[M+H]^+$ calcd for $C_{25}H_{42}N_7O_6$: 536.319, found: 536.287.

*3.2.9. Reduction of **13** for tokaramide A (**1**).* To a solution of **13** (10 mg, 0.018 mmol) in THF (1 ml) was added $LiAlH_4$ (5 mg, 0.13 mmol) at 0 °C. After stirring for 1 h, the mixture was added to H_2O and 1 M NaOH. The crude residue was filtered through Celite pad and evaporated in vacuo. The residue was purified by preparative RP-HPLC (CH_3CN/H_2O) to give tokaramide A (**1**) (5 mg, 0.011 mmol, 60%).

*3.2.10. Solid-phase synthesis of tokaramide A (**1**).* A solution of Fmoc-Arg(Mtr)–OH (807 mg, 2.0 mmol), DIPC (252 mg, 2.0 mmol), and HOAt (272 mg, 2.0 mmol) in DMF (2 ml) was added to Weinreb AM resin (200 mg, 0.20 mmol scale). The cartridge was gently rocked on a shaker table for 2 h. After the solution was removed, the resin was rinsed with DMF. To the resultant resin was added 20% piperidine in DMF and the mixture was stirred for 30 min. Fmoc-Val–OH (170 mg, 0.50 mmol), Fmoc-Val–OH (170 mg, 0.50 mmol), and *p*-MEMOPhCO_2H (**6**) (114 mg, 0.50 mmol) were successively condensed in this resin using DIPC (63 mg, 0.50 mmol)/HOAt (68 mg, 0.50 mmol) for 2 h. To the resultant resin was added TFA (2 ml) and the mixture was stirred for 2 h. After the solution was removed, the resin was rinsed with DMF and Et_2O. $LiAlH_4$ (20 mg, 0.53 mmol) in THF (2 ml) was added to the resultant resin. After stirring for 30 min, the reaction mixture was poured into H_2O, filtered, and subsequent evaporation gave crude peptide. The residue was purified by preparative RP-HPLC (CH_3CN/H_2O) to give **1** (11 mg, 0.024 mmol, 12%) as a colorless oil.

Acknowledgements

The authors thank Prof. Shigeki Matsunaga of the Graduate School of Agriculture and Life Science, the University of Tokyo for spectral data of the natural product. This work was supported in part by a Grant-in-aid from the Japan Society for the Promotion of Science (21689004) and Chisso Corporation Fund.

References and notes

1. Fusetani, N.; Fujita, M.; Nakao, Y.; Matsunaga, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3397–3402.
2. Rosenthal, P. J. *Int. J. Parasitol.* **2004**, *34*, 1489–1499.
3. (a) Schirmeister, T. *J. Med. Chem.* **1999**, *42*, 560–572; (b) Kato, D.; Boatright, K. M.; Berger, A. B.; Nazif, T.; Blum, G.; Ryan, C.; Chegade, K. A. H.; Salvesen, G. S.; Bogyo, M. *Nat. Chem. Biol.* **2005**, *1*, 33–38.
4. (a) Hook, V. Y. H.; Kindy, M.; Reinhechel, T.; Peters, C.; Hook, G. *Biochem. Biophys. Res. Commun.* **2009**, *386*, 284–288; (b) Hook, V. Y. H.; Kindy, M.; Hook, G. *J. Biol. Chem.* **2008**, *283*, 7745–7753.
5. Wu, H.; Ge, J.; Yang, P.-Y.; Wang, J.; Uttamchandani, M.; Yao, S. Q. *J. Am. Chem. Soc.* **2011**, *133*, 1946–1954.
6. Konno, H.; Kubo, K.; Makabe, H.; Toshiro, E.; Hinoda, N.; Nosaka, K.; Akaji, K. *Tetrahedron* **2007**, *63*, 9502–9513.
7. (a) Tanaka, M.; Oishi, S.; Ohno, H.; Fujii, N. *Int. J. Pept. Res. Ther.* **2007**, *13*, 271–279; (b) Ede, N. J.; Bray, A. M. *Tetrahedron Lett.* **1997**, *38*, 7119–7122; (c) Pothion, C.; Paris, M.; Heitz, A.; Rocheblave, L.; Rouch, F.; Fehrentz, J. A.; Martinez, J. *Tetrahedron Lett.* **1997**, *38*, 7749–7752.
8. (a) Dinh, T. Q.; Armstrong, R. W. *Tetrahedron Lett.* **1996**, *37*, 1161–1164; (b) Fehrentz, J.-A.; Paris, M.; Heitz, A.; Velek, J.; Liu, C.-F.; Winternitz, F.; Martines, J. *Tetrahedron Lett.* **1995**, *36*, 7871–7874.
9. (a) Castro, B.; Dormoy, J. R.; Evin, G.; Seive, C. *Tetrahedron Lett.* **1975**, *16*, 1219–1222; (b) Seyer, R.; Aumelas, A.; Caraty, A.; Rivaille, P.; Castro, B. *Int. J. Pept. Protein Res.* **1990**, *35*, 465–472.
10. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
11. Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mugge, C.; Wenschuh, H.; Klose, J.; Beyerman, M.; Bienert, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 442–445.
12. Rush, J.; Bertozzi, C. R. *Org. Lett.* **2006**, *8*, 131–134.