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Surugamides A–E, Cyclic Octapeptides with Four D-Amino Acid Residues, from a Marine *Streptomyces* sp.: LC–MS-Aided Inspection of Partial Hydrolysates for the Distinction of D- and L-Amino Acid Residues in the Sequence

Kentaro Takada,[†,§] Akihiro Ninomiya,[†,§] Masato Naruse,[†] Yi Sun,[†] Masayuki Miyazaki,[‡] Yuichi Nogi,[‡] Shigeru Okada,[†] and Shigeki Matsunaga*[,†]

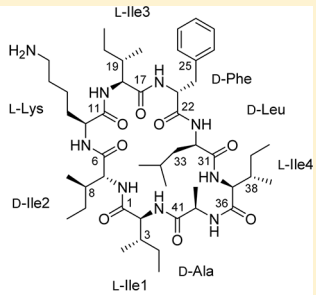
[†]Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

[‡]Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Natsushima, Yokosuka, Kanagawa 237-0061, Japan

Supporting Information

ABSTRACT: Surugamides A–E (**1**–**5**), cyclic octapeptides with four D-amino acid residues, were isolated from the broth of marine-derived *Streptomyces* sp. Their planar structures were determined by analyses of spectroscopic data, and the absolute configuration of constituent amino acid residues was determined by the Marfey's method. Differentiation of D-Ile and L-Ile in the sequence was established by chiral analysis of fragment peptides obtained from the partial hydrolysate, whose identification was conducted by LC–MS/MS.

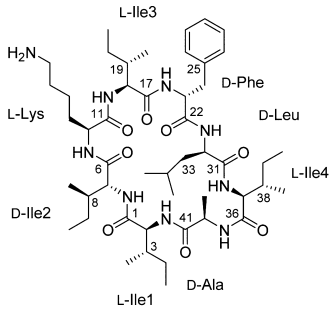
Streptomyces sp. JAMM992



surugamide A (**1**)

Terrestrial actinomycete bacteria are one of the most prolific sources of secondary metabolites, from which a large number of peptide antibiotics with a variety of bioactivities have been isolated.[1] Recent genome sequencing studies of actinomycetes revealed that a significant number of peptides of nonribosomal peptide synthetase (NRPS) or hybrid of NRPS/polyketide synthase (PKS) origin are coded but have not been isolated, indicating their higher metabolic potentials than those inferred from the past records of natural products discovery.[2] In the course of our exploration of bioactive metabolites from marine-derived actinomycetes, we found five new cathepsin B inhibitory cyclic peptides, surugamides A–E (**1**–**5**), from a marine *Streptomyces* sp. Four of eight residues in surugamides are in the D-form. In this contribution, we describe the structures of surugamides, which comprise a new class of cyclic peptides, with particular reference to the use of LC–MS to facilitate identification of partial hydrolysis products. This process is indispensable for establishing total configurational assignment of cyclic peptides in which both D- and L-forms of one or more amino acid residues are present.

The crude extract of the mycelia was subjected to solvent partitioning, ODS flash chromatography, and reversed-phase HPLC to afford surugamides A (**1**, 9.8 mg), B (**2**, 1.2 mg), C (**3**, 1.2 mg), D (**4**, 1.3 mg), and E (**5**, 1.0 mg) (Figure S1 in Supporting Information [SI]). The molecular formula of **1** was



surugamide A (**1**)

1: L-Ile -D-Ile -L-Lys-L-Ile -D-Phe-D-Leu-L-Ile -D-Ala
2: L-Ile -D-Val-L-Lys-L-Ile -D-Phe-D-Leu-L-Ile -D-Ala
3: L-Ile -D-Ile -L-Lys-L-Val-D-Phe-D-Leu-L-Ile -D-Ala
4: L-Val-D-Ile -L-Lys-L-Ile -D-Phe-D-Leu-L-Ile -D-Ala
5: L-Ile -D-Ile -L-Lys-L-Ile -D-Phe-D-Leu-L-Val-D-Ala

determined to be $C_{48}H_{81}N_9O_8$ by HRESIMS. ^{1}H NMR spectrum in DMSO-d_6 exhibited eight each of amide- and α-protons, indicating the peptidic nature of the compound. Analyses of COSY, TOCSY, and HSQC data revealed that **1** was composed of eight proteinogenic amino acid residues: Ala (1 residue), Ile or *allo*-Ile (designated as Ile unless specifically differentiated) (4), Leu (1), Lys (1), and Phe (1) (Table S1 in SI). The presence of eight amide bonds was deduced from the molecular formula, which indicated **1** was cyclic form. Eight sequential NOE correlations, δ 8.50 (NH, Ile1) /δ 4.27 (αH, Ala), δ 7.98

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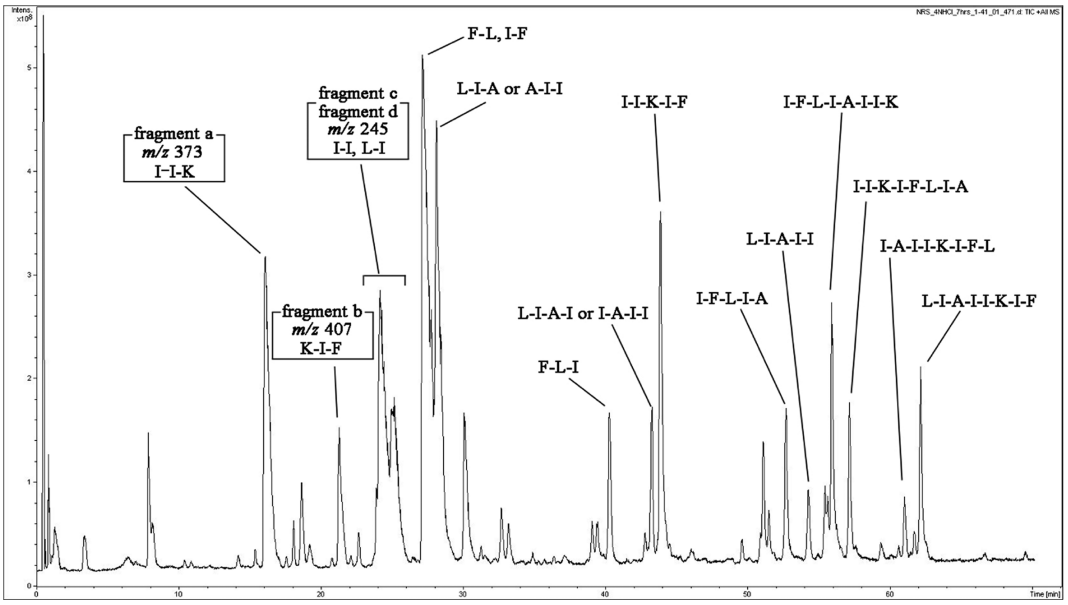

Figure 1. Total ion current (TIC) chromatogram of the partial hydrolysate of **1**. Amino acid sequences of the fragments were determined on the basis of the MS/MS data. For the HPLC conditions, see the Experimental Section.

(NH, Ile2) /δ 4.14 (αH, Ile1), δ 7.45 (NH, Lys) /δ 4.13 (αH, Ile2), δ 7.91 (NH, Ile3) /δ 4.31 (αH, Lys), δ 8.45 (NH, Phe) /δ 3.82 (αH, Ile3), δ 7.72 (NH, Leu) /δ 4.35 (αH, Phe), δ 7.00 (NH, Ile4) /δ 4.20 (αH, Leu), and δ 7.73 (NH, Ala) /δ 4.06 (αH, Ile4), permitted us to sequence **1** as cyclo[-Ile-Ile-Lys-Ile-Phe-Leu-Ile-Ala-].

Surugamides B (**2**)−E (**5**) all had the molecular formula of $C_{47}H_{79}N_9O_8$ which was smaller than that of **1** by a CH_2 unit. TOCSY and HSQC data of **2**−**5** showed that one of the Ile residues was replaced by a Val residue (Table S2 in SI). Interpretation of the 2D NMR data revealed that Ile2, Ile3, Ile1, or Ile4 in **2**−**5**, respectively, was substituted for Val.

The Marfey's analysis[3] of the total hydrolysate of **1** showed the presence of L-Lys, D-Phe, D-Leu, and D-Ala. DAA (2,4-dinitrophenyl-5-L-alanine amide) derivatives of D-Ile and D-*allo*-Ile were hardly separable in reversed-phase HPLC as were those of L-Ile and L-*allo*-Ile. Therefore, we used 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)[4] for the derivatization of the hydrolysate and analyzed by LC−MS, allowing us to conclude that **1** contained one residue of D-Ile and three residues of L-Ile, but none of D-*allo*-Ile or L-*allo*-Ile (Figure S24 in SI).

In order to complete the structure elucidation of **1**, we had to distinguish between D- and L-Ile in the amino acid sequence. This process is, in general, the most time- and sample-consuming step in structure elucidation of peptides.[5] Isolation of suitable fragments by partial hydrolysis is prerequisite for the distinction of optical isomers of amino acids in a peptide sequence,[6] and it is hard to predict whether desirable fragments can be generated and acquirable under certain hydrolysis conditions. Therefore, in order to facilitate this step of structure elucidation we used LC−MS/MS analysis, which worked out successfully to set up proper hydrolysis conditions and to identify peptide fragments. Because most of constituent amino acids in **1** were nonpolar and bulky, **1** was expected to be sluggish toward acid hydrolysis. From this inspection, we chose harsher conditions of 4 N HCl at 100 °C and optimized the reaction time (Figure S25 in SI). We hoped to isolate smaller peptides to determine the chirality of Ile in the fragments and set the hydrolysis time for 4 h on the basis of the LC−MS data (Figure 1). The partial hydrolysate of **1** thus obtained was subjected to a LC−MS-guided fractionation, in which 20% of the effluent was directed to the MS spectrometer, and the remaining portion was collected. The LC−MS/MS data allowed us to identify fragments that contain Ile residues of specific positions, i.e., Ile-Ile-Lys (fragment **a**), Lys-Ile-Phe (fragment **b**), Ile-Ile (fragment **c**), and Leu-Ile (fragment **d**), together with other fragments and several linear octapeptides (Figure 1). Marfey's analyses of fragments **a** and **b** indicated that fragment **a** contained one residue each of D-Ile, L-Ile, and L-Lys and fragment **b** contained L-Ile, D-Phe, and L-Lys (Figure S26 in SI). Therefore, we had to determine the configurations of the contiguous Ile residues. Due to the bulkiness of Ile we did not find any fragment cleaved between these two Ile residues. Therefore, we used the fraction, which was a mixture of fragments **c** and **d**, to determine the configuration of Ile in question; we were not able to separate these peptides nor distinguish them by MS/MS data. Due to the scarcity of the material we sought to differentiate optical isomers of fragments **c** and **d** chromatographically. We first prepared all of their possible isomers (D-Ile-L-Ile-OMe, D-Ile-D-Ile-OMe, L-Ile-L-Ile-OMe, L-Ile-D-Ile-OMe, D-Leu-L-Ile-OMe, and D-Leu-D-Ile-OMe) and converted each peptide to the methyl ester DAA derivative. The mixture of fragments **c** and **d** was also derivatized in the same way and subjected to the LC−MS analysis, which demonstrated that fragments **c** and **d** were L-Ile-D-Ile-OMe and D-Leu-L-Ile-OMe, respectively (Figure 2), allowing us to determine the position of D-Ile in the sequence. From these observations we assigned the structure of **1** as cyclo[-L-Ile-D-Ile-L-Lys-L-Ile-D-Phe-D-Leu-L-Ile-D-Ala-].

The absolute configurations of **2**−**5** were determined in the same manner. The Marfey's analysis of their total hydrolysates showed that the configurations of L-Lys, D-Phe, D-Leu, and D-Ala were conserved among **2**−**5**. The configuration of **2** was assigned from the Marfey's analysis alone, because remaining amino acids were one D-Val and three L-Ile. However, **3**−**5** contained one D-Ile and two L-Ile residues, necessitating the determination of

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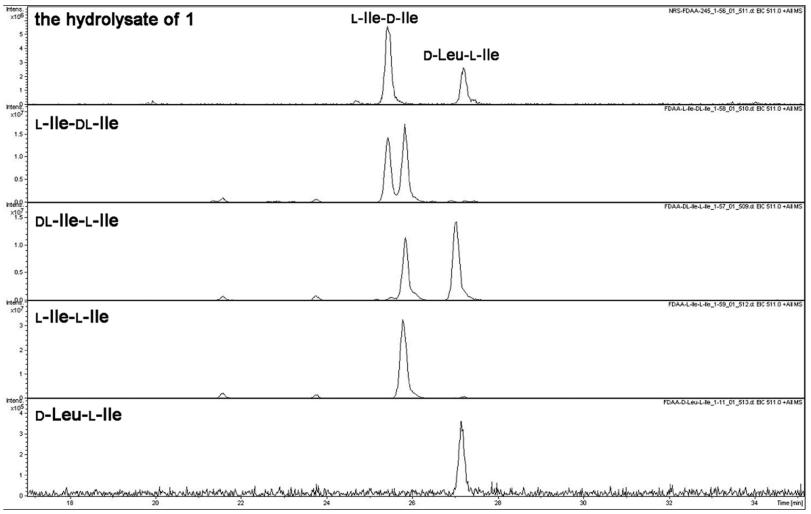

Figure 2. LC−MS analyses of DAA derivatives of the partial hydrolysate and synthetic standards. For the HPLC conditions, see the Experimental Section.

the position of D-Ile residue. The positions of D-Ile in **3** and **5** were determined as described above: LC−MS analyses of DAA derivative of fragments **c** and **d** obtained by partial hydrolysis. In the case of **4**, we anticipated that the Ile residue in the Val-Ile fragment was in the D-form and prepared L-Val-D-Ile-OMe and L-Val-L-Ile-OMe. Their presence in the partial hydrolysate was examined by the LC−MS analysis after converting to the methyl ester DAA derivative. This analysis demonstrated the presence of L-Val-D-Ile, permitting us to locate the position of D-Ile in **4** (Figure S27 in SI).

The total asymmetry of the molecule was conserved throughout the five peptides. We speculated that the formation of the minor congeners **2**−**5** was due to the permissive specificities of the adenylation domain of NRPS. In fact, replacements of Ile by Val (or vice versa) are observed in nonribosomal peptides such as actinomycins from *Streptomyces* sp.[7] and surfactins from *Bacillus subtilis*.[8] Surugamides **1**−**5** showed inhibitory activity against bovine cathepsin B, a cysteine protease implicated in invasion of metastatic tumor cells,[9] with IC_{50} values of 21 μM, 27 μM, 36 μM, 18 μM, and 16 μM, respectively.

A large number of cyclic octapeptides have been reported from marine sponges[10] and terrestrial plants.[11] They are remarkably different from surugamides, because almost all of them contain one or more Pro residues and composed only of L-amino acids. Cyclic octapeptides are rare among microbial secondary metabolites.[12] To the best of our knowledge surugamides comprise a new class of cyclic peptide natural products. In the course of LC−MS analyses of our extract library of actinomycetes culture broths, we found that several strains of *Streptomyces* sp. isolated from deep sea sediments collected at diverse locations around Japan including Suruga Bay (see Experimental Section) produced surugamide A (**1**). 16S rRNA phylogenetic analyses indicated that all the strains were *Streptomyces* sp. The partial 16S rRNA sequences (1410 bp) of three of them were 100% identical to those of *S. coelicolor* NBRC12854 and *S. somaliensis* NBRC12916. It is interesting to note that biosynthetic gene cluster of **1** is widely distributed in marine *Streptomyces* species collected around Japan.

EXPERIMENTAL SECTION

General Experimental Procedure. Optical rotation and UV spectra were measured in MeOH. NMR spectra were recorded on a 600 MHz NMR spectrometer at 293 K for all compounds. ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 2.49 and δ_C 40.0 for DMSO-d_6.

The Strains Producing Surugamides. Deep-sea sediment was collected by the unmanned ROV HYPER-DOLPHIN system from Kinko Bay (−106 m) in 2005. The sediment sample was stored in a sterilized sampler and frozen with liquid nitrogen. Then the sample was transported to the laboratory, where it was kept frozen until processed. The *Streptomyces* sp. JAMM992 was isolated from this sample. The taxonomy of the strains was determined by 16S rRNA phylogenetic analyses using universal 27F and 1492R primers. The *Streptomyces* sp. JAMM1350, JAMM2700, and JAMM2709 were isolated from the deep-sea sediment collected from Sagami Bay, Japan in 2006 and 2008. The *Streptomyces* sp. ACT198 was isolated from the deep-sea sediment collected by the unmanned ROV KAIKO system from Suruga Bay (−2409 m) in 2001. The strains JAMM992, JAMM2700, and JAMM2709 had the same 16S rRNA sequences (1410 bp). The sequences from JAMM1350 and ACT198 were 99.8 and 99.4% identical with that from JAMM992, respectively.

Fermentation, Extraction, and Isolation. Fermentation of the strain *Streptomyces* sp. JAMM992 was performed in PC-1 medium (1.0% starch, 1.0% polypeptone, 1.0% meat extract, 1.0% molasses, pH 7.2, 5 L) for 5 days at 30 °C with agitation and aeration. After the filtration of this culture, the mycelia were extracted with acetone and MeOH. The dried extract was purified by RP-HPLC (Cosmosil ARII ϕ 10 mm × 250 mm) after the ODS flash chromatography to afford surugamides A (**1**, 9.8 mg), B (**2**, 1.2 mg), C (**3**, 1.2 mg), D (**4**,1.2 mg), and E (**5**, 1.0 mg)

*Surugamide A (**1**):* pale yellow; $[\alpha]^{20}_D$ −0.8 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.98); ^{1}H NMR data (DMSO-d_6), see Table S1 in SI; ^{13}C NMR data (DMSO-d_6), see Table S1 in SI; HRMS (ESI-TOF)m/z: $[M + Na]^+$ Calcd for $C_{48}H_{81}N_9NaO_8$ 934.6106; Found 934.6089, Δ −1.7 mmu.

*Surugamide B (**2**):* pale yellow; $[\alpha]^{20}_D$ +3.3 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.85); ^{1}H NMR data (DMSO-d_6), see Table S2 in SI; ^{13}C NMR data (DMSO-d_6), see Table S2 in SI; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{47}H_{79}N_9NaO_8$920.5949; Found 920.5940, Δ −1.0 mmu.

*Surugamide C (**3**):* pale yellow; $[\alpha]^{20}_D$ −5.2 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.84); ^{1}H NMR data (DMSO-d_6), see Table S2 in SI; ^{13}C NMR data (DMSO-d_6), see Table S2 in SI; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{47}H_{79}N_9NaO_8$ 920.5949; Found 920.5923, Δ −2.1 mmu.

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Surugamide D (4): pale yellow; $[\alpha]^{20}_{D}$ −2.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.87); ^{1}H NMR data (DMSO-d_6), see Table S2 in SI; ^{13}C NMR data (DMSO-d_6), see Table S2 in SI; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ Calcd for $C_{47}H_{79}N_9NaO_8$ 920.5949; Found 920.5928, Δ −2.1 mmu.

Surugamide E (5): pale yellow; $[\alpha]^{20}_{D}$ +5.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.92); ^{1}H NMR data (DMSO-d_6), see Table S2 in SI; ^{13}C NMR data (DMSO-d_6), see Table S2 in SI; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ Calcd for $C_{47}H_{79}N_9NaO_8$ 920.5949; Found 920.5923, Δ −2.6 mmu.

Total Hydrolysis and Derivatization with FDAA and GITC. A portion of **1** (100 μg) was hydrolyzed in 6 N HCl at 110 °C overnight. The solution was dried under a stream of N_2 and dissolved in H_2O (100 μL). To a half portion of the solution were added 100 μL of 1% FDAA in acetone and 20 μL of 1 M $NaHCO_3$ and allowed to react at 50 °C for 30 min. The mixture was quenched with 10 μL of 2 N HCl. To the other half was added 10 μL 6% triethylamine and 1% GITC in acetone and allowed to react at rt for 10 min. The mixture was quenched with 10 μL of 5% AcOH. Surugamides B−E (**2**−**5**) were also hydrolyzed, and the hydrolysates were derivatized with FDAA in the same manner.

LC−MS Analyses of the FDAA and GITC Derivatives of Amino Acids. The DAA derivatives were analyzed by reversed-phase HPLC (Cosmosil 2.5C18-MSII 2.0 mm× 100 mm) using a gradient elution of 10 to 50% MeCN containing 0.5% AcOH for 30 min. Retention times for standard amino acids (min): L-Ala (10.5), D-Ala (12.4), L-Val (13.6), D-Val (16.1), L-Ile (15.6), D-Ile (18.2), L-*allo*-Ile (15.6), D-*allo*-Ile (18.2), L-Leu (16.1), D-Leu (18.5), L-Phe (16.1), D-Phe (18.1), L-Lys (17.2), D-Lys (18.5). Retention times (R_t) for the acid hydrolysate of **1** (min): 12.4 (*m*/*z* 342, Ala), 15.6 (*m*/*z* 384, Ile), 17.1 (*m*/*z* 651, Lys), 18.0 (*m*/*z* 418, Phe), 18.2 (*m*/*z* 384, Ile), 18.4 (*m*/*z* 384, Leu); **2**: 12.2 (*m*/*z* 342, Ala), 15.6 (*m*/*z* 384, Ile), 16.1 (*m*/*z* 372, Val), 17.1 (*m*/*z* 651, Lys), 18.0 (*m*/*z* 418, Phe), 18.3 (*m*/*z* 384, Leu); **3**: 12.2 (*m*/*z* 342, Ala), 13.6 (*m*/*z* 372, Val), 15.5 (*m*/*z* 384, Ile), 17.1 (*m*/*z* 651, Lys), 18.0 (*m*/*z* 418, Phe), 18.1 (*m*/*z* 384, Ile), 18.3 (*m*/*z* 384, Leu); **4**: 12.2 (*m*/*z* 342, Ala), 13.5 (*m*/*z* 372, Val), 15.5 (*m*/*z* 384, Ile), 17.0 (*m*/*z* 651, Lys), 18.0 (*m*/*z* 418, Phe), 18.1 (*m*/*z* 384, Ile), 18.3 (*m*/*z* 384, Leu); **5**: 12.2 (*m*/*z* 342, Ala), 13.6 (*m*/*z* 372, Val), 15.5 (*m*/*z* 384, Ile), 17.1 (*m*/*z* 651, Lys), 17.9 (*m*/*z* 418, Phe), 18.1 (*m*/*z* 384, Ile), 18.3 (*m*/*z* 384, Leu)

The GITC derivatives were analyzed by RP-HPLC (Cosmosil 2.5C18-MSII 2.0 mm × 100 mm) using a gradient elution of 21 to 26% MeCN containing 0.5% AcOH for 120 min. Retention times for standard amino acids (min): L-Ile (31.9), D-Ile (42.6), L-*allo*-Ile (30.7), D-*allo*-Ile (42.3). Retention times of the pertinent derivatives from the acid hydrolysate of **1** (min): 31.9 (L-Ile), 42.6 (D-Ile).

LC−MS-Guided Fractionation of the Partial Hydrolysate. An aliquot of **1** (200 μg) was hydrolyzed in 4 N HCl at 100 °C for 4 h. The dried hydrolysate was redissolved in 20 μL of MeOH. A 15 μL portion was separated by RP-HPLC (Cosmosil 2.5C18-MSII 2.0 mm × 100 mm) using a gradient elution from 0 to 35% MeCN containing 0.5% AcOH. The eluate was split with a microsplitter bulb (GL Science) located between the UV detector and the MS detector. One fifth of the eluate was directed to ESIMS for the MS/MS analyses, and the remaining effluent was collected every 12 s for 48 min into 96-well deep plates. After the assignments of peptide sequences by extensive MS/MS analyses, the fractions containing Ile-Ile-Lys, Lys-Ile-Phe, a mixture of Ile-Ile and Leu-Ile, and linear octapeptides were identified.

Marfey's Analyses of Ile-Ile-Lys and Lys-Ile-Phe. A fraction containing a peptide was hydrolyzed in 6 N HCl at 110 °C overnight. The solution was dried, converted to the methyl ester DAA derivative, and analyzed as described above. Retention times for standard amino acids (min): L-Ile (15.7), D-Ile (18.2), L-Phe (16.1), D-Phe (18.1), L-Lys (17.2), D-Lys (18.5). Retention times of the derivatives from the hydrolysate (min): fragment **a** (Ile-Ile-Lys): 15.8 (*m*/*z* 384, Ile), 17.1 (*m*/*z* 651, Lys), 18.2 (*m*/*z* 384, Ile); fragment **b** (Lys-Ile-Phe): 15.6 (*m*/*z* 384, Ile), 17.1 (*m*/*z* 651, Lys), 18.0 (*m*/*z* 418, Phe).

Synthesis of Dipeptide Methylesters. L-Ile (50 mg) was dissolved in 10% HCl−MeOH (1 mL) and heated at 100 °C for 2 h to yield L-Ile-OMe which was dissolved in DMF (1 mL). To the solution were added Boc-L-Ile (75 mg), WSCI (75 mg), HOBt (50 mg), and Et_3N (200 μL) and stirred at rt for 1h. The product was diluted with H_2O and extracted with EtOAc. The EtOAc layer was dried, dissolved in TFA (0.5 mL), and left at rt for 1 h. The solvent was removed to afford L-Ile-L-Ile-OMe which was used without further purification. Boc-DL-Ile, Boc-D-Leu, DL-Ile-L-Ile-OMe, L-Leu-L-Ile-OMe. L-Ile-DL-Ile-OMe, L-Val-DL-Ile-OMe, and L-Val-L-Ile-OMe were prepared in the same manner.

LC−MS Analyses of DAA Derivatives of Dipeptide Methyl Esters. A mixture of fragments **c** and **d** (Ile-Ile and Leu-Ile) was treated with TMS diazomethane. The reaction mixture was dried under a stream of N_2 and converted to the DAA derivatives which were analyzed by RP-HPLC (Cosmosil 2.5C18-MSII 2.0 mm × 100 mm) using a gradient elution of 20 to 100% MeCN containing 0.5% AcOH. Retention times for standard dipeptide methyl esters (min): L-Ile-D-Ile-OMe (25.6), L-Ile-L-Ile-OMe (25.8), D-Ile-L-Ile-OMe (27.0), D-Leu-L-Ile-OMe (27.2). Retention times of the derivative from partial hydrolysate of **1**: 25.6, 27.2. Each partial hydrolysate of **2**−**5** was treated in the same way. Each product was analyzed by RP-HPLC using a gradient elution from H_2O−75% MeCN containing 0.5% AcOH. Retention times of standard dipeptide methyl esters (min): L-Val-L-Ile-OMe (33.5), L-Val-D-Ile-OMe (35.3), L-Ile-D-Ile-OMe (37.0), L-Ile-L-Ile-OMe (37.4), D-Ile-L-Ile-OMe (38.5), D-Leu-L-Ile-OMe (38.7). Retention times of the pertinent derivatives from partial hydrolysates (min): **3**, 37.1 and 38.7; **4**, 35.4; **5**, 37.0.

Cathepsin B Inhibitory Assay. Cathepsin B inhibitory assay was performed according to a modification of the method of Hisawa et al.[13] The enzyme (cathepsin B from bovine spleen, Sigma C6286) was stocked at 1 unit/mL in 50 mM MES pH 6.0 and 0.1% Brij-35.The enzyme solution was diluted by 100 times with the buffer before use. The mixture of 4 μL test sample solution, 100 μL of the enzyme solution, and 50 μL of 25 μM fluorescent substrate (*Z*-Arg-Arg-AMC, Peptide Institute, Inc.) in DMSO was incubated at 37 °C for 30 min. The fluorescence of the liberated AMC was measured with an excitation at 345 nm and emission at 440 nm. The experiments were conducted in triplicate. IC_{50} values (μM) of 1, 2, 3, 4, 5, and E-64 (>98% pure, Peptide Institute, Osaka) were as follows: 21 ± 1, 27 ± 2, 36 ± 3, 18 ± 1, 16 ± 2, and 0.014 ± 0.002, respectively.

■ ASSOCIATED CONTENT

Supporting Information

NMR data for **1**−**5** and the results of Marfey analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: assmats@mail.ecc.u-tokyo.ac.jp. Tel.: 81-3-5841-5297. Fax: 81-3-5841-8166.

Author Contributions

§K.T. and A.N. contributed equally to this paper.

Notes

The authors declare no competing financial interest.

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