JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Total Synthesis and Biological Evaluation of PF1171A, C, F, and G, Cyclic Hexapeptides with Insecticidal Activity

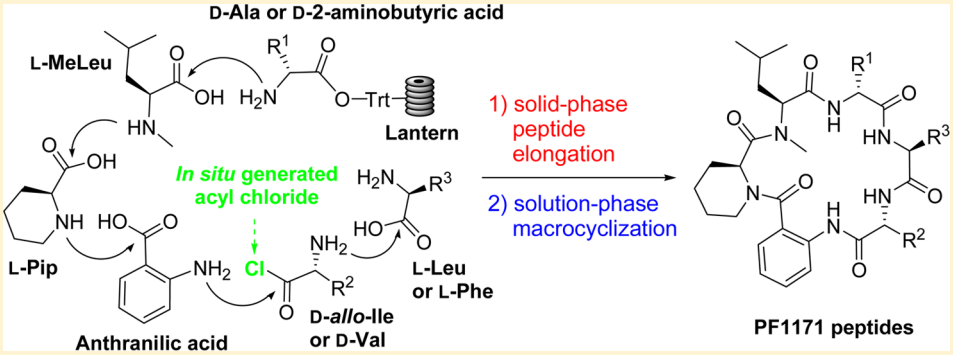
Yuichi Masuda,[†] Ren Tanaka,[†] Kenji Kai,[‡] A. Ganesan,*[,§] and Takayuki Doi*[,†]

[†]Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8578, Japan

[‡]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

[§]School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, United Kingdom

Supporting Information



ABSTRACT: The total synthesis of the cyclic hexapeptides PF1171A, C, F, and G has been achieved by solid-phase synthesis of a linear precursor and solution-phase macrolactamization. The synthesis includes a solid-phase peptide coupling with the weakly nucleophilic amino group of an anthranilic acid residue. This was efficiently achieved by in situ generation of an Fmoc-amino acid chloride using triphosgene. The natural products exhibit potent paralytic activities against silkworm larvae, whereas *epi*-PF1171A and *epi*-PF1171C, bearing L-Ala instead of D-Ala, were relatively inactive. X-ray crystallographic analysis indicates that intramolecular hydrogen bonds in PF1171 peptides are critical for maintaining their active conformations.

INTRODUCTION

Natural products have been, and continue to be, an exciting source of biologically active small molecules as molecular probes[1,2] and leads for drug discovery.[3−6] A particularly diverse class of natural products are the cyclic peptides and depsipeptides of terrestrial and marine origin.[7−9] These compounds often incorporate nonproteinogenic amino acids and attract considerable attention as novel pharmaceutical and agrochemical leads due to their wide variety of unique and potent biological activities. A number of cyclic peptides and depsipeptides are marketed for medicinal and veterinary applications such as the antibacterial vancomycin, the antifungal caspofungin, the immunosuppressant cyclosporine, the anticancer agent FK228 and the antihelmintic emodepside.[8,9] A recent addition to naturally occurring cyclopeptides is the PF1171 family of cyclic hexapeptides isolated from the fermentation extract of the fungus *Hamigera avellanea* and disclosed in a patent by Meiji Seika Kaisha.[10] The peptides, exemplified by PF1171A (**1**) and C (**3**) (Figure 1), were reported to suppress apolipoprotein B secretion in HepG2 cells and increase the contractile force of isolated guinea-pig hearts. A decade later, Opatz and colleagues identified anthranicine, which is identical to PF1171A (**1**), from a mycophilic *Acremonium* species.[11] More recently, Hayashi and colleagues isolated PF1171F (**5**) and G (**6**) (Figure 1) in addition to PF1171A (**1**) and C (**3**) from extracts of the unidentified ascomycete OK-128 fermented with okara (the insoluble residue left during the production of soybean milk and tofu).[12,13] The peptides were demonstrated to have a paralyzing effect on larvae of the silkworm *Bombyx mori*.[12,13] It has been reported that *Bombyx mori* produces paralytic peptide (PP), which has a variety of biological effects such as paralysis accompanying muscle contraction and morphologic alterations of hemocyte subtypes.[14,15] Quite recently, Sekimizu and coworkers suggested that PP could be an insect cytokine that regulates innate immune responses in multiple tissues and contributes to self-defense.[16−18] Since PF1171 peptides also exhibit the paralytic activities similar to PP, we were interested in them not only as an agrochemical lead but also as a possible agonist/antagonist of insect cytokine.

The PF1171 natural products contain three amino acid residues in common: *N*-methylleucine (MeLeu) and the nonproteinogenic anthranilic acid (Ant) and pipecolinic acid

Received: April 17, 2014
Published: August 7, 2014

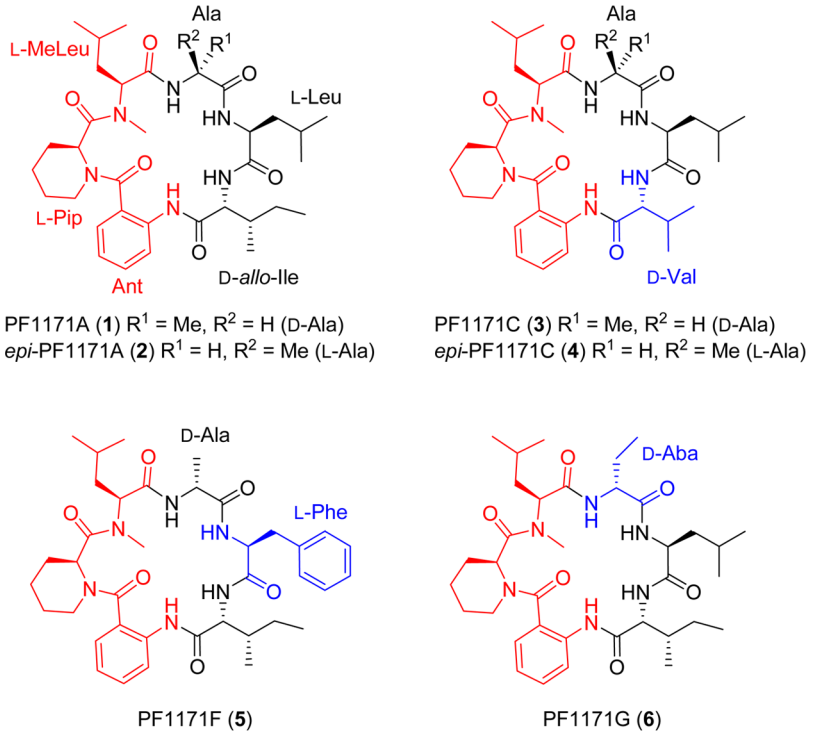
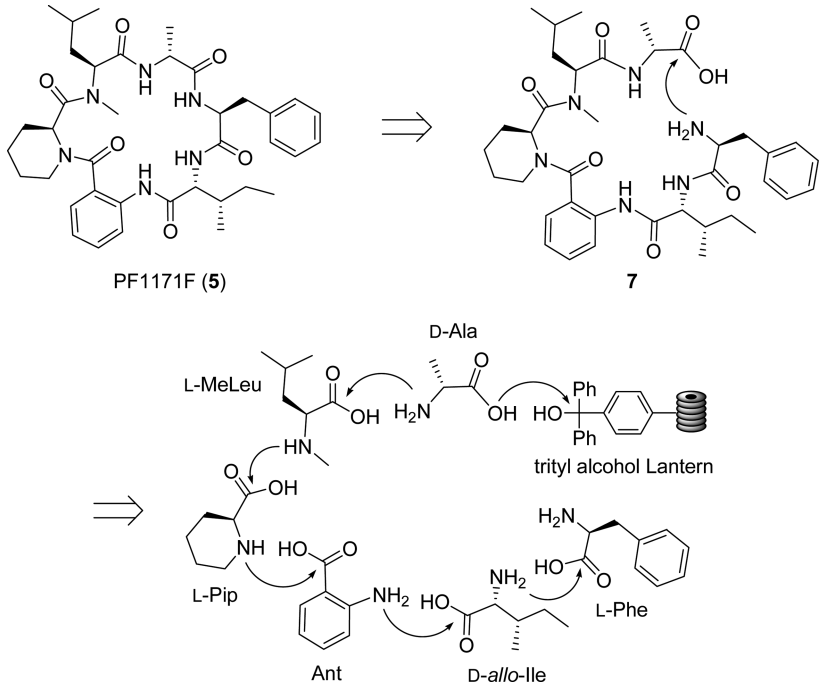


Figure 1. Chemical structures of PF1171 peptides. The invariant and the altered amino acids are shown in red and blue, respectively.

Scheme 1. Retrosynthetic Analysis of PF1171F (5)

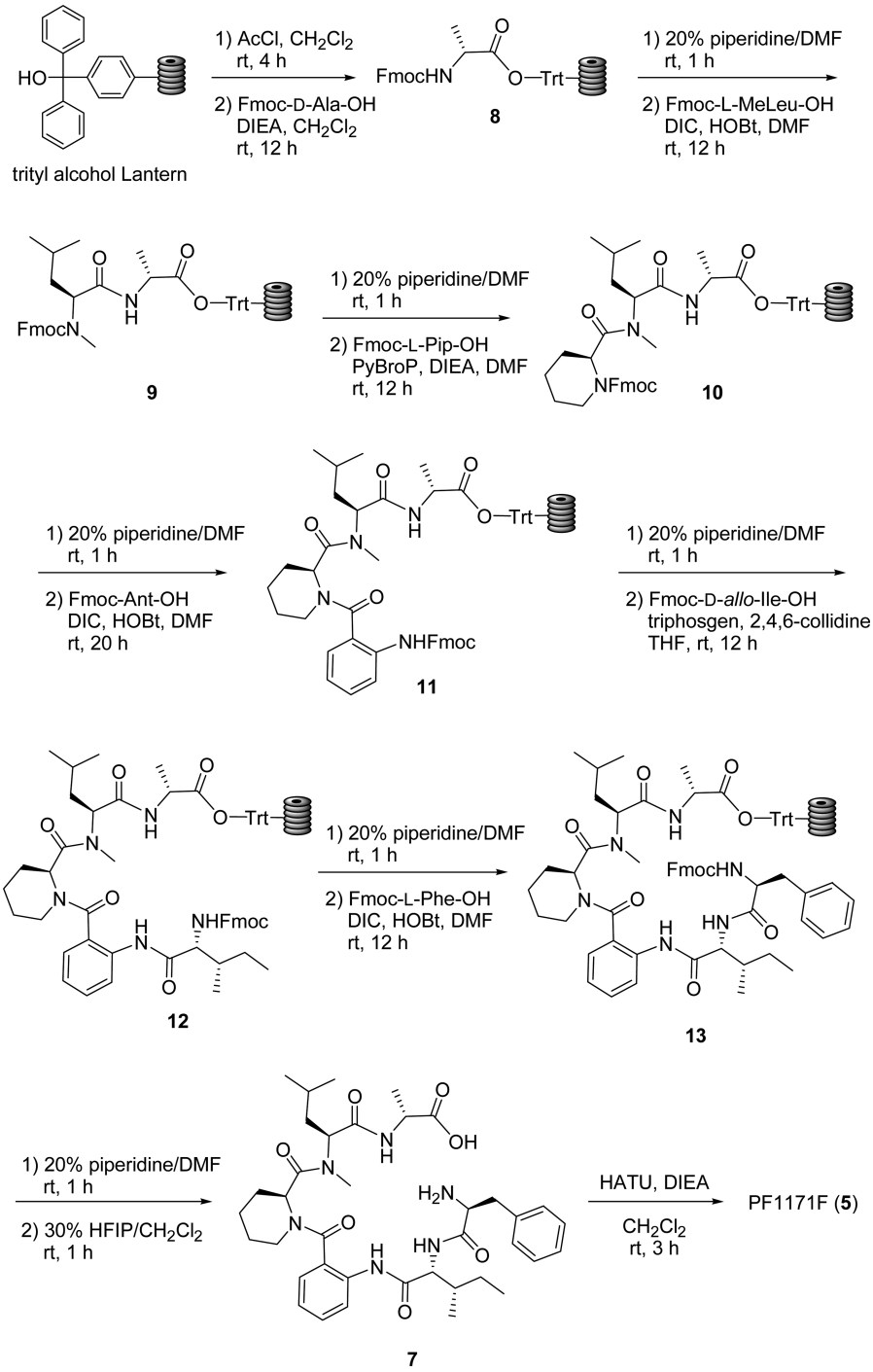


(Pip); both Ant[19−25] and Pip[26−31] are relatively uncommon in natural products and it is even rarer to find them together. In addition, the PF1171 peptides have three variable amino acids with hydrophobic side-chains. All in all, the natural products incorporate several design features that are instructive for enhancing the bioavailability of a peptide scaffold. First, the macrocyle enforces a rigid conformation that can improve target affinity and reduce promiscuity relative to a linear analogue, while promoting intramolecular hydrogen bonding to decrease aqueous solvation and improve cell permeability by passive diffusion.[32] Furthermore, the cyclic nature and the presence of D-amino acids reduces the susceptibility to metabolic breakdown.[32] Finally, the inclusion of two *N*-alkyl amino acids brings the number of hydrogen bond donors down to 4, compliant with Lipinski's rule[33] for orally bioactive small molecules.[32]

As described above, PF1171 peptides possess interesting structures and potent biological activity. However, there were

dx.doi.org/10.1021/jo500861k | J. Org. Chem. 2014, 79, 7844−7853

Scheme 2. Solid-Phase-Assisted Total Synthesis of PF1171F (5)



no reports of synthetic studies on the PF1171 natural products when we commenced our investigations. Herein, we report a synthesis of PF1171A (**1**), C (**3**), F (**5**), and G (**6**) achieved via solid-phase linear peptide synthesis and solution-phase macrolactamization.

RESULTS AND DISCUSSION

Synthetic Strategy. The reported absolute configurations of Ala residues in the PF1171 peptides were ambiguous in the literature. Opatz and colleagues assigned the absolute configuration of the Ala residue as the D-enantiomer in PF1171A (**1**).[11] However, Hayashi and colleagues reported that PF1171A and C contain L-Ala,[12] whereas PF1171F and G contain D-Ala and D-2-aminobutyric acid (Aba), respectively.[13] One explanation could be that the high-performance liquid chromatography (HPLC) retention times of the Marfey derivatives of D-Ala and L-Ala were too close for resolution in Hayashi's first publication (the retention times for the Marfey's derivatives were L-Ala, 19.4 min; D-Ala, 20.0 min).[12] Because of this uncertainty, we initially targeted PF1171F (**5**) for total synthesis as the stereochemical assignment in the later publication was based on a greater difference in retention times for the Marfey's derivatives (L-Ala, 10.3 min; D-Ala, 13.1 min).[13]

dx.doi.org/10.1021/jo500861k | J. Org. Chem. 2014, 79, 7844−7853

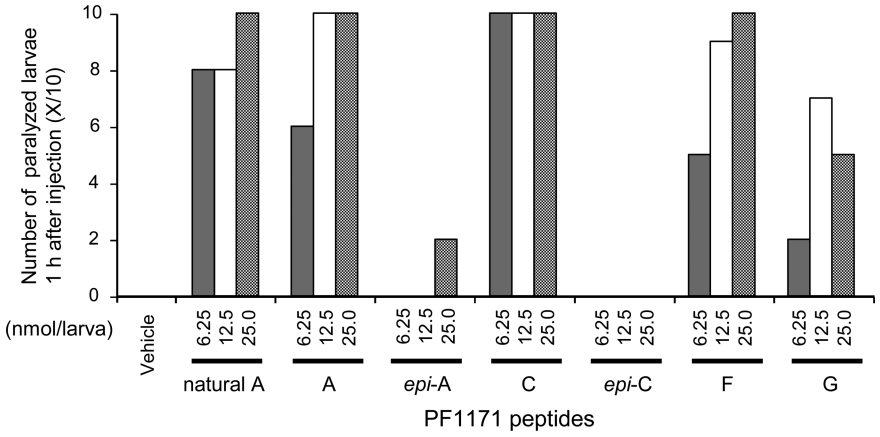


Figure 2. Paralytic activities of PF1171 peptides against larvae of silkworms. Each peptide dissolved in dimethylsulfoxide was injected into fourth-instar larvae. The number of paralyzed larvae was monitored an hour after the injection.

Our retrosynthetic strategy for PF1171F (**5**) is illustrated in Scheme 1. The natural product is envisioned to arise from the linear precursor **7** via macrolactamization. The amide bond between L-Phe and D-Ala was chosen as the cyclization site because the reactive primary amine of L-Phe should easily attack the activated carboxyl group of D-Ala bearing a sterically unhindered methyl side-chain. In addition, we anticipated that the successive Pip and Ant residues would be turn-inducing and help in bringing the N-terminus into a reactive conformation proximal to the C-terminus. The cyclization precursor **7** can be obtained by the sequential coupling of six fragments, D-Ala, L-MeLeu, L-Pip, Ant, D-*allo*-Ile,[34] and L-Phe by solid-phase peptide synthesis using an acid-labile trityl linker. We adopted the SynPhase Lantern[35] based on our past experience with this solid support for the construction of natural product-based combinatorial libraries.[36,37]

In our synthetic planning, the presence of the Ant residue is noteworthy due to the low nucleophilicity of the aniline nitrogen. In the total synthesis of avellanins A and B, Ant-containing cyclic pentapeptides, Shioiri and colleagues found that Fmoc-amino acid chlorides coupled with methyl anthranilate in good yield without racemization.[38] We also used Fmoc-amino acid chlorides in our solution and solid-phase syntheses of the fumiquinazoline alkaloids.[39,40] Meanwhile, Ellman and colleagues accomplished the coupling of 2-aminobenzophenones on solid-support with Fmoc-amino acid fluorides in the presence of the hindered base 4-methyl-2,6-di-*tert*-butylpyridine.[41]

Total Synthesis. Our solid-phase synthesis (Scheme 2) of the PF1171F precursor (**7**) commenced with attachement of Fmoc-D-Ala-OH onto the trityl alcohol Lantern. Trityl alcohol SynPhase Lantern was treated with a solution of acetyl chloride in CH_2Cl_2 to afford trityl chloride Lantern, which was immediately used for the immobilization of Fmoc-D-Ala to provide polymer-supported **8**. In this and subsequent steps, acidic cleavage from the polymer support and isolation of the product enabled quantitative monitoring of each coupling. The immobilization yield of Fmoc-D-Ala-OH was found to be 78% by gravimetric analysis after cleavage from the polymer support (30% hexafluoroisopropyl alcohol (HFIP)/CH_2Cl_2, rt, 1 h). After removal of the Fmoc group in **8** with 20% piperidine/DMF, acylation of the resulting amine with Fmoc-L-MeLeu-OH was performed using *N*,*N*′-diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt) to afford dipeptide **9** with high purity. The reaction was monitored by reverse-phase HPLC–MS analysis (UV 214 nm, Figure S1, Supporting Information (SI)) after cleavage of the dipeptide from the polymer support. After removal of the Fmoc group in **9** (20% piperidine/DMF, rt, 1 h), coupling of Fmoc-L-Pip-OH to the secondary amino group of MeLeu was not completed by DIC/HOBt even after two repetitions of the coupling. We thus utilized bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP)[42]/diisopropylethylamine (DIEA) to provide tripeptide **10** in a high yield (purity, 99%, UV 214 nm, Figure S1 (SI)). In the subsequent acylation with Fmoc-Ant-OH, the conditions such as DIC/4-(dimethylamino)pyridine (DMAP), PyBroP/DIEA, and 7-azabenzotriazol-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)[43]/DIEA were hampered by competing partial deprotection of the Fmoc group on Ant under the basic conditions. Instead, a DIC/HOBt mediated coupling resulted in a high yield of the tetrapeptide **11** (purity 99%, UV 214 nm, Figure S1 (SI)). Next came the challenging coupling with the Ant amine that has been accomplished with Fmoc amino acid chlorides and fluorides as discussed above. We devised a simplified procedure utilizing the in situ generation of the Fmoc-amino acid chloride, thus avoiding the need to purify and handle these racemization-prone acylating agents.[44] The reaction of the Fmoc-amino acid (3 equiv) with triphosgene and 2,4,6-collidine[45] served to produce the acid chloride and this was coupled with the Ant residue on solid-phase to afford pentapeptide **12** in high yield without epimerization of the α-position (99%, UV 214 nm, Figure S1 (SI)). After removal of the Fmoc group in **12**, coupling with Fmoc-L-Phe-OH using DIC/HOBt gave the hexapeptide **13** (purity, 99%, UV 214 nm, Figure S1 (SI)). Removal of the Fmoc group with 20% piperidine/DMF and cleavage from the support under mildly acidic conditions gave the cyclization precursor **7**. In the subsequent macrolactamization, we tried various coupling reagents (EDCI/HOBt/DIEA, EDCI/HOAt/DIEA, HBTU/DIEA, and HATU/DIEA) under high dilution conditions (1 mM). We found that HATU/DIEA achieved the fastest coupling with the least byproducts, resulting in the high yield of the desired macrocycle **5**. Overall, the yield of **5** from the Fmoc-D-Ala-Lantern was found to be 39%. Characterization by spectroscopy and specific rotation confirmed that it was identical to the natural product PF1171F (Figures S2 and S3, Table S1 (SI)).

With the successful total synthesis of PF1171F (**5**) (Scheme 2) completed, we applied the route to the preparation of other PF1171 peptides (**1**–**4** and **6**). To verify the absolute

dx.doi.org/10.1021/jo500861k | J. Org. Chem. 2014, 79, 7844–7853

configuration of the Ala residue in PF1171A and C, we synthesized these peptides with either D-Ala (**1** and **3**) or L-Ala (**2** and **4**). Elongation of the peptides was carried out in parallel on the SynPhase Lantern. Cleavage from the solid-support and the subsequent macrolactamization provided each cyclic peptide in the same manner as PF1171F (**5**). The spectroscopic data of the synthetic PF1171A (**1**), C (**3**), and G (**6**) were in good agreement with those of the corresponding natural PF1171A, C, and G, respectively (Figures S4−S9, Tables S2−S4 (SI)). On the other hand, the spectroscopic data of *epi*-PF1171A (**2**) and *epi*-PF1171C (**4**) showed clear differences from those of the natural PF1171A and C, respectively (Figures S4−S7 (SI)). Our data demonstrate that the Ala residues in natural PF1171A and PF1171C are the D-enantiomers, in accordance with Opatz's assignment. In the NMR spectra of *epi*-PF1171A (**2**) and *epi*-PF1171C (**4**), the NMR signals were broad, and several conformers were observed (Figures S4−S7 (SI)). This indicates that the conformations of *epi*-PF1171A (**2**) and *epi*-PF1171C (**4**) are substantially different from those of PF1171A (**1**) and C (**3**), respectively.

Biological Evaluation. The paralytic activities of PF1171 peptides against silkworms were evaluated by direct injection into the fourth-instar larvae (Figure 2). A sample of the natural PF1171A, isolated from okara media fermented with ascomycete OK-128, was used in the assay as a standard. The paralytic activity of the synthetic PF1171A was found to be almost equal to that of the natural PF1171A. PF1171C (**3**) was slightly more potent than PF1171A (**1**), whereas PF1171F (**5**) and G (**6**) were less potent than PF1171A (**1**). It is noteworthy that *epi*-PF1171A (**2**) and *epi*-PF1171C (**4**) exhibited little activity. This indicates the importance of the Ala configuration for the paralytic activity of PF1171 peptides. Since the NMR spectra of *epi*-PF1171A (**2**) and *epi*-PF1171C (**4**) were very different from those of PF1171A (**1**) and PF1171C (**3**) (Figures S4−S7 (SI)), respectively, it is likely the epimers could not adopt the active conformations required for biological activity. McAlpine and colleagues have reported that the difference between an L- and a D-amino acid in a cyclic peptide significantly impacts on biological activity presumably through modification of a 3D macrocyclic conformation.[46] In the structure−activity relationship study of sanguinamide B, they demonstrated that the analogues with an L-Phe and a D-Phe form single conformations different from one another.[47]

X-ray Crystallographic Analysis. In addition to the NMR data above, X-ray crystallographic analysis and computational analysis are popular methods for investigating the 3D conformation of cyclic peptides.[48−50] We succeeded in preparing a single crystal of PF1171F (**5**) and its 3D structure was determined by X-ray crystallographic analysis (Figure 3A). Despite PF1171F (**5**) containing an *N*-methyl amino acid (MeLeu) and a cyclic amino acid (Pip) that can favor *cis*-amide rotamers, all the amide bonds were found to be in the *s*-trans forms. The distance and angle information between the amide N−H and C−O bonds indicate the existence of four intramolecular hydrogen bonds in the crystalline PF1171F (**5**) (Table 1). The hydrogen bonds at Ant-CO···NH-D-Ala and Pip-CO···NH-Phe form two β-turns[51] at the Ant-Pip-MeLeu-D-Ala and Pip-MeLeu-D-Ala-Phe portions of the peptide, respectively (Figure 3B,C). On the other hand, the hydrogen bond at D-Ala-CO···NH-D-*allo*-Ile forms a γ-turn[51] at D-Ala-Phe-D-*allo*-Ile (Figure 3D). It is noteworthy that the hydrogen bond at Ant-CO···NH-Ant seems to maintain a bend structure at D-*allo*-Ile-Ant-Pip residues (Figure 3E). This unique

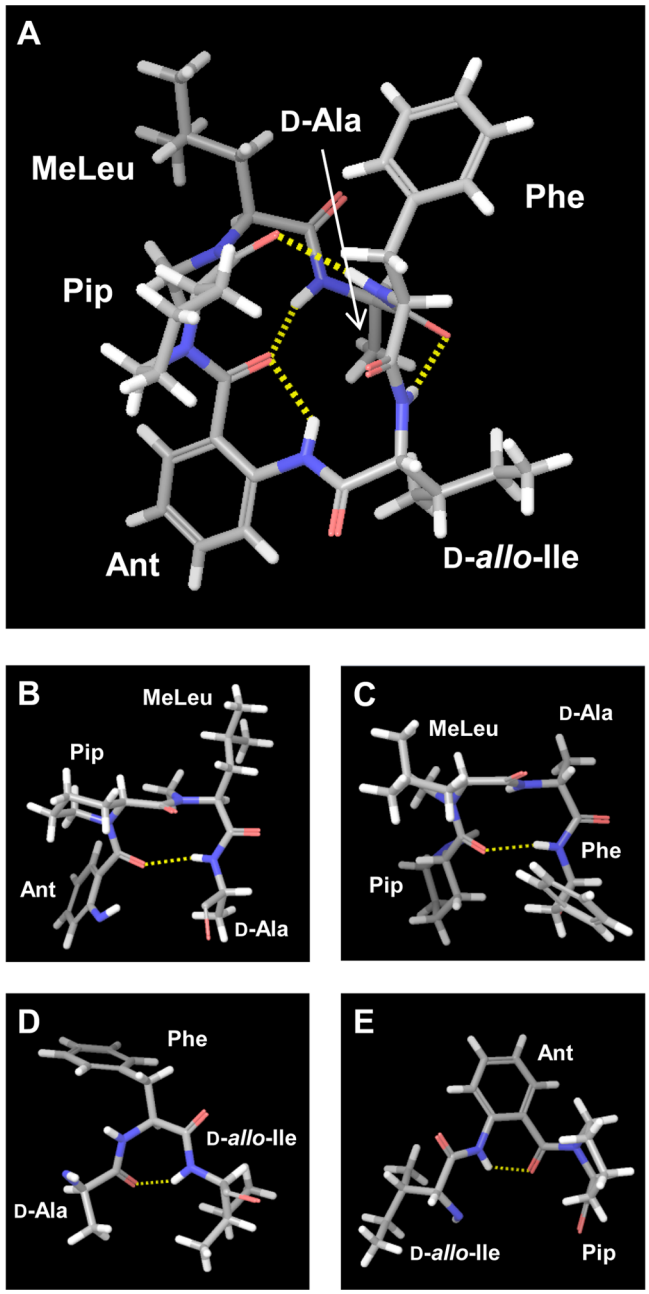


Figure 3. X-ray crystal structure of PF1171F (**5**) in an overall view (A) and segmentary views of turn or bend structures (B−E). (B) A β-turn at Ant-Pip-MeLeu-D-Ala stabilized by a hydrogen bond at Ant-CO···NH-D-Ala. (C) A β-turn at Pip-MeLeu-D-Ala-Phe stabilized by a hydrogen bond at Pip-CO···NH-Phe. (D) A γ-turn at D-Ala-Phe-D-*allo*-Ile stablized by a hydrogen bond at D-Ala-CO···NH-D-*allo*-Ile. (E) A bend structure at D-*allo*-Ile-Ant-Pip stablized by a hydrogen bond at Ant-CO···NH-Ant. Yellow dotted lines indicate intramolecular hydrogen bonds.

hydrogen bond of Ant has been reported in the study of cycloaspeptide E, an Ant-containing cyclic peptide.[23]

As shown in Tables S1−S4 (SI), the chemical shifts for PF1171A (**1**), C (**3**), and G (**6**) were similar to those of PF1171F (**5**). This suggests that all of their conformations in $CDCl_3$ are similar to that of PF1171F (**5**). On the other hand, the chemical shifts of *epi*-PF1171A (**2**) and *epi*-PF1171C (**4**) were different from PF1171F. This indicates that the epimers could not adopt the conformation of PF1171A (**1**), C (**3**), F

dx.doi.org/10.1021/jo500861k | J. Org. Chem. 2014, 79, 7844−7853

Table 1. Intramolecular Hydrogen Bonds[a] in Crystalline PF1171F (5)

		distance		angle	
donor	acceptor	$d_{N\cdots O}$ (Å)	$d_{H\cdots O}$ (Å)	∠(N−H···O)	∠(C═O···H)
D-Ala-NH	Ant-CO	2.88	2.13	143.1°	138.7°
Phe-NH	Pip-CO	2.94	2.08	167.4°	118.8°
D-*allo*-Ile-NH	D-Ala-CO	2.85	2.06	147.9°	87.9°
Ant-NH	Ant-CO	2.79	2.12	132.9°	90.4°

[a]H-atoms were constructed with N−H = 0.88 Å

(5), and G (6). The amide proton chemical shift of an amino acid residue has been recognized as an indicator for hydrogen bond strength; increasing hydrogen bond strength causes a downfield amide proton chemical shift.[52,53] In the ^{1}H NMR spectra (Figures S4 and S6 (SI)), NH signals of Ant and Leu in *epi*-PF1171A (2) and *epi*-PF1171C (4) move upfield compared with those of PF1171A (1) and PF1171C (3). This indicated that *epi*-PF1171A (2) and *epi*-PF1171C (4) could not form the intramolecular hydrogen bonds which are important for maintaining the active conformation required for binding to the biological target. We suspect that the active conformation also enhances the bioavailability of PF1171 peptides in vivo. The PF1171 peptides comply with three out of four of Lipinski's guidelines (four H-bond donors, six H-bond acceptors, C log P 1.20−2.26[54]), but their molecular weights are larger than 500. The observed four hydrogen bonds would not only reduce their number of donors and acceptors to decrease aqueous solvation but also make their molecular sizes compact, resulting in good membrane permeability. In fact, natural PF1171A, C, F, and G when administered orally induce paralysis against silkworms.[12,13]

CONCLUSION

In summary, we have accomplished the first total synthesis of all the PF1171 cyclic hexapeptide natural products, namely, A (1), C (3), F (5), and G (6), by a combination of solid-phase peptide synthesis and solution-phase macrolactamization. The in situ generation of Fmoc-amino acid chlorides using triphosgene was found to be an efficient means of coupling to the weakly nucleophilic amino group of Ant and this method can be recommended for the synthesis of other peptides with such labile residues. Our total synthesis unambiguously determined that the Ala residues in PF1171A and PF1171C have the D configurations. The natural products PF1171A (1), C (3), F (5), and G (6) were all found to exhibit paralytic activities against the larvae of silkworms. Meanwhile, *epi*-PF1171A (2) and *epi*-PF1171C (4) with L-Ala instead of D-Ala had weak activity as they adopt alternative conformations. The NMR and X-ray crystallographic analysis indicates that intramolecular hydrogen bonds in PF1171 peptides are critical for maintaining their active conformation and uptake in vivo.

EXPERIMENTAL SECTION

General Techniques. All commercially available reagents were used as received. Trityl alcohol SynPhase Lantern (surface: polystyrene, loading: 35 μmol) was purchased from Mimotopes. Fmoc-Ant-OH, Fmoc-L-Pip-OH, Fmoc-D-*allo*-Ile-OH, and Fmoc-D-Val were prepared from the corresponding free amino acids as reported previously.[55−58] All reactions in solution-phase were monitored by thin-layer chromatography performed on glass-packed silica gel plates (60F-254) with UV light, and visualized with ninhydrin solution. Flash column chromatography was performed with silica gel (40−100 μm) with the indicated solvent system. ^{1}H NMR spectra (400 MHz, 600 MHz) and ^{13}C NMR spectra (100 MHz, 150 MHz) were recorded in the indicated solvent. Chemical shifts (δ) for ^{1}H NMR spectra are referenced to signals for internal tetramethylsilane (0 ppm) and residual nondeuterated solvents (chloroform 7.26 ppm; methanol-d_4 3.30 ppm; dimethyl sulfoxide (DMSO)-d_6 2.49 ppm). Chemical shifts (δ) for ^{13}C NMR spectra are referenced to signals for residual deuterated solvents (chloroform-*d* 77.0 ppm, methanol-d_4 49.0 ppm; DMSO-d_6 39.5 ppm). The ^{1}H and ^{13}C chemical shifts were assigned using a combination of COSY, HMQC, and HMBC. Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), dt (double triplet), brs (broad singlet). Coupling constants (J) are represented in hertz (Hz). High-resolution mass spectra were measured on TOF-MS with ESI probe. Only the strongest and/or structurally important absorptions of IR spectra were reported in wavenumbers (cm^{-1}). Specific rotations were measured on a polarimeter at 589 nm. Melting points were measured on a melting point apparatus and are not corrected. Analysis of the synthetic peptides was performed by Kaiser test, chloranil test, and reversed-phase high performance liquid chromatography (RP-HPLC) on a LC−MS system with a photodiode array detector and a quadrupole mass analyzer. RP-HPLC conditions were as follows: column, X-Bridge ODS-3.5 μm, 4.6 × 75 mm; flow rate, 1.1 mL/min; elution method, solvent A/solvent B = 90:10−5:95 linear gradient (0.0−4.0 min), solvent A/solvent B = 5:95 isocratic (4.0−11.0 min), solvent A/solvent B = 90:10 isocratic (11.0−15.0 min), (solvent A: 0.1% HCOOH/H_2O, solvent B: 0.1% HCOOH/MeOH). The purity was determined with peak area at UV 214 nm. Preparative RP-HPLC was carried out on a HPLC system equipped with a UV detector (monitoring at 214 and 254 nm).

General Procedure for Loading of Fmoc-D-Ala, Fmoc-L-Ala, or Fmoc-D-Aba to the Trityl Alcohol Lantern. Trityl alcohol SynPhase Lantern (D-series, 35 μmol/unit) was treated with a solution of acetyl chloride in CH_2Cl_2 (1:10, v/v) at room temperature. After the Lantern was shaken at the same temperature for 4 h, the mixture was filtered. The Lantern was rinsed with CH_2Cl_2 and washed five times each with CH_2Cl_2 to afford trityl chloride Lantern. The resulting Lantern was immediately used for the immobilization of Fmoc amino acid. The trityl chloride Lantern was treated with a solution of Fmoc amino acid (Fmoc-D-Ala, Fmoc-L-Ala, or Fmoc-D-Aba) (0.100 M) and DIEA (0.200 M) in CH_2Cl_2 (1.00 mL/Lantern) at room temperature and then shaken at the same temperature. After being shaken for 12 h, the reaction mixture was filtered. The resulting Lantern was rinsed with CH_2Cl_2, and then washed with CH_2Cl_2 (3 min × 5), Et_2O (3 min × 1). The washed Lantern was dried in vacuo to afford polymer-supported amino acid. The immobilization yields were found to be 78% (Fmoc-D-Ala), 70% (Fmoc-L-Ala), and 52% (Fmoc-D-Aba) by gravimetric analysis after cleavage with 30% HFIP/CH_2Cl_2 (room temperature, 1 h) from the polymer support.

General Procedure for Acylation with Fmoc-L-MeLeu-OH on the Polymer Support. The *N*-Fmoc protected amino acid-supported Lantern was treated with a solution of 20% piperidine in DMF at room temperature. After being shaken for 1 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 5). The washed Lantern was immediately used for the next reaction. To a suspension of Lantern, Fmoc-L-MeLeu-OH (0.100 M), and HOBt (0.150 M) in DMF (1.00 mL/Lantern) was added DIC (0.100 M), and the mixture was shaken at room temperature. After being shaken for 12 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 3), THF−H_2O (3:1) (3 min × 3), MeOH (3 min × 2), and CH_2Cl_2 (3 min × 2). The washed Lantern was dried in vacuo to afford the polymer-supported dipeptide.

General Procedure for Acylation with Fmoc-L-Pip-OH on the Polymer Support. The *N*-Fmoc protected dipeptide-supported Lantern was treated with a solution of 20% piperidine in DMF at room temperature. After being shaken for 1 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 5). The washed Lantern was immediately used for the next condensation. To a suspension of Lantern, Fmoc-L-Pip-OH (0.100 M), and DIEA (0.200

dx.doi.org/10.1021/jo500861k | J. Org. Chem. 2014, 79, 7844−7853

M) in DMF (1.00 mL/Lantern) was added PyBroP (0.100 M) at room temperature and the flask was purged with argon. After being shaken at the same temperature for 12 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 3), THF−H_2O (3:1) (3 min × 3), MeOH (3 min × 2), and CH_2Cl_2 (3 min × 2). The washed Lantern was dried in vacuo to afford the polymer-supported tripeptide.

General Procedure for Acylation with Fmoc-Ant-OH on the Polymer Support. The *N*-Fmoc protected tripeptide-supported Lantern was treated with a solution of 20% piperidine in DMF at room temperature. After being shaken for 1 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 5). The washed Lantern was immediately used for the next reaction. To a suspension of Lantern, Fmoc-Ant-OH (0.100 M), and HOBt (0.150 M) in DMF (1.00 mL/Lantern) was added DIC (0.100 M), and the mixture was shaken at room temperature. After being shaken for 20 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 3), THF−H_2O (3:1) (3 min × 3), MeOH (3 min × 2), and CH_2Cl_2 (3 min × 2). The washed Lantern was dried in vacuo to afford the polymer-supported tetrapeptide.

General Procedure for Acylation with Fmoc-D-*allo*-Ile or Fmoc-D-Val on the Polymer Support. The *N*-Fmoc protected tetraptide-supported Lantern was treated with a solution of 20% piperidine in DMF at room temperature. After being shaken for 1 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 5), THF (3 min × 2). The washed Lantern was immediately used for the next reaction. To a solution of Fmoc amino acid (Fmoc-D-*allo*-Ile or Fmoc-D-Val) (0.100 M) and triphosgene (0.033 M) in THF (1.00 mL/Lantern) was added 2,4,6-collidine (0.250 M) to give a white suspension. After 1 min, the suspension was added to the Lantern, and the mixture was shaken at room temperature. After being shaken for 12 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 3), THF−H_2O (3:1) (3 min × 3), MeOH (3 min × 2), and CH_2Cl_2 (3 min × 2). The washed Lantern was dried in vacuo to afford the polymer-supported pentapeptide.

General Procedure for Acylation with Fmoc-L-Leu or Fmoc-L-Phe on the Solid Support. The *N*-Fmoc protected pentapeptide-supported Lantern was treated with a solution of 20% piperidine in DMF at room temperature. After being shaken for 1 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 5). The washed Lantern was immediately used for the next reaction. To a suspension of Lantern, Fmoc amino acid (Fmoc-L-Leu or Fmoc-L-Phe) (0.100 M), and HOBt (0.150 M) in DMF (1.00 mL/Lantern) was added DIC (0.100 M), and the mixture was shaken at room temperature. After being shaken for 12 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 3), THF−H_2O (3:1) (3 min × 3), MeOH (3 min × 2), and CH_2Cl_2 (3 min × 2). The washed Lantern was dried in vacuo to afford polymer-supported hexapeptide.

General Procedure of Deprotection of Fmoc Group and Cleavage from Polymer Support. The *N*-Fmoc protected hexapeptide-supported Lantern was treated with a solution of 20% piperidine in DMF at room temperature. After being shaken for 1 h, the reaction mixture was filtered and the Lantern was washed with DMF (3 min × 5), CH_2Cl_2 (3 min × 2). The washed Lantern was dried in vacuo. The dried Lantern was immediately treated with 30% HFIP/CH_2Cl_2 at room temperature. After being shaken for 1 h, the reaction mixture was filtered and the Lantern was washed with CH_2Cl_2 (3 min × 3). The combined filtrate was concentrated in vacuo, and the crude cyclization precursor was used for the next reaction after it was passed through a short pad of silica gel.

Cyclization Precursor of PF1171A (H_2N-L-Leu-D-allo-Ile-Ant-L-Pip-L-MeLeu-D-Ala-OH). HPLC retention time 8.3 min, purity 92%, LRESIMS calcd for $C_{35}H_{56}N_6O_7$ $[M + H]^+$ 673.4, found 673.4.

Cyclization Precursor of epi-PF1171A (H_2N-L-Leu-D-allo-Ile-Ant-L-Pip-L-MeLeu-L-Ala-OH). HPLC retention time 8.5 min, purity 99%, LRESIMS calcd for $C_{35}H_{56}N_6O_7$ $[M + H]^+$ 673.4, found 673.4.

Cyclization Precursor of PF1171C (H_2N-L-Leu-D-Val−Ant-L-Pip-L-MeLeu-D-Ala-OH). HPLC retention time 8.2 min, purity 99%, LRESIMS calcd for $C_{34}H_{54}N_6O_7$ $[M + H]^+$ 659.4, found 659.4.

Cyclization Precursor of epi-PF1171C (H_2N-L-Leu-D-Val−Ant-L-Pip-L-MeLeu-L-Ala-OH). HPLC retention time 8.3 min, purity 99%, LRESIMS calcd for $C_{34}H_{54}N_6O_7$ $[M + H]^+$ 659.4, found 659.4.

Cyclization Precursor of PF1171F (H_2N-L-Phe-D-allo-Ile-Ant-L-Pip-L-MeLeu-D-Ala-OH). HPLC retention time 8.5 min, purity 99%, LRESIMS calcd for $C_{38}H_{54}N_6O_7$ $[M + H]^+$ 707.4, found 707.4.

Cyclization Precursor of PF1171G (H_2N-L-Leu-D-allo-Ile-Ant-L-Pip-L-MeLeu-D-Aba-OH). HPLC retention time 8.4 min, purity 95%, LRESIMS calcd for $C_{36}H_{58}N_6O_7$ $[M + H]^+$ 687.4, found 687.4.

General Procedure for Macrolactamization. To a solution of crude cyclization precursor (1.0 equiv) in dry CH_2Cl_2 (1.0 mM) was added DIEA (6.0 equiv), then HATU (3.0 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 3 h, the reaction mixture was poured into saturated aq $NaHCO_3$ and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was passed through a short pad silica gel and purified by RP-HPLC (column, YMC-Pack R&D ODS-A 20 mm × 150 mm; flow rate, 12.0 mL/min; elution method, H_2O/MeOH = 25:75−10:90 linear gradient (0.0−15.0 min)).

*PF1171A (**1**).* Yield: 29% (11.4 mg, 17.4 μmol) over 13 steps from Fmoc-D-Ala-Lantern; white solid; mp 229−230 °C [lit. mp 225−226 °C];[10] ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.43 (1H, s, Ant-NH), 8.32 (1H, d, J = 8.4 Hz, Ant-3), 8.00 (1H, d, J = 8.0 Hz, Leu-NH), 7.63 (1H, d, J = 10.0 Hz, Ala-NH), 7.47 (H, m, Ant-4), 7.39 (1H, d, J = 7.6 Hz, Ile-NH), 7.19 (1H, dd, J = 7.7, 1.5 Hz, Ant-6), 7.12 (1H, m, Ant-5), 4.81 (1H, m, Ala-α), 4.55 (1H, m, Leu-α), 4.44 (1H, dd, J = 7.6, 3.2 Hz, Ile-α), 4.14 (1H, m, Pip-ε), 3.70 (1H, dd, J = 11.4, 2.7 Hz, Pip-α), 3.48 (1H, dd, J = 9.1, 4.7 Hz, MeLeu-α), 3.20 (3H, s, MeLeu-*N*-Me), 3.16 (1H, dt, J = 13.2, 2.5 Hz, Pip-ε), 2.42 (1H, m, Ile-β), 2.19 (1H, m, MeLeu-β), 2.08 (1H, m, Pip-β), 2.05 (2H, m, Pip-γ), 1.99 (1H, m, Leu-β), 1.93 (1H, m, MeLeu-β), 1.78 (2H, m, Leu-β, Leu-γ), 1.65 (1H, m, MeLeu-γ), 1.57 (1H, m, Pip-δ), 1.52 (1H, m, Pip-β), 1.40 (2H, m, Ile-γ), 1.29 (3H, d, J = 7.2 Hz, Ala-β), 1.27 (1H, m, Pip-δ), 0.98 (6H, m, MeLeu-δ, δ'), 0.96 (3H, m, Leu-δ'), 0.94 (3H, m, Ile-δ), 0.91 (3H, d, J = 6.8 Hz, Ile-γ'), 0.89 (3H, d, J = 6.4 Hz, Leu-δ); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2 (Ala-CO), 174.0 (Leu-CO), 171.0 (Ile-CO), 170.2 (Ant-CO), 169.2 (MeLeu-CO), 168.8 (Pip-CO), 137.1 (Ant-C2), 131.7 (Ant-C4), 127.0 (Ant-C6), 123.8 (Ant-C3), 123.3 (Ant-C5), 122.7 (Ant-C1), 65.1 (MeLeu-α), 61.5 (Pip-α), 57.2 (Ile-α), 52.6 (Pip-ε), 50.9 (Leu-α), 47.8 (Ala-α), 37.82 (MeLeu-*N*-Me), 37.78 (MeLeu-β), 36.4 (Ile-β), 36.2 (Leu-β), 28.1 (Pip-γ), 27.4 (Pip-δ), 26.9 (Ile-γ), 25.6 (MeLeu-γ), 24.5 (Leu-γ, Pip-β), 23.3 (MeLeu-δ'), 23.1 (Leu-δ'), 22.1 (MeLeu-δ), 21.7 (Leu-δ), 18.4 (Ala-β), 13.9 (Ile-γ'), 11.8 (Ile-δ); IR (neat) 3340, 3011, 2958, 2934, 2871, 1684, 1644, 1618, 1594, 1520, 1450, 1292, 1207, 1161, 756; $[\alpha]_D^{28}$ +25.8 (*c* 0.301, CH_3OH) [lit. $[\alpha]_D^{25}$ +25.4 (*c* 0.500, CH_3OH)];[9] HRESIMS calcd for $C_{35}H_{54}N_6O_6$ $[M + Na]^+$ 677.4003, found 677.3997.

*epi-PF1171A (**2**).* Yield: 39% (14.8 mg, 22.6 μmol) over 13 steps from Fmoc-L-Ala-Lantern; white solid; mp 148−150 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.88 (1H, s, Ant-NH (major)), 8.53 (1H, d, J = 9.5 Hz), 8.48 (1H, s, Ant-NH (minor)), 8.21 (1H, d, J = 8.3 Hz, Ant-2 (major)), 7.90−7.83 (1H, m, Ant-2 (minor), Ala-NH), 7.42 (1H, m, Ant-3), 7.35 (1H, d, J = 7.6 Hz, Ant-5 (minor)), 7.25−7.08 (3H, m, Ant-5, Ant-C4, Ile-NH), 6.85 (1H, m, Leu-NH), 5.88 (1H, brs), 5.31 (1H, m), 4.57−4.39 (2H, m, Ala-α, Ile-α (minor)), 4.25 (1H, m, Ile-α (major)), 4.10−3.95 (2H, m, Leu-α, Pip-ε), 3.85−3.65 (1H, m, Pip-α), 3.52 (1H, m, MeLeu-α), 3.29−3.15 (3H, s, MeLeu-*N*-Me (major), Pip-ε), 2.80 (1H, s, MeLeu-*N*-Me (minor)), 2.39−2.25 (1H, m, Ile-β (major)), 2.20−2.10 (1H, m, Ile-β (minor)), 2.04−1.22 (16H, m, Ala-β, MeLeu-β, MeLeu-γ, Pip-β, Pip-γ, Pip-δ, Ile-γ, Leu-β, Leu-γ), 1.04−0.68 (1H, m, MeLeu-δ, Ile-δ, Ile-γ', Leu-δ); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.7, 173.6, 173.3, 172.3, 172.0, 170.7, 170.5, 170.4, 170.3, 170.2, 170.0, 136.1, 134.3, 131.1, 130.2, 127.3, 127.0, 126.8, 125.6, 124.2, 124.1, 123.7, 65.0, 59.1, 58.1, 57.7, 52.2, 51.5, 51.3, 50.0, 45.4,

dx.doi.org/10.1021/jo500861k | J. Org. Chem. 2014, 79, 7844−7853

38.6, 38.0, 37.6, 37.5, 36.9, 28.9, 27.7, 26.7, 26.6, 25.5, 25.0, 24.8, 24.7, 23.6, 23.4, 23.1, 23.0, 22.6, 22.42, 22.1, 21.5, 18.4, 18.3, 16.7, 16.5, 14.3, 14.0, 11.7; IR (neat) 3335, 2959, 2872, 1662, 1617, 1524, 1449, 1404, 1388, 1369, 1279, 1266, 755 cm^{-1}; $[\alpha]_D^{28}$ −48.5 (*c* 0.175, CH_3OH); HRESIMS calcd for $C_{35}H_{54}N_6O_6$ $[M + Na]^+$ 677.4003, found 677.3997.

*PF1171C (**3**).* Yield: 41% (17.3 mg, 27.0 μmol) over 13 steps from Fmoc-D-Ala-Lantern; white solid; mp 138−140 °C [lit. mp 127−128 °C];[10] ^1H NMR (600 MHz, $CDCl_3$) δ 9.41 (1H, s, Ant-NH), 8.30 (1H, d, *J* = 8.4 Hz, Ant-3), 8.00 (1H, d, *J* = 8.0 Hz, Leu-NH), 7.63 (1H, d, *J* = 9.8 Hz, Ala-NH), 7.46 (1H, m, Ant-4), 7.42 (1H, d, *J* = 7.5 Hz, Val-NH), 7.19 (1H, dd, *J* = 7.6, 1.4 Hz, Ant-C6), 7.13 (1H, m, Ant-5), 4.82 (1H, m, Ala-α), 4.57 (1H, m, Leu-α), 4.32 (1H, dd, *J* = 7.6, 3.4 Hz, Val-α), 4.13 (1H, m, Pip-ε), 3.70 (1H, dd, *J* = 11.4, 2.8 Hz, Pip-α), 3.48 (1H, dd, *J* = 9.0, 4.7 Hz, MeLeu-α), 3.20 (3H, s, MeLeu-*N*-Me), 3.15 (1H, dt, *J* = 13.0, 2.3 Hz, Pip-ε), 2.68 (1H, m, Val-β), 2.20 (1H, m, MeLeu-β), 2.08 (1H, m, Pip-β), 2.04 (2H, m, Pip-γ), 2.00 (1H, m, Leu-β), 1.94 (1H, m, MeLeu-β), 1.77 (1H, m, Leu-γ) 1.76 (1H, m, Leu-β), 1.64 (1H, m, MeLeu-γ), 1.55 (1H, m, Pip-δ), 1.52 (1H, m, Pip-β), 1.29 (3H, d, *J* = 7.2 Hz, Ala-β), 1.26 (1H, m, Pip-δ), 1.06 (3H, d, *J* = 6.8 Hz, Val-γ′), 0.97 (6H, m, MeLeu-δ, δ′), 0.96 (3H, m, Leu-δ′), 0.93 (3H, m, Val-γ), 0.87 (3H, m, Leu-δ); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.3 (Ala-CO), 174.1 (Leu-CO), 170.6 (Val-CO), 170.1 (Ant-CO), 169.2 (MeLeu-CO), 168.8 (Pip-CO), 137.1 (Ant-C2), 131.6 (Ant-C4), 127.1 (Ant-C6), 123.9 (Ant-C3), 123.3 (Ant-C5), 122.7 (Ant-C1), 65.1 (MeLeu-α), 61.5 (Pip-α), 59.2 (Val-α), 52.5 (Pip-ε), 50.9 (Leu-α), 47.8 (Ala-α), 37.82 (MeLeu-*N*-Me), 37.77 (MeLeu-β), 36.2 (Leu-β), 29.9 (Val-β), 28.1 (Pip-γ), 27.3 (Pip-δ), 25.6 (MeLeu-γ), 24.5 (Pip-β), 24.4 (Leu-γ), 23.3 (MeLeu-δ′), 23.1 (Leu-δ′), 22.1 (MeLeu-δ), 21.7 (Leu-δ), 19.7 (Val-γ′), 18.4 (Ala-β), 16.1(Val-γ); IR (neat) 3340, 3011, 2959, 2871, 1685, 1644, 1618, 1595, 1520, 1450, 1293, 1162, 755 cm^{-1}; $[\alpha]_D^{27}$ +22.0 (*c* 0.103, CH_3OH) [lit. $[\alpha]_D^{16}$ 40.5 (*c* 0.200, CH_3OH)];[9] HRESIMS calcd for $C_{34}H_{52}N_6O_6$ $[M + Na]^+$ 663.3846, found 663.3841.

*epi-PF1171C (**4**).* Yield: 41% (14.1 mg, 22.0 μmol) over 13 steps from Fmoc-L-Ala-Lantern; white solid; mp 176−178 °C; ^1H NMR (600 MHz, $CDCl_3$) δ 8.69 (1H, s, Ant-NH (major)), 8.55 (1H, d, *J* = 9.2 Hz), 8.50 (1H, s, Ant-NH (minor)), 8.19 (1H, d, *J* = 8.0 Hz, Ant-2 (major)), 7.87 (1H, d, *J* = 7.6 Hz, Ant-2 (minor)), 7.77 (1H, d, *J* = 8.0 Hz, Ala-NH (major)), 7.41 (1H, m, Ant-3), 7.35 (1H, d, *J* = 7.2 Hz, Ant-5 (minor)), 7.25−7.10 (3H, m, Ant-5, Ant-4, Val-NH), 6.73 (1H, m, Leu-NH), 5.91 (1H, brs), 5.31 (1H, m), 4.60−4.40 (3H, m, Ala-α), 4.35 (1H, m, Val-α), 4.30−4.18 (1H, m, Leu-α), 4.00−3.88 (1H, m, Pip-ε), 3.88−3.60 (1H, m, Pip-α), 3.62−3.46 (1H, m, MeLeu-α), 3.33−3.18 (3H, s, MeLeu-*N*-Me (major), Pip-ε), 2.79 (1H, s, MeLeu-*N*-Me (minor)), 2.55−2.42 (1H, m, Val-β (major)), 2.48 (1H, m, Val-β (minor)), 2.35 (1H, m, MeLeu-β), 2.10−1.10 (14H, m, Ala-β, MeLeu-β, MeLeu-γ, Pip-β, Pip-γ, Pip-δ, Leu-β, Leu-γ), 1.03−0.78 (18H, m, MeLeu-δ, Val-γ, Leu-δ); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.7, 173.3, 173.3, 172.2, 172.0, 171.1, 170.2, 172.2, 170.1, 169.8, 135.9, 134.5, 131.0, 130.3, 127.1, 126.8, 125.3, 124.5, 124.1, 123.7, 123.5, 64.8, 60.2, 59.1, 56.4, 51.9, 51.5, 51.4, 49.1, 45.4, 38.9, 38.0, 37.6, 37.0, 29.9, 29.5, 28.9, 27.6, 26.3, 25.5, 25.1, 24.8, 24.7, 23.6, 23.4, 23.1, 23.0, 22.7, 22.4, 22.2, 22.1, 21.5, 19.6, 19.4, 18.3, 17.1, 16.8, 16.6; IR (neat) 3334, 3010, 2959, 2934, 2871, 1662, 1619, 1524, 1449, 1403, 1369, 1266, 1236, 755; $[\alpha]_D^{27}$ −34.9 (*c* 0.150, CH_3OH); HRESIMS calcd for $C_{34}H_{52}N_6O_6$ $[M + Na]^+$ 663.3846, found 663.3841.

*PF1171F (**5**).* Yield: 39% (37.3 mg, 52.7 μmol) over 13 steps from Fmoc-D-Ala-Lantern; white solid; mp 232−234 °C; ^1H NMR (600 MHz, $CDCl_3$) δ 9.43 (1H, s, Ant-NH), 8.29 (1H, d, *J* = 7.8 Hz, Ant-3), 8.23 (1H, d, *J* = 7.8 Hz, Phe-NH), 7.66 (1H, d, *J* = 10.0 Hz, Ala-NH), 7.47 (1H, m, Ant-4), 7.40 (1H, d, *J* = 7.9 Hz, Ile-NH), 7.38 (2H, d, *J* = 7.2 Hz, Phe-2, 6), 7.28 (1H, d, *J* = 7.2 Hz, Phe-3, 5), 7.21 (1H, m, Ant-6), 7.19 (1H, m, Phe-4), 7.14 (1H, t, *J* = 5.2 Hz, Ant-5), 4.70 (1H, m, Ala-α), 4.65 (1H, m, Phe-α), 4.44 (1H, dd, *J* = 7.5, 3.6 Hz, Ile-α), 4.16 (1H, m, Pip-ε), 3.75 (1H, m, Pip-α), 3.52 (1H, dd, *J* = 9.0, 4.6 Hz, MeLeu-α), 3.37 (1H, dd, *J* = 14.4, 5.4 Hz, Phe-β), 3.27 (1H, dd, *J* = 14.4, 9.6 Hz, Phe-β), 3.23 (3H, s, MeLeu-*N*-Me), 3.18 (1H, dt, *J* = 13.3, 2.6 Hz, Pip-ε), 2.41 (1H, m, Ile-β), 2.29 (H, m, MeLeu-β), 2.16 (2H, m, Pip-γ), 2.13 (1H, m, Pip-β), 2.04 (1H, m, MeLeu-β), 1.70 (1H, m, MeLeu-γ), 1.60 (1H, m, Pip-δ), 1.57 (1H, m, Pip-β), 1.35 (1H, m, Ile-γ), 1.31 (1H, m, Pip-δ), 1.24 (3H, d, *J* = 7.3 Hz, Ala-β), 1.00 (6H, m, MeLeu-δ, δ′), 0.90 (6H, m, Ile-δ, Ile-γ′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.4 (Ala-CO), 173.4 (Phe-CO), 170.8 (Ile-CO), 170.2 (Ant-CO), 169.0 (Pip-CO), 168.9 (MeLeu-CO), 138.3 (Phe-C1), 137.0 (Ant-C2), 131.7 (Ant-C4), 129.5 (Phe-C2, C6), 128.3 (Phe-C3, C5), 127.0 (Phe-C4), 126.4 (Ant-C6), 124.0 (Ant-C3), 123.4 (Ant-C5), 122.8 (Ant-C1), 65.4 (MeLeu-α), 61.5 (Pip-α), 57.3 (Ile-α), 54.6 (Phe-α), 52.6 (Pip-ε), 47.7 (Ala-α), 37.8 (MeLeu-*N*-Me, MeLeu-β), 36.4 (Ile-β), 33.9 (Phe-β), 28.0 (Pip-γ), 27.4 (Pip-δ), 26.8 (Ile-γ), 25.7 (MeLeu-γ), 24.5 (Pip-β), 23.4 (MeLeu-δ′), 22.1 (MeLeu-δ), 18.2 (Ala-β), 13.9 (Ile-γ′), 11.7 (Ile-δ); IR (neat) 3341, 2958, 1685, 1641, 1618, 1594, 1517, 1449, 1292 cm^{-1}; $[\alpha]_D^{28}$ +15.7 (*c* 0.150, $CHCl_3$) [lit. $[\alpha]_D^{28}$ +18 (c 0.150, $CHCl_3$)];[10] HRESIMS calcd for $C_{38}H_{52}N_6O_6$ $[M + Na]^+$ 711.3846, found 711.3841.

*PF1171G (**6**).* Yield: 29% (18.0 mg, 26.9 μmol) over 13 steps from Fmoc-D-Aba-Lantern; white solid; mp 250−252 °C; ^1H NMR (600 MHz, $CDCl_3$) δ 9.44 (1H, s, Ant-NH), 8.30 (1H, d, *J* = 8.4 Hz, Ant-3), 7.96 (1H, d, *J* = 7.6 Hz, Leu-NH), 7.63 (1H, d, *J* = 10.0 Hz, Aba-NH), 7.46 (2H, m, Ant-4), 7.43 (2H, m, Ile-NH), 7.19 (1H, dd, *J* = 7.6, 1.2 Hz, Ant-6), 7.12 (1H, t, *J* = 7.2 Hz, Ant-5), 4.78 (1H, m, Aba-α), 4.55 (1H, m, Leu-α), 4.44 (1H, dd, *J* = 7.2, 2.8 Hz, Ile-α), 4.12 (1H, m, Pip-ε), 3.69 (1H, dd, *J* = 10.4, 2.4 Hz, Pip-α), 3.51 (1H, dd, *J* = 8.8, 4.4 Hz, MeLeu-α), 3.20 (3H, s, MeLeu-*N*-Me), 3.16 (1H, dt, J =12.8, 2.0 Hz, Pip-ε), 2.43 (1H, m, Ile-β), 2.20 (1H, m, MeLeu-β), 2.09 (1H, m, Pip-β), 2.04 (2H, m, Pip-γ), 1.96 (1H, m, Leu-β), 1.94 (1H, m, MeLeu-β), 1.89 (1H, m, Aba-β), 1.78 (2H, m, Leu-β, Leu-γ), 1.66 (1H, m, Aba-β), 1.55 (1H, m, Pip-δ), 1.53 (H, m, Pip-β), 1.64 (1H, m, MeLeu-γ), 1.39 (2H, m, Ile-γ), 1.27 (1H, m, Pip-δ), 0.96 (6H, m, MeLeu-δ, δ′), 0.95 (3H, m, Leu-δ′), 0.93 (3H, m, Ile-δ), 0.91 (3H, m, Ile-γ′), 0.88 (3H, d, *J* = 6.4 Hz, Leu-δ), 0.73 (3H, t, *J* = 7.6 Hz, Aba-γ); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.0 (Leu-CO), 173.4 (Aba-CO), 170.9 (Ile-CO), 170.1 (Ant-CO), 169.8 (MeLeu-CO), 168.8 (Pip-CO), 137.1 (Ant-C2), 131.7 (Ant-C4), 127.1 (Ant-C6), 123.8 (Ant-C3), 123.3 (Ant-C5), 122.5 (Ant-C1), 65.1 (MeLeu-α), 61.5 (Pip-α), 57.2 (Ile-α), 52.9 (Aba-α), 52.5 (Pip-ε), 50.8 (Leu-α), 37.9 (MeLeu-*N*-Me, MeLeu-β), 36.3 (Ile-β, Leu-β), 27.9 (Pip-γ), 27.4 (Pip-δ), 26.9 (Ile-γ), 25.5 (MeLeu-γ), 24.7 (Pip-β), 24.5 (Aba-β, Leu-γ), 23.3 (MeLeu-δ), 23.0 (MeLeu-δ), 22.1 (Leu-δ′), 21.7 (Leu-δ), 14.0 (Ile-γ′), 11.7 (Ile-δ), 9.7 (Aba-γ); IR (neat) 3338, 3012, 2958, 2871, 1686, 1645, 1618, 1512, 1450, 1293, 1162, 754 cm^{-1}; $[\alpha]_D^{28}$ +26.2 (*c* 0.300, $CHCl_3$) [lit. $[\alpha]_D^{28}$ +26 (c 0.150, $CHCl_3$)];[10] HRESIMS calcd for $C_{36}H_{56}N_6O_6$ $[M + Na]^+$ 691.4159, found 691.4154.

Assay of Paralytic Activity against Larvae of Silkworms. Eggs of silkworm *Bombyx mori* were obtained from Ueda Sanshu Kyogyo Kumiai (Ueda, Japan) and cultured on an artificial diet SilkMate 2S purchased from Nosan Corporation. Each PF1171 peptide was dissolved in DMSO at concentrations of 1.25, 2.5, and 5 mM. A 5 μL of each solution was injected into open vessels of fourth-instar larvae (ca. 1 g weight), resulting in the dose of 6.25, 12.5, and 25.0 nmol/larva, respectively. A total of 10 larvae were injected with each dilution and the number of paralyzed larvae was counted 1 h after the injection. We confirmed that the paralyzed silkworms were alive and creeping 24 h later, indicating that the paralysis is reversible.

X-ray Crystallographic Analysis of PF1171F (5). Suitable colorless single crystals were obtained from a solution of PF1171F (**5**) (1 mg) in a mixture of $CHCl_3$ (5 μL), Et_2O (650 μL), and hexane (350 μL) at room temperature. CCDC 1006825 contains the supplementary crystallographic data for PF1171F (**5**) in this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supporting Figures S1−S10, Tables S1−S4, and copies of ^1H and ^{13}C NMR spectra for **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

dx.doi.org/10.1021/jo500861k | J. Org. Chem. 2014, 79, 7844−7853

AUTHOR INFORMATION

Corresponding Authors

*E-mail: a.ganesan@uea.ac.uk.
*E-mail: doi_taka@mail.pharm.tohoku.ac.jp.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Scientific Research (B) (No. 23310145 for T.D.) and for Young Scientists (B) (No. 26850068 for Y.M.), and Excellent Graduate Schools, and Platform for Drug Discovery, Informatics, and Structural Life Science from MEXT, Japan. We thank Prof. Masahiko Yamaguchi at the Graduate School of Pharmaceutical Sciences in Tohoku University for allowing us to use the X-ray diffractometer. We greatly thank Dr. Hidehiro Uekusa and Mr. Kohei Johmoto at Department of Chemistry and Materials Science in Tokyo Institute of Technology for determination of the X-ray crystal structure. We are grateful to Prof. Hideo Hayashi at Osaka Prefecture University for helpful discussions.

REFERENCES

(1) La Clair, J. J. *Nat. Prod. Rep.* **2010**, *27*, 969.
(2) Schmitt, E. K.; Moore, C. M.; Krastel, P.; Petersen, F. *Curr. Opin. Chem. Biol.* **2011**, *15*, 497.
(3) Newman, D. J.; Cragg, G. M.; Snader, K. M. *Nat. Prod. Rep.* **2000**, *17*, 215.
(4) Ortholand, J. Y.; Ganesan, A. *Curr. Opin. Chem. Biol.* **2004**, *8*, 271.
(5) Ganesan, A. *Curr. Opin. Chem. Biol.* **2008**, *12*, 306.
(6) Cragg, G. M.; Newman, D. J. *Biochim. Biophys. Acta* **2013**, *1830*, 3670.
(7) Thorstholm, L.; Craik, D. J. *Drug Discovery Today: Technol.* **2012**, *9*, e13.
(8) Schwarzer, D.; Finking, R.; Marahiel, M. A. *Nat. Prod. Rep.* **2003**, *20*, 275.
(9) Nagarajan, M.; Maruthanayagam, V.; Sundararaman, M. *J. Appl. Toxicol.* **2012**, *32*, 153.
(10) Umagome, K.; Nagase, K.; Harimaya, K.; Nakayama, F.; Yaguchi, T.; Sato, E.; Hoshiko, S.; Kamito, N.; Soneda, T.; Hachisu, M. Japan patent 11021297, 1999.
(11) Liermann, J. C.; Thines, E.; Anke, H.; Opatz, T. *Z. Naturforsch.* **2009**, *64b*, 727.
(12) Kai, K.; Yoshikawa, H.; Kuo, Y.-H.; Akiyama, K.; Hayashi, H. *Biosci., Biotechnol., Biochem.* **2010**, *74*, 1309.
(13) Kuo, Y.-H.; Kai, K.; Akiyama, K.; Hayashi, H. *Tetrahedron Lett.* **2012**, *53*, 429.
(14) Ha, S.-D.; Nagata, S.; Suzuki, A.; Kataoka, H. *Peptides* **1999**, *20*, 561.
(15) Nakahara, Y.; Kanamori, Y.; Kiuchi, M.; Kamimura, M. *Arch. Insect Biochem. Physiol.* **2003**, *52*, 163.
(16) Ishii, K.; Hamamoto, H.; Kamimura, M.; Sekimizu, K. *J. Biol. Chem.* **2008**, *283*, 2185.
(17) Ishii, K.; Hamamoto, H.; Kamimura, M.; Nakamura, Y.; Noda, H.; Imamura, K.; Mita, K.; Sekimizu, K. *J. Biol. Chem.* **2010**, *285*, 28635.
(18) Ishii, K.; Hamamoto, H.; Imamura, K.; Adachi, T.; Shoji, M.; Nakayama, K.; Sekimizu, K. *J. Biol. Chem.* **2010**, *285*, 33338.
(19) Yamazaki, M.; Horie, Y.; Bae, K.; Maebayashi, Y.; Jisai, Y.; Fujimoto, H. *Chem. Pharm. Bull.* **1987**, *35*, 2122.
(20) Kobayashi, R.; Samejima, Y.; Nakajima, S.; Kawai, K.; Udagawa, S. *Chem. Pharm. Bull.* **1987**, *35*, 1347.
(21) Dalsgaard, P. W.; Larsen, T. O.; Frydenvang, K.; Christophersen, C. *J. Nat. Prod.* **2004**, *67*, 878.
(22) Dalsgaard, P. W.; Blunt, J. W.; Munro, M. H.; Larsen, T. O.; Christophersen, C. *J. Nat. Prod.* **2004**, *67*, 1950.
(23) Lewer, P.; Graupner, P. R.; Hahn, D. R.; Karr, L. L.; Duebelbeis, D. O.; Lira, J. M.; Anzeveno, P. B.; Fields, S. C.; Gilbert, J. R.; Pearce, C. *J. Nat. Prod.* **2006**, *69*, 1506.
(24) Zhang, Y.; Liu, S.; Liu, H.; Liu, X.; Che, Y. *J. Nat. Prod.* **2009**, *72*, 1364.
(25) Zheng, J.; Zhu, H.; Hong, K.; Wang, Y.; Liu, P.; Wang, X.; Peng, X.; Zhu, W. *Org. Lett.* **2009**, *11*, 5262.
(26) Martin, J. H.; Hausmann, W. K. *J. Am. Chem. Soc.* **1960**, *82*, 2079.
(27) Umehara, K.; Nakahara, K.; Kiyoto, S.; Iwami, M.; Okamoto, M.; Tanaka, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1983**, *36*, 478.
(28) Toda, S.; Sugawara, K.; Nishiyama, Y.; Ohbayashi, M.; Ohkusa, N.; Yamamoto, H.; Konishi, M.; Oki, T. *J. Antibiot.* **1990**, *43*, 796.
(29) Darkin-Rattray, S. J.; Gurnett, A. M.; Myers, R. W.; Dulski, P. M.; Crumley, T. M.; Allocco, J. J.; Cannova, C.; Meinke, P. T.; Colletti, S. L.; Bednarek, M. A.; Singh, S. B.; Goetz, M. A.; Dombrowski, A. W.; Polishook, J. D.; Schmatz, D. M. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 13143.
(30) Hedge, V. R.; Puar, M. S.; Dai, P.; Pu, H.; Patel, M.; Anthes, J. C.; Richard, C.; Terracciano, J.; Das, P. R.; Gullo, V. *J. Antibiot.* **2001**, *54*, 125.
(31) Thornburg, C. C.; Cowley, E. S.; Sikorska, J.; Shaala, L. A.; Ishmael, J. E.; Youssef, D. T.; McPhail, K. L. *J. Nat. Prod.* **2013**, *76*, 1781.
(32) Bockus, A. T.; McEwen, C. M.; Lokey, R. S. *Curr. Top Med. Chem.* **2013**, *13*, 821.
(33) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2001**, *46*, 3.
(34) Whether D-Ile (2*R*,3*R*) or D-*allo*-Ile (2*R*,3*S*) was the reference standard was not mentioned in references 11−13, but Hayashi's group confirmed in a private communication that they used D-*allo*-Ile for configuration analysis by Marfey's method.
(35) Rasoul, F.; Ercole, F.; Pham, Y.; Bui, C. T.; Wu, Z.; James, S. N.; Trainor, R. W.; Wickham, G.; Maeji, N. J. *Biopolym. (Pept. Sci.)* **2000**, *55*, 207.
(36) Yoshida, M.; Takeuchi, H.; Ishida, Y.; Yashiroda, Y.; Takagi, M.; Shin-ya, K.; Doi, T. *Org. Lett.* **2010**, *12*, 3792.
(37) Doi, T.; Numajiri, Y.; Takahashi, T.; Takagi, M.; Shin-ya, K. *Chem.—Asian J.* **2011**, *6*, 180.
(38) Nakao, K.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1989**, *37*, 930.
(39) Wang, H.; Ganesan, A. *J. Org. Chem.* **2000**, *65*, 1022.
(40) Wang, H.; Ganesan, A. *J. Comb. Chem.* **2000**, *2*, 186.
(41) Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997.
(42) Frérot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259.
(43) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
(44) Carpino, L. A.; Cohen, B. J.; Stephens, K. E.; Sadat-Aalaee, S. Y.; Tien, J. H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732.
(45) Falb, E.; Yechezkel, T.; Salitra, Y.; Gilon, C. *J. Pept. Res.* **1999**, *53*, 507.
(46) Rodriguez, R. A.; Pan, P.-S.; Pan, C.-M.; Ravula, S.; Lapera, S.; Singh, E. K.; Styers, T. J.; Brown, J. D.; Cajica, J.; Parry, E.; Otrubova, K.; McAlpine, S. R. *J. Org. Chem.* **2007**, *72*, 1980.
(47) Wahyudi, H.; Tantisantisom, W.; Liu, X.; Ramsey, D. M.; Singh, E. K.; McAlpine, S. R. *J. Org. Chem.* **2012**, *77*, 10596.
(48) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512.
(49) Heller, M.; Sukopp, M.; Tsomaia, N.; John, M.; Mierke, D. F.; Reif, B.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 13806.
(50) Beck, J. G.; Chatterjee, J.; Laufer, B.; Kiran, M. U.; Frank, A. O.; Neubauer, S.; Ovadia, O.; Greenberg, S.; Gilon, C.; Hoffman, A.; Kessler, H. *J. Am. Chem. Soc.* **2012**, *134*, 12125.
(51) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1.
(52) Wagner, G.; Pardi, A.; Wuethrich, K. *J. Am. Chem. Soc.* **1983**, *105*, 5948.

(53) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *J. Mol. Biol.* **1991**, *222*, 311.
(54) C log P values for PF1171 peptides were estimated using the log P add-on for ChemSketch ver. 14.0 (Advanced Chemistry Development, I., Toronto, ON, Canada). The calculated C log P values were as follows: PF1171A, 1.73; PF1171C, 1.20; PF1171F, 2.02; PF1171G, 2.26.
(55) Kisfaludy, L.; Schön, I. *Synthesis* **1983**, 325.
(56) Schmidt, U.; Kroner, M.; Griesser, H. *Synthesis* **1989**, 832.
(57) Mjalli, A. M. M. US patent 59149, 2004.
(58) Lüttenberg, S.; Sondermann, F.; Scherkenbeck, J. *Tetrahedron* **2012**, *68*, 2068.