NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH Public Access

Author Manuscript

Planta Med. Author manuscript; available in PMC 2010 April 1.

Published in final edited form as:
Planta Med. 2009 April ; 75(5): 528–533. doi:10.1055/s-0029-1185332.

Largamides A–C, Tiglic Acid-Containing Cyclodepsipeptides with Elastase-Inhibitory Activity from the Marine Cyanobacterium *Lyngbya confervoides*

Susan Matthew[1], **Valerie J. Paul**[2], and **Hendrik Luesch**[1]

[1] Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610, USA

[2] Smithsonian Marine Station, 701 Seaway Drive, Fort Pierce, Florida 34949, USA

Abstract

Three unusual tiglic acid-containing cyclodepsipeptides, possessing the revised regioisomeric structures for largamides A–C (**1**–**3**),have been isolated from the marine cyanobacterium *Lyngbya confervoides* collected from southeastern Florida. The two-dimensional structures were determined by NMR spectroscopy and the absolute configurations by chiral HPLC analysis of degradation products. Compounds **1**–**3** are moderate inhibitors of mammalian elastase activity *in vitro* with IC_{50} values ranging from 0.53 to 1.41 μM.

Keywords

Marine cyanobacteria; *Lyngbya confervoides*; cyclodepsipeptides; elastase

Introduction

Marine cyanobacteria produce secondary metabolites with unusual structural features most commonly incorporated in a peptidic framework or in peptide–polyketide hybrids [1]. The presence of unconventional or highly modified amino acid units is a characteristic biosynthetic signature of cyanobacterial metabolites [1,2]. We initiated screening of various collections of marine cyanobacteria from coastal Florida with the aim of discovering novel protease inhibitors. A preliminary bioassay-guided fractionation of the organic extract of *Lyngbya confervoides* led us to identify several active fractions. Our systematic chemical investigations of the active fractions of *Lyngbya confervoides* that previously yielded the potent elastase and chymotrypsin inhibitors lyngbyastatins 4–6 [3,4], trypsin inhibitor pompanopeptin A [5], and putative carboxypeptidase inhibitor pompanopeptin B [5] now also yielded largamides A–C (**1**–**3**), along with largamides D–H [6]. Here we describe the isolation, biological evaluation and revision of the proposed structures of largamides A–C (**1**–**3**) [6]. The natural products were tested for inhibitory activity against three proteolytic enzymes, namely elastase, chymotrypsin and trypsin. Compounds **1**–**3** demonstrated moderate inhibition of porcine pancreatic elastase (PPE), but no activity against the other two serine proteases up to 50 μM.

Correspondence Prof. Dr. Hendrik Luesch, Department of Medicinal Chemistry, University of Florida, 1600 SW Archer Road, Gainesville, Florida 32610, USA. Email: luesch@cop.ufl.edu, Phone: +1 (352) 273-7738, Fax: + 1 (352) 273-7741.

Supporting Information
^{1}H NMR, ^{13}C NMR, and 1D TOCSY spectra of compounds **1**–**3** are available at http://www.thieme-connect.de/ejournals/toc/plantamedica.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Elastase is associated with tissue destruction and inflammation characteristic of chronic obstructive pulmonary disease (COPD) such as emphysema. COPD is the fourth leading cause of death in the USA [7]. In a murine model of emphysema, PPE was capable of recruiting monocytes to the lung, increasing lung macrophage content to generate an inflammatory cell infiltrate [7]. PPE causes direct proteolytic injury to the lung and induces airspace enlargement; however, greater lesions develop related to subsequent endogenous inflammation and destruction [7]. Because of the critical role of elastase in inflammatory processes, elastase inhibitors might be of importance in the treatment of inflammation related syndromes.

Materials and Methods

General Experimental Procedures

Optical rotation was measured on a Perkin Elmer 341 polarimeter. UV spectra were recorded using a SpectraMax M5 (Molecular Devices). ^{1}H and 2D NMR spectra were recorded in DMF-d_7 on a Varian 500 MHz spectrometer operating at 500 MHz and 125 MHz using residual solvent signals as the internal standard (δ_H 8.02, δ_C 162.7). HSQC experiments were optimized for $^1J_{CH}$ = 145 Hz, and HMBC experiments were optimized for $^nJ_{C,H}$ = 7 Hz. HRMS data were obtained using an Agilent LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector.

Porcine pancreatic elastase (Elastase-high purity; EC134) and *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide (NS945, purity >95%) were purchased from EPC, Missouri, USA. Trypsin from porcine pancreas (T0303,Type IX-S, 13,000–20,000 BAEE units/mg protein), α-chymotrypsin from bovine pancreas (C4129, Type II, ≥40 units/mg protein), *N*-succinyl-Gly-Gly-Phe-*p*-nitroanilide (S1899, purity >98%), *N*-α-benzoyl-DL-arginine 4-nitroanilide hydrochloride (B4874, purity ≥98%), and PMSF (P7626, purity ≥98.5%) were procured from Sigma, USA. All other reagents and solvents used were of reagent grade or HPLC grades.

Biological material

Samples of *Lyngbya confervoides* were collected off the coast of Broward County (Fort Lauderdale, Florida, USA (26°01.1414′N, 80°05.9973′W; 26°15.134′N, 80°03.908′W) in July 2004 and August 2005. S. Golubic identified the cyanobacterium [8]. A voucher specimen is retained at the Smithsonian Marine Station.

Extraction and Isolation

The freeze-dried sample (539.7 g)of *Lyngbya confervoides* was extracted with EtOAc–MeOH (1:1) and EtOH–H_2O (1:1) to afford VPL–NP (78.8 g) and VPL–P (113.8 g), respectively. The crude extracts VPL–NP (26.6 g) and VPL–P (95.9 g) were then combined, suspended in H_2O (500 mL) and defatted with hexanes (300 mL × 3; 0.67 g). The dried aqueous layer (~118 g) was further partitioned between *n*-BuOH (250 mL × 3) and H_2O to afford *n*-BuOH extract (6.3 g) and an aqueous extract (~110 g) enriched with salt. The concentrated *n*-BuOH extract was applied on a column packed with diaion HP-20 (Supelco) polymeric resin (60 g), fractionated with H_2O and increasing concentrations of MeOH, and then with MeCN to yield 7 fractions [Fr. 1: H_2O (100%, 2 L, ~3 g); Fr. 2: H_2O–MeOH (75:25 and 50:50), 2 L, 350 mg); Fr. 3: H_2O–MeOH (25:75, 1 L, 175 mg); Fr. 4: H_2O:MeOH (25:75, 1 L, 155 mg); Fr. 5: H_2O:MeOH (25:75, 1 L, 368 mg); Fr. 6: MeOH (100%, 1 L, 496 mg); Fr. 7: MeCN (100%, 1 L, 162 mg)]. The fractions (Fr. 5 and Fr. 6) were subjected to reversed-phase preparative HPLC [Phenomenex Luna 10u (C18), 100 × 21.20 mm, 10.0 mL/min; UV detection at 220 and 240 nm, using a MeOH–H_2O linear gradient (30–100% over 40 min and then 100% MeOH for 10 min)]. Fractions eluted with t_R 16–23 min (58–70% MeOH) were then subjected to repeated semi-preparative reversed-phase HPLC (YMC-Pack ODS-AQ, 250 × 10 mm, 2.0 mL/min; UV detection at 220 and 240 nm) using a 0.05% TFA in MeOH (60–90% for 25 min, then 90–

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

100% for 10 min and finally 100% MeOH for 10 min), to give largamide A (**1**), t_R 21.0 min (1.2 mg), largamide B (**2**), t_R 18.5 min (5.0 mg), and largamide C (**3**), t_R 20.5 min (1.8 mg).

Largamide A (1): colorless, amorphous solid; $[\alpha]^{20}_D$ –55 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.34), 280 (3.32) nm; IR (film) ν_{max} 3317 (br), 2959, 2924, 1725, 1659 (br), 1548, 1443, 1204 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 1; HR-ESI/APCI-MS *m/z* $[M + H]^+$ 842.4303 (calcd for $C_{41}H_{60}N_7O_{12}$, 842.4300).

Largamide B (2): colorless, amorphous solid; $[\alpha]^{20}_D$ –43 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.09), 280 (3.09) nm; IR (film) ν_{max} 3331 (br), 2958, 2923, 1721, 1656, 1640, 1597 (br), 1461 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 2; HR-ESI/APCI-MS *m/z* $[M + H]^+$ 920.4404 (calcd for $C_{46}H_{62}N_7O_{13}$, 920.4406).

Largamide C (3): colorless, amorphous solid; $[\alpha]^{20}_D$ –46 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.27), 280 (2.97) nm; IR (film) ν_{max} 3318 (br), 2967, 2924, 1725, 1662, 1641, 1597 (br), 1465 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 2; HR-ESI/APCI-MS *m/z* $[M + H]^+$ 934.4559 (calcd for $C_{47}H_{64}N_7O_{13}$, 934.4562).

Absolute Configuration

Compounds **1**, **2**, and **3** (0.1 mg each) were treated with 6 N HCl (0.3 mL) and heated at 110 ºC for 24 h. The hydrolyzates were evaporated to dryness and the residue resuspended in H_2O (100 μL) and then subjected to chiral HPLC analysis (Phenomenex, Chirex 3126 *N*,S-dioctyl-(D)-penicillamine, 250 mm × 4.60 mm, 5 μm; solvent, 2 mM $CuSO_4$–MeCN, 95:5); UV detection 254 nm; flow rate 1.0 mL/min). The retention times of amino acids in the hydrolyzates were compared with those of authentic standards. The retention times for the standards were as follows (t_R, min): L-Ala (6.8), D-Ala (8.5), L-Thr (7.2), L-*allo*-Thr (9.4), D-Thr (8.0), D-*allo*-Thr (9.4), L-Val (17.2), D-Val (22.2), L-Tyr (53.0), D-Tyr (60.5), L-Leu (47.0), D-Leu (58.0), L-Glu (53.0), and D-Glu (57.0). The hydrolyzates of **1**–**3** yielded peaks corresponding to L-Ala (6.8) and L-Thr (7.2), L-Val (17.2), D-Glu (57.0) and D-Tyr (60.5). The hydrolyzate of compound **1** also generated an additional peak (t_R 47.0 min), thus verifying the presence of L-Leu.

Compounds **2** and **3** (0.1 mg each) were each dissolved in 3 mL of MeOH and ozone was bubbled through the solution at 25 °C for 30 min. The solvent was evaporated, and the products were suspended in H_2O_2–HCOOH (1:2) and heated for 20 min at 70 oC. Then the mixtures were concentrated to dryness and subjected to acid hydrolysis and chiral HPLC analysis as described above, confirming the presence of L-Ala, L-Val, L-Thr, and D-Glu in both compounds. An additional peak for D-Asp due to ozonolysis (t_R 35.5 min; solvent, 2 mM $CuSO_4$–MeCN, 95:5) must have originated from Tyr, indicating D configuration of Tyr in the parent molecules (t_R of L-Asp standard, 28.0 min). Furthermore, L-2-aminoadipic acid (t_R 58.5 min; solvent, 2 mM $CuSO_4$–MeCN, 95:5) and L-2-aminopimelic acid (t_R 41.0 min; solvent, 2 mM $CuSO_4$–MeCN, 90:10) were detected in the degradation product mixtures of compounds **2** and **3**, respectively. The corresponding D-isomers were not present. Retention times of standards (t_R, min) using the matching solvent systems: D-2-aminoadipic acid (84.0), D-2-aminopimelic acid (53.0).

Serine Protease Inhibition Assays

Compounds **1**–**3** dissolved in DMSO were tested for serine protease-inhibitory activity against porcine pancreatic elastase, α-chymotrypsin from bovine pancreas, or trypsin from porcine pancreas, in the presence of chromogenic substrates (*N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide for elastase, *N*-succinyl-Gly-Gly-Phe-*p*-nitroanilide for chymotrypsin, *N*-α-benzoyl-DL-arginine 4-nitroanilide hydrochloride for trypsin). Activity was determined [3–5] by measuring

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

the increase in absorbance at 405 nm over 30 min in a microplate reader using phenylmethylsulfonyl fluoride (PMSF) and lyngbyastatin 4 (purity ≥ 95%)[3] as positive controls. All assays were performed in quadruplicate and the data were expressed as mean ± S.D. and IC_{50} values calculated.

Results

The organic extract of *Lyngbya confervoides* collected near Ft. Lauderdale (Florida, USA) was subjected to HP-20 chromatography, crude reversed-phase fractionation and two HPLC purification steps to yield compounds **1**–**3** as colorless, amorphous solids. The planar structures of **1**–**3** (Figure 1) were determined by a combination of NMR (^{1}H, ^{13}C, COSY, 1D TOCSY, ROESY, HSQC, and HMBC) spectroscopic analysis and mass spectrometry as described below.

Compound **1** gave a $[M + H]^+$ peak at *m/z* 842.4303 in the HR-ESI/APCI-MS, which, combined with ^{13}C NMR data, suggested a molecular formula of $C_{41}H_{59}N_7O_{12}$. A detailed ^{1}H NMR, COSY, HSQC and HMBC analysis in DMF-d_7 (Table 1) indicated that compound **1** was a heptapeptide, consisting of six regular amino acid units (alanine, valine, glutamic acid, tyrosine, threonine, leucine) and one modified amino acid moiety, 2-amino-2-butenoic acid (Abu). Remaining ^{1}H NMR signals for two deshielded methyl groups (δ_H 1.80 and 1.71) and one olefinic methine proton (δ_H 6.42) could be rationalized by an additional carboxylic acid residue, 2-methyl-2-butenoic acid, supported by COSY and HMBC data. In particular, signal multiplicity (qq) and coupling constants (7.0 and 1.0 Hz) for the olefinic methine proton H-3 (δ_H 6.42) were indicative of vicinal and allylic coupling to two methyl groups. Lack of a ROESY correlation between both the 2-methyl group resonating at δ_H 1.80 and the methine resonating at δ_H 6.42 within this carboxylic acid unit in combination with a weak ROESY correlations between both methyl groups (δ_H 1.71, 1.80) indicated *E* configuration and thus the trivial name tiglic acid (Tig) for this unit. The geometry of the Abu unit was deduced as Z based on an NOE between the Abu NH (δ_H 10.16) and Abu methyl group (δ_H 1.76). HMBC supported by ROESY correlations unambiguously established the linear sequence of the amino acid units and tiglic acid moiety. The cyclic depsipeptide core was proposed due to the low-field shift of the Thr β proton (δ_H 5.37) indicative of an ester functionality which involved the C-terminal leucine moiety, consistent with weak ROESY correlations between both units (Thr $\delta_{H\text{-}2}$ 4.74 and Leu $\delta_{H\text{-}2}$ 4.67). Furthermore, an absorption at ν_{max} 1725 cm^{-1} characteristic of an ester carbonyl stretching vibration was observed in the IR spectrum of **1** in addition to the strong amide stretching bands for peptides (ν_{max} 1659 cm^{-1}), supporting the proposed planar structure.

An $[M + H]^+$ peak at *m/z* 920.4404 in the HR-ESI/APCI-MS of **2** in conjunction with ^{13}C NMR data suggested a molecular formula of $C_{46}H_{61}N_7O_{13}$ for compound **2**. The ^{1}H NMR spectrum indicated that compound **2** is closely related to **1**. Further NMR analysis (^{1}H, COSY, HSQC, HMBC, ROESY) as carried out for **1** and additional 1D TOCSY experiments in DMF-d_7 (Figure 2) revealed the presence of eight spin systems as for **1**, except that the leucine moiety was replaced by a 2-amino-5-(4′-hydroxy-phenyl)pentanoic acid (Ahppa) residue (Table 2).

The ^{1}H NMR spectrum of **3** was virtually identical to the one of **2**. HR-ESI/APCI-MS analysis for compound **3** provided an $[M + H]^+$ peak at *m/z* 934.4559, suggesting a molecular formula of $C_{47}H_{63}N_7O_{13}$ and thus that compound **3** differs from **2** only by the presence of an additional methylene group. Closer inspection of NMR data revealed that the Ahppa unit was homologated to a 2-amino-5-(4′-hydroxy-phenyl)hexanoic acid (Ahpha) unit in **3** (Table 2), in agreement with the molecular formula requirements. HMBC analysis verified the units' connectivity and the proposed planar structure.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The absolute configuration of all amino acid units in compounds **1**–**3** was deduced by chiral HPLC analysis of the acid hydrolysis products, revealing D configuration for glutamic acid and tyrosine and L configuration for all other usual amino acids. For compounds **2** and **3**, additionally, acid hydrolysis was preceded by ozonolysis with oxidative work-up, thereby allowing us to detect 2-aminoadipic acid and 2-aminopimelic acid instead of Ahppa and Ahpha, respectively, to take advantage of commercially available standards. This analysis confirmed that L-Leu in **1** was replaced by L-Ahppa in **2** and L-Ahpha in **3**.

Compounds **1**–**3** were tested for serine protease-inhibitory activity. They showed moderate activity against porcine pancreatic elastase activity *in vitro* in a dose-dependent manner with IC_{50} values of 1.41 ± 0.28, 0.53 ± 0.19, and 1.15± 0.46 μM, respectively. To largely eliminate the possibility that the elastase activity is due to trace amounts of the potent inhibitors lyngbyastatins 4, 5, or 6, [3,4] compounds **1**–**3** were repeatedly purified by HPLC without loss of activity. Compounds **1**–**3** were inactive against trypsin and chymotrypsin (tested up to 50 μM). They also did not affect the viability of any of the cancer cell lines tested (HT29, U2OS, IMR-32) for growth inhibitory activity.

Discussion

A tiglic acid moiety present as a key feature in **1**–**3** had not been previously described in a cyanobacterial metabolite; however, it has been found in the bacterial metabolite cytosaminomycin D from *Streptomyces* sp. KO-8119 [9]. It is commonly encountered in higher plants [10] where it is presumably biosynthesized from isoleucine [11]. However, compounds **1**–**3** differ from largamides A–C, recently isolated from an *Oscillatoria* sp. from Key Largo (Florida), only by the presence of the tiglic acid instead of the senecioic acid residue [6]. The physical description of the largamide-producing cyanobacterium closely matches the morphology and appearance of the organism we have been investigating. Close inspection and comparison of NMR data in DMF-d_7, DMSO-d_6 and CD_3OD (Supporting Information) with those reported for largamides A–C in CD_3OD and DMSO-d_6 [6] suggested that the senecioic acid residue in these compounds was assigned incorrectly, that compounds **1**–**3** and largamides A–C are identical [12] and that DMF-d_7 was a superior solvent since there was no signal overlap in the low-field region of the ^{1}H NMR spectra. Furthermore, our collections yielded a common set of largamides (D–H) [6], while lyngbyastatins 4–6 and pompanopeptins A and B may be unique to our samples from the Ft. Lauderdale area [3–5]. This high secondary metabolite content of a single organism demonstrates the enormous biosynthetic potential of cyanobacteria.

The elastase-inhibitory activity of largamides A–C (**1**–**3**) is inferior to the activity of lyngbyastatins 4–6, which bear the 3-amino-6-hydroxy-2-piperidone (Ahp) residue critical for their serine protease-inhibitory activity [3–5]. Yet the largamide A–C core structure represents a new structural scaffold for elastase inhibition. Since numerous fatty acids have been shown to inhibit elastase [13], it is possible that the carboxylic acid moiety of the glutamic acid residue in largamides A–C (**1**–**3**) may play a role.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was funded by the Florida Sea Grant College Program with support from NOAA, Office of Sea Grant, U.S Department of Commerce, Grant No. NA06OAR4170014; and NIGMS grant P41GM086210. We thank L. Fisher and K. Banks (Broward County Environmental Protection Department) and C. Ross, A. Capper, and R. Ritson-Williams (Smithsonian Marine Station) for assistance with collections and W. Yoshida (University of Hawaii) for providing

NMR data. We also thank C. Bewley for helpful discussions and comparison with authentic largamides A–C from *Oscillatoria* sp. from Key Largo. Mass spectral analyses were performed at the UCR Mass Spectrometry Facility, Department of Chemistry, University of California at Riverside. This is contribution # 759 from the Smithsonian Marine Station at Fort Pierce.

References

1. Gerwick WH, Tan LT, Sitachitta N. Nitrogen-containing metabolites from marine cyanobacteria. Alkaloids Chem Biol 2001;57:75–184. [PubMed: 11705123]
2. Welker M, von Döhren H. Cyanobacterial peptides – nature's own combinatorial biosynthesis. FEMS Microbiol Rev 2006;30:530–563. [PubMed: 16774586]
3. Matthew S, Ross C, Rocca JR, Paul VJ, Luesch H. Lyngbyastatin 4, a dolastatin 13 analogue with elastase and chymotrypsin inhibitory activity from the marine cyanobacterium *Lyngbya confervoides*. J Nat Prod 2007;70:124–127. [PubMed: 17253864]
4. Taori K, Matthew S, Rocca JR, Paul VJ, Luesch H. Lyngbyastatins 5–7, potent elastase inhibitors from Floridian marine cyanobacteria, *Lyngbya* spp. J Nat Prod 2007;70:1593–1600. [PubMed: 17910513]
5. Matthew S, Ross C, Paul VJ, Luesch H. Pompanopeptins A and B, new cyclic peptides from the marine cyanobacterium *Lyngbya confervoides*. Tetrahedron 2008;64:4081–4089.
6. Plaza A, Bewley CA. Largamides A–H, Unusual cyclic peptides from the marine cyanobacterium *Oscillatoria* sp. J Org Chem 2006;71:6898–6907. [PubMed: 16930043]
7. Houghton AM, Quintero PA, Perkins DL, Kobayashi DK, Kelley DG, Marconcini LA, et al. Elastin fragments drive disease progression in a murine model of emphysema. J Clin Invest 2006;116:753–759. [PubMed: 16470245]
8. Paul VJ, Thacker RW, Banks K, Golubic S. Benthic cyanobacterial bloom impacts the reefs of South Florida (Broward County, USA). Coral Reefs 2005;24:693–697.
9. Shiomi K, Haneda K, Tomoda H, Iwai Y, Omura S. Cytosaminomycins, new anticoccidial agents produced by *Streptomyces* sp. KO-8119. II. Structure elucidation of cytosaminomycins A, B, C and D. J Antibiot (Tokyo) 1994;47:782–786. [PubMed: 8071123]
10. Mann, J. Secondary Metabolism. Vol. 2. Oxford: Clarendon Press; 1987. p. 95-172.
11. Rhee SW, Leete E, McGaw BA. Stereospecificity of enzymic dehydrogenation during tiglate biosynthesis. J Am Chem Soc 1980;102:7344–7348.
12. Bewley C. Personal communication.
13. Rennert B, Melzig MF. Free fatty acids inhibit the activity of *Clostridium histolyticum* collagenase and human neutrophil elastase. Planta Med 2002;68:767–769. [PubMed: 12357383]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

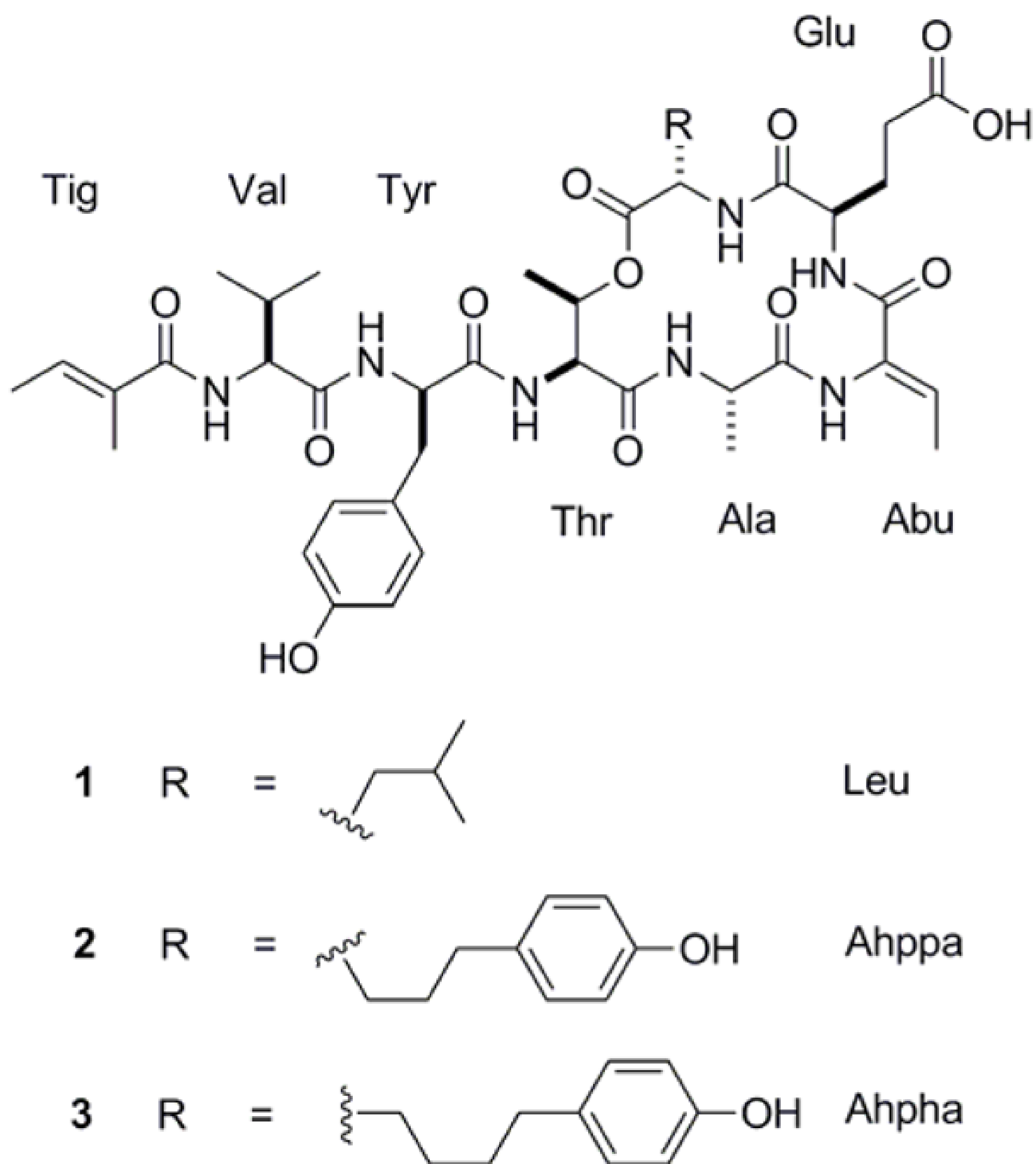

Fig. 1.
Structures of largamides A–C (**1**–**3**)

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

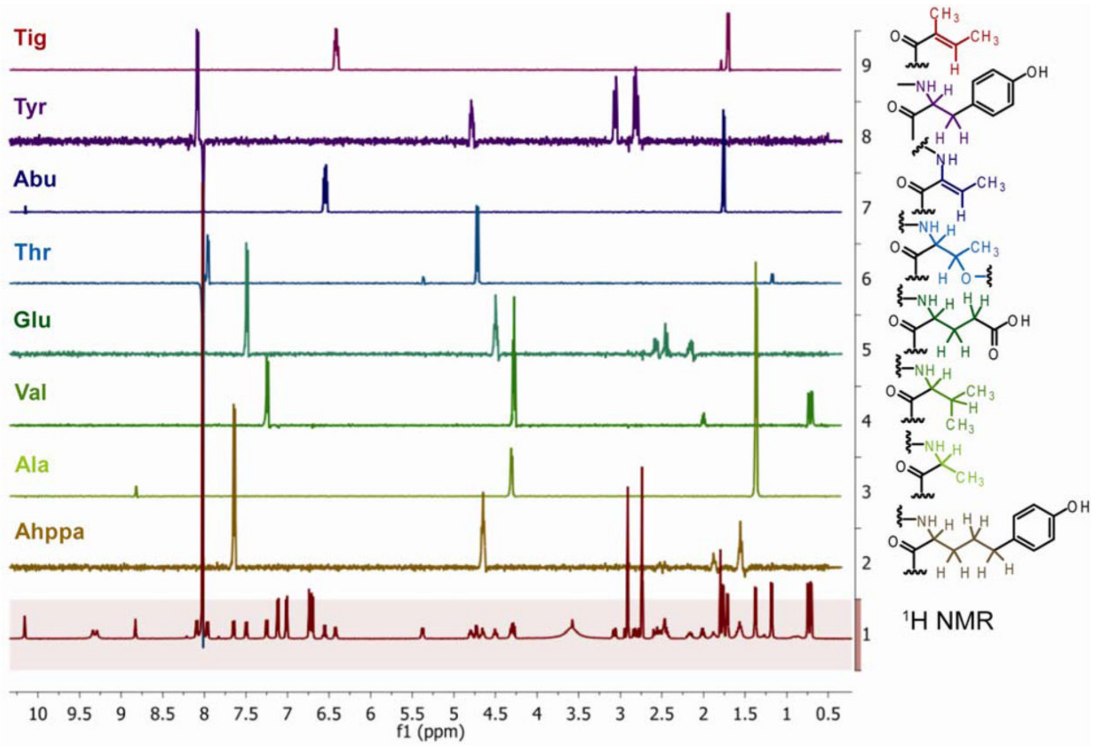

Fig. 2.
^{1}H NMR (front) and 1D TOCSY spectra of largamide B (**2**) in DMF-d_7.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 1

NMR data for largamide A (**1**) in DMF-d_7 (500 MHz)

Unit	C/H no.	δ_H (*J* in Hz)	δ_C, mult.	HMBC[a]
Leu	1		172.0, qC	
	2	4.67, ddd (10.0, 9.5, 4.5)	49.8, CH	
	3	1.70, m	40.9, CH_2	2, 4
		1.49, m		
	4	1.58, m	24.8, CH	
	5	0.86, d (6.5)	23.6, CH_3	3, 4, 6
	6	0.89, d (6.5)	21.7, CH_3	3, 4, 5
	NH	7.62, d (9.5)		1 (Glu)
Glu	1		171.6, qC	
	2	4.51, ddd (9.5, 9.0, 4.5)	53.0, CH	1, 4
	3	2.53, m	27.0, CH_2	5
		2.14, m		
	4	2.54, m	31.1, CH_2	5
		2.42, m		
	5		175.2, qC	
	OH	not observed		
	NH	7.54, d (9.0)		1 (Abu)
Abu	1		164.0, qC	
	2		130.9, qC	
	3	6.54, q (7.0)	129.1, CH	1
	4	1.76, d (7.0)	12.7, CH_3	1, 2, 3
	NH	10.16, s		1 (Ala)
Ala	1		175.8, qC	
	2	4.33, qd (6.5, 2.5)	50.7, CH	1, 1 (Thr)
	3	1.38, d (6.5)	16.8, CH_3	1, 2
	NH	8.83, d (2.5)		1 (Thr)
Thr	1		170.3, qC	
	2	4.74, dd (8.5, 3.0)	55.8, CH	1, 1 (Tyr)
	3	5.37, qd (6.3, 3.0)	72.9, CH	4
	4	1.18, d (6.3)	16.1, CH_3	2, 3
	NH	7.90, d (8.5)		1 (Tyr)
Tyr	1		172.7, qC	
	2	4.78, ddd (10.0, 9.5, 4.5)	55.9, CH	1, 4
	3	3.07, dd (−14.0, 4.5)	38.1, CH_2	2, 4, 5/9
		2.82, dd (−14.0, 10.0)		
	4		128.6, qC	
	5/9	7.12, d (8.5)	131.1, CH	7
	6/8	6.70, d (8.5)	115.6, CH	7
	7		157.1, qC	
	OH	9.34, s		7

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Unit	**C/H no.**	**δ_H (*J* in Hz)**	**δ_C, mult.**	**HMBC[a]**
	NH	8.10, br		1 (Val)
Val	1		171.9, qC	
	2	4.29, dd (9.0, 7.0)	59.3, CH	1, 3, 4, 5
	3	2.01, m	31.6, CH	2, 4, 5
	4	0.75, d (6.5)	19.7, CH_3	2, 3
	5	0.72, d (6.5)	18.4, CH_3	2, 3
	NH	7.26, brd (7.0)		1 (Tig)
Tig	1		169.3, qC	
	2		132.7, qC	
	3	6.42, qq (7.0, 1.0)	130.4, CH	1
	4	1.71, br d (7.0)	13.8, CH_3	2, 3
	5	1.80, br s	12.5, CH_3	1, 2, 3

[a]Protons showing long-range correlation with indicated carbon.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 2

NMR data for largamides B (**2**) and C (**3**) in DMF-d_7 (500 MHz)

		Largamide B (2)			Largamide C (3)		
Unit	**C/H no.**	**δ_H (*J* in Hz)**	**δ_C, mult.**	**HMBC[a]**	**δ_H (*J* in Hz)**	**δ_C, mult.**	**HMBC[a]**
Ahppa[b]/Ahpha[c]	1		171.7, qC			171.7, qC	
	2	4.65, ddd (9.5, 9.5, 5.5)	51.1, CH	1, 3, 4	4.61, ddd (9.5, 9.0, 5.0)	51.2, CH	1, 3, 4, 4a
	3	1.88, m 1.56, m	31.2, CH_2	2, 4, 5	1.91, m 1.57, m	31.4, CH_2	5
	4	1.60, m 1.58, m	27.9, CH_2	2, 3, 5, 6	1.36, m	25.6, CH_2	
	4a[c]	–	–		1.47, m 1.53, m	32.0, CH_2	2, 3, 5
	5	2.56, m 2.48, m	34.7, CH_2	3, 4, 6, 7	2.43, m	35.1, CH_2	4, 4a, 6, 7/11
	6		133.1, qC			133.4, qC	
	7/11	7.01, d (8.5)	129.9, CH	5, 6, 8/10, 9	7.01, d (8.5)	129.7, CH	5, 8/10, 9
	8/10	6.73, d (8.5)	115.7, CH	6, 7/11, 9	6.74, d (8.5)	115.6, CH	6, 9
	9		156.5, qC			156.4, qC	
	OH	9.29, s[d]			9.28, s		8/10, 9
	NH	7.65, d (9.5)		1, 1 (Glu)	7.64, d (9.5)		
Glu	1		171.6, qC			171.6, qC	
	2	4.50, ddd (10.0, 9.0, 5.0)	53.2, CH	1, 3, 4, 1 (Abu)	4.52, ddd (10.0, 8.5, 4.5)	53.1, CH	1, 3, 4, 1 (Abu)
	3	2.51, m 2.18, m	27.0, CH_2	2, 4, 5	2.50, m 2.17, m	27.0, CH_2	1, 2, 4, 5
	4	2.59, m 2.47, m	31.0, CH_2	2, 3, 5	2.57, m 2.46, m	31.0, CH_2	2, 3, 5
	5		175.1, qC			175.1, qC	
	OH	12.51, br s			not observed		
	NH	7.50, d (9.0)		1 (Abu)	7.51, d (8.5)		2, 1 (Abu)
Abu	1		164.1, qC			164.0, qC	
	2		131.1, qC			130.9, qC	
	3	6.55, qd (7.0, 1.0)	129.2, CH	1, 4	6.56, qd (7.0, 1.5)	129.2, CH	1, 4
	4	1.77, d (7.0)	12.7, CH_3	1, 2, 3	1.76, d (7.0)	12.5, CH_3	1, 2, 3

Unit	C/H no.	Largamide B (2)			Largamide C (3)		
		δ_H (*J* in Hz)	δ_C, mult.	HMBC[a]	δ_H (*J* in Hz)	δ_C, mult.	HMBC[a]
	NH	10.16, s		1, 1 (Ala)	10.16, s		1, 1 (Ala)
Ala	1		175.8, qC			175.7, qC	
	2	4.30, qd (7.0, 2.5)	50.7, CH	1, 3	4.32, qd (7.0, 2.5)	50.6, CH	1, 3
	3	1.37, d (7.0)	16.8, CH_3	1, 2	1.38, d (7.0)	16.7, CH_3	1, 2
	NH	8.83, d (2.5)		1, 2, 3	8.85, d (2.5)		1, 2, 3
Thr	1		170.4, qC			170.3, qC	
	2	4.73, dd (8.5, 2.5)	56.0, CH	1, 1 (Tyr)	4.73, dd (8.5, 3.0)	55.8, CH	1, 1 (Tyr)
	3	5.37, qd (6.5, 2.5)	73.4, CH	1, 4, 1 (Ahppa)	5.38, qd (6.5, 3.0)	73.3, CH	1, 1 (Ahpha)
	4	1.18, d (6.5)	16.2, CH_3	2, 3	1.18, d (6.5)	16.1, CH_3	2, 3
	NH	7.96, d (8.5)		2, 1(Tyr)	7.91, d (8.5)		2, 1 (Tyr)
Tyr	1		172.8, qC			172.7, qC	
	2	4.79, ddd (10.0, 8.5, 4.5)	55.5, CH	1, 3, 4, 1 (Val)	4.78, ddd (10.0, 8.0, 4.5)	55.5, CH	1, 4, 1 (Val)
	3	3.07, dd (–14.0, 4.5) 2.82, dd (–14.0, 10.0)	38.2, CH_2	1, 2, 4, 5/9	3.07, dd (–14.0, 4.5) 2.82, dd (–14.0, 10.0)	38.0, CH_2	1, 2, 4, 5/9
	4		128.6, qC			128.5, qC	
	5/9	7.11, d (8.5)	131.0, CH	6/8, 7	7.11, d (8.5)	131.0, CH	3, 6/8, 7
	6/8	6.70, d (8.5)	115.6, CH	5/9, 7	6.70, d (8.5)	115.6, CH	5/9, 7
	7		157.1, qC			157.0, qC	
	OH	9.34, s[d]			9.35, s		6/8, 7
	NH	8.09, d (8.5)		1, 2, 1 (Val)	8.08, d (8.0)		1, 1 (Val)
Val	1		172.0, qC			172.0, qC	
	2	4.28, dd (8.5, 7.0)	59.2, CH	1, 3, 4, 5, 1 (Tig)	4.28, dd (8.5, 7.0)	59.2, CH	1, 3, 4, 5, 1(Tig)
	3	2.01, m	31.6, CH	1, 2, 4, 5	2.01, m	31.5, CH	1, 2, 4, 5
	4	0.74, d (7.0)	19.8, CH_3	2, 3, 5	0.74, d (7.0)	19.6, CH_3	2, 3, 5
	5	0.71, d (7.0)	18.3, CH_3	2, 3, 4	0.72, d (7.0)	18.2, CH_3	2, 3, 4
	NH	7.25, d (8.5)		1, 2, 1 (Tig)	7.26, d (8.5)		1, 2, 1 (Tig)
Tig	1		169.3, qC			169.3, qC	
	2		132.7, qC			132.6, qC	

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Unit	C/H no.	Largamide B (2) δ_H (J in Hz)	Largamide B (2) δ_C, mult.	Largamide B (2) HMBC[a]	Largamide C (3) δ_H (J in Hz)	Largamide C (3) δ_C, mult.	Largamide C (3) HMBC[a]
	3	6.42, qq (6.7, 1.5)	130.4, CH	1, 4, 5	6.42, qd (7.0, 1.0)	130.4, CH	1, 4, 5
	4	1.71, dq (6.7, 1.5)	13.8, CH_3	2, 3	1.71, dq (7.0, 1.0)	13.7, CH_3	2, 3
	5	1.79, br s	12.6, CH_3	1, 2, 3	1.80, br s	12.4, CH_3	1, 2, 3

[a]Protons showing long-range correlation with indicated carbon.

[b]Refers to compound **2**.

[c]Refers to compound **3**.

[d]No HMBC observed. Assigned by comparison of data for **2** with data for **1** and **3**.