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Turnagainolides A and B, Cyclic Depsipeptides Produced in Culture by a *Bacillus* sp.: Isolation, Structure Elucidation, and Synthesis

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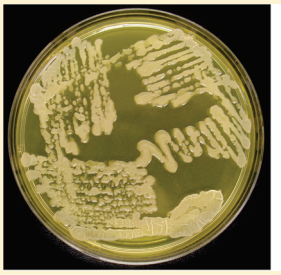
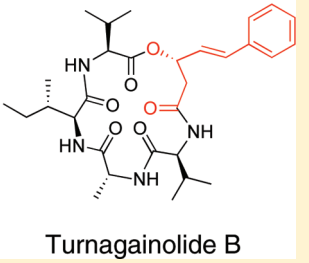
Supporting Information

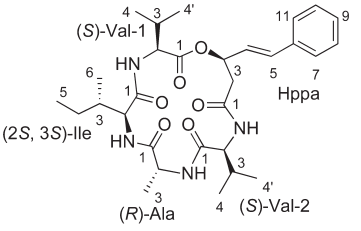
ABSTRACT: Two new cyclic depsipeptides, turnagainolides A (**1**) and B (**2**), have been isolated from laboratory cultures of a marine isolate of *Bacillus* sp. The structures of **1** and **2**, which are simply epimers at the site of macrolactonization, were elucidated by analysis of NMR data and chemical degradation. A total synthesis of the turnagainolides confirmed their structures. Turnagainolide B (**2**) showed activity in a SHIP1 activation assay.



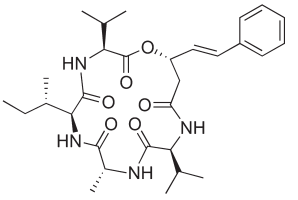


The phosphatidylinositol-3-kinase (PI3K) signaling pathway is a crucial regulator of many important cellular processes, including growth, proliferation, survival, and motility.[1−3] A key second messenger in the pathway is phosphatidylinositol-3,4,5-triphosphate (PI-3,4,5-P_3), which is present in low amounts in unstimulated cells but is rapidly formed from PI-4,5-P_2 by phosphatidylinositol-3-kinase in response to diverse extracellular stimuli. The cellular concentration of PI-3,4,5-P_3 is tightly controlled in normal cells by a balance between the activities of PI3K and the lipid phosphatase PTEN, which converts PI-3,4,5-P_3 back to PI-4,5-P_2. Aberrant activation of the phosphoinositide pathway by mutations leading to upregulated PI3K activity or loss of PTEN function contributes to inflammatory diseases and cancers.[1−3] The acknowledged role of unregulated PI3K signaling in disease has made the pathway a high-value target for small-molecule therapeutic intervention. To date, much of the drug discovery effort aimed at modulating PI3K signaling has focused on finding potent and isoform-selective PI3K inhibitors that would prevent the formation of the second messenger PI-3,4,5-P_3.[4] An alternate approach to dampening aberrant PI3K signaling would be to activate the inositol 5-phosphatase SHIP1, a negative regulator of the pathway found only in hematopoietic cells, which turns the signal off by converting the second messenger PI-3,4,5-P_3 to PI-3,4-P_2.[5] If this alternate approach is viable, small-molecule activators of SHIP1 should have potential as drugs for the treatment of inflammatory disorders and cancers of the blood.

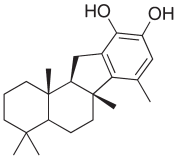
Several years ago we discovered that the sponge meroterpenoid pelorol was an in vitro activator of SHIP1.[6] Pelorol was the first small molecule, other than the endogenous activator PI-3,4-P_2, known to activate SHIP1. Synthetic pelorol analogues inhibited PI3K signaling in stimulated mast cells and macrophages, showed in vivo anti-inflammatory activity in standard mouse models of ear edema, and showed in vitro cytotoxicity toward multiple myeloma cells, providing proof of principle support for the use of SHIP1 activators as anti-inflammatory and anticancer drugs.[5,7] More recently, we reported that the soft coral diterpenoid australin E was also an in vitro activator of SHIP1.[8]



1 Turnagainolide A



2 Turnagainolide B



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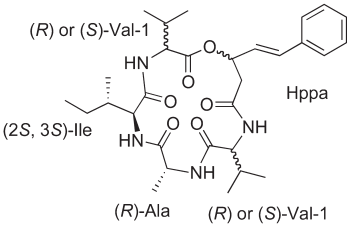

18 EGM-556

As part of an ongoing program aimed at discovering new marine natural products that modulate PI3K signaling,[6,8,9] we

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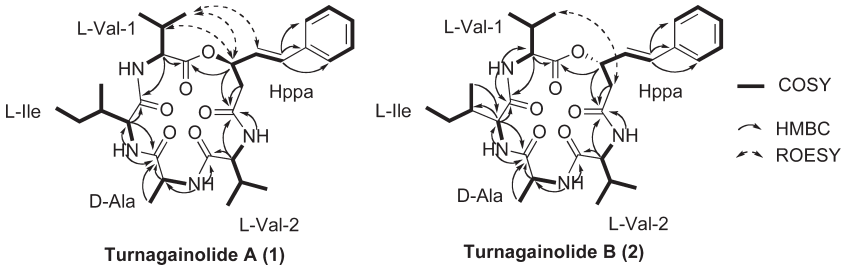


Figure 1. Selected 2D NMR correlations observed for turnagainolides A (**1**) and B (**2**).

found that extracts of a laboratory culture of a marine isolate of the bacterium *Bacillus* sp. were active in a kinetic enzyme assay for SHIP1 activation. Assay-guided fractionation of the extract led to the identification of the two epimeric cyclic depsipeptides turnagainolides A (**1**) and B (**2**). Turnagainolide B (**2**) was active in the SHIP1 activation assay, while the epimer turnagainolide A (**1**) was inactive. The turnagainolides have a rare β-hydroxy carboxylic acid-containing moiety of mixed biogenetic origin, and they are notable because this residue is found with opposite configurations in the two naturally occurring cyclic depsipeptides. Details of the isolation, structure elucidation, and synthesis of turnagainolides A (**1**) and B (**2**) are described below.

RESULTS AND DISCUSSION

Microbial strain RJA2194 was isolated from a sediment sample collected at a depth of 100 m using a grab sampler near Turnagain Island in Howe Sound, British Columbia, and it was identified as a *Bacillus* sp. by 16S RNA analysis. Laboratory cultures of RJA2194 were grown as lawns on solid agar that were harvested by lyophilizing the entire culture, cells and agar, and extracting the dry residue exhaustively with MeOH. The combined MeOH extracts were concentrated in vacuo to give a gum, which was partitioned between EtOAc and H_2O. Fractionation of the EtOAc-soluble portion by sequential application of HP20 chromatography and normal-phase Si gel flash chromatography gave pure samples of turnagainolides A (**1**) and B (**2**).

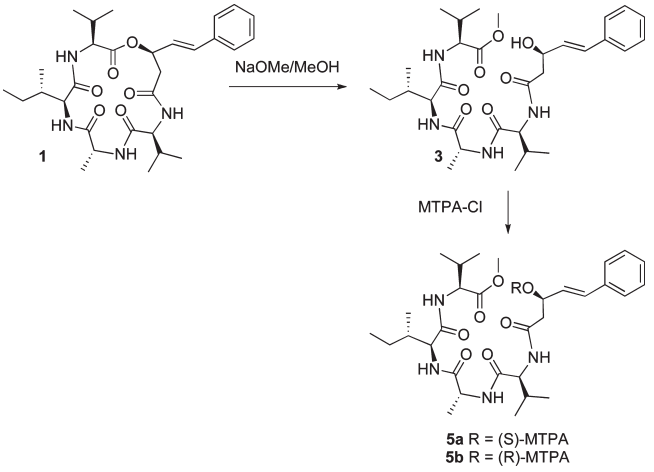
Turnagainolide A (**1**) was isolated as an optically active, white powder that gave a $[M + Na]^+$ ion at m/z 579.3152 in the HRESIMS spectrum, consistent with a molecular formula of $C_{30}H_{44}N_4O_6$, requiring 11 sites of unsaturation. The ^{13}C NMR spectrum of **1** contained only 28 resolved resonances, suggesting that there were elements of symmetry in the molecule. COSY, HSQC, and HMBC data showed that ^{13}C NMR resonances at δ 135.7 (Hppa-C-6), 126.5 (Hppa-C-7/11), 128.7 (Hppa-C8/10), and 128.2 (Hppa-C9) could be assigned to a monosubstituted phenyl ring, which accounted for the symmetry required by the ^{13}C NMR resonance count and four of the sites of unsaturation. A series of ^{13}C resonances at δ 168.6 (Hppa-C-1), 168.7 (Val1-C-1), 170.3 (Ile-C-1), 172.2 (Val2-C-1), and 172.9 (Ala-C-1) were assigned to amide or ester carbonyls, and two resonances at δ 126.7 (Hppa-C-4) and 132.5 (Hppa-C-5) were assigned to olefinic methines, accounting for a total of six additional sites of unsaturation. The lack of ^{13}C NMR evidence for a further unsaturated functionality required that turnagainolide A (**1**) must contain one more ring.

In addition to the carbonyl resonances, the ^1H and ^{13}C NMR spectra of turnagainolide A contained resonances characteristic of amino acid α-methine and side-chain functionalities, suggesting the molecule contained a peptide component (Supporting Information). Consistent with this observation, detailed analysis of the COSY, HSQC, and HMBC data led to the routine identification of alanine, isoleucine, and two valine residues (Figure 1, Table 1). Subtraction of the atoms attributed to the four amino acids residues ($C_{19}H_{34}N_4O_4$) from the molecular formula of **1** ($C_{30}H_{44}N_4O_6$) showed that the remaining fragment of the molecule had an elemental composition of $C_{11}H_{10}O_2$. Elucidation of this fragment started with the observation of COSY correlations between a carbinol methine resonance at δ 5.49 (Hppa-H-3) and an olefinic methine resonance at δ 6.27 (Hppa-H-4) and a pair of geminal methylene proton resonances at δ 2.40 (Hppa-H-2) and 2.87 (Hppa-H-2′). These correlations demonstrated that the carbinol methine was flanked by olefinic and aliphatic methylene functionalities. The downfield chemical

Table 1. NMR Data for Turnagainolides A (1) and B (2) (600 MHz ^1H; 150 MHz ^{13}C, DMSO-d_6)

residue	position	**1** δ_C	**1** δ_H (J in Hz)	**2** δ_C	**2** δ_H (J in Hz)
Val-1	1	168.7		169.0	
	2	58.2	4.23, dd (9.6, 6.3)	59.7	4.05, dd (9.6, 8.4)
	3	28.4	2.24, m	29.0	2.42, m
	4	18.9	0.89, d (7.2)	19.6	1.95, d (6.6)
	4′	19.6	0.89, d (7.2)	19.4	0.94, d (7.2)
	NH		7.59, d (9.6)		7.86, d (8.4)
Ile	1	170.3		170.9	
	2	57.1	4.26, m	57.5	4.22, dd (8.4, 3.6)
	3	35.5	2.03, m	35.4	2.01, m
	4	23.5	1.24, m	23.6	1.30, m
			1.24, m		1.30, m
	5	11.9	0.80, t (7.2)	12.1	0.82, t (7.2)
	6	15.5	0.81, d (6.8)	15.7	0.85, d (6.0)
	NH		8.10, d (9.6)		8.22, d (8.4)
Ala	1	172.9		173.7	
	2	48.7	4.32, m	49.3	4.36, m
	3	16.3	1.18, d (6.6)	16.1	1.23, d (7.2)
	NH		8.56, d (5.4)		8.87, d (5.4)
Val-2	1	172.2		172.6	
	2	57.4	4.12, dd (8.4, 6.8)	57.0	4.19, t (8.3)
	3	29.8	1.95, m	30.6	1.80, m
	4	18.4	0.87, d (7.3)	18.8	0.87, d (6.6)
	4′	19.4	0.87, d (7.3)	19.1	0.88,d (7.2)
	NH		7.74, d (8.4)		7.08, d (8.3)
Hppa	1	168.6		167.7	
	2	40.1	2.87, dd (14.2, 11.4)	41.2	2.79, dd (13.8, 4.8)
			2.40, dd (14.2, 2.2)		2.31, dd (13.8, 3.6)
	3	73.0	5.49, m	71.0	5.63, dd (8.4, 4.2)
	4	126.7	6.27, dd (16.1, 7.1)	126.5	6.20, dd (16.2, 6.0)
	5	132.5	6.67, d (16.0)	129.3	6.32, d (16.2)
	6	135.7		136.4	
	7, 11	126.5	7.44, d (7.4)	126.6	7.46, d (7.4)
	8, 10	128.7	7.34, t (7.4)	128.4	7.29, t (7.4)
	9	128.2	7.27, t (7.4)	127.5	7.21, t (7.4)

Scheme 1



shift of the carbinol methine proton resonance (δ 5.49) and the absence of COSY correlations to an OH resonance suggested that the carbinol methine was attached to an acylated alcohol. An additional COSY correlation was observed between the olefinic methine at δ 6.27 and the second olefinic methine in the ^{1}H NMR spectrum at δ 6.67 (Hppa-H-5), which was in turn correlated in the HMBC spectrum to the aromatic carbon resonances at δ 135.7 (Hppa-C-6) and 126.5 (Hppa-C7/11), establishing a connection between the disubstituted olefin and the phenyl ring. HMBC correlations between the Hppa-H2 (δ 2.40) and Hppa-H-2′ (δ 2.87) resonances and a ^{13}C resonance at δ 168.6 (Hppa-C-1) indicated that the methylene was attached to a carbonyl. The 16 Hz scalar coupling observed between the two olefinic methine resonances established the *E* configuration for the olefin. Thus, the final residue in turnagainolide A (**1**) was identified as a derivative of (*E*)-3-hydroxy-5-phenylpent-4-enoic acid (Hppa).

The linear sequence of turnagainolide A (**1**) was established from ROESY and HMBC data. ROESY correlations between the Ala-NH (δ 8.56) and Val2-αH (δ 4.12) resonances, between the Ile-NH (δ 8.12) and Ala-αH (δ 4.32) resonances, and between the Val2-NH (δ 7.74) and Hppa-H-2′ (2.87) resonances identified the Hppa-CO/Val2-NH, Val2-CO/Ala-NH, and Ala-CO/Ile-NH amide bonds. An HMBC correlation between the Hppa-H-3 resonance at δ 5.49 and the Val1-CO resonance at δ 168.7 established an ester linkage between the C-3 carbinol methine in Hppa and Val1. Finally, the Ile residue had to be inserted between Val1 and Ala to give the macrocylic ring needed to satisfy the unsaturation number required by the molecular formula of **1**.

Hydrolysis of **1** in 6 N HCl followed by Marfey's analysis established the configurations of the amino acids in turnagainolide A as (*S*)-Val, (*R*)-Ala, and (2*S*,3*S*)-Ile.[10] NaOMe-catalyzed methanolysis of the ester linkage in the macrocycle gave the turnagainolide A seco-acid methyl ester **3** (Scheme 1), which was converted to the *S* and *R* Mosher esters **5a** and **5b**. Mosher ester analysis (Table 2 and Figure 2) established that the configuration at Hppa-C-3 in **3** was *R*, and therefore, the complete structure of turnagainolide A is as shown in **1**.[11]

Turnagainolide B (**2**) was also isolated as an optically active, white powder that gave a $[M + Na]^+$ ion at *m*/*z* 579.3151 in the HRESIMS spectrum, consistent with a molecular formula of $C_{30}H_{44}N_4O_6$, identical to the molecular formula of turnagainolide

Table 2. ^{1}H NMR Data for Hppa Residue of Compounds 3, 5a, and 5b (600 MHz, $CDCl_3$)

	position	**3** δ_H (*J* in Hz)	**5a** δ_H (*J* in Hz)	**5b** δ_H (*J* in Hz)
Hppa	2	2.47, m	2.84, m	2.80, m
	3	4.74, m	6.08, m	6.08, m
	4	6.24, dd (16.2, 6.0)	6.09, dd (14.4, 6.6)	6.26, dd (14.4,6.6)
	5	6.69, d (16.2)	6.65, d (14.4)	6.80, d, (14.4)

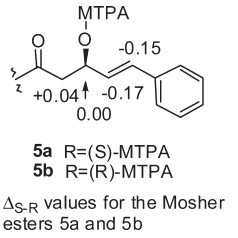
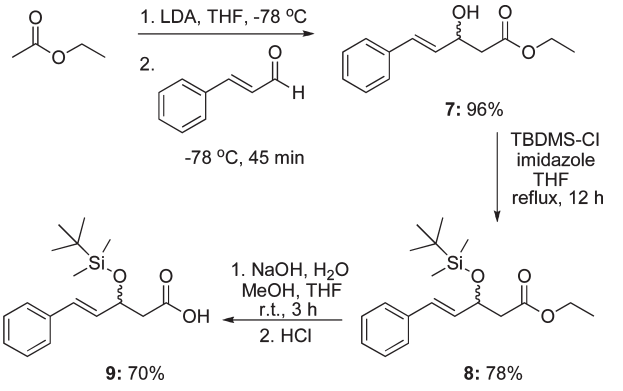


Figure 2. Mosher ester analysis of the Hppa residue in **5**.

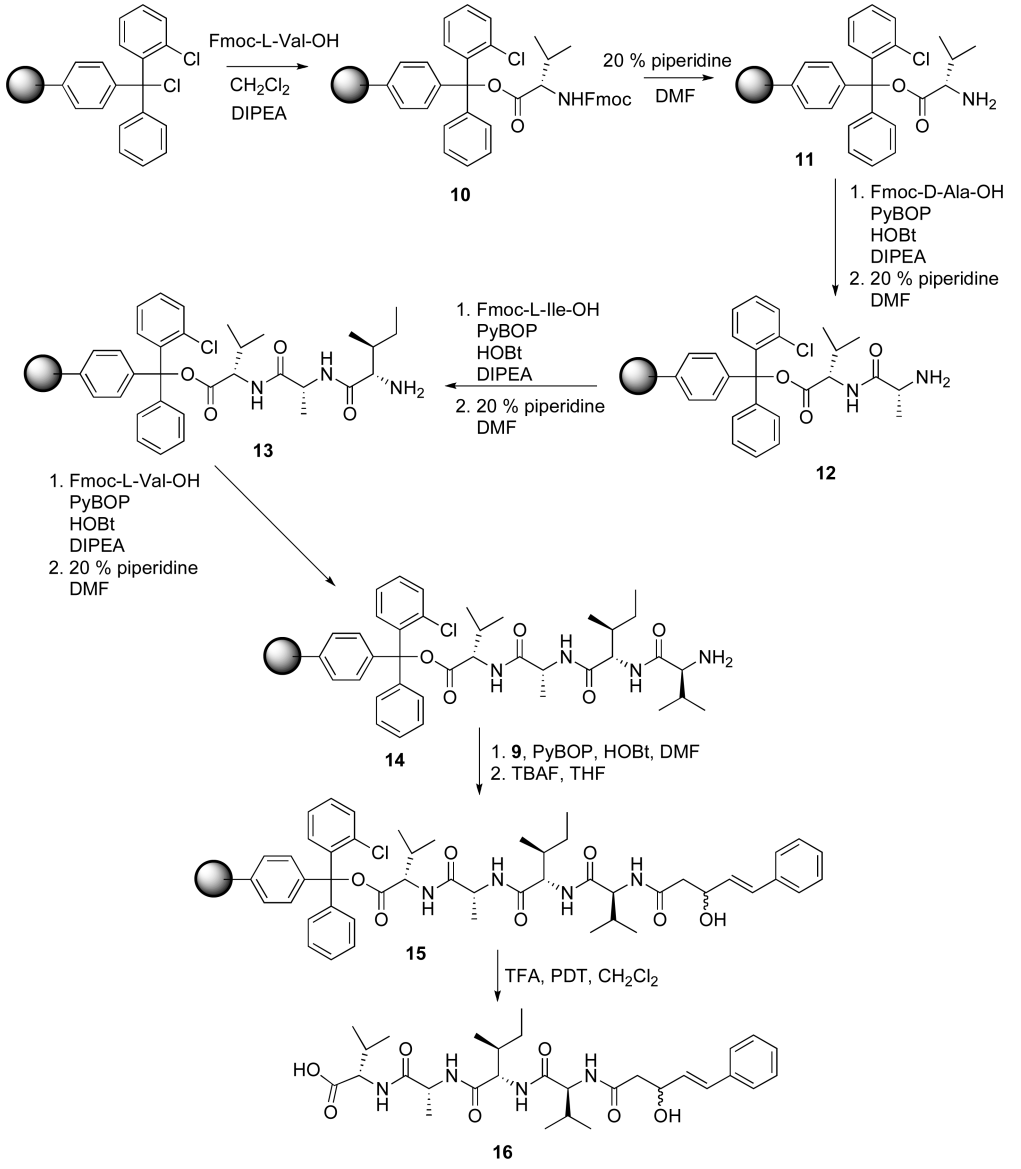
Scheme 2



A (**1**). Analysis of the 1D and 2D NMR data collected for **2** (Figure 1; Table 1) confirmed that it had the same constitution as **1**, and therefore, it had to be a stereoisomer of turnagainolide A. Comparison of the ^{1}H NMR chemical shifts observed for **2** with those observed for **1** (Table 1) showed that the biggest differences were in the Hppa, Val1, and Val2 residues, suggesting that the difference in configuration in **1** and **2** was likely located in these residues. Hydrolysis of **2** with 6 N HCl followed by Marfey's analysis[10] established the configurations of the amino acids in turnagainolide B as (*S*)-Val, (*R*)Ala, and (2*S*,3*S*)-Ile, the same as in turnagainolide A (**1**). Therefore, the difference in configuration had to be located in the Hppa residue, which led to the assignment of the absolute configuration at C-3 of the Hppa residue in **2** as *S*. This assignment was confirmed by total synthesis (vide infra).

We undertook the total synthesis of turnagainolides A (**1**) and B (**2**) in order to confirm their structures, particularly their epimeric relationship at Hppa-C-3. The synthesis started with preparation of the protected Hppa residue **9** as shown in Scheme 2. Base-catalyzed condensation of EtOAc with (*E*)-cinnamaldehyde gave the β-hydroxy ester **7** in high yield as a racemic mixture. Reaction of the alcohol **7** with TBDMS-Cl gave

Scheme 3



the TBDMS derivative **8**, which was hydrolyzed with aqueous NaOH to give the desired racemic acid **9**.

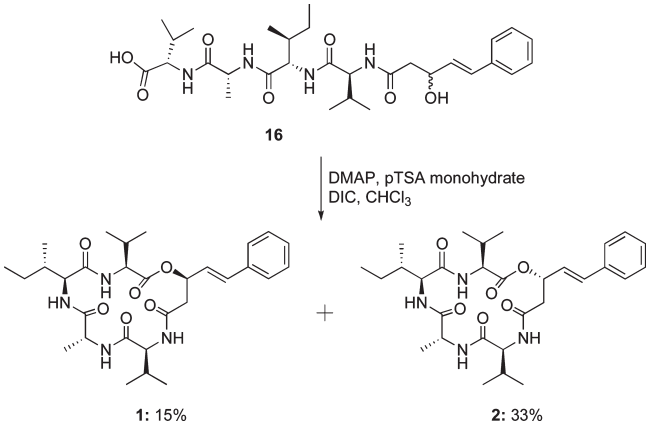
Standard solid-phase peptide synthetic methodology using a 2-chlorotrityl resin was employed to prepare the polymer-bound tetrapeptide resin-O-Val1-Ile-Ala-Val2-NH_2 (**14**) as shown in Scheme 3. PyBOP- and HOBt-catalyzed coupling of the protected Hppa fragment **9** with the resin-bound tetrapeptide **14**, followed by removal of the silyl protecting group with TBAF, gave the resin-bound turnagainolide seco acids **15**. Treatment of the resin-bound seco acids **15** with TFA gave the free seco acids **16**. Macrocyclization of the mixture of epimeric seco acids **16** was carried out in refluxing CH_2Cl_2 with DMAP and DIC catalysis to give a mixture of turnagainolide A (**1**) and turnagainolide B (**2**) in 15% and 33% yield, respectively, which was separated by Si gel chromatography and reversed-phase HPLC to give pure compounds (Scheme 4). It is interesting to note that the chemical macrocyclization gave turnagainolide B (**2**) as the major product (≈2:1), whereas turnagainolide A (**1**) was the major product isolated from the microbial cultures (≈8:1). The ^1H and ^{13}C NMR data recorded for the synthetic turnagainolides matched the data collected for the natural products (Supporting Information), confirming the structures assigned to the natural products. The synthetic turnagainolides **1** and **2** were each separately hydrolyzed using NaOMe to give the methylated seco acids **3** and **4**, respectively, which were subjected to Mosher's ester[11] analysis to confirm the assignments of the configurations at Hppa-C-3 as *R* for **1** and *S* for **2**.

Natural and synthetic samples of turnagainolide B (**2**) were active in the SHIP1 activation assay, while turnagainolide A (**1**) was inactive. The observed efficacy of turnagainolide B (**2**) was comparable to that found for the previously reported SHIP activator MN100 (**17**) (Figure 3).[5]

■ CONCLUSIONS

The two new cyclic depsipeptides turnagainolides A (**1**) and B (**2**) contain a mixture of *R* and *S* amino acids and the (*E*)-3-hydroxy-5-phenylpent-4-enoic acid fragment Hppa, which are

Scheme 4



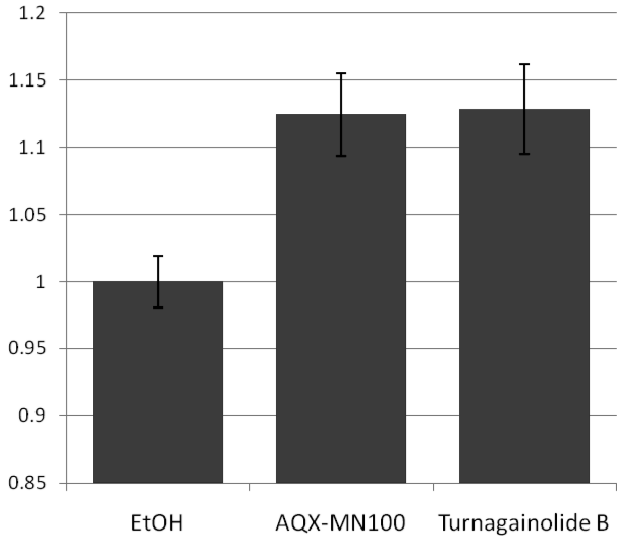


Figure 3. AQX-MN100 (**17**),[5] turnagainolide B (**2**), or EtOH control (10 μM each) was incubated with SHIP1 enzyme at 23 °C for 10 min. Then 80 μM IP4 was added, and the reaction was incubated at 37 °C for 10 min prior to the addition of malachite green containing stop solution. The wells were read at 650 nM, and enzyme activity is expressed as fold increase over the activity seen in the EtOH control wells.[5]

hallmarks of nonribosomal peptide biosynthesis.[12] Hppa has been previously reported as an acyl substituent in marinoid E, an iridoid glucoside isolated from the mangrove plant *Avicennia marina*.[13] Biogenetically, Hppa appears to be derived from a (*E*)-cinnamoylCoA residue that has been extended with an acetate unit to give a β-ketoester. Non-stereoselective reduction of the ketone would lead to the opposite configurations observed at the Hppa-C-3 site of macrolactonization in turnagainolides A (**1**) and B (**2**). We have not been able to find other examples of natural cyclic depsipeptides co-occurring as epimers at the macrocyclic lactone carbinol carbon.

Vervoort et al. have recently reported the isolation of EGM-556 (**18**) from cultures of the marine fungal isolate *Microascus* sp.[14] EGM-556 (**18**) has the same constitution as turnagainolides A (**1**) and B (**2**), but the configuration of one of the two valine residues has been assigned as *R*.[14] The positions of the *R* and *S* valine residues in EGM-556 (**18**) and the configuration at C-3 of the Hppa residue were not assigned. Comparison of the NMR data for EGM-556 (**18**) with the NMR data recorded for turnagainolide A (**1**), and a reversed-phase HPLC comparison of authentic samples of both compounds, showed that they are identical, requiring that the structure of EGM-556 should be revised to **1**.[15] It is also interesting that EGM-556 is produced by a fungal culture and the turnagainolides are produced by a *Bacillus* sp. culture. We previously found that rhizoxins are produced by a marine Pseudomonad, even though all previous reports of their isolation were from fungal cultures.[16] Later it was shown that rhizoxin production by cultures of fungi in the genus *Rhizopus* was due to the presence of a symbiotic bacterium identified as a *Burkholderia* sp.[17] Therefore, it is possible that the production of EGM-556 by *Microascus* cultures is also related to the presence of a symbiotic/associated bacterium.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Jasco P-1010 polarimeter with sodium light (589 nm). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-600 spectrometer with a 5 mm CPTCI cryoprobe. ^{1}H chemical shifts are referenced to the residual DMSO-d_6, $CDCl_3$, or acetone-d_6 signal (δ 2.49, 7.24, or 2.04 ppm, respectively), and ^{13}C chemical shifts are referenced to the DMSO-d_6, $CDCl_3$, or acetone-d_6 solvent peak (δ 39.5, 77.0, or 206.0 ppm, respectively). Low- and high-resolution ESI-QIT-MS data were recorded on a Bruker-Hewlett-Packard 1100 Esquire−LC system mass spectrometer. Reversed-phase HPLC purifications were performed on a Waters 600E System Controller liquid chromatography system attached to a Waters 996 photodiode array detector with a CSC-Inertsil 150A/ODS2 column. All solvents used for HPLC were Fisher HPLC grade.

Isolation and Cultivation of Strain RJA2194. Strain RJA2194, identified as a *Bacillus* sp., was isolated from a sediment sample collected on Oct 11, 2007, at a depth of 104 m near Turnagain Island in Howe Sound, British Columbia, at N 49°,31.473°; W 123°,59.014° and grown on ISP2 at 15 °C. NCBI blastn analysis of the partial 16S rRNA sequence of *Bacillus* sp. RJA2194 (deposited in GenBank with an accession no. JF827033) is 99% identical to *Bacillus atrophaeus* strain EHFS2_S04Ha (GenBank accession no. EU071547).

Production cultures of strain RJA2194 were grown as lawns on solid ISP2 medium (per liter: yeast extract, 4 g; malt exract, 10 g; glucose, 4 g; agar, 15 g) in sixty 8 × 12 × 1 inch pans at room temperature (rt) for 14 days. The mature cultures, cells and media together, were lyophilized in the growth pans.

Extraction and Purification. The lyophilized solid agar (8 L) and cells were exhaustively extracted with MeOH (3 × 4 L). The combined MeOH extract was concentrated in vacuo and then partitioned between EtOAc and water. The EtOAc-soluble portion was fractionated on HP20 using gradient elution from H_2O to acetone. The SHIP active fraction (300 mg, 80% acetone) was chromatographed on Si gel eluting with CH_2Cl_2/MeOH (20:1) to yield turnagainolide A (**1**) (40 mg) and turnagainolide B (**2**) (5 mg).

Turnagainolide A (***1***): white powder; $[\alpha]^{20}_D$ −54 (*c* 0.05, 1:1 CH_2Cl_2/MeOH); UV (1:1 CH_2Cl_2/MeOH) λ_{max} (log ε) 202 (4.23), 253 (4.36) nm; ^{1}H and ^{13}C NMR see Table 1; (+)-HRESIMS *m*/*z* $[M + Na]^+$ 579.3152 (calcd for $C_{30}H_{44}N_4O_6Na$, 579.3159).

Turnagainolide B (***2***): white powder; $[\alpha]^{20}_D$ −90 (*c* 0.05, 1:1 CH_2Cl_2/MeOH); UV (1:1 CH_2Cl_2/MeOH) λ_{max} (log ε) 209 (4.14), 252 (4.27) nm; ^{1}H and ^{13}C NMR see Table 1; (+)-HRESIMS *m*/*z* $[M + Na]^+$ 579.3151 (calcd for $C_{30}H_{44}N_4O_6Na$, 579.3159).

Acid Hydrolysis of Compounds 1 and 2. Purified compounds **1** (0.2 mg) and **2** (0.2 mg) were hydrolyzed separately in 0.5 mL of 6 M

HCl at 108 °C for 16 h in screw-top vials. After cooling, the solvent was removed under N_2 and the residue was dissolved in 100 μL of H_2O.

Derivatization of Amino Acids with Marfey's Reagent and HPLC Analysis. To a 0.5 mL vial containing 2.0 μmol of the pure amino acid standard in 40 μL of H_2O was added 2.8 μmol of FDAA in 80 μL of acetone followed by 20 μL of 1 M $NaHCO_3$. The mixture was heated for 1 h at 40 °C. After cooling to rt, 10 μL of 2 M HCl was added, and the resulting solution was filtered through a 4.5 μm filter and stored in the dark until HPLC analysis.

The hydrolysate mixture of **1** (40 μL) and **2** (40 μL) was treated with FDAA as described above, respectively. The resulting solutions were analyzed by employing an Alltech Econosil C_{18} HPLC column eluting with 60% mixed buffer in MeCN (flow rate: 1 mL/min). The mixed buffer was prepared by mixing an aqueous solution of sodium borate, potassium chloride, citric acid, and tris(hydroxymethyl)aminomethane (each 25 mM) and 0.05 M HCl in a 3:2 ratio (pH 2−3).

Retention times in minutes for the derivatized amino acid standards were as follows: (*S*)-Ala 7.94; (*R*)-Ala 9.95; (*S*)-Val 14.83; (*R*)-Val 19.89; (2*S*,3*S*)-Ile 23.90; (2*R*,3*R*)-Ile 41.86. The retention times for compound **1** were 9.94, (*R*)-Ala; 14.75, (*S*)-Val; 23.43 (2*S*,3*S*)-Ile. For compound **2** they were 9.99, (*R*)-Ala; 14.73 (*S*)-Val; 23.57, (2*S*,3*S*)-Ile.

Methanolysis of 1 to Ester 3. Compound **1** (3 mg) was dissolved in 5% NaOMe/MeOH (2 mL) and stirred for 1.5 h at rt. The reaction mixture was neutralized with 1 M aqueous HCl, and then the aqueous phase was extracted three times with EtOAc. The residue, after solvent removal, was purified by C_{18} reversed-phase HPLC using 11:9 MeCN/H_2O (t_R = 12.58 min) to yield compound **3** (1.8 mg).

Compound ***3****:* white powder; $[\alpha]^{20}_D$ −62.5 (*c* 0.12, 1:1 CH_2Cl_2/MeOH); UV (1:1 CH_2Cl_2/MeOH) λ_{max} (log ε) 210 (4.14), 250 (4.22) nm; ^{1}H and ^{13}C NMR see Supporting Information Table S1; (+)-LRESIMS *m*/*z* $[M + Na]^+$ 611.3.

Mosher Esters 5a and 5b. Compound **3** (0.6 mg) was dissolved in pyridine (1 mL), and dimethylaminopyridine (1 mg) and (*R*)-MTPA-Cl (10 μL) were added in sequence. The reaction mixture was stirred for 5 h at rt, and two drops of H_2O were then added. The resulting solution was concentrated under reduced pressure to dryness. The residue was purified by C_{18} reversed-phase HPLC eluting with 13:7 MeCN/H_2O (t_R = 29.72 min) to yield compound **5a** (0.4 mg). In the same fashion, a solution of **3** (0.6 mg) in pyridine was treated with dimethylaminopyridine (1 mg) and (*S*)-MTPA-Cl (10 μL) to afford, after isolation as above (t_R = 29.02 min), 0.3 mg of **5b**. ^{1}H NMR see Table 2.

Compound 7. A flask containing a stir bar and THF (5 mL) under N_2 was cooled to −78 °C, and to this flask was added a solution of LDA in THF (10.0 mL of a 1.8 M solution, 18.0 mmol). EtOAc (1.0 mL, 10.2 mmol) was added dropwise, followed by (*E*)-cinnamaldehyde (2.0 mL, 15.9 mmol). The reaction mixture was stirred for 45 min at −78 °C before being quenched by the addition of saturated aqueous NH_4Cl. The mixture was extracted with CH_2Cl_2, dried in vacuo, and purified by silica gel chromatography (eluent: gradient from 1:1 CH_2Cl_2/hexane to CH_2Cl_2) to give compound **7** (2.17 g, 96%).

Compound 8. To a solution of compound **7** (2.17 g, 9.88 mmol) in THF were added TBDMS-Cl (1.65 g, 10.9 mmol) and imidazole (1.02 g, 15.0 mmol), and the reaction mixture was refluxed for 12 h. The reaction mixture was dried *in vacuo* and purified by Si gel chromatography (eluent: 1:3 CH_2Cl_2/hexane to 1:1 CH_2Cl_2/hexane) to give compound **8** (2.56 g, 78%).

Compound 9. To a solution of compound **8** (2.56 g, 7.65 mmol) in THF (5 mL) and MeOH (5 mL) was added 1 M aqueous NaOH (5 mL), and the reaction mixture was stirred for 3 h at rt. The reaction mixture was acidified with 1 M aqueous HCl and extracted with CH_2Cl_2. The organic layer was dried *in vacuo* and purified by Si gel chromatography (eluent: gradient from CH_2Cl_2 to 1:19 MeOH/CH_2Cl_2) to give **9** (1.64 g, 70%): ^{1}H NMR (400 MHz, acetone-d_6) δ 7.44 (2H, d, *J* = 7.3 Hz, H-7/11), 7.33 (2H, t, *J* = 7.5 Hz, H-8/10), 7.24 (1H, m, H-9), 6.67 (1H, d, *J* = 15.9 Hz, H-5), 6.34 (1H, dd, *J* = 15.9, 6.7 Hz, H-4), 4.83 (1H, m, H-3), 2.57 (2H, m, H-2), 0.91 (9H, s, H-13/14/15), 0.12 (3H, s, H-16), 0.09 (3H, s, H-17); ^{13}C NMR (100 MHz, acetone-d_6) δ 172.2 (C, C-1), 137.8 (C, C-6), 132.8 (CH, C-5), 130.7 (CH, C-9), 129.5 (CH, C-7/11), 128.5 (CH, C-4), 127.4 (CH, C-8/10), 71.7 (CH, C-3), 44.1 (CH_2, C-2), 26.3 (CH_3, C-13/14/15), 18.8 (C, C-12), −4.0 (CH_3, C-16), −4.7 (CH_3, C-17).

Compound 16. To CH_2Cl_2-washed polymer-bound 2-chlorotrityl chloride (572 mg) under N_2 was added Fmoc-(*S*)-Val-OH (330 mg, 0.97 mmol) in CH_2Cl_2 (10 mL), followed by DIPEA (0.3 mL, 1.72 mmol). The reaction mixture was stirred for 1 h at rt before the solid-phase resin was washed with CH_2Cl_2. The solid-phase resin was capped by adding a solution of CH_2Cl_2/MeOH/DIPEA (ratio 17:2:1, 10 mL) and stirring for 1 h at rt. The solid-phase resin was then washed with CH_2Cl_2, followed by DMF. The Fmoc protecting group of (*S*)-Val was removed by treatment with 20% piperidine in DMF (5 mL) for 5 min at rt. This reaction was repeated twice more with 20% piperidine in DMF (5 mL), followed by washing the solid-phase resin with DMF, CH_2Cl_2, and DMF again. The coupling of the next amino acid ((2*S*,3*S*)-Ile) was performed by adding a solution of Fmoc-(2*S*,3*S*)-Ile-OH (330 mg, 0.93 mmol), PyBOP (560 mg, 1.08 mmol), and HOBt (2.0 mL of a 0.5 M solution, 1.0 mmol) in DMF (10 mL), followed by the addition of DIPEA (0.2 mL, 1.15 mmol), and stirring for 1 h at rt.

The above deprotection/coupling procedure was repeated for (*R*)-Ala and (*S*)-Val using approximately 1 mmol of each reagent as described above. The final coupling step was performed by adding a solution of **9** (306 mg, 1.0 mmol), PyBOP (560 mg, 1.08 mmol), and HOBt (2.0 mL of a 0.5 M solution, 1.0 mmol) in DMF (10 mL), followed by the addition of DIPEA (0.2 mL, 1.15 mmol), and stirring for 1 h at rt. The solid-phase resin was then washed with DMF, followed by THF. Deprotection of the silyl protecting group was accomplished by treatment with a solution of TBAF in THF (10 mL of a 0.2 M solution, 2.0 mmol) for 1 h at rt. This reaction was repeated once more for an additional hour, followed by washing the solid-phase resin with THF and then CH_2Cl_2.

Cleavage from the solid-phase resin was accomplished by treatment with CH_2Cl_2/PDT/TFA (ratio 98:1:1, 10 mL) for 1 h at rt under N_2. This procedure was repeated for an additional hour at rt, followed by washing the solid-phase resin with CH_2Cl_2 and MeOH. The washings were combined and dried *in vacuo* to give the crude linear peptide (**16**). The peptide was washed with CH_2Cl_2 and purified by Si gel chromatography (eluent: gradient from 1:9 MeOH/EtOAc to 1:3 MeOH/EtOAc) to give the linear peptide **16** (387 mg) as a mixture of diastereomers.

Synthetic Turnagainolides A (1) and B (2). A solution of DMAP (208 mg, 1.70 mmol), pTSA monohydrate (162 mg, 0.85 mmol), and DIC (0.75 mL, 4.79 mmol) in $CHCl_3$ (200 mL) was heated to reflux. To this reaction mixture at reflux was added a solution of the linear peptide (**16**) (95.8 mg, 0.167 mmol) in DMF (20 mL) dropwise over the course of 10 h, and heating was continued at reflux overnight. The reaction mixture was then cooled, dried *in vacuo*, and purified by Si gel flash chromatography (eluent: gradient from CH_2Cl_2 to 1:19 MeOH/CH_2Cl_2) and Si gel chromatography (eluent: gradient from CH_2Cl_2 to 1:1 acetone/CH_2Cl_2) to give crude turnagainolide B (**2**) (31.5 mg, 33%) and crude turnagainolide A (**1**) (14.0 mg, 15%) (combined yield = 48%). Portions of the crude turnagainolides A and B were further purified separately by C_{18} reversed-phase HPLC (eluent: 3:2 MeOH/H_2O) to give pure turnagainolide A (**1**) and pure turnagainolide B (**2**). Turnagainolide A (**1**) was converted to the Mosher esters **5a** and **5b** as described above for the natural product **1**.

Methanolysis of Synthetic 2 to Ester 4. Synthetic **2** (6.1 mg) was dissolved in 2 mL of 5% NaOMe/MeOH and stirred overnight at rt. The reaction was acidified with 1 M aqueous HCl, diluted with H_2O, and extracted with CH_2Cl_2. The organic layer was purified by C_{18} reversed-phase HPLC using 3:2 MeOH/H_2O to give compound **4** (3.2 mg).

Mosher's Esters 6a and 6b. Compound **4** (0.4 mg) was dissolved in CH_2Cl_2 (1 mL), and to this solution were added one crystal of dimethylaminopyridine and (*R*)-MTPA-Cl (10 μL). The reaction mixture was stirred overnight at rt and purified by Si gel chromatography using a gradient from CH_2Cl_2 to 1:3 acetone/CH_2Cl_2 to yield compound **6a**. In the same fashion, a solution of **4** (0.4 mg) in CH_2Cl_2 (1 mL) was treated with one crystal of dimethylaminopyridine and (*S*)-MTPA-Cl (10 μL). The reaction mixture was stirred overnight at rt and purified by Si gel chromatography using a gradient from CH_2Cl_2 to 1:3 acetone/CH_2Cl_2 to yield **6b**.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra and data for compounds **1** to **3** are available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Bunney, T. D.; Katan, M. *Nat. Rev. Cancer* **2010**, *10*, 342–352.
(2) Martelli, A. M.; Evangelisti, C.; Chiarini, F.; Grimaldi, C.; Manzoli, L.; McCubrey, J. A. *Expert Opin. Investig. Drugs* **2009**, *18* 1333–1349.
(3) Harris, S. J.; Parry, R. V.; Westwick, J.; Ward, S.G. J. *Biol. Chem.* **2008**, *283*, 2465–2469.
(4) Pereira, A. R.; Strangman, W. K.; Marion, F.; Feldberg, L.; Roll, D.; Mallon, R.; Hollander, I.; Andersen, R. J. *J. Med. Chem.* **2010**, *53*, 8523–8533.
(5) Ong, C. J.; Ming-Lum, A.; Nodwell, M.; Ghanipour, A.; Yang, L.; Williams, D. E.; Kim, J.; Demirjan, L.; Qasimi, P.; Ruschmann, J.; Cao, L. P.; Ma, K. W.; Chung, S. W.; Duronio, V.; Andersen, R. J.; Krystal, G.; Mui, A. L. F. *Blood* **2007**, *110*, 1942–1949.
(6) Yang, L.; Williams, D. E.; Mui, A.; Ong, C.; Krystal, G.; van Soest, R.; Andersen, R. J. *Org. Lett.* **2005**, *7*, 1073–1076.
(7) Kennah, M.; Yau, T. Y.; Nodwell, M.; Krystal, G.; Andersen, R. J.; Ong, C. J.; Mui, A. L. F. *Exp. Hematol.* **2009**, *37*, 1274–1283.
(8) Williams, D. E.; Amiani, A.; Dewi, A. S.; Patrick, B. O.; van Ofwegen, L.; Mui, A. L. F.; Andersen, R. J. *Aust. J. Chem.* **2010**, *63*, 895–900.
(9) Marion, F.; Williams, D. E.; Patrick, B. O.; Hollander, I.; Mallon, R.; Kim, S. C.; Roll, D. M.; Feldberg, L.; van Soest, R.; Andersen, R. J. *Org. Lett.* **2006**, *8*, 321–324.
(10) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
(11) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(12) Jones, A. C.; Monroe, E. A.; Eisman, E. B.; Gerwick, L.; Sherman, D. H.; Gerwick, W. H. *Nat. Prod. Rep.* **2010**, *27*, 1048–1065.
(13) Sun, Y.; Ouyang, J.; Deng, Z.; Li, Q.; Lin, W. *Magn. Reson. Chem.* **2008**, *46*, 638–642.
(14) Vervoort, H. C.; Draskovic, M.; Crews, P. *Org. Lett.* **2010**, *13*, 410–413.
(15) An authentic sample of EGM-556 was provided by Prof. P. Crews.
(16) Roberge, M.; Cinel, B.; Anderson, H. J.; Lim, L.; Jiang, X. X.; Xu, L.; Bigg, C. M.; Kelly, M. T.; Andersen, R. J. *Cancer Res.* **2000**, *60*, 5052–5058.
(17) Partida-Martinez, L. P.; Hertweck, C. *Nature* **2005**, *437*, 884 888.